

Detection of streptomycin residues in local meat of bovine and ovine

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Abstract

From meat retails in Mosul province, forty-five meat samples of local ovine and bovine (23 bovine samples and 22 ovine samples) were collected. The period of collection was during November 2010 to May 2011, by means of multistage random sampling for detection of streptomycin residues. Enzyme linked immunosorbent assay (ELISA) was used for detection of streptomycin residues. The results revealed that eleven ovine meat samples (50%) were positive to streptomycin residue, with a mean value 35.06 $\mu\text{g kg}^{-1}$, while 14 bovine meat samples (60.86%) were positive to residual streptomycin with a mean value 59.56 $\mu\text{g kg}^{-1}$. From the results, it is clear that all tested meat samples (ovine and bovine) were safe enough for human consumption.

Keywords: Streptomycin, local meat, ELISA.

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الكشف عن بقايا (ثمالات) الستربتومايسين في اللحوم المحلية للأبقار والضأن

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الخلاصة

تم جمع ٤٥ عينة للحوم الأبقار والأغنام المحلية (٢٣ عينة للحوم الأبقار و ٢٢ عينة للحوم الأغنام) من مختلف أسواق اللحوم في مدينة الموصل خلال الفترة الواقعة بين تشرين الثاني ٢٠١٠ و أيار ٢٠١١ وبصورة متعاقبة وعشوائية للكشف عن بقايا المضاد الحيوي ستربتومايسين. استخدمت تقنية الأدمصاص المناعي المرتبط بالأنزيم في الكشف عن بقايا المضاد الحيوي (الستربتومايسين). أوضحت النتائج أن ١١ عينة للحوم الأغنام (٥٠%) كانت موجبة لبقايا المضاد وبمعدل ٣٥,٠٦ مايكرو غرام /كغم وان ١٤ عينة للحوم الأبقار كانت موجبة (٦٠,٨٦%) لبقايا المضاد وبمعدل ٥٩,٥٦ مايكرو غرام/كغم. من النتائج المتحصل عليها يتضح أن جميع عينات لحوم الأبقار والأغنام صالحة للاستهلاك البشري.

Introduction

Meat is one of the most important constituents of the human diet as it provides protein, energy, vitamins and minerals (1). Due to the demand for increasing meat production, several agents employed for animal treatment and for growth promotion. These include various types of antibiotics, sulfonamides and synthetic as well as natural anabolic agents, which could be a source of health hazards (2).

The use of veterinary drugs for food producing animals can affect the public health and international trade of food

products, because of the presence of residues of the drugs, or their metabolites in edible products. Depending on withdrawal period, other factors, which determine the occurrence of residues, are the route of administration, contamination of food or water, physicochemical properties, metabolism of the drug and the physical condition of the animal, all these factors are considered to be an access for assurance of food safety, and to take regulatory action after identification of chemical residues as stated by (3).

Streptomycin (STR), produced by *Streptomyces griseus*, is an aminoglycoside antibiotic, which shows activity

against aerobic gram – negative bacteria and is widely used in treatment of infectious diseases in farm animals (4). STR residues reported to be present in meat, liver, kidney, milk and other food commodities (5).

According to the European Union (EU) maximum residues limits (MRLs) for STR in food producing animals is 500 µg Kg⁻¹ in muscles, skin, fat and liver; 1000 µg Kg⁻¹ in kidney and 200 µg Kg⁻¹ in milk (6,7).

Unintentional consumption of antibiotics leads to resistance of bacteria that are pathogenic to human, which considered one of the most serious threats to human health (8). However, STR has the potential for severe side effects, such as allergic reaction and inhibition of marrow growth and may cause damage in the vestibular and auditory functions (9,10).

At present, three methods for antibiotic residue detection can be applied: the first one is the microbiological assay (11), but it is slow, with low sensitivity (12). 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) (13). These methods are sensitive and need highly skilled analysts, time consuming, expensive and not suitable for routine analysis of large -scale samples. The third method is immunoassay has been an alternative to the instrument and microbiological methods for accurate measurements of antibiotic residues in complex matrices, and because it is highly sensitive and specific, it can be conducted on long scale, with low cost, a combined with rapid outcome. Unlike the instrument methods, immunoassays do not require samples pre – concentration and extraction (14). Immunoassay for measuring STR residues in animal samples based on polyclonal antibodies and monoclonal antibodies have been described by (15,16).

