

Detection of antibiotic residues among raw beef in Erbil City (Iraq) and impact of temperature on antibiotic remains

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Abstract

The presence of antibiotic residues in beef is considered a serious threat to public health. This study aimed to detect antibiotic residues in raw beef and the impact of low and high temperature treatments on residues persistence. A total of 250 samples were collected from retail markets in Erbil city (Iraq) and analyzed microbiologically in plates pre-inoculated with *Bacillus subtilis*. The overall occurrence of antibiotics residues was (10.8%). The highest rate was detected in January (16.7%). Cooking for thirty minutes completely deactivate antibiotic residues against the challenged bacterium. In conclusion, the presence of antibiotic residues in beef samples in Erbil city was high and their persistence is markedly reduced by cooking.

Introduction

Antibiotics are secondary metabolites from certain bacterial and fungal species with harmful properties against other bacterial species. These compounds play pivotal roles in agricultural, veterinary, and clinical settings (Schwarz *et al.*, 2001). Furthermore, they are widely used in food-producing animals for therapeutic, prophylactic, and metaphylactic purposes (Baynes *et al.*, 2016; Sarker *et al.*, 2018). Antibiotic residues term refers to molecules that remain in meat and organs of slaughtered animals that have been given antibiotics earlier without adherence to withdrawal period of the antibiotic (Darko *et al.*, 2017; Aidara-kane *et al.*, 2018). Globally, antibiotics have been extensively used in the field of animal farming in due to their availability and low price. Moreover, antibiotics have been employed to enhance the growth and productivity (Tang *et al.*, 2017; Kirchhelle, 2018).

Most humans are vigilant toward consumption of meat from farm industry that massively uses antibiotics in their production process. This cautious attitude resulted from the fact that antibiotic residues or their

derivatives remain in meat. It is worth mentioning that various antibiotics require different time periods to be eliminated from the body. This time period is identified as withdrawal period (WP) for the particular antibiotic. The length of WP depends on the dosage form, antibiotic type, and method of administration. The withdrawal time ranges from only few hours to numerous days or weeks, so this period must be observed in ante mortem inspection of cattle (Al-mashhadany *et al.*, 2018; Okocha *et al.*, 2018). Lack of knowledge between animal producers about withdrawal periods of antibiotics along with improper use of antibiotics are the main reasons that contribute to the problem. The presence of antibiotic residues above the Maximum Residue Limits (MRLs) in food produced from these animals is considered globally, by various public health authorities, as illegal practice (Jayalakshmi *et al.*, 2017; Regea, 2018). The hazardous threats of such residues including direct toxicity, drug allergy, hypersensitive reaction and development of antibiotic resistant bacteria that have been known as a global health challenge in the 21st century. Moreover, antibiotic residues may also influence starter cultures in food industry leading to economic losses (Beyene, 2016; Prajwal *et al.*, 2017; Manyloh *et al.*, 2018). The presence of antibiotic residues in different types of meat has been widely investigated in several countries (Alla *et al.*, 2011; Babapour *et al.*, 2012; Mangsi *et al.*, 2014; Mgonja *et al.*, 2017; Ramatla *et al.*, 2017; Ashraf *et al.*, 2018).

Bacterial infections in cattle seem to be often associated with rainy season. In Saudi Arabia, the highest incidence rate of mastitis in cattle was found during the cold season (summer) in a three-year study (Shathele, 2009). Moreover, cattle brucellosis in Uganda was found to peak during seasons of heavy rains (Mwebe *et al.*, 2011). On the other hand, the highest rate of diarrheal infections in calves of cattle in northern India was reported after the onset of rains during spring and summer seasons (Malik *et al.*, 2012). On the contrary, a study from Uganda found that dry season is associated with increase in bacterial infections in cattle but not in poultry. This observation was explained by the movement of cattle in search for food (Byaruhanga *et al.*, 2017). Additionally, a recent study from Bangladesh has found that the highest prevalence of various bacterial diseases in bovine occurs in the rainy summer season (Mohammed *et al.*, 2017).

