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Occurrence and levels of multiple veterinary antibiotics in broiler chicken processed and sold in Southern Mozambique

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Antibiotic residues in chicken meat pose a health risk to the general public, particularly to sensitive consumers. The aim of this study was to determine the level of antibiotic residues in on-the-market dressed chicken in the Southern Region of Mozambique. A total of 160 samples were randomly collected from eight markets located in five districts in the provinces of Maputo, Gaza, and Inhambane. The residues of β -lactams, quinolones, tetracyclines, and sulfonamides in chicken breast muscle were separated, identified, and quantified using high-performance liquid chromatography (HPLC), coupled with ultraviolet light (UV-VIS). Results revealed that 26.9% of the analyzed samples displayed detectable antibiotic residues, with 14.4% classified as mono-contaminated and 12.5% as poly-contaminated. Oxytetracycline prevailed (19.4%) and had the highest concentration (84–636 $\mu\text{g}/\text{kg}$), followed by trimethoprim (9.4%) and sulfamethoxazole (7.5%). Although the majority of samples adhered to the criteria established by Codex and EU, 100% of positive sample, exceeded the maximum residue limits for trimethoprim, with an average contamination level of 197 $\mu\text{g}/\text{kg}$. The presence of antibiotics in chicken breast meat at levels above maximum residue limits represents a risk to public health, reflecting even higher concentrations in risky parts like gizzard and liver, which are delicacies in Mozambique. This study suggests the implementation of a rigorous monitoring system across the chicken value chain (including farms, transportation, and slaughter facilities) to prevent the indiscriminate use of antibiotics in chickens.

KEYWORDS

antibiotics, broiler, chicken breast muscle, HPLC, Mozambique, UV-VIS

1 Introduction

In chicken production, antibiotics are used to prevent, treat, and control infectious diseases. Globally, 63 MT of antibiotics are annually used in animal production. Nearly 80% of these antibiotics are used to treat or prevent diseases, and some proportions are used as growth promoters (Mulchandani et al., 2023; Van Boeckel et al., 2015). Consequently, a Tanzanian study found that 60% of farmers use antibiotics for disease prevention, 26% for growth promotion, and 14% for disease treatment (Mdegela et al., 2021). In 2017, 93,309 tons of veterinary

antimicrobials were mainly (93.8%) used for animals (chickens, cattle, and pigs), which is estimated to increase by 11.5% to 104,079 tons by 2030 (Mulchandani et al., 2023; Tiseo et al., 2020). This illustrates that on average 68 mg/population correction units (PCU) of antimicrobials are employed in chickens, representing a 33% global increase in antimicrobial intake. Compared to other continents, Africa used a low level of antimicrobials (4,606 tons); by 2030, it is expected to increase by 37%, accounting for 6.1% of global use.

Efficient animal husbandry will reduce antibiotic use as chickens experience a low prevalence of diseases. With limited use of such practices in developing countries like Mozambique, where chickens are raised in confinement/backyards with poor facilities, unsanitary conditions, improperly stored feed, and minimal disease control (Makary et al., 2018; Ndlovu et al., 2024; Shanab and Amer, 2025; Yami et al., 2024), outbreaks of animal diseases are widespread and frequent, indicating that the use of antimicrobials for prevention and treatment will increase (Klaharn et al., 2022; Li et al., 2024).

Unfortunately, the majority of smallholder farmers lack knowledge of GAHP. Indiscriminate use of antibiotics is most likely because farmers prioritize the health of chickens over the safety of poultry and its products. Lack of compliance with good animal management practices (GAHP), treatment regimes, and drug withdrawal periods is a major cause of antimicrobial residues in meat and poultry products (Arafa et al., 2024; Makary et al., 2018; Ramtahal et al., 2022). Overuse of antibiotics impairs the liver's capacity to process drugs, leaving animals with insufficient drug elimination (Caneschi et al., 2023; Islam et al., 2023; Tarantino and Citro, 2024; Thapa, 2021).

Antibiotic residues in food may cause gastrointestinal problems, hypersensitivity reactions, tissue damage, and neurological disorders (McKenna, 2017; Osorio et al., 2023; Ramatla et al., 2017), as well as cancers and obesity (Arafa et al., 2024; Lu et al., 2022; Oladeji et al., 2025). Tetracycline residues in meat are associated with allergic reactions in people and the emergence of resistant bacterial strains in the digestive system of chickens (Arafa et al., 2024; Buczkowska et al., 2025; Mylostyyvi et al., 2025). In addition, the widespread and excessive use of antibiotics in the food chain may lead to antimicrobial resistance in some microorganisms. On the one hand, antimicrobial resistance genes and antibiotic-resistant bacteria can be transmitted from food to humans through contact or ingestion of animal products (Arafa et al., 2024; Endale et al., 2023; Jammoul and El Darra, 2019; Osorio et al., 2023). On the other hand, antibiotic residues contribute to antibiotic resistance in pathogenic bacteria, reducing the effectiveness of antibiotics in treating and curing diseases (Bitas and Samanidou, 2018; Manyi-Loh et al., 2018; Sabeq et al., 2022).

Currently, there are no effective decontamination techniques for antibiotic residues in meat and poultry products. However, high heat and refrigeration can inactivate antibiotic residues (Elbayoumi et al., 2018; Kamouh et al., 2024). Moreover, sterilization, activated charcoal, resins, and UV irradiation, heating, and refrigeration can break down oxytetracycline, penicillin, macrolide, and sulfonamide residues, but they do not completely remove them (Rana et al., 2019; A. A. Sani et al., 2023). Apparently, cooking does not change the residual state of oxytetracycline (Abd El Razik et al., 2025; Elbayoumi et al., 2018; Ferdous et al., 2020; Kamouh et al., 2024).

Therefore, to ensure public health, antibiotic residues in meat and meat products should be regularly examined (Mylostyyvi et al., 2025; Ndlovu et al., 2024; Vostrikova et al., 2025). The public health risk of antibiotic residues is significant in developing countries where awareness of stakeholders and regulatory control along the food value chain are inadequate (Ali et al., 2024; Nonga et al., 2013).

Mozambique has limited chicken meat antibiotic residue quantification studies. A previous study using the ELISA technique found fluoroquinolone in 6.7% of broiler chicken meat from industrial slaughterhouses in Maputo, while tetracyclines were detected below the maximum advised limit (Cumbula, 2017). Therefore, this study used HPLC coupled with the ultraviolet (UV-VIS) technique to determine the residue concentrations of amoxicillin, trimethoprim, ciprofloxacin, oxytetracycline, chlortetracycline, sulfamethoxazole, and sulfadiazine in the breast muscle of broiler chickens from Southern Mozambique. Additionally, the study compared the results with the maximum residue limits (MRLs) set by the European Union (EU) and the Codex Alimentarius Commission. The Mozambican Standard (No. 440/2013) advises using the Codex Alimentarius MRLs. In this context, we hypothesized that a significant proportion of chicken meat samples from local markets may contain antibiotic residues (oxytetracycline, chlortetracycline) above the EU and Codex MRLs due to antibiotic use as growth promoters and insufficient adherence to withdrawal periods. The findings of this study will serve to elucidate the safety of chicken meat in terms of antibiotic residue levels compared to the recommended MRLs, thereby contributing to the monitoring of antibiotic residues in broiler chicken meat processed and sold in Southern Mozambique markets.

2 Materials and methods

2.1 Study area

The study area was the Southern Region of Mozambique, which includes the capital (Maputo) and two other provinces (Figure 1). The areas are major producers of broiler chicken (Southern Mozambique accounts 77.1%) and contain big markets where chickens are slaughtered and dressed (Chunga et al., 2025). Laboratory analysis was conducted in the Chemistry Laboratory, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Morogoro, Tanzania.

