

Quantification of antibiotic residues from green edible beef offal (tripe) from abattoirs and butcheries in Gaborone, Botswana

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Abstract

Antibiotics, one of the greatest medical discoveries of the 20th century, have significantly reduced infectious diseases. However, prolonged use can lead to antibiotic resistance, making it essential to monitor their residues in food such as tripe. This study aimed to identify and quantify antibiotic residues in tripe samples from butcheries and abattoirs in Gaborone, Botswana, using LC-MS/MS. Method validation included calibration, trueness, precision, and coefficient of variation. Thirty tripe samples were analyzed, 12 from abattoirs and 18 from butcheries. Trace amounts of doxycycline were found in all samples, followed by cloxacillin and penicillin G, detected in 37% and 23% of samples, respectively. Notably, ampicillin was present in samples S4 (36.9 µg/kg) and S6 (32.4 µg/kg), while enrofloxacin was found in S13 (17 µg/kg) and S17 (24.7 µg/kg). Sulfamethazine was detected in S28 at 23.8 µg/kg. All detected levels were below the maximum residue limits. This is the first study in Botswana to detect antibiotic residues in tripe using LC-MS/MS. The findings highlight the need for longer withdrawal periods before slaughter to ensure antibiotics are eliminated from animal tissues, thereby improving food safety and reducing the risk of antibiotic resistance in consumers.

1. Introduction

Antibiotics were the greatest discovery of modern medicine in the 20th century, and they have played a major role in controlling infectious diseases. Antibiotics are used in veterinary medicine to improve herd health, and to increase the productivity of food-producing animals. Similarly, they reduce the morbidity and mortality rates amongst livestock [1]. Antibiotics are also used as feed additives. The most common antibiotic substances in veterinary medicine include; aminoglycosides, β -lactams (penicillins and cephalosporins), macrolides, phenicols, quinolones, sulfamides, glycopeptides and tetracyclines [2]. Due to the continual consumption of antibiotics from food and water [3], humans have developed resistance to many commonly consumed antibiotics because of continual exposure. However, over the years research has shown that proper cooking methods are able to break down antibiotic residues from food items [4, 5]. Like other animals, cattle get sick and are treated with antibiotics, leading to the accumulation of these drugs in their tissues. Antibiotics residues remaining in animal-derived human foods may pose potential human health hazards toxicologically, microbiologically or immunopathologically. Thus, there is a need to close the knowledge gap on the antibiotic residues found in edible beef green offals.

Edible offals are sold in local butcheries to be cooked into delicacies such as *serobe*, *mogodu*, trotters, liver, tail and *mokoto*. These delicacies are consumed in many households in Botswana as part of diet since regular meat cut prices are unaffordable to a lot of households. The preferred alternative of animal protein source being edible offals, which are relatively affordable. Edible offals have a good nutrient profile, containing nutrients such as proteins, carbohydrates, fatty acids, vitamins and minerals [6], which are important in human nutrition. Locally, commonly consumed edible beef offals include tripe, liver, intestines, tongue, heart, kidney, spleen, lungs, trotters and tail [7], thus, the focus of the current study in terms of tripe material.

Tripe refers to the muscle walls (with the interior mucosal lining removed) of the first three chambers of a ruminant's gastrointestinal tract, being the rumen, reticulum and omasum. Tripe processing occurs after evisceration, usually in a facility separate from the carcass processing chain, and includes removal of the ingesta, trimming out of the rumen pillars and cutting the tripe into pieces. Tripe is a good source of high quality proteins, vitamin B12, proline, hydroxyproline, glycine, tryptophan and tyrosine [8]. *Serobe* is a favorite dish for many Batswana made up mainly of green offal (intestines and tripe), supplemented with heart, lungs and pancreas,

depending on the taste and preference of the individual, family and or community preparing it. It is normally eaten as part of relish in many households, and it is also prepared during weddings and funerals as well as other traditional ceremonies. Other tripe dishes include *mala mogodu*, which is a traditional South African dish consisting of stewed intestines (*mala*) and tripe (*mogodu*). A similar dish is also prepared in Botswana called *mala le mogodu*, Namibian dish called *matangara* prepared from goat or cow tripe, *ichifu* of Zambia and a Zimbabwean dish called *matumbu/ezanga phakathi*. Meat samples have been found to have antibiotic residues [9, 10], thus there is a need to find out if the residues are also present in offals. Many traditional dishes are produced from tripe hence; the main aim of this study was to document and quantify the antibiotics residues from tripe samples purchased from butcheries and Gaborone, Botswana using LC-MS/MS using Liquid Chromatography with tandem mass spectrometry (LC-MS/MS).

