

Monitoring of antibiotic residues among sheep meats at Erbil city and thermal processing effect on their remnants

D.A. Al-Mashhadany

Department of Medical Lab Science, College of Science, Knowledge University, Erbil, Kurdistan Region, Iraq
Email: dhary.ammed@knu.edu.iq, Tel: +9647733565479

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Abstract

The incidence of antibiotic residues in meat is considered a serious hazard to public health. This work was conducted to survey the incidence of antibiotics residues among lambs' meats and mutton in Erbil city. The effect of thermal processing on residues was also addressed. From July to December 2018, a total of 280 samples were collected from butcher shops in Erbil city and analyzed microbiologically in plates pre-inoculated with *Bacillus subtilis* by direct and extraction methods. The overall incidence of antibiotics residues was 10.4% and 13.2% according to direct and extraction methods respectively. The highest incidence rate was detected in December 22.2%. We found that thermal processing, cooking for 45 minutes leads to the conversion of antibiotic residues into completely inactive residues against the bactericidal bacteria. In conclusion, the presence of antibiotic residue among sheep meats in the city of Erbil is high and their persistence is easily eliminated by cooking

Keywords: Antibiotic residues, Lamb meat, Mutton, Erbil, Iraq

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تواجد بقايا المضادات الحيوية بين لحوم الأغنام في مدينة أربيل وتأثير المعالجة الحرارية على بقاياها

ضاري عليوي المشهداني

قسم التحليلات المرضية، كلية العلوم، جامعة نولج، أربيل، إقليم كردستان، العراق

الخلاصة

يُعد تواجد بقايا المضادات الحيوية في اللحم خطراً كبيراً على الصحة العامة للمجتمع. لقد هدفت هذه الدراسة لتقصي تواجد بقايا المضادات الحيوية في لحوم الخراف اليافعة والبالغة في مدينة أربيل. وكذلك دراسة تأثير المعاملة الحرارية على بقاء هذه المركبات في اللحم. ففي الفترة الزمنية ما بين تموز وكانون الأول ٢٠١٨، تم جمع ٢٨٠ عينة لحم من محلات القصابية وخللت مايكروبيولوجياً للكشف عن تواجد بقايا المضادات الحيوية في أوساط زرعية ملقحة مسبقاً ببكتيريا *Bacillus subtilis* من خلال الطريقة المباشرة وطريقة الاستخلاص. أظهرت النتائج بأن التواجد الكلي لهذه المركبات كان بنسبة ١٠,٤٪ و ١٣,٢٪ طبقاً للطريقة المباشرة وطريقة الاستخلاص على التوالي. وقد سُجِّلت أعلى نسبة للتواجد خلال شهر ديسمبر ٢٢,٢٪. ومن ناحية التأثير الحراري على بقاء مركبات المضادات الحيوية أظهرت النتائج أن الطبخ في درجة الغليان أبطلت فعالية المركبات كلياً عند اختبارها ضد البكتيريا المذكورة. وإجمالاً، نسبة تواجد بقايا المضادات الحيوية في لحم الخراف في مدينة أربيل كانت مرتفعة وبقائها بصورتها الفعالة اختفى تماماً عند طبخ هذه اللحوم.

Introduction

Antibiotics, also known as antibacterial agents, are powerful secondary metabolites that have properties to fight certain infections by destroying bacterial cells or by

preventing their replication. These metabolites play essential roles in veterinary, agricultural, and clinical fields. Moreover, they are extensively used in food-producing animals for prophylactic, therapeutic, and metaphylactic purposes (1-3). Red meat refers to all mammalian muscle