2.2 Research design

A cross-sectional study was carried out from October 2023 to April 2024 in the urban and peri-urban districts of KaNhamakulu, KaMavota, KaMubukwana, Xai-Xai, and Massinga. Mozambique's southern region has 43 districts. The National Directorate of Animal Health provided a list of broiler farmers, with five districts purposefully selected as the largest producers of broiler chicken. Eight markets were also purposefully selected from the list provided by the National Directorate of Commerce. The market inclusion

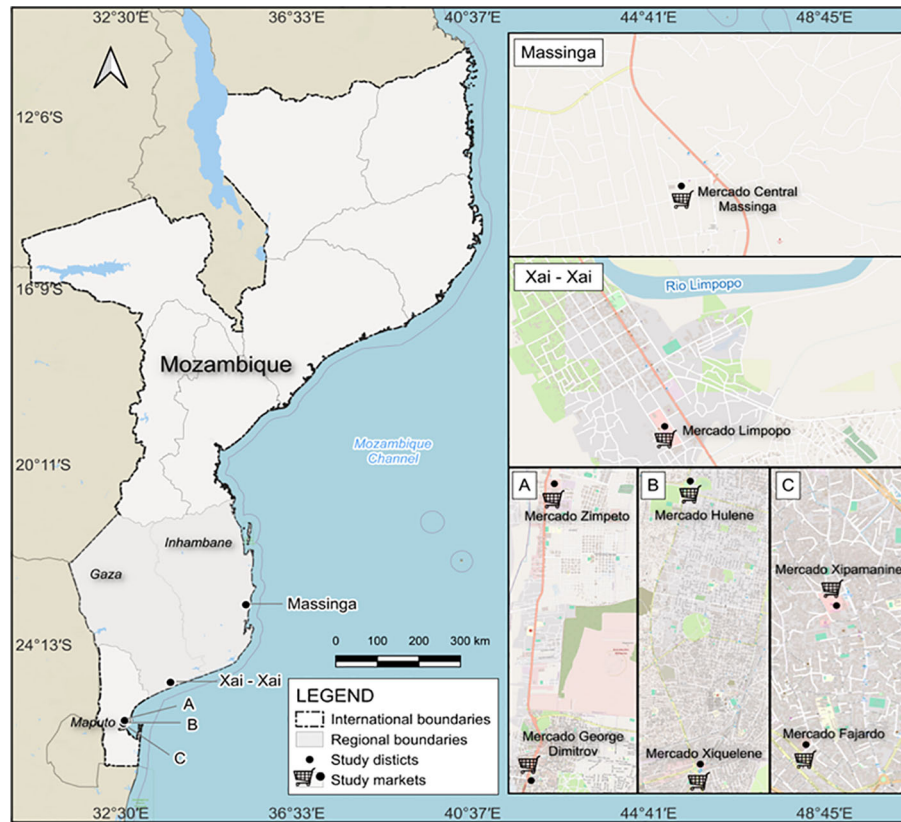


FIGURE 1

Map of the sampled retail markets across Southern Mozambique. The map illustrates the study area covering the provinces of Inhambane, Gaza, and Maputo City/Province: (A) Northern Cluster (Maputo): Represents Mercado Zimpeto and Mercado George Dimitrov; (B) Central Cluster (Maputo): Indicates Mercado Hulene and Mercado Xiquelene and (C) Southern Cluster (Maputo): Includes Mercado Xipamanine and Mercado Fajardo. Regional Markets: Mercado Central Massinga (Inhambane Province) and Mercado Limpopo (Gaza Province) are identified within their respective provincial boundaries. Cartographic Elements: Black circles denote the study districts within the three provinces. Wheelbarrow icons indicate the specific markets surveyed. For orientation, a North Arrow is provided in the upper left, and a Scale Bar (300 km) is included in the lower right to indicate regional geographical proportions.

criteria required that broiler chickens be processed and sold. The eight selected markets are referred to as M1 through M8 (Figure 2).

2.3 Ethical clearance

The Vice Chancellor of Sokoine University of Agriculture (SUA), Tanzania, was requested for a research permit, and the Directorate of Postgraduate Studies, Research, Technology Transfer and Consultancy (DPRTC) of SUA (reference: SUA/ADM/R.1/8/1070) authorized the conduct of the research. The study was also approved by the Ministry of Agriculture and Food Security of Mozambique, through the National Directorate of Animal Health (Note: number 1635/SAECM/DAP/11/23) and the Municipal Directorate of Markets and Fairs (Ref: number 84/2023). Prior to data collection, each participant gave their verbal and informed consent after the processors were informed of the purpose of the study.

2.4 Sample size

The overall sample size was determined using the formula for prevalence in a large population (Equation 1). This design adheres to the standards specified in ISO 2859-1:1999/Amd 1:2011, which specifies sampling strategies based on the Acceptance Quality Limit (AQL) for lot-by-lot inspection. This method was selected to

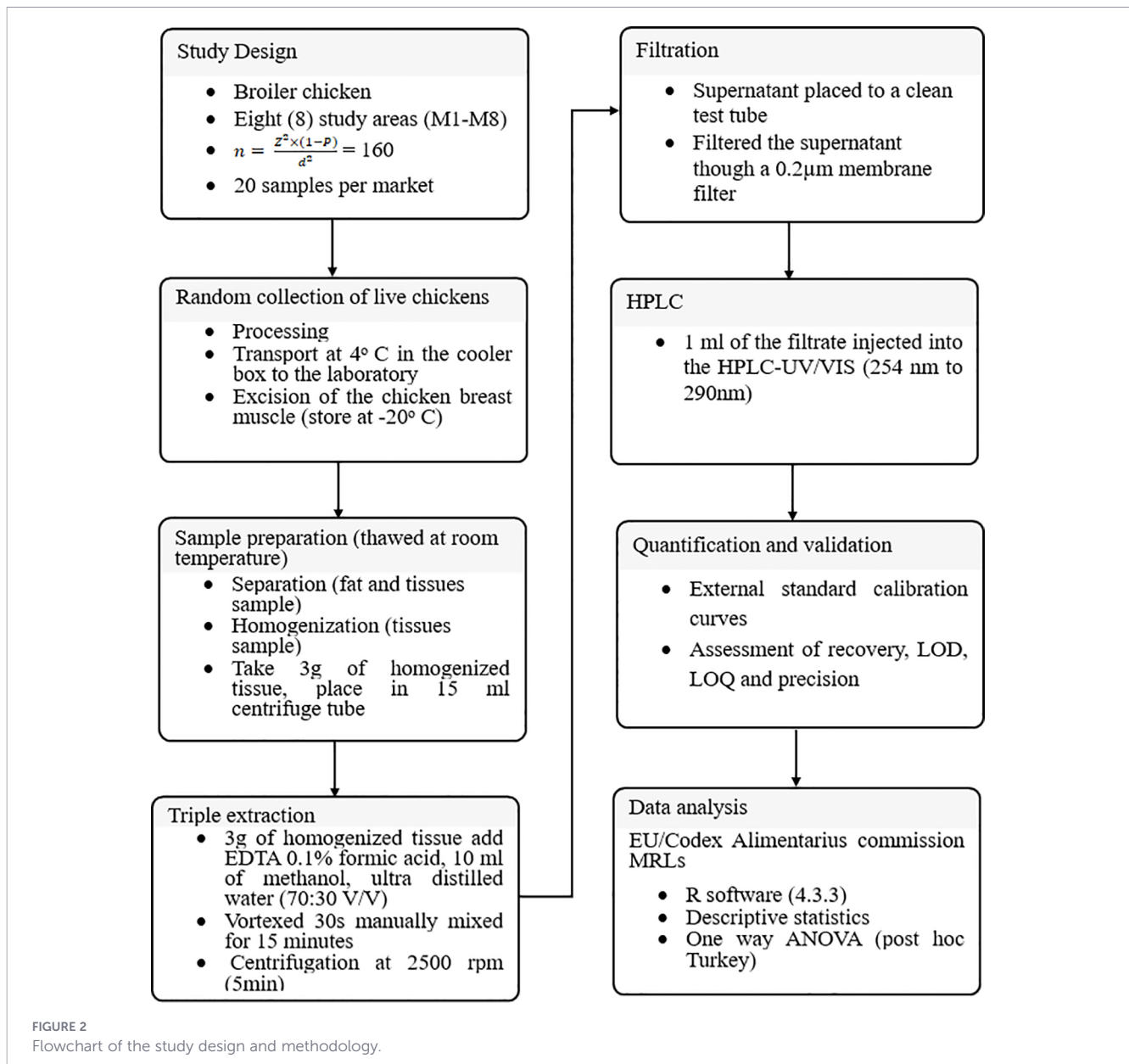
provide a robust framework for sampling techniques involving unknown lot sizes, with the objective of evaluating antibiotic residues in food products (Standardization, 1999).

