Antimicrobial Residues in Cattle Tissues Processed at Slaughterhouses in Kilosa District, Morogoro, Tanzania

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SUMMARY

The inappropriate use of antimicrobial drugs, limited knowledge among livestock stakeholders, and practices such as self-medication contribute to the accumulation of antimicrobial residues in edible animal tissues. This study aimed to investigate the presence of antimicrobial residues in cattle tissues using microbial screening methods at slaughterhouses in Kilosa District. A cross-sectional study was conducted from April to June in three wards: Kimamba, Dumila, and Kilosa. A total of 90 tissue samples, 30 each of muscle, liver, and kidney were collected. The samples were packaged in sterile polystyrene bags and transported to the laboratory at Sokoine University of Agriculture for analysis. Microbial inhibition methods were used to screen for the presence of antimicrobial residues. Data were analyzed using SPSS version 25. The chi-square test was employed to assess differences among tissue types, while Fisher's exact test was used to evaluate associations. Overall, 28.9% (26/90) of the tissue samples tested positive for antimicrobial residues. Liver tissues showed the highest prevalence at 46.7% (14/30), followed by muscle tissues at 40.0% (12/30). No antimicrobial residues were detected in kidney samples. The differences in residues occurrence among the tissue types were statistically significant ($p = 0.000$). Residues occurrence in muscle tissues varied significantly by ward ($p = 0.000$), indicating a strong geographic influence. However, the distribution of residues in liver tissues did not show a statistically significant variation among wards ($p = 0.314$), suggesting a more uniform presence. These findings highlight the urgent need to strengthen regulatory frameworks, promote responsible antibiotic use in livestock production, and enhance surveillance systems. Addressing these issues is critical to protecting public health and combating the growing threat of antimicrobial resistance.

Keywords: Microbial screening methods, antimicrobial residues, antimicrobial, Kilosa district.

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INTRODUCTION

Antimicrobials are commonly used in veterinary medicine, and their residues may persist in foods derived from animals, potentially posing adverse health effects to consumers (Chanda *et al.*, 2014). The term antimicrobial residues refers to molecules that remain in the meat and organs of slaughtered animals that had previously been treated with antibiotics, particularly when the withdrawal period has not been observed (Aidara-Kane *et al.*, 2018; Darko *et al.*, 2017). The presence of these residues in food products such as meat, milk, and eggs may result in the consumption of sub-therapeutic doses of antimicrobials, thereby contributing to the development of antimicrobial-resistant microorganisms (Dipeolu & Alonge, 2002; Kabir *et al.,* 2004; Muriuki *et al.,* 2019). In addition to promoting antimicrobial resistance, unacceptable levels of antimicrobial residues in food can cause hypersensitivity reactions, gastrointestinal disturbances, autoimmunity, carcinogenicity, mutagenicity, bone marrow toxicity, and other health-related issues (Al-mashhadany, 2019a; Nisha, 2008; Shankar *et al.*, 2010; Toldrá & Reig, 2007).

Globally, developed nations have implemented comprehensive regulatory frameworks to monitor the effects of antimicrobial residues on human health (Byarugaba, 2004; Kirchhelle, 2018; Tang *et al.*, 2017). The Codex Alimentarius Commission (CAC) recommends that animals treated with antimicrobials, such as oxytetracycline, should not be slaughtered within 28 days to allow for adequate withdrawal and to prevent harmful residues in food (MacNeil & Kay, 2016). The presence of antibiotic residues above the Maximum Residue Limits (MRLs) is considered illegal by public health authorities worldwide (Jayalakshmi *et al.*, 2017.; Regea *et al.*, 2018.). Despite these regulations, in many developing countries, including Tanzania, enforcement remains weak (Nonga *et al.*, 2009; Katakweba *et al.,* 2012;). The irrational use of antimicrobials in livestock is worsened by their widespread availability without effective regulation, limited awareness, poor attitudes, and inadequate knowledge among livestock keepers regarding proper drug use (Nisha, 2008). These challenges are further compounded by the lack of strict control measures and educational interventions (Nonga *et al.*, 2009; Katakweba *et al.*, 2012).

