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Impact of heat treatment on the antimicrobial residues in raw goat's milk

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Abstract

Although antibiotics are valuable drugs for treatment of bacterial and some parasitic infections, their presence in animal products have a potential public health hazard. This study investigated antibiotic residues in goat raw milk and thermal effect on residues. Samples were collected randomly from different farms and retail outlets in Erbil city from January 1st to June 30th 2019. The residues were detected by disc diffusion assay against Bacillus subtilis bacteria on Muller Hinton agar. The occurrence rates among milk samples was 14.9%, also these residues in the samples was 13.5% and 16.4% in farm samples and retail store samples, respectively. No significant differences were found between sampling sites (farms vs sale points). Regarding the seasonal variations, spring was found to be associated with gradual decrease in antibiotic residues frequency in milk. Boiling for 5 minutes was the most effective treatment (among pasteurization and microwave heating) that inactivated antibiotic residues in 57.7% of positive samples. Such occurrence rate of residues is alarming and require authorities to observe and validate the quality of raw milk introduced to markets for consumers. Further evaluation of antibiotic stability period in raw milk is highly recommended.

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Introduction

Milk is a natural food source for mammals that contains valuable nutrients to support body growth. It contains various key nutrients such as; calcium, phosphorus, vitamin A, vitamin D, riboflavin, vitamin B_{12}, proteins, potassium, zinc, and magnesium. Dairy products, including milk, are among the richest dietary sources of calcium (1). The possible adverse health effects occasionally associated with milk are represented by the antimicrobial and hormones residues mostly when breeders do not comply the withdrawal period of the antimicrobials or hormones (2). Basically, antimicrobial agents including antibiotics, antifungals, and antiprotozoals are widely, and often injudiciously, used for therapeutic and prophylactic purposes to kills pathogens or stops their growth. Practically, almost all the types of antimicrobials that are used for humans are also being used for food-producing animals (3). Beside therapeutic goals, antibiotics are also used in livestock industry for growth promotion and weight gain. After the administration of antimicrobials to animals, their remnants last in tissue for different periods depending on the drug, its pharmacokinetic properties, and the tissue. Foods derived from treated animals contain such remnants collectively called antimicrobial/antibiotic residues (ARs) (4). These residues can have a negative impact on human health, mostly as GIT disturbance, cancer inducing effects, as well as emergence of drug-resistant bacteria (5,6). Moreover, economic losses associated with milk containing antibiotic residues are apparent in failure of fermentation processes due to inhibition of starter bacteria (7). Goat milk has been an important part of human nutrition for millennia, in part because of the greater similarity of goat milk to human milk, higher proportion of small milk fat globules, softer curd formation, and low allergenic characteristics compared to cow milk (8). Safety and quality of goat milk and its dairy products is a central part of the current trends in health and food standards. Goat milk contains higher amount of P, Ca, and Mg exceeding the levels present in human's or cow's milk. Medium chain triglycerides which is more in goat milk has been documented as unique lipid of health benefits. The soft curd of goat milk may be beneficial to adult humans suffering from gastrointestinal disorders and ulcers (9). The involvement of goats in supplying milk and milk products is high and it has significant role in village economy and health. The global dairy goat population was estimated to be 218 million in 2017 with approximately 18.7 million tons are of goat origin. That covers around 2 % a share of the global milk production (10). To detect ARs, agar disk-diffusion assay developed in 1940, is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) (11).

There were insufficient data on the scale of ARs among milk at Erbil governorate. Hence, this work was carried out to determine the degree of antibiotic residues in raw goat milk at Erbil governorate. The relation between months and detection of ARs among goat milk was also investigated along with the impact of different heat processing methods on degradation of ARs.

Materials and methods

Sample collection

Three hundred and fifty samples of goat raw milk were collected from different regions in the Erbil governorate for the period between January and June of 2019. Samples distributed between 180 milk samples from goats breeding fields and 165 milk samples from the local markets of Erbil Governorate, where the samples were collected in sterile glass containers of 250 ml capacity and then transferred to college of science at Knowledge University. Transport conditions and storage were in accordance with previously published method (12).

Detection of antibiotic residues

Spores of *Bacillus subtilis* (obtained from Central Veterinary Laboratory, Erbil) was prepared at the required density according to a previously published method (13). Muller-Hinton agar was prepared as recommended by the manufacturing company (HiMedia, India). After cooling to approximately 45°C, an inoculum of 0.1 ml of spore suspension was mixed with 100 ml of the molten agar before solidification. The mixture was poured into Petri dishes and allowed to solidify at room temperature. The presence of ARs in milk was tested as described previously (14).

