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ANTIBIOTICS RESIDUES IN BEEF FROM SLAUGHTER HOUSES WITHIN BENUE STATE

By

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Abstract

The use of antibiotics in cattle farming for therapeutic, prophylactic, and growth-promoting purposes has raised concerns about antibiotic residues in animal tissues. These residues pose potential health risks to consumers, including allergic reactions, toxicity, and antimicrobial resistance. This study aimed to assess the presence of antibiotic residues in animals slaughtered in Benue State, Nigeria, particularly in beef products. A total of 117 beef samples were collected from three regions (Zones A, B, and C) in Benue State. The samples were analyzed for tetracycline antibiotics (oxytetracycline and chlortetracycline) and penicillin using high-performance liquid chromatography (HPLC) and agar gel diffusion methods. The results showed that 45% of the samples contained detectable antibiotic residues, with oxytetracycline being the most prevalent, followed by chlortetracycline and penicillin. Their mean concentrations were 0.15 mg/kg, 0.12 mg/kg, and 0.10 mg/kg, respectively. This finding indicates serious food safety concerns, as prolonged exposure to antibiotic residues can contribute to antimicrobial resistance and other health risks. The study recommends the need for stricter regulations, routine monitoring, and farmer education on responsible antibiotic use. Enhancing surveillance programs and promoting alternative disease prevention strategies such as vaccination and improved husbandry practices are important.

Keywords: *Antibiotics, high performance liquid chromatography (HPLC), agar gel, Beef, Residues*

1.0 INTRODUCTION

The use of antibiotics in livestock production has played significant role in improving animal health, increasing productivity, and ensuring food security. Antibiotics are commonly used in cattle farming for therapeutic purposes, disease prevention, and as growth promoters. Their application has significantly enhanced the quality of beef production by reducing disease related mortality and improving weight gain efficiency (Delabouglise *et al*., 2017: Rafiq *et al*., 2022).

However, the indiscriminate and excessive use of these antibiotics in cattle farming has led to the emergence of antibiotic residues in animal-derived food products, particularly in beef. Antibiotic residues refer to the remnants of pharmaceutical compounds that persist in animal tissues after treatment. These residues, when present in meat products beyond permissible limits, pose serious public health risks, contribute to antibiotic resistance, and impact international trade in livestock products (Mouliom Mouiche *et al*., 2024).

Globally, antibiotic residues in food animals have become a critical concern due to their potential to induce antimicrobial resistance (AMR). AMR occurs when bacteria evolve to resist the effects of antibiotics, making bacterial infections more difficult to treat in both animals and humans. The World Health Organization (WHO) has identified AMR as a major public health threat that requires urgent action (Vishnura *et al*., 2016). In many developing countries, including Nigeria, inadequate regulatory enforcement and weak monitoring systems exacerbate the issue of antibiotic residues in animal products (Alhaji and Isola, 2018). The presence of these residues in beef can lead to serious health risks, including allergic reactions, toxicity, disruption of gut microbiota, and

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the development of resistant bacterial strains in consumers (Arsène *et al*., 2022).

Benue State, often regarded as the "Food Basket of the Nation," is also producer of livestock. The state's vast agricultural land and conducive climatic conditions make cattle rearing an activity within the state. Benue state has faced challenges related to open grazing leading to the implementation of an anti-open grazing law. This law aimed at managing conflicts between crop farmers and pastoralists (Gever, 2018).

With an increasing demand for beef, cattle farmers and traders frequently resort to the use of antibiotics for therapeutic, prophylactic, and growth promoting purposes. However, the improper administration, lack of veterinary supervision, and non-compliance with withdrawal periods have led to concerns regarding antibiotic residues in beef (Okocha *et al.*, 2018).This study aims to access the presence of antibiotic residues in kidney, liver and muscles of food animals slaughtered in Benue state.

