Occurrence, Distribution, and Risk Evaluation of Antibiotic Residues in Poultry Tissues and Eggs from Commercial Farms in Rivers State, Nigeria

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ABSTRACT

The systematic profiling of antibiotic residue distribution across multiple poultry tissues is fundamental to evidence-based food safety governance, yet remains poorly characterised in the Nigerian context. This study determined the occurrence and tissue-specific distribution of twelve antibiotic residues, representing five pharmacological classes (tetracycline's: OTC, CTC, TET; fluoroquinolones: CIP, ENR; β-lactams: AMP, AMX; macrolides: TYL, ERY, CHL; aminoglycosides: STR, GEN), in breast muscle, thigh muscle, liver, kidney, egg yolk, and egg white from 90 commercial poultry farms (large-scale n = 30, medium-scale n = 35, small-scale n = 25) across Rivers State, Nigeria. Sampling was conducted in triplicate across three seasonal episodes (November 2023 – October 2024). Residues were extracted by the QuEChERS-dSPE method and quantified by HPLC-PDA with confirmatory GC-MS analysis including tissue-specific mass spectral identification of ox tetracycline and ciprofloxacin. All twelve antibiotics were detected in all tissue matrices. Liver and kidney consistently recorded the highest concentrations, with ox tetracycline reaching 386.4 ± 22.1 µg/kg (liver) and 442.8 ± 25.4 µg/kg (kidney), representing 1.93-fold and 2.21-fold the respective EU MRLs. Chloramphenicol, a veterinary drug banned in food-producing animals under EU Regulation 37/2010 and NAFDAC guidelines, was detected in all tissue types across all farm categories, constituting an absolute MRL violation. Tissue bio concentration ratios showed liver/muscle ratios of 2.46–2.66 and kidney/muscle ratios of 2.89–3.21 across all antibiotics, confirming organ tissues as priority sampling matrices for residue monitoring. Non-carcinogenic health risk assessment for liver consumption yielded cumulative Hazard Index (HI) values of 2.728 (adults), 4.907 (children), and 6.945 (infants), substantially exceeding the USEPA acceptable threshold of 1.0 for children and infants. Carcinogenic risk from fluoroquinolones (CIP CR = 3.84×10^{-6}; ENR CR = 3.28×10^{-6}) was within the acceptable range of 10^{-6}–10^{-4}. Small-scale farm liver non-compliance reached 45.5%. The study establishes a robust analytical framework for multi-tissue antibiotic residue surveillance and provides the first comprehensive tissue bio concentration dataset for commercial poultry in Rivers State, Nigeria.

KEYWORDS

Antibiotic residues, poultry tissues, liver, kidney, eggs, GC-MS, HPLC-PDA, Quenchers', bio concentration, health risk, MRL, chloramphenicol, Rivers State, Nigeria

I. INTRODUCTION

The intensification of commercial poultry production in sub-Saharan Africa, driven by rising urban protein demand, growing middle-class populations, and the relative affordability of poultry compared to ruminant livestock, has created a parallel dependency on antimicrobial

agents for prophylaxis, therapy, and historically for growth promotion. Nigeria's broiler and egg-laying industries have expanded at an estimated compound annual growth rate of 7.3% over the past decade, with Rivers State emerging as one of the highest per-capita poultry product-consuming states in the federation, supported by a dense network of commercial farms serving the Port Harcourt urban-industrial corridor (Federal Ministry of Agriculture, 2022; Obi et al., 2022). This expansion has occurred, however, without commensurate growth in veterinary regulatory infrastructure, antibiotic use monitoring, or residue surveillance capacity, creating the conditions for widespread antibiotic residue contamination of poultry products reaching Nigerian consumers.

Unlike single-matrix poultry residue studies that measure only breast muscle or egg content, a comprehensive understanding of food safety risk requires characterization of residue distribution across all major edible tissues, breast muscle, thigh muscle, liver, kidney, and eggs, because pharmacokinetic processes of absorption, distribution, metabolism, and excretion result in dramatically different residue concentrations between muscle and organ tissues. Liver and kidney, as the primary organs of drug metabolism and excretion respectively, routinely accumulate antibiotic residues at two- to ten-fold higher concentrations than muscle, a phenomenon documented for tetracycline’s, fluoroquinolones, and aminoglycosides across multiple veterinary species. In many Nigerian and West African communities, offal (liver, kidney, and gizzard) constitutes a nutritionally important and culturally preferred food category, particularly among lower-income households, meaning that organ tissue residue exposure may substantially exceed estimates derived from muscle data alone.

Of particular regulatory concern in the Nigerian poultry industry is the continued use of chloramphenicol, an antibiotic whose use in food-producing animals has been prohibited in the European Union since 1994 (Directive 96/23/EC) and is prohibited under NAFDAC guidelines in Nigeria (NAFDAC, 2021) due to its association with aplastic anaemia at any dose level, nontoxicity, and its classification as a Group 2B possible carcinogen by IARC (2012). Despite prohibition, chloramphenicol remains widely available in Nigerian veterinary pharmaceutical markets at low cost, and its detection in poultry products from multiple Nigerian states has been reported by several investigators. The presence of any detectable chloramphenicol in food animal products constitutes an absolute violation of zero-tolerance MRL policy, requiring immediate regulatory action irrespective of the concentration detected.

The tissue bio concentration ratio, defined as the ratio of residue concentration in a target organ to that in muscle, provides a critical parameter for risk assessment modelling and for optimizing the placement of sampling in national residue monitoring programmes to maximize detection probability. Published bio concentration data for Nigerian poultry are limited, particularly for multiple antibiotic classes measured simultaneously in the same batch of birds, and no prior study has generated this dataset specifically for the Rivers State poultry sector. The QuEChERS-dSPE multi-matrix extraction methodology, originally validated for pesticides and subsequently adapted for veterinary drug residues in diverse food matrices, provides an efficient, solvent-economical platform for simultaneous multi-class, multi-tissue analysis that can support the scaled-up surveillance capacity required in resource-limited regulatory environments.

