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Antimicrobial residues in some slaughtered food animals

F. A. Khalafalla*, Fatma H. M. Ali, K. A. Abd-Allah

Department of Food Hygiene, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt.

A total of two hundreds of slaughtered animals; 50 each of cattle buffaloes, sheep and buffalo calves were collected from slaughterhouse Giza. Each animal was represented by muscular part, prenephric fat, liver, and kidneys. The Four Plate Technique (FPT) is intended to detect antimicrobial residues in collected samples. The liver samples showed high detection incidences (30, 16, 16 and 8%) in cattle, buffaloe, sheep and buffaloe calves respectively as compared to kidney samples (16, 12, 10 and 6% respectively). The lowest incidence was detected in fat samples (0%) followed by muscle samples (4%). High incidence was detected in medium I followed by medium II and III, finally medium IV as well as medium V which failed to be showed any incidence of detection. The tetracycline residues in tissues of slaughtered animals depend on husbandry as well as on withdrawal time after use. The liver (67 ± 15, 50 ± 14, 54 ± 10 and 3 ±0.8) and kidney (63 ± 16, 46 ± 12, 56 ± 12 and 8 ± 2) samples in cattle, buffaloe, sheep and buffaloe calves respectively showed high residual levels as compared with muscles and fat. The detection of sulfonamide residues in tissues of slaughtered animals may be attributed to misuse of these compounds for long term in animal feed as well as result in accumulation in animal tissues.

Drug residues in animal tissues above the standard tolerance clearly have an impact on human health tolerance which represents maximal level or time of slaughter.The existing evidence for specific health hazards of certain residues in meat and poultry above the established tolerance was showed (Concordet and Toutain, 1997; Paturkar, *et al.,* 2005). Most residues of veterinary drugs occur in food at such low levels that they rarely pose a chronic or long-term health hazard to consumers. Antimicrobials are used for mass treatment against infectious diseases (Paige *et al.,* 1997) or continuously in feed at very low doses (parts per million) for growth promotion (Wang *et al.,* 2006), particularly in animal production. Microbiological inhibition tests are considered as multi-residue screening tests for antibiotics in meat or other animal tissues. An inhibition test method is useful for detection of an antibiotic or a group of antibiotics, if the detection limits of these antibiotics are below safe levels or maximal residue limits (MRL) (Korkeala *et al.,* 1982; Concordet and Toutain, 1997; Korsrud *et al.,* 1998). The presence of drug or antibiotic residues in meat is illegal (Serratosa *et al.,* 2006; Nouws and Ziv, 2008). The potential problems associated with antibiotics residues may be classified into two broad categories (Gibbons *et al.,* 1996; Paige *et al.,* 1997). First, aesthetics; most consumers do not like the idea of foreign substances being present in food. The second problem is associated with potential health risks, including allergic reactions direct toxic effects, and a change in the resistance patterns of bacteria exposed to antibiotics. Food safety remains a major challenge contemporary society. Therefore, this work was carried out to assess the antimicrobial residues in slaughtered food animals (cattle, sheep and buffaloes calves) collected from Giza slaughterhouse, as well as public health significance of such residues was discussed.

Materials and methods

Detection of veterinary drug residue in muscles and organs.

Collection of samples. A total of two hundreds of slaughtered animals, 50 each of cattle, buffaloes, sheep and buffalo calves were collected from Giza slaughterhouse. Each animal was represented by muscular part, prenephric fat, liver and kidneys. Each sample was separately collected in clean polyethylene bag, then identified and rapidly transferred to laboratory for detection of antimicrobial residues.

Experimental techniques. The Four Plate Technique (FPT) described in manual of reference materials and methods to detect veterinary drug residues (Veterinary Drug Residues, 1994), was intended to detect antimicrobial residues in muscular tissue and organs of slaughtered animals (cattle, buff aloe, sheep and buffaloe calves).

Five different inoculated media were used for antibiotic detection.

Medium I, test agar pH 6, seeded with B. subtilis;

Medium II, test agar pH 7.2, with trimethoprim (Sigma, St. Louis, MO, USA; No. T-7883) added to a final concentration of 0.05 mg, and seeded with B subtilis;

Medium III, test agar pH 8, seeded with B. subtilis.

* Corresponding author. Tel.: +20 082 2322066;
Fax: +20 082 2327982
E-mail address: khalafalla@bsu.edu.eg
(Fathy A. khalafalla)

Medium IV, test agar pH 8 seeded with M. luteus.
Medium V, test agar pH 6, seeded with E. coli (0.1 ml of the diluted suspension). Sterile Petri dishes (diameter 90 mm) were filled with 5 ml of the prepared and seeded media.
Antibiotic standards. Antibiotic standards from Sigma were used, which were obtained from the pharmaceutical companies Bayer (Leverkusen, Germany), and pharmacia and Upjohn (puurs, Belgium).
Calibration technique. Detection limits of antibiotics were determined as follows.

