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RAO designed the study, performed statistical analysis. IL help with some literature, ONO and IL wrote and proofread the paper for publication. All authors read and approved the final manuscript.

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Assessment of Antibiotic Residues in Goat Meat from Slaughter Houses in Benue State

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Abstract:

The widespread use of antibiotics in livestock farming has raised concerns about antibiotic residues in meat, posing risks such as antimicrobial resistance (AMR), allergic reactions, and toxicity in consumers. This study investigates the incidence of tetracycline, oxytetracycline, chlortetracycline, and penicillin residues in goat meat from slaughterhouses in Benue State, Nigeria. A total of 117 samples were collected from slaughterhouses across three senatorial zones in Benue State. Kidney, liver, and muscle tissues were randomly sampled and preserved under appropriate conditions for laboratory analysis. The goat meat samples underwent microbiological analysis followed by high-performance liquid chromatography (HPLC) for precise quantification of tetracycline, oxytetracycline, chlortetracycline, and penicillin residues. The results revealed that chlortetracycline and penicillin residues were present above the Codex Maximum Residue Limits (MRL) in all tested organs. The highest mean concentration of antibiotic residue was detected in the liver (1694±390.9 µg/kg), followed by the kidney (1435±624.2 µg/kg) and muscle (339.8±110.7 µg/kg). The presence of penicillin in muscle tissue was particularly concerning, exceeding the permissible limit by up to 19 times. Hazard quotient calculations indicated that while some antibiotic residues were within acceptable limits, prolonged consumption of contaminated meat poses potential health risks. The study confirms the presence of antibiotic residues in goat meat, with certain residues exceeding permissible limits, posing risks to human health. To mitigate the risks associated with antibiotic residues in meat, the study recommends strict regulatory monitoring of antibiotic use in livestock farming, routine screening of meat products, and public awareness campaigns on the dangers of consuming contaminated meat.

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INTRODUCTION

Antibiotics play very important roles in livestock farming by preventing and treating bacterial infections, thereby improving animal health and increasing meat production efficiency (Iqbal and Ashraf, 2020). However, the excessive and improper use of antibiotics in food-producing animals has led to growing concerns regarding antibiotic residues in meat and their associated health risks to consumers (Ashraf *et al.,* 2020; Zhang *et al*., 2022). Residual antibiotics in meat beyond permissible limits pose significant threats, including antimicrobial resistance (AMR), hypersensitivity reactions, toxicity, and disruption of gut microbiota in humans (Sharma *et al*., 2024; Shawish *et al*., 2020).

Goats, like other livestock, are susceptible to bacterial infections such as pneumonia, mastitis, and gastrointestinal disorders. Antibiotics and anthelmintic drugs are commonly administered to treat these infections, ensuring optimal health and productivity (Muhammad *et al.,* 2015). However, the indiscriminate use of these drugs without adherence to withdrawal periods can result in antibiotic residues in meat, posing health hazards to consumers (Herawati *et al*., 2023).

Antibiotic residues are traces of antimicrobial compounds that remain in animal tissues after treatment. Consumption of meat containing antibiotic residues above permissible limits can contribute to antimicrobial resistance (AMR), allergic reactions, and toxicity in humans (Canton *et al*., 2021; Saleem *et al.,* 2020). Regulatory bodies such as the Codex Alimentarius Commission have established Maximum Residue Limits (MRLs) to ensure food safety, but non-compliance remains a challenge in many regions, including Nigeria (Delatour *et al*., 2018).

High-Performance Liquid Chromatography (HPLC) is a widely employed analytical technique for detecting and quantifying antibiotic residues in food products due to its high sensitivity, precision, and reliability (Gupta *et al*., 2022; Patel *et al*., 2024). Additionally, agar gel method, including agar well diffusion and agar gel precipitation techniques, is a microbiological approach used to detect antibiotic residues in meat samples. These methods rely on bacterial growth inhibition to indicate the presence of antimicrobial compounds, making them effective for preliminary screening before confirmatory tests like HPLC (Valgas *et al*., 2007).

