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EFFECT OF COOKING AND STORAGE CONDITIONS ON CIPROFLOXACIN AND GENTAMICIN RESIDUES IN BALADI AND BROILER CHICKENS MEAT

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ABSTRACT

The great invasion of antibiotics in our food has become a tough problem due to the misuse of them in our farms for treatment of infection or as a food additives to improve growth and poultry output inducing sever harmful health problems on human. Despite using antibiotics as growth promotors in Egypt as a developing country, there is shortage in studies concerning gentamicin and ciprofloxacin residues in poultry. This study aimed to evaluate the residues of two of the most commonly used antibiotics in the poultry farms and effect of cooking and freezing on these residues. A total of 120 chicken samples were collected from chicken markets at Fayoum Governorate, Egypt. The results revealed that the incidence of antibiotic residues detected in all liver samples of both baladi and broiler chicken samples, while breast and thigh muscle the result was different. Ciprofloxacin and Gentamicin residues in positive samples were detected and quantitified using HPLC. The liver had the highest residual level of ciprofloxacin and gentamicin residues, whereas the breast and thigh muscles had the lowest concentrations. Cooking methods and freezing reduce antibiotic residues in experimentally spiked chicken meat with varying percentages 57.9 and 31.3% by boiling, 92.3% and 86.2% by microwaving, 44.9 and 47.5 % by freezing for one month, and 75.14 % and 81.2%by freezing for2 months in ciprofloxacin and gentamicin respectively, also the antibiotic was detected in broth of cooked meat samples. The estimated daily intakes for liver in Baladi and broiler was exceeded the acceptable daily intake (ADI) of ciprofloxacin residues. The EDI of gentamicin residues were higher than the ADI in all broiler samples, while all Baladi samples were within the ADI. Despite the fact that the calculated estimated daily intakes (EDIs) for antibiotics presented showed lower exposure levels than the fixed values of acceptable daily intakes (ADIs), the presence of these residues, even at lower ADIs, predisposes consumers to drug resistance and allergic reactions in the long run.

KEYWORDS: ciprofloxacin, gentamicin, residues, HPLC, chicken meat.

1. INTRODUCTION

Chicken is one of the most important protein source among the food products of animal origin because of higher nutritive value, less content of fat and the lower cost of production. In Egypt, chicken meat production is on the rise, intensive poultry farming has grown in recent years, with white meat production increasing dramatically, making the price of this product reasonable and appealing to consumers.[1]

Antibiotics are commonly used in the veterinary industry as growth promoters in poultry feed at low doses to improve feed conversion, increase growth rates, and minimise mortality in growing chickens, while much greater dose levels were administered for prophylaxis or treatment. Approximately 80% of all food-producing animals are medicated for some or all of their lives.[2] Fluroquinolones are powerful options among the antimicrobial agents used for curing bacterial caused poultry diseases.[3] Ciprofloxacin is effective for microorganisms resistant to aminoglycosides, tetracycline, macrolids and β-lactams.[4] Gentamicin is an aminoglycoside antimicrobial with bactericidal activity against most Gram-negative bacteria. It has problems of toxicity and resistance when misused. Gentamicin residue in foods of animal origin is of great public health importance worldwide, mainly due to the health risk associated with it, especially its nephrotoxic and ototoxic effect, and it is also known to deleteriously affect the immune system leading to the development of drug-resistant microorganisms in animals and humans.[5]

Antibiotics are still widely used in the veterinary field and on poultry farms with little regulation and an

ignorance of withdrawal periods in slaughtered poultry resulting in residue deposition in meat tissues and other animal-derived products, causing toxicological, microbiological, and immunological effects.[6]

Therefore, the present study was under taken to investigate the prevalence of antibiotic residues in random marketed chicken meat with effect of cooking and freezing on antibiotic residues after experimentally spiking antibiotic (ciprofloxacin and gentamicin) in muscular tissue of chicken meat. Calculation of the estimated daily intake (EDI) and Hazard index (HI) of antibiotic residues for consumers.

2. MATERIALS AND METHOD

2.1 Chemicals

Phosphate buffer, pH 8.0 (± 0.1), Methanol (MeOH) and Trichloroacetic Acid (TCA) LC-grade, with purity of 99.9%, Hydrochloric acid (HCl) concentrated, Ethylenediamine tetraacetic acid, disodium salt (Na2EDTA) and sodium Hydroxide (NaOH) analytical grade with purity of 98% were purchased from Sigma (St. Louis, MO, USA).

