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# Assessment of Tetracycline Residues in Broiler Chicken Meat Sold at Mwanakwerekwe and Darajani Markets in Zanzibar

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## Abstract

**Introduction:** The increased use of antibiotics in livestock production, particularly poultry, has prompted serious public health concerns due to the possibility of antibiotic residues in the food supply chain. This study examined the levels of tetracycline residues in broiler chicken meat samples including liver, small intestine, and thigh muscle tissues collected from two major markets in Zanzibar namely, Mwanakwerekwe and Darajani. An enzyme-linked immunosorbent assay test was used to detect tetracycline residues in 90 broiler chicken samples from the two markets. **Results:** Tetracycline residues were detected in 91% (29) of liver samples, 72% (23) of small intestine samples, and 100% (26) of thigh muscle samples. The liver samples from Mwanakwerekwe and Darajani showed the highest tetracycline concentrations of 2413.8 µg/kg and 1375.7 µg/kg, respectively. The small intestine samples from Darajani market had the highest concentrations of 2867 µg/kg and 124.2 µg/kg from Mwanakwerekwe market. Thigh muscle samples showed lower but substantial quantities of tetracycline residues, with the highest concentration of 1600 µg/kg. Compared to the Codex Alimentarius Commission's Maximum Residue levels (MRLs) of 600 µg/kg for liver samples and 1200 µg/kg for muscles. Overall, 69% of all samples exceeded the tolerated levels, especially liver and thigh muscle tissues. Small intestines had no reference standard from the Codex Alimentarius Commission (CAC) MRLs because it is not considered as a consumable part of a chicken. Furthermore, the investigation discovered that all samples exceeded the Acceptable Daily Intake (ADI) of 0 - 30 µg/kg for tetracycline residues, suggesting a public health concern to consumers. **Conclusion:** The findings highlight the need for urgent control measures and better monitoring of antibiotic usage in poultry production to ensure food safety and public health are safeguarded. The results also emphasize

the necessity of strengthening antimicrobial stewardship programs through education and awareness to farmers and consumers on the potential risks of antibiotic residues in the food supply chain. The high levels of tetracycline residues in broiler chicken meat sold at Zanzibar's markets demand immediate regulatory action to successfully implement MRLs and ADIs from the required authorities.

## Subject Areas

Public Health

## Keywords

Antimicrobial Residues, Antimicrobial Resistance, Maximum Residue Limit, Acceptable Daily Intake, Tetracycline Residues

## 1. Background Information

Livestock production has greatly expanded since intensive farming technologies make it possible to supply the rising population's demand for proteins of animal origin in an effective and economically sustainable manner [1]. Antimicrobial use (AMU) in animals has been raised to improve economic returns, productivity, and health by lowering disease incidence, morbidity, and mortality from therapeutic levels. The current global estimates indicate that the global average annual consumption of antimicrobials per kilogram of animal produce was estimated at 45 mg/kg for cattle, 148 mg/kg for chickens, and 172 mg/kg for pigs. From these figures, it is anticipated that between 2010 and 2030, global antimicrobial consumption will increase by 67%, from 63,151 ± 1,560 tons to 105,596 ± 3,605 tons [2].

In veterinary medicine, β-lactams such as ampicillin, benzathine penicillin, dicloxacillin, oxacillin, and tetracycline are commonly used antibiotics for the treatment and prevention of bacterial infections in animals [3]. To prevent and treat bacterial infections in broiler chickens, tetracycline, a broad-spectrum antibiotic, is frequently administered. These antibiotics are easily accessible, frequently unlimited, and can be purchased from veterinary supply stores and distributors [4].

Previous research has demonstrated that most farmers lack the necessary knowledge and adopt inappropriate attitudes and practices regarding the prudent use of antibiotics, with a lack of veterinary officers and consultation services [5]. The overuse of antimicrobials including tetracycline has been associated with the development and spread of antimicrobial resistance [6] and other health problems such as allergic reactions, carcinogenic and mutagenic effects in the human body [7].

Therefore, understanding the Maximum Residue levels (MRLs) that are set to limit the maximum residues limits that are allowed to be consumed by the human

body is of paramount importance. The FAO/WHO Joint Meeting on Pesticide Residues (JMPR) the Codex Committee on Pesticide Residues (CCPR) sets the MRLs and ADIs for various animal food stuffs. The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) collaborated to form the Codex Alimentarius Commission, which sets Maximum Residue Limits (MRLs) for tetracycline and other antibiotics in various animal tissues. The MRLs for tetracycline are 200 μg/kg for the kidney, 600 μg/kg for the liver, and 1200 μg/kg for muscle. The Acceptable Daily Intake (ADI) for tetracycline ranges from 0 to 30 μg/kg. These restrictions are meant to guarantee that food contains safe amounts of antibiotic residues for human consumption.

