SVU-IJVS-7(4): 1-10 Print ISSN: 2535-1826 Online ISSN: 2535-1877

Research Article Open Access

Oxytetracycline Residues in Cattle and Pig Carcasses: A Study of Muscle, Liver, and Kidney

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https://doi.org/10.21608/svu.2024.320210.1342
Submit Date: 2024-09-11, Revise Date: 2024-10-03, Accept Date: 2024-10-04
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ABSTRACT

This study aimed to evaluate the levels of oxytetracycline (OTC) residues in cattle and pig carcasses in Kaduna metropolis, Nigeria. A total of 300 samples (150 each for cattle and pigs) comprising of liver, kidney, and thigh muscles were randomly collected from cattle and pigs in selected abattoirs and slaughter slabs in Kaduna metropolis. An adapted microbial inhibition test using *Bacillus subtilis* and high-performance liquid chromatography (HPLC) was used for residue analysis. The results showed that 40.0% of cattle and 26.6% of pig samples tested positive for antimicrobial residues. The sampled tissues had both liver and kidney from Kakuri as the highest at 53.3% (8/15) followed by muscle at 46.6% (7/15) for cattle while liver from Ungwan-Television and Kurminmashi had the highest 33.3% (5/15) followed by liver and kidney from Narayi 30.0% (3/10) in pigs. The highest mean concentrations of OTC residues in cattle tissues were found in the kidney from Kakuri (1541 ± 417.3 µg/kg) and in the liver from Kakuri (1526 ± 314.2 µg/kg) though not significant ($p > 0.05$). In pigs, the highest OTC residue concentration was in the liver (470.5 µg/kg) which was below the maximum recommended limit. A retention time of 1.96 min was observed for the HPLC analysis of samples. The study highlights the significant presence of OTC residues in livestock, raising concerns over potential public health risks, and antibiotic resistance. The findings emphasize the need for stringent regulatory measures and residue monitoring to ensure food safety and public health protection.

Keywords: Cattle, Kidney, Liver, Oxytetracycline, Muscle, Pig

INTRODUCTION

The use of antibiotics for the treatment of clinically sick animals, disease prevention, and promotion of growth is on the increase due to the intensification of farming practices in developing countries including Nigeria (Adesokan *et al.*, 2013). Oxytetracycline (OTC), a broad spectrum and bacteriostatic antibiotic is widely used to treat gram-positive (*Streptococcus* spp.; *staphylococcus* spp.) and gram-negative (*Escherichia coli*) bacteria, rickettsiae (*Anaplasma*, *Cowdria*, and *Ehrlichia*spp.), chlamydiae (*Psittacosis*, *Trachoma*, *lymphogranuloma venereum*) and some protozoa (*Theileria* and *Babesia* spp.) diseases in animals (Lei *et al*., 2017; Jetty *et al*., 2023). The long-acting oxytetracycline (LOTC), because of its convenience of administration, wide antibacterial activity, and the challenges of accurate bacterial diagnosis under field practice, is widely used to treat a wide range of diseases, often indiscriminately. A single injection of LOTC can keep high concentrations in plasma above minimum inhibitory concentration for several days thereby decreasing the number of administrations per treatment (Lei *et al*., 2017). As a result of the ready availability of the product at informal markets and the lack of restrictions on usage being imposed, OTC has been used indiscriminately by laymen and non-professionals alike in the treatment of various kinds of diseases. Thus, OTC has become a source of concern for residue monitoring authorities around the world (Mohammed *et al*., 2022). Consuming meat with high levels of OTC residues could lead to gastrointestinal disturbances, hypersensitivity, bone and teeth problems in children, and the development of bacterial resistance (Mohammed *et al*., 2022). This is because improper dosage of OTC especially at sub-therapeutic levels can result in acute or chronic public health problems that could be toxicological, microbiological, or immunological (Josiah *et al*., 2018). There is a dearth of data on the levels of OTC residues in cattle and pig carcasses slaughtered within the Kaduna metropolis. The information from the

