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ANTIBIOTIC RESIDUE LEVELS IN CAMEL, CATTLE AND SHEEP TISSUES USING LC-MS/MS METHOD

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ABSTRACT

Antimicrobials are very essential in prevention and control of infectious diseases for both humans and animals. However, the unregulated use of the antimicrobials may lead to several adverse health effects, such as development of multidrug resistant microbial strains, allergic and anaphylactic reactions. Saudi Arabia is one of the major countries in Middle East and Arabian Peninsula with a drastic increase in the livestock production. However, there is a clear lack of information about the current situation of antimicrobial residues in meat and edible offal intended for human consumption in Saudi Arabia. Therefore, this study screened the residue levels of nine antimicrobials with the most common use in Saudi Arabia veterinary medical field. These antimicrobials were enrofloxacin, ciprofloxacin, tylosin, erythromycin, tetracycline, oxytetracycline, chlortetracycline, sulfamethazine and sulfaquinoxaline. The tested samples included muscles, livers and kidneys of camel, cattle and sheep slaughtered at Al-Ahsa, Saudi Arabia. Antimicrobial residues in the tested samples was quantitatively estimated using liquid chromatography tandem mass spectrometry (LC–MS/MS). Sulfamethazine was detected at higher levels compared with the maximum residual limits (MPL) established by the regulatory authorities. Therefore, the effect of different cooking methods on sulfamethazine residues was investigated. The achieved results indicated occurrence of antimicrobial residues in the examined samples at levels below the recommended MPL, except for sulfamethazine that was detected at higher levels. Six antimicrobial residues were detected in samples collected from sheep; however, only three antimicrobials were detected in camel and cattle. Efficient heat treatment of the meat contaminated with sulfamethazine significantly reduced the residue load of sulfamethazine. In conclusion, efficient cooking of meat and observing the withdrawal periods of different antimicrobials are advisable to reduce human exposure to antimicrobial residues via meat consumption.

Key words: Camel, Cattle, Sheep, tissues, LC-MS/MS, antimicrobials.

INTRODUCTION

Meat producing animals like cattle, camel and sheep are exposed to xenobiotics during their lifetime. Of these, antibiotics and sulfonamides are major chemicals that are directly administered to livestock as a routine practice during the production cycle. These chemicals can subsequently find their way into human body via consumption of contaminated meat and other edible animal byproducts (Darwish *et al*., 2010).

Antimicrobials play pivotal role in human life via its beneficial effects in fighting disease-causative agents. In addition, these chemicals affect agricultural economy via their use to enhance animal growth, in prevention and control of infectious diseases. On the same time, the abuse and unobserving the withdrawal times of different antimicrobials might lead to production of drug resistant microbial strains, anaphylaxis and other toxicological implications such as mutagenesis and carcinogenesis (Baynes *et al*., 2016).

Tetracyclines are group of broad-spectrum antibiotics that are widely available, cheap and efficient in the treatment of respiratory, digestive and septicemic diseases. Oxytetracyclines are classified as the first dominant prescribed antibiotics in Africa. The frequency of their usage was established at 41% of cases, followed by β-lactams at 18% (Darwish *et al*., 2013).

Quinolones and fluoroquinolones (FQs) are frequently used in both veterinary field and in human medicine. For instances, enrofloxacin and ciprofloxacin are widely used in the veterinary field as they are characterized by their wide range activity against both Gram's positive and negative organisms. However, using the same drug or its metabolite in both human and veterinary fields is of a high risk in transmission of drug resistant pathogens. In addition, consumption of animal byproducts contaminated with these antimicrobials may result in nephropathy, anaphylaxis and teratogenesis (Kools *et al*., 2008).

Sulfonamides are commonly used in poultry farms and livestock for prevention and control of many

bacterial diseases especially that related to respiratory, genital diseases and mastitis. These compounds are characterized by their rapid absorption and distribution into different tissues of the animal and subsequently transferred into animal byproducts (Kan and Petz, 2000; Weiss *et al*., 2007).

European Union had banned the use of antibiotics as feed additives or growth promoters for livestock production (EU 2010). In addition, world health organization, European Union and other regulatory authorities had set maximum permissible limits (MPLs) for antimicrobial residues in food of animal origin; food products that contain higher levels of antimicrobials than MPLs are unsuitable for human consumption (EU 2010; WHO 2011). Therefore, continuous screening of antimicrobial residues in meat is mandatory in meat hygiene and food safety sectors for the sake of human health.