2. Materials and Methods

2.1 Sample collection

The tripe samples (*mogodu* and *ntshothwane*) were sampled from local abattoirs and butcheries in the Gaborone, Botswana. A total of 30 tripe samples were collected as follows: 12 samples were from abattoirs and 18 samples from butcheries (BBS mall, Block 3, Block 5, Block 6, Block 8, Gaborone west, Gaborone west industrial, Extension 4, Ledumang, Maruapula, Old Naledi, Partial, Phakalane, Phase 2, Phase 4, Sebele and Station [taxi rank]). The samples were collected from different cattle, different abattoirs and different butcheries, put into sterile ziplock bags, transported at 4°C and stored at – 20°C until analysis. All analysis was carried out within 6 to 8 hours to sample collection to avoid degradation of the antibiotic residues from the samples.

2.2 Chemicals and reagents

Acetonitrile, ammonia solution, citric acid monohydrate, dibasic sodium hydrogen phosphate, and LC-MS grade methanol were purchased from VWR (Radnor, PA, USA) for chemical extraction. The methanol that was used as the solvent in LC-MS was procured from Wako Chemicals (Osaka, Japan). Anhydrous magnesium sulphate (MgSO_4), sodium chloride (NaCl) and acetonitrile were purchased from (Sigma-Aldrich, USA), while formic acid and dimethyl sulfoxide (DMSO) were purchased from Fisher Scientific (Waltham, MA, USA). A total of 43 antibiotics belonging to 16 different antibiotic classes were used for this study. Doxycycline, ampicillin, penicillin G, penicillin V, oxacillin, cloxacillin, dicloxacillin, nafcillin, cefalonium, cefapirin, cefalexin, cefquinome, danofloxacin, difloxacin, enrofloxacin, flumequine, ciprofloxacin, marbofloxacin, oxolonic acid, sarafloxacin, sulfamerazine, sulfamethazine, sulfamonomethoxine, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfadimethoxine, sulfachloropyridazine, sulfadiazine, sulfamethoxypyridazine, trimethoprim, sulfisoxazole, sulfamethizole, amoxicillin, erythromycin, tilmicosin, tylosin, tulathromycin, gamithromycin, lincomycin, pirlimycin and tildipirosin were purchased from Fluka (St. Louis, MO, USA), MP Biomedicals (Irvine, CA, USA) and Sigma-Aldrich (Darmstadt, Germany).

2.3 Stock Standard Solutions

Individual stock standard solutions (200 µg/mL) of sulfonamides, fluoroquinolones, and macrolide antibiotics and trimethoprim were prepared in methanol using polypropylene volumetric flasks and stored in the freezer at – 20°C until use. The same concentration of β -lactam antibiotics was prepared using distilled water and stored in the refrigerator at 4°C for no more than 2 weeks.

2.4 Sample Preparation

Samples were prepared according to the method for LC-MS/MS [11]. About 50g of tripe was chopped into small pieces using a sanitized mini food chopper, and thereafter, homogenized. From the homogenized samples, 2.0 ± 0.02 g was weighed into 50 mL centrifuge tubes. Extra 5 blank samples were included for calibration curve and extra 3 blank samples for quality control. Calibration samples were fortified with reference standard at 0.0, 0.25, 1.0, 1.5 and 2.0 maximum residue limit (MRL) concentrations, while the quality control samples were fortified with reference standard at 1.0 MRL concentrations. Samples were allowed to stand for 30 minutes before the next step. Acetonitrile of 10 mL was added to the weighed samples and homogenized for 30 seconds using probe homogenizer. Four grams anhydrous magnesium sulphate (MgSO_4) and 1.0 g sodium chloride (NaCl) were added to the sample. The samples were homogenized by spin mixing using multi-reax homogenizer for 20 minutes and then centrifuged at a speed of 8000 rpm with centrifuge temperature at 4°C . The samples were placed in -40°C freezer for 30 minutes to freeze the fat. The supernatant was immediately decanted into 15 mL centrifuge tube containing 1.0 g of MgSO_4 and 500 mg of C18 bulk solvent and vortexed for 10 minutes. The sample was centrifuged at 4000 rpm, 4°C for 10 minutes. From the resultant supernatant, 6.0 mL was transferred into 15 mL drying tubes containing 100 μL of 2% dimethyl sulfoxide (DMSO) and concentrated using turbovap LV (Biotage, Sweden) concentration workstation under the flow of nitrogen. The sample was reconstituted with 900 μL filtered with 0.45 μm filtration nylon syringe filters (Sartorius, Germany) into 2 mL HPLC vials and stored in the fridge before LC-MS/MS.