Water, LC grade -House distilled water passed through Waters MilliQ deionization system then passed through 0.45 mm filterwas purchased from Cronus Filter (UK). Acetic acid, glacial (HOAc) analytical grade with purity of 98% and Potassium phosphate monobasic (KH2PO4) LC-grade with purity of 99.9% were purchased from fisher scientific (Leicestershire, UK).

Gentamycin sulfate and ciprofloxacin standards were purchased from Sigma (St. Louis, MO, USA) with purity of 99%.

2.2 Microorganisms Used for the Bioassay System

a) *Staphylococcus epidermidis (S. epidermidis)*, ATCC 12228 (SE)
b) *Bacillus subtilis* spores (*B. subtilis)*, ATCC 6633 (BS)

2.3Antibiotic sensitivity disks

1) ciprofloxacin (Cip 5ppm)
2) Gentamycin (G10ppm)

2.4 Collection of samples

A total of 120 samples (60baladi) and (60 broiler) of breast muscles, thigh muscles and chicken liver tissues 20 for each were collected from poultry markets at period from October 2021 to January 2022. Each sample was wrapped on a separate labelled poly ethylene bag and transported in an insulated ice box at 4°C ± 1°C to central lab of Animal Health Research Institute, Dokki, Giza. All samples were analyzed within 5days not more for detection of flouroquinolones and aminoglycoside residues according to United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) (2011).

2.5 Sample Preparation

- In one labeled bag, 5g of tissue sample was added to 20 ml phosphate buffer at pH 7.2, then muscle homogenized for 60seconds and liver for 30seconds. Mix, extract.

2.6 Performing the plates of Bioassay (Qualitative evaluation)

Two hundred µl buffered sample extract was inoculated into wells and 200 µl of antibiotic standard in one well. Plate for flourquinolones prepared with *B. subtilis* (ATCC 6633) incubated at 29ºC and plate for aminoglycosides prepared with S. epidermidis incubated at 37 ºC for 16-18 hours.

The inhibition zone (IZ) of each plate (or absence of zones) on each of the two plates were read, recorded and compared with the standard curve.

2.7Quantitative evaluation of antibiotic residues by HPLC

- Estimation of two antibiotics (Gentamicin and Ciprofloxacin) residues by HPLC (high performance liquid chromatography) in the positive collected tissue samples.

2.7.1 Ciprofloxacin residues

Ciprofloxacin residues were determined as described by.[7] Briefly, 2 g of ground meat was weighed in a 50 ml polypropylene centrifuge tube, homogenized for 2 min and then 8 ml of 5% Trichloroacetic Acid (TCA) was added. Vortex for 1 minute, rotary agitated for 10minutes, then centrifuged for 5 minutes at 14000 rpm. After filtering through a 0.45 µm nylon filter, 25 µl of solution was injected into HPLC for analysis. The mobile phase consisting of 0.1% formic acid: Acetonitrile operated in agradient mode at a flow rate of 0.3 ml/min.

2.7.2 Gentamicin residues

The residues of gentamicin were determined using the method of.[8] In brief, 2 g of ground meat was weighed in a 50 ml polypropylene centrifuge tube, homogenized for 2 min, then 10 ml phosphate buffer/TCA added, homogenized for 30-60 seconds, shake vigorously for 10 minutes and centrifuged at 4000 rpm for 10 minutes. Decant the supernatant solution into a clean 50 ml tube. The tissue pellet was re-extracted using 10 mL of extracting solution, and the supernatants were mixed together. pH of the combined extract was adjusted to 7.5–8.0 (This requires approximately 0.16 ml 30% NaOH) and repeat centrifugation. Cleanup was performed by SPE cartridge (Agela Cleaner C18) attached to vacuum manifold using 5 ml methanol followed by 5 ml water. The residues were eluted at 1–3 ml/minute with 3 ml 10% acetic/methanol in a 15 ml glass centrifuge tube, and centrifuged at 2500 rpm for 10 minutes. The extract was transferred to a 0.6 ml polypropylene micro-centrifuge tube and centrifuged at approximately 16000 rpm for 10 minutes. After filtering through a 0.45 µm nylon filter, 50 µl of solution was injected into HPLC for analysis. The mobile phase

consisting of 0.1M TFA acid: methanol, 92:8 was pumped at a flow rate of 1.5 ml/min.

