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DETECTION OF ANTIBIOTIC RESIDUES AMONG POULTRY MEAT IN ERBIL CITY AND IMPACT OF THERMAL PROCESSING ON REMNANTS

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ABSTRACT: This study was conducted to survey the occurrence of antibiotic residues in poultry meat in Erbil city and study the effect of thermal processing on these remnants. A total of 135 Breast muscle, Thigh muscle, and Liver (45 for each) were examined by direct microbiological method. The incidence rate was (11.1%), the highest rate were found in Liver (17.8%), then Thigh (8.9%), and Breast (6.7%). The value of ($p< 0.05$) (0.000) showed significant differences between the presence of antibiotic residues. January, December, and February take the more detection rate (20.8%), (18.5%) and (12.5%)respectively. The result indicated that there were significant differences ($p<0.05$) in the relationship between months and incidence of antibiotic remnants in total samples of poultry meat during interval of research. This study registered that the cooling at 4°C for a period of 3 days and the freezing at (-18°C) for a period of 4 weeks gave the affect on the remains of antibiotic in different percentages. As for cooking (boiling) for 30 minutes showed full affect on the existence of remains antibiotics. From this study we concluded that the occurrence of antibiotic remnants in poultry meat in Erbil city was high. The importance of public health hazards was discussed.

KEYWORDS: Antibiotic residues , Poultry meat , Detection , Erbil city, Thermal Processing , Remnants .

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1.INTRODUCTION

Fowls have been on the ground for over 150 million years. The poultry section was the most active meat sector, because there are different advantage of farming poultry, specially broilers, over the

animals, such as growth rotation which allows for repeated production during the year, the return from the investment in broiler is fast , high feed conversion efficiency (FCE), and the consumer demand for poultry meat was high[1-3].Antibiotics, also called antibacterial, are a type of antimicrobial drug widely used in food producing animals as they are in human medicine, and nearly all the classes of antimicrobials that are used for humans are also being used for food producing animals including the most modern classes of drugs, some antibiotics are produced by a variety of germs (Bacteria and Fungi), but most of them are now manufactured artificially [4-6]. Antibiotics are used in poultry output not only for remedial purpose, some producers nowadays manage sub-therapeutic concentrations of antibiotics for different goals, such as growth enhance objectives , hindrance or decline of disease outbreaks, improving digestion , rushing of weight gain, and increase feed conversion ratio (FCR), and these antibiotics include a large number of compounds which can be administered in the feed or drinking water, or may be individually depending on the reason of prescription [7-9].The misuse and overuse of these antibiotics lead to collection of violative levels of unfit for human consumption poultry tissues and organs as residues intended for human consumption , especially with the lack of knowledge between poultry producers about withdrawal periods of antibiotics, and the presence of antibiotic residues in food of animal origin above the maximum residue limits (MRLs)is recognized worldwide by various public health authorities as being unlawful [10-12].The eating of poultry meat containing antibiotic remnants poses a major threats and hazards to the public health including direct toxicity, expansion of antibiotic resistance among bacterial pathogens that have been known as a worldwide health difficulty and escalated by major world health organizations to one of the peak health gauntlet facing the 21st century, remedy hypersensitivity which characterized by a spectrum of reactions ranging from mild skin rashes to angioedema or life-aggressive anaphylaxis, disorder of the normal gastrointestinal microbiota , carcinogenic effect, bone marrow despair, mutagenesis and teratogenic effect of the antibiotic remnants on human being[13-15].Residues of antibiotics have been detected in poultry meat by various investigators in several countries [16-25].In recent decades, there has a significant increase in antibiotic residues in food of animal origin, including poultry meat, therefore the aims of this research were to investigate the occurrence of antibiotic residues in poultry meat in Erbil city and to determine the relationship between occurrence of antibiotic residues in poultry meat with months during the period of research, as well as study the effect of thermal treatment on these residues. Also high lights on the importance of antibiotic residues in poultry meat help in the establishing public health action that could halt the misuse of these type of antimicrobial drugs, and to focusing on the withdrawal periods of antibiotics.

2. MATERIALS AND METHODS

2.1. Collection of samples

A total of 135 Breast muscle, Thigh muscle and Liver (45 for each) were randomly collected from

different retail markets in Erbil city, Erbil, Kurdistan region, during the period from December 2016 to May 2017. The collected sample were put in separate plastic bags and transported to pathological analysis department, college of Science, Knowledge University, under chilling for further examinations. In the laboratory, samples divided into 3 parts.

