



Detection of multiple presence of antibiotic residues in slaughtered sheep at Duhok abattoir, Iraq

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Abstract

The aim of this study is to investigate the presence and/or multi-presence of tetracycline, penicillin G, streptomycin, and gentamicin residues in slaughtered sheep carcasses at Duhok abattoir using ELISA technique. Eighty-eight samples were collected from different sites of twenty-two sheep carcasses. These samples included *Longissimus dorsi*, diaphragmatic muscles, liver and kidney. The samples were prepared, homogenized, extracted and assayed according to ELISA kit instructions. The results revealed that all 22 carcasses were contained at least one type of tested antibiotics. Multiple antibiotic residues were found in 17(77.27%) of examined carcasses. The study indicated that local ovine meat sold in Duhok province generally contained residues of antibiotics. Animals slaughtered might have been treated with veterinary drugs and the proper withdrawal period was not respected before slaughtering.

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Introduction

Meat is an esteemed article of human diet as it provides proteins, energy, minerals and most B complex vitamins (1). Animal food industries are rapidly growing with consequent demand of healthy wholesome animal (2). In spite of their merits, meat could be a source of health hazards if it contains harmful material such as pathogens, toxins, residues of chemical agents as a parent compound or its metabolites (3). Residues in meat may result from many sources such as pesticides or from veterinary drugs which used to prevent or treat diseases, or to promote growth (4). Other disadvantages are that withdrawal time in relation to the maximum residue limit (MRL) is not taken into account (5-8). Nowadays, antibiotics play a major role to rise agriculture and to enhance feed conversion in livestock industries (2). The most commonly used antimicrobials in food producing animals are the β -Lactames, aminoglycosides, macrolides, sulfonamides and lincosamides (3). Public health hazards associated with these residues include direct toxic effects on

consumers like allergic reactions and indirect problems through induction of resistant strains of pathogenic bacteria (9). Residues from these antibiotics may be present in edible tissues, milk and eggs and may exert different levels of toxicity on consumers when consuming them (2). Thus, easy, rapid and sensitive tests are really needed for an effective at line use (10). At present, there are various ways for detection of antibiotic residues which include microbiological, instrumental and immunoassay methods (11). A great proportion of livestock in Duhok province is reared by the herdsmen who administer animal pharmaceutical agents particularly incorrect dosage of antibiotics without veterinary prescription and with ignorance of withdrawal periods (12,13).

The aims of this study is to detect and determine the residues of single or multiple presence of tetracycline, penicillin G, streptomycin and gentamicin in meat and various edible tissues of sheep slaughtered at Duhok province, Iraq.

Materials and methods

Sampling

Eighty-eight samples have been collected from 22 sheep carcasses which were slaughtered within one week of August 2013 in Duhok abattoir to analyze for antibiotic residue by ELISA test. The samples were intentionally collected from organs and tissues suffering and affected by different pathological lesions. Four samples of 200 gm of *Longissimus dorsi*, diaphragmatic muscle, liver and kidney were taken and packed in clean plastic bags. Samples of the current study were intentionally taken from certain clinical cases and those suffering from pathological changes. The identified samples were transported immediately to the Faculty of Veterinary Medicine, Public Health laboratory, Duhok University in an ice box with a minimum period of delay. The samples were stored at -20°C in the freezer.

Sample preparation

Samples were finely homogenized to create a uniform form and were then extracted for each investigated antibiotic i.e. tetracycline, penicillin G, streptomycin and gentamicin according to the protocol recommended by the kit supplier (Shenzhen Lvshiyuan Biotechnology Co., Ltd, China). ELISA kits stored at 4°C. The product codes are LSY-10006, LSY-10019, LSY- 100024-2 and LSY-10023 for tetracycline, penicillin g, streptomycin and gentamicin, respectively.

Calculation of antibiotics concentrations

The concentrations of antibiotics in the samples were calculated according to the following formula, Percentage of absorbance value = $B/B_0 \times 100$. In which B is the optical density value of samples and B_0 is the optical density value of the standard solution. The values were then multiplying by the dilution factor as suggested by the kit manual. The standard and test absorbencies were entering manually to IPC software-version 4 from IPC Company, which then interpreted test results. Then the values were compare with the Maximum residue level (MRL) according EU standards (14).

Table 1: MRL of different organs according EU standards (ppb)

Type of antibiotic	Muscles	Liver	Kidney
Tetracycline	100	300	600
Penicillin G	50	50	50
Streptomycin	500	500	1000
Gentamicin	50	200	750

Results

The results of ELISA test for the examined samples of *Longissimus dorsi* muscle showed that there were different

concentrations of antibiotics residues at a rate (ppb) of 138.4522, 355.6462, 227.1922 and 897.0317 with a percentage of 40.9%, 40.9%, 45.45% and 13.63% for tetracycline, penicillin, streptomycin and gentamicin, respectively (Table 2 and 3).