$$n = \frac{Z^2 \times P(1 - P)}{d^2} \quad (1)$$

Where n is the sample size, Z is the confidence level (1.96 for a 95% confidence interval), and P is the expected prevalence. Due to the lack of previous empirical data for this region, P was established at 0.5 (50%) to maximize the sample size, and d the margin of error was defined at 0.08 (8%). The calculation resulted in a minimum necessity of 151 samples. This study collected 160 samples (20 from each of the eight markets) to enhance the statistical power and guarantee equitable representation across all sampling points.

2.5 Data collection

A total of 160 samples were obtained from markets spread across the five districts (Figure 1). Twenty broiler chickens were randomly sampled from each market's vendors prior to slaughter. Following the slaughtering process, the resulting carcasses were immediately placed into specific, sterile, plastic bag and labeled properly. After that, the samples were stored in an isothermal cool box maintained between 4°C and 10°C and transported to the laboratory within less than 4 hours



of collection. Upon arrival at the laboratory, 200 g of breast muscle was aseptically excised from each carcass. Performed using sterilized surgical scissors and forceps to prevent cross-contamination. The excised tissue was immediately transferred into a new, sterile, labeled airtight zip-lock bags, ensuring no contact with the non-sterile exterior and were stored at -20° C until further processing. All procedures were conducted following aseptic techniques to ensure the representativeness of the chemical profiles.

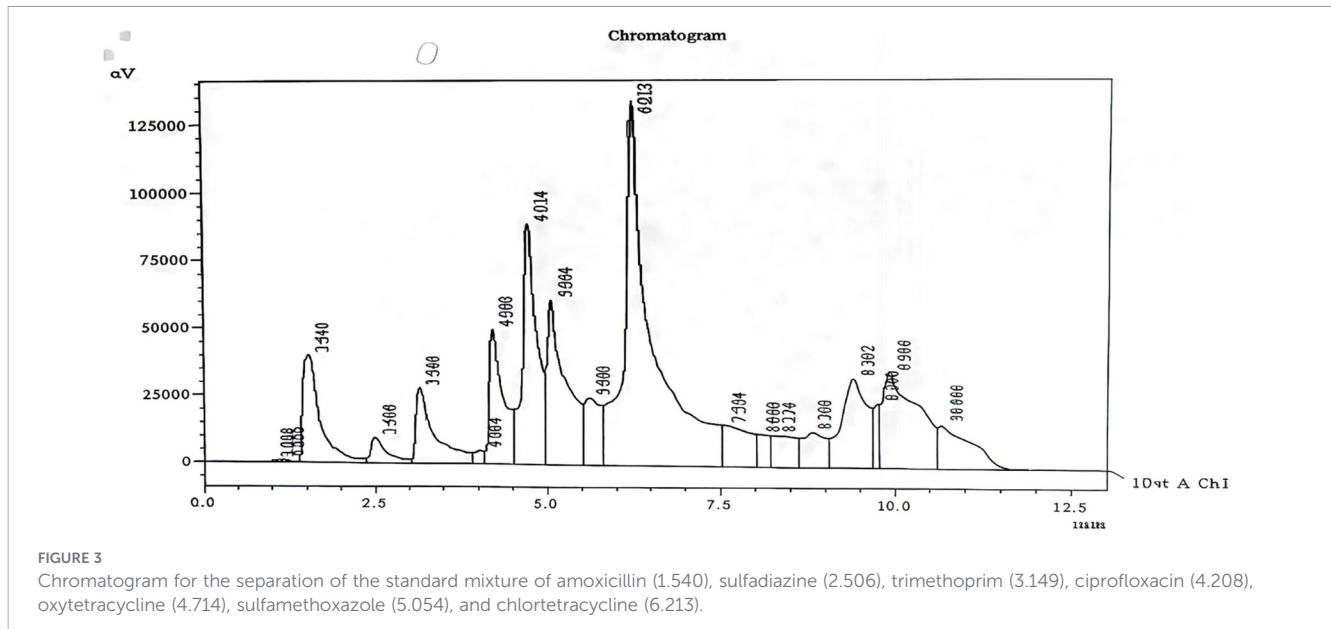
2.6 Reagents and equipment

HPLC-grade methanol (Central Drug House Ltd., New Delhi, India, 99.8%), analytical grade formic acid (Sigma Aldrich, St. Louis, MO, USA, 98%), water for HPLC (Fisher Scientific, Waltham, MA, USA), and acetonitrile (Alpha Chemika Ltd.) were used. Analytical grade ammonium formate (Honeywell Ltd., Mumbai, India), citric acid monohydrate (Griffen Fire Chemicals Ltd.), disodium EDTA (Sisco Research Laboratories Pvt. Ltd.), lead acetate (Loba Chemie Ltd.), and

disodium hydrogen phosphate (Span Sc Chemie) were also used. Analytical standards of amoxicillin trihydrate, trimethoprim, sulfadiazine, oxytetracycline hydrochloride, sulfamethoxazole, ciprofloxacin, and chlortetracycline hydrochloride (Sigma Aldrich, USA) with purity above 99% were used. Chromatography was performed using the Shimadzu high-performance liquid chromatography (HPLC) system, comprised of a Prominence degasser (DGU-20A), a binary pump (LC20AD), a Prominence autosampler (SIL-20AHT), a thermostatted column compartment (CTO-20A), and a UV/VIS detector (SPD-20A).

2.7 Antibiotic standard preparations

Stock solutions were prepared by dissolving each analytical standard in methanol and ammoniated methanol for ciprofloxacin (MeOH), making 1,000 mg/L (Berendsen et al., 2015; Lakew et al., 2022). Stock solutions were stored at -20°C and utilized within 3 weeks of preparation. Working standard solutions were prepared by



diluting the stock standard solutions (Cetinkaya et al., 2012) with the mobile phase consisting of HPLC water and methanol at a ratio of 80:20 v/v% (A. A. Sani et al., 2023).

2.8 Optimization of the HPLC method

Chromatographic conditions were optimized to improve the separation, sensitivity, and selectivity of the analytes. Separations were performed on a Thermo Scientific Acclaim 120 analytical column: C18 (4.6 mm × 150 mm; 5 μm particle size). The aqueous phase used HPLC water, whereas the organic phase used methanol. Various concentrations of methanol, formic acid, and ammonium forms, as well as flow rate, were adjusted to improve chromatographic resolution and peak shapes while reducing total analysis time (Patyra et al., 2020; Sharkawi et al., 2024). The analytes were separated using gradient elution with HPLC water as mobile phase (A) and methanol as mobile phase (B), both of which contained 2 mM of ammonium and 0.16% formic acid.

The elution gradient was 20% B for 1 min (0–1 min); then increased to 45% B in 5 min (1–5 min), 68% B in 2.5 min (5–7.5 min), and 90% B for 1.5 min (7.5–8 min); was kept constant for 1.5 min (8–9.5 min); reduced to 20% B in 0.5 min (9.5–10 min); and allowed to settle for 3 min. Different flow rates (0.5 to 1.5 mL/min) were tested, and the optimal flow rate was determined to be 1.5 mL/min. The injection volume used was 10 μL, with a column temperature of 40°C (Hong et al., 2024; Oyedeji et al., 2021). The retention times for amoxicillin, sulfadiazine, trimethoprim, ciprofloxacin, oxytetracycline, sulfamethoxazole, and chlortetracycline were 1.5, 2.5, 3.1, 4.2, 4.7, 5.1, and 6.2, respectively (Figure 3).