Poultry production is a significant industry in Zanzibar that supports both the livelihood economy and food security of many Zanzibaris. On the other hand, the safety of broiler meat sold in markets has come under scrutiny due to the use of antibiotics, specifically tetracycline, in chicken production systems. Prior research has shown that poultry products from different regions may include antibiotic residues, however, there is little information that is unique to Zanzibar. The purpose of this study was, therefore, to determine the concentrations of tetracycline residues in broiler chicken meat sold at Mwanakwerekwe and Darajani, two of the main markets in Zanzibar. The findings from this study are envisaged to contribute to shedding light on the safety of broiler meat with regards to tetracycline residues concentrations in the liver, small intestine, and thigh muscle in comparison to known MRLs and ADIs in Zanzibar that would inform on appropriate public health measures.

## 2. Research Objectives

1) To assess the tetracycline residues in broiler chicken meat sold at Mwanakwerekwe market in Unguja, Zanzibar.

2) To assess the tetracycline residues in broiler chicken meat sold at Darajani market in Unguja, Zanzibar.

## 3. Materials and Methods

### 3.1. Study Area

The study was conducted in February 2024 at Mwanakwerekwe and Darajani markets in Mjini Magharibi Region in Unguja, Zanzibar, found in its Mjini and Magharibi B districts. Geographically, Zanzibar Island is in the Indian Ocean and is situated on the Swahili coast adjacent to the Tanzania mainland. The island is about 85 kilometers long and 39 kilometers wide, with an area of 1,464 $km^2$ (565 sq. mi). Unguja one of the islands forming part of the Zanzibar Archipelago is mainly low-lying, with its highest point being 120 meters (390 ft.) [8]. According to Tanzania's national census 2022 results, Zanzibar has a population of 1,889,773 people. The National Sample Census of Agriculture 2019 shows that indigenous chicken were raised by 99.3% of total Zanzibar households, whereas, 2.9% accounted for layers and 0.6% broilers.

### 3.2. Study Design and Sample Size

A cross-sectional study design was used for the collection of liver, intestinal, and thigh muscle tissues from the broiler meat sold at the two markets of Mwanakwerekwe and Darajani in February 2023. The sample size was determined by using Cochran's formula, that $n = Z^2P\ (1 - P)/E^2$, where n = is the number of the sample size required, Z = is the constant for z score (1.96), P = is the estimated proportion of the population which has the attribute for prevalence, the P used was 90.5%, E = was the marginal error allowed, which is 5%, resulting in a 95% confidence interval. Therefore, the total sample size was 132, but this study only obtained 90 samples from consenting broiler sellers at the two markets.

### 3.3. Sample Techniques and Collection

Samples were collected using a random sampling technique, whereby three parts of the broiler chicken were taken from the markets: the liver, the small intestine (ileum), and the muscles (thighs). Liver samples were collected since they serve as the metabolic hub for most substances, such as food and drugs. The ileum, part of the small intestine, was collected since it plays a significant role in the digestion and absorption of starch, minerals, and water in fast-growing broiler chickens. Lastly, muscles (thighs) were collected since they are among the parts mostly eaten by people but also to represent other broiler body muscles. Therefore, 90 samples were collected from Darajani and Mwanakwerekwe markets, where 32 were liver samples, 32 small intestines (ileum), and 26 muscles (thighs). Muscles were obtained in fewer numbers compared to the other sample types because of their physiological nature and role in the digestive system, which does not deal with the absorption of the feed components such as nutrients and chemicals, especially the thigh muscles.

### 3.4. Tetracycline Residue Extraction from the Samples

Analysis of tetracycline residues from the collected samples was performed as per the manufacturer's instructions on the ELISA antibiotic kit with Lot No: 20231101, which was purchased from Shenzhen Lvshiyuan Biotechnology Company, Shenzhen, China. Briefly, the tissue samples (liver, thighs, and small intestines) were homogenized using mortar and pestle to obtain a homogeneous mixture of the samples for subsequent tetracycline extraction.