3.0 MATERIALS AND METHOD

3.1 Study Area

Benue state lies in the North central zone of Nigeria between latitudes 6°25'N and 8°8'Nand longitudes 7°47'E and 10°E'. It covers an area of 30, 955km^2. The state shares border with 6 states (Nasarawa to the North, Taraba to the East, Kogi to the Westand Enugu, Ebonyi and Cross River to the South) and Cameroun Republic to the Southeast. Based on the 2006 Census, the population of Benue State is 4,219,244 with 2,164,058 males and 2,055,186 females. A greater percentage of these live in rural areas (NPC, 2006). Makurdi is the capital of Benue State of Nigeria. The city is located in central Nigeria along the Benue River. As of 2007, Makurdi had an estimated population of 500,797. Agriculture is the mainstay of the economy of the state as most of the populace is farmers. The state is acclaimed “the food basket of the Nation” because of its diverse agricultural produce which includes rice, beans, cassava, yam, maize. Millet, among other foods. Few people engage in livestock farming such as pigs, sheep, goats and cattle-especially the Muturu breed of cattle (Gbaka, 2014).

Benue experiences two (2) distinct seasons.-The rainy season which lasts from April to October with annual rainfall in the range of 100-200mm and the dry season begins in November and ends in March. Temperatures fluctuate between 21-37^{0}C in the year. Benue state has three senatorial zones (figure 1) namely Zone A comprising of eight local government areas including Katsina-ala and Kwande from where samples were collected. Zone B comprises of seven Local government areas and samples for zone B were collected from Makurdi and Gboko local government areas. Zone C has nine local government areas and samples were collected from Otukpo and Ogbadibo local government areas.

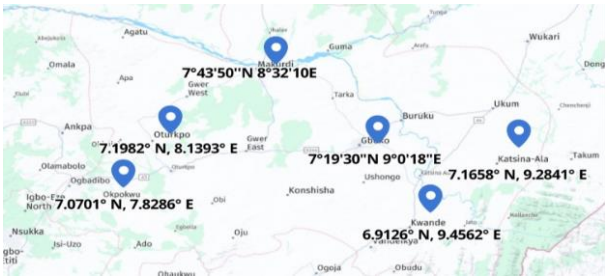

Figure 1: Map of Benue State Nigeria indicating the sites of sample collection

3.2 Determination of the presence and Prevalence of Tetracyclines and Penicillin residues in beef.

3.2.1 Sample size determination.

Sample size was calculated based on the work by Nhung *et al*., (2018) with mean prevalence rate of 7.3% obtained from beef using the same formular of Thrushfield (2005):

$$N\frac{Z^2pq}{d^2}$$

$$N = \frac{1.96 \times 0.073 \times (1-0.073)}{0.05^2}$$

$$N = \frac{0.14308 \times 0.927}{0.0025}$$

N = 53.05m

For the purpose of this work, 117 samples were collected.

3.2.2 Screening by Agar gel diffusion method and High performance liquid chromatography

3.2.2.1 Sampling

Samples of kidney, liver and muscle (50g) were collected from each cattle slaughtered for human consumption at selected slaughter slabs in the three health zones (zone A, B and C) of Benue state. The samples were collected from two slaughter slabs in each zone. The sample sites were Katsina-Ala and Adikpo in Zone A, Wadata and Wurukum in Makurdi town and Gboko in Zone B, and Otukpo and Ugbokolo in zone C.

About one hundred and seventeen (117) samples were collected using the systematic random sampling method during the dry season (from October to March 2018 and October to March of 2019) each site of collection was visited once in two weeks and a minimum of 18 samples were collected. The sample was collected separately in sterile polyethene bags, kept in ice packs and taken to the laboratory for extraction. After extraction, samples were kept in the freezer at -4^{o}C until processed.

3.3 Laboratory Analysis

3.3.1 Sample extraction

Samples were extracted using the methods described by Mc Donald and Bouvier (2001) and used by Abbasi *et al.*, (2009) and Ezenduka and Ugwumba (2012) as follows: The samples were cut into fine pieces and blended using a simple kitchen blender. After each blending, the blender was wiped with 70% alcohol and rinsed with distilled water to avoid sample mix-up and possible transfer of drug residues. Two point five grams (2.5g) was weighed into a set of centrifuge tubes. One point

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eight milliliters (1.8 ml) of 5N hydrochloric acid was dispensed and mixed properly. McIlvain solution was prepared with 1.97g of phosphate buffer (KH_2PO_4), 2.8ml sodium hydroxide (NaOH) and 5M sodium metabisulphite $Na_2S_2O_5$ in 200ml water, giving a pH of 7.6 and used in the ratio of 2:1:4 respectively was poured into the samples and covered properly, shaken for 15 min and then centrifuged for 20 min at 4500 rpm. The supernatant was collected and filtered using 0.45µm filter paper into plastic vials for the determination using HPLC.