Furthermore, different wavelengths were tested to determine the high detection of UV/VIS detectors ranging from 254 to 290 nm (Jansen et al., 2019; Oyedeji et al., 2021), with good peak separation at 280 nm. Data were processed with the LC solution program. Figure 2 depicts a representative chromatogram for the analyte.

2.9 Establishment of the calibration curve

Calibration standards were prepared using a standard mixture with different concentrations at nine concentration levels (30–270

μL); each level was prepared in three replicates and diluted using mobile phase (HPLC water and methanol in a ratio of 80:20 v/v%) to determine repeatability and precision of the detection method developed. The curves were best fitted using a least squares linear regression model, $y = mx + b$, in which y is the peak area of the analyte, m is the slope of the calibration curve, b is the y -axis intercept of the calibration curve, and x is the analyte concentration (Figure 4). Linearity, limit of detection (LOD), and limit of quantitation (LOQ) are presented in Table 1.

The LOD and LOQ for each standard were determined using the calibration curves and associated linear regression equations, based on the linear correlation between the HPLC response y and the concentration of the standard inside the model. The sensitivity of b , LOD, and LOQ can be articulated by the equation. Therefore, the subsequent formulae ($\text{LOD} = 3\text{Sa}/b$, $\text{LOQ} = 10\text{Sa}/b$) can be employed to mathematically define the LOD and LOQ, where Sa represents the standard deviation of the instrument response, determined by the standard deviation of either the y -intercepts of the regression lines or the y residuals, and b means the slope of the calibration curve. When sample concentrations exceeded the top limit of the calibration curve, the samples were diluted and reanalyzed.

The accuracy and precision of the extraction were assessed by recovery tests by introducing known concentrations of reference standards to unexposed chicken samples to antibiotic treatment. Samples were spiked at three specific concentrations (25, 50, and 100 μg/kg) in accordance with the MRL for various antibiotics and conducted in triplicate. The spiked samples were incubated in the dark for 12 h after being agitated in a swirling water bath at 10°C for 30 min. The recovery percentage (%R) of the analytes was calculated as specified in Equation 4 below:

$$\%R = \frac{\text{Concentration Found} - \text{Concentration Unspiked}}{\text{Concentration Added}} \times 100 \quad (4)$$

Where C_F represents the concentration detected in the spiked sample, C_U is the concentration detected in the sample prior to spiking, and C_A indicates the concentration that was genuinely

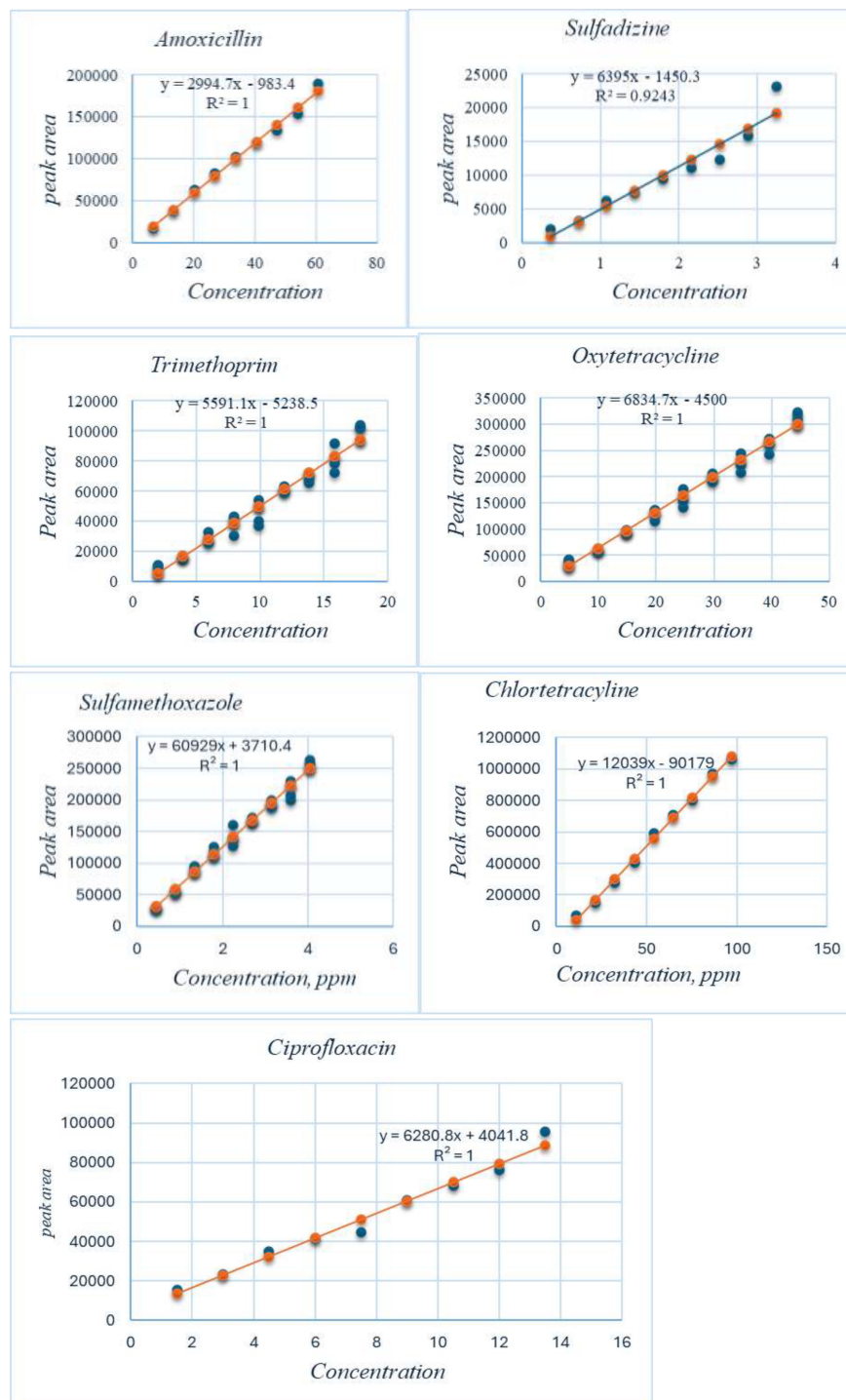


FIGURE 4
Calibration curves.

added. The percentage bias was calculated by comparing the findings of the analysis of the spiked samples. The reproducibility of the analytical results was evaluated by reanalyzing a randomly selected sample after every 10 analyses.

2.10 Sample preparation

Chicken breast muscle samples were stored at -20°C , thawed at room temperature for an hour, and processed using procedures as

described by [Arslanbaş \(2018\)](#). After separating the fat and skin tissues, 3 g of minced sample was put into 15 mL polypropylene centrifuge tubes, and 200 μL of EDTA (0.1 M) with 0.1% formic acid was added, followed by 10 mL of methanol and ultra-distilled water (70:30, v/v) mixture. The resulting mixture was vortexed for 30 s and manually mixed for 15 min using either shaker.

After centrifugation at $2,500\times g$ for 5 min, the supernatant was transferred to a clean test tube and filtered through a 0.2- μm membrane filter. Finally, 1 mL of the filtrate was introduced into the HPLC system

TABLE 1 Calibration table for antimicrobial residues.

