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A Study of some quality indicators and effect of freezing on antibiotic residue in chicken meat

Ahmed Morad Asaad*, Mostafa Mahmoud Enakib, Nesreen Zakaria Eleiwa***, Ahmed Hussein****, Esraa Mohamed Nadi*****

*Clinical Microbiology & Immunology, Coordinator of IC Master
**Program, Microbes and Infectious Diseases Faculty of Medicine, Zagazig University.
***Professor of microbiology and Immunology, Military Medical Academy
****Professor of food hygiene and technology, Animal Health Research Institute, Agriculture Research Center
*****Armed Forces Laboratories for Medical Research and Blood Bank. Cairo, Egypt

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ABSTRACT

This study meticulously investigated antibiotic residue profiles of fresh and frozen chicken meat collected from major Egyptian governorates. We assessed the presence of some antibiotic residues, (Gentamicin, Oxytetracycline, and Amoxicillin), and our findings underscore the widespread presence of critical antibiotic residues in poultry meat. This raises significant concerns regarding food safety and the potential development of antimicrobial resistance (AMR), alongside possible toxicological effects on humans.

Furthermore, freezing samples for extended periods significantly decreased most antibiotic residues for gentamicin and oxytetracycline and on the other hand amoxicillin residues was undetected after 3 months of freezing

These findings stress the necessity of controlled storage conditions to reduce residue persistence.

These findings collectively pose a significant concern for public health, given the established links between antibiotic exposure through food and the escalating crisis of AMR, as well as the direct presence of antibiotic residues.

INTRODUCTION

Chicken meat is recognized globally as a rich source of high-quality protein due to its low collagen content, which enhances digestibility compared to red meats. According to the European Food Safety Authority **(EFSA, 2017).** chicken meat also contains lower fat levels and a higher proportion of desirable un-

Corresponding author: Ahmed M. Asaad, Clinical Microbiology & Immunology, Coordinator of IC Master , Egypt
Email address: dr_ah_hussien@yahoo.com
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saturated fatty acids **(Marangoni et al. 2015)**, making it an ideal food for athletes, children, and the elderly **(Cavani et al. 2009)**

Antibiotics whether natural, synthetic, or semi-synthetic are used to inhibit or kill microorganisms **(Catteau et al. 2018).** Their use in veterinary medicine is nearly unavoidable, particularly for treating infections, preventing disease, and promoting growth in livestock **(Arsene et al. 2021)**. Alarmingly, global antibiotic usage in animals significantly exceeds that in humans (**Aarestru, 2012).** In veterinary practice, antibiotics serve three primary roles: therapeutic treatment of diseased animals, prophylactic administration to prevent potential infections, and use as growth promoters to enhance feed efficiency and weight gain. utilization and production for their growth promoting properties, they are routinely used at subtherapeutic levels as animal feed additives **(Okerman et al. 1998)** In poultry production, antibiotics are often misused, leading to the accumulation of antimicrobial residues in edible tissues **(Kamouh. heba et al. 2024).**

To ensure consumer safety, the Maximum Residue Limit (MRL) defines the permissible concentration of a drug in animal-derived food products. If levels remain below the MRL, food is considered safe for consumption and manufacturing processes **(Lee et al. 2001).** A withdrawal period is also required to allow drug residues to deplete before slaughter **(Beyene, 2016).**

Antimicrobial resistance (AMR) is now one of the most pressing global health threats. The Centers for Disease Control and Prevention (CDC) has warned about the increasing number of antibiotic-resistant infections, many of which no longer respond to first-line treatments **(Singh et al. 2014).** Infections such as pneumonia, gonorrhea, and tuberculosis have become more difficult and costly to treat. The World Health Organization **(WHO, 2019)** Projections estimate that AMR could result in up to 10 million deaths annually by 2050.