Preliminary Screening of Samples for Multi Drug Residues

The samples were screened for drug residues using the modified agar diffusion method of Kirby and Bauer, 2007, as follows: Work benches were swabbed with 70% alcohol before the procedure.

Three colonies each of *Escherichia coli* NC101 and *Staphylococcus aureus* CC398 (both obtained from NAFDAC Zonal laboratory, Agulu) were picked with sterile wire loop each and emulsified in 9ml of sterile nutrient broth. These were incubated at 37^{o}C for 24hrs. After this, dilutions of the samples were made and compared with turbidity standard equivalent to 0.5 Mc Farland's standard. A sterile swab stick was dipped in each of the dilutions removing excess liquid by pressing against the inner surface of the bottle and spread appropriately on Mueller Hinton agar plates rotating the plates approximately at 60^o to ensure even distribution of test organism- *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram positive). Holes of 8mm were made in the center of each Mueller Hinton agar plates using a cork borer. 0.5ml each of the various test samples was introduced into the wells, using a different syringe for each sample It was allowed to stand for about 1hr undisturbed to allow for diffusion of test samples into the agar. The plates were then incubated at 37^{o}C for 18 hours. The presence of clear zones of inhibition indicate the activity of antibiotics present in the test samples. Using a ruler, the diameter of the zones was recorded. The zones of inhibition were calculated as follows for each sample;

Zone (cm) x 10(to mm) – 8 (cork borer) =mm (S.I unit for zone of inhibition is millimeter)

Samples which were seen to inhibit the growth of the test organisms were set aside as samples containing multidrug residues and were used to carry out HPLC for determination of tetracycline, oxytetracycline, chlortetracycline and penicillin.

3.4 Drug Residue Determination using High performance Liquid Chromatography

3.4.1 Preparation of standard curves

A calibration curve was made using five (5) point levels and a graph plotted giving 99.9% accuracy, in order to obtain the concentration of each standard. Standard solutions were prepared using the method described by McDonald and Bouvier (2001), by dissolving 25mg of each reference standard of Tetracycline, Oxytetracycline, Chlortetracycline and Penicillin (obtained from sigma Aldrich) in a mixture of methanol, acetonitrile and hydrochloricic acid in a ratio of 10:20:70, v/v. Serial dilutions were made using 25mg of the reference standard in 25ml volumetric flask as the stock, followed by a serial dilution of 1mg of dissolved reference standard from stock made up to 10ml using the mobile phase, 2mg of dissolved reference standard made up to 10ml, 4mg of dissolved reference standard made up to 10ml, 6mg of dissolved reference standard made up to 10ml, 8mg of dissolved reference standard made up to 10ml and 10mg of dissolved reference standard made up to 10ml, to obtain a concentration of 2, 4, 6 8 and 10mg/ml concentrations, which were injected into the HPLC machine and used to construct standard curves. The peak areas were plotted against the corresponding concentrations and the best line of fit was plotted using Microsoft Excel programs.

3.4.2 High performance liquid chromatography (HPLC) system and procedure

The analysis and quantification of tetracycline, oxytetracycline, chlortetracycline and penicillin residues was carried out at the HPLC laboratory of the National Agency for Food and Drug Administration (NAFDAC) Zonal office Agulu, Anambra state using a HPLC KNAUER system (HITACHI, Japan). The system is equipped with a quaternary pump (K-1000), a BIOTECH model 2003 degasser, a Spark Triathlon autosampler and a RF-551 fluorescence detector. Data processing was performed using the Chromgate V3.1 software.