Nigeria, including Benue State, is a major producer and consumer of goat meat. However, studies have reported varying levels of antibiotic residues in locally sourced meat, raising concerns about food safety and public health risks. Factors such as inadequate regulation, lack of awareness among farmers, and improper withdrawal periods contribute to the presence of antibiotic residues in goat meat (Oloso *et al*., 2018; Njoga *et al*., 2021). Assessing antibiotic contamination in goat meat from slaughterhouses in Benue State is essential for addressing these concerns and ensuring compliance with food safety standards. The aim of this study was to investigate the occurrence of tetracycline, oxytetracycline, chlortetracycline, and penicillin residues in goat meat from slaughter houses in Benue State, Nigeria.

MATERIALS AND METHODS

Sample collection

Two slaughter slabs in each of the three health zones, zone A (Katsina-Ala and Adikpo), zone B (Wadata and Wurukum in Makurdi town and Gboko), and zone C (Otukpo and Ugbokolo) of Benue state were visited and 50g samples of kidney, liver, and muscle were taken from every goat slaughtered for human consumption.

The systematic random sampling method was used to collect around 117 samples during the dry season (October to March 2018 and October to March 2019). At least 18 samples were taken from each collecting location, which was visited once every two weeks. Separate samples were taken in sterile polyethene bags, stored in ice packs, and transported to the lab for extraction. Samples were stored in a freezer at -4°C until processing was completed after extraction.

Screening for antibiotic residues in goat meat

Microbiological method (four-plate test)

This is a formal procedure for examining meat from livestock. It uses agar gel diffusion to reveal residues of antibiotic substances (Creff-Froger, 2002).

Drug residue confirmation by a quantitative method (HPLC)

Positive samples of certain residues were identified and quantified using a physical/chemical method: high-performance liquid chromatography (HPLC).

Sample extraction

Samples were extracted in accordance with the previous research (Abasi *et al.*, 2009; Ezenduka and Ugwumba, 2012; McDonald and Bouvier, 2001). The samples were chopped into small pieces and blended with a basic kitchen blender to prevent sample mix-up and potential drug residue transfer. The blender was cleaned with 70% alcohol and rinsed with distilled water after each blending. 2.5 grams were weighed into a set of centrifuge tubes and 1.8 ml of 5N hydrochloric acid was dispensed and thoroughly mixed. The McIlvain solution, which was made with 1.97 grams of phosphate buffer (KH_2PO_4), 2.8 millilitres of sodium hydroxide (NaOH), and 5M sodium metabisulphite $Na_2S_2O_5$ in 200 millilitres of water, resulting in a pH of 7.6. The samples were then properly covered, shaken for fifteen minutes, and centrifuged for twenty minutes at 4500 rpm. The samples were then properly covered, shaken for fifteen minutes, and centrifuged for twenty minutes at 4500 rpm. For the HPLC analysis, the supernatant was collected and passed through 0.45µm filter paper into plastic vials.

Preparation of standard curves

The concentration of each standard was determined by creating a calibration curve with five (5) point levels and plotting a graph with 99.9% accuracy (McDonald and Bouvier, 2001). Standard solutions were made by dissolving 25 mg of each reference standard of Tetracycline, Oxytetracycline, Chlortetracycline, and Penicillin (obtained from Sigma Aldrich) in a mixture of methanol, acetonitrile, and hydrochloric acid in a ratio of 10:20:70, v/v. A 25 mg reference standard was used as the stock in a 25 ml volumetric flask, and then 1 mg of dissolved reference standard was diluted from stock up to 10 ml using the mobile phase, 2 mg of dissolved reference standard was diluted up to 10 ml, 3 mg of dissolved reference standard was diluted up to 10 ml, 4 mg of dissolved reference standard was diluted up to 10 ml, and 5 mg of dissolved reference standard was made up to 10 ml. These concentrations were then injected into the HPLC machine and used to create standard curves, with the peak areas plotted against the corresponding concentrations and the best line of fit plotted using Microsoft Excel programs.

