



Assessment of oxytetracycline and ampicillin residues in sheep carcasses

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ABSTRACT

This study was conducted for assessment of oxytetracycline and ampicillin residues in ninety sheep carcasses (meat, liver & kidney 30 of each) collected from two different abattoirs (urban and rural area 45 of each) in Menofia governorate, by using four plate test (FPT) and high performance liquid chromatography (HPLC) technique. The results revealed that the incidence of oxytetracycline and ampicillin residues in the examined samples of sheep meat, liver and kidney were 6.67%, 20% & 20% and 0%, 13.33% & 13.33% with application of FPT and 13.33%, 20% & 33.33% and 13.33%, 20% & 26.67% by using HPLC from urban area, respectively. While, 13.33%, 26.67% & 33.33% and 6.67%, 20% & 20% for those samples meat, liver and kidney with application of FPT and 20%, 33.33% & 33.33% and 13.33%, 26.67% & 33.33% by using HPLC from rural area, respectively. However, the mean values of oxytetracycline and ampicillin residues ($\mu\text{g}/\text{kg}$) were 109.5 ± 17.3 , 584.7 ± 41 & 1115.2 ± 98.7 and 17.5 ± 1.1 , 50 ± 4.8 & 72.3 ± 6.2 for samples in urban area and 178.3 ± 22.8 , 697.2 ± 59.1 & 1291.6 ± 117.5 and 28.5 ± 1.9 , 57.7 ± 5.2 & 85.8 ± 9.4 for those in rural area, respectively. Accurately 0%, 13.33% & 13.33% and 6.67%, 20% & 26.67% of both samples from urban and rural areas were unaccepted based on their contents of oxytetracycline according to FAO/WHO (2004), respectively. In addition, accurately 6.67% & 20% and 13.33% & 20% of liver and kidney samples for urban and rural areas were unaccepted, while all meat samples were accepted according to FAO/WHO (2004), respectively. The public health significance of antibiotics residues and the suggestive recommendations to avoid the presence of such residues were discussed in this study.

Keywords: Sheep meat, offals, oxytetracycline, ampicillin.

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1. INTRODUCTION

Meat is one of the most important constituents of the human diet as it provides protein, energy, vitamins and minerals. However, sheep meat is generally considered healthier because of its combination of protein, omega fatty acids (the good kind). Adding more lamb meat into your diet will naturally yield several different benefits, only one of which is the boost in protein intake. Due to the demand for increasing meat production, several drugs employed for food producing animals for treatment, prevention and control of diseases were also used as growth promoters. These include various types of antibiotics as tetracyclines (TCs) and penicillin (PEN),

which cause health hazards as it can remain in animal's body even after slaughtering as residues in its carcass if the antibiotic withdrawal period is insufficient. The presence of these residues in meat can influence the metabolic activity of the intestinal microflora of the consumer, may cause allergic reactions in sensitive individuals and affect on the bacterial composition leading to the development of resistant strains of bacteria due to the ingestion of sub therapeutic doses of these antibiotics (Mehran *et al.*, 2012). Presence of drugs or antibiotics residues in food above the maximum level recognized world wide by various public authorities is illegal

(Kemp and Verachtert, 2000). Monitoring of antibiotic residues is very important in controlling the safety of foods for human consumption so several analytical methods are available for determination of antibiotic residues in animal tissue. The first one is the microbiological assay, but it is slow with low sensitivity. The second method is the instrumental methods, which includes gas chromatography (GC), liquid chromatography with mass spectrometric detection (LC-MS) and high performance liquid chromatography (HPLC). These methods are sensitive and need highly skilled analysts, expensive and not suitable for routine analysis of large scale samples. (Abdullah *et al.*, 2012).

Antibiotic resistance is an emerging public health problem especially due to the continuous use of antibiotics that selects more aggressive and resistant species (Messano and Petti, 2011). However, In Egypt, OTC and APC compounds are the most common antibiotics used in sheep treatment due to low cost, easily accessible, readiness availability. Misuse of antibiotics in treatment of animal producing meat especially oxytetracycline and ampicillin without any control and observed withdrawal period was the start point for this study. Therefore, this study was planned out for the assessment of oxytetracycline (OTC) and ampicillin (APC) residues levels in sheep carcasses (meat, liver and kidney) in Menofia Governorate (urban and rural abattoirs) using FPT and HPLC.

