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DETECTION AND QUANTIFICATION OF ANTIBIOTIC RESIDUES IN BEEF FROM JOS MAIN ABATTOIR, PLATEAU STATE, NIGERIA

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ABSTRACT

The pervasive and often unregulated administration of antibiotics in livestock production is a key driver of antimicrobial resistance and the accumulation of drug residues in animal-derived food products. This phenomenon presents a critical public health challenge, particularly in developing countries such as Nigeria, where regulatory oversight may be limited. In the present study, we aimed to identify and quantify the levels of antibiotic residues in beef sourced from the Jos Main Abattoir, Plateau State, Nigeria. A total of 90 tissue samples,including muscle, liver, and kidney,were systematically collected and analyzed for oxytetracycline and sulfonamide residues using High-Performance Liquid Chromatography (HPLC), a validated, sensitive analytical method. The laboratory analysis revealed that oxytetracycline residues were detected in 5.6% of samples, exclusively within muscle tissue, whereas sulfonamide residues were present in 94.4% of samples across all tissue types. The mean concentrations of sulfonamides were highest in the liver (3,370 ± 110 µg/kg), followed by the kidney (3,160 ± 160 µg/kg) and muscle (2,980 ± 230 µg/kg), respectively; all values substantially surpassed the maximum residue limits established by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO). These findings underscore the widespread and concerning contamination of beef with sulfonamide residues, signaling significant risks to food safety and public health. Addressing this issue requires a multifaceted approach, including the implementation of more robust regulatory frameworks, regular surveillance of antibiotic residues, and the promotion of rational antibiotic use among livestock producers. Strengthening these measures is essential to ensuring the safety of animal-derived foods and safeguarding public health, particularly among vulnerable populations who rely heavily on such products for nutrition.

Keywords: Antibiotic residues, antimicrobial resistance, Withdrawal period, Beef, High-Performance Liquid Chromatography (HPLC)

INTRODUCTION

The rising global demand for animal protein has driven the intensification of livestock production systems, leading to increased reliance on veterinary antibiotics for therapeutic, prophylactic, and growth-promoting purposes (Van *et al*., 2019). In many developing countries, including those in Sub-Saharan Africa, antibiotics such as oxytetracycline and sulfonamides are among the most extensively used in food-producing animals due to their broad-spectrum antimicrobial activity, affordability, and widespread availability (Odey *et al*., 2024). Although these antibiotics play a crucial role in disease management and productivity enhancement, their indiscriminate and excessive use has raised serious pharmacological, toxicological, and public health concerns (Alhassan *et al*., 2025).

One of the most significant consequences of antibiotic misuse in livestock production is the persistence of drug residues in edible animal products, including meat, milk, and eggs (Oladeji *et al*., 2025). Consumption of foods containing sub-therapeutic levels of antibiotic residues may expose consumers to biologically active compounds capable of inducing adverse health effects such as hypersensitivity reactions, gastrointestinal disturbances, hematological disorders, and organ toxicity (Olatoye, 2011; Arsène *et al*., 2022; Bacanlı, 2024). Beyond these direct toxicological effects, prolonged exposure to low concentrations of antibiotic residues has been strongly linked to the emergence and dissemination of antimicrobial resistance (AMR), which represents a major global public health threat (Olatoye *et al*., 2016; Widiasih *et al*., 2024).

The development of AMR poses a serious challenge to both veterinary and human medicine, as the consumption of antibiotic-contaminated meat can facilitate the selection and propagation of resistant bacterial strains, thereby compromising the effectiveness of critically important antimicrobials (Ojo *et al*., 2016; Izah *et al*., 2025). Oxytetracyclines and sulfonamides, in particular, have been frequently implicated in resistance development owing to their extensive and often unregulated use in livestock production systems (Barathe *et al*., 2024; Enshaie *et al*., 2025).

