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Asimwe Mukalunyoisa Mugyabuso
asimwemugyabuso2@gmail.com
Sokoine University of Agriculture
Isaac Makundi
Sokoine University of Agriculture
Christopher John Mwankuna
Sokoine University of Agriculture
Faith Philemon Mabiki
Sokoine University of Agriculture
Abubakar Shaaban Hoza
Sokoine University of Agriculture

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Determination of antimicrobial residue concentration levels in raw cow's milk from dairy farms in Mvomero district, Tanzania

Asimwe M. Mugyabuso[1*], Isaac Makundi [1], Christopher J. Mwankuna[2], Faith P. Mabiki[2], Abubakar. S. Hoza[1]

[1]Department of Veterinary Microbiology, Parasitology & Biotechnology, Sokoine University of Agriculture, P.O. BOX 3019, Morogoro, Tanzania.

[2]Department of Chemistry and Physics, College of Natural and Applied Sciences, Sokoine University of Agriculture, P.O. BOX 3000, Morogoro, Tanzania.

[*]Corresponding author: asimwemugyabuso2@gmail.com

Abstract

Background

Veterinary antibiotics are essential for maintaining dairy cow health and preventing infections. However, improper use can leave residues in milk, which may pose public health risks. Understanding the occurrence of these residues and their potential impact on consumers is critical for safe milk production. This study determined the residue concentration levels and potential health risks of oxytetracycline, tetracycline, and tylosin residues in raw cow milk in Mvomero district, Tanzania.

Methods

A cross-sectional study was conducted in Dakawa and Mtibwa wards of Mvomero district, where raw cow milk samples were collected. Samples were first screened for antimicrobial residues using an inhibition assay and confirmed through High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS/MS). Residue concentrations were compared against the Maximum Residue Limits (MRLs) established by the Codex Alimentarius Commission, specifically those outlined in the Codex Alimentarius MRLs for Veterinary Drugs in Foods (CXM 2-2018) [1]. Also reference was made to national standards, including guidelines from the Tanzania Medicines and Medical Devices Authority and the Ministry of Livestock and Fisheries Tanzania [2], [3]. Human health risk was assessed using Hazard Quotient (HQ) based on the estimated daily intake [4], [5].

Results

Out of the 380 raw cow milk samples screened using a microbial inhibition assay, 36 (9.5%) tested positive for antimicrobial residues. Confirmatory HPLC-MS/MS analysis of the 36 positive samples and 16 randomly selected negative samples revealed that OTC had the highest mean concentration (95.37 ± 12.51 mg/kg), followed by TTC (0.63 ± 0.07 mg/kg) and tylosin (0.23 ± 0.06 mg/kg). Residue levels of oxytetracycline and tetracycline generally exceeded the 100 µg/kg MRLs, while only 16 of the samples exceeded the permissible limits for tylosin. The estimated daily intake (EDI) values were 0.054, 0.0004, and 0.0001 mg/kg body weight/day for oxytetracycline, tetracycline, and tylosin, respectively, with corresponding Hazard Quotient (HQ) values of 1.80, 0.01, and 0.00. These findings indicate a potential health risk associated

with oxytetracycline exposure through milk consumption, whereas tetracycline and tylosin are unlikely to pose significant chronic health risks at average intake levels. However, prolonged exposure to antimicrobial residues may contribute to antimicrobial resistance and long-term public health concerns.

Conclusion

Findings from this study highlight critical gaps in antimicrobial use practices and regulatory compliance within dairy production systems that may compromise milk safety and consumer health. Strengthening antimicrobial stewardship, improving farmer awareness on responsible drug use, adherence to withdrawal periods, and enforcing routine residue monitoring are essential to ensure the safety of milk and milk products and to mitigate the growing public health threat associated with antimicrobial resistance.

Key words: Drug residues; milk; Maximum residue limits, antimicrobials; HPLC; LC-MS; public health risk

Introduction

Milk and dairy products are among the most important animal-source foods, providing essential nutrients to people of all age groups and playing a critical role in food security and nutrition worldwide [6]. Global demand for milk is estimated to rise by 80% in 2030, mainly driven by population growth, urbanization, and advances in dairy production technologies [7], [8]. As milk consumption increases, ensuring its safety has become a major public health priority, particularly in low and middle-income countries where regulatory oversight may be limited [9].

To sustain high productivity and manage infectious diseases in dairy cattle, antibiotics are widely used, especially for treatment and prevention of conditions such as mastitis, respiratory infections, and gastrointestinal diseases [10]. However, inappropriate use of antibiotics, including incorrect dosing, self-prescription, and failure to observe withdrawal periods, can result in the persistence of antimicrobial residues in milk [11], [12]. These residues pose significant health risks to consumers, including hypersensitivity reactions, gastrointestinal disturbances, and disruption of beneficial gut micro flora [13]. Long-term exposure to antibiotic residues has also been linked to more serious chronic effects, such as toxicity to vital organs and increased selective pressure for antimicrobial resistance (AMR) [14], [15], [16].

Globally, it is estimated that more than 70% of antibiotics are used in food-producing animals, and antimicrobial use in livestock is projected to continue increasing in the coming decades [17]. In sub-Saharan Africa, including Tanzania, antibiotics are often readily accessible without a prescription, and their use is frequently poorly regulated [18], [19], [20]. Limited veterinary services, inadequate farm hygiene, weak enforcement of regulations, and low farmer awareness of withdrawal periods further exacerbate the risk of antibiotic residues entering the milk supply chain [21], [22].

Among the commonly used veterinary antimicrobials, tetracycline-class antibiotics, particularly oxytetracycline, are extensively used in livestock production due to their broad-spectrum activity, affordability, and widespread availability [23], [24]. Oxytetracycline is frequently administered to dairy cattle, often without adequate veterinary supervision, making it one of the most commonly detected antibiotic residues in milk worldwide [12], [25]. In addition, tylosin, a

macrolide antibiotic, is widely used in the dairy sector for the treatment of mastitis and respiratory infections, which are prevalent in smallholder dairy systems. Although these antibiotics are effective in disease control, their frequent and sometimes indiscriminate use increases the likelihood of residue persistence in milk [26], [27][26] .

To protect consumers, Food and Agriculture Organization (FAO) of the United Nations, the World Organization for Animal Health (WOAH), the World Health Organization (WHO), and the Codex Alimentarius Commission have established Maximum Residue Limits (MRLs) for veterinary antibiotics in milk (FAO/WHO; 2021). For oxytetracycline, tetracycline, and tylosin, the recommended MRLs are 100μg/kg; therefore, exceeding these limits may not only pose direct health risks but also interfere with trade and dairy fermentation processes by inhibiting beneficial lactic acid bacteria [28],[38]. Despite the existence of these standards, compliance remains a challenge in many developing countries, including Tanzania, due to weak regulatory enforcement, over-the-counter antibiotic sales, poor biosecurity practices, and limited routine monitoring of milk attributable to poor laboratory capacities [23], [29], [30].

Studies from different parts of the world have reported varying prevalence of antibiotic residues in milk, with particularly high levels documented in regions where antimicrobial stewardship is weak (Lweendo *et al*., 2025; Simbine-ribisse *et al*., 2024). In Kenya and Rwanda several studies have reported the presence of tetracycline residues in raw milk, often exceeding MRLs [33], [34]. In Tanzania, available data indicate that antimicrobial residues in milk are not uncommon, especially in smallholder dairy systems; however, comprehensive and up-to-date data on specific antibiotics such as oxytetracycline, tetracycline, and tylosin remain limited [24], [35].