Part 1: Also divided into two parts, first part used to detection of antibiotic residues by direct method, while second part used to study the effect of cooking (Boiling)(100°C/30minutes) on remnants.

Part 2: preserved in refrigerator at 4°C.After 3 days, these samples used to study the effect of cooling (4°C) on remnants.

Part 3: preserved at (-18°C /4 weeks). These samples used to study the effect of freezing (-18°C) on remnants.

2.2. Preparation of the spore suspension. This step was done according to (26) .

2.3. Determination of spore's concentration. Done according to (27) .

2.4. preparation of test plates.

a- Muller-Hinton medium was left to cool to (45-50°C),and then it was inoculate with the spore suspension of Bacillus subtilis (0.1ml / 100 ml medium).

b- The ingredients was poured on petri dishes and left for complete solidification, and either used in the same day or stored in a refrigerator at (4°C)and used within one week.

2.5. Detection of antibiotic residues in samples

a- Technique of (16) is adopted for detection of antibiotic remnants in poultry meat samples.

b- Antibiotic residues were analyzed by direct plate method using *Bacillus subtitles* as the test microorganism.

c- Result would be indicated by measuring the diameter of inhibition zones of the growth of the *Bacillus subtitles* around meat samples.

d- The zone which is more than or equal to 2mm would be recorded as positive result.

e- The zone which is from 1-2 mm would be recorded as suspicious result.

f-The zone which is less than 1mm would be considered as negative result.

2.6. Effect of thermal treatment on positive samples

The positive raw samples were selected for cooling,freezing and cooking methods then we performed the test for processed samples just like raw samples after complete thermal processing of them.

a. Cooling: Samples which showed positive results in the direct method were kept in the refrigeration at (4°C). After 3 days examined for the detection of antibiotic residues by using of direct method.

b. Freezing: Samples which showed positive results in the direct method were kept in the freezing at (-18°C).After 4 weeks examined for the detection of antibiotic residues by using of direct method.

c. Cooking(Boiling): A15-20grams of (Breast muscle, Thigh muscle and liver) samples which showed positive results in direct method were placed into a strainer, immersed in 100ml of water bath preheated to 100°C , and cooked for 30 minutes, then removed and allowed to cool and examined for

the detection of antibiotic residues by using of direct method(28 , 29).

2.7. Statistical Analysis: Statistical analysis using Chi – Square test and SPSS software version 15.

3.RESULTS AND DISCUSSION

3.1. Incidence of antibiotic residues in poultry meat samples

From 135 samples of different types of poultry meat,15(11.1%)were gave a positive results for presence of antibiotic residues. This result include 3 (6.7%) positive samples from breast muscle, 4(8.9%) positive samples from thigh muscle and 8 (17.8%) positive samples from liver (Table1).The value of ($p< 0.05$) (0.000)showed significant differences between the presence of antibiotic residues in different type of samples.

Table (1) :- Incidence of antibiotic residues in poultry meat samples

Type of samples	No. of samples	Positive samples		Negative samples	
		No.	%	No.	%
Breast muscle	45	3	6.7	42	93.3
Thigh muscle	45	4	8.9	41	91.1
Liver	45	8	17.8	37	82.2
Total	135	15	11.1	120	88.9

3.2. Relationship between months and incidence of antibiotic residues in total samples of poultry meat.

When we study the relationship between months and incidence of antibiotic residues in total samples of poultry meat during period of research, we noticed that the highest rate of detection of antibiotic residues were found in January5(20.8%),approximately to this rate in December 5(18.5%), then in February 3 (12.5%) (Table 2).The result indicated that there were significant differences ($p< 0.05$) in the relationship between months and incidence of antibiotic residues in total samples of poultry meat during period of research .

Table (2):-Relationship between months and incidence of antibiotic residues in total samples of poultry meat.

Month	Breast muscle		Thigh muscle		Liver		Total No	Total positive	
	No. examined	No. positive	No. examined	No. positive	No. examined	No. positive		No	%
December	9	2	9	1	9	2	27	5	18.5
January	8	0	8	2	8	3	24	5	20.8
February	8	1	8	1	8	1	24	3	12.5
March	6	0	6	0	6	1	18	1	5.6
April	7	0	7	0	7	1	21	1	4.8
May	7	0	7	0	7	0	21	0	0
Total	45	3	45	4	45	8	135	15	11.1

3.3. Effect of cooling (4°C/3days) on antibiotic residues in poultry meat samples

This study showed that the effect of cooling (4°C/3 days) on antibiotic residues in poultry meat samples was influential on the incidence of antibiotic residues in samples (Table3).