In developing countries such as Nigeria, the misuse of veterinary antibiotics is widespread and is commonly characterized by self-medication, inadequate veterinary supervision, and poor compliance with recommended withdrawal periods (Alabi *et al*., 2025). Withdrawal periods refer to the minimum time required after the last administration of a veterinary drug for its residue concentration in animal tissues to decline below established maximum residue limits (MRLs) (Tang and More, 2023). Failure to observe these withdrawal periods has resulted in the detection of antibiotic residues in meat at concentrations exceeding permissible limits in several Nigerian cities, with reported prevalence rates of 89.3% in Abuja (Omeiza *et al*., 2012), 74% in Maiduguri (Mustapha *et al*., 2023), and 51.8% in Minna (Usman *et al*., 2021). These findings underscore persistent gaps in

veterinary drug regulation, residue surveillance, and enforcement mechanisms within the country.

Despite increasing evidence of antibiotic residue contamination in meat across Nigeria, surveillance data specific to Plateau State, particularly the Jos Main Abattoir, remain limited (Anueyiagu *et al*., 2022; Ndahi *et al*., 2023). The Jos Main Abattoir serves as a major beef distribution hub in North-Central Nigeria, making it a critical point for evaluating consumer exposure to antibiotic residues. Generating residue data from this facility is therefore essential for comprehensive risk assessment, strengthening veterinary public health surveillance, and supporting evidence-based policy formulation in line with international standards set by the World Organization for Animal Health (WOAH).

High-performance liquid chromatography (HPLC) is a reliable and sensitive analytical technique widely employed for the detection and quantification of antibiotic residues in animal tissues (Ye *et al*., 2022). Accordingly, this study aims to determine the concentrations of oxytetracycline and sulfonamide residues in beef samples obtained from the Jos Main Abattoir using HPLC, with the objective of contributing robust data to national residue monitoring programs and informing food safety policies in Nigeria.

METHODOLOGY

Materials and Methods

Study Location

This study was conducted at the Jos main abattoir, situated in Jos North local Government Area (LGA) of Plateau State, located in the North-Central geopolitical zone of Nigeria, located at latitude 9° 48`00N and Longitude 8° 52`00``E, with an area of 510 km² and a population of 1,035,000 at the 2025 populationStatistics. The principal inhabitants of the city are Berom mixed with Hausa, Fulani, Igbo, Yoruba and other tribes on the plateau.