The mobile phase was a mixture of methanol, acetonitrile and 50mol Hydrochloric acid 10:20:70 v/v as described by Abbasi *et al.* (2009). The prepared mobile phase was filtered through a 0.45µ filter paper using vacuum pump and then degassed by sonication for 5 min before application. Detection was carried out using 365 nm as excitation and emission wavelength. A Phenomenex Luna C-18 column (Torrance, CA, USA) (particle size 5µm; 4.6mm x 250) was used for elution of the analytes with mobile phase. The machine was flushed at regular intervals with blank methanol and the mobile phase was allowed to run through the machine for equilibration and conditioning during which stable baseline was obtained on the recorder monitor. The column and tubing were regularly checked to ensure that there is no leakage. Twenty microliters (20µl) of analyte from each sample was injected into the column when the machine gave instruction "waiting for pulse injection". The antibiotic was eluted on the C-18 column and resolution occurred in the detector resulting in peaks (chromatographs) shown on the monitor with the peak areas and retention times recorded by the computer. The spikes measure the concentration of each antibiotic. The flow rate was 1.0 ml/min with retention times of 3.0-8.0 for tetracyclines and 4.0-5.0 for penicillin based on the results obtained from spiking the reference standards (Abbasi *et al.*, 2009).

3.4.3 Specificity

The specificity of the liquid chromatography method was evaluated to ensure that there was no interference from the excipients present in the pharmaceutical product. This was

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studied by injecting ethanol, acetonitrile hydrochloric acid and the standard solution of tetracycline, oxytetracycline, chlortetracycline and penicillin respectively. (Oyedeji *et al.*, 2021)

3.4.4 Linearity

Five different concentration levels (2 mg/ml, 4mg/ml, 6 mg/ml, 8 mg/ml and 10 mg/ml) were prepared from standard solution. Then 20 µl from each solution was injected into the HPLC using auto-sampler the analyses were monitored at 365 nm and repeated four times. The average peak areas were plotted against concentrations. The linearity of the proposed method was evaluated by using calibration curve to calculate coefficient of correlation, slope and intercept values. Standard curves for the assessed drugs as follows:

i. Standard Curve for Tetracycline

The results of the standard concentrations and the peak areas were plotted as the standard curve (figure 2) using linear regression equation The linear equation Y = a + b X was obtained where Y = peak area (cm2), a = Y-intercept, b = the slope, X = concentration of the tetracycline, y = 1.2285+008x + 0.0000 and the goodness of fit (R^2) value of 0.9716, where y is the peak area and x is the concentration in µg/g. The R^2 value > 0.9 showed the linearity. The detection limit for tetracycline was 0.01ppm while the retention time ranged between 4.4 to 5.8 minutes with the peak retention time being 4.7 minutes.

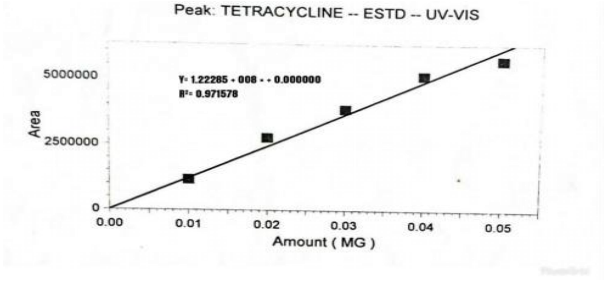

Figure 2: Standard curve for tetracycline

ii. Standard Curve for Oxytetracycline

For oxytetracycline, y = 1.21411 +008x + 0.0000 and the goodness of fit (R^2)value of 0.998835, where y is the peak area and x is the concentration in µg/g. The R^2 value > 0.9 showed the linearity. The detection limit for oxytetracycline was 0.01ppm while the retention time ranged between 4.0 to 5.0 minutes with the peak retention time being 4.2 minutes.

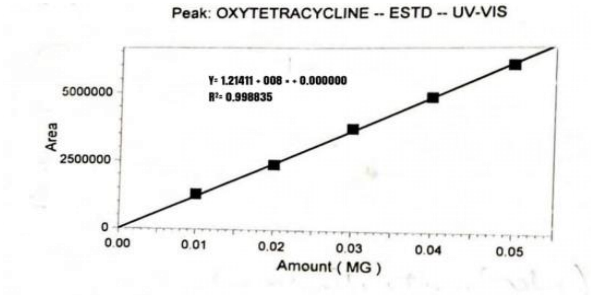

Figure 3: Standard curve for Oxytetracycline

iii. Standard Curve for Chlortetracycline

For chlortetracycline, y = 1.2584 +008x + 0.0000 and the goodness of fit (R^2)value of 0.979616, where y is the peak area and x is the concentration in µg/g. The R^2 value > 0.9 showed the linearity. The detection limit for chlortetracycline was 0.1mg while the retention time ranged between 7.0 to 8.0 minutes with the peak retention time being 7.9 minutes.