The global scientific and medical commits are increasingly emphasizing the critical need to cut back on the widespread use of antibiotics. This is not just a performance but It considered a vital strategy to slow down the risk of rising antibiotics-resistance pathogens, a dorminant threat that has become a major public health concern. To overcome such risk, research and industry efforts are now mainly focused on finding an alternative, natural and sustainable that can induce effective microbial control while minimizing the dependency on conventional antibiotics.

This switching aims to preserve the efficacy of existing antibiotics until they are be truly essential and have the full capacity to protect global health from the consequences of uncontrolled resistant pathogens.

Several studies have reported antibiotic residues exceeding MRLs in poultry products worldwide. For instance, in South Africa, tetracycline and other residues were detected in chicken livers using ultrahigh-performance liquid chromatography–tandem mass spectrometry **(Adesiyun et al. 2021).** Freezing at -20°C was found to gradually degrade antibiotic residues in rabbit tissues **(Ahmed-Gehad, 2002),** with similar reductions noted in liver, kidney, and muscle tissues stored for six months **(Chlopkiewicz et al. 2005).**

Residues of β-lactam antibiotics, such as cephalosporins and penicillins, have been linked to adverse health effects in humans, including allergic reactions and gastrointestinal symptoms **(Kyuchukova, 2020).** High tetracycline levels have also been associated with fetal developmental issues, autoimmune disorders, and possible carcinogenic effects **(Arsène et al. 2022).**

Specifically, it aims to: (1) assess selected quality indicators of chicken meat quality. (2) evaluate the presence and levels of antibiotic residues in poultry meat, and (3) investigate the impact of freezing on the persistence of these residues.

These objectives highlight the urgent need for improved monitoring and control strategies to ensure food safety and public health

MTERIALS AND METHODS

3.1 Sample Collection and Preparation

A study examined a total of 15 fresh chick-

en samples were collected from three poultry slaughterhouses in Alexandria, Al-Dakahlia, and Al-Qalubia governorates in Egypt. Each location provided five chicken samples, comprising breast, thigh, and liver (five of each). Fresh chicken samples were transported under cooled conditions to the Animal Health Research Institute for detection and quantification of antibiotic residues. High-Performance Liquid Chromatography (HPLC) was used to analyze Oxytetracycline, Gentamycine, and Amoxicillin.

Subsequently, samples were frozen at –20° C and stored for up to three months for re-analysis. This comparison aimed to evaluate the effect of freezing on antibiotic residue stability in poultry meat and liver.

3.2. Quantitative detection of antibiotic residues by High-Performance Liquid Chromatography (HPLC)

Oxytetracycline, Gentamycine, and Amoxicillin.

3.1.1. Chemicals:

Standards for Oxytetracycline, Gentamicin, and Amoxicillin were supplied by sigma- Aldrich.
Methanol and acitonitrile were of HPLC grade
Citrate buffer pH 4 for OTC, Phosphate buffer/ TCA for Gentamicin, and KH2PO4 buffer (pH 2.5) for Amoxicillin

3.2.2.Apparatus:

Agilent series 1200 quaternary gradient pump, series 1200
quto sampler, series 1200 UV
Vis detector, and HPLC 2D
Chemstation software

3.2.3. Oxytetracycline (Preparation and Extraction) (Shama et al, 2018)

Fortification of samples:

The negative chicken samples were spiked with a OTCs standard solution at two levels: 100 ng and 200 ng per gram of meat, and 300 ng and 600 ng per gram of liver. Fortified samples were allowed to stand at 4°C for at least 30 minutes before analysis.

Extraction Procedure: Weigh 5 g of the samples and blend in a high-speed tissue blender. Add 3mL of citrate buffer and vortex the mixture at high speed for 5 minutes. Incubate for 5 minutes at room temperature, then centrifuge at 3,500 rpm for 10 minutes in a cooling centrifuge. Repeat the extraction by adding 2 mL of citrate buffer.

Clean-up Technique: The supernatant was filtered and loaded onto an SPE cartridge, previously conditioned with 3 mL of methanol and 2 mL of water. The cartridge containing the sample was washed with 5 mL of water, and then oxytetracyclines were eluted. Subsequently, 1 mL of eluent was filtered through a 0.45 µm nylon filter; 100 µL of the aliquot was injected into the HPLC system.