meat. In gastronomy, it means any meat that is dark colored before and after cooking compared to white meat which is light in color prior to and after cooking. Sheep meat in its first year is called "lamb". This type of meat is characterized by little fat content and variation in color from a mild pink to a pale red, while the meat of an adult sheep is called "mutton". Adult sheep are typically slaughtered around two to three years of age (4-6). Antibiotic residues remain in tissues and organs of slaughtered animals and birds that have been given antibiotics previously without adherence to the withdrawal period (WP) of the antibiotic. Moreover, such residues may also be found in milk and eggs. The European Union (EU) and the Center for Veterinary Medicine (CVM), an agency under the Food and Drug Administration (FDA) in the USA, define the residues as "pharmacologically active substances (whether active principles, recipients or degradation products) and their metabolites which remain in foodstuffs obtained from animals to which the veterinary medical products (VMPs) in question has been administered" (7-9). Almost 80% of world antibiotic production is used in animal industry. In fact, some animal producers nowadays manage sub-therapeutic concentrations of antibiotics for different objectives, such as growth enhancement, impediment or decrease of disease outbreaks, enhancing digestion, acceleration of weight gain, and to rise feed conversion ratio (FCR). Therefore, residues of veterinary drugs may be present in food of animal origin even if their use is fully regulated. Indeed, such regulation is critical to ensure the proper use of veterinary drugs to protect consumers from undesirable antibiotics, and to ensure the supply of high-quality safe food products (10-12). The misuse and overuse of antibiotics in veterinary field lead to accumulation of large amounts of residues posing a serious threat to human health and economy after consuming contaminated meat tissues and organs. This problem is exacerbated by the lack or shortage of information among animal breeder about withdrawal periods of antibiotics. The incidence of antibiotic leftover in food of animal origin above the maximum residue limits (MRLs) is considered globally by different public health authorities as being illegal (13-15). Consequently, persistence of antibiotic residues in food of animal origin have been studied by different researchers in numerous countries (7,16-24). The consumption of red meat containing antibiotic residues could lead to direct toxicity, hypersensitivity; which characterized by extent of reactions ranging from mild skin rashes to life-threatening anaphylaxis, disturbance of the normal GIT microbiota, bone marrow desolation, teratogenic and mutagenesis effects. Additionally, most of antibiotics used in animal farming can lead to emergence of antibiotic-resistant bacteria that can then be transmitted to humans through food or contact. Indeed, bacterial resistance to antibiotics is a major global health problem that has been declared by WHO as one of the major challenges facing the 21st century (25-

27). Regulatory agencies and governmental authorities have established criteria regarding maximum residue limit (MRL) in various food matrices of animal origin in order to reinforce industry and food workers to control veterinary drug residues in the food chain. Nowadays, about 200 veterinary drug residues from several families, mainly with anti-parasitic, antibiotic, and anti-inflammatory behaviors are regulated in a diversity of food matrices such as milk, meat, and eggs (7,21,28).

Recently, there has been a considerable increase in antibiotic residues among food of animal origin, including sheep meat. Therefore, the objectives of this study were to detect the incidence of antibiotic residues among sheep meat in Erbil city using direct and extraction methods. The association between months and incidence of antibiotic residues in sheep meat was also investigated. The effect of thermal processing on remnants was also addressed.

Materials and methods

Samples collection

A total of 280 sheep meat samples of 100 gram from carcasses of lamb and mutton (135 lamb and 145 mutton) were aseptically and randomly collected from diverse butcher shops in Erbil city, during the period from July to December 2018. The collected samples were put in separate plastic bags and transported to Pathological Analysis Department, Knowledge University, under cold condition.

In the laboratory, each sample was divided into five parts; Part 1 and 2: Used for the detection of the antibiotic residues by the direct and extraction methods. Part 3: Ten grams of each positive samples were preserved in a refrigerator at 4 °C. After 3 days, to study the effect of chilling at 4°C on residues. Part 4: Ten grams of each positive samples were preserved at -18 °C for 6 weeks. These samples used to investigate the effect of freezing at -18 °C on residues. Part 5: Ten grams of each positive samples were used to study the effect of thermal processing (cooking) at 100 °C/45 minutes on antibiotic residues.

Detection of antibiotic residues: Direct method

The direct detection of antibiotic residues was carried out following a previously published technique (29). Briefly, a disc-shaped meat sample of 8 mm in diameter and 2 mm thick was prepared and placed on the surface of Muller-Hinton (MH) agar (HiMedia, India), pre-inoculated by spore suspension of a susceptible strain of *Bacillus subtilis* (0.1 ml/100 ml medium). *Bacillus subtilis* suspension was prepared at preferred concentration according to standard methods (30,31). Plates were incubated aerobically at 37 °C for 24 hrs. After incubation, diameters of inhibition zones around meat disks were measured in mm. Zone equal or more than to 2 mm was considered as a positive result. While zones smaller than 1 mm was considered as negative results.