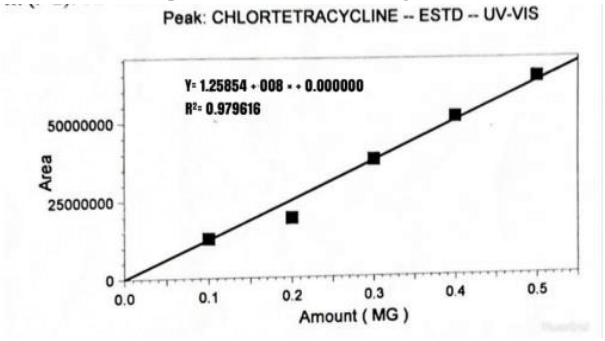

Figure 4: Standard curve for chlortetracycline.

iv. Standard Curve for Penicillin

For penicillin, y = 1.61380 +007x + 0.0000 and the goodness of fit (R^2)value of 1.00000, where y is the peak area and x is the concentration in mg/g. The R^2 value > 0.9 showed the linearity. The detection limit for penicillin was 0.1mg while the retention time ranged between 4.3 to 5.5 minutes with the peak retention time being 7.9 minutes

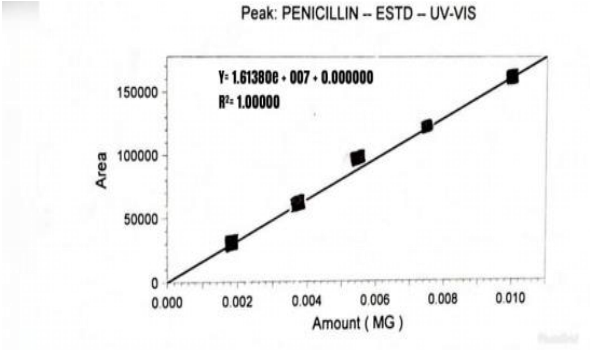

Figure 5: Standard curve for Penicillin

3.4.5 Accuracy/ recovery

The accuracy of an analytical method expresses the nearness between the expected value and the value obtained. It is expressed by calculating the percent recovery (%R) of analyte recovered. In this case, to evaluate the accuracy of the proposed method, successive analysis (n=3) for three different concentrations (6mg/ml, 8mg/ml and 10 mg/ml) of standard solutions of tetracycline, oxytetracycline, chlortetracycline and penicillin were carried out using the proposed method. The data of the experiment were analyzed using the formula [% Recovery = (Recovered conc./Injected conc.) x 100] to study the recovery and validity of the proposed method.

3.4.6 Precision/reproducibility

Precision of the assay was assessed with respect to both repeatability and reproducibility. The precision of an analytical method is the degree of agreement among individual test results where the method is applied repeatedly to multiple samples. It was checked by intra and inter day repeatability of responses after replicate injections and expressed as RSD % amongst responses using the formula

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[RSD (%) = (Standard deviation/Mean) x 100 %]. The precision was determined by five replicate analyses at the concentration of 8 mg/ml of standard solutions of all the antibiotics used.

3.6 Statistical Analysis

Descriptive statistics (percentages) was used to analyze the demographics for the questionnaire survey and to determine which antibiotics are the most commonly used in Benue state. Chi square (χ^2) was used to analyze the association between the demographics and the different practices that could lead to drug residues in meat and significant p-values were further tested using multivariate analysis (odds-ratio). Percentages and Analysis of variance (ANOVA) were used to analyze the screening test. Mean antibiotic concentrations in the different tissues were determined using excel and compared using ANOVA. Statistically significant results were followed by Turkeys multiple comparison test at a confidence limit of 95% (p=0.05) using graph pad prism 5.0 (Graph pad software inc. San Diego, CA).