2.5 LC-MS/MS analysis

The Acquity I-Class ultra-high-performance liquid chromatographic system by Waters (Milford, MA, USA), coupled with the QTRAP® 6500 + MS system from AB Sciex (Framingham, MA, USA), was used for LC-MS/MS analysis. A ZORBAX Eclipse plus C18 column (2.1×150 mm, 5 μm) (Agilent Technologies, USA) was used for separation, and the column oven temperature was set at 40°C . The elution gradient (solvent A: 0.1% aqueous formic acid, solvent B: acetonitrile/methanol 50/50 v/v + 0.1% formic acid) was set up as follows: 0 minutes, 0% B; 0.1 minute, 0% B; 1 minute, 10% B; 6.5 minutes, 50% B; 7 minutes, 100% B; 8 minutes, 100% B. This gradient was re-equilibrated to 0% B for 2 minutes after each run. The flow rate was 0.5 mL/minutes, and the injection volume was 5 μL . In terms of electrospray ionization, the parameters were the following: dwell time (4 msec), curtain gas (35), entrance potential (10 volts); collision gas (medium); electrospray voltage (4500 V); ion source temperature (450°C); ion source gas 1 (50 psi) and ion source gas 2 (60 psi). Table S1 gives the detailed description of the parameters of multiple reaction monitoring (MRM) condition and retention times of the antibiotics.

2.6 Method validation

The analytical method was validated according to the European Commission directive 2021/808 [12]. The method validation was evaluated using various parameters: calibration function, trueness, precision and coefficient of variation. Since 0.25 MRL is the lowest validation level, it was included in the calibration curve. The goodness-of-fit of the data (coefficient of determination, R^2) to the curve and the mathematical formula of the curve ($y = mx + c$), where m is the gradient of the line (how steep the line is) and c is the y-intercept, the point in which the line crosses the y-axis, are both described. To assess the trueness of the analytical method, recovery study was conducted, considering also that certified reference materials (CRMs) not available. The following equation was used to calculate each sample's trueness.

$$\text{Trueness (\%)} = (\text{mean recovery} - \text{corrected concentration detected}) \times \frac{100}{\text{fortification level}}$$

2.7 Statistical analysis

In this study, a comprehensive data analysis of antibiotic detection across various samples was conducted utilizing several methods using SPSS (IBM SPSS Statistics, 30.0.0, USA). Descriptive statistics, to summarize central tendencies and variability, while maximum concentrations highlighted peak levels of presence. Data visualization techniques, primarily through detailed tables, presented antibiotic concentrations and detection frequencies. Hierarchical clustering categorized antibiotics into three distinct groups based on detection consistency namely, a high presence group, a moderate presence group, and a group with predominantly zero concentrations. The mean and standard deviation of the spiked recoveries were calculated through Excel (Microsoft Excel 2019, USA). Results were expressed as mean $\pm$ standard error.

3. Results and Discussion

3.1 Method validation

3.1.1 Calibration function

A standard curve, sometimes referred to as a calibration curve, is a common technique for figuring out how much of a material is present in an unknown sample as adapted from the Commission Implementing Regulation (EU) 2021/808 [12]. At least five equidistant levels (0.0, 0.25, 0.5, 1.0, 1.5, and 2.0 MRL) were used in the development of the extracted matrix calibration curves, which are used for quantification. The coefficient of determination (R^2) was found to be over the minimal requirement of 0.98, with a range of 0.9889 to 0.9995 (Table 4). Research shows that R^2 values can be below 0.990 because of enzymatic/chemical instability or the extreme polarity of the compounds but this was not the case in the current study [13]. The coefficient of correlation revealed good linearity in the concentration range for each compound.

3.1.2 Trueness

Trueness is the proximity of agreement between the average value obtained from a large series of test outcomes and an accepted reference value. Trueness was determined on basis of fortified samples. Since the approved reference material was unavailable, experiments employing a fortified blank matrix were used to ascertain the method's veracity. Six replicates of the analysis were run for each batch, with the blank matrix being a certified reference standard that was fortified at 0.0, 0.25, 1.0, 1.5 and 2.0 the MRL at each level. The samples adhered to the extraction and analysis protocol. The multi-quant program was utilized to compute the concentration found in every sample. According to Table 1 and Commission Implementing Regulation (EU) 2021/808 of March 22, 2021 [12], the trueness ranges from 72.4% (cefquinome) to 114.8% (penicillin G) (Fig. 1, Table 1 and Table 3), which was within the permissible range of 80–120% for concentrations $\geq 10 \mu\text{g/kg}$. Table 1 below displays the mean trueness as a percentage recovery (Mean % Rec) for all batches as well as the total trueness.

