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Oxytetracycline Residues in Eggs from Commercial Poultry Farms in Ilorin, Nigeria

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ABSTRACT

Background: The risks posed by indiscriminate antibiotic use in Nigeria are high and of serious public health and food implications. Unrestricted usage of oxytetracycline can lead to the accumulation of antibiotic residues in animal products like eggs.

Objective: The study investigated oxytetracycline residues in eggs from 20 commercial poultry farms in Ilorin, Nigeria.

Methods: Samples were randomly collected from poultry farms in Ilorin, Nigeria and analysed using high-performance liquid chromatography (HPLC).

Results: Oytetracycline residue was detected in pooled egg samples from 15 farms (prevalence = 75%). The mean concentration of 398.30±186.73 µg/kg was obtained with six samples (30%) exceeding the recommended maximum residue limit (MRL) of 400µg/kg. Standard curve analysis showed linearity ($r^2 = 0.98$).

Conclusions: This study revealed a high prevalence of oxytetracycline residues in the eggs produced by commercial poultry farms in Ilorin, Nigeria. The study underscores the need for better regulation and oversight of antibiotic use in poultry farming to mitigate health risks associated with antibiotic residues and antimicrobial resistance.

Keywords: Food safety, antibiotics, public health, tetracycline, laying birds

Introduction

The indiscriminate use of antibiotics in food-producing animals poses significant risks to human health and food safety. In Nigeria, nearly 80% of food-producing animals receive medication throughout their lives (Alhaji et al., 2018), and the prevalence of antibiotic residues, particularly oxytetracycline, in poultry farming is a pressing concern (Lee et al., 2001). Antimicrobials are utilized in animals for therapeutic and prophylactic purposes, as well as for growth enhancement (Nisha 2008). Tetracyclines are the most commonly used and misused drug in the Nigerian livestock and poultry production industry (Adesokan et al., 2015; Ayeni et al., 2016; Alhaji & Isola, 2018; Alhaji et al., 2019, 2023; Odey et al., 2024). Thus, it is important to ascertain if the residues of these antibiotics are found in food products consumed in Nigeria, such as eggs from poultry.

Antibiotics are administered without proper oversight and are readily available over the counter to poultry farmers. This unrestricted usage leads to the accumulation of antibiotic residues in animal products like eggs, meat, and fish (Adetunji et al., 2012a, 2012b), raising concerns about antimicrobial resistance (AMR) and its potential impact on public health. Medications administered orally or through parental routes to birds can accumulate in tissues, particularly if birds are slaughtered without adhering to a withdrawal period, or if eggs are collected during the drug's withdrawal period (Coulibaly et al., 2022; Owusu-Doubreh et al., 2023).

Studies conducted in Nigeria have revealed elevated levels of antibiotic residues in food-producing animals, attributed to the indiscriminate or excessive utilization of antimicrobials (Adetunji 2008; Idowu et al., 2010). Reports indicate a growing emergence and dissemination of

resistant strains of bacterial pathogens due to the indiscriminate use of antibiotics in food animals, presenting a significant challenge to the health of both animals and humans (Adesokan et al., 2014).

Antimicrobial resistance is recognized as a worldwide issue, emphasizing the crucial role of reliable national surveillance systems. The continual use of antibiotics in veterinary medicine, alongside the presence of other selective agents in livestock production settings, could co-select for multi-drug resistance among bacteria, which may persist longer in the environment (Mamza et al., 2017). Studies have indicated that consumption of antimicrobial residues via food animal products could lead to the transmission of resistant microorganism strains to humans, as well as disruptions in intestinal microflora and conditions like bone marrow depression, among other pathologies. (Jafari et al., 2007; Nisha, 2008). By 2050, AMR could become the leading cause of mortality. Global estimates indicate that the number of deaths directly attributed to AMR surpassed 1.2 million in 2019 (Antimicrobial Resistance Collaborators, 2022). The worldwide mortality rate due to AMR is anticipated to reach nearly 700,000 annually. Without adequate measures to control AMR, this figure is expected to rise to over 10 million annually by 2050 (O'Neill, 2014). The burden is more worrisome in sub-Saharan Africa, especially in Nigeria and other LMICs such as the Central African Republic, Zimbabwe, Mozambique, and Eritrea, where the burden is still soaring (Antimicrobial Resistance Collaborators, 2024).