Calibration table for antimicrobial residues			
Compound	Calibration equation	LOD ($\mu\text{g}/\text{mL}$)	LOQ ($\mu\text{g}/\text{mL}$)
Amoxicillin	$y = 2994.7x - 983.4$	0.10	0.33
Sulfadiazine	$y = 6395x - 1450.3$	0.32	1.08
Trimethoprim	$y = 5591.1x - 5238.5$	0.16	0.54
Oxytetracycline	$y = 6834.7x - 4500$	0.10	0.34
Ciprofloxacin	$y = 6280.8x + 4041.8$	0.17	0.56
Sulfamethoxazole	$y = 60929x + 3710.4$	0.09	0.29
Chlortetracycline	$y = 12039x - 90179$	0.07	0.25

LOD, limit of detection; LOQ, limit of quantitation.

for analysis. To determine analyte recovery, the sample fortification method was used, in which pre-spiked samples were spiked with 1 mL of standard mixture per 3 g of meat sample before being processed (after mincing). The post-spiked samples were similarly processed as the unspiked meat samples, with the exception that they were spiked with a standard mixture after the filtration stage. Recovery for each analyte was calculated using Equation 2. The recovery percentage of analytical standards was satisfactory, as shown in Figure 5.

$$\text{Recovery}(\%) = \frac{\text{peak of prespiked sample}}{\text{peak of postspiked sample}} \times 100\% \quad (2)$$

Recoveries for all analytes were above 75%, as shown in Figure 3; hence, reliable detection can be performed for all the compounds.

3 Statistical analysis

Statistical analysis was conducted using R software (version 4.3.3) to evaluate the presence, concentration levels, and

distribution of antibiotic residues in 160 chicken breast muscle samples. Descriptive statistics were used to summarize detection frequencies, mean concentrations, and exceedance rates relative to the established EU and Codex MRLs. To classify contamination levels, samples were categorized as non-contaminated, mono-contaminated, or poly-contaminated based on the number of antibiotic residues detected. One-way ANOVA was employed to assess variations in antibiotic residue levels across different market locations, with a *post-hoc* Tukey test applied to identify statistically significant differences. Data manipulation, visualization, and tabulation were performed within R to generate summary tables and support interpretation.

3.1 Data normality testing

Prior to conducting parametric statistical tests (ANOVA), the normality of the residue concentration data was assessed using the Shapiro–Wilk test. The Shapiro–Wilk test served as the primary statistical approach for evaluating normality. This test evaluates the hypothesis that data were derived from a normally distributed population, thereby justifying the use of parametric procedures. The test statistics were as follows:

- Null hypothesis (H_0): Data follow a normal distribution.
- Decision rule: If the p -value $>$ 0.05, we fail to reject H_0 (data are normally distributed).
- Application: It is applied to each antibiotic residue dataset separately.

In instances of non-normality, data were analyzed via non-parametric alternatives to satisfy model assumptions.

3.2 Statistical model framework

The data analysis employed a linear model (LM) framework implemented by one-way ANOVA to evaluate variations in

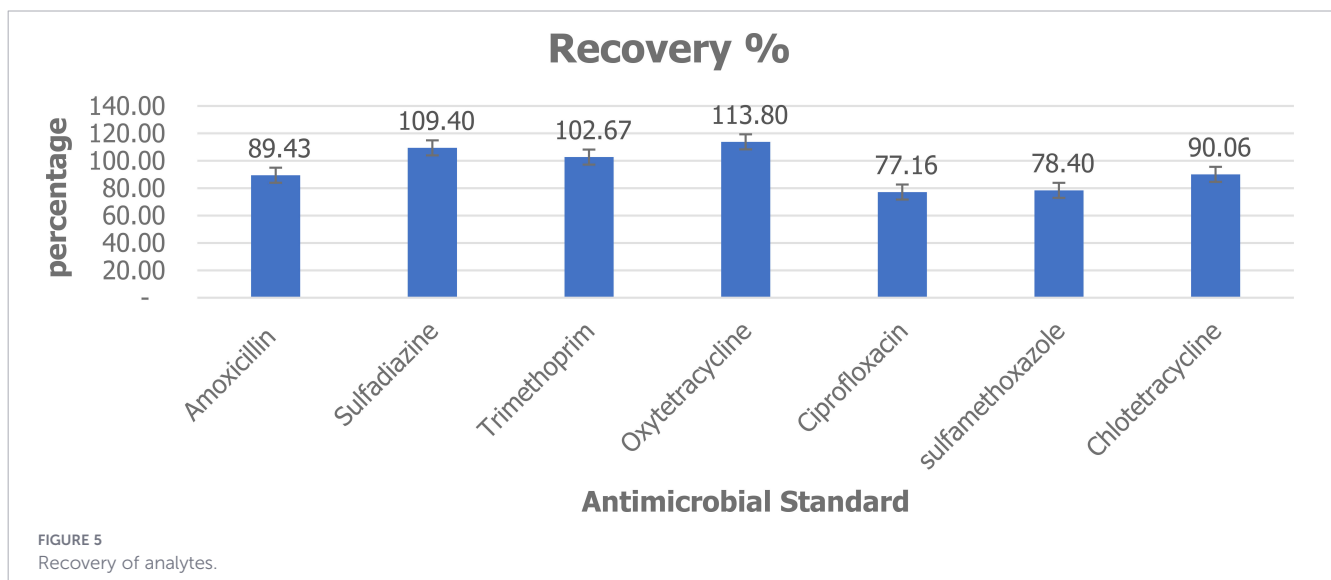


FIGURE 5
Recovery of analytes.

TABLE 2 Occurrence of antibiotic residues in chicken breast muscle samples ($n = 160$).

Antibiotics	Positive samples (%)	Mean ($\mu\text{g/kg}$)	Concentration range ($\mu\text{g/kg}$)	STDEVA ($\mu\text{g/kg}$)	EU MRL ($\mu\text{g/kg}$)	Codex MRL ($\mu\text{g/kg}$)	Samples above MRL (%)
Sulfadiazine	3.12	102.11	36.95–210.65	68.63	100	100	60.00
Sulfamethoxazole	7.50	129.06	44.00–234.00	62.38	100	100	75.00
Ciprofloxacin	<DL	<DL	<DL	<DL	100	150	<DL
Oxytetracycline	19.38	165.71	84.00–636.00	115.17	100	200	77.42
Chlortetracycline	6.88	150.57	80.40–311.70	63.43	100	200	81.82
Amoxicillin	<DL	<DL	<DL	<DL	50	50	<DL
Trimethoprim	9.38	196.95	97.60–341.64	80.27	50	100	100

maximum residue limits (MRL) established by the European Union (EU) (2010) and Codex Alimentarius. <DL, below the detection limit.

antibiotic residue levels across different market locations. The model is defined by the following equation:

General Linear Model

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

Where:

Y_{ij} = Antibiotic residue concentration ($\mu\text{g/kg}$) for the j -th sample from the i -th market

μ = Overall grand mean of antibiotic residue concentrations

α_i = Effect of the i -th market location ($i = M1, M2, M3, M4, M5, M6, M7, M8$)

ε_{ij} = Random error term, assumed to follow $N(0, \sigma^2)$

i = Market location index (1 to 8)

j = Sample index within each market

3.3 Hypothesis testing and model fit

The analysis tested the following hypotheses:

- Null hypothesis (H_0): $\alpha_1 = \alpha_2 = \alpha_3 = \dots = \alpha_8 = 0$ (no difference in mean residue levels across markets)
- Alternative hypothesis (H_1): At least one $\alpha_i \neq 0$ (at least one market differs significantly)

The F -test statistic was calculated (Equation 3) as the ratio of the mean square between groups (MS_{between}) to the mean square within groups (MS_{within}): $F = MS_{\text{between}}/MS_{\text{within}}$

$$F = \frac{MS_{\text{between}}}{MS_{\text{within}}} = \frac{SS_{\text{between}}/(K - 1)}{SS_{\text{within}}/(N - K)} \quad (3)$$

Where:

MS_{between} = Between-group mean square = $SS_{\text{between}}/(k - 1)$

MS_{within} = Within-group mean square = $SS_{\text{within}}/(N - k)$

k = Number of markets (8)

N = Total number of samples (160)

All statistical computations were performed using R software version 4.3.3, with *post-hoc* Tukey comparisons applied, where significant F -values were observed.

