Original Article

Oxytetracycline Residues in Eggs From Commercial Poultry Farms in Ilorin City, Nigeria

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ABSTRACT

Background: The risk of indiscriminate antibiotic use in Nigeria is high and has serious public health and food implications. The unrestricted usage of oxytetracycline can lead to the accumulation of antibiotic residues in animal products, such as eggs.

Objectives: This study investigated oxytetracycline residues in eggs from 20 commercial poultry farms in Ilorin City, Nigeria.

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

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

Conclusion: This study revealed a high prevalence of oxytetracycline residues in eggs produced by commercial poultry farms in Ilorin City, Nigeria. It 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 (AMR).

Keywords: Food safety, Antibiotics, Public health, Tetracycline, Laying birds

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Introduction

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

Antibiotics are administered without proper oversight and are readily available to poultry farmers. This unrestricted usage leads to the accumulation of antibiotic residues in animal products, such as eggs, meat, and fish (Adetunji et al., 2012a; Adetunji et al., 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 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).

AMR is recognized worldwide, emphasizing the crucial role of reliable national surveillance systems. The continual use of antibiotics in veterinary medicine and other selective agents in livestock production settings could co-select for multidrug resistance among bacteria, which may persist longer in the environment (Mamza et al., 2017). Studies have indicated that consumption of antimicrobial residues via animal food products can lead to the transmission of resistant microorganism strains to humans, as well as disruptions in intestinal microflora and conditions, such as bone marrow depression, among other pathologies. (Jafari et al., 2007; Nisha, 2008). AMR can become the leading cause of mortality by 2050. Global estimates indicate that the deaths directly attributed to AMR surpassed 1.2 million in 2019 (Antimicrobial Resistance Collaborators, 2022). The worldwide mortality rate of AMR is anticipated to reach approximately 700000 annually. Without adequate measures to control AMR, this figure is expected to rise to over 10 million annually by 2050 (O'Neill, 2016). 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) is extensively used for the precise quantification of diverse antibiotic residues in food products, offering excellent sensitivity and specificity (Olatoye & Ehinmowo, 2010; Khorrami et al., 2022; Sadighara et al., 2024). Therefore, this study assesses the oxytetracycline residues in eggs, employing HPLC for precise quantification. The results of this study are expected to 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 conducted on randomly selected poultry farms in Ilorin City, Kwara State, North-Central Zone, Nigeria. Ilorin is a major commercial poultry hub in the north-central part of the country, serving other neighbouring cities and their environs. The study area, comprising Ilorin South, Ilorin East, Ilorin West, Moro, and Asa Local Government Areas (LGAs), was selected due to the 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 of poultry farmers in the state.

Sampling of eggs

Egg samples were aseptically collected from selected poultry farms in Ilorin City 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). The number of eggs available at each farm was numbered from the first to the last crate. The egg at the top left corner of the first crate was assigned the number one until the last egg in the bottom right corner. Ten eggs were randomly selected from the total number of eggs by simple random sampling. All samples were stored at -4 ˚C until they were transported to the laboratory for analysis (Kabir et al., 2004).

Preparation of reagents

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

Preparation of standard solutions

The actual weight (g) of the antimicrobial standard (oxytetracycline HCl) was determined using a weighing balance (Mettler Toledo®, Australia) and dissolved in 10 mL of methanol in a volumetric flask to produce a standard stock solution of 10 ppm. 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 0.25 ppm. These concentrations were injected into HPLC to obtain correspondent concentrations which were used to prepare the calibration curve.

Extraction

Solid-phase extraction (SPE) was carried out according to Olatoye and Saraye (2012). Ten egg samples were pooled and homogenized. Then, 5 g of the specimen was blended with 50 mL of 0.1 M Na_2EDTA-McIlvaine buffer (pH 4.0) and centrifuged at 4000 rpm. The supernatant was applied to a Bakerbond SPE C18 cartridge (JT Baker, Deventer, Netherlands), which was activated overnight with methanol and water. The cartridge was washed with 20 mL of water, and the analyte (residue) was eluted with 10 mL of 0.01 M formic-oxalic acid solution and collected in a 10 mL 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). The elution of oxytetracycline from the analyte was performed on a Nucleosil C18 (4.5×150 mm, 5 µm 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 volumes of the analytes from each sample were injected in duplicate to obtain the average peak area of the positive sample, which corresponded to the retention time of 10.02 to 10.32 minutes of the reference standard. Figure 1 shows the chromatogram of the sample.