2.8 Effect of cooking and freezing storage on chicken samples inoculated with ciprofloxacin and gentamicin

Fifty samples of chicken breast muscles proved negative for ciprofloxacin and gentamicin were spiked with known concentrations of ciprofloxacin and gentamicin. The samples were subdivided into 5 groups (10 samples each). The first group was cooked by boiling, the second by microwaving, the third stored by freezing at -18 ºC for one month, the fourth stored freezed for 2 months at-18 ºC and the last group was left raw.

- Muscle samples subjected to the technique recommended by.[9]

2.8.1 Boiling

- A twenty g sample (chicken ball) obtained from local market which was measured to confirm that no antibiotics were presented, then mixed accurately with 5ml of antibiotic then immersed in a water bath at 100°C boiled for 15-20 minutes for muscles, 10 minutes for liver. The cooked meat had a "well done" appearance on the outside by reaching grayish coloration of meat, brownish coloration of liver, juicy and by measuring internal temperature > 71Cº by using digital thermometer.

-A 20 ml of chicken meat broth also taken for detection of antibiotic residues in it.

2.8.2 Microwaving

Chicken meat ball were micro waved at 800 W with 1 spoonful of sunflower oil for 5-10 minutes for muscles and 3-5 minutes for liver. The cooked meat had a "well done" appearance on the outside by reaching grayish coloration of meat, brownish coloration of liver, juicy and by measuring internal temperature > 71Cº by using digital thermometer.

2.8.3 Freezing

Chicken meat samples were preserved after spiking with antibiotic in deep freezer at -18°C for one month and 2 months then the samples were measured again to detect the amount of drugs residues.

2.9 Statistical Analysis

All values are presented as means ± standard error. The antibiotic residues were expressed as part per million (ppm). The statistical analysis for this study was performed using (SPSS 16, 2007). The data was evaluated for substantial changes in antibiotic residues in chicken meat as a result of cooking techniques.

3. RESULTS AND DISCUSSION

Antibiotic residues in chicken and their edible offals have a widespread concern among national and international public health organizations as humans absorb varying amounts of these medications.[10] The findings of the microbiological inhibition experiment revealed that the inhibition zone (I.Z) produced by liver samples was higher than that of thigh and breast samples in both baladi and broiler chicken. This result is parallel to those achieved by[11] who detected antibiotic residues using Microbiological Inhibition Assay Technique on120 random frozen chicken samples at Dakahlia Governorate, Egypt. Their results revealed that (I.Z) produced by liver samples was higher than that of thigh and breast.

Table 1: Mean values of ciprofloxacin and Gentamicin residues (µg/g) in baladi and broiler chicken meat samples.

	Baladi		Broiler	
	Ciprofloxacin	**Gentamicin**	**Ciprofloxacin**	**Gentamicin**
Liver	36.96 ± 4.192*	26.94 ± 2.745*	49.23 ± 4.544*	102.2 ± 13.87*
Breast	8.965 ± 2.735*	4.166 ± 1.819	10.26 ± 3.311*	74.44 ± 14.26*
Thigh	17.11 ± 3.015*	5.277 ± 1.828	17.92 ± 3.311*	86.66 ± 8.520*

(*) significant differences

p <0.05 by using ANOVA test

Accepted and un accepted samples according to the maximum residual limits (MRL) established by National organization of Food Safety Resolution (2020)

Regarding the residual levels of ciprofloxacin and gentamicin(Table 1) the highest mean values of ciprofloxacin and gentamicin residues were recorded in liver, thigh then breast respectively, with mean values 36.96 ± 4.192, 49.23 ± 4.544(µg/g) for ciprofloxacin residues in liver samples of baladi and broiler chicken respectively, and 26.94 ± 2.745, 102.2 ± 13.87(µg/g) for gentamicin residues in liver samples of baladi and broiler chicken respectively. More over broiler chicken meat has the highest concentration of antibiotic residues comparing with the Baladi chicken meat. These results were in harmony with results reported by[12] who recorded the highest concentration of ciprofloxacin in liver,[13] who concluded that liver and kidney were the major sites showing the highest proportion of ciprofloxacin quinolones residues, and[14] who showed that the concentration of ciprofloxacin residues were higher in liver than in muscles, and[15] who stated that ciprofloxacin residues were detected in chicken tissues "thigh, breast and liver " by using HPLC. Their results reflected a significant difference variation among liver, breast and thigh in examined samples as *p* values <0.05 indicating that liver was the site of accumulation of ciprofloxacin residues with highest residual level of ciprofloxacin found in liver with mean value 211.1+/-20.6 µg/kg Whereas the lowest concentrations were