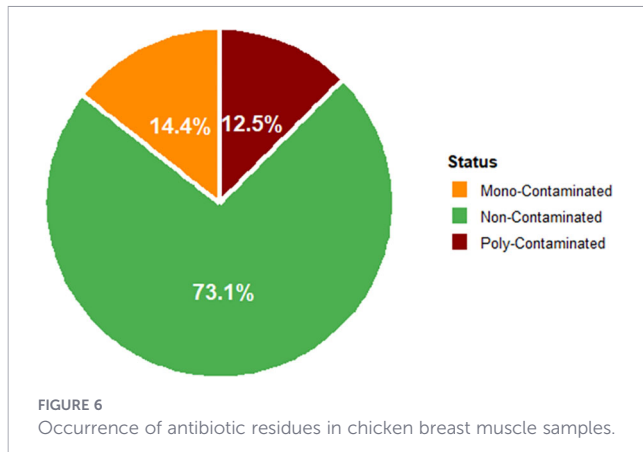
4 Results

The model (Table 1) displays the LOD and LOQ for the different standards, ranging from 0.10 to 0.17 and 0.25 to 1.08 $\mu\text{g/mL}$, respectively, indicating the model's suitability for drug detection and quantification because the LOQ values are below the maximum permitted residue limits for the drugs in chicken meat.

Subsequently, optimizing chromatographic conditions, specific antibiotics were eluted separately from the column, and their retention times were determined. A mixed standard of antibiotics at 0–500 ng/mL was then prepared. The seven targeted antibiotics eluted by retention time were amoxicillin (AMO), sulfadiazine (SDZ), trimethoprim (TRI), ciprofloxacin (CIP), oxytetracycline (OTC), sulfamethoxazole (SMX), and chlortetracycline (CTC) (Figure 2). A 15-point calibration curve was generated using the standard's retention time and the integrated peak area of the chromatograms to obtain the linear equations shown in Table 1. Sample extracts were spiked with a 50- ng/mL standard mixture to enhance the analyte signals and highlight significant peaks.

The target antibiotics were identified based on peak areas, and the spiked values were subsequently deducted from the matching concentration values. Figure 2 presents an overlay of extract chromatograms beside the standards at a specific wavelength. The elution sequence of various antibiotics, in relation to their polarity, is contingent upon the kind of column and other chromatographic parameters, including solvent composition and gradient (Chico et al., 2008; Combs et al., 1997; Oyedeji et al., 2021). The elution sequence identified in this study aligned with the results of Oyedeji et al. (2021), who employed a similar column, but with variations in retention time due to differing chromatographic conditions. Table 1 presents the accuracy results of the extraction process for several analytes. The average recovery percentage for all antibiotics exceeded 75%.

The analysis of 160 chicken breast muscle samples revealed the presence of different antibiotic residues, with oxytetracycline, trimethoprim, and sulfamethoxazole identified at the highest frequencies of 19.38%, 9.38%, and 7.5%, respectively (Table 2). Trimethoprim showed the highest mean concentration (196.95 μg /



kg) and had 100% of positive samples exceeding the EU MRL. Oxytetracycline and chlortetracycline were also frequently detected, with 77.42% and 81.82% of their positive samples surpassing the MRLs. The MRLs in muscle are 200 µg/kg according de CODEX for tetracycline class, which includes chlortetracycline and oxytetracycline, with an Acceptable Daily Intake (ADI) established at 0–30 µg/kg body weight. The MRLs in muscle are 100 µg/kg for sulfonamides (including sulfadiazine and sulfamethoxazole) while the ADI is generally recognized at 0–50 µg/kg body weight.

The presence of antibiotic residues above the MRLs in broiler chicken samples highlights potential issues related to the misuse or overuse of antibiotics in poultry farming and improper withdrawal periods. Stricter oversight and regulatory enforcement are required to guarantee food safety, given the possible health hazards, including consumer allergic responses and antimicrobial resistance (AMR). In contrast, ciprofloxacin and amoxicillin were not detected in any of the samples. The absence of amoxicillin and ciprofloxacin might indicate either their proper use in compliance with withdrawal periods or their lesser prevalence in poultry production.

4.1 Mono-contaminated and poly-contaminated chicken muscle samples

Out of the 160 chicken breast muscle samples analyzed, 43 (26.88%) had antibiotic residues (Figure 6). Among these, 23 samples (14.37%) were mono-contaminated, indicating the

TABLE 3 Mean concentrations of sulfonamides and tetracycline in chicken breast muscle samples.

Antibiotic	Mean (µg/kg)	Min (µg/kg)	Max (µg/kg)	n Positive	% Positive
Sulfadiazine	102.11	36.95	210.65	5	3.12
sulfamethoxazole	129.06	44.00	234.00	12	7.5
Trimethoprim	196.95	97.6	341.64	15	9.38
Oxytetracycline	165.71	84	636.95	31	19.38
Chlortetracycline	150.57	80.4	311.7	11	6.88

presence of a single antibiotic, while 20 samples (12.51%) were poly-contaminated, meaning they contained residues from two or more antibiotic families.

4.2 Mean concentrations of sulfonamides and tetracycline in chicken breast muscle samples

Table 3 shows that antibiotic classes such as sulfonamide (sulfadiazine, sulfamethoxazole, and trimethoprim) and tetracycline (oxytetracycline and chlortetracycline) were detected in chicken breast muscle samples tested. Trimethoprim demonstrates the highest mean concentration at 196.95 µg/kg, with values ranging from 97.6 to 341.64 µg/kg. Elevated levels in muscle tissue indicate the misuse and over administration of this antibiotic in chicken production. This analysis reveals that the maximum concentration of oxytetracycline (636.95 µg/kg) significantly surpasses its average (165.71 µg/kg), indicating a skewed distribution characterized by a small number of samples displaying extremely high, potentially dangerous values. The mean concentrations of tetracyclines (165.71 and 150.57) and maximum values (up to 636.95) surpass the MRL established by the EU, showing noncompliance with withdrawal periods. Similarly, the findings for the sulfonamide class demonstrate that the mean average of Sulfamethoxazole (129.06) and Trimethoprim (196.95) exceed the safety thresholds set by the EU and FAO/WHO Codex Alimentarius.

Trimethoprim was detected in 15 samples, representing a 9.38% contamination rate. The readings ranged from 97.6 to 341.64 µg/kg. In this study, 31 samples (19.38%) contained oxytetracycline at levels ranging from 84 to 636.95 µg/kg, with a mean of 165.71 µg/kg. Chlortetracycline was detected in 11 samples (6.88%) with concentrations ranging from 80.4 to 311.7 µg/kg, with a mean of 150.57 µg/kg.

4.3 Antibiotic residues across different market locations

The ANOVA results indicated that chlortetracycline exhibited a statistically significant difference ($p < 0.05$), indicating that its residue levels varied significantly across locations (Table 4). Chlortetracycline exhibited significant differences among markets, with M7 having the highest mean residue (311.70 µg/kg), significantly different from M1, M2, M4, and M6 (denoted by different *post-hoc* letters “ab”). The levels of residue from markets M3, M6, and M8 (sharing the letters “a” and “b”) were significantly different from M1, M2, and M4. The observed variations in antibiotic residue levels among the markets suggested differences in poultry sources, antibiotic usage patterns, non-compliance with withdrawal periods, or regulatory compliance. For sulfadiazine, the M3 mean level was higher than M2, and trimethoprim demonstrated a high level in M5 and M7. The differences in the levels between markets showed a lack of uniform farming practices. The relatively uniform levels of oxytetracycline across markets suggested widespread use in poultry farming, reinforcing the need for stricter regulation and monitoring.

TABLE 4 Mean antibiotic residue levels ($\mu\text{g}/\text{kg}$) across different market locations.