High performance liquid chromatography (HPLC) system and procedure

Tetracycline, oxytetracycline, chlortetracycline, and penicillin residues were analyzed and quantified at the National Agency for Food and Drug Administration (NAFDAC) Zonal office Agulu, Anambra state, using an HPLC KNAUER system (HITACHI, Japan) that has a quaternary pump (K-1000), a BIOTECH model 2003 degasser, a Spark Triathlon autosampler, and an RF-551 fluorescence detector. Chromgate V3.1 software was used to process the data. The mobile phase was a mixture of methanol, acetonitrile, and 50mol hydrochloric acid 10:20:70 v/v. The prepared mobile phase was filtered through a 0.45µ filter paper using a vacuum pump, and it was then degassed by sonication for five minutes prior to application. The detection was conducted using 365 nm as the excitation and emission wavelength. The analyte was eluted with a Phenomenex Luna C-18 column (Torrance, CA, USA) with a particle size of 5µm; 4.6mm x 250. The machine was flushed with blank methanol at regular intervals, and the mobile phase was allowed to run through the machine for equilibration and conditioning, during which a stable baseline was obtained on the recorder monitor. The column and tubing were frequently checked to ensure that there is no leakage. The machine instructed the user to inject 20 microliters (20µl) of analyte

from each sample into the column while it was "waiting for pulse injection." The antibiotic was eluted on the C-18 column, and resolution occurred in the detector, resulting in peaks (chromatographs) displayed on the monitor, with the peak areas and retention times recorded by the computer. According to the results of spiking the reference standards, the flow rate was 1.0 ml/min, and the retention times were 3.0-8.0 for tetracyclines and 4.0-5.0 for penicillin (Abasi *et al.,* 2009).

Standard Curve for Tetracycline

The linear regression equation was used to plot the standard curve (Figure 1) based on the findings of the standard concentrations and peak regions.

The results of the standard concentrations and the peak areas were plotted as the standard curve (figure 2) using linear regression equation.

$Y = a + b X$ ---------- 1

Y = peak area (cm^2), a = Y-intercept, b = the slope,

X = conc. of tetracycline, y = 1.2285+008x + 0.0000

and the goodness of fit (R^2) value of 0.9716.

The R^2 value > 0.9 revealed the linearity. The detection limit for tetracycline was 0.01ppm while the retention time spanned from 4.4 to 5.8 minutes with the highest retention time being 4.7 minutes.

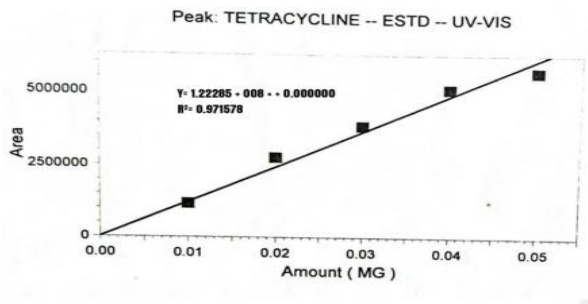

Fig. 1. Standard curve for tetracycline.

Standard Curve for Oxytetracycline

For oxytetracycline, y = 1.21411 +008x + 0.0000 and the goodness of fit (R^2) value of 0.998835. The R^2 value > 0.9 revealed the linearity. The detection limit for oxytetracycline was 0.01ppm while the retention time spanned from 4.0 to 5.0 minutes with the highest retention time being 4.2 minutes (Figure 2).

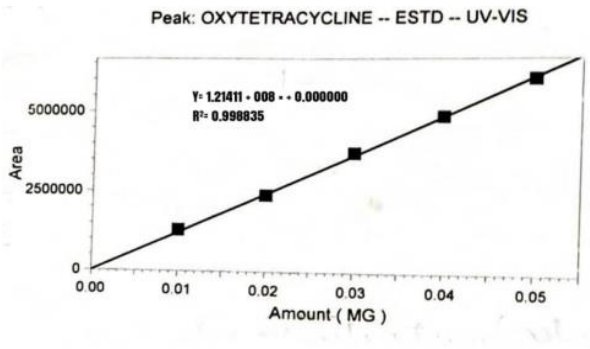

Fig. 2. Standard curve for Oxytetracycline.

Standard Curve for Chlortetracycline

For chlortetracycline, y = 1.2584 +008x + 0.0000 and the goodness of fit (R^2) value of 0.979616. The R^2 value > 0.9 revealed the linearity. The detection limit for chlortetracycline was 0.1mg while the retention time spanned from 7.0 to 8.0 minutes with the highest retention time being 7.9 minutes (Figure 3).