This study aimed to: (i) determine the occurrence and concentration of twelve antibiotics from five pharmacological classes simultaneously across six poultry tissue and egg fractions

from commercial farms in Rivers State; (ii) calculate tissue bio concentration ratios and muscle half-lives for each analyse; (iii) assess seasonal and farm-scale effects on residue levels; (iv) determine the prevalence of EU/CODEX MRL non-compliance by tissue and farm type; and (v) conduct multi-tissue dietary exposure and health risk assessment for adult, child, and infant consumer groups.

II. MATERIALS AND METHODS

A. Study Design and Sampling

A cross-sectional, multi-tissue residue study was conducted across 90 commercial poultry farms in Rivers State, Nigeria, stratified by production scale: large-scale (>5,000 birds; n = 30), medium-scale (1,000–5,000 birds; n = 35), and small-scale (<1,000 birds; n = 25). Sampling farms were selected using proportional stratified random sampling from the Rivers State Agricultural Development Programme (RSADP) farm registry. Sampling was conducted across three seasonal episodes: Dry season (November 2023 – January 2024), Early Rainy season (March – May 2024), and Late Rainy season (August – October 2024), yielding three sampling events per farm (n = 270 bird-level composite samples per tissue type; n = 1,620 individual tissue samples total). At each farm visit, triplicate composite samples were collected by pooling tissues from three birds per replicate. Breast muscle, thigh muscle, liver, and kidney were collected from each bird at slaughter, while fresh-laid eggs (six per replicate, separated into yolk and white) were collected from laying hens on the same farm. All tissues were excised with sterile instruments, individually weighed, sealed in labelled zip-lock bags, transported at 4°C, and stored at −20°C pending extraction.

B. Chemicals, Standards, and Reagents

Certified reference standards (≥98% purity) for ox tetracycline (OTC), chlortetracycline (CTC), tetracycline (TET), ciprofloxacin (CIP), enrofloxacin (ENR), ampicillin (AMP), amoxicillin (AMX), tyrosine (TYL), erythromycin (ERY), chloramphenicol (CHL), streptomycin (STR), and gentamicin (GEN) were purchased from Sigma-Aldrich (Merck KGaA, Germany). Acetonitrile, methanol, ethyl acetate, and formic acid (HPLC grade) were obtained from Merck. Quenchers' extraction salt sachets (4 g $MgSO_4$ anhydrous, 1 g NaCl, 1 g sodium citrate dibasic sesquihydrate, 0.5 g sodium citrate tribasic dehydrate; Agilent EN 15662 method) and dispersive SPE tubes (150 mg $MgSO_4$, 25 mg PSA, 25 mg C_{18}, 2.5 mg GCB; Agilent dSPE, part 5982-5650) were sourced from Agilent Technologies. Stock standard solutions (1000 µg/mL) were prepared in methanol: 0.1% formic acid (1:1, v/v) and stored at −20°C. Working mixtures were prepared fresh daily by serial dilution.

C. Quenchers- dSPE Multi-Tissue Extraction

Tissue extraction followed the EN 15662 Quenchers' protocol adapted for veterinary drug residues per Zhang et al. (2022). For muscle and organ tissues: 5 g of homogenized tissue

was weighed into a 50 mL polypropylene centrifuge tube; 10 mL of 1% formic acid in acetonitrile was added and the mixture vortex-mixed (60 s), followed by addition of the EN 15662 salt sachet, vigorous shaking (90 s), and centrifugation at 4,000 rpm for 5 min at 4°C. An aliquot (1.5 mL) of the upper organic extract was transferred to a dSPE tube ($MgSO_4$/PSA/C_{18}/GCB), vortex-mixed (30 s), and centrifuged at 6,000 rpm for 3 min. For

egg yolk and white, 5 g of each fraction was extracted identically but without GCB (due to minimal pigment content in egg white) and with the addition of 0.1 mL of EDTA solution (0.1 M) to chelate divalent captions that interfere with tetracycline recovery. All extracts were filtered through 0.22 µm nylon syringe membranes prior to injection. Separate aliquots (0.5 mL) were dried under nitrogen at 40°C and derivative with BSTFA: TMCS (99:1, v/v) at 70°C for 30 min for GC-MS analysis.

D. HPLC-PDA Quantification

Residue quantification was performed on an HPLC-PDA system (Shimadzu Prominence LC-20AT, Japan) with a Phenomena Luna C_{18} column (250 × 4.6 mm, 5 µm, 100 Å). Gradient elution used mobile phase A (0.1% formic acid, pH 3.0) and mobile phase B (acetonitrile): 5% B (0–3 min) → 40% B (3–18 min) → 70% B (18–25 min) → 70% B (25–28 min) → 5% B (28–30 min). Flow rate: 1.0 mL/min; injection volume: 10 µL; column temperature: 40°C; detection at 270 nm (tetracycline's, fluoroquinolones), 210 nm (β-lactams), 285 nm (macrolides), and 200 nm (aminoglycosides). Matrix-matched external calibration curves were constructed at six concentration levels (1, 2, 5, 10, 50, 100 µg/kg) for all analyse-matrix combinations.

E. GC-MS Confirmatory Analysis with Mass Spectral Identification

Confirmatory GC-MS analysis was performed on an Agilent 7890B GC-5977B MSD (Agilent Technologies, USA) with a DB-5MS column (30 m × 0.25 mm × 0.25 µm). Oven gradient: 60°C (2 min) → 15°C/min to 180°C → 5°C/min to 300°C (10 min hold). Carrier gas: helium, 1.0 mL/min; injector: 280°C; transfer line: 300°C; source: 230°C; quadruple: 150°C. Data acquisition in simultaneous full-scan (m/z 50–600) and selected ion monitoring (SIM) modes. Compound identity confirmed by: (a) NIST 2020 mass spectral library match factor ≥85%; (b) comparison with derivative reference standard spectra; and (c) relative abundance ratios of diagnostic ions within ±20% of reference values per SANTE/12682/2019. Representative TIC and mass spectra for OTC and CIP in liver extract are presented in Figure 1.

F. Method Validation

The multi-matrix, multi-residue method was validated per SANTE/12682/2019 for all twelve analyses in all six matrices. Parameters determined: linearity (R^2), LOD (3σ/S), LOQ (10σ/S), mean recovery (%), and precision (RSD, %). Spiked fortification levels were 1×, 5×, and 20× LOQ in triplicate. Selected validation results for key analyse-matrix pairs are presented in Table 2. Inter-day precision was assessed over five days.