In Tanzania, several studies have reported a high prevalence of antimicrobial residues in animal derived food products such as beef, chicken meat, milk, and eggs, with rates ranging from 35% to 100% (Mmbando, 2004; Karimuribo *et al.,* 2005; Kurwijila *et al.*, 2006; Nonga *et al.*, 2009 Mgonja *et al.*, 2016, 2017; Mdegela *et al.*, 2021). Despite these findings, regulatory authorities currently rely merely on withdrawal periods indicated by pharmaceutical manufacturers, without establishing independent guidelines. Although antimicrobial residues have previously been detected in various food products using quantitative analytical techniques, limited attention has been given to the application of microbial screening methods for routine detection of antimicrobial residues in cattle tissues at slaughterhouses, particularly in resource-limited settings such as Kilosa District, Morogoro, Tanzania. Microbial screening methods offer a simple, cost-effective, and practical approach for preliminary surveillance of antimicrobial residues where advanced quantitative techniques may not be readily available. Therefore, this study aimed to investigate the presence of antimicrobial residues in cattle tissues using microbial screening methods at slaughterhouses in Kilosa District, Morogoro.

MATERIALS AND METHODS

Study area

The study was conducted in Kilosa District (5°55’–7°53’S, 36°30’–37°30’E) in the Morogoro Region of eastern Tanzania, which has an average population density of 31 people per square kilometer. The region has a sufficient number of cattle stakeholders, including cattle keepers, traders/butcher owners, livestock officers, and consumers. Three wards Dumila, Kimamba, and Kilosa were involved in this study, as shown in Figure 1, because they have adequate numbers of livestock keepers and more than four livestock markets (Katakweba *et al.,* 2012).