In Saudi Arabia, intensive livestock production is increasing with high consumption rates of red meat with an average of 43.0±1.3 g among different age groups (Moradi-Lakeh *et al*., 2017). However, there is no available information about the residual levels of antimicrobials in meat intended for human consumption in Saudi Arabia. In sight of these facts, this study was undertaken to estimate the residual concentrations of nine antimicrobials, commonly prescribed in veterinary field in Saudi Arabia, in meat (muscle, liver and kidney) of camel, cattle and sheep slaughtered in Al-Ahsa slaughterhouse. The tested antimicrobials were enrofloxacin, ciprofloxacin, tylosin, erythromycin, tetracycline, oxytetracycline, chlortetracycline, sulfamethazine and sulfaquinoxaline (Fig. 1). It is a fact that meat in Saudi Arabia like many other countries are not consumed raw, therefore, the effects of different cooking methods (boiling, pan-frying and barbecuing) on antibiotic residues were investigated.

MATERIALS AND METHODS

All experiments were done according to the regulations and guidelines of King Faisal University.

Chemicals: Ciprofloxacin, enrofloxacin, sulfamethazine, sulfaquinoxaline, tetracycline, oxytetracycline, chlortetracycline, erythromycin and tylosin were purchased from Sigma–Aldrich, (95%; European Pharmacopoeia HPLC assay, product of China; Shanghai, Trading Co., Ltd.). Formic acid (purity 98%, w/w) was from Sigma-Aldrich (St. Louis, MO, USA). McIlvaine buffer was prepared (12.0g sodium hydrogen phosphate (Na2HPO4), 3.72g EDTA and 11.9g anhydrous citric acid in 1L deionized water), and the pH was adjusted to 4 using concentrated phosphoric acid. Methanol (HPLC grade solvent) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (AcN) was obtained from Merck, Darmstadt, Germany. Deionized water was obtained from a Milli-Q water system (Millipore, Bedford, MA, USA). Disposable 0.45 µm nylon membrane filter filters (used for extract filtration) were obtained from Millipore (MA, USA). Agilent C18 cartridges (500 mg per 3 mL) were used for solid phase extraction (SPE) for sample preparation.

Collection of Samples: Hundred-eighty samples consisted of muscles, livers and kidneys (n = 60 each tissue) were collected randomly from slaughtered cattle, camels, and sheep (n = 20 from each animal species). Samples were collected from Al-Ahsa central slaughterhouse, Saudi Arabia, directly after slaughter. Samples were collected during the period of July to December 2017. The average ages of the slaughtered camel, cattle and sheep were estimated to be 31.65±24.27, 24.66±8.17 and 12.45±7.94 months, respectively. All animals were apparently healthy, active, and free from any disease. Owners claimed that no history of antibiotic given within one month prior to slaughter. Sampled tissues for antibiotic residue measurements weighed 250 g each and were divided into pieces (50 g/each) and stored in falcon tubes at −20°C until extraction and measurement. Samples with extremely high content of antibiotics (camel muscle (n = 5) with high content of sulfamethazine) were further exposed to heat treatment (boiling, pan-frying and electric grilling) in order to investigate the effect of heat treatments on sulfamethazine residues. Heat treatment was conducted according to the method reported before (Darwish *et al*., 2015a). In brief, boiling was conducted in a covered pan filled with distilled water and heated to 100°C for 30 min. Deep pan-frying was done in corn oil until browning of the samples (well-done). Electric grilling was done in an electric griller; the temperature was set to 180ºC for 15 min. All analyses were done in the Veterinary Laboratories Department, Ministry of Agriculture, Saudi Arabia.

UPLC/MS/MS system and operating condition analysis: An ultra-performance liquid chromatography (UPLC) system Acquity (Waters, Mildford, MA, USA) was interfaced to a triple quadrupole mass spectrometer (UPLC/MS/MS) (TQDTM, Waters Micromass, Manchester, UK) using an orthogonal Z-spray electrospray interface. The UPLC separation was performed using an Acquity UPLC BEH C18 analytical column, 1.7 lm particle size, 2.1mm × 50 mm (Waters), at a flow rate of 300 µL/min. The mobile phase consisted of three eluents, solvent A (ultrapure water with 0.15% formic acid), solvent B (AcN), and solvent C (methanol), delivered at a flow rate of 0.3 mL/min. A gradient elution system was employed with different ratios of the three eluents A, B and C as follows: 0 min, 78:2:20; 4 min, 75:5:20; 8 min, 70:10:20; 10 min, 42:38:20; 6 min hold,

42:38:20; 16.5 min, 78:2:20; 23 min, 78:2:20 (Wen *et al*., 2012).