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

Results

Assessment of oxytetracycline residues in eggs using HPLC

Out 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. In contrast, the highest (792.81±53.49 µg/kg) was observed with samples from a farm at Ilorin West LGA. Five other samples were below the detection limit of this method (0.001 ppm). In this study, six pooled egg samples (one from the Ilorin East, two from the Ilorin South, and three from the Ilorin West LGAs) had mean concentrations higher than the maximum residue limit (MRL) standard set by the Codex Alimentarius Commission 2023.

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

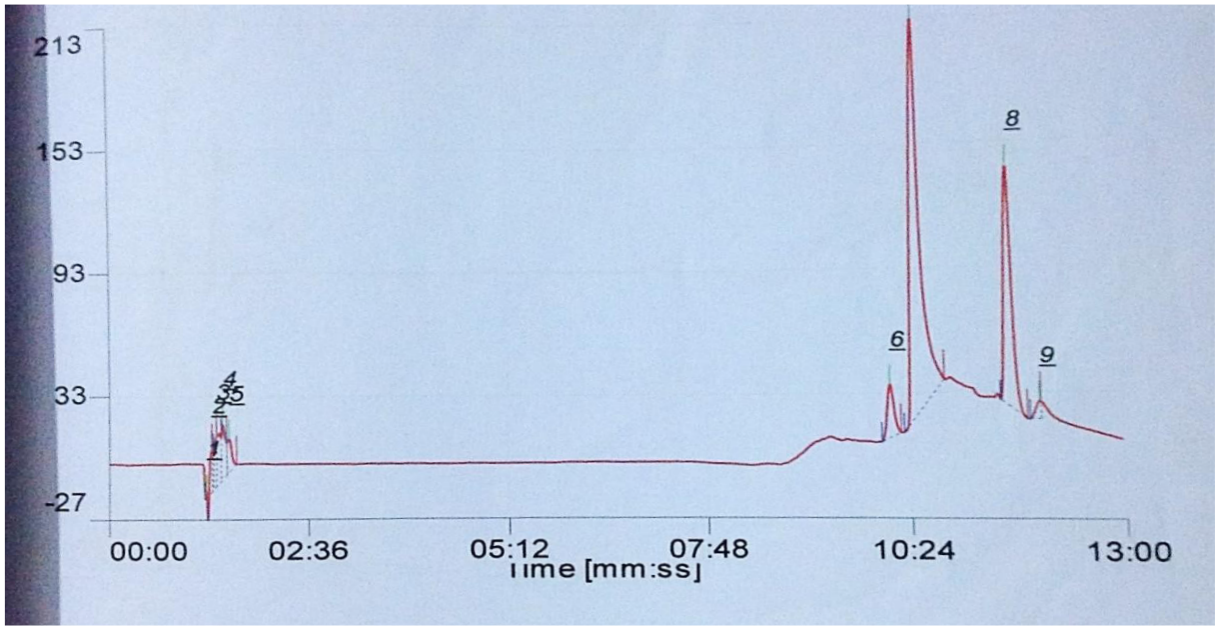

Figure 1. Chromatogram of oxytetracycline residue in egg samples

A total of 15 pooled egg samples (75% prevalence) contained oxytetracycline residues at concentrations ranging from 156.55 to 792.81 µg/kg. Forty percent of the positive samples contained oxytetracycline residues at levels higher than the recommended MRL.

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 400 µg/kg in eggs (Codex Alimentarius, 2023). The findings of this study high-