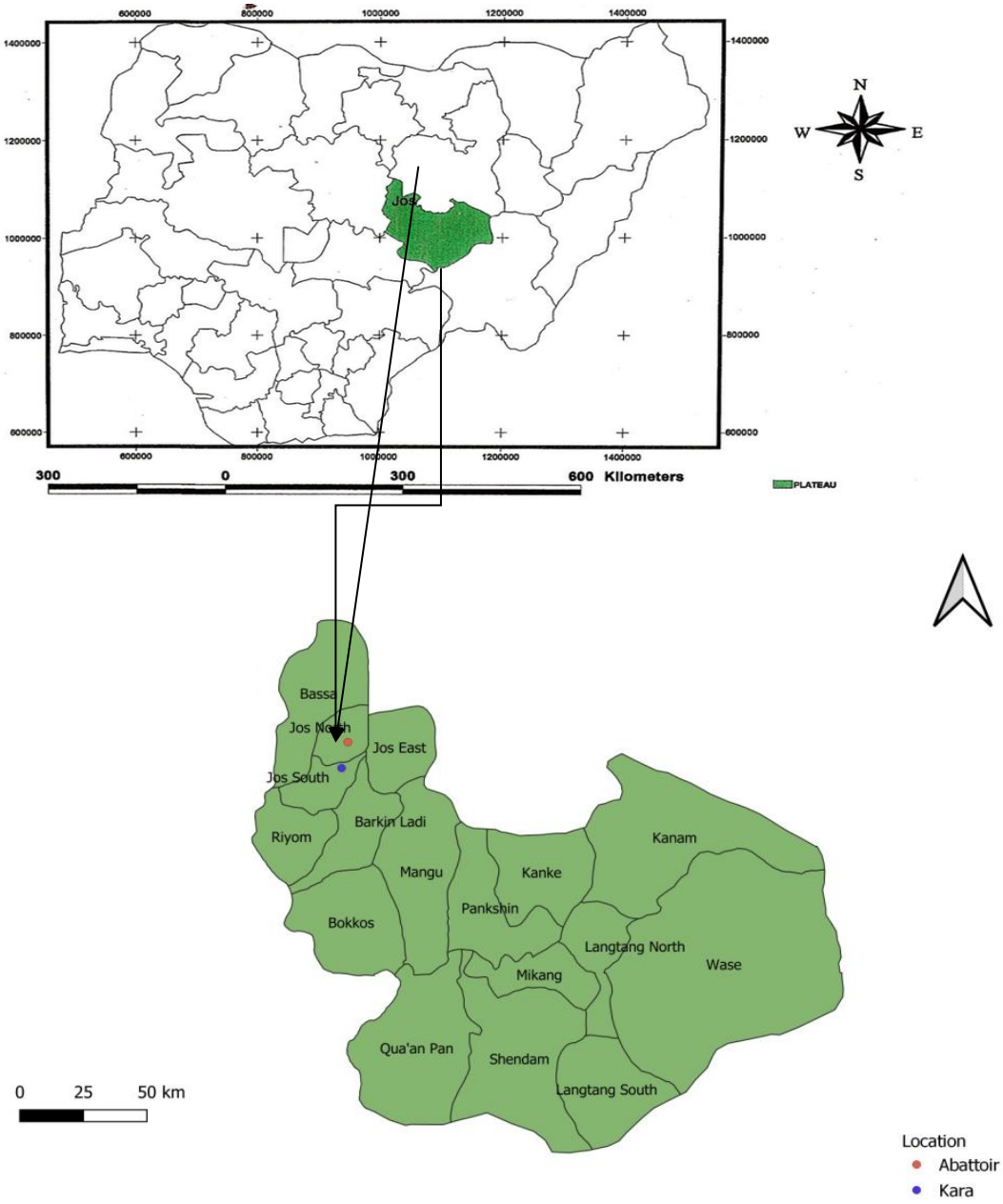

Figure 1. Map of Plateau State showing sampling locations (www.researchgate.net and QGIS)

Study Design

A cross-sectional study design was employed at Jos main abattoir. Sample collection was carried out over 12 months (2023–2024) to capture routine slaughter activities across different times of the year. However, samples were not stratified or analyzed based on seasonal variation

Sample Size Determination

The Thrusfield (2018) method was used to calculate the sample size

$$n = \frac{Z^2 . P(1-P)}{d^2}$$

Where

- n = required sample size
- Z = 1.96 (95% confidence)
- P = expected prevalence
- d= desired absolute precision (10%)
- Expected prevalence (P): 64% (0.64) based on Ezenduka (2019)
- $n = \frac{(1.96)^2 \cdot 0.64(1-0.64)}{(0.10)^2}$

$= 90$

Sampling approach:

- Total samples: 90 tissue samples
- Stratified equally among muscle, liver, kidney (30 each)
- Ensures balanced representation of major edible tissues

Precision achieved: ±10% at 95% confidence

- Sample size is practical for field collection

Ethical Clearance

Ethical approval for this study was obtained from the Ahmadu Bello University Zaria(ABU) Committee on Animal Use and Care (ABUCAUC) with the Approval Number: ABUCAUC/2025/061.

Sample Collection

A total of 90tissues (30 each of Liver, kidney, and muscle) samples were collected from the Jos main abattoir, Plateau State, Nigeria. Each sample was placed into a separate plastic container. The collected samples were stored at -20°C and transferred to ABU Zaria Multi-User laboratory in an ice cooler.