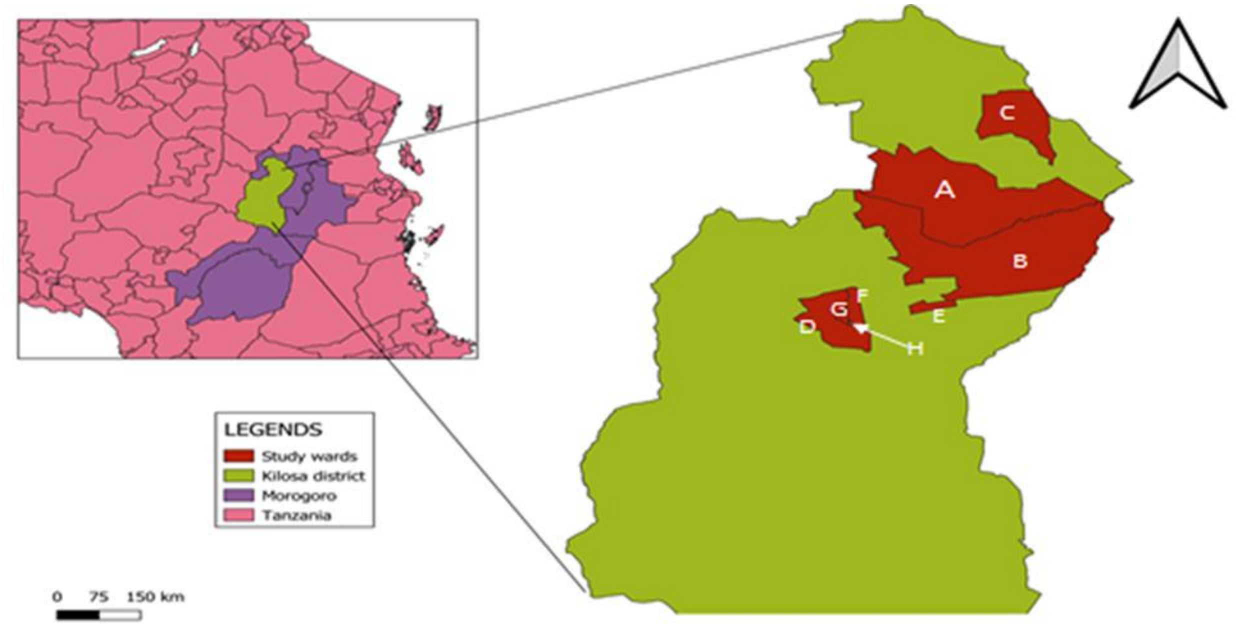

Figure 1: A map illustrating the study areas in Kilosa district, highlighting three wards involved in the study. The wards are represented by letters as follows: Dumila (C), Kimamba (E), and Kilosa (B, F, G, and H).

Study design, sample size and sampling procedure

A cross-sectional study was conducted between April and June 2025 to detect the presence of antimicrobial residues in cattle tissues. A total of 90 tissue samples were randomly collected from slaughterhouses in three wards of Kilosa District; Dumila, Kilosa, and Kimamba with 30 samples obtained from each ward. The selection of wards was based on their slaughterhouse activity, ensuring a representative distribution of samples. From each ward, 30 tissue samples were randomly selected, consisting of 10 kidney, 10 liver, and 10 muscle samples. Each sample, weighing approximately 100 grams, was placed in a cleaned, sterilized separate polythene bag, stored on ice at 4°C, and immediately transported to the microbiology laboratory at Sokoine University of Agriculture for analysis.

Sample preparation

For each tissue sample (liver, muscle, and kidney), a 5-gram portion was weighed and placed into a sterile plastic bag. The samples were then homogenized using a stomacher at high speed for 2 minutes. This process was repeated three times to ensure thorough mixing. The resulting homogenate was centrifuged at 5000 rpm for 10 minutes. After centrifugation, the supernatant was carefully decanted and stored at -20°C for further analysis

Preparation of the spore suspension

A spore suspension of *Bacillus subtilis* (ATCC 6633) was prepared following standard methods, as described by Al-mashhadany, (2019). Briefly, 0.1 mL of the standard organism was added to 20 mL of molten nutrient agar in each Petri dish, thoroughly mixed, and allowed to solidify for 10 minutes on a leveled surface. A heavy inoculum of *Bacillus subtilis* was then streaked onto the surface of nutrient agar plates (HiMe dia, India), which were incubated at 30°C for 10 days to induce sporulation. After incubation, the colonies were harvested into 10 mL of sterile normal saline and subjected to heat treatment at 70°C for 10 minutes to kill any vegetative cells. The heated suspension was centrifuged at 3000 rpm for 10 minutes, and the clear supernatant was discarded. An additional 10 mL of sterile saline was added to the pellet to wash away debris from lysed vegetative cells. The suspension was centrifuged again under the same conditions. This washing process was repeated twice to ensure the purity of the endospore suspension. Finally, the turbidity of the resulting spore suspension was adjusted to match a 0.5 McFarland standard solution (equivalent to approximately 1.5×10^8 CFU/mL).

Preparation of Mueller–Hinton Agar Plates

Muller-Hinton agar was prepared according to the manufacturer's instructions (HiMedia, India). Once the medium cooled to approximately 45°C, it was poured into sterile Petri dishes and allowed to solidify at room temperature. After solidification, 0.1 mL of a pure *Bacillus subtilis* spore suspension adjusted to match the 0.5 McFarland standard (1.5×10^8 CFU/mL) was evenly inoculated onto the surface of each agar plate using a sterile cotton swab. The inoculated plates were then stored in a refrigerator for later use.