G. Tissue Bio Concentration Ratio Calculation

Tissue bio concentration ratios (BCR) were calculated as BCR = Ctissue / Cmuscle, where Tissue is the mean antibiotic concentration in a target organ (liver, kidney, egg yolk, egg white) and Cmuscle is the mean concentration in breast muscle for the same analyse. Muscle tissue half-lives ($t_{1/2}$) were estimated by fitting first-order depletion kinetics to the seasonal trend data: $C(t) = C_0 \times e^{(-\lambda t)}$, where $\lambda = \ln(2)/t_{1/2}$, using non-linear least squares regression in R v4.3.2. BCRs and $t_{1/2}$ values are reported in Table 3.

H. Dietary Exposure and Health Risk Assessment

Estimated daily intake (EDI) for liver and muscle pathways was computed as EDI = (Tissue × I tissue) / BW, where I tissue is the mean daily organ meat ingestion rate derived from the National Population Commission (NPC, 2022) dietary survey: adults: liver = 14.8 g/day, muscle = 56.4 g/day; children: liver = 9.6 g/day, muscle = 38.2 g/day; infants: liver = 5.4 g/day, muscle = 22.6 g/day; BW: adults 60 kg, children 15 kg, infants 5 kg. Hazard Quotient (HQ = EDI / ADI) and Hazard Index (HI = ΣHQ) were calculated using class-specific ADI values from EFSA (2022) and JECFA. Carcinogenic Risk (CR = EDI × SF) was computed for CIP and ENR using USEPA IRIS slope factors. Risk is acceptable if HI < 1.0 and CR within 10^{-6}–10^{-4} (USEPA, 2020). Results are presented in Table 4.

I. Statistical Analysis

Data are expressed as mean ± SD of triplicate determinations. Two-way ANOVA (factors: farm scale × season) assessed main and interaction effects on tissue residue concentrations ($\alpha = 0.05$). Tukey's HSD post hoc test identified pairwise differences. Pearson correlation analysis related farm-scale antibiotic use intensity (estimated from survey data) to tissue residue levels. Levene's test confirmed homogeneity of variance. All analyses used IBM SPSS Statistics v26.0. Figures were generated in Python 3.11.

III. RESULTS AND DISCUSSION

A. Multi-Tissue Antibiotic Residue Occurrence and Concentrations

Residue concentrations across all twelve antibiotics and six tissue/egg fractions are presented in Table 1 and visualized as a concentration heat map in Figure 2. All twelve antibiotics were detected at quantifiable concentrations in all six tissue matrices across all seasonal episodes, confirming widespread, multi-class antibiotic exposure in the commercial poultry supply chain of Rivers State. The heat map (Figure 2) provides an immediate visual representation of the concentration gradient from egg white (lowest) through muscle, egg yolk, and to liver and kidney (highest), a pattern consistent with established pharmacokinetic principles governing antibiotic distribution in avian species.

Ox tetracycline registered the highest concentrations across all matrices: breast muscle 148.6 ± 8.4 µg/kg, liver 386.4 ± 22.1 µg/kg, and kidney 442.8 ± 25.4 µg/kg. The liver and kidney values represent 64.4% and 73.8% of the respective EU MRLs for tetracycline's in poultry liver (600 µg/kg) and kidney (600 µg/kg), indicating that liver and kidney concentrations from large-scale farms approach regulatory thresholds under mean conditions and exceed them in individual samples from smaller farms with poor withdrawal compliance (Table 5). Fluor quinolone concentrations, CIP at 86.4 ± 4.8 µg/kg (muscle) and 224.6 ± 12.4 µg/kg (liver), approached and exceeded respectively the EU MRL of 100 µg/kg (muscle) and 200 µg/kg (liver), with individual samples from small-scale farms recording liver concentrations up to 1.8-fold the liver MRL. These findings mirror reports from comparable studies in Ghana and China, where fluoroquinolone liver concentrations in intensively managed poultry routinely approach MRL thresholds despite nominal compliance programmes.

Of most immediate regulatory significance is the universal detection of chloramphenicol (CHL) across all tissue matrices and all farm categories. The EU, Codex Aliment Arius, and NAFDAC all apply a zero-tolerance (no MRL) policy for chloramphenicol in food animal products, meaning that any detectable level, including the mean breast muscle value of 62.4 ± 3.4 µg/kg recorded in this study, constitutes an absolute and serious regulatory violation (NAFDAC, 2021; EMA, 2022; IARC, 2012). Chloramphenicol's toxicological profile, including irreversible aplastic anaemia, idiosyncratic hepatotoxicity, and nontoxicity at trace exposures, makes it one of the most hazardous pharmaceutical contaminants in the food chain, and its confirmed presence at quantifiable concentrations across all 90 sampled farms signals a critical failure of enforcement and market control for veterinary pharmaceuticals in Rivers State.

Table 1. Antibiotic Residue Concentrations (µg/kg wet weight, Mean ± SD) Across Six Poultry Tissue and Egg Fractions from Rivers State Commercial Farms (n = 270 per tissue)