The results referred that there were different concentrations of antibiotics residues of diaphragmatic muscle samples at a rate ppb of 72.868, 397.5671, 465.6912 and 428.12 with a percentage of 59.09, 50, 50 and 22.72% for tetracycline, penicillin, streptomycin and gentamicin, respectively (Table 4 and 6).

The findings also indicated different concentrations of liver samples, where recorded at a rate ppb of 108.6313, 593.4464, 343.4162 and 839.074 in a percentage of 54.54, 22.72, 27.27 and 13.63% for tetracycline, penicillin, streptomycin and gentamicin respectively (Table 5 and 7).

The results also showed that there were different concentrations of kidney samples at a rate of 72.868, 397.5671, 465.6912 and 428.12 with a percentage of 59.09%, 50%, 50% and 22.72% for tetracycline, penicillin, streptomycin and gentamicin respectively (Table 8 and 10). The table revealed co- and multiple presence of more than one antibiotic residue in sheep carcasses as shown in table 9.

Table 2: Antibiotics residues levels (ppb) levels in *Longissimus dorsi* muscle by using ELISA technique

Carcass No.	<i>Longissimus dorsi</i> muscle antibiotic residues (ppb)			
	Tetracycline	Penicillin G	Streptomycin	Gentamicin
1			5.005	926.133*
2	29.641			
3		721.621*	34.351	
4		298.001*	159.993	
5		288.952*	82.183	
6				847.038*
7	145.348*			
8		469.330*	3.672	
9				
10	114.266*			
11	175.192*			
12		645.102 *	690.307*	
13		23.843	690.307*	
14				917.924*
15	254.109*			
16				
17				
18	195.051*	252.473*	22.183	
19	189.181*	482.366*	580.249*	
20	111.018*			
21	32.264	19.128	3.672	
22				

* Over the MRL according EU standards.

Table 3: Mean and range of antibiotics residues levels and their positivity in *Longissimus dorsi* muscle by using ELISA technique

Samples	No. of Positive samples	Range of Str. conc. ppb	Mean (ppb)	Positive (%)
Tetracycline residues level (ppb)	9	29.641- 254.109	138.4522	40.9
Penicillin G residues level (ppb)	9	19.128 - 645.102	355.6462	40.9
Streptomycin residues level (ppb)	10	3.672 - 690.307	227.1922	45.45
Gentamicin residues level (ppb)	3	847.038 - 926.133	897.0317	13.63

Table 4: Antibiotics residues levels (ppb) levels in diaphragmatic muscle by using ELISA technique

Carcass No.	diaphragmatic muscle antibiotic residues (ppb)			
	Tetracycline	Penicillin G	Streptomycin	Gentamicin
1		33.325	39.962	922.825*
2	8.668			
3		579.859*	680.306*	
4	16.923	601.547*	581.030*	
5	9.857	274.853*	581.030*	
6				36.032
7	79.361			
8	168.328*	479.718*	584.667*	
9				
10	122.931*		149.656	
11	58.107			
12		768.773*		
13	66.443			
14				368.421*
15	176.845*			
16		552.940*	690.306*	
17	100.505*			572.305*
18		702.189*	580.214*	241.017*
19	13.007	86.964*	672.113*	
20	68.207	242.010*	486.288	
21	58.107			
22		51.06*	77.031	

* Over the MRL according EU standards.

Table 5: Antibiotics residues levels (ppb) levels in in local sheep carcasses in liver by using ELISA technique

Carcass No.	Liver antibiotic residues (ppb)			
	Tetracycline	Penicillin G	Streptomycin	Gentamicin
1				914.245*
2	129.841			
3		647.441*	35.601	
4				
5	105.354	590.589*	65.801	
6				628.772*
7	150.033			
8	124.766	399.862*	584.667*	
9				974.205*
10	111.266			
11	137.995	667.147*	680.206*	
12		662.193*	690.211*	
13	77.235			
14				
15	224.150			
16				
17	35.982			
18	105.854			
19	88.233			
20	12.866			
21			4.011	
22				

* Over the MRL according EU standards.