Recognizing the severity of the situation, reliable national surveillance systems and stringent regulatory measures are imperative to control antibiotic use in veterinary medicine. High-performance liquid chromatography (HPLC) methods are extensively used for the precise quantification of diverse antibiotic residues in food products, offering excellent sensitivity and

specificity (Olatoye et al., 2010; Khorrami et al., 2022; Sadighara et al., 2024). Therefore, this study assesses the oxytetracycline residues in eggs, employing high-performance liquid chromatography (HPLC) for precise quantification. It is expected that the findings from this study will contribute to a deeper understanding of the prevalence of oxytetracycline residues in poultry products and suggest strategies to safeguard food safety and combat AMR.

Materials and methods

Study area

This study was undertaken in randomly selected poultry farms situated in Ilorin, Kwara State, North-Central Zone, Nigeria. Ilorin is a major commercial poultry hub in the Northcentral part of the country servicing other neighbouring cities and its environs. The study area, comprising Ilorin South, Ilorin East, Ilorin West, Moro, and Asa Local Government Areas (LGAs), was selected due to their significant concentration of registered poultry farmers, as indicated by data from the Poultry Association of Nigeria (PAN), Kwara State chapter. These five LGAs have the highest percentage share of poultry farmers in the state.

Sampling of eggs

Egg samples were collected aseptically from the selected poultry farms in Ilorin and transported to the Food Safety Laboratory of the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan for sample preparation.

Commercial egg samples (n = 200) were collected from the selected commercial poultry farms (10 egg samples from each farm). Crates of eggs available at each farm were numbered from the first to the last crates. The egg at the top left corner of the first crate was assigned number one until the last egg in the bottom right corner. A total of 10 eggs were then randomly

selected from the total number of eggs using simple random sampling. All samples were stored at -4˚C until they were transported to the laboratory for analyses (Kabir et al., 2004).

Preparation of reagents

Oxytetracycline hydrochloride as procured from Sigma Chemical. All remaining chemicals were of analytical grade. To prepare the oxytetracycline standard, a solution containing oxytetracycline at a concentration of 1mg/ml was created by dissolving oxytetracycline hydrochloride in methanol. For HPLC calibration purposes, the initial solution was diluted with methanol to generate standard solutions ranging from 0.1 to 10.0pg/ml. All solutions, including the stock solution and the various standard solutions, were stored at approximately 4°C. The oxalic acid was obtained by dissolving 1.126g oxalic acid salt in 1L of deionized water to make a 0.01M solution while the Mcllvaine buffer comprised the citric acid and disodium hydrogen phosphate (citrate-phosphate buffer).

Preparation of standard solutions

The actual weight (g) of the antimicrobial standard (oxy-tetracycline HCl), was determined using a weighing balance (Mettler Toledo®, Australia) and was dissolved in 10ml of methanol in a volumetric flask, to produce the standard stock solution of 10ppm. Then this stock solution was serially diluted with equal parts of methanol, acetonitrile, and deionized water to produce 5.0, 2.5, 1.0, 0.5 and 0.25ppm. These concentrations were injected to obtain to the HPLC to obtain correspondent concentrations which were used to prepare calibration curve.

Extraction

Solid-phase extraction (SPE) was carried out according to Olatoye and Saraye (2012). Ten

egg samples were pooled and homogenized. Then, 5g of the specimen was blended with 50mL of 0.1M Na_2EDTA-Mcllvaine buffer (pH 4.0) and centrifuged at 4000rpm. The supernatant was applied to a Bakerbond SPE C_{18} cartridge (JT Baker, Deventer, The Netherlands) that was activated overnight with methanol and water. The cartridge was washed with 20mL of water, and the analyte (residue) was eluted with 10mL of 0.01M formic-oxalic acid solution and collected in a 10mL volumetric flask.

HPLC analysis of oxytetracycline residue

Oxytetracycline residue was detected and quantified from the analyte using an HPLC apparatus equipped with a constant flow pump and a variable wavelength U.V detector according to Olatoye and Basiru (2013). Elution of oxytetracycline from the analyte was performed on a nucleosil C18 (4.5 × 150 mm, 5µm ID) column with formic-Acetonitrile- 0.01M aqueous oxalic acid solution (1- 1.5:2.5) at pH 2.0 as the mobile phase. Twenty microlitre injection volume of the analytes from each sample was injected in duplicate to obtain the average peak area of the positive sample corresponding to the retention time of 10.02 to 10.32 mins of the reference standard. The sample chromatogram is presented in Fig 2.

For validation, five serial diluted standard solutions of oxytetracycline were cleaned up and eluted on SPE (Sep-Pak C18). The mean recovery of oxytetracycline was 87% (cv 4-0%). The retention time of oxytetracycline was about 10 minutes. The calibration curve was linear with a correlation coefficient (R^2) of 0.9808.