found in breast then thigh muscles. Whereas these achieved results were quietly higher than those achieved by[16] who detected the ciprofloxacin residues in chicken samples by 44% in liver, 42% in kidneys, 34% in thigh muscles and 30% in breast muscles. On other words our obtained results were disagreed with those reported by[17] who reported that ciprofloxacin residues were not found in the breast muscle, but detected in the liver at concentrations below MRL. This finding is in agreement with[18] who identified gentamicin residues in goat muscle, liver, and kidney samples, with the highest amount of gentamicin residue found in the kidney, followed by liver and muscle samples.

Table (2): Ciprofloxacin and gentamicin residues in the examined chicken samples compared with maximum residual limits (MRL).

Ciprofloxacin					
Organization			**Liver**	**Breast**	**Thigh**
National organization of Food Safety Resolution (2020)	MRL (µg/g)		0.4	0.2	0.2
	Within MRL	No.	0	8	6
		%	0	40	30
	Above MRL	No.	20	12	14
		%	100	60	70
European Commission EU (2010)&European Medicine Agency EMEA (2002)	MRL (µg/g)		0.2	0.1	0.1
	Within MRL	No.	0	8	6
		%	0	40	30
	Above MRL	No.	20	12	14
		%	100	60	70
Gentamicin					
Organization			Liver	Breast	Thigh
National organization of Food Safety Resolution (2020)	MRL (µg/g)		0.6	0.6	0.6
	Within MRL	No.	0	12	10
		%	0	60	50
	Above MRL	No.	20	8	10
		%	100	40	50
Health Canda (2015)	MRL (µg/g)		0.1	0.1	0.1
	Within MRL	No.	0	12	10
		%	0	60	50
	Above MRL	No.	20	8	10
		%	100	40	50

According to the maximum residual level established by[19] &[20] and[21] all liver samples were above the MRL of ciprofloxacin residues while in thigh and breast 70% and 60% of samples were exceeded the MRL respectively. More over100% of liver samples, 50% of thigh samples and 40% of breast samples were exceeded the MRL of gentamicin residues established by[19] and[22] (table 2). This result was nearly similar those achieved by[23] who stated that 33.7%, 47.3%, 60% and 77.3% respectively of examined chicken thigh, breast, liver and kidney samples were exceeded the MRL of.[21]

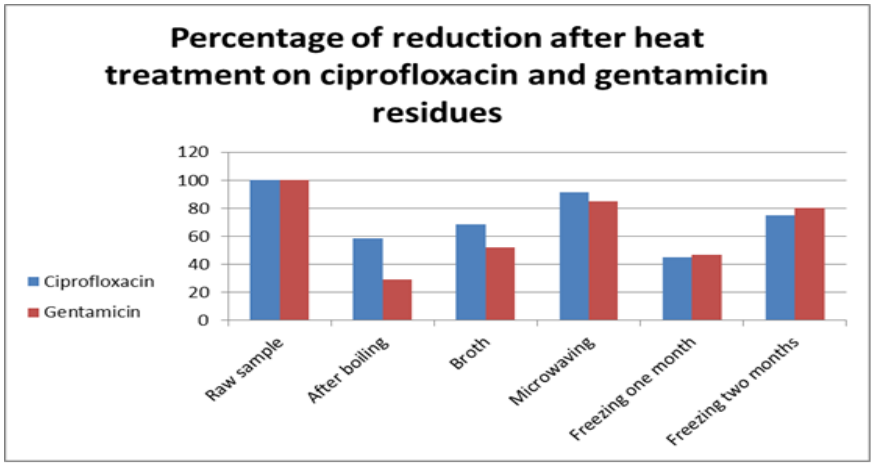

Figure 1: Effect of some heat treatment on the mean values of ciprofloxacin and gentamicin residues (µg/gm) in the examined experimental samples.

Effect of cooking methods on antibiotic residues

The majority of animal-derived food is boiled or processed with food additives to improve digestibility, sensory qualities, appealing quality, and shelf-life.[24] HPLC has recently been used to assess changes in veterinary medication residue during processing.[25] The percentage of reduction after cooking and freezing storage calculated by equation R= (concentration of raw – concentration in cooked or freezed) ÷ (concentration in raw) × 100.