The injection volume was 7 µL. Drying gas as well as nebulizing gas was nitrogen. The gas flow was set to 1200 L/h. For operation in MS/MS mode, collision gas was Argon 99.995% (Praxair, Valencia, Spain) with a pressure of approximately 2.103 mbar in the T-wave collision cell. Capillary voltage of 3.5 kV in positive electrospray ionization mode was applied. The interface temperature was set to 500°C and the source temperature to 120°C. Temperature column was set to 40°C. Dwell times of 30 ms/scan were chosen. Mass lynx v 4.1(Waters, Manchester, UK) software was used to process the quantitative data obtained from calibration standards and samples. MRM transitions of the antibiotics were as following: chlortetracycline, *m/z* 479.4 → 444.1 and *m/z* 479.4 → 462.1; ciprofloxacin, *m/z* 332.5 → 314.5 and *m/z* 332.5 → 231.4; enrofloxacin, *m/z* 360.0 → 432.0 and *m/z* 360.0 → 286.0; sulfamethazine, *m/z* 279.1 → 92.0 and *m/z* 279.1 → 124.0; sulfaquinoxaline, *m/z* 301.0 → 156.0 and *m/z* 301.0 → 108.0; tetracycline *m/z* 445.4 → 154.0 and *m/z* 445.4 → 410.2; oxytetracycline *m/z* 461.2 → 426.2 and *m/z* 461.2 → 443.1; erythromycin *m/z* 735.1 → 158.2 and *m/z* 735.1 → 576.2; tylosin, *m/z* 916.5 → 174.0 and *m/z* 916.5 → 100.9, were the qualifier and quantifier ions, respectively (Han *et al*., 2015). Mass spectrometry operating parameters were displayed in Table 1.

Extraction Procedure: Extraction procedures were done according to the method of Kwenga *et al.* (2015) with slight modifications. In brief, two grams of each sample were blended at a high-speed (15,000 rpm) tissue blender for 2 min and transferred into 25 ml centrifuge tubes. 10 mL of McIlvaine -Na2EDTA buffer (pH 4) were added to each sample, the mixture was vortexed at high speed and the samples were centrifuged at 4000 rpm for 15 min. The supernatants were pooled, then vortexed at high speed for 5 s and centrifuged at 3500 rpm for 10 min. Thereafter, they were filtered through 0.22 µm nylon membrane filter for SPE clean up. The solid phase extraction cartridges were conditioned by using 5 mL methanol solution, followed by 5 mL of de-ionized water. The SPE cartridges were loaded with 3 mL of the filtered sample, followed by washing with 5 mL of deionized water, and left to dry under vacuum for approximately 3 min to remove any residual moisture. The contents of the tube were discarded following elution with 4 mL of 10 mmol L–1 Methanolic oxalic acid (0.63 g oxalic acid dehydrated in 500 mL methanol HPLC grade) at pH 1.8. The eluent was evaporated gently at room temperature using a stream of nitrogen gas to dryness and the residue reconstituted with 1 mL of 50:50 mobile phase A (ultrapure water with 0.15% formic acid) and mobile phase C (methanol). The resultant solution was filtered using a Whatman 0.45 µm syringe filter and transferred to an UPLC auto-sampler vial. The samples were then ready for injection into the UPLC-MS system.

Matrix-matched calibration curves, Quality assurance and control: Matrix-matched calibration curves were prepared for control and quantification purposes according to Ghoneim *et al.* (2017) and Han *et al.* (2015). The meat samples were used for calibration curves. Samples were fortified by spiking the meat with different aliquots of standard solutions of the tested antibiotics at a wide range of concentrations ranging from 0 to 250 ng/g. The linearity of the method performed by preparation of calibration curves by using a wide range of concentrations (Fig. 2). Quantitation was made based on the linear standard curve (R^2>0.990). Limits of detection (LOD) and quantification (LOQ) were defined as lowest concentrations with a signal-to-noise (S/N) ratio of ≥3 for LOD or ≥10 for LOQ. Accuracy was evaluated by comparing found values with standard additions in spikes.

Statistical analysis: Statistical significance was evaluated using Dunnett's test *at* p<0.05 considered as significant (JMP program, SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

The extensive use of antimicrobials in livestock production might lead to several public health implications when contaminated meat is consumed. This study screened the residue levels of nine of the most commonly used antimicrobials in livestock production in Saudi Arabia.

Fluoroquinolones (FQs): The recorded results in Table 2 and Fig. 3 declared that FQs were detected only in the examined sheep tissues with incidence percentages of 1.66% and 4.98% for enrofloxacin and ciprofloxacin, respectively. Enrofloxacin was detected only in the kidney (5%), while ciprofloxacin was detected in 5% of the examined muscles, livers and kidneys of the sheep. The recorded concentrations of the detected FQs ranged from 10.51 to 87.99 ng/g in sheep samples. These concentrations were within the recommended maximum residue limits of FQs set by European Union (EU 2010) (100-300 ng/g) (Table 3). The recorded concentrations of FQs in the sheep samples were comparable to that recorded in the muscles of livestock in China (Wang *et al*., 2017). Unlikely, Lee *et al.* (2018) did not detect any FQs in the edible tissues of lambs collected from 2011 to 2015 in Taiwan. Enrofloxacin and ciprofloxacin were not detected in the examined cattle and camel samples. This result disagree with Barreto *et al.* (2017), who detected these compounds in bovine and swine muscles slaughtered in Brazil at a concentration range from 96.4 to 102.1 ng/g

Macrolides: Macrolides are a large group of natural antibiotics, which have antibacterial and antifungal activities (Song *et al*., 2016). In the present study, two macrolides, namely erythromycin and tylosin, were screened for their residues in the examined animal edible tissues. Erythromycin was not detected in any of the examined samples. However, erythromycin was previously detected in the milk of the sheep in Spain (García-Mayor *et al*., 2015). Similarly, it was the most detected antibiotic in raw and cooked meat in Hong Kong (Li *et al*., 2017).