Detection of antimicrobial residues in samples

A previously published method by Al-mashhadany, (2019) was adapted for the detection of antimicrobial residues in cattle tissue samples. The agar well diffusion technique was used for this purpose. Sterile Muller-Hinton agar plates, previously inoculated with the sensitive test organism *Bacillus subtilis*, were prepared. Using a sterile cork borer, four wells were made in each plate. Three wells were used to test different tissue samples, while the fourth well served as a negative control. Using a sterile micropipette, 2 mL of each homogenized tissue sample was carefully dispensed into the corresponding wells. The plates were then incubated aerobically at 37°C for 24 hours. After incubation, the presence of a clear (transparent) zone around a well indicated antimicrobial activity. A zone of inhibition measuring 2 mm or more was recorded as a positive result. While those less than 1 mm were regarded as negative results. The diameter of the inhibition zones for positive samples was measured using a ruler. Since no inhibition zones were observed around the control wells, the inhibition zones of the test samples were considered valid and recorded for further evaluation.

Statistical data analysis

Data on antimicrobial residues were coded and entered into Microsoft Excel 2010, then analyzed using SPSS version 25. Descriptive statistics were applied to calculate frequencies and percentages. The Chi-square test was used to assess differences between tissue samples, while Fisher's exact test evaluated the association between locations and antimicrobial residues. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Distribution of antimicrobial residues in cattle tissues across the wards

The analysis of 90 tissue samples collected from Dumila, Kilosa, and Kimamba wards revealed the presence of antimicrobial residues predominantly in liver and muscle tissues, while kidneys showed no detectable residues. Overall, 46.7% (14/30) of liver samples and 40% (12/30) of muscle samples tested positive for residues, whereas all kidney samples (0/30) tested negative, as indicated in the table 1 below.

Table 1. Detection of antimicrobial residues in muscle, kidney, and liver tissue samples collected from cattle. Positive (+) indicates the presence of residues, while negative (−) indicates no detectable residues.

Ward	**Sample ID**	**Muscle (N=30)**	**Kidney (N=30)**	**Liver (N=30)**
Dumila	DM1	+	-	+
	DM2	+	-	-
	DM3	-	-	-
	DM4	-	-	-
	DM5	+	-	+
	DM6	+	-	-
	DM7	+	-	+
	DM8	+	-	-
	DM9	+	-	+
	DM10	-	-	+
Kilosa	KM1	-	-	-
	KM2	-	-	-
	KM3	+	-	+
	KM4	-	-	+
	KM5	-	-	-
	KM6	-	-	+
	KM7	-	-	-
	KM8	-	-	-
	KM9	-	-	-
	KM10	+	-	+
Kimamba	MM1	-	-	-
	MM2	-	-	-
	MM3	-	-	+
	MM4	+	-	-
	MM5	-	-	+
	MM6	+	-	+
	MM7	+	-	-
	MM8	-	-	+
	MM9	-	-	+
	MM10	-	-	-

The prevalence of antimicrobial residues

As shown in Table 2, a total of 26 out of 90 tissue samples tested positive for antimicrobial residues. Residues were detected in liver and muscle tissues, whereas no residues were detected in kidney samples. A statistically significant association was observed between tissue type and the occurrence of antimicrobial residues ($\chi^2 = 57.375$, $p < 0.001$).

Table 2. Distribution of antimicrobial residue-positive samples by tissue type and the association between tissue type and residue detection.

Types of Samples	Number of Samples	Positive Samples		*P- value*
		No	(%)	
Muscles	30	12	40	
Kidney	30	00	00	0.000
Liver	30	14	46.7	
Total	90	26	28.9	

Association between antimicrobial residue occurrence and wards in cattle tissues.

The results presented in Table 3 reveal significant variation in the occurrence of antimicrobial residues among wards in muscle tissues ($p < 0.001$), whereas no significant differences were observed in liver tissues ($p > 0.314$). No antimicrobial residues were detected in kidney samples from any ward.

Table 3. Shows the frequency of positive samples and their association with sampling location.