Table (3): Effect of cooling (4°C/3days) on antibiotic residues in poultry meat samples.

Types of samples	No. of positive samples before cooling	No. of affected samples after cooling	Percentage of affected samples
Breast muscle	**3**	**2**	**66.7%**
Thigh muscle	**4**	**2**	**50.0%**
Liver	**8**	**4**	**50.0%**

3.4. Effect of freezing (-18°C/ 4 weeks) on antibiotic residues in poultry meat samples

Also the effect of freezing (-18°C/4 weeks) on antibiotic residues in poultry meat samples,were followed up.As shown in (Table 4) the effect of this process was influential on the incidence of antibiotic residues in samples.

Table (4): Effect of freezing (-18°C/4 weeks) on antibiotic residues in poultry meat samples

Types of samples	**No. of positive samples before freezing**	**No. of affected samples after freezing**	**Percentage of affected samples**
Breast muscle	3	2	66.7%
Thigh muscle	4	3	75.0%
Liver	8	5	62.5%

3.5. Effect of boiling at (100°C/30 minutes) on antibiotic residues in poultry meat samples

Data presented in (Table 5) declared that all processed samples were affected by boiling at (100°C / 30 minutes).

Table (5): Effect of boiling at (100°C/30 minutes) on antibiotic residues in poultry meat samples

Types of samples	**No. of positive samples before boiling**	**No. of affected samples after boiling**	**Percentage of affected samples**
Breast muscle	3	3	100%
Thigh muscle	4	4	100%
Liver	8	8	100%
Total	15	15	100%

DISCUSSION

Chicken meats represents about 33% of worldwide meat output, and the global average per capita consumption of Chicken meat has virtually fourfold since the 1960s (11 Kg in 2003 comparison with 3 Kg in 1963[30,31].Poultry meat are trendy between both developed and developing countries,

providing high value protein, significant vitamin and minerals, also little in fat, simplicity of preparation, flexibility, and well fitting for fast service [32,33].In the recent years , antibiotic remnants are on the top of most important concern for the public health references all over the world because of increasing food safety significance and the WHO confirmed that antibiotic resistance is international tribulation[34,35].Diverse antibiotics take dissimilar time periods to be secreted from the body. This time period is identified as withdrawal period (WP) for the particular antibiotic, and this intervals depending on the antibiotic product, dosage form, and method of administration, the withdrawal time vary from only some hours to numerous days or week, so this period must be noticed in ante mortem inspection of birds [19,36]. In the study at hand, one hundred and thirty five(135) poultry meat samples were collected. These samples included 45 breast muscle,45thigh muscle, and 45 liver. The incidence of antibiotic residues in total poultry meat samples were 15(11.1 %).The highest rate of detection of antibiotic residues was found in liver 8(17.8%),then thigh muscle 4(8.9%),while the lowest rate of detection of antibiotic residues was from the breast muscle3(6.7%)(Table1).Statistical analysis using Chi – Square showed a significant difference at the level of 0.05 for the incidence of antibiotic residues in total poultry meat samples with level of significance 0.000 (0.05).Our result were consistent with Pavlovetal.[37] in Bulgaria,whom found that the incidence of antibiotic residues at enterprise I were 4% and 17% in Breast muscle and Liver respectively. Ezendukaet al. [19] in Nigeria, found that 11% muscle samples were positive for antibiotic residues.Also our result were compatible with the study conducted by Morshdy etal.[29] in Egypt,whom found that the occurrence of antibiotic residues in chicken meat samples were (15%), (12.5%) and (15%) in breast muscle,thigh muscle and liver respectively. Amro etal.[38]in Egypt,revealed that the incidence percentage were(40%),(10%),and (10%) in chicken liver,thigh muscle and gizzard respectively.Many searchers were inconsistently with our finding.Tajick and Shohreh[39]in Iran found that more than 50% of chicken meat samples had noticeable antibiotic residues.Buket et al.[40]in Turkey mentioned that one hundred eighteen of 231 (51.1%) examined chicken meat and beef samples were found to contain quinolone antibiotic residue,among the chicken meat and beef samples,58 (45.7%) of chicken meat samples and 60 (57.7%) of beef meat samples were positive for quinolones,respectively.In Khartoum State,Sudan,Elnasri et al.[18]found that the total number of positive samples was 27 % , the highest percentage of positive samples was detected in the muscle tissue (29.2 %) and the least was in the kidney . AL-bayoumi [21]in Gaza strip reported that (24.1%)of poultry meat samples were positive for one or more of antibiotic residues.Our result (11.1%) consider lesser than results showed by Sahu and Saxena [41] in India whom found that the antibiotic residues were present in 23% of chicken meat samples.Currently,Chaiba et al.[2]reported that 36.15% of poultry meat samples from intensive livestock were positive to antibiotic residues,while all samples from traditional livestock were negative.AlsoWang et al.[42]in China mentioned that the overall prevalence of antibiotic residues in chicken meat was 22.2 % ,while