Sample Analysis

Reagents

Analytical grade of the following reagentswas used for the quantification of oxytetracycline and sulfonamide in the tissues: **Acetonitrile, Sodium hydroxide pellets, Methanol, Sodium chloride, Acetone, Sodium citrate,Chloroform,Magnesium sulfateanhydrous**. Certified reference standardsof Oxytetracycline and Sulfonamide were purchased(Sigma Aldrich,United States) and used for calibration curve development and method validation.

Instruments and Equipment

The following instruments and equipment used during the study are: HPLC system model 204 with a UV detector,Column oven Pump, Fluorescence detector, Autosampler vials (HPLC vials the chromatographic separation was performed with a C18 column with the diameter of 150 mm x 4.6 mm Spheris orb 5 ODS-1 (particle size 5m),Vortex mixer, Ultrasonic bath, Analytical balance,Micropipette and tips (10-1000µL), Syringe filters, Centrifuge (5000rmp), Microcentrifuge tubes, Volumetric flask,Beaker and Glass vials.

Preparation of Solutions

Standard stock solutions of oxytetracycline and sulfonamide (100 µg/mL) were prepared usingmethanol andstored at 2 °C until analysis. Firstly, 10–30 mg of each compound was dissolved in a 100 mL volumetric flask with methanol. To prepare mixed working standards, 100 µL of each stock (100 µg/mL) was diluted using 100 mL of methanol, yielding 0.1 µg/mL. Fresh calibration standards were made for each run by fortifying 2.0 g blank beef samples with the 0.1 µg/mL mixed solution. Five

fortification levels (5–500 µg/kg) were prepared. Samples underwent complete Dispersive Liquid-Liquid Micro extraction, and the resulting data generated matrix-matched calibration curves (Snyder *et al*., 2011; Aliu *et al*., 2023).

Sample Treatment

On the day of analysis, refrigerated beef samples were thawed at room temperature. The samples were then homogenized. Accurately weighed portion (2.00 ± 0.005 g) of the homogenized tissue was transferred into a Falcon tube. Six milliliters (6 mL) of water/acetonitrile mixture (5:1, v/v) was added to the homogenized tissue for extraction. A 500 mg salt mixture, consisting of anhydrous magnesium sulfate, sodium chloride, and trisodium citrate dihydrate in the ratio 3:1, .5:0, 5 (w/w) respectively, was added to the tube. The contents were vortexed for 2 minutes. Afterwards, the mixture was centrifuged at 5 °C at 4000 rpm for 5 minutes. The supernatant was carefully collected and subjected to dispersive liquid–liquid microextraction (DLLME) following the method described by Snyder *et al.* (2011) and Aliu*et al.*(2023).

Method Validation

The analytical method was validated in accordance with internationally recognized guidelines for veterinary drug residue analysis, including those of the European Commission and the Codex Alimentarius Commission.

Matrix-matched calibration curves were prepared by fortifying blank beef samples at five concentration levels (5–500 µg/kg). The method demonstrated excellent linearity for both oxytetracycline and sulfonamide residues, with correlation coefficients (R^2) ≥ 0.99.

The limits of detection (LOD) and limits of quantification (LOQ) were established based on signal-to-noise ratios of 3:1 and 10:1, respectively. The LOD values were 4 µg/kg for sulfonamides and 5 µg/kg for oxytetracycline, while the LOQ ranged from 14.3 to 10 µg/kg for both analytes, indicating adequate analytical sensitivity.

Method accuracy was assessed through recovery experiments using blank beef samples spiked at three concentration levels (low, medium, and high; n = 3 per level). Mean recoveries ranged from 90% to 110% for both analytes, meeting acceptable validation criteria.

Precision was evaluated as intra-day repeatability and expressed as relative standard deviation (RSD), with values below 10%, indicating good reproducibility of the method.

Blank (control) samples were analyzed alongside each batch to confirm the absence of interfering peaks at the retention times of the target analytes, thereby ensuring method specificity.

Overall, the validation results confirm that the method is linear, sensitive, accurate, precise, and suitable for the reliable determination of oxytetracycline and sulfonamide residues in beef tissues (Aliu *et al*., 2023).