Chromatographic Conditions: Gradient elution was applied using a mixture of methanol (A), acetonitrile (B), and 0.03M oxalic acid (C). The chromatographic column was a reversed-phase column (Extend-C8, Zorbax column, 4.6 mm, 250 mm, 5 µm), adjusted at 35° C. The flow rate was 1 mL/min, and the injection volume was 100 µL. Detection and quantitation were performed using a UV detector at 351 nm, with quantification integrated by HPLC 2D Chemstation software interfaced to a personal computer.

3.2.2. Gentamycine (Preparation and Extraction) (Ragab-Marwa et al, 2015)

Fortification of samples: the negative Chicken samples were spiked with gentamycine standard solution at two levels: 100 ng and 200 ng per gram of meat, and 300 ng and 600 ng per gram of liver. Fortified samples were allowed to stand at 4°C for at least 30 minutes before analysis.

The spiked samples were left for 30 minutes in the dark at room temperature before starting the extraction process.

Extraction procedure: Approximately 2 grams of each sample were weighed and homogenized with 10 mL of phosphate buffer containing trichloroacetic acid (TCA). The homogenates were sealed and subjected to vigorous shaking for 10 minutes using a mechanical shaker. Following this, samples were centrifuged at 4000 rpm (≈3000 g) for 10 minutes. The resulting supernatant was transferred into

a clean 50 mL tube. The remaining pellet was extracted again using an additional 10 mL of buffer under identical conditions, and both supernatants were pooled.

The pH of the combined extract was adjusted to 7.5–8.0, using approximately 0.16 mL of 30% NaOH, with fine adjustments made using 1 N HCl or 1 N NaOH, and confirmed via pH meter. The solution was then centrifuged again under the same conditions.

Cleanup technique: For sample cleanup, solid -phase extraction (SPE) cartridges were preconditioned with 5 mL methanol followed by 5 mL distilled water. After stopping the vacuum, the prepared extract was loaded onto the cartridge and eluted at a flow rate of 1–3 mL/ minute. The cartridge was washed with 4–5 mL of water, then dried under vacuum (~10 in Hg) for at least 5 minutes.

Gentamicin residues were eluted using 3 mL of 10% acetic acid in methanol into clean 15 mL centrifuge tubes. The eluent was evaporated under nitrogen at 40 °C to a final volume of ~0.1 mL, then further dried at room temperature. The dried residue was reconstituted in 0.4 mL of 0.1 M trifluoroacetic acid, vortexed, sonicated for 15 minutes, and centrifuged at 2500 rpm (≈1200 g) for 10 minutes. Approximately half of the supernatant was transferred into polypropylene auto-sampler vials for further analysis. **(Office of Public Health Science, 2011)**

Chromatographic condition:

Isocratic elution using (0.1 m) of triflouroacitic acid: methanol (92:8 v/v).

Chromatographic separation was performed with reversed phase column (c18, 4.6 mm i.d., 250mm, 5mm). the column temperature adjusted at 25 centigrade at flow rate of 105 ml/min. the injection volume was maintained at 50 ml. detection was performed using UV detector at 280 nm.

3.2.3. Amoxicillin (Preparation and Extraction) (Wang et al,. 2009)

Fortification of samples:

Negative chicken samples were fortified with gentamicin standard solution at two concentration levels: 100 ng/g and 200 ng/g for muscle tissue, and 300 ng/g and 600 ng/g for liver samples. The enriched samples were stored at 4 °C for a minimum of 30 minutes prior to analysis and were kept at room temperature in the dark for an additional 30 minutes before commencing the extraction procedure.