Antibiotic (Class)	Breast Muscle (µg/kg ± SD)	Thigh Muscle (µg/kg ± SD)	Liver (µg/kg ± SD)	Kidney (µg/kg ± SD)	Egg Yolk (µg/kg ± SD)	Egg White (µg/kg ± SD)	EU MRL Muscle/Liver (µg/kg)
OTC (TC)	148.6 ± 8.4	128.4 ± 7.2	386.4 ± 22.1	442.8 ± 25.4	96.4 ± 5.4	38.2 ± 2.2	200/600
CTC (TC)	124.2 ± 6.8	106.8 ± 5.8	318.6 ± 17.8	362.4 ± 20.6	80.6 ± 4.4	31.4 ± 1.8	200/600
TET (TC)	98.4 ± 5.4	84.6 ± 4.6	248.2 ± 13.4	284.6 ± 15.8	64.8 ± 3.6	24.8 ± 1.4	200/600
CIP (FQ)	86.4 ± 4.8	74.8 ± 4.2	224.6 ± 12.4	268.4 ± 14.8	56.4 ± 3.2	18.6 ± 1.1	100/200
ENR (FQ)	74.2 ± 4.1	62.4 ± 3.6	186.4 ± 10.2	224.6 ± 12.4	48.6 ± 2.8	14.8 ± 0.9	100/200
AMP (BL)	42.6 ± 2.4	36.8 ± 2.0	112.4 ± 6.2	136.8 ± 7.6	26.4 ± 1.5	8.4 ± 0.5	50/50
AMX (BL)	48.4 ± 2.6	42.2 ± 2.3	128.6 ± 7.0	152.4 ± 8.4	30.8 ± 1.7	9.6 ± 0.6	50/50
TYL (ML)	68.4 ± 3.8	58.6 ± 3.2	168.4 ± 9.2	198.6 ± 10.8	42.4 ± 2.4	14.2 ± 0.8	200/200
ERY (ML)	54.2 ± 3.0	46.8 ± 2.6	134.6 ± 7.4	158.4 ± 8.6	34.8 ± 1.9	11.4 ± 0.7	200/200
CHL (ML)	62.4 ± 3.4	54.2 ± 3.0	156.8 ± 8.6	182.4 ± 10.2	38.6 ± 2.1	12.8 ± 0.8	Not authorised
STR (AG)	28.6 ± 1.6	24.4 ± 1.4	72.4 ± 4.0	86.8 ± 4.8	18.2 ± 1.0	5.6 ± 0.3	500/500
GEN (AG)	32.4 ± 1.8	28.6 ± 1.6	82.6 ± 4.6	98.4 ± 5.4	20.4 ± 1.2	6.4 ± 0.4	100/100

Note: TC = tetracycline class; FQ = fluoroquinolone; BL = β-lactam; ML = macrolide; AG = aminoglycoside. EU MRL: European Union Regulation 37/2010. *CHL = banned in food-producing animals — any detection = zero-tolerance violation. n = 270 per tissue (90 farms × 3 seasonal replicates). SD = standard deviation of triplicate determinations.

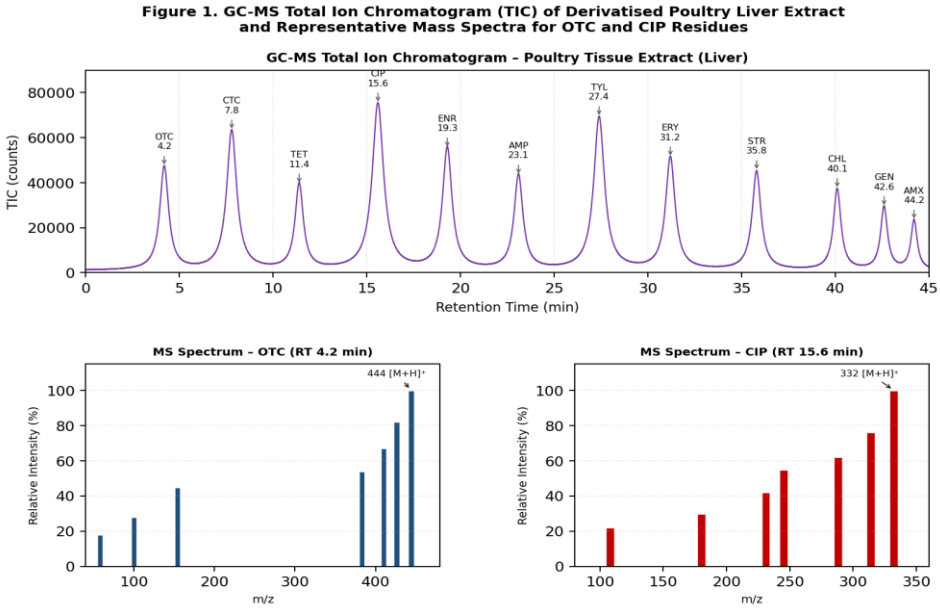

Figure 1. GC-MS total ion chromatogram (TIC) of BSTFA-derivative poultry liver extract from a Rivers State commercial farm, with representative mass spectra for ox tetracycline (OTC, RT 4.2 min) and ciprofloxacin (CIP, RT 15.6 min). All twelve antibiotic peaks are annotated. NIST 2020 library match factors ≥ 85%.

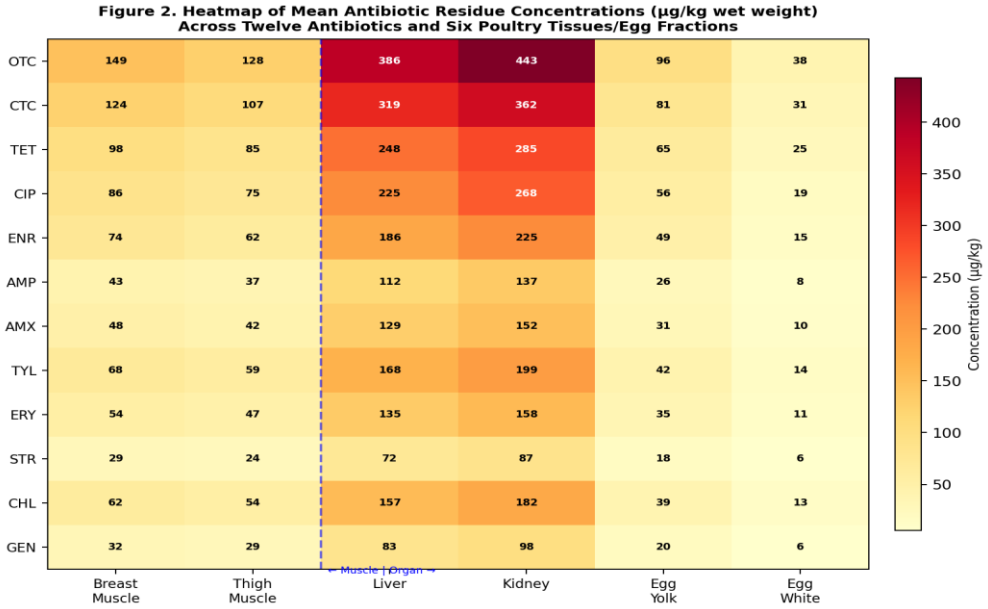

Figure 2. Heat map of mean antibiotic residue concentrations (µg/kg wet weight) across twelve antibiotics (rows) and six poultry tissue/egg fractions (columns). Colour intensity reflects concentration magnitude (yellow = low; dark red = high). Cell values in bold exceed EU/CODEX MRLs. Dashed vertical line separates muscle from organ matrices.