Cooking by boiling resulted in considerable reductions in ciprofloxacin and gentamicin residues, with reduction percentages of 57.9% and 31.3 percent, respectively, in this investigation as shown in (figure 1).

These results were slightly lower than those published by[26], who reported that boiling can reduce gentamicin residues in poultry muscles by up to 90%.[27] revealed a 100 % reduction in residual gentamicin concentration after boiling, which is higher than the findings of this investigation. The antibiotic residue also detected in the broth of chicken meat with a reduction percentage 69.2 % for ciprofloxacin and 52.5 % for gentamicin. This result is in parallel to those reported by.[2] The result revealed that cooking by microwaving induce a remarkable reduction on ciprofloxacin and gentamicin residues with a reduction percentages reach to 92.3 % and 86.2% respectively. These results were in parallel to[28] who recorded a significant reduction in gentamicin residues in muscle samples of poultry reach to 56.2%.

Effect of freezing

Ciprofloxacin residues degraded to their metabolites by freezing process at -18ºC for one month by 44.9% and for 2 months by75.14% in chicken tissues samples. These results were slightly lower than those obtained by[29] who stated that frozen storage (-18°C) for broiler had a marked effect on ciprofloxacin residue in breast, thigh, liver and fat samples. She concluded that in liver samples the drug failed to be detected at 5th week of storage at -18°C whereas in fat and breast muscle samples, no drug residue could be detected at 4" week and at 3 week of storage, thigh muscles samples were free from any drug residue.

Table 3: Estimated daily intake (EDI) of ciprofloxacin and gentamicin residues (µg.kg-1body weight) from consumption of Baladi and broiler chicken meat samples

Chicken samples	EDI(µg/kg/day) of Ciprofloxacin		ADI µg/kg/day)	HI of Ciprofloxacin		EDI(µg/kg/day) of Gentamicin		ADI (µg/kg/day)	HI of Gentamicin	
	Baladi	Broiler		Baladi	Broiler	Baladi	Broiler		Baladi	Broiler
Liver	14.46	19.27	7.1	2.036	2.71	10.54	40.01	20	0.527	2.005
Breast	3.507	4.017		0.49	0.56	1.630	29.13		0.08	1.4
Thigh	6.701	7.015		0.94	0.98	2.065	33.92		0.1	1.6

ADI (µg/kg/day) of Ciprofloxacin according to AUSTRALIAN GUIDELINES, (2008).
ADI (µg/kg/day) of Gentamicin according to FAO (2010).

The antibiotic residues estimated daily intake (EDI) was calculated to determine the extent to which persons have been exposed to its residues in chicken meat. Calculating estimated daily intakes (EDIs) for antibiotic based on the integration of data from analysis of antibiotics, chicken consumption rates and body weight of Egyptian adults. EDI (µg/kg/day) for gentamicin and ciprofloxacin obtained using the following equation: EDI= (Cm×FIR)/BW, described by the Human Health Evaluation Manual where Cm is concentration of antibiotic in sample (mg/kg.wet weight)[30], calculated FIR is the food (chicken meat) ingestion rate in Egypt which was 27.4 g/day; BW is Egyptian adults' body weight, which was estimated to be 70 kg.The hazard index (HI) is the ratio of EDI to ADI (HI = EDI/ADI) to estimate the potential health risk for each antibiotic. When the HI is less than 1 (HI<1), there is no risk associated with the consumption of poultry products. However, when 1≤HI≤10 indicates that risk exists, but does not require immediate action, while when HI > 10, it shows that the risk is unacceptable.[31] Our result revealed that EDI for cipro floxacin residues were 14.46 , 19.27 in liver, 3.507, 4.017 in breast and 6.701, 7.015 µg.kg-1body weight in thigh muscle samples of baladi and broiler chicken respectively, while, EDI for gentamicin residues were 10.54, 40.01in liver, 1.630, 29.13 in breast and 2.065, 33.92 µg.kg-1body weight in thigh muscle samples of baladi and broiler chicken respectively. The estimated daily intakes for liver in Baladi and broiler was exceeded the acceptable daily intake (ADI) of ciprofloxacin residues (7.1 µg/kg/day) established by.[32] while, (EDI) in breast and thigh muscle samples were within the acceptable daily intake. Theses result is nearly similar those achieved by[33] who estimated dietary intakes of ciprofloxacin in chicken meat which was 44.90ng/kg body weight/day resulting in the hazard index of 0.0063 for the consumption of ciprofloxacin residues in chicken meat by the adult population in Indonesia. The EDI of gentamicin residues were higher than the ADI (20 µg/kg/day) recommended by[30] in all broiler samples, while all Baladi samples were within the ADI. These results are higher than those achieved by[28] who recorded the estimated daily intake of gentamicin residues in liver and thigh muscle 0.37 and 0.26 respectively.