Tissue	**Dumila**		**Kilosa**		**Kimamba**			
	No sample	Positive (%)	No sample	Positive (%)	No sample	Positive (%)	Total samples	*P-value*
Muscle	10	7 (70)	10	2 (20)	10	3 (30)	30	0.000
Liver	10	5 (50)	10	4 (40)	10	5 (50)	30	0.314
Kidney	10	0 (00)	10	0 (00)	10	0 (00)	30	•
Total	30	12 (40)	30	6 (20)	30	8 (26.7)	90	

- *Indicating no statistical analysis was performed for kidney samples due to constant outcome (all negative)*

DISCUSSION

The presence of antibiotic residues in cattle meat and other animal-derived food products can pose serious health risks to consumers due to their potential toxic effects.

In this study conducted in Kilosa District, microbial screening methods detected the presence of antimicrobial residues in cattle meat intended for human consumption. These findings provide preliminary evidence of possible antimicrobial residue contamination and demonstrate the usefulness of microbial screening as an initial surveillance tool for identifying samples that may require further confirmatory quantitative analysis. Although antimicrobials are essential in cattle farming and beef production, their use must strictly adhere to established regulatory frameworks to prevent misuse and overuse. (Correa *et al.,* 2018; Shang *et al.,* 2018). One major concern is the contamination of food products with antibiotic residues, which can contribute to the development of antimicrobial resistance. In response, the Codex Alimentarius Commission (CAC) has recommended that food products derived from animals treated with veterinary drugs should contain antimicrobial residue levels below the established Maximum Residue Limits (MRLs) to ensure consumer safety (MacNeil and Kay, 2016).

In the current study, (28.9%) of the 90 tissue samples tested positive for antimicrobial residues. This prevalence is comparable to findings from a study conducted in Sudan, where 27% of samples were found to contain antimicrobial residues (Elnasri *et al.*, 2014), Such results suggest possible misuse of antimicrobials by veterinarians and cattle keepers, as well as insufficient monitoring systems for antimicrobial residues in slaughterhouses. In contrast, lower prevalence rates have been reported in studies conducted in Iran (22.8%), Sudan (17.33%), Philippines (16.7%), and Iraq (10.8%) (Babapour *et al.,* 2012; Alla *et al.,* 2011.; Tabon & Domondon, 2019; Al-mashhadany, 2019b) respectively. These variations in prevalence may be attributed to differences in antimicrobials usage practices, including non-compliance with recommended pre-slaughter withdrawal periods and the indiscriminate or excessive use of antimicrobials by livestock farmers (Khatun *et al.*, 2018).

There is continuing argument regarding the most appropriate tissue for the detection of antimicrobial residues. Among edible tissues, muscle is generally considered the primary tissue for monitoring, followed by the liver and kidneys. In the present study, a higher proportion of antimicrobial residues was detected in liver and muscle tissues, whereas no residues were identified in kidney samples. These findings contrast with those reported by Pavlov *et al*., (2008), Elnasri *et al.*, (2014), and Ezenduka *et al.*, (2014), who observed the highest frequency of antimicrobial residues in kidney tissues, followed by the liver and muscle, particularly in poultry. Furthermore, the prevalence of antimicrobial residues in this study was higher than that reported by Shahid *et al.*, (2007) and Elnasri *et al.,* (2014) and , who documented an incidence ranging from 21.4% to 28.3% across liver, kidney, and muscle tissues of poultry in Khartoum State, Sudan. The higher levels of antimicrobial residues observed in livestock tissues in the present study may be attributed to the relatively recent introduction of antibiotic use in animal production within the study area (Kimera *et al.*, 2015). Many farmers remain unfamiliar with appropriate antibiotic administration practices, which likely contributes to misuse and the subsequent accumulation of residues in edible tissues (Sindato *et al*., 2020; Mpina and Mgonja, 2024;).