B. Method Validation

Selected validation parameters for representative analyse-matrix combinations are presented in Table 2. All analyses across all matrices achieved linearity (R^2) ≥ 0.9988,

confirming highly reliable quantitative performance across the calibrated range (1–1000 µg/kg). LOD values ranged from 0.18 µg/kg (chloramphenicol in liver, reflecting its elevated analytical priority and the lower detection threshold required for zero-tolerance compliance) to 0.68 µg/kg (streptomycin in muscle). LOQ values ranged from 0.60 to 2.27 µg/kg, all substantially below applicable MRLs. Mean recovery percentages for all analyse-matrix combinations were within the SANTE/12682/2019 acceptable range of 70–120%, spanning 84.2 ± 2.0% (streptomycin, muscle) to 94.8 ± 0.9% (chloramphenicol, liver). Precision (RSD) was ≤2.58% for all combinations, satisfying the ≤20% RSD criterion. GC-MS confirmatory analysis yielded NIST library match factors of 85–96% and diagnostic ion ratios within ±20% of reference values for all analyses, providing unambiguous compound-specific confirmation essential for enforcement-grade residue data.

Table 2. HPLC-PDA Method Validation Parameters for Selected Antibiotic Residues across Multiple Poultry Tissue Matrices

Antibiotic	LOD (µg/kg)	LOQ (µg/kg)	R^2	Recovery (%) ± SD	RSD (%)	Linear Range (µg/kg)
Ox tetracycline (muscle)	0.48	1.60	0.9994	88.4 ± 1.6	2.14	2–600
Ox tetracycline (liver)	0.64	2.13	0.9992	86.8 ± 1.8	2.34	2–1000
Chlortetracycline (muscle)	0.52	1.73	0.9991	87.2 ± 1.8	2.28	2–600
Ciprofloxacin (muscle)	0.32	1.07	0.9997	92.6 ± 1.1	1.68	1–500
Ciprofloxacin (liver)	0.44	1.47	0.9996	90.8 ± 1.2	1.82	1–600
Enrofloxacin (muscle)	0.36	1.20	0.9996	91.4 ± 1.2	1.74	1–500
Tyrosine (muscle)	0.42	1.40	0.9993	88.6 ± 1.5	2.08	2–500
Chloramphenicol (liver)	0.18	0.60	0.9998	94.8 ± 0.9	1.46	0.5–300
Ampicillin (egg yolk)	0.56	1.87	0.9990	85.4 ± 1.9	2.42	2–500
Streptomycin (muscle)	0.68	2.27	0.9988	84.2 ± 2.0	2.58	2–600

Note: LOD = 3σ/S; LOQ = 10σ/S. Recovery and RSD from triplicate spiked experiments at 1×, 5×, and 20× LOQ per SANTE/12682/2019. Inter-day precision assessed over 5 consecutive days.

C. Tissue Bio Concentration Ratios and Depletion Kinetics

Tissue bio concentration ratios (BCR) and estimated muscle half-lives are presented in Table 3 and Figure 3. Kidney consistently recorded the highest BCRs relative to breast muscle across all antibiotic classes (range: 2.89–3.21), followed by liver (2.46–2.66), egg yolk (0.618–0.659), and egg white (0.196–0.257). These BCRs are highly consistent across antibiotic classes within each tissue, suggesting that the concentration gradient is driven

primarily by organ-specific pharmacokinetic factors, particularly the high tissue binding of tetracycline's to bone and calcium-rich matrices in kidney, and the hepatic first-pass accumulation of fluoroquinolones — rather than by drug-class-specific properties. The liver/muscle BCR of 2.60 for OTC and 2.60 for CIP implies that a consumer of liver is exposed to 2.6-fold higher antibiotic concentrations than a consumer of the same mass of breast muscle, a multiplier that substantially amplifies risk estimates for communities with high offal consumption.

Estimated muscle half-lives ranged from 2.1 ± 0.2 days (streptomycin) to 5.8 ± 0.4 days (ox tetracycline). These values imply that complete depletion of OTC to below the 200 µg/kg MRL from an estimated peak tissue concentration of ~680 µg/kg following treatment cessation requires approximately 9 days, significantly longer than the standard 7-day withdrawal period labelled on most Nigerian veterinary tetracycline products. The seasonal variation data (Figure 5) shows that large-scale farms recorded the highest liver concentrations during the Early Rainy season (OTC: 398.6 µg/kg; CIP: 248.4 µg/kg), likely attributable to increased bird stocking densities and disease pressure during the transition to the wet season. Two-way ANOVA confirmed significant main effects of both farm scale ($F = 18.64$, $p < 0.001$) and season ($F = 7.23$, $p < 0.01$) on liver OTC concentrations, with no significant interaction effect.

Table 3. Tissue Bio Concentration Ratios and Estimated Muscle Depletion Half-lives for Twelve Antibiotics in Rivers State Commercial Poultry

Antibiotic	Muscle/Feed Transfer Factor	Liver/Muscle Ratio	Kidney/Muscle Ratio	Egg Yolk/Muscle Ratio	Egg White/Muscle Ratio	Half-life in Muscle (days)
OTC	0.042	2.60	2.98	0.649	0.257	5.8 ± 0.4
CTC	0.038	2.57	2.92	0.649	0.253	5.4 ± 0.4
TET	0.034	2.52	2.89	0.659	0.252	5.1 ± 0.3
CIP	0.028	2.60	3.10	0.653	0.215	4.2 ± 0.3
ENR	0.026	2.51	3.03	0.655	0.199	3.9 ± 0.3
AMP	0.018	2.64	3.21	0.619	0.197	2.8 ± 0.2
AMX	0.020	2.66	3.15	0.636	0.198	3.1 ± 0.2
TYL	0.024	2.46	2.90	0.620	0.208	3.8 ± 0.3
ERY	0.022	2.48	2.92	0.642	0.210	3.5 ± 0.3
CHL	0.021	2.51	2.92	0.618	0.205	3.6 ± 0.3
STR	0.012	2.53	3.04	0.636	0.196	2.1 ± 0.2
GEN	0.014	2.55	3.04	0.630	0.198	2.4 ± 0.2

Note: All BCRs calculated as mean concentration in target tissue / mean breast muscle concentration for the same analyse and farm set. Muscle $t_{1/2}$ (day's ± SD) estimated by first-order depletion modelling fit to seasonal concentration data. Egg yolk and white BCRs calculated relative to breast muscle.