The elevation of antibiotic residues in liver samples could be explained by the fact that the liver's microsomal enzymes are involved for antibiotic metabolism and detoxification. The significant variation among thigh,

breast and liver in examined chicken samples indicated that liver is the harbor site of ciprofloxacin residues. The highest residual level of ciprofloxacin in liver whereas the lowest concentration were in breast then thigh muscles. This refers to liver is the organ of metabolism in the body, all metabolic processes occur in liver beside detoxification of these toxic substances then drug metabolites excreted through bile and kidneys. The elevations in our study may be due to chicken samples may came from farms which had wrong excessive use of antibiotic program, without following the recommended dose and method of drug administration as applied drug in both drinking water and mixing with feed, neglecting withdrawal periods of the ciprofloxacin. Also may be due to randomly administration of ciprofloxacin before transportation to avoid emergency death of chicken. Broiler chicken meat has the highest concentration of antibiotic residues comparing with the Baladi chicken meat, this could be due to a variety of factors including drug dosage, bird type and age, food, disease condition, inadequate treatment, off-label drug use, withdrawal duration, and route of administration.[34]

concerning effect of cooking, the differences in our results comparing with other studies could be due to various factors such as: test method, variable apparatus, cooking temperature or cooking time which involved during the process of cooking may also affect the antibiotics residue concentration.[35] During boiling treatment, some quinolones were extracted into broth and in cooking procedure with water loss, quinolone residues were increased. The decrease in antibiotic residue concentration during boiling could be attributed to antibiotic residues migrating from the chicken meat to the boiling water. Cooking processes cannot destroy the total amounts of antibiotic residues but it could only decrease their amount in boiled samples as compared to raw samples.[2]

The moisture in the chicken meat leached out and was replenished by the oil during the microwaving procedure. Antibiotics could be removed from chicken flesh through the exchange of moisture and oil.[36], and this explain why the microwaving process had a remarkable reduction effect on the antibiotic residues. The reduction in concentration of gentamicin residues after microwaving may be attributed to the nature of gentamicin which is water soluble, highly polar compounds, contain no chromophores or fluorophores and thermally labile.[37] The drop in antibiotic residue concentrations after boiling and microwaving was attributed to antibiotic residue migration from the chicken flesh slices to the cooking medium (water and oil).[2] The reduction of ciprofloxacin concentration after freezing may refers to ciprofloxacin are water soluble molecules and little binding to plasma protein which on freezing with slow thawing, small ice particles may increase exposure of protein to ice liquid surface area as protein denaturation occurred ciprofloxacin molecules may left protein, entrapped with ice crystals and descend together in the thawing fluid.[38] [39]

4. CONCLUSION

Liver samples have the highest antibiotic residual level. Broiler chicken samples have the highest concentration of antibiotics residues comparing with Baladi chicken samples. Microwaving was the most effective in reduction of antibiotic residues. It worth to be noted that increase period of storage to 2 months freezing at -18c could increase the percentage of reduction in antibiotic residues. In general, cooking techniques do not ensure complete breakdown of antibacterial medication residues found in poultry and can only reduce their levels. Because the majority of residues are expelled from tissue into the cooking fluid during the boiling process, it is recommended to remove any meat broth to prevent residue exposure. Although calculated estimated daily intakes (EDIs) and (HI) for antibiotics presented showed lower exposure levels than the fixed values of acceptable daily intakes (ADIs), the presence of these residues even in lower ADIs on long run predisposes consumers to drug resistance and allergic reactions.

