**Original Research Article**

**ANTIMICROBIAL RESIDUES, RESISTANT BACTERIA AND RESISTANT GENES ISOLATED IN MILK AND BEEF MARKETED IN KABETE, KENYA**

ABSTRACT

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| This study investigated the presence of antimicrobial residues, antimicrobial-resistant bacteria, and resistance genes in raw milk and beef sold in Kabete, Kenya. A total of 120 samples (60 milk, 60 beef) were analyzed using high-performance liquid chromatography (HPLC) and PCR-based molecular techniques. Antimicrobial residues were detected in 8.3% of milk and 5% of beef samples, with oxytetracycline and β-lactam antibiotics such as penicillin G and amoxicillin being the most common. Although residue levels were in low concentrations, their presence indicates lapses in observing drug withdrawal periods. Microbiological analysis revealed bacterial contamination in 10% of beef and 18.3% of milk samples. Escherichia coli was the most frequently isolated pathogen in milk, while Staphylococcus aureus and one methicillin-resistant S. aureus (MRSA) strain were detected in beef. Antimicrobial susceptibility testing showed widespread resistance to ampicillin, with some isolates exhibiting multidrug resistance (MDR) against up to five antibiotic classes. Molecular testing identified several resistance genes, including blaZ, blaTEM, blaCTX-M, mecA, tetA, and sul1. Notably, multidrug resistance gene combinations such as blaCTX-M/tetA/sul1 were found in milk isolates, highlighting the potential for transmission of highly resistant bacteria through the food chain. Strong positive correlations were observed between antimicrobial residues, bacterial contamination, and resistance gene presence. The findings underscore significant public health concerns linked to the consumption of contaminated animal products and emphasize the need for routine surveillance, strict enforcement of veterinary drug use regulations, and enhanced farmers’ education on antimicrobial stewardship. This study supports the One Health approach in addressing antimicrobial resistance and calls for integrated measures to ensure food safety and combat the spread of resistance from livestock to humans.***Keywords****: antimicrobial residues, antimicrobial-resistant bacteria, resistance genes, milk, beef, Kabete.* |

**1. INTRODUCTION**

Antibiotics are widely used in dairy farming for treating and preventing diseases, promoting growth, improving digestion, and enhancing milk production and feed efficiency ([1](#Redding)). Though most antibiotics are excreted in urine and to a lesser extent in feces, residues may persist in animal products such as milk, meat, and eggs ([2](#Rashid),[3](#ALMASHHADANY)). Regulatory agencies like the Food and Drug Authority(FDA) and European Union(EU) classify these residues as pharmacologically active substances in food of animal origin ([4)](#Meklati). Their presence in food violates safety standards, often due to farmers' ignorance or insufficient guidance from drug manufacturers ([5](#Food)). To mitigate this, farmers are required to observe a withdrawal period, the time between the last antibiotic dose and when the animal or its products can be safely consumed ( [6](#VELAZ)).

Failure to observe this period may result in health risks, including allergic reactions, disruption of gut microbiota, bone marrow toxicity, congenital defects, cancer, and antibiotic resistance ([7](#VIEIRA)). Residues also affect the dairy industry by inhibiting beneficial fermentation microbes ([7](#Kjedgaard), [8](#Zarzeka)). A study in Dagoretti, Kenya, found antibiotic residues in 4% of household milk samples ([9](#Ekuttan)). Given this context, the study sought to detect antibiotic residues in raw milk and beef sold in Kabete, Kenya. Globally, antimicrobial resistance (AMR) accounted for 1.27 million deaths and contributed to 4.95 million deaths in 2019 ([10)](#Murray). Antibiotics used in livestock lead to residues in animal-derived products, which can contribute to AMR ([11](#Arsene)). As these residues enter the food chain and environment, resistant bacteria can spread, posing a risk to public health. Some foodborne pathogens like *Staphylococcus spp.*, *Salmonella spp.*, and *Campylobacter spp.* have shown increased resistance in humans due to the consumption of contaminated animal products ([12](#Lekshmi)).