Results

Assessment of oxytetracycline residues in eggs using HPLC

Out of a total of 20 pooled egg samples, 15 were found to contain oxytetracycline drug residues with a total prevalence of 75% and a mean concentration of 398.30 ± 186.73 µg/kg (Table 1).

The lowest oxytetracycline drug residue concentration (156.55 ± 15.73 µg/kg) was observed from the egg samples pooled from a farm at the Ilorin South LGA, while the highest (792.81 ± 53.49 µg/kg) was seen with samples from a farm at Ilorin West LGA. Five other samples were below the detectable limits of the method (0.001 ppm). In this study, a total of six pooled egg samples (one from Ilorin East, two from Ilorin South, and three from Ilorin West LGAs) had mean concentrations higher than the MRL standard set by the Codex Alimentarius Commission (CAC) 2021.

The calibration curve obtained from the standard oxytetracycline is shown in Fig. 1 with the linear equation y= 4.4954x + 3.3266 where y = peak area (mAu) and x = concentration of oxytetracycline (ppm) and the correlation coefficient (r^2) = 0.9808 showing the linearity.

On the overall, a total of 15 pooled egg samples (prevalence of 75%) contained oxytetracycline residue in concentrations ranging from 156.55 – 792.81 µg/kg. Forty percent of the positive samples contained oxytetracycline residue at a level higher than the recommended MRL.

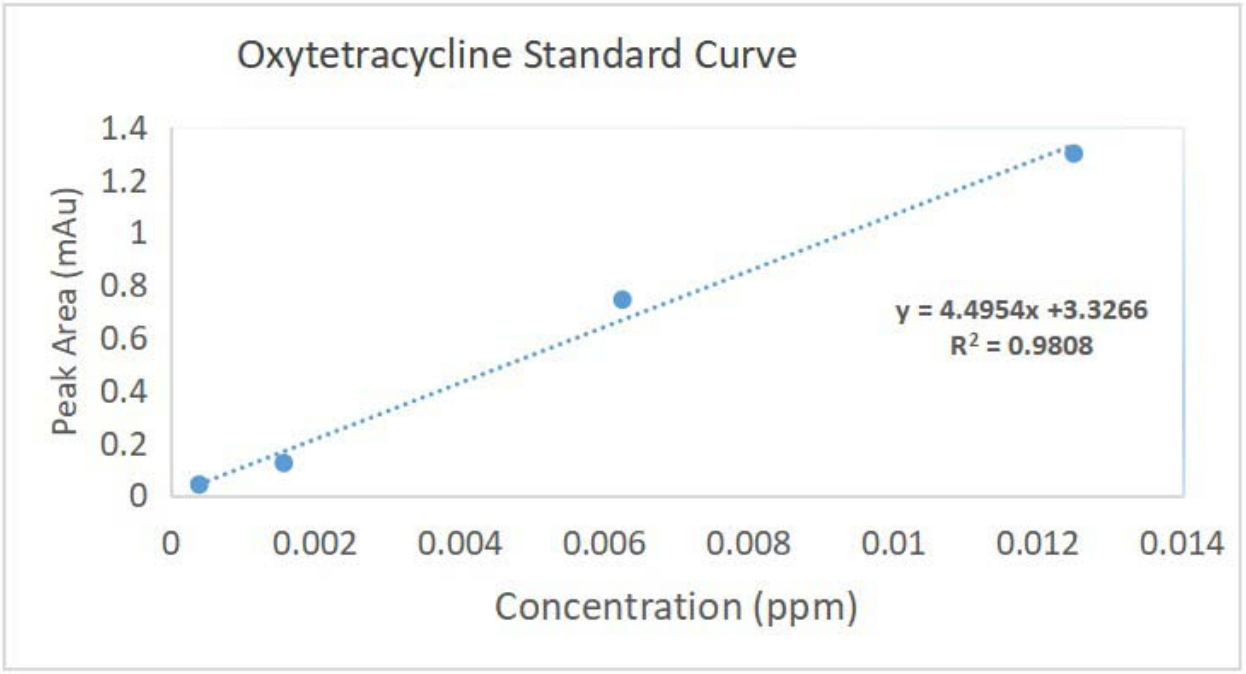

Figure 1. Calibration curve of oxytetracycline standard solution

Chromatogram Report

Tetracycline uv 3.125% for calibration (\PowerStream Support\DUAL MULTI-RESIDUE\UV-LINE\ )

