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DETECTION OF OXYTETRACYCLINE RESIDUES IN EXPERIMENTALLY INJECTED CHICKENS FROM DAKAHLIA POULTRY FARMS, EGYPT

Tamer M. M. Gad*, Alaa El-Din M. A. Morshdy and Adel I. M. El-Atabany****

** Educational Veterinary Hospital, Faculty of Veterinary Medicine, Mansoura University, Egypt*
*** Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Egypt*

ABSTRACT

Antibiotics residues in chicken meat have a particular public health significance in the field of food safety due to its related resistance and emergence of antimicrobial-resistant microorganisms. The objectives of this study were firstly, to investigate the incidence of oxytetracycline residues in chicken muscles (pectoral and thigh) and its organs (liver and kidney) which were collected from Dakahlia poultry farms, Dakahlia governorate, Egypt. Secondly, to reduce these residues in chicken meat and its organs. The public health significance of oxytetracycline residues was discussed. Thigh muscles had the biggest inhibition zone in the examined samples on 1st and 2nd days after intramuscular injection of oxytetracycline in chickens followed by pectoral muscles, livers and kidneys. After that, inhibition zone reduced gradually in all examined samples on 3rd, 4th, 5th and 6th days till disappeared on 7th day from thigh and breast muscles together while on 9th and 10th days, it disappeared from livers and kidneys respectively.

Both deep freezing at (-18:-20oc) for 14 successive days and boiling at 100oc for 60 minutes of examined samples are an efficient strategy in reducing oxytetracycline residues in chicken meat. Thus, strict instructions and precautions during using antibiotics for prophylaxis or treatment in poultry farms should be followed to reduce its residues in meat.

INTRODUCTION

Chicken meat is a very important source for animal derived proteins, essential amino acids, vitamins and minerals. Chickens meat is one of the most highly perishable food products, but the misuse of antibiotics in poultry feeds or treatment leads to emergence of antibiotics residues ***(Salehzadeh et al., 2006)***. Tetracyclines are among various antibiotics widely used in livestock ***(Nonga et al., 2009)***.

The presence of antibiotic residues in animal meat and organs caused very harmful effect to consumers, carcinogenic effect, allergic effect and microbial resistance caused by antibiotic residues ***(Kay , 1993)***.

Tetracyclines including the oxytetracycline are relatively nontoxic to human and animals, but the therapeutic concentration, they are occasionally associated with peripheral blood changes, discoloration of bones and teeth and allergic reaction in humans ***(David and Scott, 1994).***

MATERIALS AND METHODS

Samples collection and preparation:

A total number of thirty chickens and three chickens as a control weighing about 1500-1750 grams from Dakahlia poultry farms, Egypt feed a balanced ration free from antibiotics for two weeks. Each chicken was represented by 4 samples (pectoral, thigh muscle, liver and kidney), the thirty chickens were injected oxytetracycline 20mg/kg body weight intramuscularly in the thigh region as a single dose for 3 successive days. Each three injected chickens (as a single group) from the examined chickens were slaughtered on 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th}, 8^{th}, 9^{th} and 10^{th} days respectively after the last dose of drug administration while the control chickens were slaughtered and examined directly before drug injection. Examined samples were taken from pectoral, thigh muscle, liver and kidney and these samples were wrapped in polyethylene bags and were put in cool boxes. The samples were subsequently transported directly under a complete aseptic condition to our laboratories. The samples were stored at -20°C until time of analysis. Cylindrical pieces (9mm diameter and 2mm thickness) were taken by sterile cork borer from such samples. Two pieces of each sample were put diagonally on the surface of freshly prepared B. subtilis plates (pH 6.0 and pH 8.0).

Plates were incubated at 30°C for 24 hr according to (***Ezhov, 1968***) and the width of inhibition zones was measured according to ***(Schoevers et al., 1994)***.

Effect of freezing at (-18: -20°C) for 14 successive days in the deep freezer on the same examined samples was studied by the same technique. Also, effect of boiling at 100°C for 60 min on the same examined samples was studied.

a) Organism used:

Bacillus subtilis (ATCC-6633) was obtained from Department of Food Hygiene, Animal Health Research Institute in Dokki-Cairo- Egypt.

b) Media used: ***(Oxoid, 1990)***

1- Standard II nutrient agar (Merck. Art. Nr. 7883) to 1% KH_2PO_4 at pH 7.2.