Two-fold dilutions were prepared and 0.01 ml of these dilutions was applied to 6 mm diameter paper disks. Each dilution was tested four times on the medium considered to be most sensitive for the antibiotic tested. Concentrations producing a twelve mm diameter inhibition zone were calculated.
Detection limits were as follows.
On medium I. Penicillin G (sodium salt), 0.4 ng;Ampicillin trihydrate, 3ng; Oxytetracycline, 8 ng;Tetracycline, 5 ng; Chlortetracycline,0.5 ng; Doxycycline, 1 ng;
On medium II. Sulfadimidin, approx. 50 ng;
On medium III. Streptomycin sulfate, 20 ng;
On medium IV. Erythromycin, 1 ng; Tylosin, 10 ng; Lincomycin, 20 ng; Penicillin G, 0.5 ng; Ampicillin, 0.8 ng;
On medium V. Enrofloxacin, 2 ng; Ciprofloxacin, 1 ng; Flumequin, 10 ng.
Determination of Antimicrobial residues. Three plates from one batch of medium were used for each concentration tested. Four paper disks (diameter 6 mm) were laid on each plate, at a distance of 10 mm from the edge of the plate, and 0.01 ml of an appropriate standard solution was added. As soon as possible, and always within 15 min, pieces of muscle tissue , liver, kidney and fat were each laid upon two paper disks, on opposite sides of the plate. Each meat species was tested twice on one plate while the other two paper disks on the plate served as controls. The muscle tissue had been bored with a cork borer of 8 mm diameter and cut into disks approximately 2 mm thick, as prescribed for the Four Plate Technique. The volume of such tissue disks is 0.1 mm^3 and the weight can be estimated about 0.1 g. Plates containing media I, II, III and V were incubated overnight at 30 °C, while plates with medium IV were incubated for 24 h at 37 °C. Diameters of inhibition zones of standard with muscle tissue were measured and compared with zones observed around standard disks without tissue.

Results and Discussion

From the obtained results recorded in the Table (1) the liver samples of slaughtered animals (cattle, buffaloe, sheep and buffaloe calves) showed high detection incidences to liver (30, 16, 16 and 8% respectively), kidney samples (16, 12, 10 and 6% respectively) followed by muscle samples (4, 0, 2 and 0% respectively) and fat (0% in all samples). However, high incidence was detected in medium I followed by medium II and III finally Medium IV and medium V which failed to be showed any incidence of detection. Antimicrobial residues in foods of animal origin are one of the sources of concern among the public and medical health professionals. Many of ant microbes used to treat bacterial infections in human also have veterinary applications; they are used to treat infection in sick and injured animals and as prophylactics and growth promoters. In the later two cases the antimicrobes are used at concentrations lower than those used for treatment, a potentially dangerous practice since it can encourage the antimicrobial resistance strain of bacteria. This was supported by (Khachatourians, 1998; Simonsen *et al.,* 1998). The differences in levels of antimicrobial residues between of slaughtered animals may be attributed to vary widely reflecting regional animal husbandry and slaughter practices as well as difference in sampling (Tittiger *et al.,* 1975). Antimicrobial residues may produce allergic or anaphylactic reactions in susceptible individuals (with sulfonamides being of particular local importance). Some antibiotics are directly toxic, e.g. Chloramphenicol which destroys blood forming tissue. Allergic reactions and toxic side effects may have fatal consequences (FDA, 1997).
Tetracyclines residues in the examined samples. The present data in Table (4) revealed that 8%, 4% and 6% of the examined liver samples of beef, buffaloe and sheep; respectively were exceeded the permissible limits recommended by (Gracey *et al.,* 1999) (300ug /kg), while 4%, 4% and 4% of the examined samples were exceeded the limits recommended by FAO/WHO (9) (600 µg); respectively. In kidney samples, 6%, 4% and 4% of the examined samples of beef, buffalo and sheep, respectively were exceeded the limits recommended by (Gracey *et al.,* 1999) (600 µg/kg) and none of the examined samples exceeded the limits recommended by FAO/WHO (1999) (1200 µg/Kg.). None of the examined muscle and fat samples of slaughtered cattle, buffalo, sheep and buffalo calves showed results exceeded the limits

recommended by Gracey *et al.,* (1999); Charm, (2003) (100 µg/Kg).