Extraction procedure:

Precisely 1 gram of homogenized muscle or liver tissue was weighed into a 20 mL centrifuge tube, followed by the addition of 0.1 M potassium dihydrogen phosphate buffer (pH 2.5). The mixture was vortexed for 5 minutes and subsequently centrifuged at 7000 rpm for 5 minutes. The supernatant was carefully transferred to a clean tube. The extraction step was repeated twice for the remaining precipitate, and all supernatants were pooled for further cleanup.

Cleanup procedure:

A certified 3 mL (500 mg) Bond-Elut C18 solid-phase extraction column was preconditioned with 4 mL of methanol and 1 mL of 0.005 M KH_2PO_4 buffer (pH 6.8). Care was taken to avoid the introduction of air onto the stationary phase. The combined extract was loaded onto the SPE column and discarded. The column was washed with 1 mL of deionized water and dried under vacuum for 20 minutes. Elution was performed using 1 mL of a 60:40 water-to-methanol mixture into a clean 10 mL Quickfit glass tube. The eluate was evaporated under a gentle nitrogen stream at 50 °C. The resulting dry residue was reconstituted in 1 mL of deionized water (pH 9.0) and passed through a 0.2 µm syringe filter (Advantec-MFD, Japan). A 20 µL aliquot was transferred into an autosampler vial for chromatographic analysis.

Chromatographic conditions:

The analysis was carried out using isocratic elution with a mobile phase consisting of 0.005 M KH_2PO_4 buffer (pH 6.8) and methanol in a 90:10 (v/v) ratio. Separation was achieved using a reversed-phase C18 column (4.6 mm internal diameter × 250 mm, 5 µm particle size) at a flow rate of 1 mL/min. Detection was performed at 210 nm using a UV detector.

RESULTS

Table 1. Statistical analysis of oxytetracycline residue (ppb) in examined chicken samples of different governorates. (n=3)

Governate/ inspection time	Sample type		
	Breast (Mean ± SD)	Thigh (Mean ± SD)	Liver (Mean ± SD)
		Alexanderia	
Fresh	133.67±3.68	231±1.63	453±2.49
1st month of freezing	57.57±2.17	201.43 ± 1.23	400.67 ± 1.70
2nd month of freezing	65.7 ± 1.07	114.67±2.05	177.33 ± 3.49
3rd month of freezing	35.33± 2.8	66.34 ± 2.05	97± 3.27
		Al- Dakahlia	
Fresh	152± 2.49	186.70 ± 2.49	305.67 ± 2.87
1st month of freezing	125± 2.05	153 ± 2.16	280 ± 2.88
2nd month of freezing	68± 1.16	97 ± 2.45	138.73 ± 2.98
3rd month of freezing	39.67 ± 2.4	55± 2.86	83 ± 2.94
		Al-Qalubia	
Fresh	178± 3.27	292± 1.24	333 ± 2.45
1st month of freezing	149 ± 1.70	211 ± 1.63	293± 2.45
2nd month of freezing	75± 1.62	122.67 ± 2.05	148± 1.61
3rd month of freezing	44 ± 2.04	77 ± 2.88	87.7 ± 2.11

Table 2+. statistical analysis of gentamicin residue (ppb) in examined chicken samples of different governorates. (n=3)

Governate/ inspection time	Sample type		
	Breast (Mean ± SD)	Thigh (Mean ± SD)	Liver (Mean ± SD)
		Alexanderia	
Fresh	160±1.63	190±.82	250±4.08
1st month of freezing	158± 3.30	180 ± 2.49	244 ± 3.68
2nd month of freezing	143.3 ± 3.27	157±3.68	218 ± 4.19
3rd month of freezing	54± 2.49	60 ± 3.40	103± 1.25
		Al- Dakahlia	
Fresh	202.67± 3.30	220.7 ± 1.69	396 ± 3.74
1st month of freezing	195.33± 1.25	217 ± 3.30	351± 2.16
2nd month of freezing	170.33± 3.86	196 ± 3.29	246 ± 2.٤٥
3rd month of freezing	65.67 ± 3.68	82 ± 1.63	158 ± 3.28
		Al-Qalubia	
Fresh	93± 2.44	122± 2.49	273.67± 2.88
1st month of freezing	88± 2.49	187 ± 2.94	262± 1.63
2nd month of freezing	71.33± 1.70	163 ± 1.69	238± 2.05
3rd month of freezing	30.67± 2.72	62± 1.63	109 ± 2.94