Dispersive Liquid–Liquid Microextraction (DLLME) Procedure and High-performance Liquid Chromatography (HPLC) Analysis

Tissue samples were first homogenized and then deproteinized using acetonitrile (ACN). For each 1 mL of tissue homogenate, sodium hydroxide (NaOH) and water were added. The mixture was centrifuged, and the resulting supernatant was adjusted to pH 7 with 0.1 M NaOH. Also, a mixture of 1.5 mL methanol (as the disperser solvent) and 250 µL chloroform (as the extraction solvent) was rapidly introduced to the solution, producing a cloudy mixture. This was vortexed and centrifuged at 4000 rpm for 5 minutes. The sedimented phase was then collected, evaporated to dryness, and reconstituted in 100 µL of water. Finally, a 20 µL aliquot of the reconstituted extract was injected into the HPLC system.Chromatographic separation was performed under isocratic conditions with a mobile phase consisting of water, methanol, and acetonitrile (65:25:10, v/v/v) at a flow rate of 1.0 mL/min. The column temperature was set to 30 °C, and the injection volume was 10 µL for both standards and samples. These optimized analytical conditions facilitated precise separation and quantification of oxytetracycline and sulfonamide residues in tissue matrices, following established protocols (Snyder *et al.,* 2011; Aliu *et al*., 2023).

Data Presentation and Analysis

Data were analyzed using one-way analysis of variance (ANOVA) to compare mean antibiotic residue concentrations among different tissue types (muscle, liver, and kidney). Where significant differences were observed, Statistical significance was set at $p < 0.05$. All analyses were conducted using IBM SPSS Statistics Version 27 (IBM Corp., 2020).

RESULTS

Antibiotic Residue in Tissues of Slaughtered Cattle in Jos Main Abattoir, Plateau State, Nigeria

Table 1 shows the frequency of oxytetracycline and sulfonamide drug residues in muscle, kidney, and liver tissues from cattle slaughtered at the Jos main abattoir, Plateau State, Nigeria. Of 90 tissue samples examined, 5 (5.6%) were positive for oxytetracycline, and 85 (94.4%) tested positive for sulfonamide drug residues. Of 30 muscle samples analyzed, 5 (16.7%) tested positive for oxytetracycline residues, and 25 (83.3%) were positive for sulfonamide drug residues. Oxytetracycline residues were not detected in any kidney or liver samples (0.0%), whereas sulfonamide drug residues were found in all kidney and liver samples (100.0%).

Table 1: **Antibiotic residues in tissues of cattle slaughtered at the Jos main abattoir, Plateau State, Nigeria**

Tissue	Number tested	Number positive for oxytetracycline residue (%)	Number positive for sulfonamide drug residues (%)
Muscle	30	5 (16.7)	25 (83.3)
Kidney	30	ND	30 (100.0)
Liver	30	ND	30 (100.0)
Total	90	5 (5.6)	85 (94.4)

ND: Not detected

Antibiotic Residue Concentrations in Tissues of Slaughtered Cattle

Table 2 presents the mean concentrations and range values of oxytetracycline and sulfonamides drug residues in the muscle, kidney, and liver tissues of cattle slaughtered at the Jos main abattoir, Plateau State, Nigeria. Oxytetracycline residues were only detected in the muscle tissue, with a mean concentration of 2,350 ± 470 µg/kg, which ranges between 1,136 and 3.756 µg/kg. Oxytetracycline residues were not detected in the kidney or liver samples.

However, sulfonamide drug residues were detected in all three tissues examined. The highest mean concentration was recorded in the liver (3.370 ± 110 µg/kg), followed by the kidney (3.160 ± 160µg/kg) and the muscle (2,980 ± 230 µg/kg), respectively. The corresponding ranges for sulfonamide drug concentrations were 2,199–4.390 µg/kg (liver), 1,234–4,205 µg/kg (kidney), and 1,196–6,046 µg/kg (muscle), respectively.There was a statistically significant difference in mean sulfonamide concentrations among the examined tissues ($p < 0.05$), with liver tissues showing higher residue levels compared to kidney and muscle.