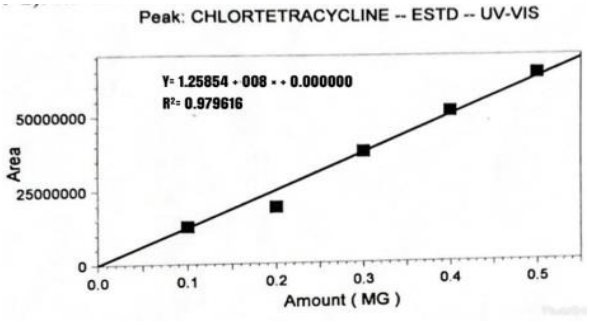

Fig. 3. Standard curve for chlortetracycline.

Standard Curve for Penicillin

For penicillin, y = 1.61380 +007x + 0.0000 and the goodness of fit (R^2) value of 1.00000. The R^2 value > 0.9 revealed the linearity. The detection limit for penicillin was 0.1mg while the retention time spanned from 4.3 to 5.5 minutes with the highest retention time being 7.9 minutes (Figure 4).

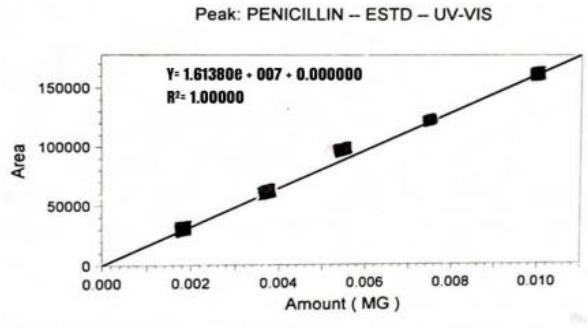

Fig. 4. Standard curve for Penicillin.

Statistical Analysis

The screening test was analysed using percentages and Analysis of Variance (ANOVA). The mean antibiotic concentrations in the various organs were calculated using Excel and compared using ANOVA. Based on statistically significant results, the Tukeys multiple comparison test was performed using Graph Pad Prism 5.0 (Graph Pad software inc., San Diego, CA) at a 95% confidence level (p=0.05).

RESULTS

Incidence of antibiotics among organs of goats in Benue state

From the screening test by agar gel diffusion, a total of 42 among 117 goat samples were positive for antibiotic residues giving a prevalence of 35.9%. Among the positive samples, 16 were kidney, 17 liver and 9 muscle samples (Figure 5). Sixteen positive samples were acquired from Zone A, 10 from Zone B and 16 from Zone C (Figure 6).

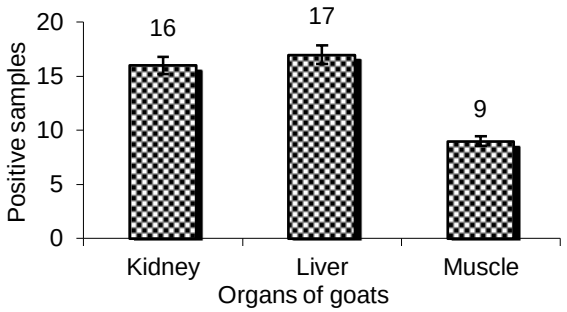

Fig. 5. Incidence of antibiotics among organs of goats in Benue state.

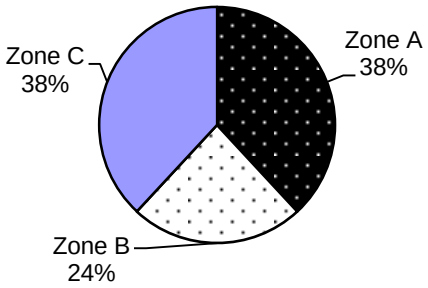

Fig. 6. Incidence of antibiotics among organs of goats in different zones of Benue state.