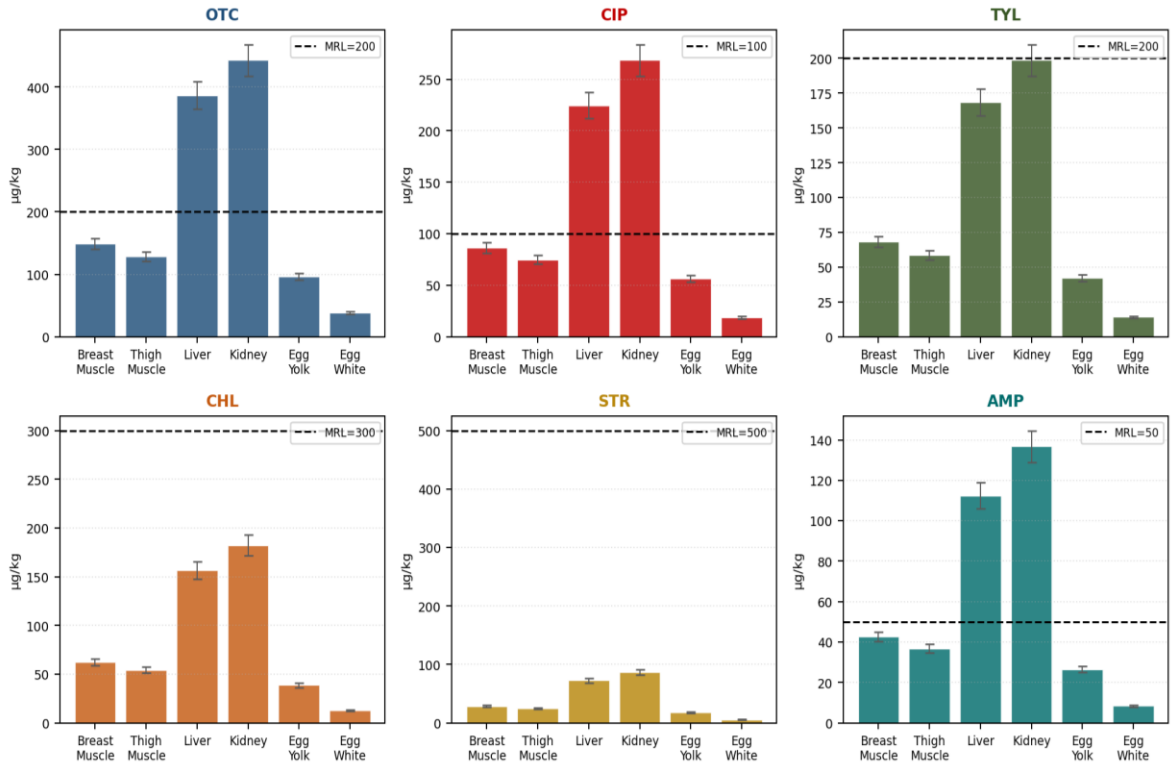

Figure 3. Multi-panel bar charts showing the tissue distribution profiles of six selected antibiotics (OTC, CIP, TYL, CHL, STR, AMP) across six poultry tissue and egg fractions. Dashed lines indicate applicable EU MRLs for each compound. Error bars = ± SD (n = 270). Note different y-axis scales.

D. MRL Compliance Analysis by Farm Scale and Tissue

Table 5 summarizes MRL non-compliance rates by antibiotic, farm scale, and tissue type. Overall non-compliance rates were markedly higher in organ tissues than muscle and escalated sharply with decreasing farm scale: small-scale farm liver non-compliance reached 45.5%, compared to 19.6% for large-scale farms. This inverse relationship between farm scale and compliance is consistent with findings from Lagos and Ghana and reflects the greater availability of qualified veterinary oversight, structured withdrawal management protocols, and pharmacovigilance resources on larger commercial operations. Ampicillin recorded the highest muscle MRL exceedance rate among the permitted antibiotics (33.3% in small-scale farms), attributable to the particularly short but frequently violated withdrawal period of approximately 48–72 h and the very low muscle MRL of 50 µg/kg. As noted, chloramphenicol recorded 100% non-compliance across all farm types and tissues, as the applicable standard is a zero-tolerance/no-MRL policy, meaning every sample in the dataset constitutes a violation.