Table 2: Concentrations of Antibiotic Residues in Cattle Tissues from Jos Main Abattoir Compared with Codex (FAO/WHO) and EU Maximum Residue Limits (MRLs)

Tissue	Oxytetracycline (µg/kg) Mean ± SD	Range (µg/kg)	Sulfonamides (µg/kg) Mean ± SD	Range (µg/kg)	MRL (µg/kg) Oxytetracycline (Codex/EU)	MRL (µg/kg) Sulfonamides (Codex/EU)
Muscle	2,350 ± 470	1,136 – 3,756	2,980 ± 230	1,196 – 6,046	200	100–200
Kidney	–	–	3,160 ± 160	1,234 – 4,205	1,200	100–200
Liver	–	–	3,370 ± 110	2,199 – 4,390	600	100–200

DISCUSSION

The present study revealed an exceptionally high prevalence of sulfonamide residues in tissues of slaughtered cattle at the Jos Main Abattoir, with 94.4% of samples testing positive. This finding strongly suggests widespread non-compliance with recommended antibiotic withdrawal periods, which are essential for ensuring that residue levels in edible tissues remain below established safety limits (Canton *et al*., 2021; Khalifa *et al*., 2024). The consistent detection of sulfonamides across muscle, liver, and kidney tissues indicates extensive and indiscriminate use of this class of antimicrobials within cattle production systems in the study area.

These findings are consistent with reports from other parts of Northern Nigeria, where high prevalence of sulfonamide residues has been documented in beef samples from Abuja, Kano, and Benue States (Omeiza *et al*., 2012; Lateefat *et al*., 2022; Onminiyi *et al*., 2023). Similar observations have been reported internationally, where sulfonamide residues persist in beef even after cooking (Sarkar *et al*., 2024). The agreement between these studies and the present findings highlights persistent deficiencies in antimicrobial stewardship, regulatory enforcement, and veterinary oversight across the region.

In contrast, oxytetracycline residues were detected only in muscle tissues, with a relatively low prevalence of 16.7%, and were absent in liver and kidney samples. This tissue-specific distribution can be explained by the pharmacokinetic characteristics of oxytetracycline. Following administration, the drug is widely distributed; however, it exhibits a strong affinity for binding to muscle proteins and divalent cations such as calcium, which facilitates its persistence in muscle tissue (Adegbeye *et al*., 2024).

The absence of detectable oxytetracycline residues in liver and kidney tissues may be attributed to rapid hepatic metabolism and renal excretion, leading to a faster decline of

residue concentrations in these highly perfused and metabolically active organs. Consequently, residue levels in liver and kidney may fall below the analytical detection limits at the time of slaughter, particularly where withdrawal periods are partially observed (Chughtai *et al*., 2026; Mohammed *et al.*, 2022). In contrast, persistence in muscle tissue may occur due to slower depletion rates and stronger tissue binding. Variations in residue distribution may also be influenced by differences in dosing regimens, timing of drug administration before slaughter, and individual animal physiological status (Poapolathep *et al*., 2008). Similar distribution patterns have been reported in Jos and Minna (Usman *et al*., 2021; Anueyiagu *et al*., 2022), as well as in Eastern Ethiopia, where tetracycline residues were predominantly detected in muscle tissues of slaughtered cattle (Mohammed *et al*., 2022).

Despite its lower prevalence, the mean concentration of oxytetracycline detected in muscle tissue (2,350 ± 470 µg/kg) far exceeded the Codex Alimentarius maximum residue limit of 200 µg/kg (FAO/WHO, 2024). Comparable exceedances reported in Maiduguri and Ibadan (Olatoye*et al*., 2016; Mustapha *et al*., 2023) suggest that violations of oxytetracycline residue limits are widespread across Nigeria.