In recent decades, there has been a significant increase of antibiotic residues in food of animal origin, including red meat, therefore this study aimed to investigate the

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occurrence of antibiotic residues in beef in Erbil city and to determine the correlation between occurrence of antibiotic residues and months during the period of research, as well as investigate the effects of low and high temperature on persistence of these residues.

Materials and Methods

Determination of carcasses age

Cattle carcasses age was determined according to previously published criteria (Tatum, 2011). Briefly, carcass's age is assigned either as young carcass (around 24 months) or adult carcass (more than 24 months) by examining the ossification of the tips or buttons of the thoracic vertebrae, the size and shape of the ribs, in addition to the color and texture of the flesh.

Sample collection

A total of 250 thigh beef samples from carcasses of young and adult cattle (125 of each) were randomly collected from slaughter houses and malls in different retail markets in Erbil city, during the period from January to June 2018. The collected samples were placed in separate plastic bags and transported to Pathological Analysis Department, College of Science, Knowledge University, under chilling condition. In the laboratory.

Preparation of the spore suspension

Spores suspension of *Bacillus subtilis* was prepared at desired concentration according to standard methods (AL-Rubeae, 2000). Briefly, Heavy inoculums of *B. subtilis* were introduced to the surface of a Nutrient agar plate (HiMedia, India). The plates were incubated at 30°C for 10 days to induce sporulation. After the incubation period, colonies were harvested into 10 mL of sterile normal saline and heated at 70°C for 10 minutes to kill the vegetative cells. The heated suspension was centrifuged at 3000 rpm for 10 minutes. The clear supernatant was discarded. Another 10 mL of sterile saline were added to wash off debris of vegetative cells. The mixture was concentrated at the same speed and duration. The process was repeated twice to obtain a pure suspension of endospores. Suspension turbidity was adjusted to match 0.5 McFarland standard solutions ($\approx 1.5 \times 10^8$ CFU/mL).

Preparation of test plates

Muller-Hinton agar was prepared as recommended by the manufacturing company (HiMedia, India). After cooling to approximately 45°C, inoculum of 0.1 mL of spore suspension was introduced to each 100 mL of the agar before solidification. The molten agar was poured into petri-dishes and allowed to solidify at room temperature. Plates were used at the same day of preparation or held at refrigerator and used within one week.

Detection of antibiotic residues in samples

A previously published technique (Al-mashhadany, 2009) was adapted for the detection of antibiotic residues in beef samples. A disc-shaped meat sample of 2 mm in thickness and 8 mm in diameter was prepared and placed on the surface of Muller-Hinton agar inoculated by the sensitive test organism (*Bacillus subtilis*). The plates were incubated aerobically at 37°C for 24 hrs. Formation of transparent zone equal or more than 2mm was considered as a positive result. However, a zone of 1 to 2 mm

was considered as suspicious one, and a zone less than 1 mm was considered as a negative result. The zone size around each positive sample was measured using Vernier caliper. Since no zone appeared in control samples, zones diameters of samples were recorded for evaluation.

Impact of food preservative methods on positive samples

The effects of preservation methods on residues persistence were evaluated as follows:

Cooling: Beef samples which showed positive results in the direct method were kept in the refrigeration at (4°C). After 3 days, samples were examined for the persistence of antibiotic residues by the direct method.

Freezing: Beef samples which showed positive results in the direct method were kept in a freezing compartment at (-18°C). After 4 weeks, antibiotic residues presence was evaluated by the direct method.

Cooking (Boiling): About 20-25 grams of each residue-positive sample were placed into a sieve and immersed in 100 mL of water bath preheated to 100°C for 30 minutes. Samples then removed and allowed to cool before re-evaluation for antibiotic residues.

Statistical analysis: All statistical calculations were done using version 15 of SPSS software. Chi-Square test was employed to assess the different responses between beef samples to preservative methods. A level was set to 0.05.

Results

Occurrence of antibiotic residues in beef samples

Out of 250 raw beef samples, 27 (10.8%) were positive for the presence of antibiotic residues (Inhibition zones diameter ranged from 2 mm to 8 mm). Of note, 9% (12/125) of samples from young carcasses were positive, while 12% (15/125) of adult carcasses samples were harboring antibiotic residues (Table 1). Based on statistical inference, at any given month, it is estimated that 7.4-14.1% (95% confidence interval) of raw beef meat in Erbil market is contaminated by antibiotic residues.