Tylosin is used as animal-feed additives and recently used for prevention and treatment of mastitis and arthritis in livestock (Song *et al*., 2016). Similar to FQs, tylosin residues was detected only in samples collected from the sheep with an incidence percentage of 5% in both liver and muscle with an overall incidence of 3.32%. The recorded concentrations of tylosin in the two positive muscle and liver samples were 2.18 and 2.19 ng/g, respectively (Table 2 & Fig. 3). These concentrations were far below the recommended maximum residue limits of tylosin (EU 2010) (Table 3). The recorded concentrations of tylosin in the present study was lower than that recorded in meat samples (32.1 ng/g) from Croatia (Kolanović *et al*., 2014). Macrolides were also detected in beef sold at wet markets of Vietnam (Nhung *et al*., 2018).

Tetracyclines: Tetracyclines including oxytetracycline, tetracycline and chlortetracycline are widely used as antibiotics especially in the veterinary field because of their broad-spectrum properties and low cost (Darwish *et al*., 2013). The results achieved in this study indicated that this group of antibiotics was highly detected in the examined samples of cattle followed by camel and finally sheep. The highest residual concentration was recorded for chlortetracycline at 92.82 ng/g (Table 2). Oxytetracycline and chlortetracycline were highly detected when compared with tetracycline with the highest incidence in the kidney (45%/each) (Fig. 3). The recorded concentrations of tetracyclines in the present study were within the permissible limits set by European Union (EU 2010). In agreement with the current investigation, tetracyclines were detected at comparable levels in the edible tissues of cattle, buffalo and pig in Ethiopia, Nigeria, Sudan, Egypt and Vietnam (Myllyniemi *et al*., 2000; Olufemi and Agboola, 2009; Eltayb *et al*., 2012; Morshdy *et al*., 2013; Nhung *et al*., 2018). Unlikely, tetracycline was detected at concentrations five-times higher in bovine offal in Kenya (Muriuki *et al*., 2001).

Sulfonamides: Sulfonamides are extensively used for prevention and control of bacterial diseases due to their bacteriostatic and bactericidal effects. However, the un-controlled use of sulfonamides might lead to disposition of excessive concentrations of either parent compounds or their metabolites in the animal byproducts, and thus regarded as food-contaminants (Farré and Barceló, 2013). Sulfamethazine and sulfaquinoxaline were only detected in the camel and sheep samples at 36.52% and 3.32% in camel samples; 28.22% and 4.98% in sheep samples, respectively (Table 2). Muscles of both camel and sheep had the highest residues of sulfamethazine (Fig. 3). The recorded concentrations (ng/g) of sulfamethazine ranged between 3.64 and 4434.82 in the camel and between 2.61 to 2554.98 in the sheep (Table 2). Such concentrations were comparatively higher than MPL of sulfonamides in 15-45% of camel samples and 10-25% of sheep samples (Table 3 & Fig. 4). The high content of sulfonamides in the examined tissues indicates a recent usage of these antimicrobials and unobserving the recommended withdrawal times of the drugs prior to slaughter. In agreement with these results, sulfonamides were detected at higher concentrations in animal edible tissues in Brazil, China, Hong Kong, Lebanon and Spain (Aguilera-Luiz *et al*., 2012; Huang *et al*., 2012; Hoff *et al*. 2015).

Health hazards of antimicrobial residues in meat and some reduction trials: The unregulated use of antimicrobials in the livestock production may lead to occurrence of antibiotic residues in the edible tissues of the exposed animals with potential health hazards if such contaminated tissues are ingested. Antimicrobial residues in food are linked to development of multidrug resistant strains, severe allergic reactions, alterations in the beneficial intestinal microflora, hematological, psychological and neuronal symptoms (Gruchalla 2003; Baran *et al*., 2011; Darwish *et al*., 2013; Hiba *et al*., 2016). For instances, tetracyclines, sulfonamides and macrolides are linked to severe allergic reactions and anaphylaxis in humans (Paige *et al*., 1997). In our previous studies, we reported development of multidrug resistant enteropathogenic *Escherichia coli, Salmonella Enterica, Staphylococcus aureus* strains isolated from the edible tissues of various avian, aquatic and large animal species (Darwish *et al*., 2015b; El-Baymoi *et al*., 2016). Therefore, reduction of usage of unnecessary antibiotic in livestock production is highly recommended. Avoidance of using same antimicrobials in the treatment of both human and animal infectious diseases is highly suggested. It notes worthy to confirm that meat is consumed cooked in Saudi Arabia. Therefore, the effect of different cooking methods on the load of antimicrobials is investigated. The effects of boiling, pan-frying and grilling on sulfamethazine, the only detected compound at extremely higher concentrations than the recommended levels, were studied. The achieved results indicated that grilling achieved the maximum reduction on sulfamethazine (65%), followed by pan-frying (52%) and finally boiling (30%) (Fig. 5). These results go in agreement with Elbagory *et al*. (2017), who recorded a complete reduction of oxytetracycline and penicillin in