Table 1
Minimum trueness of quantitative
methods as adapted from the Commission
Implementing Regulation (EU) 2021/808
[12]

Mass Fraction	Range
≤ 1 µg/kg	–50% to + 20%
> 1 µg/kg to 10 µg/kg	–30% to + 20%
≥ 10 µg/kg	–20% to + 20%

3.1.3 Precision

Precision (test findings' standard deviation or coefficient of variation) is the degree to which independent test results acquired under specific conditions are close to one another. The formula for precision is reproducibility (% CV). When test results are achieved using the same procedure on identical test objects in different laboratories with different operators using different equipment, reproducibility is defined as precision under those conditions. A series of samples with identical blank matrices from caprine, ovine, and bovine liver were fortified with the analyte to produce concentrations equal to 0.0, 0.25, 1.0, 1.5 and 2.0 times the MRL in order to assess repeatability. Under within-laboratory repeatability conditions, the coefficient of variation (CV) for the repeated examination of a fortified material does not surpass the threshold determined by the Horwitz equation $CV = 2^{(1 - 0.5 \log C)}$. This study had very good coefficient of variation (% CV) ranging from 3.3 to 13.1% (Table 3). The coefficient of variation for analyses conducted under repeatable settings is less than two thirds of the values shown in Table 2. For all investigated compounds, the CV for precision was acceptable and in agreement with the criteria of Commission Implementing Regulation (EU) 2021/808 [12].

Table 2
Acceptable coefficient of variation as adapted from the Commission Implementing
Regulation (EU) 2021/808 [12].

Mass fraction	Reproducibility CV (%)
> 1 000µg/kg	16 (adapted from Horwitz equation)
> 120 µg/kg – 1 000µg/kg	22 (adapted from Horwitz equation)
10–120 µg/kg	25*
< 10 µg/kg	30*
*The CV (%) presented is a guideline and should be as low as reasonably possible.	

3.1.4 Decision limit for confirmation (CC_α)

The decision limit for confirmation, or CC_α, is the upper limit at which a sample is deemed non-compliant with an error probability of α; the value 1 - α indicates the percentage statistical certainty that the allowed limit has been exceeded. The calibration curve method in accordance with the Commission Implementing Regulation (EU) 2021/808 [12] was used to calculate the decision limit for confirmation (CC_α), also known as the critical value of the net state variable. This instance involved the use of blank material that was fortified at 0.0, 0.25, 1.0, 1.5 and 2.0 MRL. The samples were removed and examined in accordance with protocol. Plotting the predicted concentration against the added concentration was done after identification. The decision limit, denoted as α =

5%, is the mean concentration at the MRL plus 1,64 times the standard deviation of the repeatability within the laboratory at the allowed limit. The current study had CC α ranging from 26.1 (cefalonium) and 1075 (cefalexin) (Table 3). The gained results showed good sensitivity because the values of the CC α are in accordance with the requirements of Commission Implementing Regulation (EU) 2021/808 [12].

Table 3

Method validation of the different antibiotics based on their MRL, recovery, reproducibility, limit of decision and coefficient of determination.

Analyte Peak Name	MRL ($\mu\text{g/Kg}$)	Recovery	Reproducibility CV (%)	CC α ($\mu\text{g/Kg}$)	Coefficient of determination (R^2)
Doxycycline	600	83.42 $\pm$ 14.20	9.6	615.4	0.9985
Ampicillin	50	103.84 $\pm$ 4.37	7.7	61.2	0.9982
Penicillin G	50	114.80 $\pm$ 5.02	6.1	55.4	0.9933
Penicillin V	50	109.80 $\pm$ 1.57	6.2	52.3	0.9945
Oxacillin	300	105.28 $\pm$ 7.75	5.5	32.3	0.9930
Cloxacillin	300	102.53 $\pm$ 14.10	8.9	312.1	0.9915
Dicloxacillin	300	73.22 $\pm$ 39.20	12.4	314.6	0.9986
Nafcillin	300	85.96 $\pm$ 11.90	9.0	325.2	0.9975
Cefalonium	20	72.43 $\pm$ 3.88	5.6	26.1	0.9889
Cefapirin	100	109.74 $\pm$ 14.6	7.9	111.5	0.9951
Cefalexin	1000	107.81 $\pm$ 11.60	6.3	1075.0	0.9955
Cefquinome	200	94.43 $\pm$ 9.71	4.6	227.2	0.9942
Danofloxacin	100	105.69 $\pm$ 2.97	10.2	105.2	0.9987
Difloxacin	800	76.52 $\pm$ 4.41	7.1	819.9	0.9952
Enrofloxacin	200	109.98 $\pm$ 4.02	7.0	213.4	0.9991
Flumequine	1000	107.32 $\pm$ 0.79	10.0	1050.1	0.9978
Ciprofloxacin	100	107.80 $\pm$ 0.30	7.4	107.5	0.9989
Marbofloxacin	100	93.77 $\pm$ 1.34	7.7	113.3	0.9987
Oxolonic acid	100	105.05 $\pm$ 0.05	8.3	107.9	0.9985