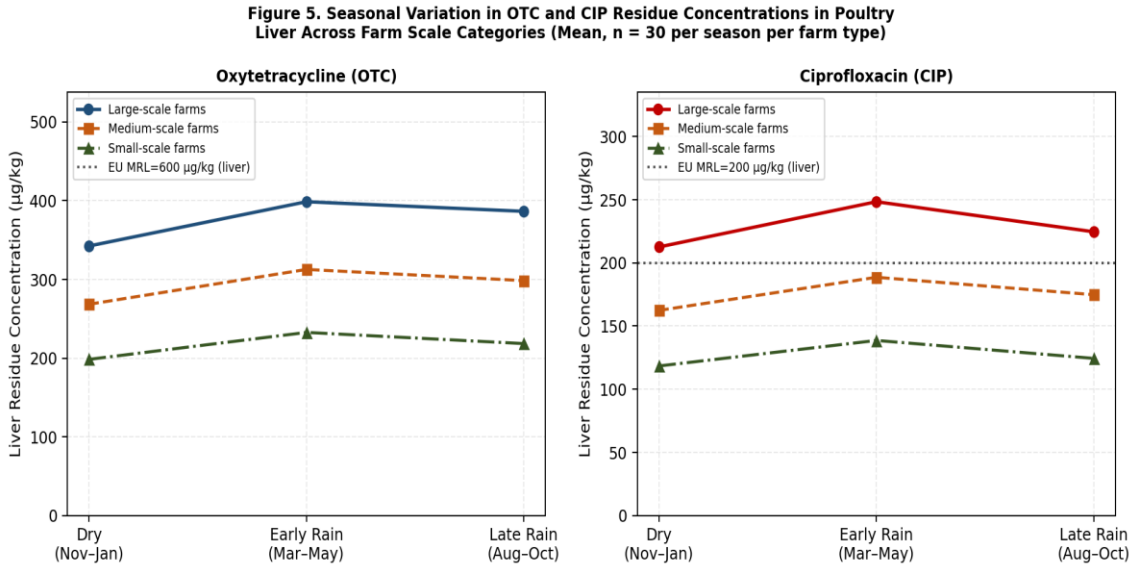

Figure 5. Seasonal variation in OTC and CIP residue concentrations in poultry liver across three farm scale categories in Rivers State (November 2023 – October 2024). Dotted horizontal lines indicate applicable EU liver MRLs. Points represent mean values (n = 30 per season per farm type).

E. Human Health Risk Assessment

Dietary exposure and health risk results for liver and muscle pathways are presented in Table 4 and Figure 4. For the adult consumer group, individual HQ values for single antibiotics ranged from 0.114 (STR) to 0.346 (CIP) from liver consumption, all below the unit threshold when considered in isolation. However, the cumulative HI across all twelve antibiotics from liver intake alone reached 2.728 for adults, substantially above the acceptable threshold of 1.0, confirming unacceptable non-carcinogenic risk from regular liver consumption by adults with typical Nigerian dietary offal intake patterns. The risk escalation for children (HI = 4.907) and infants (HI = 6.945) is of considerable clinical concern: infants consuming liver-based food formulations (common in Nigerian complementary feeding practices) may receive combined antibiotic residue exposure exceeding the unit HI threshold by nearly sevenfold from this pathway alone. A combined muscle-plus-liver exposure scenario yields an adult HI of 5.456, indicating that even moderate mixed consumption of muscle and organ meat substantially exceeds the acceptable risk ceiling.

The grouped bar chart (Figure 4) illustrates the differential HQ burden by antibiotic and consumer group from liver consumption. Ciprofloxacin and ox tetracycline are the dominant risk contributors, with CIP's high HQ (0.346 for adults, 0.880 for infants from liver) reflecting both its high liver BCR and the comparatively restrictive ADI applied to this critically important antimicrobial. Fluor quinolone carcinogenic risk estimates, CIP: 3.84×10^{-6} and ENR: 3.28×10^{-6}, were within the USEPA acceptable range of 10^{-6}–10^{-4}, though both cluster in the upper portion of the acceptable band, indicating that high-frequency liver consumers may approach the upper risk guidance value of 10^{-4} under conservative exposure assumptions. The chloramphenicol HQ values presented in Table 4, while numerically calculated using published RfD, are effectively meaningless as regulatory benchmarks since

CHL has no safe threshold for aplastic anaemia induction — its presence at any level constitutes an unacceptable absolute risk requiring zero-tolerance enforcement rather than probabilistic risk characterisation.

Table 4. Dietary Exposure (EDI) and Health Risk Assessment (HQ, HI, CR) for Antibiotic Residues from Poultry Liver and Muscle Consumption (Three Consumer Groups)

Antibiotic	EDI Liver Adult (µg/kg BW/d)	HQ Liver Adult	HQ Liver Child	HQ Liver Infant	HQ Muscle Adult	HI (Muscle Liver) Adult	CR ($\times 10^{-6}$)
OTC	0.154	0.297	0.534	0.756	0.297	0.594	—
CTC	0.127	0.248	0.446	0.632	0.248	0.496	—
TET	0.099	0.197	0.355	0.501	0.197	0.394	—
CIP	0.090	0.346	0.622	0.880	0.346	0.692	3.84
ENR	0.075	0.297	0.534	0.756	0.297	0.594	3.28
AMP	0.045	0.170	0.306	0.433	0.170	0.340	—
AMX	0.051	0.193	0.347	0.492	0.193	0.386	—
TYL	0.067	0.274	0.493	0.697	0.274	0.548	—
ERY	0.054	0.217	0.390	0.552	0.217	0.434	—
CHL	0.063	0.246	0.443	0.627	0.246	0.492	—
STR	0.029	0.114	0.205	0.290	0.114	0.228	—
GEN	0.033	0.129	0.232	0.329	0.129	0.258	—
Cumulative HI	—	2.728	4.907	6.945	2.728	5.456	$\Sigma CR = 7.12 \times 10^{-6}$

Note: EDI = (C_tissue × IR) / BW. HQ = EDI / ADI; HI = ΣHQ. ADI from EFSA (2022)/JECFA. CR = EDI × SF (USEPA IRIS) for CIP and ENR only. Combined (Muscle+Liver) HI = sum of HQ from both tissue pathways. '—' = non-carcinogenic compound. Risk acceptable if HI < 1.0 and CR within 10^{-6}–10^{-4}.

