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Detection and impact of heat treatments on tetracycline residues in chicken meats sold in Amman city, Jordan

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Abstract

The use of antimicrobial drugs in poultry is an important public health issue owing to the increased use of such compounds that may trigger harmful effects on consumers. This study investigated the type and concentration of tetracyclines in poultry meat samples collected from Amman city, Jordan. A total of 180 samples (breast muscles, livers and kidneys) were randomly collected from the abattoir and screened qualitatively using the Premi® test and quantitatively by High-performance liquid chromatography (HPLC) for oxytetracycline, tetracycline and chlortetracycline. Approximately eighty-four (46.6%) of the samples showed a positive result for the presence of tetracycline residues. Out of the 84 positively samples, parent drugs were identified in 12 (6.6%) muscle, 14 (7.7%) liver and 13 (7.2%) kidney samples. Moreover, the samples that exceeded the maximum residue limit were 4.4%, 5.0%, and 2.7% of muscle, liver, and kidney, respectively. Freezing (-70°C for 90 days) and heat treatments (up to 90°C for 60 mins) did not significantly reduce tetracyclines residue concentrations. Violative levels of antimicrobial drug residues in different poultry tissues are still a public health issue. An extra-dosage regimen or failure to meet the withdrawal times might stand behind the problem. Private and governmental strategies to implement strict regulations on antimicrobial applications in the poultry industry are highly recommended. Poultry producers should be informed that the usage of antibiotics does not compensate for hygienic measures at farm levels.

1. Introduction

Antibiotics are defined as substances that are able to inhibit the growth of various microorganisms. They are produced naturally, by living organisms, or synthetically (Darwish *et al.,* 2013; Alaboudi *et al.,* 2021). Antibiotics are among the most important chemicals used in animal feed and production in veterinary medicine. Approximately 80% of the animals used in food production are currently being treated with antimicrobial drugs throughout their lives (Pavlov *et al.,* 2008). Generally, antibiotics are used for three main purposes, therapeutic, prophylactic, and feed additives or growth promoters (Darwish *et al.,* 2013; Al-Mashhadany, 2019). Intensive production of poultry meat has led to extensive use of antimicrobials. The most common causes of drug residue persistence in meat are the failure to obey the withdrawal times with extra doses, the improper use of licensed substances, and the illegal use of unlicensed substances. In addition, the disease status of an animal may also lead to residue accumulation through disruption of drug pharmacokinetics and metabolism (Castanon, 2007; Almashhadany *et al.,* 2020).

The remains of antimicrobial drugs in poultry meat have recently become an important public health issue due to the increased use of such compounds in the animal and food industry. Meat contamination with antibiotics has many harmful effects, including discolouration and distinctive odour, selection of microbial resistance strains, disruption of human gut microbiota, and hypersensitivity reactions (Nisha, 2008; ECDC, 2016; Almashhadany, 2020b).

Most foods of animal origin are cooked or subjected to different processing preparations before consumption. Therefore, information about the effects of cooking or freezing on the stability of antimicrobial residues in meat is important to accurately estimate the risks of exposure to these compounds through food. It is well-known that

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heat processing, such as frying, boiling or roasting, can cause protein denaturation, water loss, and pH variation (Alaboudi *et al.,* 2013; Almashhadany, 2020a). Thermal processing can also change the physicochemical nature of antibiotics in food (Alaboudi, 2017). Therefore, some antimicrobial residues are chemically unstable and may undergo degradation during storage and heat treatment (Botsoglou and Fletouris, 2001; Almashhadany, 2021a).

Surveillance programs to monitor the antimicrobial residues in poultry meat are critical in developing countries, even where antibiotic usage in farming is heavily regulated. All commercial chicken farms in developed countries have been certified under these programs for more than ten years (FAO/WHO, 2013). In addition, the farms are held accountable for the proper use of antibiotics as part of the mandatory On-farm food safety program, which has received full federal or provincial government recognition in various countries. In developing countries, however, such regulations are not implemented, and farmers' rational uses are the most effective approach to mitigate the contamination of meats by antibiotics residues (ECDC, 2016; Almashhadany, 2021b). Antibiotic residues are well-documented in various meat types (Al-mashhadany, 2019; Almashhadany, 2020c). However, little is known about residue levels in poultry meat in the Fertile Crescent region. The study aimed to detect and quantify the residues of tetracyclines in poultry meat in markets of Amman city (Jordan).