All the homogeneous sample tissues were weighed to obtain a weight of 2 ± 0.05 grams using a beam balance and put in a 50 ml falcon tube then 3 ml of diluted sample extract A from the kit was added and mixed thoroughly by manually tilting the tubes containing the homogeneous sample and the diluted sample extract A for 3 min. This was followed by the addition of 600 µl 1 M NaOH and 2.4 ml diluted extract B, which was also mixed thoroughly by tilting for 3 min. The mixture was then centrifuged at 4000 rpm at room temperature (20˚C - 25˚C) for 5 min. After centrifugation, 50 µl of the supernatant was transferred and added to 450 µl of diluted sample diluent and mixed evenly by tilting. Finally, a volume of 50 µl was used for Tetracycline residues analysis, for each sample two extractions

were carried out to obtain maximum recovery.

### 3.5. Determination and Confirmation of Tetracycline Residues in the Samples

Briefly, a volume of 50 µL of each standard solution obtained from the manufacturer's kit, and 50 µL of each prepared extracted samples were added to each of the 96 wells plate followed by addition of 50 µL of the anti-antibiotic solution to prevent possible bacterial contaminations in the wells. Then, the plates were incubated at room temperature for 1 hour to allow the binding of the solutions and then washed three times with 250 µL of washing buffer to remove the unbound components such as proteins and antibodies. Then, it was followed by adding 100 µL of enzyme conjugate to each well, to allow binding of the antigen (tetracycline residues) in the samples. The plate was then incubated for 15 minutes to allow the binding process to take effect. The plate was washed thrice with 250 µL of washing buffer to remove the excess unbound antigens.

After the second wash step, 50 µL of each substrate A (hydrogen peroxide) and substrate B (alkaline phosphate) followed by 50 µL of chromogen, were added to each well, resulting in a color change to pink in wells containing tetracycline residues. The plate was then incubated in the dark for 15 minutes at room temperature to allow the coloration process. Finally, 100 µL of stop solution was added in each well to stop the reaction, then the plate was inserted in the ELISA machine, followed by an absorbance reading at 450 nm within 30 min. A calibration curve was then plotted for standard concentration and Optical Density (OD). The mean values of the absorbance values were calculated as follows: percentage of absorbance value = B/B0 × 100%, where B is the average OD value of the sample or the standard solution, B0 is the average OD value of the 0 ng/mL standard solution.

### 3.6. Data Analysis

Raw data from the ELISA machine was collected, entered, and analyzed using Microsoft Excel to find the range and means of the concentrations, Statistical Package for Social Sciences (SPSS) version 20 software was used to compare the means of tetracycline residues concentrations from the samples of the two markets tested by using unpaired t-test and one-way analysis of variance (ANOVA) where p-value of less than 0.05 was considered significant.

## 4. Results

Of the 90 samples collected (32 livers, 32 small intestines, 26 thigh muscles) and tested, 29 (91%) liver samples were positive for tetracycline residues. The highest concentration of tetracycline residues in the positive samples were (2413.81 ug/kg) and the lowest concentration of (0 ug/kg) with an average concentration of (846.2984 ug/kg). Whereas the small intestine samples, 23 (72%) were positive. The highest concentration of the tetracycline residues for the positive small intestine samples was (2866.999 ug/kg) and the lowest concentration of (0 ug/kg) with

an average concentration of (732.1541 ug/kg). The results for the thigh muscles showed that 26 (100%) of all the samples tested were positive for the tetracycline residues. The highest concentration in the thigh muscles being (1600.019 ug/kg) and the lowest concentration of (97.917 ug/kg) and the average concentration of (655.6412 ug/kg), the results are as shown in Figure 1.

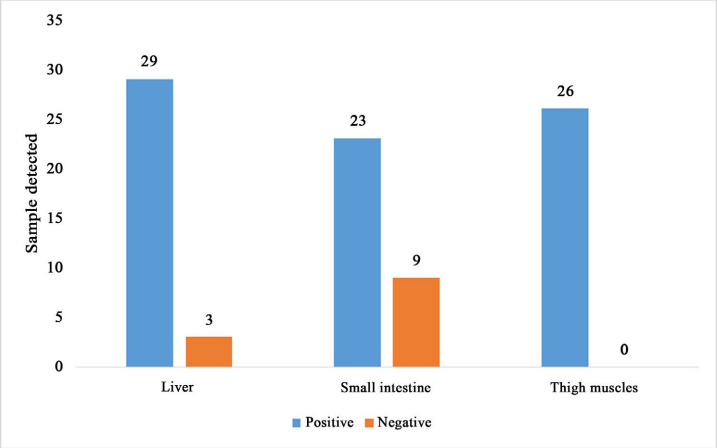


Figure 1. Proportion of Tetracycline residues in liver, small intestine and thigh muscles from Mwanakwerekwe and Darajani markets.