Given the public health risks associated with antibiotic residues in food of animal origin and their contribution to antimicrobial resistance (AMR), continuous monitoring of milk is essential. Beyond detection of residues, assessing dietary exposure and associated health risks provides important evidence for informed public health and regulatory decisions. This study also aligns with the NAP-AMR 2023–2028 Strategic Objective 5, which focuses on strengthening AMR surveillance and generating evidence to support risk-based decision-making and policy development in Tanzania [36]. By determining antimicrobial residue levels in raw cow's milk and evaluating human health risks using the Hazard Quotient approach, the study contributes to national efforts to improve food safety monitoring and control AMR [5].

Therefore, this study aimed to determine the presence and concentration levels of oxytetracycline, tetracycline, and tylosin residues in raw cow milk using a screening-confirmation analytical approach, and to evaluate the potential health risks associated with milk consumption using the Hazard Quotient (HQ) method.

Materials and Methods

Study area

The study was carried out in Mvomero District, Morogoro Region, Tanzania (Figure 1). Mvomero District is located between latitudes 05°45′ S - 07°26′ S and longitudes 37°09′ E - 38°07′ E, covering an area of approximately 6,631 km². The district is predominantly rural and characterized by mixed crop-livestock farming systems that form the mainstay of the local livelihood economy. Mvomero experiences a warm, humid tropical climate with diverse agro-

ecological zones that favour dairy production but also contribute to the prevalence of cattle diseases, leading to frequent use of veterinary drugs, including antimicrobials. Livestock keeping is widespread, with smallholder dairy farmers, including Maasai pastoral communities, serving as the primary milk producers. Farmers predominantly keep indigenous and crossbred dairy cattle under extensive, semi-intensive, and intensive management systems.

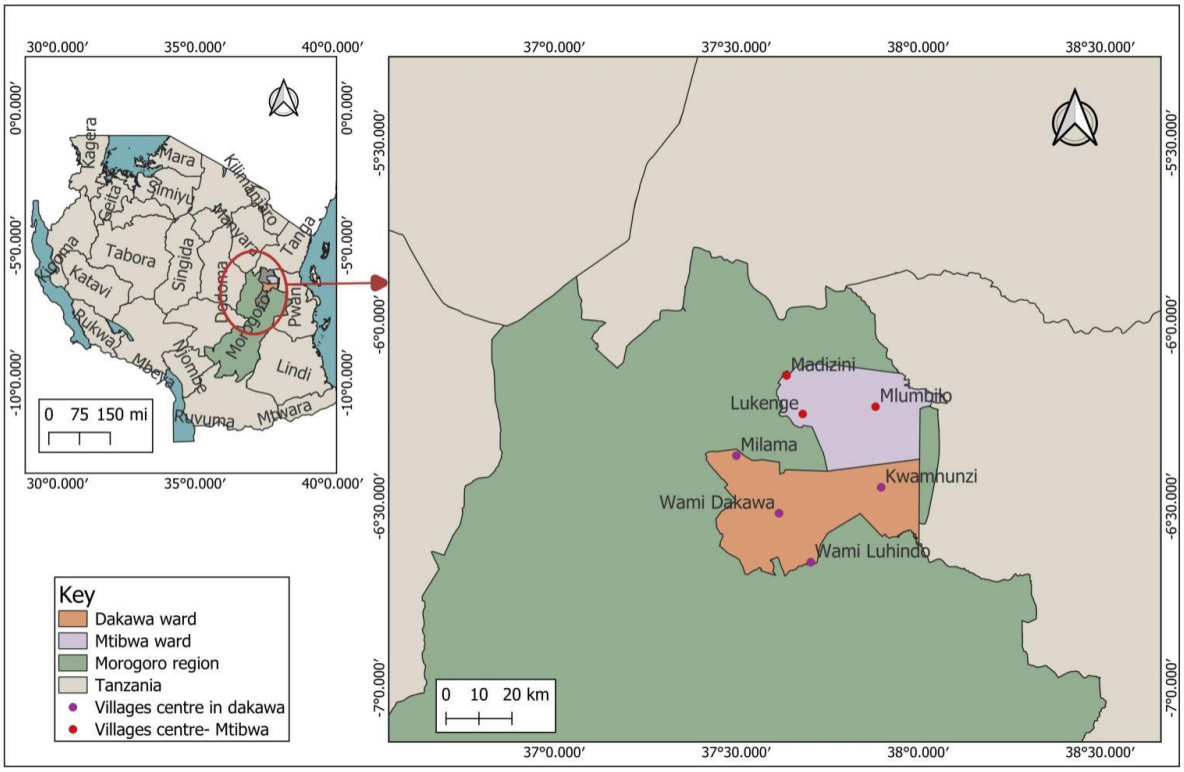


Figure 1: A map of Mvomero District, Morogoro Region, Tanzania, showing the wards of Dakawa and Mtibwa, and villages where milk samples were collected for this study. (Map adapted from [40]).

Study design and sample size determination

A cross-sectional study was conducted between February and May 2025. Milk samples were collected from dairy farms within the district during the study period. The required sample size

was determined using Cochran's formula $n = \frac{z^2 p(1-p)}{d^2}$ for estimating proportions in a population with unknown prevalence; the minimum required sample size used was 380 milk samples (Cochran, 1977). Only lactating cows that were neither under antimicrobial treatment nor under withdrawal period observation were selected for sampling in this study.

Sample collection and processing

Milk samples were collected directly from lactating cows using clean, sterile 50 mL Falcon tubes. Prior to sampling, the udder and teats were cleaned using clean tap/well water to remove visible dirt and minimize the risk of contamination. Pooled milk samples from all four quarters of each cow were obtained during routine milking, and approximately 30 mL of milk was collected per cow.

A total of 380 milk samples were collected, comprising 182 samples from Mtibwa Ward and 198 samples from Dakawa Ward. In Mtibwa Ward, 53 samples were collected from Madizini village, 63 from Mlumbilo, and 82 from Lukenge. In Dakawa Ward, 35 samples were collected from Wami Luhindo village, 60 from Kwa Mhunzi, 65 from Wami Dakawa, and 22 from Milama. The distribution of samples across villages was determined based on the number of dairy cattle available in each village and the consent of cattle owners for sample collection.

Each sample was immediately placed in a cool box containing ice packs under approximately −24 °C. The samples were transported on the same day to the Microbiology Laboratory at the College of Veterinary Medicine and Biomedical Sciences, at Sokoine University of Agriculture (SUA). Upon arrival, all milk samples were kept at −20 °C freezer until further analysis. In addition to milk sampling, farm- and animal-level information was collected using a structured

observational checklist and a semi-structured questionnaire. These were used to document key variables, including production system (intensive, semi-intensive, or extensive), treatment records, vaccination status, breed type, management practices, and approximate age of the animal. These data were obtained through direct observation and confirmation from the animal owner or farm attendant at the time of sampling.