2. MATERIAL AND METHODS

2.1. Collection of samples

90 random samples of sheep meat, liver and kidneys (30 of each) were collected from two different abattoirs (urban and rural areas) in Menofia governorate. All collected samples were transferred in an icebox to the laboratory for determination of their contents oxytetracycline and ampicillin residues.

2.2. Determination of antibiotic residues

2.2.1. Four Plate Test(FPT)

According to Levetzow and Weise (1979) for qualitative detection of antibiotic drug residue in the examined samples.

Preparation of the spore suspension: Nutrient agar was inoculated with heavy suspension of *Bacillus subtilis* and incubated at 30°C for 10 days then centrifuged at 3000rpm /10minutes then diluted with normal saline to achieve a density of 10^7 spores/ml of diluent.

Preparation of test plates: The media was divided into 2 portions. The first was adjusted at PH 6 for detection of β -lactam, tetracycline and penicillin residues and the second was adjusted at PH 8 for detection of amino glycoside residues. Maintenance of *B. subtilis* culture: Incubation for nutrient agar slopes inoculated with *Bacillus subtilis* at 30°C overnight. Application of microbial inhibition test: Which gave annular zones of inhibition of a specified minimum size.

Interpretation of the results: The results were indicated by measuring the diameter of inhibition zones of the growth of the *Bacillus subtilis* cells around meat samples. A zone more than or equal 2 mm was recorded as positive result. A zone from 1 to 2 mm was recorded as suspicious.

A zone less than 1 mm was considered as negative.

2.2.2. High Performance Liquid Chromatography (HPLC):

According to (Heitzman,1994) for quantitative analysis of oxytetracycline and ampicillin in the examined samples.

Sample preparation for extraction of the drug with a specific solvent: Extraction: Accurately, 5 ± 0.01 g of the sample were put into 50 ml capped polypropylene centrifuge tube and 15 ml of acetonitrile /water (15:2) were added. Complete homogenization and centrifugation at 4000rpm. Finally, the supernatant was filtered through regenerated cellulose. Actually, 10 ml of the extract were loaded onto the Agilent Sampli Q OPT 6 mL/150 mg cartridge.

Purification: (Ball, 2008). The cartridge was washed with 0.1% formic acid in water and then pH 8.5 potassium phosphate buffer. Finally, the sample was eluted with 3 ml. acetonitrile. The sample was filtered with 13 mm, 45 ml PTFE syringe filter . The eluent was dried under nitrogen at room temperature. The residue was resuspended in mobile phase to 1.0 ml. The sample was vortexed for 2 minutes and then transferred to a 2 ml, auto sampler vial.

Solid-phase extraction and final sample preparation: Add 25 ml of methanol, and then 25 ml of water, and then 40 ml 0.01 M calcium hydroxide. Then apply sample (3 ml) to cartridge. A flow rate of not more than 2 drops/s. The cartridge was flushed with 40 ml of distilled water, and then applies 10 ml of acetonitrile. Then elution was performed successively with 40 ml of 2.5% acetic acid including 50% methanol .Then, the collected eluate was evaporated at 45°C till complete dryness. Finally, the dried residue was reconstituted in 3 ml of the mobile phase. The samples were mixed and filtered through 0.2 µm filters before injection into the LC system

Separation of the antibiotics on the solid phase by HPLC: It was an Agilent 1100 HPLC system with quaternary pump model G 1311A, UV detector (Model G 1314A) set at 254nm wavelength, auto sampler (model G1329A VP-ODS) (Shimadzu, Kyoto, Japan). Accordingly, tetracycline and ampicillin residues were estimated by using their standard solutions specific for each of them. Operating conditions for analysis of oxytetracycline eluant at 36°C, flow rate 1 ml/min., injection volume, 50µl; wave length 312nm and for ampicillin eluant at ambient temperature, flow rate 0.5ml/min ,injection volume, 10µl; wave length 239nm.