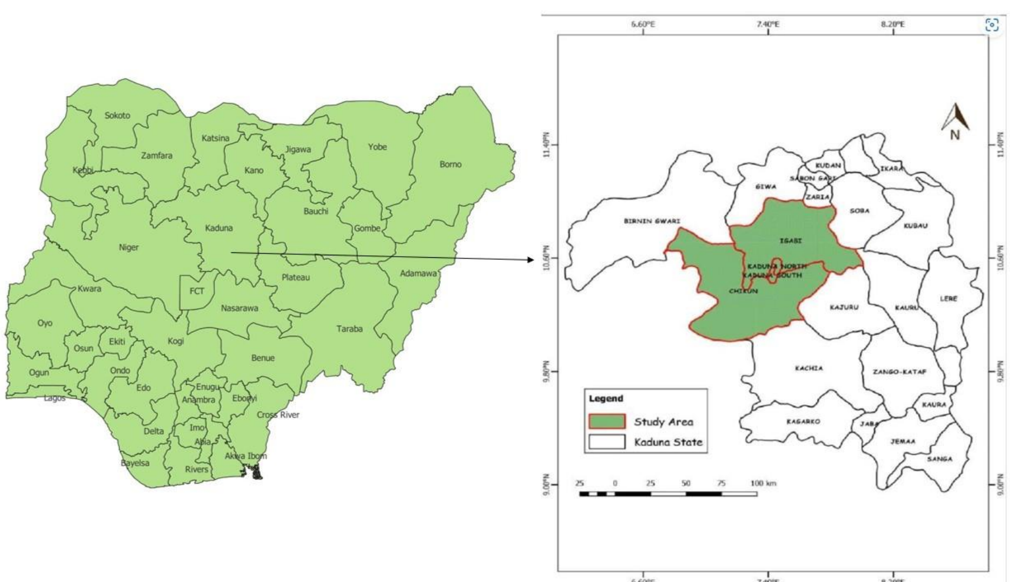

Fig. 1. Map showing the location of the study sites (abattoirs/slaughter slabs) within Kaduna metropolis

study can inform regulatory authorities and guide decision-making on some edible livestock products.

MATERIAL AND METHODS

Study Area

Kaduna is situated in the Northern Guinea savannah zone between latitudes 11˚ and 12˚N and longitudes 7^0 and 8^0E at an elevation of 650 m above sea level (Fig. 1). The average annual maximum and minimum temperatures are between 31.0 ± 3.2 and 18.0 ± 3.7˚C. The average annual rainfall is 1100 mm lasting from May to October with a mean relative humidity of 72%. The dry season lasts from November to April with mean daily temperature ranging from 15^0C - 36˚C and mean relative humidity 20% - 37% (Garuba *et al*., 2021). The sampling points were selected based on high slaughtering activity and availability of cattle and pigs. Kakuri, Kawo, and Tudun-Wada abattoirs were selected for cattle while Barakallahu, Kurminmashi, Narayi, and Ungwan-Television slaughter slabs were selected for pigs.

Study design and sample collection

Animals were selected randomly across the abattoirs and slaughter slabs. Three samples (liver, kidney, and thigh muscle) of 100-200 g each were obtained from 10-15 cattle and pigs yielding a total of 30-45 tissues each per collection. To increase randomization, samples were collected weekly over a period of five weeks yielding 150 samples each for cattle and pig carcasses. Each collected sample was packaged in a zip-lock bag and placed on ice in a cooler box before being transported to the laboratory. The samples were stored in a deep freezer at -20˚C for approximately a week before analysis.

Sample analysis

Samples were prepared and analyzed at the Department of Public Health Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Samples were removed from the freezer and placed on the laboratory bench to thaw at room temperature (25˚C). An adapted microbial inhibition test using *Bacillus subtilis* agar was utilized to screen samples for evidence of antimicrobial drug residue as described by Ezenduka and Ugwunba (2012). The presence of antimicrobial agents in each of the samples was confirmed by a complete zone of inhibition of microbial growth in an annular zone not less than 2 mm around the samples. Less than a 2 mm inhibitory zone indicates a negative result.