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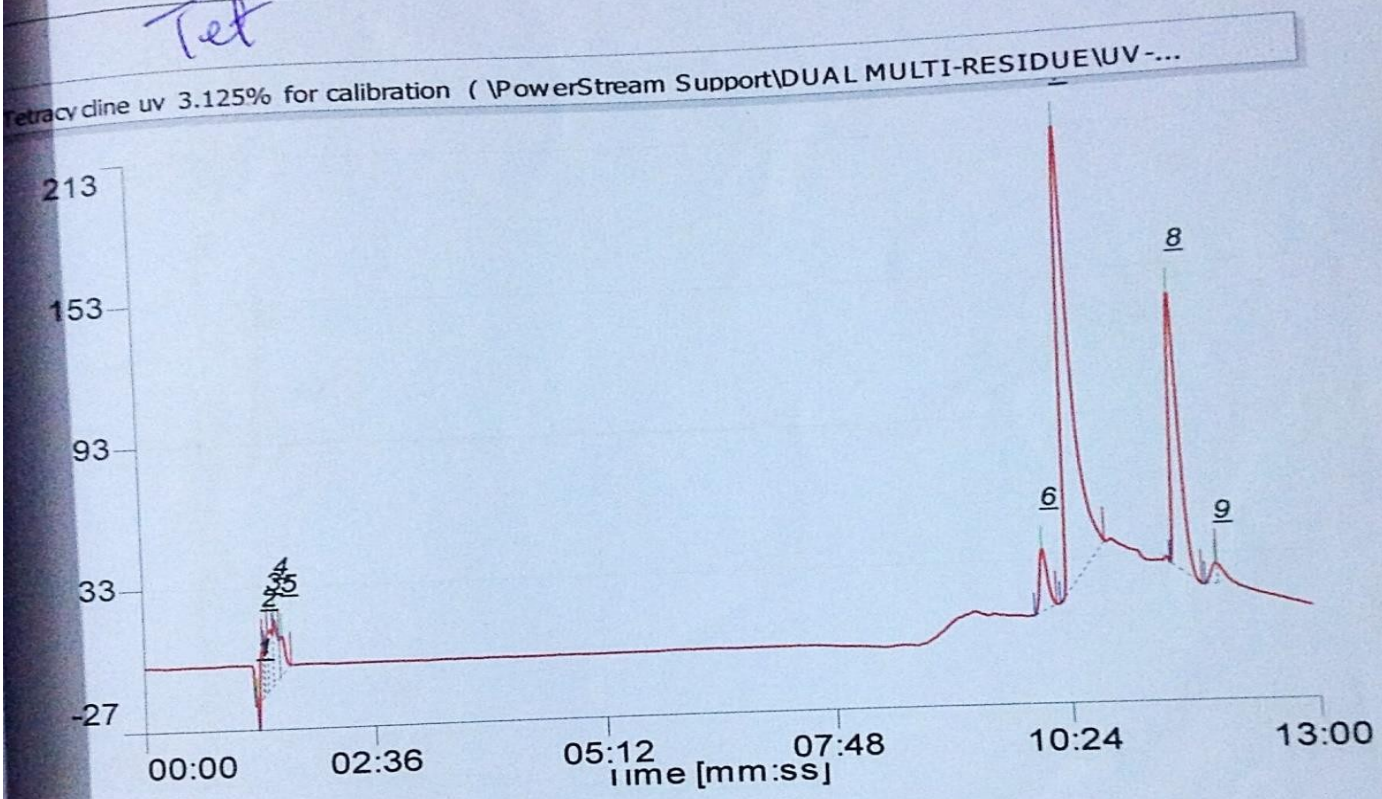

Peak Name	Ret. Time [mm:ss]	Start Time [mm:ss]	End Time [mm:ss]	Area [mAs]	Height [mA]	Quantity
	01:17.1	01:16.4	01:18.3	-3.0	2.6	N/A
	01:21.2	01:18.3	01:21.6	30.5	20.5	N/A
	01:24.9	01:22.7	01:25.1	51.3	24.6	N/A
	01:29.9	01:28.6	01:33.0	96.5	27.4	N/A
	01:34.9	01:33.0	01:40.6	68.4	16.6	N/A
	10:09.1	10:02.4	10:17.1	151.7	25.7	N/A
TETRACYCLINE	10:28.4	10:19.4	10:49.9	1649.9	191.4	N/A
	11:37.7	11:31.4	11:50.8	738.9	114.8	N/A
	11:59.8	11:52.6	12:00.5	37.1	8.5	N/A

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Figure 2. Chromatogram of oxytetracycline residue in egg samples.