Concentration of antibiotics in organs of goats in Benue state based on HPLC

These 42 samples were confirmed by High performance liquid chromatography. The mean concentration of tetracycline in kidney, liver and muscle of goat was 61.5±27.3µg/kg, 69.4±33.0µg/kg and 56.7±21.5µg/kg respectively. The mean concentration of oxytetracycline in kidney, liver and muscle of goat was 56.9±19.4µg/kg, 20.1±7.5µg/kg and 26.7±7.5 µg/kg respectively. The mean concentrations were not significantly different (p=0.120). The mean concentration of chlortetracycline in kidney, liver and muscle of goat was 1435±624.2µg/kg, 1694±390.9µg/kg and 339.8±110.7µg/kg respectively. The mean concentrations were not significantly different (p=0.124), while that of penicillin was 729.9±352.8µg/kg, 325.3±118.9µg/kg and 481.0±264.7µg/kg for kidney, liver and muscle respectively. The mean concentrations were also not significantly different (p=0.540) (Table 1).

Comparison of antibiotics residue between different organs of goats in Benue State by HPLC

The mean concentrations of antibiotic residues in organs of goats in Benue State by HPLC are presented in table 2. The concentrations of tetracycline, oxytetracycline, chlortetracycline and penicillin residues in the kidney of goat were 61.5±27.3 µg/kg, 56.9±19.4 µg/kg, 1435±624.2 µg/kg and 729.9±352.8 µg/kg respectively. These mean concentrations were found to be statistically significantly different. The difference lied with the concentrations of chlortetracycline and penicillin which were found to be higher (p=0.0252). The concentrations of tetracycline, oxytetracycline, chlortetracycline and penicillin residues in the liver of goat were 69.4±33.02 µg/kg, 20.1±7.5µg/kg, 1694±390.9µg/kg and 325.3±118.9µg/kg respectively. These mean concentrations were found to be statistically significantly different. The difference lied with the concentrations of chlortetracycline and penicillin which were found to be higher (p=0.0001). In the muscles of goats, the mean concentrations of tetracycline, oxytetracycline, chlortetracycline and penicillin residues were 56.7±21.5µg/kg, 26.7±6.5µg/kg, 339.8±110.7 µg/kg and 481±264.7µg/kg respectively. The mean concentrations were not statistically significantly different (p=0.0604).

Table 1: Mean Concentration (µg/kg) of antibiotics residues in goat organs slaughtered in Benue State.

Organs	Concentration of antibiotics (µg/kg)			
	Tetracycline	**Oxytetracycline**	**Chlortetracycline**	**Penicillin**
Kidney	61.5±27.3	56.9±19.4	1435±624.2	729.9±352.8
Liver	69.4±33.0	20.1±7.5	1694±390.9	325.3±118.9
Muscle	56.7±21.5	26.7±6.5	339.8±110.7	481.0±264.7
P-value	0.952	0.120	0.124	0.540

Table 2. Comparison of antibiotics residue between different organs of goats in Benue State by HPLC.

Organs	Concentration of antibiotics (µg/kg)				p-value
	Tetracycline	**Oxytetracycline**	**Chlortetracycline**	**Penicillin**	
Kidney	61.5±27.3	56.9±19.4	1435±624.2	729.9±352.8	0.0252
Liver	69.4±33.0	20.1±7.5	1694±390.9	325.3±118.9	0.0001
Muscle	56.7±21.5	26.7±6.5	339.8±110.7	481.0±264.7	0.0604

Antibiotic residues above CODEX maximum limit (MRL) in goats

Chlortetracycline and penicillin had residues which were above maximum residue limits in all the organs of goats. Penicillin had residues above maximum residue limit more than chlortetracycline with a mean concentration of 949.8 ± 423.9µg/kg in the muscle (19 times above MRL) (Table 3).

Table 3. Mean concentration of antibiotic residues in goats above maximum residue limit (MRL).

Organs	Kidney		Liver		Muscle		p-value
Residues	**CLTC**	**PCN**	**CLTC**	**PCN**	**CLTC**	**PCN**	
n	4	12	9	8	5	4	
mean±SEM	4374 ± 1669	906.3±429	2571394.3	502.6±185	1094±491	949.8±424	0.0007
CODEX MRL (µg/kg)	1200	50	600	50	200	50	

CLTC: chlortetracycline, PCN: Penicillin, n: Number of samples.