### 4.1. Comparison of Tetracycline Residues Sampled from Mwanakwerekwe and Darajani Markets

The comparison of the results of the tetracycline residue levels from Mwanakwerekwe and Darajani markets revealed that, of the 90 samples collected, 45 samples were from Mwanakwerekwe market and 45 samples from the Darajani market. All 45 (100%) samples from Darajani market were positive, while for Mwanakwerekwe market, only 33 (73%) samples were positive. Results further show that the difference was statistically significant, with p-value = 0.00 in tetracycline residue concentration among the broiler samples between the two markets. (See Figure 2)

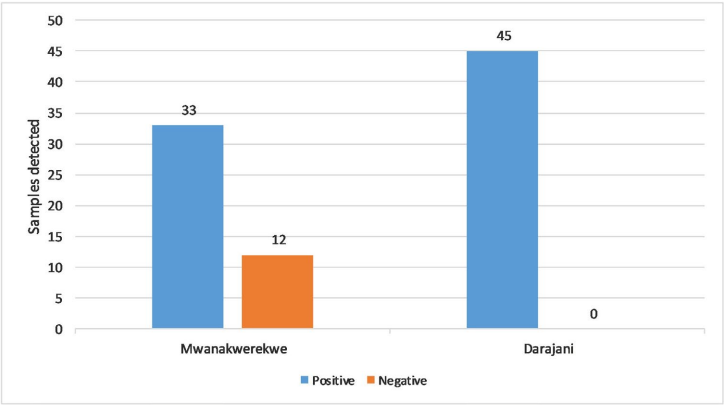


Figure 2. Comparison of Tetracycline residues between Mwanakwerekwe and Darajani markets.

### 4.2. Comparison of Tetracycline Residue Concentration in Different Tissues between Market Locations

The highest average concentration was observed from small intestine samples from Darajani and the lowest average concentration was observed from small intestine samples from the Mwanakwerekwe market as shown in Table 1. Comparison of the tetracycline residues from the samples in the two markets showed that the thigh muscle samples had no significant difference in their concentration (p-value = 0.83) between the two markets. However, the liver and small intestine had significant differences in their concentration of (p-value = 0.00) between the two markets.

Table 1. Comparison of the tetracycline residues from the samples in the two markets.

| | Mwanakwerekwe | | | Darajani | | | |
|---|---|---|---|---|---|---|---|
| **Tissue** | Frequency | Highest (in µg/kg) | Lowest (in µg/kg) | Frequency | Highest (in µg/kg) | Lowest (in µg/kg) | Sig. |
| **Liver** | 15 | 791.10 | 0 | 17 | 2413.81 | 429.28 | 0.001 |
| **Small intestine** | 15 | 537.70 | 0 | 17 | 2867 | 2.89 | 0.001 |
| **Thighs** | 15 | 920.23 | 97.92 | 11 | 1600 | 179.54 | 0.83 |

### 4.3. Comparisons of Tetracycline Levels in Tissues and Organs with the Maximum Residue Limit

Based on the WHO/FAO Codex Alimentarius Commission MRLs for broiler meat for different antibiotics, for tetracycline, the MRLs are 200, 1200, and 600 µg/kg for muscle, kidney, and liver, respectively, we compared our results of tetracycline residues in the liver and thigh muscle tissues with the required MRLs. The results of the comparison of the liver and thigh muscle tissues in our study from the required MRLs showed that 14 out of 32 (44%) liver samples from Mwanakwerekwe and only 2 out of 32 (6%) samples from the Darajani market had concentrations below the set MRL for the liver. On the other hand, 2 out of 26 (8%) thigh muscle samples, one from each market, had a concentration below the set MRLs for the thigh muscle tissues. The details are indicated in Table 2. The results of acceptable daily intake for tetracycline residues in different broiler tissue samples in this study showed that all samples contained tetracycline residues that exceeded the ADI standards of 0 - 30 µg/kg set by the FAO and WHO.