2.3. Statistical Analysis:

According to Feldman *et al.*, (2003) and (SPSS, 1993).

3. RESULTS

Table (1) showed that the incidence of oxytetracycline in the examined samples of sheep meat, liver and kidneys by using FPT in both urban and rural areas were 6.67% , 20% & 20% s and 13.33%, 26.67% & 33.33% ,respectively. By performing HPLC, the oxytetracycline residues in sheep meat, liver and kidney were detected in 13.33%, 20% & 33.33% in urban area and 20%, 33.33% & 33.33% in rural area, respectively. Also, it was detected that results in table(2) showed that the highest mean value of oxytetracycline was $1115.2 \pm 98.7 \mu\text{g/kg}$ in kidney samples ,while the lowest mean value was $109.5 \pm 17.3 \mu\text{g/kg}$ in meat samples at urban area. On the other hand, at rural area the highest mean value was $1291.6 \pm 117.5 \mu\text{g/kg}$ in kidney samples, while the lowest mean value was $178.3 \pm 22.8 \mu\text{g/kg}$ in meat samples. There was high significant difference ($p < 0.01$). Results achieved in table (3) reported that 13.33% & 13.33% of liver and kidney samples exceeded the maximum permissible limits ,while all examined meat samples collected from urban area were accepted based on their contents of oxytetracycline. At rural area 6.67%, 20% & 26.67% of the examined samples of sheep meat, liver and kidney were unaccepted, respectively and exceeded the permissible limit stipulated by FAO/WHO (2004). In addition, the results in table (4) showed the incidence of ampicillin residues in the examined samples of sheep meat, liver and kidneys by using FPT in urban and rural areas were 0%, 13.33% & 13.33% and 6.67%, 20% & 20% for meat, liver and kidney samples, respectively. Moreover, by performing HPLC, the levels of ampicillin at both urban and rural areas were 13.33%, 20% & 26.67% and 13.33%, 26.67% & 33.33% for meat, liver and kidney samples, respectively. And also, it is evident from the results recorded in table (5) that the mean values of ampicillin ($\mu\text{g/kg}$) were 17.5 ± 1.1 , 50 ± 4.8 & 72.3 ± 6.2 for meat, liver and kidney samples at urban area,

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Table (1): Incidence of oxytetracycline residues in the examined samples of sheep meat and offals (n=15)

Slaughter area Sheep Tissues	Urban				Rural			
	FPT*		HPLC**		FPT*		HPLC**	
	No.	%	No.	%	No.	%	No.	%
Meat	1	6.67	2	13.33	2	13.33	3	20.00
Liver	3	20.00	3	20.00	4	26.67	5	33.33
Kidneys	3	20.00	5	33.33	5	33.33	5	33.33
Total (45)	7	15.56	10	22.22	11	24.44	13	28.89

* FPT: Four Plate Test, ** HPLC: High Performance Liquid Chromatography

Table (2): Statistical analytical results of oxytetracycline residues ($\mu\text{g}/\text{kg}$) in the examined samples of sheep meat and offal (n=15).

Slaughter area Sheep Tissues	Urban			Rural		
	Min.	Max.	Mean \pm S.E	Min.	Max.	Mean* \pm S.E
Meat	82	137	109.5 \pm 17.3	94	259	178.3 \pm 22.8
Liver	275	861	584.7 \pm 41.0	401	983	697.2 \pm 59.1
Kidneys	746	1450	1115.2 \pm 98.7	793	1710	1291.6 \pm 117.5

S.E= Standard error of mean, *= High significant difference ($p < 0.01$)

Table (3) Acceptability of the examined samples of sheep meat and offal according to their contents of oxytetracycline residues (n=15).