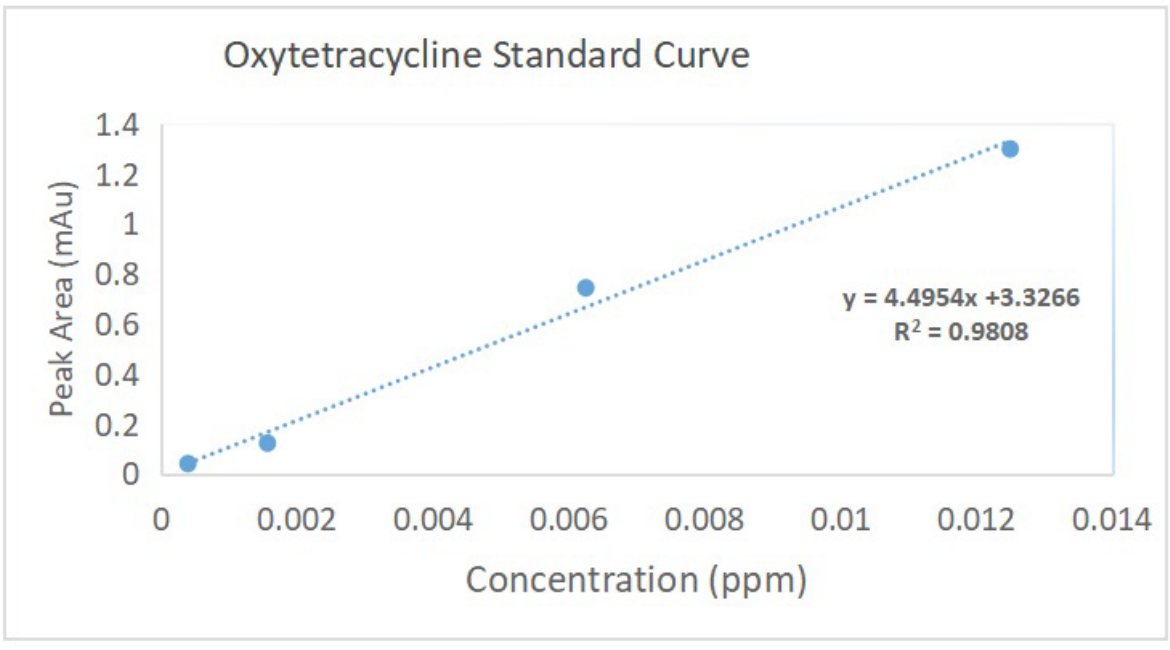

Figure 2. Calibration curve of oxytetracycline standard solution

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

Local Government Area	Sample ID	Mean±SD
		Concentration (µg/kg)
Ilorin South	AD	485.81±23.6
	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

BDL: Below detection limit.

lighted 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. The results showed a high residual presence of oxytetracycline (75%), with 30% of the pooled egg samples exceeding the acceptable maximum residue levels recommended for poultry eggs by the World Health Organization (WHO) and Food and Agriculture Organization (FAO). This underscores the continuous misuse of antibiotics in the region. Antimicrobial agents in food-producing animals have become a public health concern, especially in developing countries, where they are administered indiscriminately (Olatoye & Basiru, 2013). The analysis of oxytetracycline residues and other antibiotics used in poultry is crucial for consumer safety and is regularly monitored in developed countries. However, no such monitoring programe exists in Nigeria despite the unregulated use of these drugs in animals and the high prevalence of antibiotic residues in several foods of animal origin across the country (Olatoye & Saraye, 2012).

The prevalence of residues obtained in this study correlates with the findings of previous studies by Olatoye and Saraye (2012) and Olatoye and Basiru (2013), which showed that a greater proportion of commercial chicken eggs being consumed in Ilorin City, Nigeria could have oxytetracycline residues greater than the MRL, for which unregulated access and indiscriminate use of antibiotics by poultry farmers could be responsible. In Enugu state,

Nigeria, about 46% of egg-producing commercial farms tested positive for oxytetracycline residues (Ezenduka et al., 2011). The 75% of eggs containing oxytetracycline residues in our study were 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 between these results and our study could be due to the differences in the residue quantification methodologies used. Hind et al. (2018) used HPLC to analyze egg samples obtained from Khartoum State, Sudan, and obtained 35% of samples containing oxytetracycline residues above the maximum allowable limit.

This study used high-throughput SPE for HPLC quantitative analysis of oxytetracycline residues in eggs sold for human consumption in Ilorin City, Nigeria. This ensures 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 poultry farmers and the absence of government policies. The use of HPLC to quantify oxytetracycline residues in our study demonstrated the efficacy of this analytical method for detecting antibiotic residues in food products. However, this 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 AMR problem. Future studies should consider broader spectrum analyses to assess the extent of antibiotic residues in poultry products and their implications for public health. Our results 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 subsequent accumulation of residues in animal products. Addressing this issue requires comprehensive regulatory reforms, including stricter enforcement of antibiotic prescription requirements and enhanced monitoring of antibiotic use in poultry farming.

This study revealed the presence of oxytetracycline residues in eggs produced by commercial poultry farms in Ilorin City, Nigeria. Therefore, it is necessary to adequately control the use of veterinary drugs to protect the public. Also, education on the adverse effects of the 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 eliminate the implementation of regulations associated with antimicrobial administration in poultry production and monitoring programs (Awogbemi et al., 2018).

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Ilorin, Nigeria (Code: UIL/FVERC/003/2018).

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Authors' contributions

Conceptualizations and study design: Ibraheem Ghali-Mohammed and Ismail Ayoade Odetokun; Data collection, analysis, interception, writing, and final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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