Changes in rate of antibiotic residues during study period

The change in occurrence rate of antibiotic residues was monitored through study period. The highest rate of residues detection was observed in late summer; January (17%) and February (14.6), while the lowest rate was found in June (5%). Table 2 summarizes the detection rates in temporal scale.

Effects of preservative methods on persistence of antibiotic residues

The effectiveness of cooling (4°C/3 days) on the occurrence of antibiotic residues in positive raw beef samples was low. Indeed, third of residues-positive beef samples of young carcasses showed clearance of the residues after cooling (Table 3). However, such difference between young and adult beef samples is not significant.

On the other hand, freezing at -18°C for

Table 1. Occurrence of antibiotic residues in raw beef samples.

Age of beef	No. samples	No. positive (%)	No. negative (%)
Young	125	12 (9.6)	113 (90.4)
Adult	125	15 (12.0)	110 (88.0)
Total	250	27 (10.8)	223 (89.2)

Table 2. Relationship between months and occurrence of antibiotic residues in beef samples.

Month	Young beef		Adult beef		Total examined	Total positive (%)
	No. exam	No. Post	No. exam	No. post		
January	20	3	22	4	42	7 (16.7)
February	21	3	20	3	41	6 (14.6)
March	20	2	20	1	40	3 (7.5)
April	23	1	21	4	44	5 (11.4)
May	21	2	22	2	43	4 (9.3)
June	20	1	20	1	40	2 (5.0)
Total	125	12	125	15	250	27 (10.8)

four weeks was less effective than cooling on the clearance of antibiotic residues in raw beef samples. Likewise, residues-positivesamples of beef from young carcasses showed more response than adult derived samples to antibiotic clearance by freezing (Table 4). Despite the fact that beef of young carcasses responds more than beef of adults to clearance by cooling or freezing methods, this difference is not statistically significant. On the contrary, heat processing (100°C for 30 minutes) showed complete (100%) deactivation of antibiotic residues against challenged bacteria (data not shown).

Discussion

Over the past decades, beef industry has become popular and increased dramatically worldwide. Antibiotics are critical components of cattle farming practice and beef industry but their uses regimen should adhere strictly to the legislated regulations (Correa *et al.*, 2018; Shang *et al.*, 2018). The World Health Organization (WHO) confirmed that antibiotic resistance is an international issue and countermeasures should be taken to mitigate the progressive spread of resistant bacteria species and the practices leading to resistance evolution (Okocha *et al.*, 2018). Antibiotic residues in animal-derived food may contribute to the development of resistant bacteria. Consequently, Codex Alimentarius Commission (CAC) recommends that Food of animal origin treated with veterinary medicines/drugs must not contain any residue (CAC, 2017).

In the present study, the occurrence rate of antibiotic residues in total beef samples was 10.8%. The highest rate (12%) was found in beef adult carcasses, while the lowest (9.6%) rate was documented in young carcasses (Table 1). These findings are consistent with an Egyptian study that found the prevalence of Oxytetracycline (OTC) residues in 10% of examined imported beef meat samples (Elbagory *et al.*, 2016). Additionally, another Egyptian report states that 16% of the examined raw meat was contaminated by antibiotics (El-Wehedy *et al.*, 2018). In contrast, only 3% occurrence rate was reported in muscles beef samples analyzed in Sudan (Alla *et al.*, 2011). Moreover, In Vietnam, the occurrence rate in beef samples was found to be 7.4% (Yamaguchi *et al.*, 2015).

On the other hand, higher rates have been reported previously. In Iran, antibiotic residues were detected in 22.8% of screened beef (Babapour *et al.*, 2012). Strikingly, a Pakistani study (Mangsi *et al.*, 2014) report-

ed a high presence rate (38.33%). Most recently, an Ethiopian study estimated that 71% to 82% of fresh beef in a northwest market harbor antibiotic residue (Agmas and Adugna, 2018). Such discrepancies could be attributed to failure to adhere to pre-slaughter withdrawal period or the improper massive use of antibiotics by farmers (Khatun *et al.*, 2018).