Inhibition zone assay

Antimicrobial residues in raw cow milk were screened using an agar well diffusion inhibition assay based on the suppression of bacterial growth on Mueller–Hinton agar (Oxoid Ltd., Basingstoke, UK) used as the culture medium and prepared according to the manufacturer's instructions. A laboratory-maintained strain of *Bacillus subtilis*, obtained from the microbiology laboratory, was used as the indicator organism due to its sensitivity to a broad range of antimicrobial agents [37]. The bacterial suspension was standardized to 0.5 McFarland turbidity, corresponding to approximately 1.5×10^8 CFU/mL, to ensure uniform inoculation. The standardized inoculum was then evenly distributed over the surface of the agar plates using a sterile cotton swab by the streak method, in which the plate was streaked in three directions while rotating it approximately 60° between each streak, followed by a final sweep around the rim to ensure complete and uniform coverage, resulting in a confluent bacterial lawn.

After inoculation, uniform wells were aseptically created in the agar using a sterile pipette tip. A 50µl of raw milk sample was dispensed into each well. Negative control (nomal saline) was included to validate assay performance. Also, an archived sample previously confirmed to contain antimicrobial residues was used as a positive control. The plates were incubated at 37 °C

for 24 hours to allow diffusion of antimicrobial residues and bacterial growth. Following incubation, plates were examined for the presence of clear zones of inhibition surrounding the wells. The diameters of inhibition zones were measured in millimetres using a Vernier calliper. Samples showing no visible inhibition were recorded as 0 mm, indicating either the absence of antimicrobial residues or concentrations below the detection limit of the assay. The size of the inhibition zone was used as a semi-quantitative indicator of antimicrobial activity, with larger zones suggesting higher levels of biologically active residues.

The agar well diffusion assay served as a preliminary screening method for antimicrobial residues. Milk samples that tested positive were subsequently subjected to confirmatory and quantitative analysis using High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS/MS).

HPLC-MS/MS instrumentation and optimization of analytical conditions

Chromatographic and mass spectrometric analyses were performed using an Agilent 1100 Series High-Performance Liquid Chromatography (HPLC) system (Agilent Technologies, Germany) coupled to a Quattro Micro API triple quadrupole tandem mass spectrometer (Waters, Micromass Limited, UK) equipped with an electrospray ionization (ESI) source operated in positive ion mode. The analyses were conducted at the Department of Chemistry and Physics at Sokoine University of Agriculture (SUA). LC system comprised a degasser (G1379A), binary pump (G1312A), autosampler (G1313A), column oven (G1316A), and diode array detector (G1315B). Instrument control, data acquisition, and processing were carried out using MassLynx version 4.1 software (Waters Corporation, USA). Nitrogen was used as both the nebulizing and

desolvation gas, while argon served as the collision gas. The mass spectrometer was operated in ESI positive mode under optimized conditions: capillary voltage 3.0 kV, source temperature 150 °C, desolvation temperature 350 °C, desolvation gas flow 550 L/h, cone gas flow 900 L/h. Data were acquired in multiple reactions monitoring (MRM) mode.

Chromatographic separation was achieved on an Xterra® MSC_8 column (2.1× 150mm: 3.5µm) and a 10mm guard column (Phenomenex USA). A flow rate of 0.3 mL/min, with the column temperature maintained at 40 °C. The mobile phase consisted of solvent A, aqueous (polar) solvent (65%), and solvent B, organic solvent (35%), delivered under gradient elution whose mobile phase composition was systematically changed over time, with a total run time of 20 minutes.

Specific parameters for MS/MS operation

For compound-specific detection, oxytetracycline, tetracycline, and tylosin were monitored as shown in Table 1 and the optimized conditions ensured selective, sensitive, and reliable detection of the target analytes.

Table 1: MS/MS parameters for compound-specific detection of target antibiotics

Compound	Precursor Ion [M+H]+ (m/z)	Cone Voltage (V)	Quantification Ion (m/z)	Confirmation Ion (m/z)
OTC	461.22	18	426 (21)	443.4 (15)
TTC	445	19	409.9 (21)	153.8 (25)
TYL	916.65	41	173.9 (39)	100.6 (48)

Method validation

Method linearity was evaluated using matrix-matched calibration standards within defined concentration ranges. Oxytetracycline demonstrated linearity over the range of 1.0–10.0 µg/mL, with the regression equation $y = 422.44x - 324.12$ and a correlation coefficient (R^2) of 0.9943. Tetracycline was linear over the concentration range of 0.04–0.80 µg/mL, following the regression equation $y = 1305.3x - 79.898$, with an R^2 value of 0.9922. Tylosin showed linearity within the range of 0.0005–0.0020 µg/mL, with the calibration equation $y = 46219x - 1.1345$ and an R^2 value of 0.999. These high correlation coefficients indicate excellent linearity of the method for all analytes.

Chemicals and reagents

HPLC-grade acetonitrile and HPLC-grade methanol (Loba Chemie Pvt. Ltd., Mumbai, India), together with ultrapure water generated from a Milli-Q purification system (Millipore Corp., Burlington, MA, USA), were used throughout the study. Formic acid and ammonium formate (Loba Chemie Pvt. Ltd., Mumbai, India) were used in the preparation of the mobile phase.

Methanol and HPLC-grade water were used for sample extraction, dilution, and preparation of standard solutions. The mobile phase used for LC-MS/MS analysis consisted of mobile phase A and mobile phase B. Mobile phase A was composed of HPLC-grade water containing 5 mM ammonium formate (NH_4COO) and 0.1% formic acid, while mobile phase B consisted of methanol containing 5 mM ammonium formate (NH_4COO) and 0.1% formic acid. Analytical reference standards of oxytetracycline hydrochloride (CAS No. 2058-46-0), tetracycline (CAS No. 60-54-8), and tylosin (CAS No. 1401-69-0; Sigma-Aldrich, Glostrup, Denmark) were used for method calibration, validation, and quantification of antimicrobial residues in raw cow's milk samples. Stock solutions of each antibiotic were prepared independently at a concentration of 1 mg/mL by dissolving 0.01 g of each standard in 10 mL of methanol. The solutions were thoroughly mixed using a vortex mixer to ensure complete dissolution. All weighing procedures were conducted using an analytical balance with clean weighing accessories to ensure accuracy and prevent contamination. The prepared stock solutions were transferred into brown glass bottles and stored at −20 °C to minimize degradation. Working standard solutions (1 µg/mL) were subsequently prepared by dilution with methanol, stored at 4 °C, and used within one week to ensure analytical stability during LC-MS analysis.

Selection and preparation of antibiotic standards for LC-MS analysis

Three antibiotic standards OTC, TTC, and TYL were selected for LC-MS analysis, representing the tetracycline and macrolide classes of veterinary antimicrobials. Oxytetracycline was prioritized because of its extensive global use in cattle due to their easily availability and affordability. Tylosin was selected due to its high level of use in the treatment of frequently occurring conditions, particularly mastitis and respiratory infections in dairy cattle, especially

within the smallholder scale production systems. Its inclusion also addresses concerns related to the growing emergence of macrolide resistance and increases the likelihood of residue occurrence in milk when withdrawal periods are not strictly observed [38].

Sample preparation procedure

Approximately 1 mL of each of the 52 milk samples analyzed by HPLC-MS/MS (comprising 36 samples that tested positive and 16 randomly selected negative samples by the microbial inhibition assay) was processed individually by mixing with 1 mL of extraction solvent consisting of acetonitrile, HPLC-grade water, and methanol in the ratio of 2:1:1, respectively. The mixture was sonicated for 5 minutes to enhance the extraction of antibiotic residues and then centrifuged at 4000 rpm for 10 minutes.