Slaughter Abattoir Sheep tissues	Maximum Residual Limit ($\mu\text{g}/\text{kg}$)*	Unaccepted samples			
		Urban		Rural	
		No.	%	No.	%
Meat	200	-	-	1	6.67
Liver	600	2	13.33	3	20.00
Kidneys	1200	2	13.33	4	26.67
Total (45)		4	8.89	8	17.78

* FAO/WHO (2004)

Table (4) Incidence of ampicillin residues in the examined Samples of sheep meat and offal (n=15).

Slaughter area Sheep tissues	Urban				Rural			
	FPT*		HPLC**		FPT*		HPLC**	
	No.	%	No.	%	No.	%	No.	%
Meat	-	-	2	13.33	1	6.67	2	13.33
Liver	2	13.33	3	20.00	3	20.00	4	26.67
Kidneys	2	13.33	4	26.67	3	20.00	5	33.33
Total (45)	4	8.89	9	20.00	7	15.56	11	24.44

*FPT = Four Plate Test. **HPLC = High Performance Liquid Chromatography

Table (5): Statistical analytical results of ampicillin residues ($\mu\text{g}/\text{kg}$) in the examined samples of sheep meat and offal (n=15).

Slaughter area Sheep tissues	Urban			Rural		
	Min.	Max.	Mean \pm S.E	Min.	Max.	Mean** \pm S.E
Meat	9	26	17.5 \pm 1.1	15	42	28.5 \pm 1.9
Liver	23	79	50.0 \pm 4.8	29	90	57.7 \pm 5.2
Kidneys	38	106	72.3 \pm 6.2	44	128	85.8 \pm 9.4

S.E= Standard error of mean. **= High significant difference ($p < 0.01$) between tissues.

Table (6): Acceptability of the examined samples of sheep meat and offal according to their contents of ampicillin residue (n=15).

Slaughter Abattoir Sheep tissues	Maximum Residual Limit ($\mu\text{g}/\text{kg}$)*	Unaccepted samples			
		Urban		Rural	
		No.	%	No.	%
Meat	50	-	-	-	-
Liver	50	1	6.67	2	13.33
Kidneys	50	3	20.00	3	20.00
Total (45)		4	8.89	5	11.11

*FAO / WHO (2004)

While 28.5 ± 1.9 , 57.7 ± 5.2 & 85.8 ± 9.4 at rural area, respectively. The significant differences between two areas were significant and high significant between tissues. Table (6) showed that 6.67% & 20% and 13.33% & 20% of the examined sheep liver and kidney samples from both urban and rural areas exceeded the maximum permissible limits so, unaccepted. All the examined meat samples from urban and rural areas were accepted according to FAO/WHO (2004).

4. DISCUSSION

Oxytetracycline is widely used in veterinary medicine for treatment and prevention of a variety of bacterial infection in food producing animals including cattle, sheep, goats, pigs, poultry and fish. (Fletouris and Papanagiotou, 2008) and may remain as residues in meat and edible parts that could be of health hazard importance (Doyle, 2006). It was clear that the highest incidence of antibiotic residues was in the examined kidney and liver samples, while the lowest was in the examined meat samples. These since organs of metabolism and excretion were expected to have higher concentrations of these residues than the muscles. This assertion is also corroborated by Landoni & Errecalde (1992), who indicated an ascending order of concentrations in the muscles, livers and kidneys, respectively, with increasing time. This is equally reflected by the increasing concentrations in MRLs recommended by (FAO, 1999) as $200 \mu\text{g}/\text{kg}$, $600 \mu\text{g}/\text{kg}$ and $1200 \mu\text{g}/\text{kg}$ in muscles, livers and kidneys particularly for oxytetracycline, respectively. In addition, the presence of residues in the muscles when compared with livers and kidneys might therefore be linked to administration of these antimicrobials to the animals shortly before slaughtering. Moreover, JECFA, (1990) reported that about 60% of ingested dose of OTC was absorbed from the gastrointestinal tract then widely distributed in the body particularly to liver, kidney, bones and