beef marketed in Egypt. In addition, Li *et al.* (2017) recorded a significant reduction in the antimicrobial load in chicken and pork tissues after boiling and steaming in Hong Kong. From these findings, efficient heat treatment of meat is highly recommended to reduce the food intake of antimicrobials.

Table 1. Mass spectrometry operating parameters.

	RT	Compound name	PI	F	QI	CE	QI	CE
1	4.16	Sulfaquinoxaline	301.1	110	92.0	29	156.0	11
2	2.66	Sulfamethazine	279.1	120	124.0	18	186.0	14
3	2.07	Ciprofloxacin	332.1	130	231.0	42	314.1	18
4	2.45	Enrofloxacin	360.0	130	316.2	18	342.1	18
5	3.05	Tetracycline	445.0	427	410.0	12	410.0	18
6	2.25	Oxytetracycline	461.4	426	443.0	10	426.0	18
7	5.1	Chlortetracycline	479.0	462	444.0	15	462.0	18
8	4.34	Tylosin	916.5	240	101.0	54	174.0	42
9	7.12	Erythromycin	734.5	170	158.1	30	576.3	14

RT: Retention time; PI: Precursor ion; F: Fragmentor (V); QI: Quantifier ion; CE: Collision energy (V): QI: Qualifier ion; CE: Collision energy (V)

Table 2. Incidence (%) and residual concentrations (ng/g) of different antibiotics in camel, cattle and sheep examined tissues.

	Camel		Cattle		Sheep	
	Incidence (%)	Residues ng/g	Incidence (%)	Residues ng/g	Incidence (%)	Residues ng/g
Enrofloxacine	0	ND	0	ND	1.66	ND-10.51
Ciprofloxacine	0	ND	0	ND	4.98	ND-87.99
Tylosine	0	ND	0	ND	3.32	ND-2.19
Tetracycline	0	ND	3.32	ND-8.22	0	ND
Oxytetracycline	3.32	ND-7.67	18.26	ND-23.44	0	ND
Chlortetracycline	0	ND	18.26	ND-92.81	1.66	ND-92.81
Sulfamethazine	36.52	ND-4434.82	0	ND	28.22	ND-2554.98
Sulfaquinoxaline	3.32	ND-2.65	0	ND	4.98	ND-6.64

ND: Not detected

Table 3. Maximum Residual Concentrations of the tested antibiotics in the edible tissues of domestic animals according to EU (2010).

	Tissue	MRL
Enrofloxacine	Muscle	100 ng/g
	Liver	300 ng/g
	Kidney	200 ng/g
Ciprofloxacine	Muscle	100 ng/g
	Liver	300 ng/g
	Kidney	200 ng/g
Tylosine	Muscle	100 ng/g
	Liver	100 ng/g
	Kidney	100 ng/g
Tetracycline	Muscle	100 ng/g
	Liver	300 ng/g
	Kidney	600 ng/g
Oxytetracycline	Muscle	100 ng/g
	Liver	300 ng/g
	Kidney	600 ng/g

Chlortetracycline	Muscle	100 ng/g
	Liver	300 ng/g
	Kidney	600 ng/g
Sulfamethazine	Muscle	100 ng/g
	Liver	100 ng/g
	Kidney	100 ng/g
Sulfaquinoxaline	Muscle	100 ng/g
	Liver	100 ng/g
	Kidney	100 ng/g