4.0 RESULTS

4.1 Prevalence of antibiotic resistance in Cattle

A total of 117 samples were analyzed, with 58 (49.6%) testing positive for antibiotic resistance and 59 (50.4%) testing negative. The distribution of resistance varied across the different tissues. In the kidney, 19 out of 39 samples (48.7%) were positive for antibiotic resistance, while 20 (51.3%) were negative. Similarly, the liver had a slightly higher prevalence, with 21 out of 39 samples (53.8%) testing positive and 18 (46.2%) testing negative. In contrast, muscle tissue showed a lower resistance rate, with 18 (46.2%) samples positive and 21 (53.8%) negative. The overall prevalence across all organs suggests a nearly equal distribution between resistance and susceptibility, with a total of 58 (49.6%) positive cases and 59 (50.4%) negative cases out of 117 samples (Table 1).

4.2 Mean Concentration of Antibiotics in organs of Cattle Slaughtered in Benue state based HPLC

The mean concentrations of tetracycline residues in the Kidney, Liver and Muscle of cattle were 73.2±23.4µg/kg, 158.2±39.3µg/kg and 42.6±17.1µg/kg respectively. These mean concentrations were statistically significantly different from each other with the difference being with the liver (ANOVA, P=0.018: Turkey's multiple comparison test). The mean concentration of oxytetracycline in kidney of cattle was (72.1±14.7µg/kg), that of liver (49.5±10.3µg/kg) and muscle (45.8±13.8µg/kg) were not significantly different (ANOVA, P=0.357) The mean concentration of chlortetracycline in Kidney, Liver and Muscle of cattle were 779.9±167.1µg/kg,1661.5±385.5µg/kg and 825.7±243.0µg/kg respectively. These means were not significantly different (ANOVA, P=0.052). The mean concentration of penicillin residues in kidney, liver and muscle of cattle was 420.1±510.1µg/kg, 446.0±675.4µg/kg and 193.1±383.3µg/kg respectively. These mean concentrations were found not to be statistically significantly different (ANOVA, P=0.137). (Table 2).

4.3 Mean concentrations of antibiotic residues in organs of cattle in Benue State by HPLC

The mean concentrations of tetracycline, oxytetracycline, chlortetracycline and penicillin in the kidney of cattle were 73.2 ± 24.6µg/kg, 72.1 ±14.7µg/kg, 779.9±167.1µg/kg and 448.3± 116.3µg/kg respectively. The concentrations of tetracycline, oxytetracycline, chlortetracycline and penicillin in the kidney were found to be statistically significantly different. The difference was with the concentrations of chlortetracycline and penicillin which were found to be significantly higher than tetracycline and oxytetracycline (ANOVA, P=0.0001: Tukey's multiple comparison test). The mean concentrations of tetracycline, oxytetracycline, chlortetracycline and penicillin in the liver of cattle were 158.2 ± 39.3, 49.5 ±10.3, 1661.5 ±385.8 and 578.6 ± 180.3 respectively. The concentrations of tetracycline, oxytetracycline, chlortetracycline and penicillin in the kidney were found to be statistically significantly different. The difference was with the concentrations of chlortetracycline and penicillin which were found to be significantly higher (ANOVA, P=0.0001: Tukey's multiple comparison test). The mean concentrations of tetracycline, oxytetracycline, chlortetracycline and penicillin in the muscle of cattle were 42.6 ± 17.1, 45.8 ±13.8, and 825.7 ± 243.0 and 201.6 ± 84.6 respectively. The concentrations of these antibiotic residues were found to be statistically significantly different. The difference was with the concentrations of chlortetracycline and penicillin which were found to be significantly higher (ANOVA, P=0.0001: Tukey's multiple comparison tests). (Table 3).

4.4 Antibiotic residues above CODEX maximum residue limit (MRL) in cattle

The CODEX maximum residue limits for antibiotics are as follows: tetracyclines in kidney - 1200µg/kg, liver 600µg/kg and muscle 200µg/kg, penicillin - 50µg/kg for all organs. In cattle, chlortetracycline and penicillin had residues which were above maximum residue limits in all the organs. Penicillin had residues above maximum residue limit more than chlortetracycline with a mean concentration of 766.7 ± 221.7µg/kg in the liver of cattle (A concentration above 15 times higher than the MRL for penicillin in liver) Table 4.