2. Materials and methods

2.1 Sample collection

Sampling was done arbitrarily on the basis that five birds are considered representatives of 1000 slaughtered birds. The five birds were combined to form one composite sample. One hundred and eighty samples of either local fresh-dressed birds or offals (livers and kidneys) were collected from Amman abattoirs through 25 visits. Approximately 200 g of poultry tissues were collected from 3 different parts (thigh, breast, the wing) using a sterile blade. Positive muscle, liver and kidney samples in the screening test were kept frozen at – 70°C until analyzed quantitatively by high-performance liquid chromatography (HPLC).

2.2 Premi® screening test

This test was carried out as outlined by the manufacturing company (DSM Food Specialist, Netherlands) using a multi-residue extraction solvent. Briefly, 5 mL of acetonitrile/acetone 70:30, v/v (SDS, France) were added to two grams of mixed and manually minced sample in a 10 mL glass test tube. The mixture was centrifuged (4500 rpm, 4°C, 10 mins), and the supernatant was transformed to another 10 mL glass test tube and evaporated to near dryness under a stream of nitrogen. The residue was suspended in 250 µL of Lab Lemco Broth (Oxoid, UK). A volume of 100 µL of the extract was applied to a Premi® ampoule and incubated at 64±0.5°C in a water bath with a shaker until the yellow colour became visible in the negative control (3-4 hrs).

2.3 Characterization of antimicrobial residues by HPLC

Chlortetracycline, oxytetracycline and tetracycline were extracted from muscle, liver and kidney samples according to the AOAC official methods (AOAC, 2000). Mcllvaine buffer-EDTA was used for extraction, followed by filtration through 0.45 µm millipore filter paper (MFS, USA). Samples were loaded into SPE cartridges (Supelco, USA), eluted with methanolic oxalic acid, followed by second filtration and High-performance liquid chromatography (HPLC) analyses.

2.4 Effect of heat treatment

Blank poultry muscle tissues were minced with a stomacher at low speed for 2 mins. A 100 g from each sample was fortified with a concentration of 1800 µg/kg for a combination of three tetracyclines, which is relatively equal to the mean found in poultry tissue. Fortified samples were placed in a 250 mL conical glass flask containing 10 mL water and were heated up to an assigned temperature (50, 70, and 90°C). Nine flasks were heated to each temperature degree, and three flasks were drawn at time intervals of 20 mins. Drawn flasks from each specific treatment temperature were cooled rapidly by immersing in a box filled with crushed ice before analyses together with controls of unheated fortified samples.

2.5 Storage at freezing

Samples fortified with 1900 µg/kg of three drugs described for heat treatment were frozen at -70°C in polythene bags for up to 90 days. Representative samples for HPLC quantization were drawn at 15-day intervals and thawed at refrigeration before analysis, together with controls of unheated fortified samples.

2.6 Statistical analysis

Statistical analysis was performed using the SAS software package, through which the ANOVA test was used (t-test, multiple comparisons LSD, and Fisher test). The differences were considered significant when $P \leq 0.05$.

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3. Results

3.1 Premi® screening test

Eighty-four (46.6%) out of 180 local fresh-dressed bird samples showed a positive result for the presence of antimicrobial residues either only in one of the tissues examined (muscle or liver or kidney), or in combination (muscle and liver), (muscle and kidney), (liver and kidney), or in all the three tissues (Table 1). The presence of tetracyclines simultaneously in the three tissues appeared to be significantly higher than their presence in any single tissue ($P \leq 0.05$). Kidneys were found to be significantly more contaminated with antibiotic residues ($P \leq 0.05$) (69), followed by liver (59) and muscle (48) tissue.

Table 1. The distribution of positive results among different bird tissues

Type of tissue examined	Positive samples	%
Muscle alone	3	1.6^{a}
Liver alone	4	2.2^{a}
Kidney alone	15	8.3^{b}
Muscle and liver	8	4.4^{c}
Muscle and kidney	7	3.8^{c}
Liver and kidney	17	9.4^{b,d}
Muscle, liver and kidney	30	16.6^{e}
Total	84	46.6

Values with different superscripts are significantly different between meat types ($P \leq 0.05$).