Analyte Peak Name	MRL (µg/Kg)	Recovery	Reproducibility CV (%)	CC α (µg/Kg)	Coefficient of determination (R^2)
Sarafloxacin	100	92.97 ± 15.60	9.2	114.4	0.9962
Sulfamerazine	100	107.42 ± 0.30	7.8	109.7	0.9985
Sulfamethazine	100	105.64 ± 5.95	9.8	116.1	0.9945
Sulfamonomethoxine	100	90.39 ± 3.37	5.9	104.2	0.9982
Sulfapyridine	100	86.26 ± 19.50	6.1	106.4	0.9993
Sulfaquinoxaline	100	86.84 ± 2.22	9.0	117.1	0.9990
Sulfathiazole	100	102.16 ± 1.51	8.5	115.2	0.9967
Sulfadimethoxine	100	102.97 ± 6.42	7.2	111.0	0.9979
Sulfachloropyridazine	100	97.72 ± 5.02	8.8	109.7	0.9987
Sulfadiazine	100	93.39 ± 7.80	5.7	103.1	0.9968
Sulfamethoxypyridazine	100	91.37 ± 5.47	4.7	107.4	0.9995
Trimethoprim	50	92.25 ± 5.51	5.7	55.3	0.9978
Sulfisoxazole	100	98.67 ± 4.80	3.6	108.5	0.9994
Sulfamethizole	100	92.45 ± 20.60	3.8	101.1	0.9988
Amoxicillin	50	106.67 ± 1.51	6.6	53.3	0.9966
Erythromycin	200	75.41 ± 12.70	6.5	215.9	0.9987
Tilmicosin	1000	93.54 ± 0.41	5.6	1034.2	0.9956
Tylosin	100	96.24 ± 2.23	6.0	112.7	0.9952
Tulathromycin	1000	101.685 ± 0.22	3.3	1023.0	0.9956
Gamithromycin	100	101.455 ± 5.13	8.5	113.8	0.9906

Analyte Peak Name	MRL (µg/Kg)	Recovery	Reproducibility CV (%)	CC α (µg/Kg)	Coefficient of determination (R^2)
Lincomycin	1000	95.29 ± 12.1	12.6	1021.2	0.9907
Pirlimycin	400	101.46 ± 5.13	7.7	423.9	0.9984
Tildipirosin	1000	95.29 ± 12.10	13.1	1016.7	0.9937

*Note: The number of samples tested for each type of antibiotic was in triplicates with recovery rates given in mean percentage ± standard deviation (Recovery %).

3.2 Antibiotics residues from tripe

The results showed that doxycycline was present in all samples at a mean concentration of 0.3 µg/kg indicating a wide use of this antibiotic by farmers, followed by penicillin G and penicillin V at concentrations of 1.4 µg/kg and 1.2 µg/kg, respectively (Table 4). Even though these antibiotics were present in the samples, their concentrations were very low. The set MRL for penicillin G and penicillin V is 50 µg/kg for each of them as seen Table 3. Penicillin G is normally not detected or is detected on beef samples at very low concentrations [14, 15]. The information provided valuable insights into usage patterns, variability, and potential public health implications related to antibiotic residues, emphasizing the necessity for ongoing monitoring and responsible usage practices. The major concern about antibiotic residues in animal products (if consumed in excess) is the possible side effects from direct toxicity or from allergic reactions in consumers. This may also lead to the emergence of resistant strains of bacteria in animals as well as passage of resistant strains via the food chain from animals to humans. Antibiotic residues have been reported to have the following negative health implications: carcinogenicity, mutagenicity, teratogenicity, nephropathy, hepatotoxicity, bone marrow toxicity and allergy [16].

Table 4
Frequency and concentration of antibiotics detected in different tripe samples

Antibiotic	Detection Frequency	Number of samples tested	Detection percentage (%)	Mean Concentration (µg/kg)	Highest Concentration (µg/kg)	Notes
Doxycycline	30 samples	30 samples	100	0.3 ± 0.01	0.3	Present in all samples; indicates wide use.
Cloxacillin	11 samples	30 samples	37	0.6 ± 0.1	0.6	Consistent low levels; reflects environmental presence.
Penicillin G	7 samples	30 samples	23	0.7 ± 0.2	1.4	Common presence; suggests therapeutic use.
Penicillin V	5 samples	30 samples	17	0.8 ± 0.2	1.2	Consistent detection; moderate presence.
Ampicillin	3 samples	30 samples	10	23.0 ± 11.4	36.9	High levels in S4; potential recent use.
Enrofloxacin	3 samples	30 samples	10	8.3 ± 4.2	24.7	Indicates effectiveness against infections.
Cefalonium	3 samples	30 samples	10	6.0 ± 3.3	9.1	High peak indicates targeted use.
Sulfamethazine	2 samples	30 samples	7	16.5 ± 5.0	23.8	Significant peak; reflects treatment use.
Cefapirin	2 samples	30 samples	7	0.05 ± 0.02	0.1	Low levels; limited usage.
Gamithromycin	1 sample	30 samples	3	0.1	0.1	Very low detection; limited use.
Pirlimycin	1 sample	30 samples	3	0.1	0.1	Low concentrations; limited use.
Tulathromycin	0 samples	30 samples	0	N/A	N/A	Not detected; indicates limited application.
Lincomycin	0 samples	30 samples	0	N/A	N/A	Not detected; suggests

Antibiotic	Detection Frequency	Number of samples tested	Detection percentage (%)	Mean Concentration (µg/kg)	Highest Concentration (µg/kg)	Notes
						limited usage.
Tildipirosin	0 samples	30 samples	0	N/A	N/A	Not detected; indicates limited use.