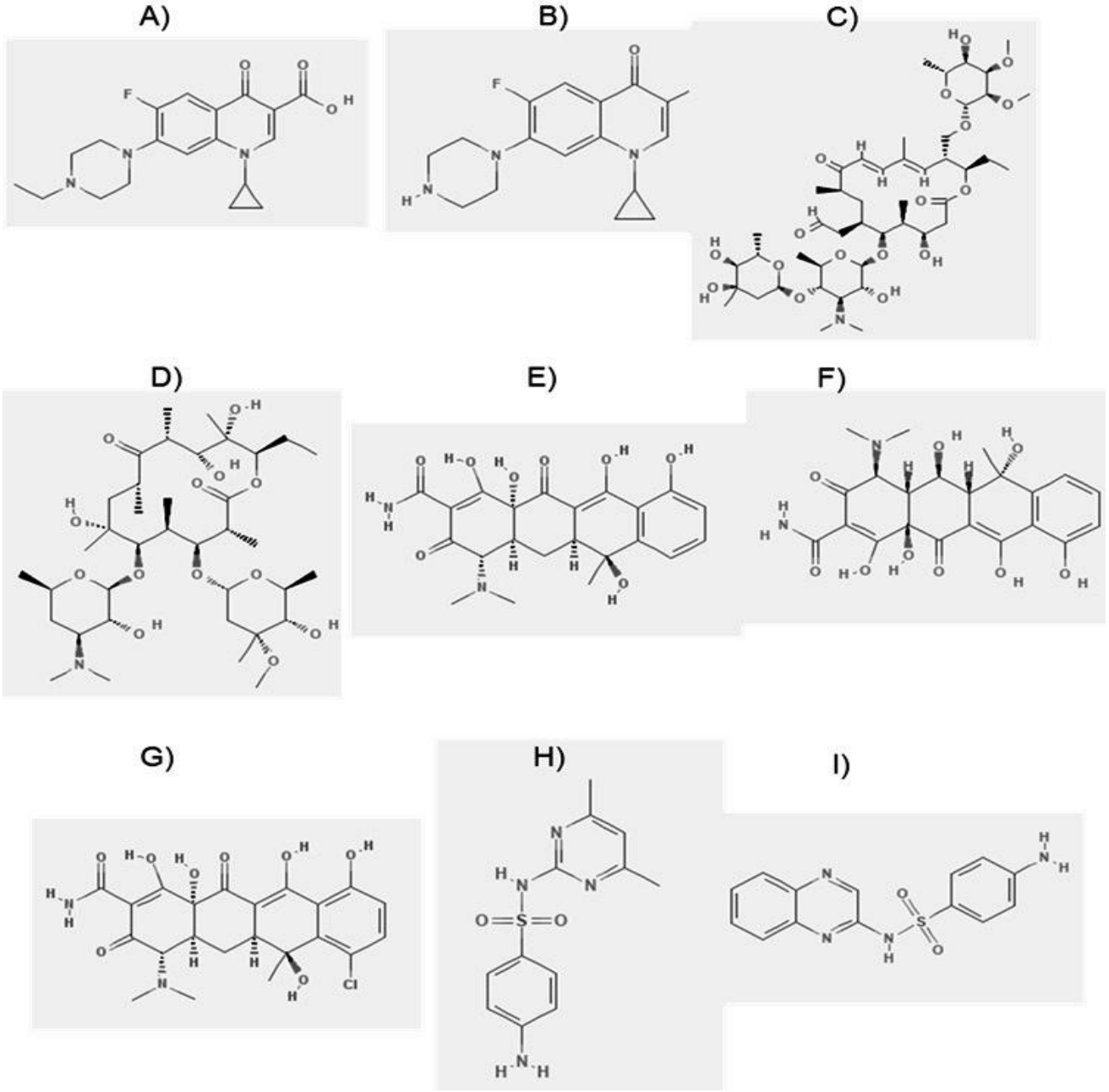

Figure 1. Chemical structure of the screened antimicrobials in the present study A) Enrofloxacin; B) Ciprofloxacin; C)Tylosin; D) Erythromycin; E)Tetracycline; F) Oxytetracycline; G) Chlortetracycline; H) Sulfamethazine; I) Sulfaquinoxaline