Table 6: Mean and range of antibiotics residues levels and their positivity in *diaphragmatic muscle* by using ELISA technique

Samples	No. of Positive samples	Range of Str. conc. ppb	Mean (ppb)	Positive (%)
Tetracycline residues level (ppb)	13	8.668 - 176.845	72.868	59.09
Penicillin G residues level (ppb)	11	33.325 - 768.773	397.5671	50
Streptomycin residues level (ppb)	11	39.962 - 690.306	465.6912	50
Gentamicin residues level (ppb)	5	36.032 - 922.825	428.12	22.72

Table 7: Mean and range of antibiotics residues levels in in liver by using ELISA technique

Samples	No. of Positive samples	Range of Str. conc. ppb	Mean (ppb)	Positive (%)
Tetracycline residues level (ppb)	12	12.866 - 150.033	108.6313	54.54
Penicillin G residues level (ppb)	5	399.862 - 667.147	593.4464	22.72
Streptomycin residues level (ppb)	6	4.011- 690.211	343.4162	27.27
Gentamicin residues level (ppb)	3	628.772 - 974.205	839.074	13.63

Table 8: Antibiotics residues levels in local sheep carcasses in kidney by using ELISA technique

Carcass No.	Kidney antibiotic residues (ppb)			
	Tetracycline	Penicillin G	Streptomycin	Gentamicin
1			19.885	346.863
2	120.539	280.113*	581.030	
3		499.352*	489.901	
4	112.627	674.609	433.871	
5	116.497	320.452*	581.030	
6				975.660*
7	166.443			
8	169.282	341.238*	143.600	
9				721.961
10	58.107			
11	85.428	515.873*	70.870	
12		138.365*	682.401	913.308*
13	9.961			
14				943.407*
15	215.934	34.108	671.578	
16		464.780*	689.421	
17	6.357			
18	75.442	241.352*	580.331	
19	82.337			
20	75.791	130.085*	496.288	
21				
22				213.510

* Over the MRL according EU standards.

Table 9: Multi-presence of antibiotic residues in local sheep carcasses

Carcass No.	Tetracycline	Penicillin G	Streptomycin	Gentamicin
1		x	x	x
2	x	x	x	
3		x	x	
4	x	x	x	
5	x	x	x	
6				x
7	x			
8	x	x	x	
9				x
10	x			
11	x	x	x	
12		x	x	x
13	x	x	x	
14				x
15	x	x	x	
16		x	x	
17	x			x
18	x	x	x	x
19	x	x	x	
20	x	x	x	
21	x	x	x	
22		x	x	x
%	63.64	72.73	72.73	36.37

X refers to the presence of the residues.

Table 10: Mean and range of antibiotics residues levels (ppb) in kidney by using ELISA technique

Samples	No. of Positive samples	Range of Str. conc. ppb	Mean (ppb)	Positive (%)
Tetracycline residues level (ppb)	13	6.357 - 215.934	103.3288	59.09
Penicillin G residues level (ppb)	11	34.108 - 674.609	351.0242	50
Streptomycin residues level (ppb)	12	19.885-689.421	449.4471	54.54
Gentamicin residues level (ppb)	6	213.510 - 975.660	780.2398	27.27

Discussion

In Iraq, the classic habit followed in which animal breeders administer a wide spectrum of veterinary drugs randomly to their animals without observing the withdrawal periods of these drugs before they are slaughtered for human consumption. Table 2 and 3 showed the percentage of samples containing tetracycline residues which referred that 50% of positive samples of *Longissimus dorsi* and diaphragm muscle exceeded recommended MRL 100 ppb as per EU standards for muscles (14).

Meat is considered as an essential feedstuff for human beings due to its high nutritional value, digestibility and palatability (1). In Iraq, livestock including sheep represent the main source of animal protein. Consequently, the emergent and increasing demand for animal products oblige many animal raisers to expand their scale of livestock

production and to increase their profitable business by using different antibiotics indiscriminately and application of "Blanket treatment" for such animals without observing and attention of withdrawal periods of drugs to clinically diseased or apparently healthy sheep before being slaughtered (15,16).

Residual tetracycline levels of liver and kidney samples were below the maximum residual limit 300 ppb for liver and 600 ppb for kidney according to EU standards for both organs (14).

However, these values were not exceeding the MRL (14), suggesting that such animals had been treated with the drug and probably were not allowed adequate withdrawal period.

Tables 4-8 showed the percentage of *Longissimus dorsi*, liver, kidney *diaphragmatic* muscle samples containing penicillin G residues. The table also showed that 85% of positive samples of *Longissimus dorsi* and diaphragm

contained which were over the MRL 50 ppb as per EU standards for all the examined organs (14).

Tables 2-7 showed the percentage of *Longissimus dorsi*, diaphragmatic muscle, liver and kidney samples containing streptomycin residues referring that 47.6% of positive for the first two organs which contained residues levels over the MRL 500 ppb for liver and below the MRL 1000 ppb for kidney according to EU standard (14).

Regarding the percentage of gentamicin residues in different tissues, table 8 and 10 showed that 87.5% of both *Longissimus dorsi* and diaphragmatic muscles had residues over the MRL 50 ppb according to EU standard for muscles (14). On the other hand, these residues were 100% and 50% in the samples of liver and kidney, respectively according to the aforementioned standard (14).