Table 1: Prevalence of antibiotic resistance in Cattle

Organs	**Cattle**		
	No Positive (%)	No Negative (%)	Total
Kidney	19 (48.7)	20 (51.3)	39 (33.3)
Liver	21 (53.8)	18 (46.2)	39 (33.3)

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Muscle	18 (46.2)	21 (53.8)	39 (33.3)	Total	58 (49.6)	59 (50.4)	117 (100)

Table 2: Mean Concentration of Antibiotics in organs of Cattle in Benue State, by HPLC.

	Concentration(µg/kg)			
Organs	tetracycline	oxytetracycline	chlortetracycline	penicillin
Kidney	73.2 ± 24.6	72.1 ±14.7	779.9±167.1	448.3± 116.3
Liver	158.2 ± 39.3	49.5 ±10.3	1661.5 ±385.8	578.6 ± 180.3
Muscle	42.6 ± 17.1	45.8 ± 13.8	825.7 ± 243.0	201.6 ± 84.6
P-value	0.018	0.357	0.052	0.137

Table 3: Mean concentrations of the antibiotic residues in organs of cattle in Benue State by HPLC

Organs	**Concentration**				**P-value**
	Tetracycline	Oxytetracycline	Chlortetracycline	Penicillin	
Kidney	73.2 ± 24.6	72.1 ±14.7	779.9±167.1	448.3± 116.3	0.0001
Liver	158.2 ± 39.3	49.5 ±10.3	1661.5 ±385.8	578.6 ± 180.3	0.0001
Muscle	42.6 ± 17.1	45.8 ± 13.8	825.7 ± 243.0	201.6 ± 84.6	0.0001

Table 4 Mean concentration of antibiotic residues in cattle above maximum residue limit (MRL)

Organs	**Kidney**		**Liver**		**Muscle**		**P-Value**
Residues	chlortetracylin	Penicillin	chlortet	penicillin	chortet	Penicillin	
No of samples (n)	4	11	13	14	10	7	
Mean±SEM	1762±401	739±150.5	2245±550	766.7±222	1385±366	667.3±211	0.0039
CODEX MRL (µg/kg)	1200	50	600	50	200	50	

5.0 DISCUSSION

The High performance chromatography (HPLC) analysis utilized a total of one hundred and forty four samples (58 samples) which were the samples positive from the agar gel diffusion method and this proved to be adequate for statistical analysis of variance method. Olatoye and Ehinmowo (2010) collected a total of 180 samples beef that screened for oxytetracycline residues. Various samples have been collected by researchers reporting on antibiotic residues in animals: Bahmani(2019) collected a total of 450 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 high mean concentration of tetracycline residues in the liver of cattle which was statistically significantly different (ANOVA<0.05) arises from the fact that the liver is the organ of metabolism of drugs including antibiotics. This could also be due to the long term use of tetracycline in cattle since concentrations were lower in the muscle. The result of this study is similar to that of Abolghait *et al.* (2020) who reported highest concentrations of tetracyclines in the liver of broilers in Egypt. Abasi *et al (*2009) in Tabriz Iran reported the mean amount of total residues tetracyclines in all meat samples triceps gluteal and diaphragm muscles, kidney and liver as 131.0µg/kg, 163.1µg/kg, 63.4µg/kg, 408.1µg/kg and

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254.9µg/kg respectively showing tetracycline to be highest in the kidney which is however contrary to our report.

The mean concentration of oxytetracycline in the kidney was higher than the concentrations in the liver and muscles of cattle. However this difference was not statistically significant (ANOVA, P>0.05). This could be as a result of long term use or abuse of oxytetracycline. This result is contrary to the findings of Olatoye and Ehinmowo (2010) who reported higher concentrations of oxytetracycline in the liver of cattle and the difference was significant.

The mean concentration of chlortetracycline in the liver of cattle was highest. However, this difference was not significant. This could be as a result of a high level of abuse in the administration of chlortetracycline to cattle. High level of chlortetracycline was detected in kidney by Abasi *et al* (2009) who reported a concentration of 408µg/kg of chlortetracycline from the kidney of cattle. The mean concentrations of penicillin residues were found to be highest in the liver. However the difference was not significant. This may be due to farmers not adhering to the withdrawal period of the antibiotic.