If no zone appeared in the control sample, plates were selected for evaluation.

Detection of antibiotic residues: Extraction method

The technique cited by Al- Rubeae (30) was adopted. Briefly, twenty ml of potassium phosphate solution were added to 10 g of meat sample. After centrifugation at 4000 rpm for 10 minutes, 0.2 ml of the supernatant was delivered into wells on pre-inoculated MH agar and incubated at 37°C for 20-24 hours.

Effect of thermal processing methods on positive samples

Chilling: Meat samples which showed positive results in the extraction methods were preserved in cooling at 4 °C. After 3 days, samples were inspected for the continuity of antibiotic residues by the extraction methods.

Freezing: Samples which showed positive results in the extraction methods were saved in a freezer at -18°C. After 6 weeks, antibiotic residues existence was estimated by the extraction methods.

Thermal processing (cooking): Nearly 50 grams of each positive sample were placed into a strainer and soaked in 250 ml of water bath preheated to 100 °C for 45 minutes. Samples then removed from water and allowed to cool before re-assessment for antibiotic residues by extraction method.

Statistical analysis

All data were analyzed by SPSS version 21.00; chi-square test was employed to assess the difference between samples and methods. Confidence intervals were calculated by normal approximation method. Association between seasons and antibiotic residues was analyzed by Fisher's exact test. P value less than 0.05 was considered significant.

Results

Incidence of antibiotic residues

Out of 280 raw sheep meat samples, positive samples comprised 10.4% (29/280) and 13.2% (37/280) when tested by direct and extraction methods respectively (Table 1). Statistically, it is estimated that 6.79% - 13.93% (95% confidence interval) of raw sheep meat in Erbil market is contaminated by antibiotic residues if tested by the direct method. While 9.25-17.18% of sheep meat is expected to harbor antibiotic residues if tested by the extraction method. There is no significant difference between the two methods used for residues screening ($P = 0.305$).

Changes in rates of antibiotic residues during study period

The change in incidence rate of antibiotic residues was observed through study period. The highest rate of residues was detected in late winter, while the lowest rate was found

in July and August (6.7%). Table 2 summarizes the detection rates in temporal scale. There is no significant difference between seasons (winter and autumn) in terms of incidence of antibiotic residues among sheep meat ($P = 0.091$). In general, the incidence of residues in sheep meat increased as summer and autumn progress ($R^2 = 0.74$).

Effect of preservative methods on persistence of antibiotics residues

The effectiveness of chilling at 4 °C for 3 days on the incidence of antibiotic residues among positive sheep meat samples was low. In fact, only 16.2% of residues-positive samples showed clearance of the residues after cooling (Table 3). In contrast, freezing at -18 °C for six weeks was even less effective than chilling on the incidence of antibiotic residues (Table 3). However, there is no significant difference between the preservative methods ($P = 0.746$). Lastly, heat processing at 100 °C for 45 minutes showed complete 100% deactivation of antibiotic residues against challenged bacteria.

Discussion

The administration of antibiotic drugs in animal farming is prevalent and often indispensable. Indeed, rearing of large number of animals in small spaces facilities transmission of various infectious diseases. In crowded farms, bacterial infections are common and require a continuous input of antibiotics for therapeutic and prophylactic purposes. However, antibiotic residues settle in animal's tissues for different periods of time. Not only meat and other tissues harbor the antibiotic residues, but also, they can be found in milk or other dairy products from treated animals (12,32).

Lamb meat is characterized by its notable nutritional value. On the other hand, mutton meat has a higher fat content and strong flavor (33,34). Recently, antibacterial residues in food of animal origin have become a concern with extensive lack of certainty about the hazards caused by antibacterial residues to consumers. One of these hazards is the continuous selection of bacterial resistant strains. Indeed, the World Health Organization (WHO) announced that antibacterial resistance is an intercontinental problem that requires effective and rapid countermeasures to diminish the increasing level of resistant bacterial species. Moreover, Abd Al-Zahra and Ahmed (35), recommends that the obtained milk or meat from food producing animals treated with veterinary medicines/drugs must not contain any residue.