Oxytetracycline residue analysis of sample

High-performance liquid chromatography (HPLC) standard methods were used for antibiotic residue analysis. The OTC concentrations were determined according to a previously described technique (Kimera *et al*., 2015). Briefly, the analysis and quantification of

Table 1. The proportion of muscle, liver, and kidney samples containing antimicrobial residues using minimum inhibitory concentration

Animal	Location (Abattoir)	Number of samples (n)	Positive Samples (%)	Muscle (%)	Liver (%)	Kidney (%)
Cattle	Kakuri	45	23 (51.1)	7/15 (46.6)	8/15 (53.3)	8/15 (53.3)
	Kawo	45	14 (31.1)	3/15 (20.0)	6/15 (40.0)	5/15 (33.3)
	Tudun-Wada	60	23 (38.3)	7/20 (35.0)	6/20 (30.0)	10/20 (50.0)
	Total	**150**	**60**	**17**	**20**	**23**
Pigs	Barkallahu	30	6 (20.0)	2/10 (20.0)	2/10 (20.0)	2/10 (20.0)
	Kurminmashi	45	12 (26.7)	3/15 (20.0)	5/15 (33.3)	4/15 (26.7)
	Narayi	30	8 (26.7)	2/10 (20.0)	3/10 (30.0)	3/10 (30.0)
	Ungwan-Television	45	14 (31.1)	4/15 (26.7)	5/15 (33.3)	5/15 (33.3)
	Total	**150**	**40**	**11**	**15**	**14**

OTC residues in the samples were done using an HPLC (Perkin Elmer, Flexar) equipped with a constant flow pump and a variable wavelength ultraviolet detector set at 350 nm and a flow rate of 1.5 ml/min. Elution of the OTC was done using isocratic reverse phase separation mode on a Perkin Elmer Brownie Bio C18 (5 mm, 150 × 4.6 mm) column using methanol, acetonitrile, and oxalic acid mixture (1:3:6) v/v, pH 3.85 as the mobile phase. An estimated 20 µL of the analyte/sample extract was injected in duplicate to obtain peak areas of positive samples corresponding to retention times of the OTC reference standard. For determination of OTC, a blank and OTC standard solutions (20 µL) of varying concentrations at maximum residue limits (MRLs): 0.5, 3.0, 4.0, and 5.0 µg/kg were injected into the HPLC equipment and their peak areas corresponding to the retention time of the reference standard were obtained for a calibration curve that was used in quantification of OTC residues in the samples.

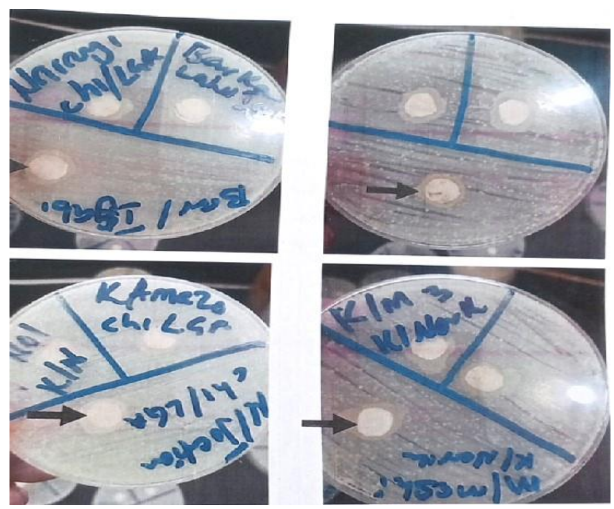

Fig. 2. Agar plates showing zones of inhibition of antimicrobial residue using Bacillus subtilis.

Data Analysis

Data were presented in tables and figures; the OTC residue quantification was presented as mean ± standard error of the mean (SEM) and subjected to the Wilcoxon test to establish if there were any significant differences in OTC residue levels in the muscle, liver, and kidney tissue samples and the level of significance was set at $p < 0.05$. GraphPad prism® for Windows (version 8; GraphPad Software, San Diego, USA) was used.

RESULTS

Antimicrobial residue positivity rate of the samples

The overall positive samples in cattle were 40.0% (60/150), Kakuri abattoir had the highest percentage positive in cattle 51.1% (23/45) when compared to Kawo abattoir which was the lowest 31.1% (14/45). The sampled tissues had both liver and kidney from Kakuri as the highest 53.3% (8/15) followed by muscle 46.6% (7/15) (Table 1). The overall positive samples in pigs were 26.6% (40/150), the highest percentage positive was in Kurminmashi 26.7% (12/45) and Narayi 26.7% (8/30). The sampled tissues had livers from Ungwan-Television and Kurminmashi as the highest 33.3% (5/15) followed by liver and kidney from Narayi 30.0% (3/10) (Table 1). The various zones of inhibition are shown in Fig. 2.