The mean concentrations of tetracycline, oxytetracycline, chlortetracycline and penicillin were significantly different in the kidney of cattle. The difference which was with the concentrations of chlortetracycline and penicillin could have arisen due to the persistent use of these antibiotics in the animals and their feeds. Guetiya *et al* (2016) reported on various levels of oxytetracycline and procain penicillin in the liver, kidney and muscles of poultry. The work reported oxytetracycline and procain penicillin in the liver and muscle but not in the kidney using thin layer chromatography.

The mean concentration of tetracycline, oxytetracycline, chlortetracycline and penicillin were significantly different in the liver of cattle. The mean concentration of chlortetracycline in the liver of cattle was highest among the antibiotics tested for. This concentration was also significantly different. The mean concentration of chlortetracycline in the muscle cattle was the highest among the four antibiotics tested for. This concentration was significantly different. High mean concentration in the muscle of cattle may be as a result of abuse.

Penicillin had the highest concentration above maximum residue limit in the liver (766.7±221.7µg/kg) a concentration about 15 times higher than the CODEX approved maximum residue limit (MRL) for penicillin in the liver of animals. Penicillin is used for the treatment of various respiratory and gastrointestinal tract infections and for the treatment of infectious mastitis in dairy cows (Canzani and Aldeek, 2017). This may be the reason for its misuse in animals in Benue state. The result of this study is in contrast to that obtained by Mohammadzadeh *et al* (2022) who reported much lower concentrations of penicillin G in (6.27±2.46 µg/kg, 8.5±2.80 µg/kg and 11.67±2.94 µg/kg) kidney, liver and liver respectively from poultry carcasses in Iran. This result agrees with that obtained by This agrees with the work of Olatoye and Ehinmowo (2010) who found about 63.2% of samples from a total of 180 samples of beef with concentrations above Maximum residue limit using HPLC.

The risk quotient of the tetracyclines and penicillin residues through meat consumption in Benue state was less than one (RQ<1) for all the antibiotic residues in the meat samples. This implies that based on this measure of risk, the meat samples were safe for human consumption or that the amount of meat consumption was not high enough to produce a risk. The recommended daily intake for meat is 70g/person/day (www.NHS.UK) but the rate of meat consumption in Nigeria is 23g/person/day (NBS, 2016). However, children weigh less and could easily generate a quotient greater than one and be at risk after consuming meat over a period of time. Elizabeta *et al.* (2011) calculated the estimated daily intakes (EDI) for the average daily consumption of 200 ml of milk for an adult in Macedonia, for the examined antimicrobials and obtained levels 2 to 100 times lower than the values of the acceptable daily intakes fixed by World Health Organization. This indicated that toxicological risk associated with the consumption of analyzed milk could not be considered as a public health issue with regards to these veterinary drugs.

According to the study of Vragovic *et al.* (2011) the acceptable daily intake for tetracycline (for a person weighing 60 kg) was 1800 µg/person/day, thus the assessed risk is negligible that is less than 1% of acceptable daily intake. Similarly in our study, the hazard quotient ranged between 0.0002 and 0.021 for oxytetracycline and chlortetracycline respectively. Therefore the risk quotient associated with the consumption of meat which contains residues in Benue State, Nigeria, is negligible to human health.

6.0 CONCLUSION

The results of this study revealed that antibiotic residues were present in beef samples collected from various slaughter houses in Benue State, Nigeria; this shows significant concerns regarding food safety. The analysis using high-performance liquid chromatography (HPLC) and agar gel diffusion methods detected residues of tetracyclines (oxytetracycline, chlortetracycline), and penicillin in different animal tissues, with the highest concentrations found in the muscle tissues. The prevalence of antibiotic residues in beef was observed across all the studied zones (A, B, and C), indicating widespread usage of antibiotics in cattle farming in the state. The presence of these antibiotic residues, particularly at detectable levels, poses public health risks, including the potential for antimicrobial resistance (AMR), which could complicate the treatment of bacterial infections in both animals and humans. This finding shows the necessity for improved regulation, veterinary oversight, and strict adherence to withdrawal periods to mitigate the risks of antibiotic contamination in meat. The study calls for increased awareness, monitoring, and enforcement of regulations to ensure the safety of beef products, protect public health, and promote sustainable practices in livestock production. Further studies are necessary to evaluate the residues of other drugs in meat, their products and the hazards associated with consumption of drug residues from foods in Benue state.

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