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5. REFERENCES

1. Alloui N. Situation actuelle et perspectives de modernisation de la filiére avicole en Algérie. Communication. Neuviémes journées de la Recherche Avicole, Tours, France, 2011.
2. Lolo M, Pedreira S, Miranda J M, Vázquez B I, Franco C M, Cepeda A, Fente C. Effect of cooking on enrofloxacin residues in chicken tissue. *Food Addit. Contam*, 2006; 23: 988-993.
3. Trouchon T, Lefebvre S. A review of enrofloxacin for veterinary use. Open J. Vet. Med, 2016; 6: 40-58.
4. Sultan I A. Detection of enrofloxacin residues in livers of livestock animals obtained from a slaughter house in Mosul City. J. Vet. Sci. Technol, 2014; 5: 2-4.
5. Tan X, Jiang Y W, Huang Y J, Hu S H. Persistence of gentamicin residues in milk after the intrama-mmary treatment of lactating cows for mastitis. *J. Zhejiang Univ. Sci. B*, 2009; 10(4): 280-284.
6. Hussein M A, Khalil S. Screening of Some Antibiotics and Anabolic Steroids Residues in Broiler Fillet Marketed in El-in El-Sharkia Governorate. Life Science J., 2013; 10: 2111-2118.
7. Verdon E, Couëdor P, Sanders P. Validation of a Multi Quinolone, Multi-Matrix, Multi-Species Method for the determination of Quinolone residues by HPLC with fluorescence detection. AFSSA;French agency for food safety,

EuroresidueV, Noodwijkerhout, The Netherlands, May 10-12, 2004.
8. Plozza T, Trenerry V C, Zeglinski P, Nguyen H, Johnstone P. The confirmation and quantification of selected aminoglycoside residues in animal tissue and bovine milk by liquid chromatography tandem mass spectrometry. Inter. Food Research J., 2011; 18: 1077-1084
9. Nguyen V, MuQing L, Khan M A, ChunBao L, Zhou G H. Effect of cooking methods on tetracycline residues in pig meat. African Journal of Pharmacy and Pharmacology, 2013; 7(22): 1448-1454.
10. Nafisi M R, Kalhor H, Zamanzad B , Karimi A, Farokhi E , Validi M. Comparison of agar screen and duplex-PCR in determination of methicillin resistant Staphylococcus aureus (MRSA) strains isolated from nose of personnel in Hajar hospital of Shahre-kord. *Arak Univ. Med. Sci. J.*, 2008; 11: 94-101.
11. Morshdy A M, El-Atabany A I, Rezk M A. Studies on Antibiotic Residues in Imported and Locally Frozen Chicken Meat. 2nd Conference of Food Safety, Suez Canal University, Faculty of Veterinary Medicine, 2015; Volume I Page 168-177.
12. El-Gendi A Y, El-Banna H A , Norag M A, Gaber M. Disposition Kinetics of danofloxacin and ciprofloxacin in broiler chickens. Deutsche Tierarztliche Wochenschrift, 2001; 108: 429-434.
13. Islam A. Detection of antibiotic residues in laying hens using microbial inhibition test (MIC) and thin layer chromatography (TLC) at Chittagong, Bangladesh. MS Thesis on Pharmacology, Department of Physiology, Biochemistry and Pharmacology, Chittagong Veterinary and Animal Sciences University, Bangladesh, 2009.
14. Sarita P , Ferrari G, Bonassa K P D, Coelho M B, Feerriera C R, De Costa H F, Jara J L P, Miguel M C V, Reyes F G R, Eberlin M N, Nogueiraa, G P, Simas RC. High precision and selectivity for quantitation of enrofloxacin and ciprofloxacin in five chicken tissues using solid phase extraction for application in monitoring residues. Analytical Methods, 2015; 7(7): 3291-3297.
15. Hasanen F S , Mousa M M, Mahomud A H , Hassan W M M, Amro F H Ciprofloxacin residues in chicken and turkey carcasses. B V M J, 2016; 31(2): 136-143.
16. Sattar S, Hassan M M, Islam S K M A, Alam M, Faruk M S A, Chowdhury S, Saifuddin, A K M. Antibiotic residues in broiler and layer meat in Chittagong district of Bangladesh. Veterinary World, 2014; 7(9): 738-743.
17. Jelena P, Baltić M, Jupić V, Stefanovic S, Stojanovic D. residues of enrofloxacin and its main metaboliteciprofloxacin in broiler chickens, Acta Veterinaria (Beograd), 2006; 56(5-6): 497-506.
18. Barge R S, Waskar V S, Sherikar A T, Paturkar A M, Zende R J. Surveillance of muscle and organ samples of ovine and caprine for the presence of residues of gentamicin sulfate by HPLC. *Journal of Bombay Veterinary College*, 2006; 14(1): 41-47.
19. National Organization of Food Safety Resolution. Egyptian Facts NO (257) 15 Nov.2020 for upgraded obligated technical rules for maximum residues limits of antibiotics.
20. European Commission (EU) pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Official Journal of European Union, 2010); 20(1): 1-72.
21. European Medicine Agency "EMEA" Committee for veterinary medicinal products. Enrofloxacin. "Extension to all food producing species ".Summary report (2002); (5).EMEA/MRL/820/02-final.
22. Health Canada Maximum residue limits (MRLs) and risk management recommendations (RMRs) for residues of veterinary drugs in foods. Its incorporated by reference in the Marketing Authorization for Maximum Residue Limits for Veterinary Drugs in Food, 2015.
23. Bayomi F H A Toxic Residues in Chicken and Turkey Carcasses. PhD, Benha University, Faculty of Veterinary Medicine,Department of Food Control, 2016.
24. De Wasch K, Okerman L, Croubels S, De Brabander H, van Hoof J, De Backer P. Detection of residues of tetracycline antibiotics in pork and chicken meat: correlation between results of screening and confirmatory test. *Analyst*, 1998; 123: 2737-2741.
25. Hassani M, Lázaro R, Pérez C, Condón S , Pagán R. Thermostability of oxytetracycline, tetracycline and doxycycline at ultrahigh temperatures. *J. Agric. Food Chem*, 2008; 56: 2676-2680.
26. *Singh T, Singh A, Kumar M, Narendra S, Kumar A.* Determination of gentamicin residues in chicken meat by high performance liquid chromatography. Indian Journal of Poultry Science, *2019;* 54(2): 155-159.
27. Fathy F M, Ahmed AM, Moursi M K. Effect of cooking methods on antibiotic residues in broiler chicken. 2nd Conference of food safety, Suez Canal University., faculty of Veterinary Medicine, 2015; Vol. I, 76-81.
28. Hussein M A, Ahmed M M , Morshedy A M. Effect of cooking methods on some antibiotic residues in chicken meat. Japanese Journal of Veterinary Research, 2016; 64(Supplement 2): S225-231.
29. ABOUL EL-NILE M O N A. Effect of boiling and freezing on ciprofloxacin residues in chicken tissues. *Assiut Veterinary Medical Journal*, 2006; *52*(110): 111-120.
30. FAO Nutrition Country Profiles – Egypt. FAO, Rome, Italy, 2010.
31. Oyedeji A O, Msagati T A M, Williams A B, Benson N U. Determination of antibiotic residues in frozen poultry by a solid-phase dispersion method using liquid chromatography-triple quadrupole mass spectrometry.Toxicol. Rep, 2019; 6: 951-956.