The high residual levels of tetracyclines in liver and kidneys as compared with muscles and fat may be attributed to that the liver and kidneys are the major storage and excretory organs of tetracyclines and are parenchymatous in nature (Warner *et al.,* 1990; Muriuki *et al.,* 2001).

On the other hand, Okerman *et al.,* (1998) mentioned that the presence of the antibiotics residues in kidney tissues of slaughtered animals might be due to the filtration and clearance of the blood from any undesirable constituents in the kidney. The wide variation in residual level from the same slaughterhouse indicates differences in animal husbandry practices from different farms and areas. Some farmers and veterinarians could treat the animals and those misuse, overdose and failure to observe the withdrawal periods can be common. The detection of tetracyclines residues in some samples of slaughtered buffaloe calves may be attributed to that the young animals are susceptible to diseases which can result in serious intestinal problems such as scouring (diarrhea) and this antibiotics mainly used in treatment. This held the view reported by (Pinnacle, 2002).

Human health problems resulting from intake of sub chronic exposure levels of tetracyclines include gastrointestinal disturbances (Baker and Leyland, 1983), poor fetal development (Cohlan and Tiamsic, 1963) and hypersensitivity (Stowe *et al.,* 1980) and other toxic effects. Tetracyclines in meat potentially may stain teeth of young children.

Sulfonamide residues in the examined samples.

In Table (5), only one sample (2%) of examined liver and kidney of slaughtered cattle and buffaloe were exceeded the permissible limits (100 µg /gm) recommended by (Council Regulation EEC, 1990; Charm 2003). In Table (5), none of the examined samples of either of slaughtered sheep and buffaloe calves were exceeded the permissible limits. Sulfonamides are used in food producing animals not only for therapeutic but also for prophylactic purposes, when used in sub therapeutic doses, they exhibits growth-promoting action and so are added to feed stuffs to increase productivity (Kozarova *et al.,* 2001).

Microbiological method for the screening of sulfonamide residues in the foods is the Four Plate Test. The presence of sulfonamide residues in the sample is expressed by the growth inhibition of the test organism (Bacillus subtilis) with subsequent production of inhibition zones. The diameter of inhibition zones is directly proportional to the concentration of sulfonamide within a concentration range of sulfonamide group (Bogarets and Wolf, 1989). Sulfonamide residues present a potential risk to human healthy. Direct toxic or allergic reaction after administrations of the therapeutic doses of sulfonamides to humans have been described by (Booth, 1988; Spoo and Riviere, 1995).The presence of sulfonamide residues in tissues of slaughtered animals may be attributed to the using of the sulfonamides compounds for long-term consumption in animal feed containing trace amounts. The accumulation of these trace amounts in edible tissues has resulted in detectable levels; this in-accordance with that reported by (Agarwal, 1992; Spoo and Riviere, 1995). However, Health *et al.,* (1975) stated that the rate of sulfonamide disappearance from various tissues were proportional to the concentration in the tissues. The kidney had the highest sulfonamide concentration followed by liver, fat, muscles and lungs. We can recommended that, In the slaughterhouses, animals suspected to be medicated with the studied drugs must be kept under quarantine to ensure the elapse of the withdrawal time of the drug before the slaughter of suspected animals. Positive/negative test (ME) must be undertaken for random samples from apparently healthy animals to ensure the wholesomeness of their flesh. A health certificate should be approved from the veterinary authority in ARE indicating that the animals send to the slaughterhouses are not suspected to be medicated with drugs. There are now several programs in the USA and Europe, which make use of HACCP principles in quality management system for farms, e.g. Milk and Dairy beef quality assurance residue Prevention Protocol. However, such recommendations necessitate a well-defined system of identification of animals intended to be slaughtered.

Table (1): Incidence of antimicrobial residues in the examined samples.

Medium	Animal	Muscles		Fat		Liver		Kidneys	
		No.	%	No.	%	No.	%	No.	%
Cattle	I	2	4e	0	0	10	20a	5	10A
	II	0	0	0	0	3	6a	2	4A
	III	0	0	0	0	2	4a	1	2A
Buffaloe	I	0	0	0	0	4	8b	3	6B
	II	0	0	0	0	3	6b	2	4B
	III	0	0	0	0	1	2b	1	2B
Sheep	I	1	2	0	0	6	12c	4	8C
	II	0	0	0	0	2	4c	1	2C
	III	0	0	0	0	0	0c	0	0C
Buffaloe calves	I	0	0	0	0	2	4d	2	4D
	II	0	0	0	0	2	4d	1	2D
	III	0	0	0	0	0	0d	0	0D

Results on medium IV and V in all tissue samples of examined animal species are negative for antimicrobial residues, a =sum of tetracycline residues in liver cattle (30%), b= 16% in liver of buffaloes, c =16% in liver of sheep, d = 8% in liver of buffaloe calves. A= 16% in kidney cattle, B = 12% in kidney buffaloe, C = 10% in kidney sheep, D = 6% in kidney buffaloe calves, e = 4% in muscle samples

Table (2): Statistical analysis of tetracycline residues in the examined samples (µg/kg).