Table 3. statistical analysis of amoxicillin residue (ppb) in examined chicken samples of different governorates. (n=3)

Governate/ inspection time	Sample type Breast (Mean ± SD)	Thigh (Mean ± SD)	Liver (Mean ± SD)
		Alexanderia	
Fresh	57.4±2.05	60.43±1.77	70.8±3.37
1st month of freezing	44.28± 2.39	49.6± 2.00	59.23± 2.30
2nd month of freezing	21.67 ± 1.43	25.61±2.25	32.13 ± 2.03
3rd month of freezing	ND	ND	ND
		Al- Dakahlia	
Fresh	53.48± 2.33	62.3± 1.42	78.93 ± 2.76
1st month of freezing	44.83± 1.91	52.23± 2.0	65.37± 1.18
2nd month of freezing	20.5± 1.76	28.34 ± 1.92	34.5 ± 2.45
3rd month of freezing	ND	ND	15.08± 2.90
		Al-Qalubia	
Fresh	52.27± 1.72	61.77± 1.67	65.13 ± 2.46
1st month of freezing	41±1.65	46.4 ± 1.51	57.3± 2.60
2nd month of freezing	17.9± 2.16	22.27 ± 1.47	29.58 ± 1.85
3rd month of freezing	ND	ND	ND

DISCUSSION

During the past few decades, there has been a notable increase in the demand for poultry meat, This growing demand increases the need to produce fast-growing broilers, which can lead to an increased use of antibiotics as antimicrobial performance enhancers (APCs).

Unfortunately, the use of this amount of antibiotics in food-producing animals can result in the accumulations of antibiotic residues in poultry organs and muscles. The residues of drugs may then potentially be transmitted to humans via the consumption of these poultry meat. **(Korsgaard et al. 2022).** his may lead to two significant threats to human health: the development of antimicrobial resistance (AMR) and potential toxicological impacts **(Bacanli and Başaran, 2019)**

This study provide a comparative evaluation of gentamycine, amoxicillin, and oxytetracycline residues in poultry tissues (breast, thigh and liver) collected from Alexandria, Dakahlia, and Qalyubia, with respect to different freezing durations (fresh to 3rd month frozen).

Tables (1), (2), and (3) clearly demonstrate the widespread presence of oxytetracycline, gentamycin, and amoxicillin residues in fresh chicken samples across all examined organs and governorates. in fresh samples, oxytetracycline and gentamycin generally presented at higher concentrations compared to amoxicillin. For instance, fresh liver samples showed oxytetracycline levels as high as 453±2.49 ppb (Alexandria) and gentamicin up to 396±4.08 ppb (Al-Dakahlia), while amoxicillin in fresh liver peaked at 70.8±3.37ppb (Al-Dakahlia).

The liver consistently had the highest residue levels, followed by thigh and breast muscles. This is expected due to the liver's role in metabolism and detoxification.

However, the rate and extent of residue reduction varied considerably among the antibiotics:

- **Amoxicillin:** Demonstrated the least persistence. In Alexandria and Al-Qalubia, amoxicillin residues became "Not Detected" (ND) in breast, thigh, and liver by the 3rd month of freezing. Even in Al-Dakahlia, while liver still showed detectable levels (15.08± 2.90 ppb), breast and thigh were ND. This indicates that amoxicillin, a beta-lactam antibiotic, is relatively unstable and degrades more readily under frozen conditions compared to the other two antibiotics. This lower persistence might make it less concerning from a long-term storage perspective, provided initial levels are not excessively high.