DISCUSSION

Forty two samples from goats were positive for the presence of antibiotic residues, 16 positive samples were acquired from Zone A, 10 from Zone B and 16 from Zone C. These samples were confirmed by High performance liquid chromatography. This indicates that some of the meat sold for human consumption in Benue State contains antibiotic residues. This result is comparable to that obtained by Mmbando (2004), who in a study analyzed muscle tissues from cattle for the presence of tetracycline in Morogoro and Dodoma Municipalities, Tanzania and got a prevalence of 41.2%. Other works have also observed residues of drugs in meat samples and have attributed the presence of these residues to lack of observation of withdrawal period and misuse of veterinary drugs in farm animals (Falowo and Akimoladun, 2019).

Various samples have been collected by researchers reporting on antibiotic residues in animals: Njoga (2012) collected a total of 285 samples of samples of cattle and pigs and determined antibiotic residues using premi test. Awenela, 2014 collected 60 samples of beef and mutton from the Kumasi metropolitan area of Ghana and determined the residues antibiotics using HPLC. The HPLC method is widely used for determination of antibiotic residues in animal tissues.

The mean concentration of tetracycline residues in the liver of goats was found to be higher than the other antibiotic residues but this difference was not significant. This is probably because farmers do not adhere to the withdrawal period of the antibiotic (Bahmani *et al*., 2019).

The mean concentration of oxytetracycline in the kidney of goats was higher than that of liver and muscle. However, the mean concentrations were not significantly different. The mean concentration of chlortetracycline in the liver of goats was high. However, this mean concentration was not statistically significant. The mean concentration of penicillin residues in the kidney of goats was higher than that of liver and muscle. There was no significant difference between the means. The reason for this may be the illegitimate use of penicillin in goats. The mean concentrations of tetracycline, oxytetracycline, chlortetracycline and penicillin were significantly different in the kidney of goats, the difference which was in the concentration of chlortetracycline and penicillin may have arisen due to the continuous use of antibiotics without observing withdrawal period.

The mean concentrations of tetracycline, oxytetracycline, chlortetracycline and penicillin were significantly different in the liver of goats. The mean concentration of chlortetracycline was highest among the antibiotics tested for. This high concentration could have arisen from the unwanton use of antibiotics. The mean concentrations of tetracycline, oxytetracycline, chlortetracycline and penicillin were significantly different in the muscle of goats. The mean concentration of penicillin was the highest. Although, this mean concentration was not statistically significant. In summary, the highest mean concentration of antibiotic residue in goats was found to be chlortetracycline (1694±390.9µg/kg), in the liver of goats, with a CODEX MRL of 600µg/kg. However, penicillin was found to have the highest residues above MRL (949.8±424µg/kg) in the muscle of goats with CODEX MRL of 50µg/kg. Therefore, it is advised to adopt all personal and general hygiene standards in order to reduce the risk of raw meat contamination (Iqbal and Iqbal, 2020). Regulatory bodies should ensure proper meat inspection and drug residues surveillance programs should be established in Benue state to ensure food safety.

CONCLUSION

This study demonstrated the presence of antibiotic residues in goat meat samples collected from slaughter slabs in Benue State, Nigeria. The results indicated that chlortetracycline and penicillin residues exceed the Codex Maximum Residue Limits (MRL), particularly in liver and muscle tissues. The high concentrations of these residues raise concerns regarding consumer health risks, including antimicrobial resistance, allergic reactions, and potential toxicity. The findings suggest that the

indiscriminate use of antibiotics in livestock, coupled with the failure to observe withdrawal periods, contributes significantly to the presence of these residues in goat meat. While some antibiotic levels were within acceptable limits, the detection of high concentrations of penicillin and chlortetracycline necessitates urgent intervention to regulate antibiotic use in animal husbandry.

To ensure food safety, routine surveillance of antibiotic residues in meat and stricter enforcement of withdrawal periods must be implemented. Additionally, veterinary authorities should improve farmer education on responsible antibiotic use, proper drug administration, and adherence to food safety standards. Failure to take immediate action may exacerbate antimicrobial resistance and compromise public health.

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CONFLICT OF INTEREST

The authors hereby state that they do not have any conflicts of interest to declare.

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