Kirrella et al .[24] in Egypt revealed that 58% of samples were positive for antibiotic residues,using the Premi test (a broad-spectrum screening test for the detection of antibiotic residues in meat)at or below the maximum residue limits.However ,Codex Alimentarius Commission[43] reported that food from animals and poultry treated with veterinary drugs / medicines must not contain any residue that might represent a hazard to the health of the consumer , also in the database of this codex the user can obtain information on Maximum Residue Limits (MRLs) and Risk Management Recommendations (RMRs) for residue of a veterinary drugs in foods.In this research,the relationship between months and occurrence of antibiotic residues in poultry meat during period of study in Erbil city was determined.Table 2 show that the highest rate of detection of antibiotic residues from different kinds of samples were found in January (20.8%), approximately close to this rate in December (18.5%).But the rate of detection were seen decrease in February(12.5%),March (5.6%),April(4.8%), while antibiotic residues not detected in May.The value of ($P< 0.05$) (0.000) for all months showed significant differences between the occurrence of antibiotic residues in poultry meat and months during period of study .In another hand , Pavlov etal.[38] in Bulgaria ,noticed that 2/45(4.4%) samples of Breast muscle , 8/45(17.8%) of liver collected from abattoir I at winter month (November to March) were found to be positive for antibiotic residues.Also Aalipour et al.[44] in Iran showed that the highest contamination rate of milk with antibiotic residues occurred in February (45%), followed by July (41%) and June (40%) .Omeiza and Nafarnda [45] in Nigeria revealed varied patterns of drug residues in chicken egg occurrences in different seasons of the year , they mentioned that the higher concentration was a consistent finding associated with dry windy and harsh weather.It is therefore rational to believe that seasons with higher incidences of poultry diseases encourage antimicrobial drugs misuse with the attendant incidences of higher residue concentrations.It is concluded that such seasons be monitored with increased effort by the relevant agencies to forestall public health risks associated with the consumption of eggs. When we study the effect of cooling (4°C/3days) on antibiotic residues in poultry meat samples,we found that the cooling process was influential on the incidence of antibiotic residues in samples (Table 3).Also the effect of freezing (-18°C/4 weeks) on antibiotic residues in poultry meat samples was influential on the incidence of residues in these samples (Table4).There is dearth of information on the effect of cooling and freezing with time on the detection of antibiotic residues in poultry meat. Data presented in Table 5 declared that all processed samples were affected by boiling at 100°C/30 minutes.These findings are in line with a study conducted byMorshdy et al.[29] ,whom reported that no antibiotic residues were detected after boiling of positive frozen chicken meat samples (breast muscles, thigh muscles and liver) at 100°C for 30 minutes,but our results are in agreement with Abou – Raya et al.[46] who found 73.6% OTC reduction in meat by boiling for 30 minutes. Mgonja et al.[47] noticed that the occurrence of violative levels of drug residues in raw meats is decreased by heating, therefore, the occurrence of violative levels of drug residues in the food may be prevented by the heating

process.Also Tian et al.[48] recorded that thermal processing results in a decrease in the concentration of parent antibiotic residues.Although antibiotic residues were degraded during boiling,but we suggest to do other studies including toxicological experiments for effect of degradation by-products on human body.As some of these products were shown to be hazardous,further investigation is needed to determine their impact on food safety and human health,as well as,most of the residue in boiling process was excreted from tissue into cooking fluid.

CONCLUSION

From this work we concluded that the occurrence of antibiotic residues in poultry meat in Erbil city was high (11.1%). The monitoring of antibiotic residues is necessary to safeguard the health of the consumers as well as minimize environmental contamination. Microbiological methods are quite suitable for the detection of antimicrobial residues especially as they are less expensive than immunochemical and chromatographic methods,and are able to screen a large number of samples at minimal cost.The withdrawal period should be observed carefully before the birds are slaughtered.Besides,the public should be educated on appropriate cooking preparation of poultry meat.In addition,we must emphasize on the proper use of antimicrobial agents in the treatment, prevention and control of diseases as well ensuring effective implementation of regulatory measures.

CONFLICT OF INTEREST

The authors have declared that they have no conflict of interest.

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