•**Oxytetracycline and Gentamicin:** In contrast, both oxytetracycline (a tetracycline) and gentamicin (an aminoglycoside) proved to be more persistent. While their concentrations significantly decreased over the three months of freezing, they were still detectable at considerable levels, especially in liver and thigh samples, even after 3 months. For example, oxytetracycline levels in the liver were still 97 ±3.27 ppb (Alexandria) and gentamicin 158 ± 3.28 ppb (Al-Dakahlia) after 3 months of freezing. This highlights their relatively greater stability or slower degradation kinetics in frozen matrices. The persistence of these residues, even after prolonged freezing, suggests that freezing alone may not be sufficient to eliminate public health risks associated with their consumption, especially if initial concentrations are high or if MRLs are stringent.

Governorate	Highest Detected Residue	Notable Trend
Qalyubia	Oxytetracycline in thigh	High initial load, drops significantly by 3rd month
Dakahlia	Gentamycin in liver	Persistently high even after 3 months
Alexandria	Oxytetracycline in liver	Rapid decline, more uniform decrease

These results agreed with **(Roca et al (2010), El-Shopary-Nermeen (2013), and and faten et al (2016).**

Lower results of reduction were obtained by (**Shaltout et al. (2019).**

But the results disagreed with **(Ezenduka, et al. 2018)** whom mentioned that freezing has no effect on anti-biotics residue.

This difference may be due to factors affecting the degradation of antibiotic drugs as (Drug formulation, pharmacodynamics, freezing temperature and time), and (shape and thickness) of cooked meat tissues.

At freezing temperatures, some chemical reactions can still occur. Certain antibiotics are more susceptible to degradation through hydrolysis, oxidation, or other chemical pathways. While freezing slows these processes, it might not completely stop them, especially over longer storage periods. **(Okerman et al. 2007)**

As food freezes, the water within it turns into ice crystals. This process can lead to the concentration of solutes (including antibiotic residues) in the unfrozen liquid phase or in interstitial spaces between ice crystals **(USDA. 2025).** More importantly, during the freezing and subsequent thawing process, there can be a redistribution or migration of the antibiotic residues. For instance, some residues might migrate into the "drip" or exudate that form when meat thaws. If this drip is discarded, it can lead to a perceived reduction in the residue concentration within the consumed food. **(Roos. 2021)**

Withdrawal periods for antibiotics used in poultry vary significantly depending on the pharmacokinetic properties of each drug. **Oxytetracycline**, for example, requires a withdrawal period ranging from **15 to 35 days**, reflecting its extended persistence in animal tissues. This wide range may also indicate variability due to dosage, route of administration, or animal metabolism.

Ampicillin requires a withdrawal period between **6 and 15 days**, which is moderate, while **tetracycline** shows a relatively shorter fixed withdrawal period of **5 days**. Interestingly, **gentamycin** is listed with a **zero-day withdrawal period**, which may be attributed to its rapid clearance or low tissue retention in certain formulations. However, this should be interpreted carefully, as other sources, such as ISO or FAO/WHO guidelines, often assign longer withdrawal times for gentamicin (e.g., up to 35 days), depending on species and use. **(Zeuko'o et al. 2019)**

In conclusion, our study revealed a concerning widespread presence of critical antibiotic residues—Gentamicin, Oxytetracycline, and Amoxicillin—in fresh chicken meat from major Egyptian governorates. Crucially, their **concentrations often exceeded the Maximum Residue Limits (MRLs)**. importantly, the study demonstrated that freezing chicken meat at –20°C for up to three months resulted in a notable reduction in antibiotic residue levels. Our results revealed that freezing decreased gentamycin and OTC residue in breast, thigh and liver significantly, but for amoxicillin residues became non detectable in most samples after 3 months of freezing

This poses a direct and significant risk to public health, contributing to potential toxicological effects and the escalating crisis of antimicrobial resistance (AMR). This research emphasizes the

urgent need for stricter monitoring and regulation of antibiotic use in poultry farming. It also highlights the necessity for routine screening of poultry products for drug residues to ensure public health safety and maintain the overall quality of meat products available in the market.

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