Oxytetracycline residue concentration in samples

The mean concentration of oxytetracycline residues in the kidney of cattle from Kakuri (1541 ± 417.3 µg/kg) was the highest followed by Tudun-Wada (1537 ± 860.2 µg/kg) and Kawo (900 ± 340.5 µg/kg) which was not significant (p > 0.05) (Table 2). Liver from Kakuri had the highest concentration (1526 ± 314.2 µg/kg) which was not significant ($p > 0.05$) in

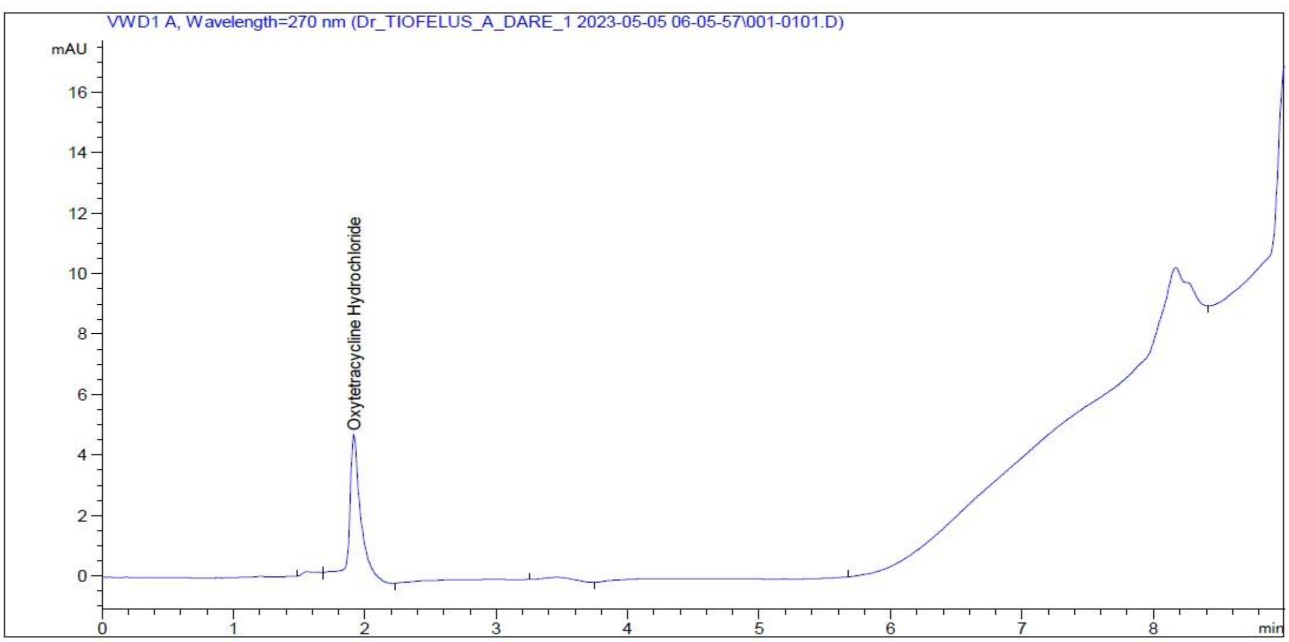

Fig. 3. HPLC chromatogram of OTC for a standard solution

Table 2. Concentration of oxytetracycline residue using high performance liquid chromatography

Animal	Location (Abattoir)	Number of samples (n)	Kidney (μg/kg)	Liver (μg/kg)	Muscle (μg/kg)
Cattle	Kakuri	45 (23)	1541 ± 417.3	1526 ± 314.2	1387 ± 102.5*
	Kawo	45 (14)	900 ± 340.5	1005 ± 750.8	582 ± 370.4
	Tudun-Wada	60 (23)	1537 ± 860.2	1305 ± 302.1	830 ± 610.6
	FAO Limit (2014)		1200	600	200
Pigs	Barkallahu	30 (6)	NA	NA	NA
	Kurminmashi	45 (12)	NA	NA	NA
	Narayi	30 (8)	NA	NA	NA
	Ungwan-Television	45 (14)	NA	NA	NA
	FAO Limit (2014)		1200	600	200

*NA: Below maximum recommended limit, μg/kg: microgram per kilogram, FAO: Food and Agriculture Organization of the United Nations, *Significant at p < 0.05.*

comparison to Tudun-Wada (1305 ± 302.1 μg/kg) and Kawo (1005 ± 750.8 μg/kg).