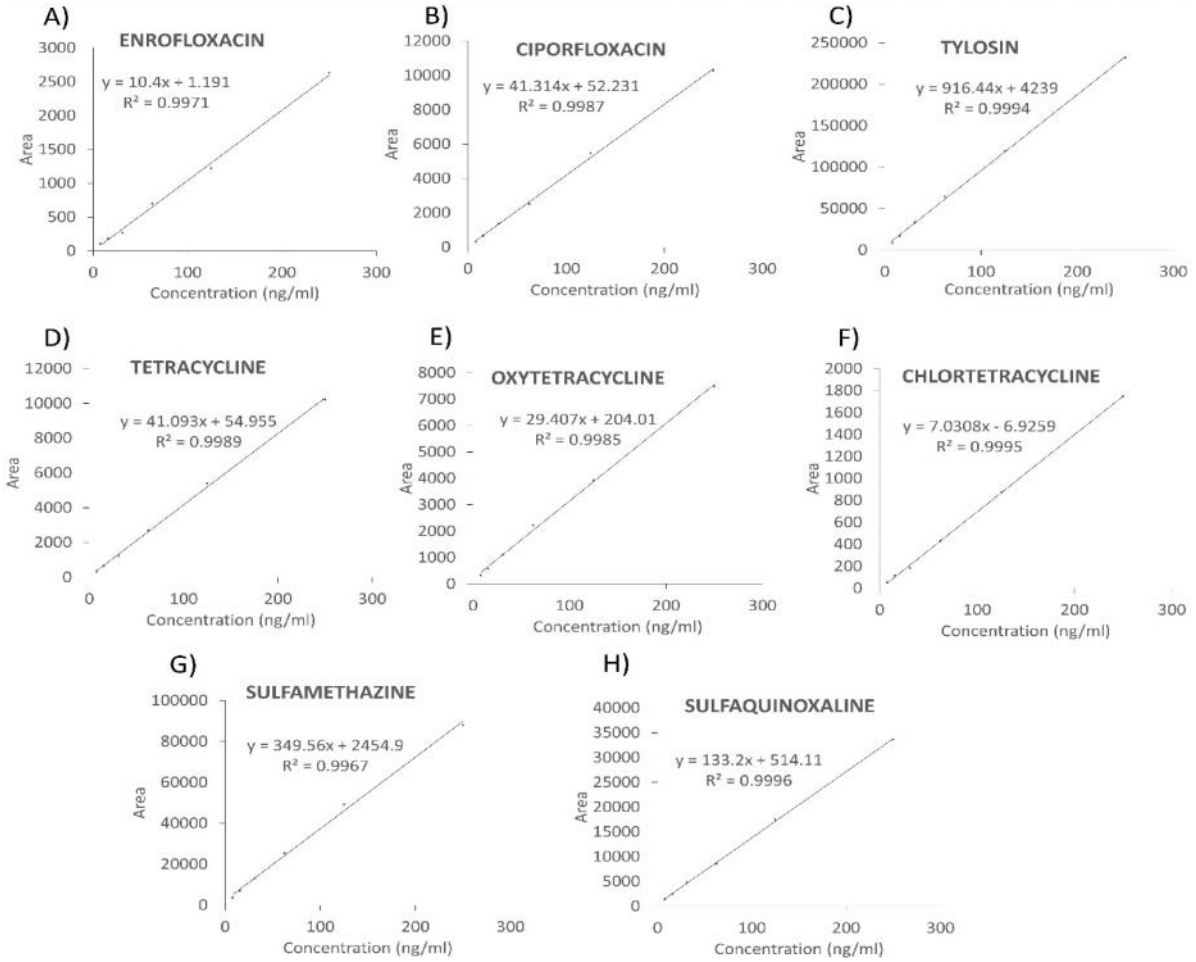

Figure 2. Standard calibration curves for the screened antimicrobials in the present study: Standard calibration curves were retrieved from the matrix-matched calibration curves for the tested antimicrobials A) Enrofloxacin; B) Ciprofloxacin; C)Tylosin; D)Tetracycline; E) Oxytetracycline; F) Chlortetracycline; G) Sulfamethazine; H) Sulfaquinoxaline

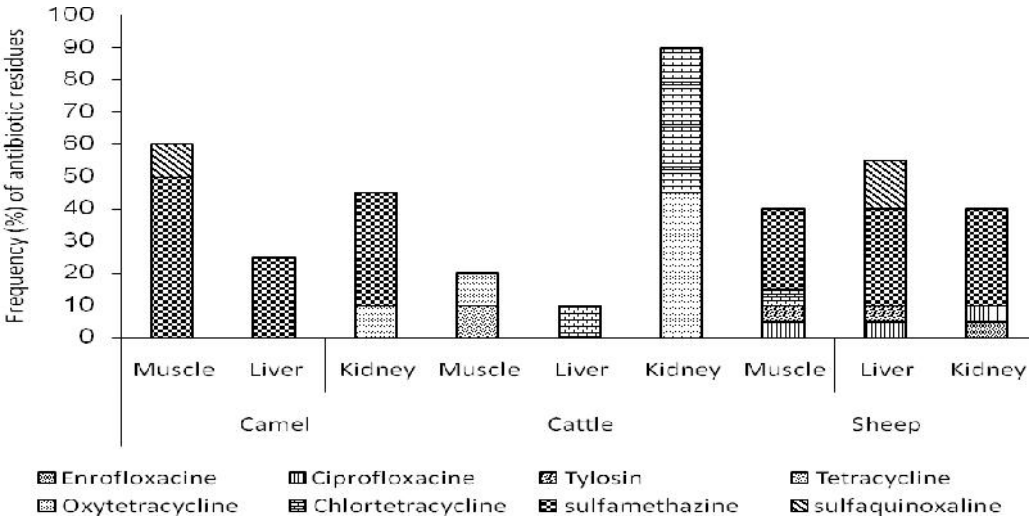

Figure 3. Frequency distribution (%) of antibiotic residues in camel, cattle and sheep offal: Frequency distribution (%) of different antibiotic residues in different edible parts (muscle, liver and kidney) of camel, cattle and sheep slaughtered at Al-Ahsa slaughterhouse, Saudi Arabia, (n = 20/each tissue from each animal species).