32. Australian Guidelines 22 for water recycling: managing health and environmental risks (Phase 2), 2008.
33. Widiastuti R, Martindah E, Anastasia Y. Detection and dietary exposure assessment of fluoroquinolonesresidues in chicken meat from the districts of Malang and Blitar , Indonesia. Tropical animal science journal, 2022; 45(1): 98-103.
34. Codex Alimentarius Commission Maximum Residue Limits for VeterinaryDrugs in Foods Updated as at the 34th Session of the Codex Alimentarius Commission, 2011.
35. Adewuyi G O, Olatoye O I, Abafe A O, Otokpa M O, Nkukut N K. High performance liquid chromatographic method for evaluation of two antibiotic residues in liver and muscle of broilers in Ibadan city, Southern Nigeria. *J. Pharm. Biomed. Sci*, 2011; (11): 1-4.
36. Ismail F M R, Jinap S, Jamilah B, Saleha A A. Effect of deep-frying atdifferent temperature and time on sulfonamide residues in chicken meat-balls. *J. Food Drug Anal*, 2008; 16: 81-86.
37. Isoherranen N, Soback S. Determination of gentamicins C (1), C (1a), and C (2) in plasma and urine by HPLC. Clinical Chemistry, 2000; 46: 837-842.
38. Silva J M B, Hollenbach C B. Fluoroquinolonas x Resistência bacteriana na medicina veterinária. Arquivos do Instituto Biológico, 2010; 77(2): 363-369.
39. Goetting V, Lee K A, Tell L A. Pharmacokinetics of veterinary drugs in laying hens and residues in eggs: a review of the literature. J. Veterinary Pharmacol Therapy, 2011; 34(6): 521-556.