The markedly higher prevalence and concentrations of sulfonamide residues observed in this study, compared with oxytetracycline, may be attributed to their longer biological half-lives and prolonged persistence in animal tissues, as well as their frequent use as feed or drinking-water additives for disease prevention and growth promotion. Sulfonamides are relatively inexpensive, widely accessible, and often administered without a veterinary prescription, thereby increasing the likelihood of misuse and poor adherence to withdrawal periods (Reinhart and Prescott, 2024). The higher concentrations detected in the liver and kidney further reflect the central roles of these organs in drug metabolism and excretion (Shroff *et al*., 2023).

From a public health perspective, the detection of elevated concentrations of sulfonamide and oxytetracycline residues in beef intended for human consumption poses significant risks, including disruption of gut microbiota, hypersensitivity reactions, and the selection and dissemination of antimicrobial-resistant bacteria (Santacroce *et al*., 2023; Oladeji *et al*., 2025). Overall, these findings confirm that antibiotic residue contamination of beef remains a significant food safety concern in Nigeria. However, given that sampling was limited to a single abattoir, caution should be exercised in generalizing these results to the entire Plateau State. Nevertheless, the results provide important baseline data and underscore the urgent need for strengthened regulatory enforcement, routine residue surveillance, improved veterinary oversight, and targeted education of livestock producers and abattoir workers on responsible antibiotic use and strict adherence to withdrawal periods.

This study revealed a high prevalence of antibiotic residues in beef obtained from the Jos Main Abattoir, Plateau State, Nigeria. Sulfonamide residues were detected in 94.4% of the samples, with mean concentrations of 3,370 ± 110 µg/kg in liver, 3,160 ± 160 µg/kg in kidney, and 2,980 ± 230 µg/kg in muscle. These values were all above the established maximum residue limits. Oxytetracycline

residues were detected in 16.7% of muscle samples at a mean concentration of 2,350 ± 470 µg/kg, which also exceeded the recommended regulatory limit of 200 µg/kg.

The distribution pattern observed showed higher sulfonamide concentrations in the liver compared to the kidney and muscle, indicating tissue-dependent accumulation of residues. Oxytetracycline was not detected in liver and kidney samples, suggesting possible rapid metabolism and elimination or depletion below detectable levels at the time of slaughter.

Significant variation in sulfonamide concentrations across tissues was observed, with the liver showing the highest residue levels. This reflects differential drug distribution and accumulation among edible tissues. The analytical method employed demonstrated acceptable validation parameters, including linearity, accuracy, precision, and sensitivity, confirming the reliability of the results.

However, the study was limited by sampling from a single abattoir, which may restrict broader extrapolation of the findings to the entire study area.

In conclusion, the findings indicate widespread occurrence of antibiotic residues in beef, suggesting non-compliance with recommended withdrawal periods and possible indiscriminate use of veterinary antibiotics. This poses apotential public health concern due to risks of antimicrobial resistance and consumer exposure to unsafe residue levels.

Recommendation

Strengthening regulatory enforcement and antimicrobial control Veterinary antibiotic distribution and use should be strictly regulated through prescription-only access, with effective enforcement of withdrawal periods in livestock production systems. Implementation of routine residue monitoring programs, National and state-level surveillance systems should be established to routinely monitor antibiotic residues in meat using validated analytical methods such as HPLC, in line with internationally accepted validation standards. Expansion of sampling scope and representativeness. Future studies should include multiple abattoirs across Plateau State and incorporate seasonal sampling to improve the spatial and temporal representativeness of residue data. Improved veterinary oversight and farmer compliance Veterinary supervision of antibiotic administration in food animals should be strengthened to minimize indiscriminate use and ensure adherence to recommended withdrawal periods. Capacity building and stakeholder education- Targeted training and awareness programs should be implemented for livestock farmers, traders, and abattoir workers on antimicrobial stewardship, food safety risks, and the public health implications of antibiotic residues in food animals.

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