It was found that, penicillin G and streptomycin 72.73% were present in a large number of the carcasses followed by tetracycline 63.64% and gentamicin 36.37%, which could be accounted to the availability of these antibiotics in local markets with low cost promoted their broad administration. It should be noted that penicillin G and streptomycin is dispersed in one combination solution called PS. Such occurrences of multiple residues in the inspected meats may be attributed to the fact that these samples were intentionally collected from animals suffered from several diseases reflected and manifested subsequently by abnormal and pathological lesions in their carcasses.

The lower percentage of gentamicin residue obtained is due to the fact that this drug has limited uses, since it is only used for urogenital infections which were actually found in the examined carcasses fulfilling those goals of the study.

In Iraq, there are limited numbers of studies on the detection of antibiotics in food of animal origin, (17) found that 52% of poultry meat samples were positive for various antibiotic residues. Subsequently, (11) reported the streptomycin residue rate as 50% and 60% in ovine and bovine meat samples, respectively. (18,19) found detectable levels of antibiotics in milk samples collected from Afak city, Al-Diwaniya and Mosul Province- Iraq, respectively. Multiple antibiotic residues 18% were found by (20) in meat from slaughtered cattle in Nigeria. However, a high percentage of antibiotic residues were found by (21) in Egypt, 58% of kidney samples and 14% of muscle in cattle samples were positive, while the percentage in buffaloes was 40% and 20%, for kidney and muscles, respectively. The lower percentage 2% of oxytetracycline residues was obtained by (22) in bovine carcasses slaughtered at Mansoura Abattoir, Egypt.

Our findings were in agreement with those of (23) from Thailand, who found that out of 130 samples, 51 (39 %) were positive for at least one of the tested antibiotic. Several authors from different developing countries detected higher proportion of antibiotic residues in various animal producing food (24-27). A similar study carried out by (28) in Iran,

showed that 14% of mutton and 22.8% of beef samples contained antibiotic residues. In Turkey, a study done by (29) revealed that 57.7% of beef samples and 45.7% of chicken meat samples collected from Ankara markets were positive for antibiotic residues. Similarly, (30) from Nigeria found that 46% of samples were positive to residues of tetracyclines. A study performed by (31) in Algeria, showed a strong presence of antibiotic residues in poultry meat, with 85.5% positive samples, most of them contained penicillin, tetracycline and aminoglycosides.

Generally, it is obvious that high rates of residues in various animal tissues and products are reported in developing countries in contrast to what is found in the developed world according to the European Community findings. Such reports revealed that positive samples for antibiotic residues in food of animal origin were less than 1%, exactly 0.19 % (32), which could be attributed to the type of animal husbandry, management and production system.

Conclusion

Consequently, in developing countries a greater proportion of animal breeding is done by uncultivated herdsmen who always purchase veterinary drugs over the counter for administration to their animals without a veterinary diagnosis, prescription and supervision, and it may still be regarded as a health hazards as they may cause allergic reactions or produce drug-tolerant bacteria. Unfortunately, this condition is an indication of lack of public health regulatory control in developing countries, which require urgent care and attention.

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Conflict of Interest

The corresponding author (on behalf of all co-authors) should submit a conflict of interest disclosure form and is responsible for the accuracy and completeness of the submitted manuscript. Conflict of Interest Disclosure form can be signed by the corresponding author on behalf of all co-authors and stating that the submitted manuscript is the authors' original work, has not received prior publication and is not under consideration for publication elsewhere, permission has been received to use any material in the manuscript such as tables, figures etc. or no permissions have necessary to publish the authors' work.

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الكشف عن التواجد المتعدد لبقايا المضادات الحيوية في الأغنام المذبوحة في مجزرة دهوك، العراق

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الخلاصة

هدفت الدراسة الحالية للكشف عن تواجد عدد من أنواع متبقيات المضادات الحيوية والتي شملت التتراسيكلين والبنسلين والستربتومايسين والجنتاميسين في لحوم الأغنام المجزورة في مدينة دهوك باستخدام تقنية

المضادات الحيوية على الأقل في العينات المفحوصة فضلا عن الوجود المتعدد لبقايا المضادات الحيوية حيث وجدت في ١٧ ذبيحة أي بنسبة ٧٧,٢٧% من الذبائح المفحوصة، تشير الدراسة إلى أن لحوم الأغنام التي تباع في الأسواق المحلية تحتوي بشكل عام على بقايا المضادات الحيوية.

الاليزا، حيث جمعت ثمان وثمانون عينة من مواقع مختلفة من ٢٢ ذبيحة من الأغنام، وشملت هذه العينات العضلة العينية الظهرية وعضلة الحجاب الحاجز والكبد والكلى، حيث تم تحضير العينات وأجريت عليها عمليات المجانسة والاستخلاص والمعايرة حسب تعليمات الشركة المصنعة لتقنية الفحص. أظهرت النتائج وجود نوع واحد من بقايا