Market	Mean residue							
	Amoxicillin	Chlortetracycline	Ciprofloxacin	Oxytetracycline	Sulfadiazine	sulfamethoxazole	Trimethoprim	
M1	<DL	92.00 ^a	<DL	115.74 ^a	<DL	44 ^a	176 ^a	
M2	<DL	138.90 ^a	<DL	187.16 ^a	63.17 ^a	157.30 ^a	213.87 ^a	
M3	<DL	180.00 ^{ab}	<DL	233.67 ^a	160.52 ^a	132.73 ^a	<DL	
M4	<DL	101.40 ^a	<DL	173.08 ^a	<DL	53 ^a	186.25 ^a	
M5	<DL	<DL	<DL	212.43 ^a	<DL	<DL	250.23 ^a	
M6	<DL	138.20 ^{ab}	<DL	100.33 ^a	<DL	<DL	<DL	
M7	<DL	311.70 ^b	<DL	119.00 ^a	<DL	<DL	262.88 ^a	
M8	<DL	176.00 ^{ab}	<DL	95.00 ^a	<DL	133.5 ^a	147.59 ^a	

Different letters between markets indicate significant differences ($p < 0.05$). Markets sharing the same letter are not significantly different ($p > 0.05$). <DL, below the detection limit.

5 Discussion

Many studies report that the reason for the occurrence of antibiotic residues is the failure to observe withdrawal periods and early depletion of animals. This study detected residues of oxytetracycline, trimethoprim, sulfamethoxazole, chlortetracycline, and sulfadiazine in chicken muscle meat. The detection of residues in chicken muscle tissue indicates the indiscriminate use of antibiotics in poultry farming, highlighting producers' failure to adhere to the withdrawal period, designed to prevent antibiotic residues prior to slaughter. The presence of residues in food poses a threat to public health due to the risk of human diseases caused by the transmission of antibiotic-resistant pathogens through the consumption of meat contaminated with antibiotics (Arsène et al., 2022; A. A. Sani et al., 2023).

According to Ali et al. (2024) and Aminatu Abubakar Sani et al. (2023), the emergence and dissemination of resistance from residues requires research on the widespread use and misuse of antibiotics in chicken farming. In addition, Arsène et al. (2022) noted that observing the withdrawal period and conducting physical-chemical analyses must be performed to ensure that the antibiotics used do not exceed the maximum residue limit before the food is commercialized.

The antibiotics that were analyzed in these studies are the most widely used for prophylaxis, growth promotion, and therapy in production animals, particularly in chicken farming, including enrofloxacin, benzylpenicillin, ampicillin, amikacin, neomycin, tetracycline, tilmicosin, and colistin sulfate (Caneschi et al., 2023; Redwan Haque et al., 2023). In particular, oxytetracycline is used in cattle and poultry for prophylaxis and therapy of various diseases, and the tetracycline class is used in feed supplements to promote growth and egg production (Ahmadi et al., 2021; Muaz et al., 2018).

In our study, a total of 43 (26.9%) samples had antibiotic residues, of which 14.4% were mono-contaminated and 12.5% were poly-contaminated. Considering local production practices, this is associated with the use of antibiotics to mitigate batch losses, improve productivity, and optimize profitability, as broiler chickens represent a significant investment for producers. In Lebanon, similar results were found, with 23.75% of chicken samples containing one antibiotic residue and 53.75% contaminated with more than one antibiotic residue (Jammoul and El Darra, 2019). Similar findings were made by S. Arab and Helal (2024), who found that 48.8% of 90 samples of chicken meat, liver, and gizzard tested positive for tetracycline and sulfadimidine antibiotic residues. According to Thapa (2021), the administration of many drugs can affect the drug's excretion from the body due to the inhibition of liver enzymes required for drug metabolism. This can impair liver or kidney function, resulting in incomplete elimination of active metabolites from the animal's body.

Furthermore, we found that oxytetracycline was detected at all research sites, followed by chlortetracycline (missing in one site) and trimethoprim (absent in two sites). The detection of antibiotic residues indicates that chickens raised and sold in these areas pose a risk to consumers due to the buildup of antibiotics in the breast muscle, the most commonly consumed portion of the meat. The adverse effect of antibiotic residues includes the transfer of antibiotic-resistant bacteria to consumers due to mobile resistance

properties (Bacanli and Başaran, 2019; Khalafalla et al., 2022). Antibiotic residues in meat products at the time of slaughter can be avoided by following label instructions when administering veterinary medications and chemicals (Thapa, 2021). In this context, Ferdous et al. (2020) highlight that when antibiotics are administered in excess of what is advised and the withdrawal period is not observed, antibiotic residues in milk, meat, and eggs may persist for a longer period of time after treatment. Additionally, critical drugs (oxytetracycline, tetracycline) should only be used for human therapy, and it should be illegal to utilize subtherapeutic amounts delivered through animal feed products to promote growth (Makary et al., 2018; Osorio et al., 2023).

In this study, oxytetracycline residues (19.4%) were highly prevalent in relation to all antibiotics analyzed, followed by trimethoprim (9.4%) and sulfamethoxazole (7.5%). This arises from the cost-effectiveness and accessibility of these drugs without a prescription at private pharmacies. Farmers often employ them in drinking water for growth promotion or disease prevention, frequently without veterinary consultation. Similarly, a study conducted in Nepal and Bangladesh also found the prevalence of doxycycline as high as 17% and 32.3% (Sarker et al., 2018; Thapa, 2021). A similar study conducted in Zanzibar, Tanzania, reported a high prevalence of tetracycline: 91% in the liver, 72% in the small intestine sample, and 100% in the thigh muscles (Ali et al., 2024). Oxytetracycline residues were also discovered in chicken meat, liver, and juice after cooking in a study carried out in Bangladesh using thin-layer chromatography (TLC), highlighting the significance of adhering to the withdrawal period and utilizing antibiotics appropriately (Ferdous et al., 2020). In contrast to these results, a study conducted in Iran found no oxytetracycline or chlortetracycline residues in samples of chicken breast muscle and liver (Ahmadi et al., 2021).

On the one hand, residues of tetracycline, sulfamethoxazole, and cefquinome can change the normal function of intestinal bacteria (Sadighara et al., 2023). On the other hand, tetracycline and β -lactam residues induce carbonylation of chicken breast, which contributes to deterioration, causes unpleasant flavors and hardening, and reduces the nutritional value of the meat (Marquez et al., 2021). According to Arafa et al. (2024); Bacanlı and Başaran (2019), and Redwan Haque et al. (2023), tetracycline residues in meat can lead to allergic reactions in individuals and produce resistance to antibiotics. Additionally, the residues might cause immunopathological effects, allergies, mutagenicity, nephropathy (gentamicin), hepatotoxicity, reproductive disorders, bone marrow toxicity (chloramphenicol), and even carcinogenicity (sulfamethazine, oxytetracycline, furazolidone) in humans (Bacanli and Başaran, 2019; Rana et al., 2019; Treiber and Beranek-Knauer, 2021). Antibiotics such as chloramphenicol and tetracycline should be properly managed because they are harmful to humans even at low concentrations (Shahrajabian and Sun, 2025; Treiber and Beranek-Knauer, 2021).

Our results align with the findings reported in Iran, where raw chicken meat and liver samples were found to contain antibiotic residues of trimethoprim, sulfachloropyrazine, and sulfadimethoxine (Ahmadi et al., 2021). In Egypt, residues of oxytetracycline, enrofloxacin, and sulfonamide were also found in

60% of broiler chicken muscle (Khalafalla et al., 2022). These results are consistent with a study done in Lebanon that found that chicken muscles had similar levels of tetracycline (17.5%) and sulfonamide (3.8%) (Jammoul and El Darra, 2019). A. A. Sani et al. (2023) also found that oxytetracycline (7.5%) was most prevalent in chicken meat, followed by ciprofloxacin (5%) and amoxicillin (5%).