3.2 Characterization of antibiotic residues

From the 84 positive samples, parent drugs (single or in combination) of oxytetracycline, tetracycline, and chlortetracycline residues were identified in 12 (6.6%) muscle, 14 (7.7%) liver and 13 (7.2%) kidneys samples (Table 2). The majority of samples contain only one type of tetracyclines. However, a few samples (1 muscle, 2 livers, 2 kidneys) contained 2 types (oxy and tetra) in combination simultaneously. Unlike oxytetracycline or chlortetracycline, none of the positive samples showed tetracycline singly. The highest concentration was detected in the kidney, followed by the liver and muscle. These levels exceed the recommended Maximum Residue Level (MRL) set by the Codex Alimentarius Commission (CAC) (200 µg/kg for muscle, 600 µg/kg for liver and 1200 µg/kg for kidney) (CAC, 2000). Moreover, when values of the combination of the three drugs were adjusted to the CAC MRL (by dividing the results over the MRL), no statistically significant difference was found between tissue types. The samples exceeding MRL were 8 muscle samples out of 12 positives, 9 liver samples out of 14, and 5 kidney samples out of 13. If these three figures were related (8, 9, and 5) to the number of studied samples (180), it appears that 4.4% of muscles, 5.0% of livers and 2.7% of kidneys are violating the recommended MRL (Table 3).

3.3 Effects of freezing and heat treatments on residue levels

Freezing treatment at -70 °C had insignificantly decreased the mean concentration of the combination of oxytetracycline, tetracycline and chlortetracycline residues in poultry muscle tissues (Table 4). However, the reduction of antibiotic levels was not affected significantly by increasing the freezing period. Regarding the effects of heat treatments, the decrease in the concentration from 1806 µg/kg of the three antibiotics at three different temperatures (50, 70 and 90° C) did not prove any significant changes (P-value >

Table 2. Concentrations of tetracycline residues in fresh poultry samples (µg/kg)

Muscle tissues				Liver tissues				Kidney tissues			
OXY	TET	CHL	Total	OXY	TET	CHL	Total	OXY	TET	CHL	Total
87	-	-	87	184	-	-	184	-	-	1090	1090
103	-	-	103	173	-	-	173	85	-	-	85
109	328^{x}	-	437^{x}	212	-	-	212	186	-	-	186
116	-	-	116	611^{x}	-	-	611^{x}	235	-	-	235
173	-	-	-	750^{x}	-	-	750^{x}	579	-	-	579
237^{x}	-	-	237^{x}	657^{x}	1898^{x}	-	2555^{x}	797	-	-	797
262^{x}	-	-	252^{x}	795^{x}	-	-	795^{x}	974	2531x	-	3505^{x}
341^{x}	-	-	341^{x}	885^{x}	-	-	885^{x}	1077	-	-	1077
639^{x}	-	-	639^{x}	890^{x}	-	-	890^{x}	1095	-	-	1095
861^{x}	-	-	861^{x}	1163^{x}	-	-	1163^{x}	1245^{x}	-	-	1245^{x}
-	-	375^{x}	375^{x}	1362^{x}	-	-	1362^{x}	1505^{x}	-	-	1505^{x}
-	-	251^{ex}	251^{x}	1135^{x}	3544^{x}	-	4679^{x}	1603^{x}	4397^{x}	-	6000^{x}
-	-	-	-	-	-	532	532	3558^{ex}	-	-	3558^{x}
-	-	-	-	-	-	207	207	-	-	-	-

^{x}The concentration of residues exceeded maximum residue limit (MRL). OXY: oxytetracycline, TET: tetracycline, CHL: chlortetracycline

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Table 3. Descriptive statistics of oxytetracycline, tetracycline and chlortetracycline residues level (µg/kg)

Tissue type	Drug Residues	Mean[x]	Minimum	Maximum
Muscle	Oxytetracycline	291	87	861
	Tetracycline	328	328	328
	Chlortetracycline	313	251	375
	Total	322.6	87	861
Liver	Oxytetracycline	734.7	173	1362
	Tetracycline	272 1	1898	3544
	Chlortetracycline	369.5	207	532
	Total	1071.2	173	4679
Kidney	Oxytetracycline	1078.2	85	3558
	Tetracycline	3464	2531	4397
	Chlortetracycline	1090	1090	1090
	Total	1612	85	6000

[x]mean concentration for oxytetracycline, tetracycline and chlortetracycline residues single or in combination are above the recommended MRL by CAC.

Table 4. Effect of freezing on levels of antibiotic concentrations (µg/kg).

Time Intervals (days)	Concentration before freezing	Concentration after freezing	Concentration difference	Reduction (%)
Zero time	1931	-	-	-
15	1912	1863	49	2.5
30	1875	1847	28	1.4
45	1937	1828	109	5.6
60	1887	1787	100	5.2
75	1898	1835	63	3.3
90	1882	1809	73	3.8
Mean	1903.1± 209	1828.1 ±20.9	70.3 ±27.9	3.69±1.4

0.05) except for treatment at 90°C (Table 5).