Animal	Parameters	Muscles	Fat	Liver	Kidneys
	Minimum			ND	ND
Cattle	Maximum	ND	ND	800	1000
	Mean± S.E			67±15	63±16
	Minimum			ND	ND
Buffaloe	Maximum	ND	ND	1000	1000
	Mean± S.E			50±14	46±12
	Minimum			ND	ND
Sheep	Maximum	ND	ND	800	1000
	Mean± S.E			54±10	56±12
	Minimum			ND	ND
Buffaloe calves	Maximum	ND	ND	100	300
	Mean± S.E			3±0.8	8±2

ND: Non-detectable level

Table (3): Statistical analysis of sulfonamides residues in the examined samples (µg/kg).

Animal	Parameters	Muscles	Fat	Liver	Kidneys
	Minimum			ND	ND
Cattle	Maximum	ND	ND	150	150
	Mean ± S.E			6.5 ± 2.5	4.5 ± 1.5
	Minimum			ND	ND
Buffaloe	Maximum	ND	ND	150	150
	Mean ± S.E			7±2.5	5±1.5
	Minimum			ND	ND
Sheep	Maximum	ND	ND	50	100
	Mean ± S.E			2 ± 0.3	2 ± 0.8
	Minimum			ND	ND
Buffaloe calves	Maximum	ND	ND	50	50
	Mean ± S.E			2 ± 0.3	1±0.4

ND: Non-detectable level

Table (4): Detection levels of tetracycline residues in the examined samples (µg/kg).

Organ	Cattle	Buffalo	Sheep	Buffalo calves	Permissible limit
Liver	50 100 (3) 300 (2) 400 (2)* 800 (2)*	300 500 700* 1000*	100 300 (2) 400* 800 (2)*	50 100	300
Kidney	50 400 700* 1000 (2)*	500 800* 1000*	300 500 1000 (2)*	100 300	600

*mean exceed the permissible limit, for liver 4 samples in cattle (8%), 2 samples (4%) in buffaloe, 3 samples in sheep (6%), for kidney 3 samples in cattle (6%), 2 samples in buffaloe (4%), 2 samples in sheep (4%). (2) = number of positive samples.

Table (5): Detection levels of sulfonamide residues in the examined samples (µg/kg).

Organ	Cattle	Buffalo	Sheep	Buffalo calves	Permissible limit
Liver	50 100 150*	100 (2) 150*	50 (2)	50 (2)	100
Kidney	75 150*	100 150*	100	50	100

* mean exceed the permissible limit.

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متبقيات مضادات الميكروبات فى بعض المذبوحات

أشتملت الدراسة علي فحص عدد ٢٠٠ حيوان مذبوح يتمثلون فى ٥٠ حيوان من كل من الأبقار، الجاموس، الأغنام وعجول الجاموس. تم تجميع العينات الممثلة لهم من مجزر الجيزة. وكل حيوان كانت تتمثل عيناته في أجزاء من العضلات، دهن الكلاوى، الكبد، الكلية والغدة المرارية). تم إستخدام Four plate technique في تحديد متبقيات المضادات الحيوية فى عضلات وأعضاء الحيوانات المذبوحة.

وجد أن عينات الكبد تحتوى على مستويات عالية من متبقيات الأدوية (٣٠، ١٦، ١٦ و ٨ %) بالنسبة للأبقار والجاموس والأغنام وعجول الجاموس على التوالى بالمقارنة مع عينات الكلى (١٦، ١٢، ١٠ و٦ %) على التوالى. أقل مستويات المتبقيات وجدت فى عينات الدهون (صفر%) ثم عينات العضلات (٤ %).

أعلى نسب من متبقيات مضادات الميكروبات تم العثور عليه باستخدام المستنبت الاول ثم المستنبت الثانى والثالث ، اما بالنسبة للمستنبتين الرابع والخامس فلم يتم العثور على اى متبقيات باستخدامهما.

تم دراسة متبقيات التتراسيكلين والسلفا فى هذه العينات وكانت النسب عالية فى عينات الكبد والكلى بالمقارنة مع عينات العضلات والدهون.