The mean concentration of oxytetracycline residue of muscle from Kakuri was the highest (1387 ± 102.5 μg/kg) and it was significant ($p < 0.05$) when compared to other locations (Table 2). For pigs, the highest concentration of OTC residue was 470.5 μg/kg found in the liver, while the mean concentrations across tissues were 109±7.05 μg/kg, 304± 0.85 μg/kg and 270 ±1.09 μg/kg for muscle, liver, and kidney, respectively (Table 2). A retention time of 1.96 min was observed for the HPLC of the samples and this corresponded with the standard OTC (Fig. 3, Fig. 4).

DISCUSSION

This study aimed to evaluate the levels of oxytetracycline (OTC) residues in cattle and

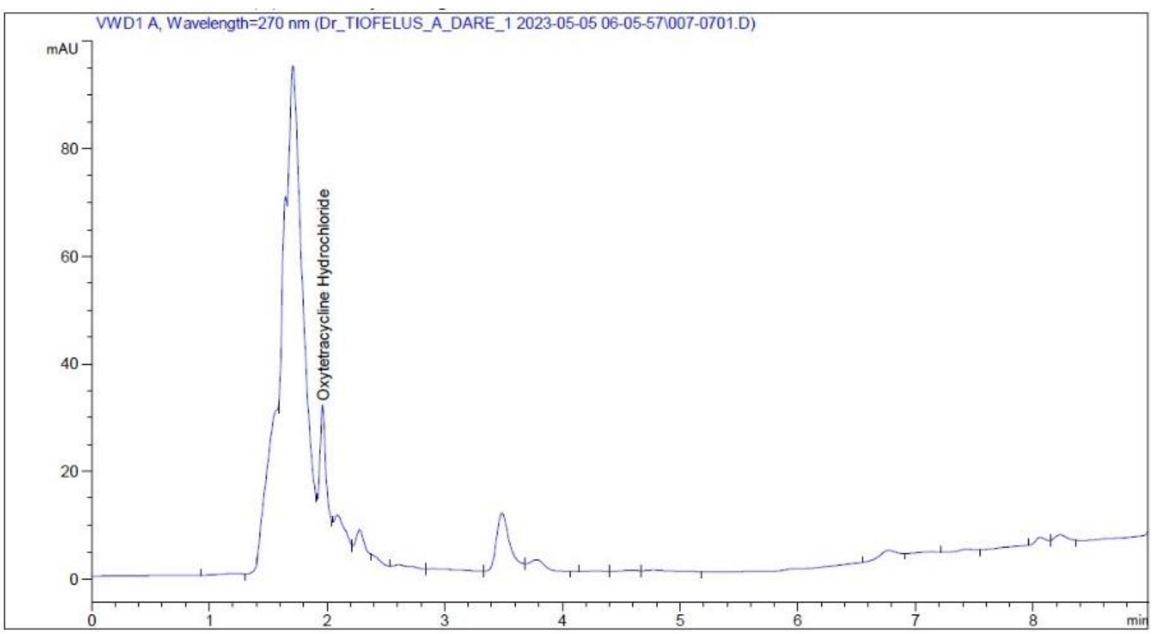

Fig. 4. HPLC chromatogram of OTC for samples

pig carcasses in Kaduna metropolis, Nigeria. This significant presence of OTC residues underscores concerns regarding the potential health risks to consumers, including gastrointestinal disturbances, hypersensitivity reactions, and the promotion of antibiotic-resistant bacteria (Chicoine *et al*., 2020). The occurrence of antimicrobial residues in meat, liver, and kidney has been reported by several authors (Adama *et al*., 2020; Kabirungi *et al*., 2022; Mohammed *et al*., 2022). However, the OTC levels in this study were lower than those reported in previous studies (Ezenduka, and Ugwumba, 2012; El-Ghareeb *et al*., 2019; Adama *et al*., 2020) although comparable to 46% in beef reported by Akinwumi (2012) and 41.2% reported by Mmbado (2004) from Tanzania. In this study, a lower concentration of OTC residues was observed in pigs when compared to cattle. Several factors may be responsible for this, the management and husbandry practices for pigs and cattle differ significantly because pigs are often raised in more controlled environments, which may include regulated feeding practices and better veterinary care, reducing the likelihood of indiscriminate antibiotic use (Ardakani *et al*., 2024). In contrast, cattle, especially those raised in extensive systems, may receive antibiotics more frequently and with less care, leading to higher residue levels in their tissues (Hosain *et al*., 2021). Another contributing factor could be the differences in the metabolism and pharmacokinetics of OTC between pigs and cattle. Pigs may metabolize and excrete OTC more efficiently than cattle, resulting in lower residue levels in their tissues (Lund *et al*., 2024).

In this study, the oxytetracycline residue concentrations were notably higher in the liver and kidney compared to muscle tissue. This can be attributed to the biological functions of these organs. The kidney is a primary organ for excreting drugs and toxins (Veiga-Matos *et al*., 2020). Many antibiotics, including OTC, are filtered from the bloodstream by the kidneys (Mog *et al*., 2020). Since the kidneys work to excrete these substances, they tend to accumulate higher concentrations of residues which explains why high levels of OTC residues were found in cattle kidneys. However, the liver plays a crucial role in metabolizing drugs. Many antibiotics, including