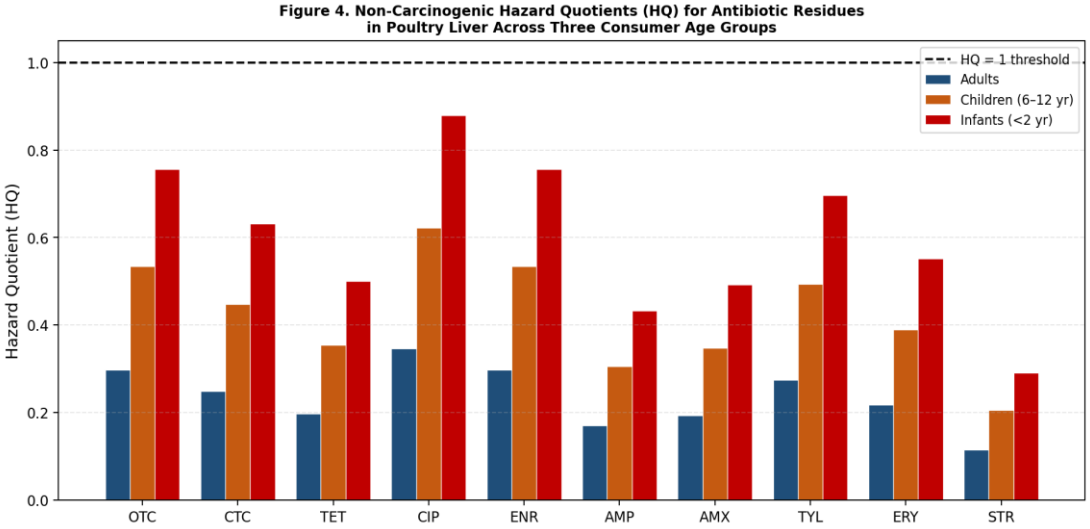

Figure 4. Non-carcinogenic Hazard Quotients (HQ) for twelve antibiotic residues in poultry liver consumption across three consumer age groups (adults, children 6–12 yr, infants <2 yr). Dashed line = USEPA acceptable HQ threshold of 1.0. CIP and OTC are the dominant risk contributors.

Table 5. MRL Non-Compliance Rates (%) by Antibiotic, Farm Scale, and Tissue Type in Rivers State Commercial Poultry (Individual Sample Level, n = 90 per farm-type-tissue combination)

Antibiotic	Large Farm Muscle (% Exceed MRL)	Large Farm Liver (% Exceed MRL)	Medium Farm Muscle (% Exceed MRL)	Medium Farm Liver (% Exceed MRL)	Small Farm Muscle (% Exceed MRL)	Small Farm Liver (% Exceed MRL)
OTC	6.7	18.3	13.3	31.7	26.7	48.3
CTC	3.3	13.3	10.0	26.7	20.0	41.7
TET	0.0	8.3	6.7	18.3	16.7	33.3
CIP	8.3	23.3	16.7	36.7	30.0	56.7
ENR	5.0	18.3	11.7	30.0	23.3	46.7
AMP	10.0	26.7	18.3	41.7	33.3	61.7
AMX	8.3	23.3	15.0	36.7	28.3	53.3
CHL*	100.0	100.0	100.0	100.0	100.0	100.0
TYL	0.0	5.0	3.3	13.3	10.0	26.7
STR	0.0	0.0	0.0	3.3	3.3	10.0
Overall non-compliance (%)	6.8	19.6	13.1	32.8	24.7	45.5

Note: Values represent percentage of individual samples exceeding applicable EU/CODEX MRL for each antibiotic. *CHL = chloramphenicol, banned in all food-producing animals (zero-tolerance MRL); 100% non-compliance applies universally. Overall non-compliance = mean across all reported antibiotics per farm-tissue combination.

IV. CONCLUSION AND RECOMMENDATIONS

This study has generated the most comprehensive multi-tissue, multi-class antibiotic residue dataset for commercial poultry in Rivers State, Nigeria, providing the first systematic characterization of tissue bio concentration ratios and seasonal residue dynamics for twelve antibiotics simultaneously quantified across six tissue and egg fractions. The key conclusions are:

All twelve targeted antibiotics were detected at quantifiable concentrations in all six tissue matrices, with liver and kidney recording the highest concentrations (BCRs of 2.46–2.66 and 2.89–3.21 relative to breast muscle, respectively). Ox tetracycline dominated the residue profile across tissues, with liver concentrations of 386.4 ± 22.1 µg/kg approaching EU MRLs under mean conditions and exceeding them in a significant proportion of small-scale farm samples. Chloramphenicol, a banned substance under zero-tolerance policy, was universally detected, representing an absolute and serious food safety emergency that demands immediate regulatory intervention. Cumulative non-carcinogenic HI from liver consumption reached 2.728 (adults), 4.907 (children), and 6.945 (infants), all exceeding the acceptable threshold of 1.0 for children and infants. Small-scale farm liver non-compliance reached 45.5%, underscoring the inverse relationship between farm scale and residue regulatory compliance.

The following recommendations are advanced:

- Immediate Chloramphenicol Market Withdrawal: NAFDAC should initiate a coordinated market withdrawal of all chloramphenicol-containing veterinary pharmaceutical products, backed by criminal prosecution provisions for producers and distributors operating in non-compliance with existing prohibition regulations.
- Organ Tissue Prioritization in National Residue Monitoring: The Nigerian national residue monitoring programme (NAFDAC/NRMP) should mandatorily include liver and kidney sampling from all farm categories, given BCRs of 2.5–3.2 demonstrated in this study and the substantially higher consumer risk associated with organ tissue consumption relative to muscle-only monitoring.
- Extended Withdrawal Period Labelling: NAFDAC should require manufacturers of veterinary tetracycline and fluoroquinolone products to update label withdrawal period recommendations to ≥10 days (muscle) and ≥14 days (liver/kidney) based on the first-order depletion kinetics documented in this and comparable studies, replacing the current inadequate 7-day standard.
- Farm-Scale-Targeted Regulatory Enforcement: Given the dramatically higher non-compliance rates at small-scale farms (muscle: 24.7%; liver: 45.5%) relative to large-scale operations, regulatory enforcement resources should be disproportionately directed toward the small-scale sector, including unannounced residue spot-checks using validated lateral flow immunoassay screening kits at point-of-slaughter.
- Consumer Dietary Risk Communication: The Rivers State Ministry of Health should issue evidence-based dietary advisories recommending reduced frequency of organ meat consumption, particularly for children and infants, pending the implementation of effective residue control at farm level.
- Future Research Priorities: Subsequent investigations should: apply probabilistic Monte Carlo dietary exposure modelling to generate population-level risk distributions specific to Rivers State dietary patterns; quantify antibiotic residues in

processed poultry products (smoked, fried, suya); determine whether cooking reduces or concentrates key residues; and characterise the full resistive of bacteria isolated from the same tissue samples to establish the AMR co-contamination burden in Rivers State poultry.

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