Table 2. Comparison of Tetracycline residue concentration against the MRLs.

| | Mwanakwerekwe | | Darajani | | |
|---|---|---|---|---|---|
| **Tissue** | Above MRL | Below MRL | Above MRL | Below MRL | Total |
| **Liver** | 1 (3%) | 14 (44%) | 15 (47%) | 2 (6%) | 32 |
| **Muscles** | 14 (54%) | 1 (4%) | 10 (38%) | 1 (4%) | 26 |
| **Total** | 15 | 15 | 25 | 3 | 58 |

## 5. Discussion

The high proportions of tetracycline residues in broiler chicken samples in this

study highlights important issues about AMU in poultry production and its consequences for food safety and public health in Zanzibar. The findings of this study demonstrate a high proportion of tetracycline residues in liver, small intestine, and thigh muscle samples compared to MRLs established by international standards of FAO, and WHO.

This study shows that Tetracycline residues were detected in 91% (29), 72% (23), and 100% (26) of the total liver, small intestine, and thigh muscle samples respectively. The highest tetracycline concentrations of the liver samples from Mwanakwerekwe were 2413.8 μg/kg and 1375.7 μg/kg from Darajani market. The thigh muscle samples had the highest concentrations of 1600 μg/kg from Darajani and 920.23 μg/kg from Mwanakwerekwe. When compared to the MRLs, 50% (16) of the total liver samples from both Mwanakwerekwe and Darajani markets had tetracycline residues above the MRLs, Mwanakwerekwe had 1 (6%) out of the 16 samples that had residues above the MRLs and Darajani had 15(93%) samples that were above the MRLs. Alternately, 24 (92%) of the thigh muscle samples had tetracycline residues above the set MRLs. Whereas 14 (58%) of the thigh muscle samples from Mwanakwerekwe had tetracycline residues above the MRLs and the remaining 10 (42%) thigh muscle samples from Darajani were above the MRLs. The small intestine samples had the highest concentrations of 2867 μg/kg from Darajani and 124.2 μg/kg from Mwanakwerekwe, but from the set MRLs, small intestines had no reference standard, because it is not considered as a consumable part of a chicken by the FAO and WHO, however it was of paramount importance to test tetracycline residue levels in the intestines because of the increased trend of consuming them amongst the poor communities owing to their low prices.

The results show that Darajani had the highest number of samples, 25 including both liver and muscles (58) that had residues above the MRLs, while Mwanakwerekwe had 15 samples of both liver and muscles that were above the MRLs. The variability in the samples for tetracycline residues above MRLs from the two markets, might be due to the absence and presence of surveillance and guidance services from the veterinary and public health authorities concerning antimicrobial use. Inappropriate use of the antimicrobials could lead to the presence of residues, hence, the provision of guidance and knowledge could reduce the public health consequences emanating from drug residues in the broiler chicken meat sold in Zanzibar markets.

This study further indicates that there is devastating tetracycline antimicrobial use in both markets, which has led to the presence of antimicrobial residues in poultry meat. Nonetheless this inconsiderate practice poses health risks in food safety supply chain, as the residues may lead to carcinogenic, allergic reactions and exacerbate antimicrobial resistance. The results of this study collate with other studies done elsewhere, including the study done by [9] in Egypt which reported that muscle and liver samples surpassed the FAO and FDA MRLs by 69% and 72% for tetracycline residues respectively. A study by [4] in Tanzania found that 100% of the liver samples had tetracycline residues above the maximum residue limits.

Both studies show that excessive tetracycline usage in chicken production is a prevalent problem. Furthermore, this study shows that the liver and small intestine samples had no significant difference in their tetracycline residues, suggesting that the birds may have been slaughtered without observing the withdrawal period or even slaughtered shortly after antibiotic treatment. This contradicts with another study by [10] in Ghana which revealed that 58% of the participants agreed to have observed the withdrawal period in keeping the broiler chicken. Another study done in Nairobi indicated that the study participants may have probably employed better withdrawal periods which explained the observed differences of 114 (45.6%) of the samples tested in that study which exhibited detectable tetracycline residues whereas in liver samples (24%), kidney samples (14%), muscle samples (7.6%). This persistent high levels across nations reflect a global pattern of inadequate regulation and enforcement of antibiotic usage in poultry farming industry [11].