*Note: N/A – antibiotic not detected in the samples.

Figure 2 shows that out of the 30 samples, 5 samples had antibiotics residues at high concentration levels. The results showed that samples S4 and S6 had ampicillin at concentration levels of 36.9µg/kg and 32.4µg/kg, respectively whereas samples S13 and S17 had enrofloxacin at concentration levels 17µg/kg and 24.7µg/kg. Lastly, sulfamethazine was detected in samples 23.8µg/kg. However, these detected antibiotics are below the maximum residual limit (Table 3). The antibiotics have been detected in other study samples such as of chicken, camel, duck meat, milk and bovine above MRL [13, 15, 17–20]. Studies have shown that antibiotic residues are the most common drug residues found in meat, with neomycin, streptomycin, penicillin, oxytetracycline, gentamicin, and sulfamethazine being the most frequently detected [2].

Ampicillin is normally used for the treatment of gram-negative bacteria such as *Escherichia coli* and metritis in cattle [21]. Ampicillin if consumed in excess causes asthma attacks, eosinophilia, leukopenia, agranulocytosis, cholestatic hepatitis, allergic reactions, exfoliative dermatitis, anemia, thrombocytopenia and thrombocytopenic [22], hence the need to monitor it in food items. Samples S4 and S6 had ampicillin at concentrations of 36.9µg/kg, 32.4µg/kg, respectively (Fig. 2). The EU maximum residue limit for this antibiotic is 50µg/kg, meaning both samples had residues below the limit (Table 3) [23]. Ampicillin is a β-lactam antibiotic that binds to specific penicillin-binding proteins in the inner surface of the bacterial cell wall acting as an irreversible inhibitor of dd-transpeptidase, an essential enzyme that bacteria use to synthesize peptidoglycan and other components of the cell wall [24]. Disruption in cell wall synthesis ultimately leads to cell lysis and death of the bacteria. There is not much information about the degradation of ampicillin through cooking, but research shows that storage at 4°C reduces this residue [25].

Enrofloxacin was detected in samples S13 and S17 at concentrations of 17µg/kg and 24.7µg/kg (Fig. 2) which were below the maximum residue limit (MRL) of 200µg/kg (Table 3) [23]. This antibiotic is usually detected in chicken meat [13, 17, 18, 26] and bovine meat at concentration of 35.22µg/kg [13]. Enrofloxacin has an acceptable daily intake (ADI) of 6.2µg/kg (high in both samples) which is the maximum amount of a substance that a person can consume daily throughout his/her life without posing any risk [27], meaning if consumed by a person at this amounts it can lead to negative health implication such as chromosomal damage [22]. The antibiotic is widely used in veterinary and human medicine against gram-positive, gram-negative and mycoplasma organisms. The action of fluoroquinolone is concentration-dependent, i.e., depending on the concentration of the drug, the duration and intensity, bacterial lysis takes place [28]. Research shows that quinolones are very stable during cooking, with almost no results obtained even by heating to ultra-high temperatures [29]. Enrofloxacin residues are not affected by cooking procedures, however, an apparent decrease in enrofloxacin concentration was observed as some of the liquid used for cooking (boiling and frying) was lost by exudation [4]. In other studies, an apparent increase in residue concentration was observed in a water-loss cooking technique (roasting and grilling) which was in contrast to the findings from the previously mentioned studies [4, 26]. Frying is an

effective way of reducing enrofloxacin residue concentrations in chicken meat [18] which can be used to reduce enrofloxacin residue in tripe. However, in Botswana tripe is mainly boiled to make dishes such as *serobe* and *mokoto*, but frying is also used in some cases.

Lastly, with sample S28, sulfamethazine was detected at a concentration of 23.8µg/kg, which was also below the EU MRL of 200µg/kg (Fig. 2 and Table 3) [23], and below an ADI of 50µg/kg [27]. This antibiotic is normally detected in food items such as chicken, milk, fish and beef meat [1, 17, 19]. Sulfamethazine is used to treat a range of bacterial infections in cattle, sheep, swine, and poultry as it is effective against certain gram-positive and gram-negative bacteria. The drug is commonly used to treat respiratory infections, urinary tract infections, and some gastrointestinal infections. However, this drug also has adverse health effects on humans which include skin reactions, blood dyscrasias and carcinogenicity [22]. Research has shown that boiling is effective in reducing sulfamethazine residue in meat [4]. Other effective cooking methods include microwaving and roasting, which have been observed to reduce sulfamethazine residues in food [30].