teeth. Also, reported that PEN is usually cleared rapidly from the blood via the kidneys where, PEN residues in kidney and liver were about 100 times higher than those in muscle. Our results in Table (1) were nearly similar to those obtained by Shahid *et al.* (2007) who found that the incidence of OTC were (39.4%), (27.3%) & (20.6%) in muscle, liver and kidney, respectively. The mean values of muscle and liver in table (2) nearly similar to those obtained by Mehran *et al.* (2012), but differs with those of kidneys samples. they recorded that the mean values of TCs antibiotic residues (TC, OTC and CTC) in the examined samples of triceps muscle, kidney and liver examined by using Solid-phase extraction (SPE) and HPLC were 176.3 ± 46.8 , 672.4 ± 192.0 and $651.3 \pm 210.1 \mu\text{g}/\text{kg}$, respectively. Additionally, 25.8% of muscle samples, 31.8% of liver samples and 22.7% of kidney samples contained amounts of TCs residues beyond MRLs. On the other side, results recorded in both tables (2)&(5) were higher than those reported by Adesokan *et al.* (2013) who recorded that the residues of OTC muscle, liver and kidney of cattle were $16.17 \pm 5.52 \mu\text{g}/\text{kg}$, $12.73 \pm 4.39 \mu\text{g}/\text{kg}$ & $9.47 \pm 3.24 \mu\text{g}/\text{kg}$ and PEN were $11.67 \pm 2.94 \mu\text{g}/\text{kg}$, $8.5 \pm 2.80 \mu\text{g}/\text{kg}$ & $6.27 \pm 2.46 \mu\text{g}/\text{kg}$, respectively. Additionally, it is of interest to note that all the tissue samples analyzed contained detectable residues of either OTC or APC. It might be due to the fact that these two antimicrobials are amongst the drugs most commonly administered in Egypt specially and Africa in general. This finding is in agreement with the recent report from a survey carried out in South Africa (Eagar and Van, 2012). Where tetracyclines constituted the second largest group of antimicrobials and PEN. The majority of the parenteral dosage forms sold. Hypersensitivity, or even toxicity and development of bacterial resistance strains are among the hazards of antimicrobial residues Corry *et al.*, (1983), Andrews *et al.*, (1988), Booth, and McDonald, (1988). Consequently, residues in meat form a great

problem facing the food hygienists in the recent time . Antibiotic residues in meat may develop an allergic reaction such as urticaria, eczema and other dermatitis as well as the increasing of resistance of the pathogenic micro-organisms in man, in addition to their bad effect on the normal micro flora and consequently they produced vitamins (Bevill, 1984 and Montanaro, 1998). To ensure the human food safety, the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have set standards for maximum residue limits (MRLs) in foods. Additionally, the European Union (EU) has set their own MRLs (Cinquina *et al.*, 2003). The acceptable MRLs for OTC and PEN G residues in muscle are 200µg/kg and 50µg/kg, respectively according to the Joint FAO/WHO Expert Committee on Food Additives (WHO/FAO, 2010). The present results allow concluding that the antibiotic residues either oxytetracycline or ampicillin in sheep meat, liver and kidney were recorded at highest concentrations in the examined samples of kidney and liver followed by meat. Furthermore, all sheep's meat, liver and kidney samples collected from a rural area had the highest levels of oxytetracycline and ampicillin residues followed by urban area. So, the use of antibiotics in food producing animals' must be under control, it should be given at recommended dose with appropriate supervision. Adequate holding period or withdrawal period should be observed in all slaughtered animals following therapeutic use of antibiotics in treating sick animals, in addition to continuous training veterinarians to know the importance of withdrawal period in avoiding tissue residues.