The results of this study show that the presence of tetracycline residues exceeding MRLs in the majority of samples suggests inadequate antibiotic control in the poultry industry and a lack of antimicrobial stewardship programs from the regulatory authorities. Tetracycline residues in the liver, thigh, and even the small intestine (an organ not normally investigated for MRL control) indicate antibiotic overuse and inability to observe withdrawal periods as corroborated by a study done in Uganda by [12] whereby only 10.7% of the participants seemed to have observed drug withdrawal period. Moreover, in the study by [13] found that in Morogoro, Tanzania some 95% of the participants did not observe a withdrawal period.

Additionally, all tested samples exceeded the ADI for tetracycline 0f 0 - 30 μg/kg, indicating that consumers are exposed to dangerous quantities of antibiotics, posing serious health concerns. This finding is consistent with earlier research that has highlighted the hazards of antibiotic resistance associated with the intake of animal products harboring antibiotic residues. Resistance can spread from farm laborers to the wider population, worsening public health risks [14]. Moreover, excessive residue levels in the small intestine, which are not regulated by MRLs, raise concerns regarding cross-contamination during food processing. In Zanzibar, where a segment of the populace consumes the small intestine, the health hazards are amplified, especially given the absence of recognized limitations for this organ. This study highlights the critical need for stricter AMU regulations and improved enforcement and follow-up in the chicken industry in Zanzibar, as indicated by the high amounts of tetracycline residues detected in this study. This study found that the confounding factors for excessive presence of residues in the broiler chicken meat could be greatly influenced by poor observation of the withdrawal period, excessive use of the drugs without prescriptions, also lack of veterinary controls and surveillance methods but also poor awareness and agricultural practices. This study contributes to the expanding amount of information pointing to antibiotic overuse in poultry production, not only in Zanzibar but worldwide.

The high amounts of tetracycline residues found in broiler chicken samples from Zanzibar's markets are similar to data from other regions in Africa, including Kenya, Uganda, Egypt, Tanzania mainland, and Ghana, highlighting the urgent need for stronger regulatory frameworks and effective enforcement. Addressing these concerns is crucial to maintaining food safety and limiting the spread of antibiotic resistance.

## 6. Limitations of the Study

Besides the observed valuable information, this study has faced a few limitations including the insufficient number of samples; whereby our expected sample size was 132 but we were able to obtain 90 samples from consented sellers, but also the study did not have sufficient funds as it was self-supported hence we couldn't push further to obtain the specified samples. Another challenge was unavailability of the set MRLs for the small intestine from FAO and WHO Codex Alimentarius Commission, which made it impossible for us to conclude and get an insight into the Tetracycline residues found in this part.

## 7. Conclusion

The current investigation focuses on the widespread presence of tetracycline residues in broiler tissues obtained from two main markets in Zanzibar. The results show that many samples exceeded the MRLs for tetracycline in muscle and liver tissues, whereas all samples were above the ADI for these antibiotic residues. Specifically, 91% of liver samples, 72% of small intestine samples, and 100% of thigh muscle samples were positive for tetracycline, with concentrations significantly exceeding the specified safety standards. The difference in residual levels between the Darajani and Mwanakwerekwe markets highlights the region's variable antibiotic usage and control procedures. These findings raise severe public health concerns, highlighting the danger of antibiotic resistance and other health issues associated with eating contaminated broiler meat.

## 8. Recommendations

To address these major challenges, it is critical to institute strict monitoring and regulation of antibiotic usage in chicken farming. Regulatory authorities should impose stricter compliance with established MRLs and ADI to ensure that broiler meat entering the food supply chain is safe to consume. Furthermore, encouraging good agricultural practices (GAP) such as observing the withdrawal period and improving veterinary controls and strengthening antimicrobial stewardship programs can considerably minimize antibiotic residues in poultry products. Public awareness initiatives are also required to educate farmers and consumers about the risks connected with antibiotic residues and the need to follow safe dosage recommendations and observe withdrawal period. By implementing these preventative actions, public health can be safeguarded, antibiotic-resistant bacteria can be prevented, and the food supply chain in Zanzibar can be guaranteed.

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## Ethical Statement

The study was approved and sought by the ethical committee and given the reference number of ZAHREC/05/ST/AUG/2023/150 from Zanzibar Health Research Institute.

## Author's Contribution

Data collection, analysis, interpretation and writing were done by the principal investigator Rai Ramadhan Ali (MSc. Candidate) whereas drafting and reviewing the article was done by Dr. Abubakar Hoza (Supervisor), Dr. Alexanda Mzula (Supervisor) and Dr. Othman Juma Othman. (Supervisor)

## Conflicts of Interest

The authors declare no conflicts of interest.

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