Table 1. Oxytetracycline residues in pooled eggs collected from commercial poultry farms in Ilorin, Nigeria

Local Government Area	Sample ID	Mean Concentration µg/kg	Standard deviation
Ilorin South	AD	485.81*	23.60
	TK	242.94	34.08
	GO	360.11	51.91
	JN	156.55	15.73
	BS	761.11*	19.66
	YK	BDL	-
	OK	242.94	34.08
Ilorin East	MC	367.34	49.55
	BL	BDL	-
	DK	BDL	-
	RM	307.83	47.19
	ZG	474.68*	31.46
Ilorin West	IK	416.84*	25.17
	JM	344.91	47.19
	BF	BDL	-
	MP	242.94	34.08

	TH	534.75*	15.73
	NH	792.81*	53.49
Asa	FB	BDL	-
Moro	TF	242.94	34.08

Discussion

We found that pooled egg samples meant for human consumption from commercial poultry farms in Ilorin, Nigeria contained oxytetracycline residues greater than the recommended MRL of 400µg/kg in eggs (Codex Alimentarius, 2021). The findings of this study highlighted the prevalence of oxytetracycline residues in eggs from commercial poultry farms in Ilorin, Nigeria, underscoring the urgent need for enhanced regulatory measures and improved antibiotic stewardship in poultry farming practices. The results showed a high residual presence of oxytetracycline (75%), with 30% of pooled egg samples being above the acceptable maximum residue levels recommended for poultry eggs by the WHO and FAO. This underscores the continuous antibiotic misuse in the region. The use of antimicrobial agents in food-producing animals has become a great public health concern, especially in developing countries where they are administered indiscriminately (Olatoye and Basiru, 2013). Analysis of oxytetracycline residue and other antibiotics used in poultry is very crucial to consumer safety and is regularly monitored in developed countries. However, in Nigeria, there is no such monitoring programme despite the unregulated use of these drugs in food animals and the high prevalence of antibiotic residues in several foods of animal origin across the country (Olatoye and Saraye, 2012).

The prevalence of residues obtained in this study correlates with the previous works of Olatoye and Saraye (2012) and Olatoye and Basiru (2013) which showed that a greater proportion of commercial chicken eggs being consumed in Ilorin, Nigeria could have oxytetracycline residues greater than the MRL for which unregulated access and indiscriminate use of antibiotic by poultry farmers could be responsible. Elsewhere in Enugu state, Nigeria, about 46% of egg-producing commercial farms were positive for oxytetracycline residues (Ezenduka et al., 2011). The 75% of eggs containing oxytetracycline residues in our study is comparable to 49% in Khartoum, Sudan (Sara et al., 2021), 17.1% in Bamako, Mali (Coulibaly et al., 2022) and 16.1% (Adesiyun et al., 2005) in Trinidad (Nonga et al., 2010) reporting lower residue rates. The disparities observed from these results compared to our study could be due to the differences in the residue quantification methodologies used. Hind et al. (2018) used HPLC to analyse eggs samples obtained from Khartoum State, Sudan and obtained 35% of samples containing oxytetracycline residues above the maximum allowable limit.

In this study, high-throughput SPE was used with HPLC quantitative analysis of oxytetracycline residues in eggs sold for human consumption in Ilorin, Nigeria. These ensure high specificity and sensitivity comparable to the Codex Alimentarius standard. This study

confirmed the lack of implementation of the recommended withdrawal times possibly due to the inadequate awareness level of the poultry farmers and the absence of government policies. The use of HPLC for quantifying oxytetracycline residues in our study demonstrated the efficacy of this analytical method in detecting antibiotic residues in food products. However, the study is limited by its focus solely on oxytetracycline residues, as other antibiotic residues may also be present in poultry products and contribute to the overall antimicrobial resistance problem. Future research should consider broader spectrum analyses to assess the full extent of antibiotic residues in poultry products and their implications for public health. Our findings also raise questions about the effectiveness of existing regulatory frameworks and veterinary oversight in Nigeria. The availability of antibiotics over the counter without prescription contributes to the lack of control over their use, leading to suboptimal antibiotic administration practices and the subsequent accumulation of residues in animal products. Addressing this issue requires comprehensive regulatory reforms, including stricter enforcement of prescription requirements for antibiotics and enhanced monitoring of antibiotic use in poultry farming.

This study has revealed the presence of oxytetracycline residues in the eggs produced by commercial poultry farms in Ilorin, Nigeria. There is a need to adequately control the use of

veterinary drugs to protect the public. Also, education on the adverse effects of indiscriminate use of antibiotics and medications especially in poultry and livestock farms is imperative. Farmers should be educated on alternative methods of infectious disease management such as vaccination, environmental sanitation, and disease containment. Responsible authorities should immediately kick off the implementation of regulations associated with antimicrobial administration in poultry production and monitoring programmes (Awogbemi et al., 2018).

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Disclosure statement

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