Impact of heat processing on AR stability

All milk samples showed a positive reaction for ARs presence were further evaluated for thermal stability of ARs by three different processes (15). Before thermal processing, a volume of 20 ml of each sample was separated to be used as a control. After heat treatment, the samples were re-evaluated by the disc diffusion assay method described above. About 40 ml of each sample was subjected to each of the following processes

Pasteurization

Milk samples were subjected to pasteurization process by heating in a water bath maintained at the 63°C for 30 minutes and were immediately removed and cooled to 5°C.

Boiling

Samples boiled at 100°C for 5 minutes and then were cooled before re-evaluation.

Microwave processing

Samples were placed in glass containers then were put in a microwave oven at 100°C for 5 minutes.

Statistical analysis

Data were analyzed using the SPSS software version 25. Confidence intervals of prevalence were estimated using "exact" Clopper-Pearson method at alpha level of 0.05. Chi square test was applied to test the different between groups.

Result

Frequency of ARs among goat raw milk

Out of 350 raw goat milk samples, 14.9% (52/350) were positive for the presence of ARs (Table 1). Statistically, 11.30% - 19.02 % (95% confidence interval) of goat raw milk sold in different markets in Erbil are expected to be contaminated with ARs. There is no significant difference in contamination rate between collection sites ($p = 0.447$, $\chi^2 = 0.578$).

Table 1. Percentages of ARs among goat milk samples (n=350)

Site	n examined	Positive n(%)	95% CI
Farm	185	25 (13.5)	8.94 - 19.30
Sale points	165	27 (16.4)	11.07 - 22.91
Total	350	52 (14.9)	11.30 - 19.02

Variations in incidence rate of ARs during study period

The highest frequency of ARs was detected in January (20.3%) and February (17.2%), while the lowest rate was found in June (10.2%) (Figure 1**)**. There was a strong association ($r^2 = 0.919$) between progress of winter-spring months and the decrease in occurrence of antibiotic residues in milk.

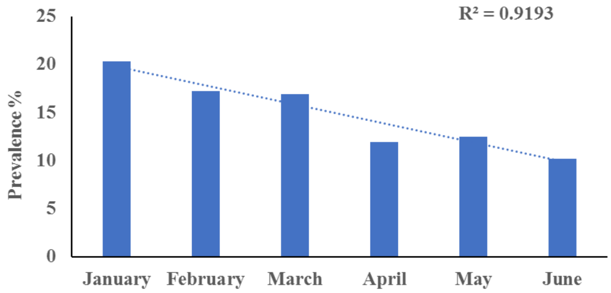

Figure 1: Temporal variations of ARs during study period

Impact of heat processes in ARs deactivation

Thermal deactivation of ARs by different processes is summarized in table 2. No significant differences were found between milk types in terms of response to various heat processing (ARs deactivation percentage). However, boiling and microwave heating were significantly more destructive than pasteurization to ARs in milk samples ($p = 0.0138$ and $p = 0.0003$, respectively). In contrast, no significant difference was found between boiling and microwave heating in terms of thermal deactivation of ARs ($p = 0.243$).

Table 2: Impact of thermal processing on ARs in milk samples

Thermal processing	Collection Site	No of positive samples		Affected samples	P value
		before treatment	after treatment	n (%)	
Pasteurization	Farm	25	20	5 (20.0)	0.617
	Sale points	27	20	7 (25.9)	
	Total	52	40	12 (23.1)	
Boiling	Farm	25	13	12 (48.0)	0.177
	Sale points	27	9	18 (66.7)	
	Total	52	22	30 (57.7)	
Microwave processing	Farm	25	16	9 (46.0)	0.493
	Sale points	27	12	15 (55.6)	
	Total	52	28	24 (46.2)	

P value less than 0.05 is considered significant.

Discussion

Presence of ARs in food is associated with negative impacts on consumer's health, food industry, environment, and public health. This article addresses the prevalence, seasonal variations, and degradation of ARs among goats' milk at Erbil governorate.

The detected proportion of contaminated milk samples was 14.9%. These findings are consistent with previously reported studies from Jordan (16), India (17), and Kenya (18) where frequency of goats' milk contamination with ARs ranged from 13% to 18%. However, lower rates (ranged from 8% to 12%) were reported from India (19), Kosovo (20), and Ethiopia (21). In contrast, higher rates (from 24% to 40%) were documented in a nearby city (Mosul, Iraq) (22), Iran (23,24), Kuwait (25), and Nigeria (26) in fresh and pasteurized milk samples. Such variations are mostly linked to the extent of adherence to withdrawal periods of administered antibiotics (27).