The detection of these antibiotics in the tripe samples is suggestive for longer withdrawal periods for animals destined for slaughter, to completely remove antibiotics from their blood stream. Proper cooking techniques could also be ideal options for degradation or reduction of the antibiotic residues so as to avoid any possible negative health effects to meat consumers. The results of the current study also demonstrate the need for farmer education in veterinary drug use in their livestock production enterprises.

Limitation

The major limitation of the current study was limited funds that could not allow for more sample collection across different districts animal processing facilities. Further, the current study is part of a PhD programme which is limited to three years, thus not allowing for a large amount of sample collection across the country. The availability of more funds and more study time would allow coverage of all 16 districts of Botswana, and a total of 84 red meat slaughter facilities. This would enable sampling from each district in both abattoirs and butcheries, giving a much larger sample size.

4. Conclusion

The LC-MS method provided a reliable approach for characterizing and quantifying antibiotic residues in beef tripe. This was the first time in Botswana antibiotic residues in tripe material were assessed using this procedure. Using this method, ampicillin, enrofloxacin and sulfamethazine were quantified from beef tripe samples, results that are vital in food quality control in the country. The quantified residue amounts were within the allowed intake limits that are considered safe to humans. The method can be used for routine monitoring and to ensure compliance with food safety regulations, both locally and internationally. Future work should focus on expanding use of the method to other edible offals evaluation, and explore ways to reduce antibiotic residues in edible offal material.

Declarations

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Author Contributions

K.M. conceptualized the study, coordinated sample collection, carried out laboratory analyses and led manuscript preparation. M.D.S. assisted in data collection and interpretation. R.T. carried out laboratory analyses and quality control. N.Z., E.M., G.M., J.M., and M.H.D.M. all supervised the project and reviewed the final manuscript. All authors read and approved the final version of the manuscript.

Data availability

The data supporting the findings of this study are available from the corresponding author, K.M., upon reasonable request. All relevant data generated and analyzed during the study are included in the manuscript and its supplementary materials, where applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable

Competing interests

The authors declare no competing interests.

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Figures

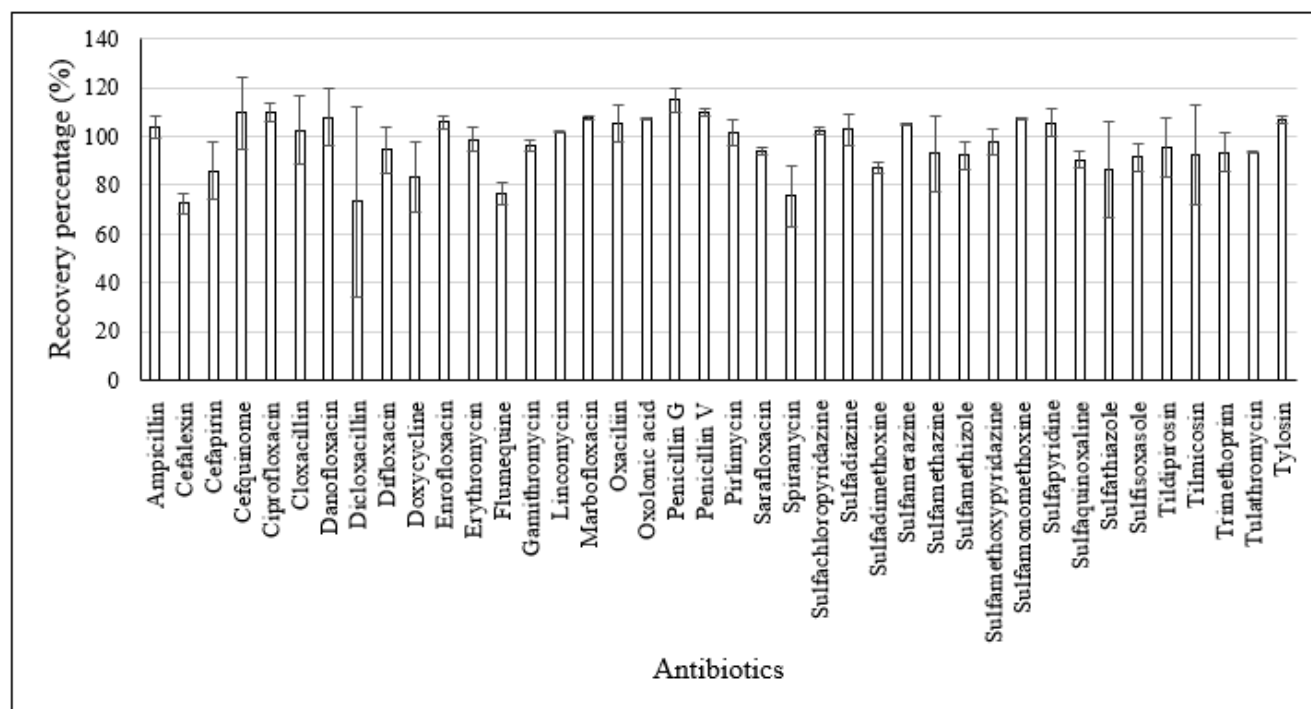


Figure 1

Spike recovery of the different antibiotics. The samples were fortified with reference standards at 0.0, 0.25, 1.0, 1.5 and 2.0 MRL concentrations.