2- Test agar at pH 6.0 (Merck. Art. Nr. 10663) and at pH 8.0 (Merck. Art. Nr. 10664).

c) Antibiotic medium No.1 (Pfizer company, Cairo, Egypt).

1-Preparation of test organism according to (*Heitzman, 1994b*).

2-Preparation of test plates according to (*Levetzow, 1971*).

3-Test used: General inhibitor test according to (***Levetzow, 197***1).

Table (1): Inhibition zone diameters in mm in the examined samples from oxytetracycline intramuscular injected chickens (20 mg/kg B.W.) for 3 successive days: (N=30)

Organ	Inhibition zone (mm) post slaughter period/day									
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day
pectoral muscles	6	5	5	4	4	3	0	0	0	0
Thigh muscles	6	6	5	5	4	3	0	0	0	0
Liver	5	5	4	4	3	3	2	2	0	0
Kidney	4	5	3	3	4	2	2	2	2	0

Table (2): Effect of deep freezing at (-18: -20oC) for 14 successive days on oxytetracycline HCL residues in the different samples of intramuscular injected chickens: (N=30)

Organ	Inhibition zone (mm) post slaughter period/day																			
	1st day		2nd day		3rd day		4th day		5th day		6th day		7th day		8th day		9th day		10th day	
	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A
pectoralmuscles	6	4	5	3	5	2	4	1	4	1	3	0	0	0	0	0	0	0	0	0
Thigh muscles	6	4	6	4	5	3	5	2	4	1	3	0	0	0	0	0	0	0	0	0
Liver	5	3	5	3	4	1	4	1	3	1	3	1	2	1	2	0	0	0	0	0
kidney	4	2	5	3	3	1	3	2	4	1	2	1	2	1	2	1	2	0	0	0

Table (3): Effect of boiling at 100oC for 60 min on oxytetracycline HCL residues in the different samples of intramuscular injected chickens: (N=30)

organ	Inhibition zone (mm) post slaughter period/day																			
	1st day		2nd day		3rd day		4th day		5th day		6th day		7th day		8th day		9th day		10th day	
	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A
pectoral muscles	6	0	5	0	5	0	4	0	4	0	3	0	0	0	0	0	0	0	0	0
Thigh muscles	6	0	6	0	5	0	5	0	4	0	3	0	0	0	0	0	0	0	0	0
Liver	5	0	5	0	4	0	4	0	3	0	3	0	2	0	2	0	0	0	0	0
Kidney	4	0	5	0	3	0	3	0	4	0	2	0	2	0	2	0	2	0	0	0

B= before **A**= after

RESULTS AND DISCUSSION

The data presented in table (1) indicated that inhibition zone diameters in mm in examined samples from oxytetracycline intramuscular injected chickens (20 mg/kg B.W.) for 3 successive days disappeared after the sixth day in both pectoral and thigh muscles together, after the eighth day in liver and after the ninth day in kidney and these disagreed with ***El-Mossalami et al., (1985)*** who mentioned that in Egypt, OTC withdrawal periods were variable, where OTC residues were detected 20 hours post-injection in both pectoral and thigh poultry muscles and completely disappeared from all the examined samples after 48 hours from the last injection. Also, the present results disagreed with ***Moustafa (1988)*** who said that OTC residues in thigh, pectoral and liver of poultry could not be detected after 36 hours, 4 days ***(Abd El-Hamid, 2000)***, 17 days ***(Hussein, 2001)***.

Liver and kidney showed higher concentration of tested antibiotic to illustrate the role of liver in metabolism and kidney in excretion of oxytetracycline and these data were in a disagreement with those obtained by ***Al-Mustafa and Al- Ghamdi (2000)*** who screened samples of market-ready chicken muscle and liver from 32 local broiler farms for antibiotic residues by microbiological assay.

On the other hand, results presented in table (1) were in an agreement with those obtained by ***Salehzadeh et al., (2006)*** who detected oxytetracycline residues in all samples (270 chicken muscle, liver and kidney samples from 90 broiler farms in Tehran province of Iran).

Also, the results presented in table (1) were in an agreement with those obtained by ***Hussein (2001)*** who recorded the recovery rate of oxytetracycline residues in broiler muscle spiked with different OTC concentration which ranged from 75.8 to 99.35% with a mean value of 86.82%.