A study comparable to ours was reported in Vietnam using the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Amoxicillin residues were found in 6.1%, oxytetracycline residues in 11%, doxycycline residues in 28%, and sulfaquinoxaline residues in 2.8% of the 360 meat broiler samples (Huong et al., 2020). Additionally, two samples had levels of sulfamethazine above the MRL (1,556.5 ng/g) required by local regulation. In this context, our analysis identified trimethoprim, chlortetracycline, and oxytetracycline as the primary contaminants exceeding established safety thresholds, according to the Codex Alimentarius Commission and EU MRLs. Exceeding the MRLs for these compounds indicates a lack of comprehension of the proper use of antibiotics, coupled with substandard chick quality, prompting producers to employ antibiotics as growth promoters. In Mozambique, farmers frequently use tetracyclines for therapeutic purposes, while other antibiotics are included in feed as growth promoters, leading to residues in the final product (Cumbula, 2017; Figuié et al., 2022).

In contrast to these findings, Al Fazari et al. (2024) found that the residue levels of levofloxacin and sulfanilamide were within the MRLs. Wen et al. (2025) discovered that while the levels of tetracycline and oxytetracycline residues in 10 and 2 chicken samples were over the MRL according to European Union regulation (EU), the levels of chlortetracycline residues in 10 chicken samples were below the MRL (3.17 to 78.36 $\mu\text{g}/\text{kg}$) according to Chinese laws. In contrast to our findings, reports from Oman indicated that although the levels of oxytetracycline and sulfamethazine antibiotic residues in chicken breast and liver samples were below the MRL, consumers were still at risk for health problems (El Tahir et al., 2021).

The results of this study show that most of the detected residues exceeded the MRLs, but trimethoprim residues exceeded the limit by 100%, indicating the excessive use of antibiotics and the lack of monitoring of antibiotic use in production. Moreover, in Mozambique, factors contributing to the accumulation of residues in meat include the lack of antibiograms and laboratory facilities for veterinary diagnostics, insufficient regulation of veterinary drug markets, and the informal trade of inappropriate and essential antibiotics (Figuié et al., 2022). According to A. A. Sani et al. (2023), it is worrying to find antibiotic residues in poultry meat and meat products above the MRLs. Antibiotic residues above the MRL in edible portions also suggest that, on average, the kidneys and liver of animals for food contain a higher percentage of antibiotic residues than do the muscle, skin, and fat (Redwan Haque et al., 2023). Using HPLC, the authors found in broiler meat a concentration of 278.96 $\mu\text{g}/\text{kg}$, which exceeded the MRLs.

According to Khalafalla et al. (2022) and Wen et al. (2025), exceeding MRLs could have negative effects for consumers, such as toxicological consequences and allergic reactions due to the consumption of chicken containing antibiotic residues. According

to Muaz et al. (2018), antibiotic residues in concentrations above acceptable limits may be linked to the lack of awareness among some farmers and the ignorance and negligence of authorities in monitoring the use of antibiotics, particularly in developing countries. Therefore, although maximum antibiotic residue limits in food help protect consumers, they do not guarantee that foods derived from animals containing antibiotic levels above the limits will not be sold. Even if maximum antibiotic residue limits in food are not exceeded, they can still cause problems in the long term (El Tahir et al., 2021; Treiber and Beranek-Knauer, 2021).

Furthermore, our study revealed that all positive samples examined adhered to the acceptable daily intake (ADI) limits for sulfadiazine, sulfamethoxazole, oxytetracycline, chlortetracycline, and trimethoprim. However, the detection of antibiotic residues signifies consumer exposure, posing substantial risks for the dissemination of antibiotic-resistant microorganisms. These findings contrast with those reported by Ali et al. (2024), as tetracycline residues exceeded the MRLs and ADI for tetracycline of 0 - 30 µg/kg/bw, indicating improper use of antibiotics in broiler chicken production. However, antibiotics such as ampicillin, tetracycline, and sulfadimidine are more frequently used in feed production in developing countries (Abd El Razik et al., 2025). Employing high-performance liquid chromatography, Islam et al. (2024) and Aminatu Abubakar Sani et al. (2023) found levels of antibiotic residues in feed below the MRL. These results revealed that producers overuse antibiotics and do not observe the withdrawal period when treating broiler chickens because the feed does not contain levels beyond the allowed limit.

Amoxicillin and ciprofloxacin were not detected in any of the chicken breast muscle samples. To ensure food safety, we analyzed these compounds in chicken breast muscle; however, they are generally easily detectable in the liver, where they are metabolized. Their significant excretion in urine and feces after oral administration, along with a half-life of 2 to 6 h, may enable complete removal, thus hindering detection during analysis. A study also did not quantify residues of oxytetracycline and chlortetracycline in samples of raw meat and chicken liver (Ahmadi et al., 2021). Jemutai et al. (2025) also did not detect amoxicillin in chicken breast muscle, and different results were found by Ramatla et al. (2017); Sarker et al. (2018), and Thapa (2021), in which ciprofloxacin residues were elevated by 9%, 44.37%, and 21.4%, respectively. Different results were found: when Gyamfi et al. (2024) used the LC-MS/MS method to identify antibiotic residues in the liver and gizzard of broiler chickens, the results showed that the levels of ciprofloxacin, oxytetracycline, and chlortetracycline were below the 0.01-mg/g acceptable limit established by the Ghanaian Standards Authority. The chicken broiler farmers in Ghana and Nigeria were not well-informed about the advantages of adhering to the withdrawal period, residues, and antibiotic resistance (Gyamfi et al., 2024; Tijani et al., 2023). This could contribute to non-compliance with the withdrawal period and lead to antibiotic residues in chicken meat. Additionally, veterinarians must ensure the appropriate use of antibiotics to treat disease and train producers on maintaining the withdrawal period after using antibiotics in broilers; these are strategies that can reduce the risk of residues in meat. Furthermore, this study will increase awareness of the careless use of antibiotics in broiler chickens and provide a more comprehensive investigation of food

safety and the presence of antibiotic residues in poultry products in Mozambique.

6 Conclusions

This study evaluated the prevalence of antibiotic residues in 160 chicken breast muscle samples from eight marketplaces in the Southern Region of Mozambique. Our findings revealed that chicken breast muscle contains substantial levels of trimethoprim, oxytetracycline, chlortetracycline, sulfamethoxazole, and sulfadiazine, which are the principal analytes surpassing the international maximum residue limits established by EU and Codex standards. The results indicate substantial non-compliance with withdrawal periods, potentially resulting in the emergence of antibiotic-resistant bacterial strains, complicating the treatment of infectious diseases in humans and posing a public health threat, particularly for consumers with drug allergies. To ensure the safety of chicken meat, it is essential that farmers, transporters, and processors of broiler chickens undergo monitoring and training through educational programs that focus on the appropriate use of antibiotics and compliance with withdrawal periods prior to processing and sale. To prevent residues during slaughter, relevant authorities must regulate and restrict the overuse of antibiotics. Future research must include multi-tissue analysis and comprehensive screening of emerging antibiotic drugs to improve public health safeguards and mitigate the risk of antimicrobial resistance. These measures will ensure that chicken meat is safe for human consumption and falls within the EU and Codex's limitations. Furthermore, an antimicrobial resistance study is necessary to ensure the safety and quality of broiler chicken meat. This research is ongoing in the laboratory as part of the same project.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by the Vice Chancellor of Sokoine University of Agriculture (SUA), Tanzania, who was also requested for a research permit, and the Directorate of Postgraduate Studies, Research, Technology Transfer and Consultancy (DPRTC) of SUA (reference: SUA/ADM/R.1/8/1070) authorized the conduct of the research. The study was also approved by the Ministry of Agriculture and Food Security of Mozambique, through the National Directorate of Animal Health

(Note: number 1635/SAECM/DAP/11/23) and the Municipal Directorate of Markets and Fairs (Ref: number 84/2023). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

NM: Conceptualization, Methodology, Data curation, Writing – review & editing, Validation, Investigation, Formal analysis, Writing – original draft, Funding acquisition, Project administration, Visualization, Resources. JK: Validation, Formal analysis, Supervision, Writing – review & editing. AI-Z: Visualization, Validation, Funding acquisition, Writing – review & editing, Supervision, Formal analysis.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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