In the present work, the incidence rate of antibiotic residues in total sheep meat samples according to direct method was 10.4%. On the other hand, the incidence rate according to extraction method was 13.2% (Table 1). These findings are consistent with an Iranian study which found an incidence rate of 14.0% in mutton (36). In contrast, lower incidence rates 6.00 and 8.3% were reported in sheep meat

samples analyzed in Yemen and Spain respectively (29,37). On the contrary, higher rates have been reported previously from different countries. For instance, in Nigeria, antibiotic residues were presented in 44% of the slaughtered cattle

(38). Additionally, higher rates were reported from Turkey 57.7% (39), Ethiopia 71.3% to 82% (40,41). Such variations may be attributed to the difference in antibiotic usage practice in animal farming.

Table1: Incidence of antibiotic residues among sheep meat samples

Meat	No. Tested	Direct method		Extraction method	
		Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)
Lamb	135	13 (9.6)	122 (90.4)	15 (11.1)	120 (88.9)
Mutton	145	16 (11.1)	129 (88.9)	22 (15.2)	123 (84.8)
Total	280	29 (10.4)	251 (89.6)	37 (13.2)	243 (86.8)

Table 2: Temporal distribution of antibiotic residues in sheep meat by extraction method

Month	Lamb		Mutton		Total examined	Total positive (%)
	No. examined	No. Positive	No. examined	No. Positive		
July	22	1	23	2	45	3 (6.7)
August	22	1	23	2	45	3 (6.7)
September	23	2	26	3	49	5 (10.2)
October	23	3	26	5	49	8 (16.3)
November	23	4	24	4	47	8 (17.0)
December	22	4	23	6	45	10 (22.2)
Total	135	15	145	22	280	37 (13.2)

Table 3. Effect of chilling & freezing on antibiotic residues persistence by extraction method

Types of samples	No. of positive samples before chilling	No. of affected sample after chilling (%)	No. of positive samples before freezing	No. of affected sample after freezing (%)
Lamb	15	4 (26.7)	15	3 (20.0)
Mutton	22	2 (9.1)	22	2 (9.1)
Total	37	6 (16.2)	37	5 (13.5)

Humidity of normal pasture in winter may lead to increase in diseases incidence rates which requires intervention practices involving antibiotics. In terms of seasonal variations of residues level, this work is in good agreement with an Iranian study reporting the highest contamination rate of milk with antibacterial residues occurred in winter and ranged from 44.5% to 65.9%. In fact, it has been confirmed that winter season is correlated with increased rate of antibacterial residues in poultry meat samples examined in Yemen and recently in Iraq (10,29).

The chilling and freezing methods created approximately comparable effects on reduction of antibiotic residues in sheep meat. There is a shortage of information on the precise effect of chilling and freezing in determining the fate of antibacterial residues in sheep meat. On the other hand, thermally-processed samples at 100°C for 45 minutes revealed total reduction in activity of antibiotic residues against test bacteria. These findings are compatible with an Egyptian study (42) which reported that antibacterial potentials disappeared in the cooked meat samples

previously harbored antibiotic residues. This is mostly attributed to the destructive effects of thermal processing. The application of various cooking methods; boiling, frying, and grilling of meat revealed significant reduction rates in concentration of residues that ranged from 81.22% by boiling for penicillin to 96.58% by grilling for oxytetracycline (18). These findings are further supported by a recent study (19) that found the reduction rate of oxytetracycline residues in beef with boiling up to 87.8% in 30 minutes and barbecued meat up to 90.9% in 20 minutes. Such slight differences are possibly resulted from various degrees of applied heat, type of antibiotic, or even the kind of meat per se. In the present work, the findings of boiling effect are in good agreement with the aforementioned reports. Moreover, microwaving and roasting were found to significantly reduce the inhibition zones produced by raw liver sample (17).

Conclusion

Findings from this study have shown that lamb meat and mutton harbor antibiotic residues in levels higher than accepted. Chilling and freezing slightly decrease the antibacterial residues in lamb meat and mutton but thermal processing successfully deactivated antibiotic residues. However, the *in vivo* fates of heat-degraded residues are unclear. The potential harm and their interactions worth studying with particular focus on the toxicological scope. Additionally, accurate analytical and screening methods in meat industry should be encouraged. Government authorities and regulatory bodies should implement the required restrictions on antibiotic utilization in animal farming.

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