The data presented in table (2) discussed the effect of deep freezing at (-18:-20^{o}C) for 14 successive days on oxytetracycline HCL residues in different samples from oxytetracycline intramuscular injected chickens and indicated that the diameters of inhibition zones decreased and these results were agreement with those obtained by ***Kindred and Hubbret (1993),*** they concluded that freezing of turkey meat for 4 weeks may help in degrading some antibiotics in muscles and liver where residues became under the detectable level like oxytetracycline but gentamicin residues had a little effect. Also, these results were in an agreement with those obtained by ***Ibrahim (1997), Mansour (2000), Hussein(2001***) and ***Mahmoud and Mohsen (2008)*** they concluded that tetracycline residues were more degraded by freezing at -18^{o}C.

The data presented in table (3) indicated the effect of boiling at 100^{o}C for 60 min on oxytetracycline HCL residues in different samples from oxytetracycline intramuscular injected chickens and indicated that the diameters of inhibition zones disappeared and these results were agreement with those obtained by ***Hassanien (1995), Ibrahim (1997)*** and ***Hussein (2001).*** The effect of heat treatment as boiling, roasting and frying on tetracycline residues demonstrated that heat treatment decreased the concentration or led to a complete inactivation according to the degree of temperature and time of exposure ***Moats (1999), Mansour (2000)*** and ***Hussein (2001).***

The heating or boiling decreases the antibacterial residues in chicken meat and organs. Moreover, antibiotics can be refractory

for heat degradation in animal tissues unless high temperature levels are maintained for considerable periods ***(El-Zeini and Atta, 1995).*** This proved that the temperature and the duration time resulted in disappearance of antibiotic residues in all edible tissues except some organs. However some antibiotics are heat stable such as chloramphenicol ***(Hamman et al., 1978)***, while others are polymerized at higher temperature (200^{o}C) and produce toxic or mutagenic products ***(Booth and McDonald, 1988)***. Boiling and roasting at 150^{o}C for 30 min (***Abd El-Hamid, 2000)*** caused full inactivation and complete degradation of OTC residues in broiler muscles.

All OTC residues were completely disappeared from all the examined samples after one week freezing at -20^{o}C as well as after boiling, frying and roasting (***Eissa et al., 1998***).

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الملخص العربى
الكشف عن بقايا عقار الأوكسي تيتراسيكلين بعد الحقن بالعضل معمليا في دواجن مزارع الدقهلية للدواجن بمحافظة الدقهلية بمصر

تامر محمد محمد جاد*، علاء الدين محمد علي مرشدي**، عادل إبراهيم محمد العتباني**

* المستشفى التعليمي البيطري، كلية الطب البيطري، جامعة المنصورة، مصر
** قسم مراقبة الأغذية، كلية الطب البيطري، جامعة الزقازيق، مصر

تؤثر المضادات الحيوية في لحوم الدواجن بمصر على صحة وسلامة الغذاء حيث إنها تترك أثرا على الصحة العامة للإنسان بما تخلفه من بقايا تؤدي إلى مقاومة الميكروبات لمثل هذه المضادات الحيوية. لذا فإن الهدف من هذه الدراسة اكتشاف بقايا عقار الأوكسي تيتراسيكلين في لحوم الدواجن بمزارع الدقهلية للدواجن بمصر ومن ثم تقليل هذه البقايا أو التخلص منها. أظهرت الفحوص الميكروبيولوجية باستخدام ميكروب الباسيلس ساتلس على عضلتي الصدر والفخذ والكبد والكلية لكل دجاجة من إجمالي ٣٠ دجاجة من دواجن مزارع الدقهلية للدواجن أن فترة سحب عقار الأوكسي تيتراسيكلين كالتالي: ٦ أيام لعضلتي الصدر والفخذ، ٨ أيام للكبد، ٩ أيام للكلية وذلك بعد الحقن العضلي للعقار في عضلة الفخذ بجرعة ٢٠ مجم/كجم لمدة ٣ أيام متتالية قبل عملية الذبح. وتم التخلص من بقايا العقار وذلك بالمعالجة الحرارية إما بالتجميد العميق عند -١٨: -٢٠ درجة مئوية لمدة ١٤ يوم متتالية أو بالغليان عند ١٠٠درجة مئوية لمدة ٦٠ دقيقة. لذا يتحتم على جميع الجهات الرقابية التأكيد على انتهاء فترة سحب الدواء قبل الذبح وإذا ماتم ذبح الطائر قبلها فإنه يتحتم على المستهلك بالمعالجة الحرارية سالفة الذكر. هذا وقد تم مناقشة الأهمية الصحية لبقايا ذلك العقار