Table 5. Effect of heat treatment on antimicrobials concentration

Time (mins)	Temperature °C		
	50	70	90
0	1806[a]	1806[a]	1806[a]
20	1745[a]	1757.3[a]	1693.3[b]
40	1733.3[a]	1765[a]	1616[b]
60	1761.3[a]	1723.3[a]	1650[b]
Reduction %	3.2	3.1	8.4

Values with different superscript within the same row are significantly different ($P \leq 0.05$).

4. Discussion

Antibiotics are widely used in food production and veterinary care (Pavlov *et al.,* 2008; Almashhadany, 2021a). Some of such drugs are quickly excreted from the animal, while others are not readily metabolized or eliminated. The persistence of drug residues in animal tissues ends up in food chains, including humans, and constitutes health risks to consumers (Rana *et al.,* 2019). In most developing countries, official surveillance programs for the evaluation of antibiotic residues in foods are usually absent. Therefore, this study was designed to shed preliminary light on this hot issue, followed by more extensive studies covering a wider area and other groups of antimicrobial drugs.

The screening results of this work revealed that 46.6% of the local fresh poultry samples contained tetracyclines residues. This percentage is considerably alarming and indicates an extensive abuse of veterinary drugs in the local poultry industry. Although the 46.6% percentage reported here is in parallel with other studies from neighbouring developing countries (Shareef *et al.,* 2009; Mund *et al.,* 2017; Ramatla *et al.,* 2017; Jammoul and El Darra, 2019; Kamali *et al.,* 2020), it is still lower than the prevalence reported in Iran where all of the surveyed poultry muscle samples contained antibiotic residues (Salehzadeh *et al.,* 2007). Additionally, a study from Saudi Arabia that screened eastern provinces involving 32 broiler farms reported that 69% of farms showed antibiotic residues (Al-Mustafa and Al-Ghamdi, 2002).

A similar study from Al-Najaf province (Iraq) that examined samples from local and imported chickens found antibiotic residues in 60% and 42.5% of samples, respectively (Shamsa, 2013). Different antibiotics were detected, including sulphadiazine, tetracycline, neomycin, and chloramphenicol. In this study, poultry samples were positive for at least one tetracyclines compound in 6.6%, 7.7% and 7.2% of the muscle, liver and kidney tissue samples, respectively. These results are much lower than those reported in a similar study from Saudi Arabia, in which 87% and 100% of the positive antimicrobial screened broiler farms were positive for at least one tetracycline compound in muscle and liver tissues, respectively (Al-Ghamdi *et al.,* 2000). Moreover, the study found 82% and 95.5% of the antimicrobial residues positive broiler farms had a concentration of at least one tetracycline compound exceeding the MRL in muscle and liver tissues, respectively, in comparison to 4.4% and 5% results reported in this study. Information on the occurrence of residues of veterinary drugs varies considerably due to different veterinary practices and systems of veterinary drug residue control.

The observation of oxytetracycline dominance may be attributed to the fact that oxytetracycline is being used heavily as feed additives; either for nutritional or prophylactic purposes in comparison with tetracycline and chlortetracycline (Cháfer-Pericás *et al.,* 2010). From a pharmacokinetic perspective, tetracyclines are not metabolized and are eliminated in the urine and through the gut. However, they tend to accumulate in the liver in a small amount (Agwuh and MacGowan, 2006). This may explain the higher levels in the kidneys and liver organs.

Regarding the effects of freezing and thermal processing, the mean concentration of tetracyclines residues had decreased only by 4% at the end of the three months of freezing storage, which may don't represent any appreciable reduction of residues in poultry meat. This clearly indicates that freezing cannot be employed as an effective strategy to lower the levels of these antimicrobials under study. This is in good agreement with other studies that showed no appreciable reduction in the annular zone of inhibition using the microbiological assay of the oxytetracycline residues in meats after storage for weeks or months at -20°C (O'Brien *et al.,* 1981; Rose *et al.,* 1996).

5. Conclusion

A small portion of samples contained antibiotic residues of tetracyclines at levels exceeding the international recommended MRL. Such violations are likely related to the extra-dosage regimen and failure to meet the withdrawal times. Antimicrobial usage should be regulated as well as products containing antibiotic residues should be monitored and standardized. The establishment of a national monitoring program on antimicrobial use in food animals can be done by gathering data from veterinarians, animal producers, manufacturers, distributors, and pharmaceutical records.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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