The resulting supernatant was carefully transferred to a sterile Eppendorf tube and subjected to further centrifugation at 3500 rpm for 10 minutes. The final supernatant was collected and used for LC-MS analysis of antibiotic residues.

Health risk assessment

Dietary exposure to antimicrobial residues through milk consumption was assessed by estimating the Estimated Daily Intake (EDI), which represents the daily amount of a contaminant ingested per unit body weight. The EDI was calculated using the following equation:

$$EDI = (C * IR) / BW$$

Where; *C* is the concentration of the contaminant in milk (mg/kg or mg/L), *IR* is the ingestion rate of milk (kg/day or L/day), and *BW* is the average body weight of the consumer (kg).

Potential non-carcinogenic health risks were evaluated using the Hazard Quotient (HQ), calculated as:

$$HQ = EDI / RfD$$

Where *EDI* is the estimated daily intake (mg/kg/day), and *RfD* is the reference dose (mg/kg/day).

Data management and analysis

Data were entered into Microsoft Excel 2021 and analysed using IBM SPSS Statistics 31.

Descriptive statistics were used to summarize the data: frequencies and percentages for categorical variables, and means, standard errors, medians, and interquartile ranges (IQR) for continuous variables. Prevalence was expressed as percentages with 95% confidence intervals.

Normality of antibiotic concentrations was assessed using Q-Q plots and the Shapiro-Wilk test. Since the data were not normally distributed, non-parametric methods were applied. Associations between variables and residue presence were tested using the Chi-square test, while logistic regression identified independent predictors. Differences in antibiotic concentrations were analysed using the Friedman test, followed by Wilcoxon signed-rank tests with Bonferroni correction for pairwise comparisons. Dietary risk was evaluated using the Estimated Daily Intake (EDI) and Hazard Quotient (HQ), where HQ > 1 indicates potential health risk.

Results

Prevalence of antimicrobial residues

The prevalence of antimicrobial residues determined by the agar well diffusion assay is shown in Figure 2. Out of 380 samples analyzed, 36 were positive, corresponding to an overall prevalence of 9.5% (95% CI: 6.5–12.4). Ward-level analysis showed a prevalence of 10.1% (20/198; 95% CI: 5.9–14.3) in Mtibwa and 8.8% (16/182; 95% CI: 4.7–12.9) in Dakawa. No significant difference in prevalence was observed between wards ($\chi^2 = 1.53$, df = 6, p = 0.957).

At the village level, the lowest prevalence was recorded in Lukenge (Mtibwa) and Milama (Dakawa) (Table 2). However, village-level differences were not statistically significant in either ward, Mtibwa ($\chi^2 = 0.38$, df = 2, p = 0.83) or Dakawa ($\chi^2 = 1.01$, df = 3, p = 0.80).

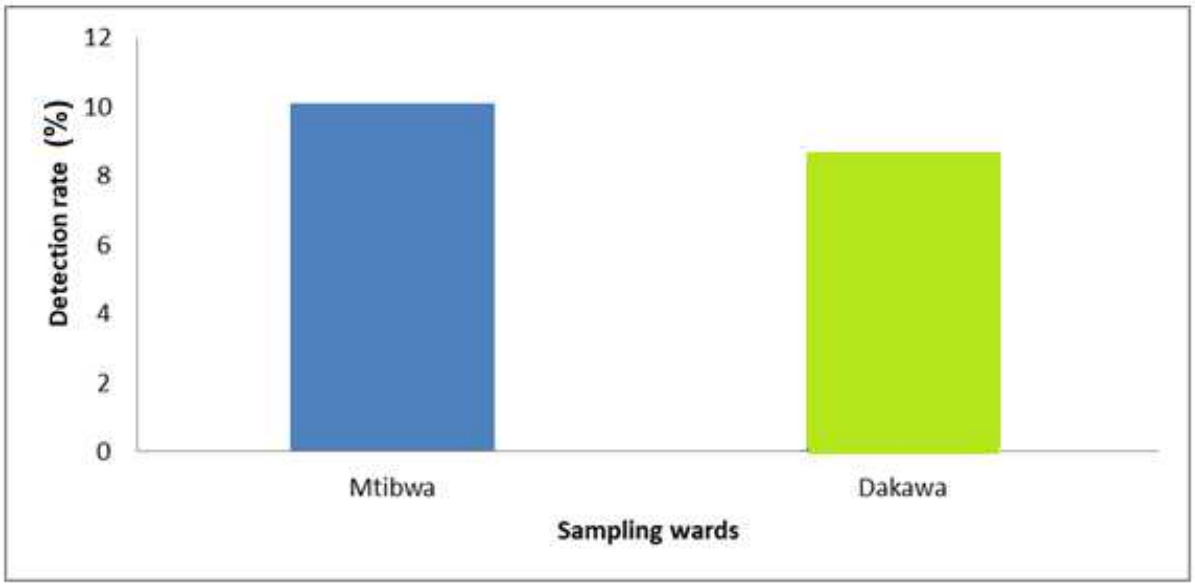


Figure 2: Prevalence of antimicrobial residues in milk samples from in Mtibwa (N=198) and Dakawa (N=182)

Table 2: Comparative results of the prevalence of antimicrobial residues among the villages

Ward	Village	Total	n	% (95%CI)
Mtibwa	Madizini	53	6	11.3 (5.3 - 22.5)
	Mlumbilo	63	7	11.1 (5.5 - 21.2)
	Lukenge	82	7	8.5 (4.2 - 16.5)
Dakawa	Wami Luhindo	35	4	11.4 (4.6 - 25.9)
	Kwa Mhunzi	60	6	10.0 (4.7 - 20.2)
	Wami Dakawa	65	5	7.7 (3.3 - 16.8)
	Milama	22	1	4.5 (0.8 - 21.8)

χ^2	df	P-value
1.53	6	0.9574

CI=Confidence Interval, n=number of positive samples

Factors associated with the detection rate of antimicrobial residue

Table 3 presents the results of the Chi-square analysis examining factors associated with the occurrence of antimicrobial residues. The detection rate of residues was highest in intensive (70.0%) and semi-intensive (52.0%) production systems, while extensive systems had a much lower rate (4.6%) ($\chi^2 = 104.8$, $p < 0.0001$). Interestingly, vaccination status did not significantly

influence residue occurrence, as both vaccinated (54.5%) and unvaccinated animals (45.5 %) showed comparable patterns ($\chi^2 = 37.43$, $p < 0.001$). Crossbred animals had a substantially higher proportion of residues (57.1%) compared to local breeds (4.6%) ($\chi^2 = 101.88$, $p < 0.001$). Regarding farmers' education level, milk from farmers with tertiary education showed lower residue prevalence (7.5%) compared to those with primary (36.4%) or informal education (18.9%) ($\chi^2 = 14.65$, $p = 0.0007$), indicating that less educated farmers were more likely to have milk with antimicrobial residues.

Table 3: Chi-square analysis results showing the differences and factors associated with residues in surveyed villages

Parameter	**Category**	**Total**	**Positive**	**%**	**χ^2**	**P - value**
Farming system	Extensive	345	16	4.6	104.8	<0.0001
	Intensive	10	7	70.0		
	Semi intensive	25	13	52.0		
Vaccination	No	360	21	5.8	37.4	<0.0001
	Yes	11	6	54.5		
Breed	Cross	35	20	57.1	101.8	<0.0001
	Local	345	16	4.6		
Farmer's Education	Informal	37	7	18.9	14.6	0.0007

Primary	332	25	7.5
Tertiary	11	4	36.4

The logistic regression analysis (Table 4) identified animal age and breed type as the primary factors associated with the presence of antimicrobial residues in milk. Older animals were significantly more likely to have residues compared to younger animals (OR = 3.14, 95% CI: 1.59–6.21, p = 0.001).