The observed variation in antimicrobial residue occurrence within muscle tissues across the sampling locations was statistically significant, with Dumila recording the highest number of positive samples compared to Kimamba and Kilosa. This suggests that practices related to drug administration, adherence to withdrawal periods, or post-slaughter monitoring may vary considerably across wards, particularly in Dumila. Also the study revealed that liver tissues had positivity rates ranging from 40% to 50% across the wards. Although slight variations were observed among the study sites, the differences were not statistically significant (P = 0.314), indicating a relatively similar occurrence of positive liver samples across the areas. Notably, no antimicrobial residues were detected in kidney tissues from any of the three locations. In contrast, a study conducted in Sudan reported that kidneys showed the highest prevalence of antimicrobial residues compared to other tissues (Elnasri *et al.*, 2014; Shahid *et al.*, 2007). The presence of antimicrobial residues in cattle tissues suggests that antimicrobial use in the study areas is mainly driven by the need to treat and prevent animal diseases under field conditions, often with limited veterinary supervision and reliance on empirical treatment. Such practices may lead to inappropriate or repeated use of antimicrobials, increasing the likelihood of residues in edible tissues. This indicates that antimicrobial use practices in the study areas may not always follow recommended guidelines, particularly regarding dosage and withdrawal periods. To reduce this burden, improved biosecurity, better animal husbandry practices, and effective vaccination programs should be encouraged as alternatives to frequent antimicrobial use. Strengthening veterinary extension services and promoting responsible drug use among livestock keepers are also important. These interventions could help reduce unnecessary antimicrobial use and minimize the occurrence of residues in food animals.

Limitation of the study

This study had some limitations. Firstly, it did not quantify antimicrobial residue concentrations and identify specific compounds. Secondly, it was also limited to three wards in Kilosa District, which may not represent the entire area, and the two-month sampling period may not reflect seasonal variation in antimicrobial use and residues occurrence. Despite these limitations, the study provides important preliminary evidence on the presence of antimicrobial residues in cattle tissues. The method used offered a rapid, low-cost, and practical screening approach for detecting the presence or absence of residues, making it suitable for routine monitoring in resource limited settings. These findings provide a useful basis for future studies employing more advanced quantitative techniques to accurately measure residue levels and identify specific antimicrobial compounds.

CONCLUSION

This study highlights the significant presence of antimicrobial residues in cattle meat from Kilosa District, with 28.9% of the samples testing positive particularly in muscle and liver tissues indicating a potential risk to public health. The variation in residue prevalence across different wards and tissue types suggests inconsistent antibiotic use practices and poor compliance with recommended withdrawal periods. While the screening method used was effective for initial detection, its inability to quantify residue levels or identify specific antibiotics limits the depth of risk assessment. Overall, these findings underscore the urgent need for stricter regulatory control, improved antibiotic stewardship in livestock production, and enhanced monitoring systems to safeguard consumers and mitigate the growing threat of antimicrobial resistance.

RECOMMENDATION

Based on the findings of this study, it is recommended that regulatory authorities strengthen the enforcement of guidelines on antimicrobial use in livestock, particularly ensuring strict adherence to drug withdrawal periods before slaughter. Training programs should be implemented to educate farmers, veterinary drug retailers, and animal health practitioners on the prudent use of antibiotics and the public health risks associated with residues. Additionally, routine surveillance and monitoring systems should be established in slaughterhouses to detect antimicrobial residues in animal tissues. Future studies should adopt advanced, quantitative analytical techniques to accurately determine residue levels and identify specific antimicrobial compounds, thereby supporting more effective control strategies to ensure food safety and protect public health.

ETHICAL APPROVAL

The ethical license for this study was granted by the Sokoine University of Agriculture Institutional Review Board under reference number SUA/DPRTC/R/186/29. All procedures for collecting samples from cattle were conducted in full compliance with Tanzanian legislation, ensuring adherence to ethical standards and national guidelines.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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