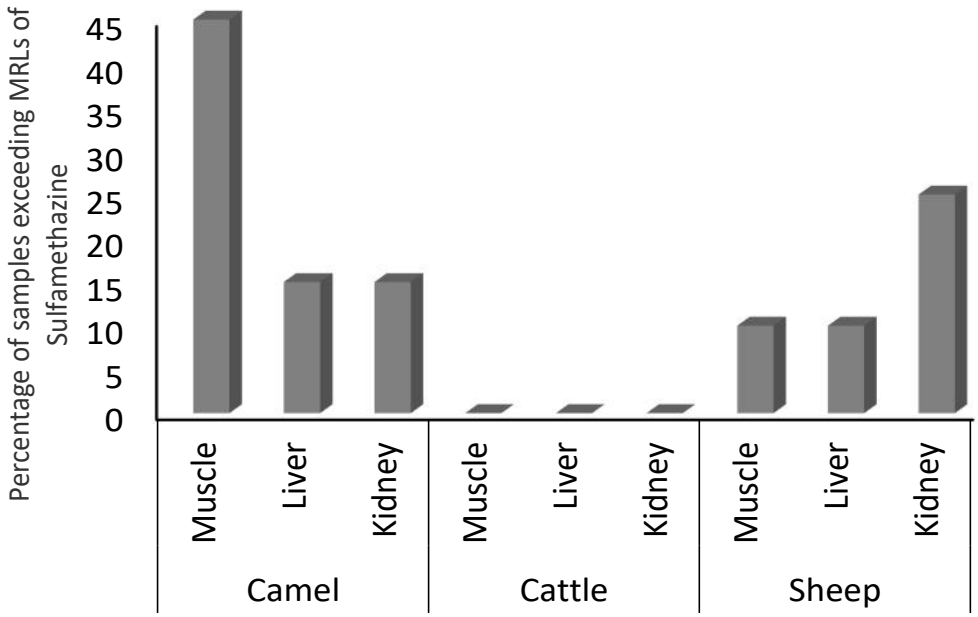

Figure 4. Percentage of edible tissue samples exceeding maximum permissible limits of sulfamethazine in camel, cattle and sheep offal: Percentage of different edible tissues (muscle, liver and kidney) of camel, cattle and sheep exceeding maximum permissible limits of sulfamethazine. Animals were slaughtered at Al-Ahsa slaughterhouse, Saudi Arabia, (n = 20/each tissue from each animal species).

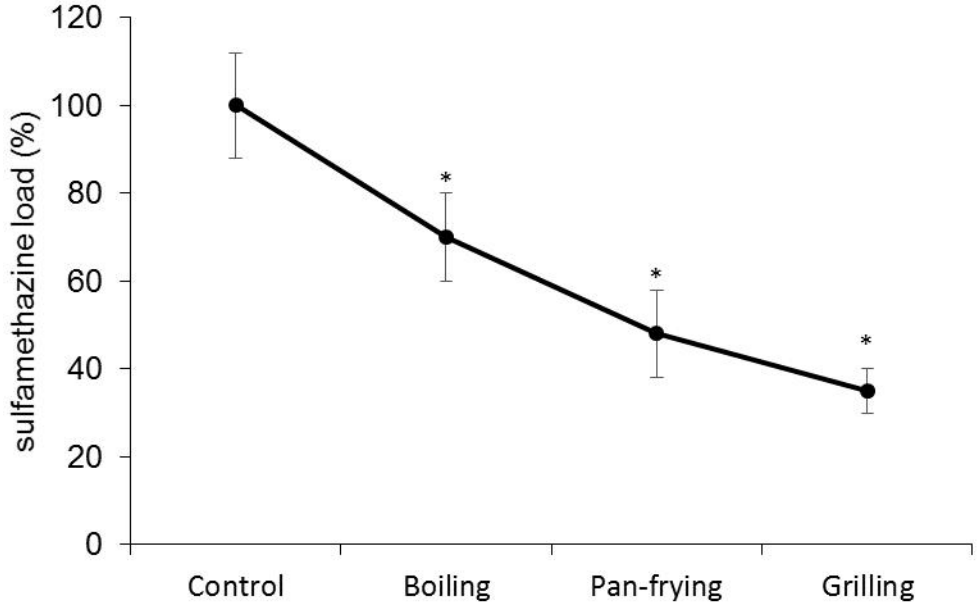

Figure 5. Effect of different cooking methods on sulfamethazine content in the muscle: Effect of different cooking methods (boiling, pan-frying and grilling) on the residual contents of sulfamethazine in the muscle tissue (n= 5/each treatment). Series carrying star mark are significantly different with the control at $p<0.05$.

Conclusions: To the best of our knowledge, this is the first study to screen antimicrobial residues in meat marketed in Saudi Arabia, camel in particular. The overall results declare that only sulfamethazine were detected at higher concentrations than the recommended levels. Efficient heat treatment of animal byproducts is highly recommended. Further investigations are still necessary to explain the inter-species differences in their retention of antibiotics.

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Conflict of interest: The authors declare that they have no conflict of interest.

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