Breed type also showed a strong association with residue occurrence. Local breeds were much less likely to have residues than crossbreeds (OR = 0.01, 95% CI: 0.003–0.040, p < 0.001), likely due to differences in management intensity.

Table 4: Logistics regression results showing age, breed and farming system as significantpredictors of antimicrobial residues

Variable	Odds Ratio	95%	C.I.	S. E.	Z-Statistic	P-Value
Age of animal	3.14	1.6	6.2	0.3	3.3	0.001
Breed (local/cross)	0.01	0.0	0.0	0.7	-6.7	0
Vaccination (yes/no)	2.13	0.3	14.2	1.0	0.8	0.4342
Education (Primary/informal)	0.28	0.1	1.4	0.8	-1.5	0.127
Farmer's education (Tertiary/informal)	0.38	0.0	5.3	1.3	-0.7	0.473
Farming system (Intensive/Extensive)	47.97	11.3	203.0	0.7	5.3	<0.0001
Farming system (semi-intensive/Extensive)	22.27	8.77	56.5	0.5	6.5	<0.0001

Levels of antibiotic residues and public health risks

A total of 52 milk samples were analysed using HPLC-MS/MS for oxytetracycline, tetracycline and tylosin residues. OTC had a mean concentration of 95.37 ± 12.51 mg/kg and a median of 76.73 mg/kg (IQR = 161.86). All 52 samples exceeded the Codex maximum residue limit (MRL) of 0.1 mg/kg for oxytetracycline in milk. Tetracycline also exceeded the MRL with a mean of 0.63 ± 0.07 mg/kg and median of 0.50 (IQR 0.67). Tylosin showed the lowest residues limit (mean 0.23 ± 0.06 mg/kg; median 0.00, IQR 0.30), with only 16 (30.8%) samples being above the MRLs. The Estimated Daily Intake (EDI) and Hazard Quotient (HQ) indicated a potential health risk for oxytetracycline (EDI = 0.054 mg/kg bw/day; HQ = 1.80), while for tetracycline

(EDI = 0.0004; HQ = 0.01) and tylosin (EDI = 0.0001; HQ = 0.00) posed negligible risk (Table 5). These results demonstrate widespread oxytetracycline contamination, moderate tetracycline exposure, and limited tylosin residues in the sampled milk.

Table 5: MRLs, EDI and HQ of oxytetracycline, tetracycline, and tylosin residues in milk samples

Antibiotic	Mean ± SE	Median (IQR)	>MRL (n, %)	EDI (mg/kg/day)	HQ
OTC	95.37 ± 12.51	76.73 (161.86)	52(100)	0.054	1.8
TTC	0.63 ± 0.07	0.50 (0.67)	52(100)	0.0004	0.01
TYL	0.23 ± 0.06	0.00 (0.30)	16(30.8)	0.0001	0

EDI = Estimated Daily Intake (mg/kg body weight/day); HQ = Hazard Quotient (EDI/ADI). HQ ≤ 1 indicates low risk; HQ > 1 indicates potential health risk. Acceptable Daily Intake (ADI) values were based on FAO/WHO JECFA guidelines.

Normality assessment of antibiotic residues

The distribution of antibiotic concentrations for oxytetracycline, tetracycline, and tylosin in milk was highly skewed (Shapiro-Wilk, $p < 0.001$). Log10 transformation improved tetracycline slightly ($p = 0.010$), but oxytetracycline and tylosin remained non-normal. Consequently, non-parametric statistical methods were used for subsequent comparisons of antibiotic concentrations (Figure 3).

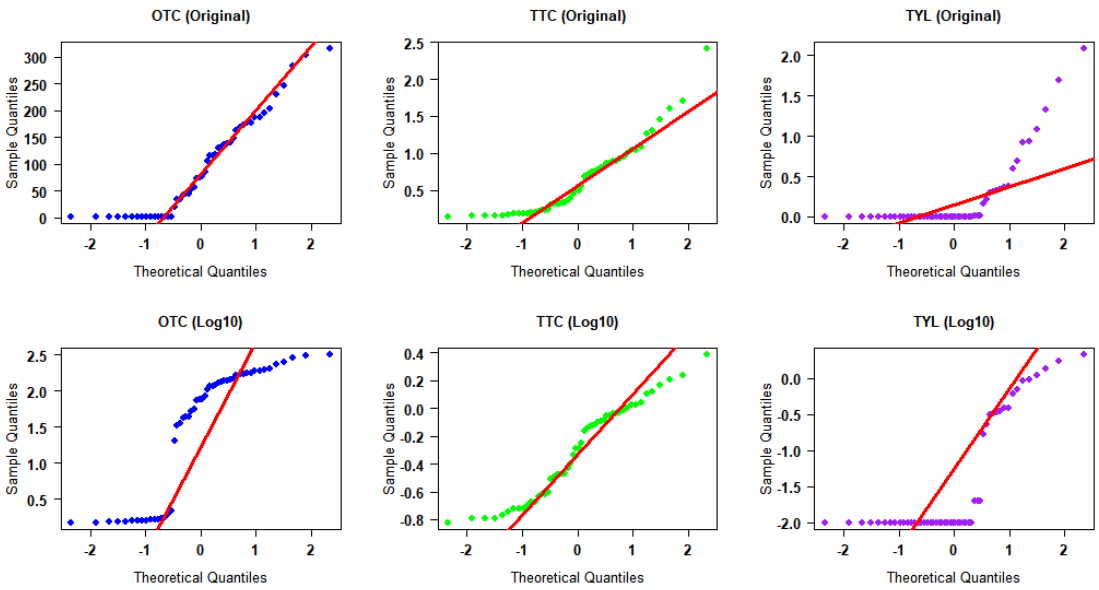


Figure 3: Distribution and normality assessment of oxytetracycline, tetracycline, and tylosin concentrations in milk samples.

Non-parametric analysis revealed significant differences in antibiotic residues among the three antibiotics. The Friedman test showed a highly significant difference ($\chi^2 = 90.21$, df = 2, $p < 0.001$). Pairwise Wilcoxon signed-rank tests with Bonferroni correction indicated that oxytetracycline concentrations were significantly higher than both tetracycline ($p < 0.001$) and tylosin ($p < 0.001$). Tetracycline was also significantly higher than tylosin ($p = 0.000075$). These results demonstrate that oxytetracycline residues dominate the samples, followed by tetracycline, while TYL is detected at substantially lower levels (Table 6).

Table 6: Comparison of Median Residue Concentrations of oxytetracycline, tetracycline, and tylosin in Raw Cow Milk Using the Friedman Test and Post-Hoc Pairwise Analysis.