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تقييم متبقيات الاوكسى تتراسيكلين والامبسيلين في ذبائح الأغنام

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الملخص العربي

أجريت هذه الدراسة لتقييم متبقيات الاوكسى تتراسيكلين والامبسيلين في ذبائح الأغنام وقد تم جمع تسعين عينة عشوائية من لحم وأكباد وكلاوى ذبائح الأغنام (30 لكل منها) من مجزرين مختلفين كل من منطقة ريفية وأخرى حضرية في محافظة المنوفية (45 لكل منها) للكشف عن متبقيات الاوكسى تتراسيكلين والامبسيلين باستخدام الطريقة الميكروبيولوجية التقليدية (FPT) للتقدير الكيفي، وكذلك الطريقة الحديثة باستخدام جهاز (HPLC) للتقدير الكمي. وقد كشفت النتائج أن معدل انتشار متبقيات الاوكسى تتراسيكلين في عينات لحوم وأكباد وكلاوى ذبائح الأغنام التي تم جمعها من المجزر الكائن بالمنطقة الحضرية كانت (6.67%، 20% و 20%) & (13.33%، 20% و 33.33%) باستخدام HPLC- FBT على التوالي. بينما نتائج معدل انتشار متبقيات الاوكسى تتراسيكلين في عينات لحوم وأكباد وكلاوى ذبائح الأغنام التي تم جمعها من المجزر الكائن بالمنطقة الريفية كانت (13.33%، 26.67% و 33.33%) & (20%، 33.33% و 33.33%) باستخدام HPLC- FBT على التوالي. كما أوضحت النتائج أن متوسط تركيز بقايا الاوكسى تتراسيكلين في عينات "اللحم والكبد والكلاوى" لذبائح الأغنام ($\mu\text{g/kg}$) (17.3±109.5)، (41±584.7) و (98.7±1115.2) التي تم جمعها من المجزر الكائن بالمنطقة الحضرية، (22.8±178.3)، (59.1±697.2) و (117.5±1291.6) للذي بالمنطقة الريفية على التوالي. وقد وجد أن جميع عينات اللحوم التي تم جمعها من المجزر الذي في المنطقة الحضرية صالحة للاستهلاك الادمي حيث أنها خالية من بقايا الاوكسى تتراسيكلين و في المقابل (13.33% و 13.33%) من عينات الأغنام "الكبد والكلاوى" التي تم جمعها من نفس المجزر وكذلك التي تم جمعها من المجزر الذي في المنطقة الريفية غير صالحة للاستهلاك الادمي حيث أن تركيز بقايا الاوكسى تتراسيكلين تعدت الحدود المسموح بها وأيضا 6.67%، 20% و 26.67% من عينات "اللحم والكبد والكلاوى" لذبائح الأغنام التي تم جمعها من المجزر بالمنطقة الريفية غير مسموح بها أيضا. ومن ناحية أخرى تبين أن معدل انتشار متبقيات الامبسيلين في عينات "لحوم وأكباد وكلاوى" ذبائح الأغنام كانت (0%، 13.33% و 13.33%) & (13.33%، 20% و 26.67%) باستخدام HPLC- FBT في المنطقة الحضرية، بينما (6.67%، 20% و 20%) & (13.33%، 26.67% و 33.33%) في العينات التي تم جمعها من المنطقة الريفية. كما أوضحت النتائج أن متوسط تركيز بقايا الامبسيلين في عينات "اللحم والكبد والكلاوى" لذبائح الأغنام (1.1±17.5)، (4.8±50) و (6.2±72.3)، بينما (1.9±28.5)، (5.2±57.7) و (9.4±85.8) في العينات التي تم جمعها من كلا المجزرين على التوالي. وكذلك جميع عينات اللحوم من كلا المجزرين صالحة للاستهلاك الادمي حيث أنها خالية من بقايا الامبسيلين وفي المقابل وجد أن (6.67%، 20%) & (13.33%، 20%) من عينات الكبد والكلاوى غير صالحة في كلا من مجزري المنطقة الحضرية والريفية على التوالي حيث أن تركيز بقايا الامبسيلين تجاوزت الحدود المسموح بها. ولقد تمت مناقشة الأهمية الصحية لبقايا المضادات الحيوية وخاصة الاوكسى تتراسيكلين والامبسيلين وتأثيرها على صحة الإنسان والمصادر المحتملة لوجودها وكذلك تقديم التوصيات والمقترحات اللازمة لإنتاج لحوم آمنة وخالية من أي أضرار صحية.

(مجلة بنها للعلوم الطبية البيطرية: عدد 27(2): 188-196, ديسمبر 2014)