OTC, undergo biotransformation in the liver (Yang *et al*., 2021). During this process, OTC can be temporarily stored or metabolized into less active forms, leading to a higher accumulation in the liver. In comparison, muscle tissue generally shows lower concentrations because it is not directly involved in the excretion or metabolism of antibiotics. Although the muscle still absorbs some OTC from circulation, the accumulation is less significant due to lower drug-binding affinity compared to liver and kidney tissue (Webb, and Archer 2020).

Local regulations and practices also play a crucial role in the observed residue levels. In Nigeria, the enforcement of regulations governing the use of veterinary drugs is often weak, leading to widespread availability and misuse of antibiotics in livestock production (Alhaji *et al*., 2023). Informal markets and the lack of strict veterinary supervision allow laymen and non-professionals to administer antibiotics without appropriate knowledge or adherence to withdrawal periods and this indiscriminate use contributes to the higher residue levels observed in cattle (Adekanye *et al*., 2020). Developing robust residue monitoring programs is crucial for ensuring food safety and protecting public health. Regular surveillance of antibiotic residues in livestock and strict adherence to withdrawal periods before slaughter can significantly reduce the risk of consumers being exposed to harmful levels of drug residues (Adekanye *et al*., 2020; Bennani *et al*., 2020). Collaborative efforts between regulatory authorities, veterinary professionals, and the livestock industry are necessary to achieve these goals and promote responsible antibiotic use in animal husbandry (Palma *et al*., 2020).

CONCLUSION

The results of this study revealed a notable percentage of oxytetracycline residues in both cattle and pig carcasses sampled from abattoirs and slaughter slabs within the Kaduna metropolis. Specifically, 40.0% of cattle and 26.6% of pig samples tested positive for antimicrobial residues, with the highest concentrations detected in kidney and liver tissues. This study emphasizes the critical issue of OTC residue contamination in livestock and its implications for public health. Addressing this problem requires a multifaceted approach, including improved regulatory measures, better veterinary practices, and increased awareness among stakeholders about the responsible use of antibiotics in livestock production.

LIST OF ABBREVIATIONS

OTC, oxytetracycline; LOTC, long-acting oxytetracycline; HPLC, high-performance liquid chromatography; µg/kg, microgram per kilogram; ^{0}C, degree celcius; g, gram; nm, nanometer; min, minutes.

ACKNOWLEDGMENTS

We appreciate the technical staff for their expertise in carrying out this research.

CONFLICT OF INTEREST

None to declare.

AUTHORS' CONTRIBUTION

TAD, SSN, JJA, and AIK designed, performed the experiment. CCU, and KOJ analyzed the data and drafted the manuscript.

All authors read and approved the manuscript.

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