Antibiotic	Median (IQR)	Friedman χ^2	df	p-value	OTC vs TTC	OTC vs TYL	TTC vs TYL
OTC	76.73 (161.86)	90.21	2	<0.001	<0.001	<0.001	7.54E-05
TTC	0.50 (0.67)	90.21	2	<0.001	<0.001	<0.001	7.54E-05
TYL	0.00 (0.30)	90.21	2	<0.001	<0.001	<0.001	7.54E-05

Discussion

The detection of antimicrobial residues in raw milk clearly indicates that veterinary antimicrobials are commonly used within dairy production systems in the study area. The relatively uniform presence of residues across wards and villages suggests that antimicrobial use practices are broadly similar throughout the area. This pattern likely reflects shared livestock management approaches, comparable access to veterinary drugs, and similar levels of awareness regarding withdrawal periods among farmers [39]. Similar observations have been reported in other low- and middle-income dairy production systems where weak regulation and limited veterinary oversight contribute to widespread antimicrobial use [40].

The presence of oxytetracycline, tetracycline, and tylosin residues in milk raises important public health concerns, particularly where detected concentrations exceed the MRLs [41]. In the present study, oxytetracycline and tetracycline exceeded the recommended MRLs in all analyzed samples, with a smaller proportion of tylosin (30.8%) exceeding the MRL of all the samples analyzed. Consumption of milk containing antimicrobial residues above recommended limits may expose consumers to adverse health effects such as allergic reactions, disruption of intestinal microbiota, and possible long-term toxicological consequences. More importantly, continuous exposure to sub-therapeutic concentrations of antimicrobial residues through dietary intake may contribute to the development and spread of AMR by exerting selective pressure on microbial populations, even when residue levels appear relatively low [25].

Human health risk assessment based on estimated daily intake (EDI) and hazard quotient (HQ) further demonstrated oxytetracycline variation in the potential risk posed by the detected antimicrobials. The calculated EDI for oxytetracycline (0.054 mg/kg bw/day) resulted in an HQ value of 1.80, exceeding the safety threshold of one and indicating a potential public health risk associated with consumption of contaminated milk. In contrast, tetracycline and tylosin showed substantially lower exposure levels, with EDI values of 0.0004 mg/kg bw/day and 0.0001 mg/kg bw/day corresponding to HQ values of 0.01 and 0.00, respectively. HQ values below unity suggest negligible toxicological risk; however, the elevated HQ observed for oxytetracycline highlights the possibility of adverse health outcomes among regular milk consumers within the study area [42], [43], [44].

The predominance of oxytetracycline residues relative to tetracycline and tylosin suggests preferential antimicrobial selection influenced by affordability, availability, and farmer familiarity rather than evidence-based prescription practices [13]. Such patterns increase the risk of residue contamination in milk and may also contribute to the environmental dissemination of antimicrobial residues and antibiotic-resistant microorganisms [20].

A significant proportion of administered antibiotics is not fully metabolized in animals and is excreted through faeces and urine, leading to contamination of soil, water sources, and pasture. In livestock production systems, especially under intensive and semi-intensive management, this environmental exposure can facilitate the persistence and spread of antimicrobial residues and resistant bacteria in the ecosystem. Animals may then be re-exposed through contaminated feed and water, creating a continuous cycle of antimicrobial exposure and resistance development [22], [40].

Management practices were identified as a major determinant of antimicrobial residue occurrence [45]. Milk obtained from cattle managed under intensive and semi-intensive production systems was more likely to contain detectable oxytetracycline, tetracycline, and tylosin residues than milk from extensively managed animals. Intensified systems are commonly associated with higher stocking densities and increased disease pressure, which often necessitate frequent antimicrobial interventions to manage disease and maintain high productivity [46]. Such frequent usage increases the likelihood of improper dosing and poor adherence to the

recommended withdrawal periods, thereby elevating the risk of antimicrobial residue contamination in milk and subsequent dietary exposure among consumers [19].

Animal-related factors also appeared to influence the occurrence of antimicrobial residues. Crossbred cattle were more likely to have detectable residues in their milk compared to indigenous breeds, likely due to differences in management intensity and veterinary input. Crossbreeds are often kept under production-oriented systems that require frequent therapeutic interventions. In contrast, indigenous breeds are generally raised under low-input systems and are better adapted to local environmental conditions.

Genetically, indigenous African cattle possess adaptive traits that enhance their resistance or tolerance to diseases and parasites. This is largely due to long-term natural selection under harsh tropical conditions, which has resulted in the development of important genetic traits such as trypanotolerance, heat tolerance, and resistance to endemic infections [47]. Studies have shown that these adaptive traits are controlled by specific genes and genomic regions associated with immune response and environmental resilience [48]. As a result, indigenous breeds often require fewer antimicrobial treatments compared to crossbreeds, which are generally more susceptible to diseases under similar conditions [49], [50].

Similarly, crossbred cattle were more likely to exhibit detectable residues than indigenous breeds due to both management practices and their higher susceptibility to diseases. Crossbreeds are less adapted to local environmental conditions such as heat stress, endemic diseases, and feed limitations, making them more prone to infections. This reduced adaptability is mainly due to

genetic factors, as crossbreeds are selected for high productivity rather than disease resistance and environmental resilience. As a result, crossbreeds require more frequent antimicrobial treatments, increasing the likelihood of residue occurrence in milk.

These findings support earlier reports that emphasize the superior adaptability and disease resistance of indigenous cattle, which may reduce the need for antimicrobial use and consequently lower the risk of residue occurrence in milk [51].

The highly skewed distribution of oxytetracycline, tetracycline, and tylosin concentrations observed in this study further suggests inconsistent antimicrobial usage practices at the farm level. The dominance of oxytetracycline residues relative to tetracycline and tylosin indicates preferential use driven by affordability, availability, and farmer familiarity rather than evidence-based veterinary prescription [12]. Non-parametric analysis confirmed significantly higher concentrations of oxytetracycline compared to tetracycline and tylosin, reinforcing concerns regarding the extensive use of tetracycline-based antimicrobials within the dairy sector. Given that cattle rearing systems and waste management practices play a critical role in the development and transmission of antibiotic-resistant pathogens from animals to humans and the environment, the widespread detection of oxytetracycline residues presents an additional risk for environmental dissemination of resistant bacteria [52].

Conclusion

These findings underscore the need to strengthen antimicrobial stewardship within dairy production systems through improved farmer education, strict adherence to withdrawal periods,

enhanced farm biosecurity, and routine monitoring of antimicrobial residues in milk. Implementation of such measures is essential for minimizing dietary exposure to antimicrobial residues, safeguarding public health, and contributing to ongoing national and global efforts aimed at mitigating antimicrobial resistance through the National Action Plans for Antimicrobial Resistance.

Recommendations

Antimicrobial stewardship should be strengthened by enforcing prescription-only access to veterinary antimicrobials and ensuring strict adherence to recommended withdrawal periods before milk enters the food chain, as described in Tanzania's NAP-AMR.

Routine monitoring and surveillance systems should be established for antimicrobial residues in raw milk at farm, market, and processing levels to support early detection, accountability, and regulatory compliance as per Tanzania's NAP-AMR.

Farmer education and capacity building should be enhanced through targeted training on prudent antimicrobial use, correct dosing, withdrawal periods, and the public health risks associated with residue-contaminated milk.

Veterinary service delivery should be improved, particularly within smallholder and intensive dairy systems, to ensure accurate disease diagnosis, appropriate antimicrobial prescribing, and proper record-keeping of drug use as described in Tanzania's NAP-AMR.

Preventive animal health strategies should be promoted, including improved farm hygiene, biosecurity measures, vaccination programs, and good husbandry practices, to reduce disease incidence and reliance on antimicrobials.