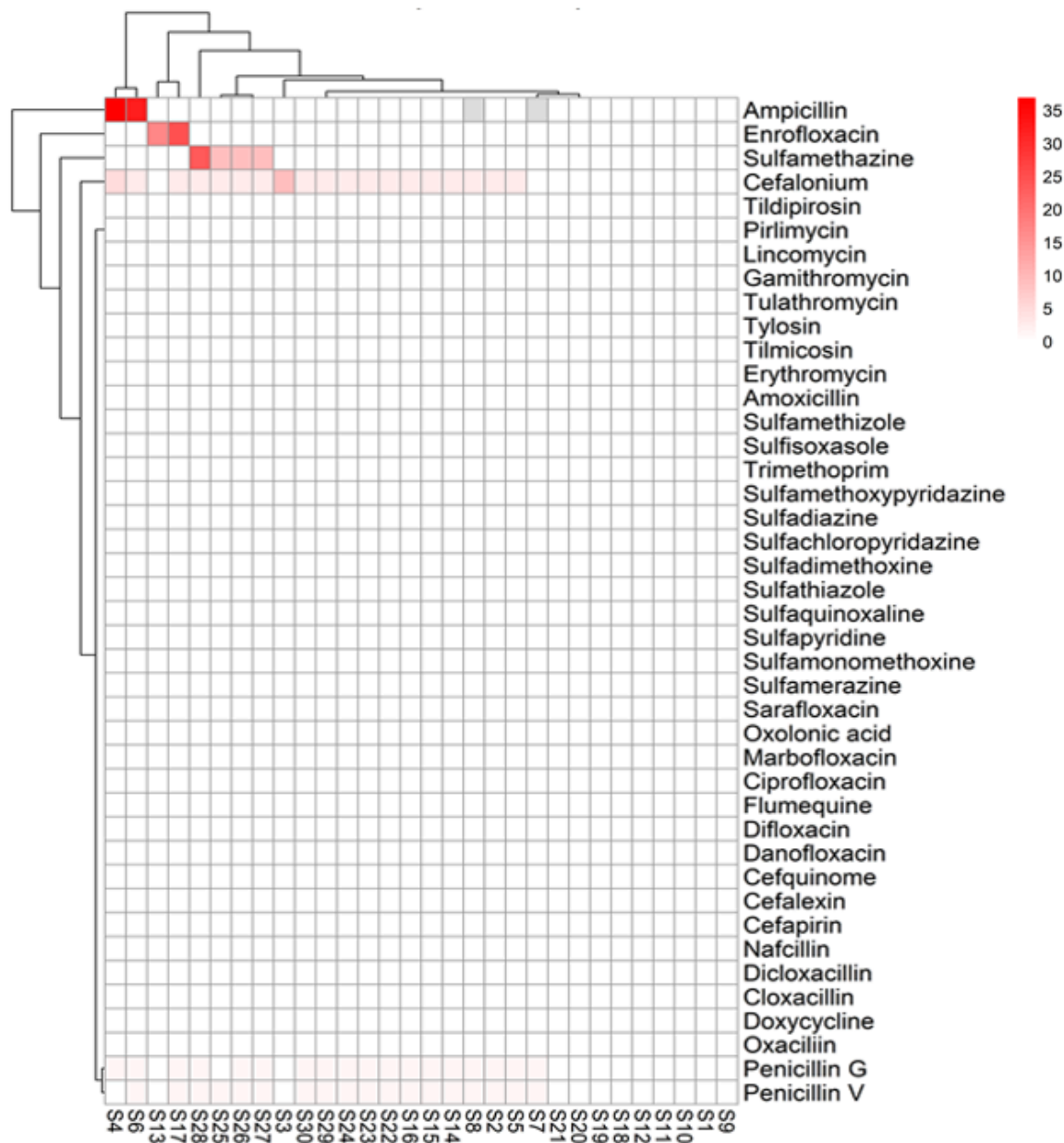


Figure 2

The quantification of antibiotics from 30 tripe samples from the South-East district of Botswana. Ampicillin, enrofloxacin and sulfamethazine were detected from S4, S6, S13, S17 and S28 at concentration levels of 36.9µg/kg, 32.4µg/kg, 17µg/kg, 24.7µg/kg and 23.8µg/kg respectively. The rest of the samples had very little to no antibiotics in them.

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