High temperature breaks covalent bonding and destabilizes ring structure of organic compounds including antibiotics, which may explain the efficiency of boiling and microwave treatments in deactivating ARs in comparison to pasteurization. However, different classes of antibiotics have different degradation rates. Indeed, sulfonamides were more heat-tolerant than β-lactams and tetracycline. Thermal degradation does not insure complete safety of milk samples that contained ARs since degradation products may still display anti-microbial activities and some of such products may were found to be more toxic. Although thermal processing results in a decrease in the concentration of parent antibiotic residues, degradation of by-products have not been properly characterized to date

Regarding the effects of heat treatment on stability of ARs, higher temperatures 100°C for 5 minutes were more effective in deactivating ARs. This observation is supported by published literature emphasizing the destructive nature of high temperatures on antibiotics (28,29). The lack of complete deactivation of ARs in boiled or microwave-processed samples is mostly attributed to concentrations and the protection conferred by the suspension medium as well as the short period of exposure to heat (17,30). Additionally, fats and proteins in milk may protect ARs from thermal degradation. It was reported that cooking time and temperature are two main factors affecting antibiotic residues and reducing several antibacterial drug activities. Indeed, increase in time of exposure to heat treatment was associated with increase of percentage of ARs deactivation. Similarly, increase of temperature from 60°C to 100°C was found to significantly decrease the half-life of residues of various β-lactams, macrolides, and quinolones residues in milk (28).

The progress of winter-spring months was associated with decrease in ARs frequency in milk. The highest frequency was detected in January 20.3% and February 17.2% where humidity and rains in Erbil is high. While the lowest rate was found during the beginning of the dry season (June 10.2%). In June, temperature increases and average rainfall levels are at the lowest level in the year (approaching zero mm). These observations are in good agreement with previously published reports stating that wet season was associated with increase in diarrheal diseases in livestock which requires more antibiotics administration than during other seasons (29). However, other studies documented a different temporal pattern in which dry season was associated with increase in ARs. This increase was linked to increased infections due animal crowding in water points and limited feeding areas. Still other studies found no association between season and frequency of ARs in milk (29).

Several approaches can be followed to mitigate the contamination of milk by ARs (3,15). Regular veterinary care of livestock to control earlier sporadic infections within the herd. If early cases were detected and treated promptly, spread of infection and demand to mass administration of antibiotics would be avoided. Educating farmers about proper administration of antibiotics and withdrawal periods is also an important factor.

Conclusions

Antimicrobial residues in milk is one of important public health challenges worldwide. The present study detected a high frequency of ARs in raw milk samples from goats collected from farms and sale points in Erbil governorate. No significant differences were found between farms and sale points in terms of ARs contamination proportions. The winter season was associated with increase in ARs in milk. As winter-spring progressed, ARs decreased. Higher temperatures for 5 minutes were found to deactivate ARs in more than half of samples. Boiling and microwave processing were significantly more effective than pasteurization process for ARs inactivation. Proper maintenance of withdrawal period after antibiotic treatment would minimize the risk of antibiotic residues in milk. In-charged authorities should insure that the screening of milk for antibiotics residues need to be strictly performed before milk reaches the consumers.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript

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تأثير المعاملة الحرارية على بقايا المضادات الحيوية في حليب الماعز الخام

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

الخلاصة

تعد المضادات الحيوية من الأدوية المهمة في علاج الكثير من المسببات المرضية، إلا أن وجودها في المنتجات الحيوانية تعتبر مشكلة في غاية الأهمية في الصحة العامة. هذه الدراسة بحثت تواجد متبقيات المضادات الحيوية في الحليب الخام للماعز ودراسة تأثير بعض المعاملات الحرارية على هذه المتبقيات. تم جمع العينات عشوائياً من محلات البيع بالتجزئة ومن مزارع في محافظة أربيل خلال الفترة من بداية كانون الثاني حتى نهاية حزيران للعام ٢٠١٩. تم الكشف عن وجود متبقيات المضادات الحيوية بطريقة الأقراص المنشرة ضد بكتيريا العصوية الرقيقة على وسط أكار مولر هنتون. أوضحت النتائج أن نسبة تواجد المتبقيات في عينات الحليب بشكل عام كانت ١٤,٩%، وان نسبة تواجد هذه المتبقيات في العينات بلغت ١٣,٥% و ١٦,٤% في عينات المزارع وعينات محلات التجزئة على التوالي، وفيما يتعلق بتأثير اختلاف الموسم على نسبة تواجد المتبقيات، أظهرت النتائج بأن فصل الربيع كان مرتبطاً بانخفاض في نسبة العينات الملوثة بالمتبقيات. أما فيما يخص المعاملات الحرارية فقد وجد أن الغليان لمدة خمس دقائق كان هو أفضل الطرق لإبطال فعالية المتبقيات في ٥٧,٧% من العينات الملوثة بالمقارنة مع البسترة أو التسخين بالمايكرويف. تعتبر هذه النسب للعينات التي تحتوي على المتبقيات مثيرة للقلق وتستوجب من الجهات ذات العلاقة بأن تراقب جودة الحليب الخام قبل أن يصل إلى المستهلك. كما نوصي بإجراء دراسات مستقبلية حول فترة استقرار متبقيات المضادات الحيوية بصورة مستقرة في الحليب الخام.