Abbreviations

ADI	Acceptable Daily Intake
AMR	Antimicrobial Resistance
CI	Confidence Interval
EDI	Estimated Daily Intake
FAO	Food and Agriculture Organization
HPLC-MS	High-Performance Liquid Chromatography-Mass Spectrometry
HQ	Hazard Quotient
LC-MS	Liquid Chromatography-Mass Spectrometry
MHA	Mueller-Hinton Agar
MRL	Maximum Residue Limit
OTC	Oxytetracycline
TTC	Tetracycline
TYL	Tylosin
WHO	World Health Organization

Study limitations

- The cross-sectional study design captured antimicrobial residue levels at a single point in time and does not account for seasonal variations in antimicrobial use or milk contamination.
- Only selected antibiotics (oxytetracycline, tetracycline, and tylosin) were analysed using HPLC-MS, while residues from other commonly used antimicrobial classes may have been present but not assessed.
- Data on antimicrobial use and farm management practices relied partly on self-reported information from farmers, which may be subject to recall bias or underreporting.
- The hazard quotient and estimated daily intake calculations were based on assumed average milk consumption and adult body weight, which may not accurately represent exposure in vulnerable populations such as children, pregnant women, or high milk consumers.

Declarations

Author's contribution

AMM conceptualized the research idea, designed the study, collected and analysed data, interpreted the results, and drafted the manuscript. CJM assisted with laboratory analyses, contributed to data interpretation, and reviewed and proofread the manuscript. FP conducted laboratory analyses, assisted with data interpretation, and reviewed the manuscript. ASH contributed to the study conceptualization, reviewed, and proofread the manuscript.

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Data availability

All data are included in this article.

Ethical approval and consent to participate

Ethical clearance approval with reference number **REF NO. TLRI/CC.21/068**, for this study was sought and obtained from Tanzania Livestock Research Institute (TALIRI). Research permits were granted by the President's Office – Regional Administration and Local Government (PO-RALG) and the respective local authorities in the study area.

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Consent for publication

Not applicable

Conflict of interest

The authors declare no conflict of interest**.**

Clinical trial number

Not applicable.

Reference

[1] S. Rome, “Codex alimentarius commission 41,” no. July, pp. 2–6, 2018.

[2] D. Tmda and D. M. C. Mcie, “tanzania medicines and medical devices authority guidelines for recall , handling and disposal of u products,” 2021.

[3] T. H. E. United, R. Of, and M. O. F. Livestock, “Standard treatment guidelines for animal diseases in tanzania,” no. February, 2022.

[4] “Chapter 6 Dietary exposure assessment for chemicals in food,” 2020.

[5] H. Pekmezci and S. Sipahi, “Health Risk Assessment of Dietary Chemical Exposures : A Comprehensive Review,” pp. 1–36, 2025.

[6] F. R. Paro, R. Davour, D. Acosta, K. Mechlowitz, C. Tiwari, and S. L. Mckune, “Improving Nutrition Security in Low- and Middle-Income Countries and the Role of Animal-Source Foods,” pp. 371–388, 2025.

[7] Y. Nozaki, “FUTURE TRENDS OF GROWING DEMAND FOR MILK AND DAIRY PRODUCTS AND MILK SUPPLY IN INDIA,” no. September, pp. 1–4, 2017.

[8] F. Rinky, A. Rahman, S. Reza, and A. Nowar, “Heliyon Residual antibiotics in milk samples : Assessing the risk and prevalence in Bangladesh,” *Heliyon*, vol. 11, no. 1, p. e41422, 2025, doi: 10.1016/j.heliyon.2024.e41422.

[9] G. Hanna, K. Rejman, and W. Laskowski, “Milk and Dairy Products and Their Nutritional Contribution to the Average Polish Diet,” 2019.

[10] D. Tomani, “Antibiotic Residues in Milk as a Consequence of Mastitis Treatment : Balancing Animal Welfare and One Health Risks,” pp. 1–21, 2025.

[11] I. P. Online, Z. Hosain, M. T. Abrar, S. M. S. Islam, and T. Islam, “Bioscience and Biotechnology An overview of antimicrobial residues in animal-originated food products in developing countries,” vol. 10, no. 3, pp. 64–76, 2025.

[12] Z. I. Kimera, S. E. Mshana, M. M. Rweyemamu, L. E. G. Mboera, and M. I. N. Matee, “Antimicrobial use and resistance in food-producing animals and the environment: An African perspective,” *Antimicrob. Resist. Infect. Control*, vol. 9, no. 1, pp. 1–12, 2020, doi: 10.1186/s13756-020-0697-x.

[13] O. M. Oladeji, L. L. Mugivhisa, and J. O. Olowoyo, “Antibiotic Residues in Animal Products from Some African Countries and Their Possible Impact on Human Health,” 2025.

[14] M. S. Rahman, M. M. Hassan, and S. Chowdhury, “Determination of antibiotic residues in milk and assessment of human health risk in Bangladesh,” *Heliyon*, vol. 7, no. 8, p. e07739, 2021, doi: 10.1016/j.heliyon.2021.e07739.

[15] R. Kyuchukova, “Antibiotic residues and human health hazard – review,” vol. 26, no. 3, pp. 664–668, 2020.

[16] F. A. Tanin, “Residue and Human Risk : A Reassessment of Aminoglycoside Residues in Edible Tissues,” vol. 3, no. 1, pp. 16–32, 2025.

[17] T. P. Van Boeckel *et al.*, “Global trends in antimicrobial use in food animals,” *Proc. Natl. Acad. Sci. U. S. A.*, vol. 112, no. 18, pp. 5649–5654, 2015, doi: 10.1073/pnas.1503141112.

[18] A. G. Orisile, R. H. M. Lim, and A. Amlogu, “JAC- Antimicrobial Resistance A systematic review of antimicrobial stewardship practices and challenges in sub-Sahara Africa (SSA) regulated retail medicine settings,” *JAC-Antimicrobial Resist.*, vol. 8, no. 1, pp. 1–15, 2026, doi: 10.1093/jacamr/dlaf235.

[19] S. A. Belachew, L. Hall, and L. A. Selvey, “Non - prescription dispensing of antibiotic agents among community drug retail outlets in Sub - Saharan African countries : a systematic review and meta - analysis,” *Antimicrob. Resist. Infect. Control*, pp. 1–15, 2021, doi: 10.1186/s13756-020-00880-w.

[20] R. H. Mdegela *et al.*, “Antimicrobial use, residues, resistance and governance in the food and agriculture sectors, tanzania,” *Antibiotics*, vol. 10, no. 4, pp. 1–23, 2021, doi: 10.3390/antibiotics10040454.

[21] G. Jaime, A. Hobeika, and M. Figuié, “Access to Veterinary Drugs in Sub-Saharan Africa: Roadblocks and Current Solutions,” *Front. Vet. Sci.*, vol. 8, no. March, pp. 1–13, 2022, doi: 10.3389/fvets.2021.558973.

[22] R. R. Ali, O. Juma, A. Mzula, and A. S. Hoza, “Assessment of Knowledge , Practices , and Attitude among Poultry Sellers on Antimicrobial Use in Mjini Magharibi Region , Zanzibar,” vol. 11, pp. 1–14, 2024, doi: 10.4236/oalib.1112458.

[23] R. Z. Sangeda *et al.*, “Consumption Trends of Antibiotic for Veterinary Use in Tanzania: A Longitudinal Retrospective Survey From 2010-2017,” *Front. Trop. Dis.*, vol. 2, no. June, pp. 1–10, 2021, doi: 10.3389/fitd.2021.694082.