Recently there has been an increasing international and local awareness of the danger of consuming meat with high levels of drug residues. Many of them now classified as carcinogenic, toxic or allergic jaundices. Some may also interfere with human and animals' natural physiological functions. Therefore, detection of these residues in meat intended for human consumption is very important for the safety of consumers. In this paper, enzyme-linked immunosorbent assay (ELISA) used to detect STR residues in ovine and bovine meat samples collected from different meat retails in Mosul province, Iraq.

Materials and methods

Sampling

A total 45 meat samples (23 bovine and 22 ovine) randomly collected from different meat retails in Mosul Province, during November 2010 – May 2011. Samples

carried to laboratory of Veterinary Public Health, and then they frozen immediately to -20 °C.

Preparation of samples and Extraction

Frozen meat samples thawed at room temperature, fat removed from the sample. Five grams from each meat sample was vigorously homogenized (using stomacher and mixer) with 20 ml of PBS-Tween-buffer for 30 minutes. (PBS-Tween-buffer, that prepared according to manufactures instructions: 0.55 g NaH₂PO₄ x H₂O + 2.85 g Na₂HPO₄ x 2 H₂O + 9 g NaCl + 0.1 % Tween 20, fill up to 1000 ml with distilled water). Samples then centrifuged for 10 minutes at a rate of 4000g at room temperature (25°C). Aliquots of the supernatant 1:10 (1+9) then diluted with sample dilution buffer (50µl supernatant +450µl buffer). Fifty µl per well were used in the assay.

Analysis of Streptomycin by ELISA

ELISA kit specific for streptomycin antibiotic was obtained from R-Biopharm AG, Germany. The streptomycin enzyme conjugate and antibody provided as aconcentrate. For reconstitution, the conjugate or antibody was diluted 1:11 (1+10) in buffer (200 µl conjugate or antibody concentrate +2ml buffer, ready to use sufficient for 4 micro-titer strips). All standards and specific streptomycin antibodies coated micro-titer plate brought to the room temperature (25°C) before use. Fifty µl of each freshly prepared samples and standard solutions added to wells and 50 µl of diluted enzyme conjugate solution added to each wells. Fifty µl of diluted antibody solution were added to each well, mixed gently by shaking the micro-titer plate manually and the plate was then incubated for 1 hour at room temperature (25°C). The liquid in the wells was completely removed and wells were washed with 250 µl washing buffer, repeated two more times and completely dried. Hundred µl of chromogen /substrate added to each well. The contents mixed and incubated for 15 minutes at room temperature in the dark. Finally, 100 µl of the stop solution added to each well, mixed gently by shaking the plate manually. The absorbance of the color was read within 30 minutes after addition of the stop solution in an ELISA reader at 450 nm. Special software, the RIDA SOFT WIN, was used for evaluation of the RIDASCREEN[®] Streptomycin ELISA kit results.

Results

Out of 45 meat samples (22 ovine and 23 bovine), 11 (50.00 %) and 14 (60.86 %) were positive for STR residues respectively (Table 1, 2).

The minimal STR concentration in ovine and bovine meat samples were 26.12 µg kg⁻¹ and 26.04 µg kg⁻¹ respectively, while the maximum concentration was

102.89 µg kg-1 for ovine samples, 282.21 µg kg-1 for bovine samples, with a mean of 35.06 µg kg-1 and 59.56 µg kg-1 respectively (Table 3).

Table 1: Concentration of residual Streptomycin (µg kg-1) in ovine meat samples by using ELISA test.