In beef, the average values of acidity and fat contents are higher than the average of poultry meats, while protein contents were high in poultry than in beef (Pereira and Vicente, 2013). Additionally, beef contains narrow muscle fibers while poultry meat contains broad fibers. However, results showed that difference in fat composition and content between beef and poultry has no effect in protecting the antibiotic residues against the thermal deactivation of

cooking. In this work, the correlation between months and incidence of antibiotic residues in beef meat during the period of study in Erbil city was monitored. Table 2 shows that the highest detection of antibiotic residues from overall samples was found in January 7/42 (16.7%), approximately close to this rate in February 6/41 (14.6%). It is obvious that there is an association (Figure 1) between increase in residues occurrence rate and other unknown environmental parameter(s) (temperature, humidity... etc.), which somehow may have affected the cattle and required antibiotic intervention by farmers.

In terms of various antibiotic residues rates and seasons, a Sudanese study (Alla *et al.*, 2011) noticed that the largest rate (24%) was documented in summer, which is in good agreement with this study.

Table 3. Effect of cooling (4°C/3 days) on antibiotic residues persistence in beef samples.

Types of samples	No. of positive before cooling	No. of affected sample after cooling (%)	Chi-Square	χ^2_t
Young	12	4 (33.3)	1.54	3.84
Adult	15	2 (13.3)		
Total	27	6 (22.2)		

Table 4. Impact of Freezing (-18°C/4 weeks) on antibiotic residues in raw beef samples.

Types of samples	No. of positive before freezing	No. of affected sample after freezing (%)	Chi-Square	χ^2_t
Young	12	2 (16.7)	0.67	3.84
Adult	15	1 (6.7)		
Total	27	3 (11.1)		



Figure 1. Correlation between study months and percentage of positive samples.

Furthermore, the finding also agrees with an Iranian study (Aalipour *et al.*, 2013) in Iran showed that the highest contamination rate of milk with antibiotic residues occurred in summer (February, 45%). Wetness of natural pasture in winter may lead to occurrence of diseases that require intervention practice including antibiotics. In fact, it has been reported that winter season is associated with increased rate of antibiotic residues in poultry meat samples examined in Kurdistan region (Al-mashhadany, 2009).

The cooling and freezing methods (Tables 3 and 4) produced roughly similar effects on reduction of antibiotic residues in beef. There is a shortage of information on the effect of cooling and freezing in determining the fate of antibiotic residues in beef. However, all heat processed samples (27 samples; 100°C for 30 minutes) showed total reduction in activity of antibiotic residues against test bacteria (Data not shown). These findings are consistent with an Egyptian study who reported that antibiotics disappeared in the cooked meat samples (El-Wehedy *et al.*, 2018). This may be attributed to the destructive effects of heat on antibiotics.

Various cooking methods (boiling, frying, and grilling) were compared based on the reduction of oxytetracycline and ampicillin residues in meat samples of chicken muscle (Elbagory *et al.*, 2016). Nonetheless, the study reported grilling and frying to be superior to boiling in reduction percentage of both antibiotics. These findings are further supported by a recent study that found a reduction of OTC with boiling up to 91% in 30 minutes and barbecued meat up to 88% in 20 minutes (Mgonja *et al.*, 2017). Such variations are most likely resulted from different degrees of applied heat, the type of antibiotics, or even the meat per se.

Due to differences in the overall immunity between young and adult cattle, different infections have been found likely to be age-related (Busato *et al.*, 1999; Nielsen and Ersbøll, 2006; Brooks-Pollock *et al.*, 2013). Consequently, different antibiotics are administered at different doses. However, no significant difference in response to thermal treatments has been found between young and adult beef samples in this study.

Conclusions

Antibiotic residues in red meat, especially beef, are one of a significant public health challenge for Iraqi Kurdistan. According to the results obtained in this study, the occurrence rate of antibiotic resi-

due in beef samples collected from Erbil city was higher than recommended. Cooling and freezing slightly reduce the antibiotic residues in beef but boiling for half an hour successfully reduces residues efficacy. However, the *in vivo* products of heat-degraded antibiotic are obscure. Their potential harms and interactions worth studying with special emphasis on the toxicological potentials.

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