[24] E. Enshaie, S. Nigam, S. Patel, and V. Rai, “Livestock Antibiotics Use and Antimicrobial

Resistance," *Antibiotics*, vol. 14, no. 6, p. 621, 2025, doi: 10.3390/antibiotics14060621.

[25] M. A. Hebbal, C. Latha, K. V. Menon, and J. Deepa, "Occurrence of oxytetracycline residues in milk samples from Palakkad , Kerala , India," vol. 13, pp. 1056–1064, 2020.

[26] A. Balala, E. L. Mayenga, A. M. Lupindu, and E. D. Karimuribo, "Prevalence and Determinants of Subclinical Mastitis in dairy Cows under Small-scale farms in Dar es Salaam , Tanzania," pp. 50–61, 2025.

[27] L. V. Costa *et al.*, "Antibiotic Residues in Raw Cow ' s Milk : A Systematic Review of the Last Decade," pp. 1–14, 2024, doi: 10.17605/OSF.IO/3ZMJC.

[28] D. M. Muloi *et al.*, "Antibiotic quality and use practices amongst dairy farmers and drug retailers in central Kenyan highlands," *Sci. Rep.*, vol. 13, no. 1, pp. 1–8, 2023, doi: 10.1038/s41598-023-50325-8.

[29] I. Consequential and P. H. Implications, *Antibiotic Use in Agriculture and Its Consequential Resistance in Environmental Sources : Potential Public Health Implications*. 2018. doi: 10.3390/molecules23040795.

[30] J. P. M. Mpatswenumugabo, *Biosecurity and milk production among smallholder dairy farmers in Rwanda*. 2024.

[31] E. O. Simbine-ribisse, N. Madalena, A. Joaquim, and C. Jo, "Antibiotic residues in dairy products in Africa : A systematic review," vol. 5, no. June, 2024.

[32] H. Lweendo, M. Judith, M. Duncan, M. Justin, and S. Cynthia, "Global Prevalence of Antibiotic Residues in Smallholder Dairy Farm Milk : A Systematic Review of Trends and Regional Disparities," vol. 7, no. 5, pp. 1–11.

[33] A. Residues, I. Milk, T. Crinad, and O. Solomon, “Metropolitan Journal of Medical and Health Research ISSN : 3006-6433 Corresponding : Author : https://www.journals.miu.ac.ug Metropolitan Journal of Medical and Health Research ISSN : 3006-6433 Vol . 4 Issue 10 , October- 2025 , Pages : 216-226 Corresponding : Author : https://www.journals.miu.ac.ug,” vol. 4, no. 10, pp. 216–226, 2025.

[34] J. Kimambo, “Antibiotic Use in Small-Scale Dairy Farming and Residue Levels of Tetracyclines and Sulphonamides in Milk from Eastern Tanzania,” pp. 1–18, 2025.

[35] J. Kimambo, Z. I. Kimera, E. Niccodem, P. Kunambi, F. X. Mgaya, and M. I. N. Matee, “Antibiotic use in small-scale dairy farming and residue levels of tetracyclines and sulphonamides in milk from Eastern Tanzania,” 2026.

[36] M. of; Health and M. of Livestock and Fisheries, “The National Action Plan on Antimicrobial Resistance 2023 -2028,” *Minist. Heal. Heal.*, vol. 136, no. 1, 2022.

[37] L. Vorlov, S. Dluhošov, and O. Hanuš, “Screening Methods for Antimicrobial Residues in the Dairy Chain — The Past and the Present,” pp. 1–28, 2024.

[38] K. Koutsoumanis *et al.*, “Maximum levels of cross-contamination for 24 antimicrobial active substances in non-target feed . Part 6 : Macrolides : tilmicosin , tylosin and tylvalosin,” vol. 19, no. September, 2021, doi: 10.2903/j.efsa.2021.6858.

[39] R. Das and N. Vyas, “International Journal of One Health Antimicrobial usage , awareness , and animal health management practices among household dairy farmers in West Bengal , India,” vol. 11, pp. 242–258, 2025, doi: 10.14202/IJOH.2025.242-258.

[40] A. M. Mugyabuso, I. Makundi, and A. S. Hoza, “Assessment of antimicrobial use and

stewardship practices among animal health practitioners , veterinary drug retailers and cattle keepers in," no. January, pp. 1–14, 2026, doi: 10.3389/frabi.2025.1688828.

[41] B. A. Odeny, G. O. Asudi, G. Omwenga, R. O. Oduor, and G. M. Karau, "Analysis of Antibiotics in Milk from Smallholder Farmers from Kenya Shows Traces Above the Recommended Maximum Residue Limits," vol. 9, no. 2, pp. 27–39, 2024.

[42] O. Region, A. Nugussa, and G. Faye, "Assessment of Human Health Risks from Aflatoxin M1 in Raw Milk : A Study from North Shewa Zone ," 2024, doi: 10.1177/11786302241304524.

[43] S. Es and L. Schoonman, "Microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania," vol. 1, no. 3, pp. 217–222, 2011, doi: 10.1016/S2221-1691(11)60030-0.

[44] S. Aytenfsu, G. Mamo, and B. Kebede, "Review on Chemical Residues in Milk and Their Public Health Concern in," vol. 6, no. 4, 2016, doi: 10.4172/2155-9600.1000524.

[45] S. H. Gwandu, H. E. Nonga, R. H. Mdegela, A. S. Katakweba, T. S. Suleiman, and R. Ryoba, "Assessment of Raw Cow Milk Quality in Smallholder Dairy Farms in Pemba Island Zanzibar , Tanzania," vol. 2018, 2018.

[46] B. A. Gemeda *et al.*, "Antimicrobial Use in Extensive Smallholder Livestock Farming Systems in Ethiopia: Knowledge, Attitudes, and Practices of Livestock Keepers," *Front. Vet. Sci.*, vol. 7, no. February, pp. 1–15, 2020, doi: 10.3389/fvets.2020.00055.

[47] O. Mwai, O. Hanotte, Y. Kwon, and S. Cho, "- Invited Review - African Indigenous Cattle : Unique Genetic Resources in a Rapidly Changing World," vol. 28, no. 7, pp. 911–

921, 2015.

[48] M. K. Bitew *et al.*, “Genomic evidence of positive selection highlights functional divergence associated with environmental resilience in central and West African indigenous cattle,” pp. 1–13, 2026.

[49] H. Dadi, “Journal of Fisheries & Selective Breeding for Enhanced Growth and Disease Resistance in Indigenous Cattle Breeds : A Genomic Approach,” vol. 13, no. 6, 2025, doi: 10.4172/2332-2608.1000666.

[50] S. Fridaïus, U. Vanvanhossou, T. Yin, G. Gorjanc, and S. König, “Evaluation of crossbreeding strategies for improved adaptation and productivity in African smallholder cattle farms,” *Genet. Sel. Evol.*, pp. 1–19, 2025, doi: 10.1186/s12711-025-00952-8.

[51] M. Tibbo, *Productivity and Health of Indigenous Sheep Breeds and Crossbreds in the Central Ethiopian Highlands*. 2006.

[52] E. Pokrant *et al.*, “Evaluation of Antibiotic Dissemination into the Environment and Untreated Animals , by Analysis of Oxytetracycline in Poultry Droppings and Litter,” 2021.