Total No. of analyzed samples	No. of Positive samples	Range of Streptomycin conc. µg kg-1	Percentage
n=22			
	2	26.13-26.71	9.1%
	3	27.13-27.98	13.7%
	4	28.14-29.71	18.1%
	2	31.00-102.90	9.1%
Total	11		50%

Half of the ovine samples were negative (50%) for STR residues, while (39.13%) was negative for bovine samples. The remaining (50%) of ovine samples were positive

(n=11) with concentration ranged between 26.12 to 50 µg kg-1. Only one sample exceeded 100 µg kg-1. In bovine meat, the positive samples were (60.86%) (n=14), with residual STR concentration ranged between 26.04 to 50 µg kg-1 (12 out of 14). The two remaining samples, one sample showed STR residues between 100 to 200 µg kg-1 and the other showed over 200 µg kg-1.

Table 2: Concentration of residual Streptomycin (µg kg-1) in bovine meat samples by using ELISA test.

Total No. of analyzed samples	No. of Positive samples	Range of Streptomycin conc. µg kg-1	Percentage
n=23			
	4	26.04-26.96	17.3%
	2	27.63-27.98	8.7%
	6	29.78-34.00	26.1%
	2	194.79-282.22	8.7%
Total	14		60.9%

Table 3: Mean, minimum and maximum level of residual streptomycin (µg kg-1) detected in ovine and bovine meat samples.

Matrix	Number of samples	Positive %	Mean µg kg-1	Minimum Concentration µg kg-1	Maximum Concentration µg kg-1
Ovine meat	22	50	35.06	26.12	102.893
Bovine meat	23	60	59.56	26.04	282.219

Discussion

The safety of meat has been at the forefront of social concerns in recent years, and indications exist that challenges to meats safety will continue in the future. One of the major meat safety issues and related challenges includes antibiotic residues (17, 18). In the current study, the high percentages of STR residues in both ovine and bovine meats samples traced primarily to the field of pre-harvest intervention of antibiotic treatments for pathogenic control, since no traditional antimicrobial intervention practiced at slaughtering or thereafter during processing of meat in Mosul abattoir. Although, all STR residual concentration were blew EU MRLs of 500 µg kg-1. This does not mean 100% clearance of STR residue in ovine and bovine carcass tissues, because elimination of STR molecule by animals needs long period of time due to the continued presence of residue concentration greater than tolerance limited to the kidneys (19,20). Moreover, Dihydrostreptomycin detected at the site of injection for a prolonged period (45 days) (21). It stressed also that STR is stable even after processing meat like cooking, baking and frying (22).

The current concentration in ovine and bovine meat samples of STR residues, although harbor no direct toxic

threat to consumer but their relevant adverse effect of exposure, seem to be those on human intestinal microflora and possible strain may be developed, which cause failure to antibiotic therapy in clinical situation (23,24). Development of resistance to residual antibiotics was expected to develop *Salmonella typhimurium* DT104, R-type ACSSuT penta – resistant, and to *Salmonella newport* R-type MDR –Ampc strain against STR (24). This does not mean, we have to stop the treatment of farm animals against infectious diseases (and simple solution at present time is not available), but common sense recommendation are not to over use or abuse antibiotic in animals.

It is interesting to note that higher percentage of positive STR residues in bovine meat samples over ovine meat samples. This trend is similar to the 10 years of observations in Ireland, through their plan in antimicrobial residual testing between 1998 to 2007 years in ovine and bovine positive percentages of antimicrobial residues. They were as follows: for ovine meat samples they were 0%, 0.35%, 0.4%, 0.35%, 0%, 0%, 0.38%, 0.68%, 0.2% and 0%; while those of bovine meat samples they were: 1.7%, 1.58%, 0.48%, 0.25%, 0.3%, 0.7%, 0.77%, 0.72%, 0.3% and 0.15%) during the years 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006 and 2007 respectively (25).

From all above, it is evident that local ovine and bovine meat sold in Mosul province generally contain residues of antibiotic agents. Although these levels are within acceptable limits, their presence may still be regarded as a health hazard as they may cause allergic reactions or produce drug tolerant bacteria. So all attempts to reduce antibiotic residues in meat should be applied through education by veterinary personnel; rapid screening procedures for the analysis of antibiotic residues and prohibition of meat containing antibiotics more than MRL.

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