Despite the evident risks, limited data exist on antibiotic usage in livestock and its implications for human resistance. Livestock are given antibiotics for treatment, prevention, and growth promotion, often without proper monitoring ([13)](#USFOOD). Commonly used antibiotics include Penicillin G, Oxacillin, Cloxacillin, Dicloxacillin, Nafcillin, Oxytetracycline, Chlortetracycline, Doxycycline, and Sulfonamides ([14](#ODENYTODAY)). Resistant bacteria of concern include *Enterobacterales* (carbapenem and cephalosporin-resistant), *Acinetobacter baumannii* (carbapenem-resistant), *Mycobacterium tuberculosis* (rifampicin-resistant), *Salmonella Typhi* and *Shigella spp.* (fluoroquinolone-resistant), *Enterococcus faecium* (vancomycin-resistant), *Pseudomonas aeruginosa* (carbapenem-resistant), *Neisseria gonorrhoeae* (cephalosporin- and fluoroquinolone-resistant), and *Staphylococcus aureus* (methicillin-resistant) ([15](#WHO22)). Once administered, antibiotic residues can accumulate in body tissues and fluids. Misuse, poor farm hygiene, and lack of withdrawal period observance increase residue levels in consumables like milk, meat, and eggs. Furthermore, 40% to 90% of antibiotics are excreted into the environment via urine and feces, further compounding ecological contamination ([16)](#FALOWO).

Milk remains a dietary staple in sub-Saharan Africa, particularly in Kenya, where it contributes significantly to nutrition and the economy ([17](#Mattielo)). However, indiscriminate antibiotic use in breeding farms, often based solely on clinical judgment, leads to contaminated milk and beef, especially when withdrawal periods are ignored ([18](#lander)). Environmental pollution also worsens due to antibiotic runoff. The withdrawal period is indicated on veterinary drug labels and prohibits the consumption or marketing of animal products during this time ([19](#Virto)). Despite high demand for milk and beef, Kenya lacks a residue monitoring system. Farmers often have unregulated access to veterinary drugs, and no clear oversight mechanism exists. Consequently, there is a critical need to gather data on antibiotic residues in milk and beef in Kenya, particularly in Kabete.

The research could influence policy on use of the antibiotics in treating animals and the period to be taken before the use of animal products. The study would avail data on antibiotic residue in dairy products and enable further research on the impact on antimicrobial residues.

**2. material and methods**

* 1. **Study Site**

This research project was carried out in Kabete constituency in Kiambu County and the laboratory analysis was conducted at Kabarak University research laboratories.

* 1. **Study Design**

This research employed a cross-sectional descriptive study design.

* 1. **Study Population**

With five wards, 140,427 people lived in the constituency, which was roughly 60.20 km³ in size. 60 Venders were sampled from every ward for milk and beef samples.

* + 1. **The Inclusion Criteria**

All raw beef and milk from livestock that have recently (not more than one week) completed a dose of antibiotic being sold within Kabete constituency.

* + 1. **Exclusion Criteria**

Processed products were not included in the study.

All raw beef and milk from livestock that are still on antibiotic treatment being sold within Kabete constituency

* 1. **Sampling Technique**

The study employed stratified proportionate random sampling, which enhances accuracy in parameter estimation and ensures a more representative sample from a generally homogeneous population ([20](#orodho)). Kiambu County was divided into five wards, each treated as a stratum. From each stratum, 60 respondents were selected through simple random sampling, ensuring equal representation and reducing selection bias. For the sampling of raw milk and beef, stratified purposive sampling was applied. Samples were purposively collected from each of the five wards based on relevance, focusing on key sites such as slaughterhouses and milk vending points. This approach ensured that the samples were both diverse and relevant to the study objectives.

* 1. **Sample Size Determination**

Fisher et al (1988) was used to calculate sample size for the study

n = t2 xp (1-p) m2

Where n = required sample size

t = confidence interval at 95% (standard value of 1.96)

p = estimated prevalence of antibiotic residue in the samples assumed to be at 4% ([9](#Ekuttan))

Margin = margin of error at 5%

 n = t2 x p (1-p)

 m2

 n = 1.962 x 0.04 (1-0.04)

 0.052

 = 60 Vendors per ward for both milk and Beef.

* 1. **Methodology**

A total of 60 (100 ml) raw milk was aseptically collected in sterile bottles. For antibiotic residue screening, 10 ml was added to a Delvotest® pre-treatment ampoule, stirred, and 100 µL pipetted onto the agar surface. After 20 minutes of pre-diffusion at room temperature, the ampoules were rinsed twice, sealed with foil, and incubated at 64°C using a DSM heating block or water bath. The test ended upon color change in the negative control ([20](#orodho)).
Similarly, 1 g of lean beef was pressed to yield 100 ml of juice, from which 10 ml was placed in a Premi® Test ampoule. After mixing, 100 µL was added to the agar surface, left to pre-diffuse for 20 minutes, rinsed, sealed, and incubated at 64°C. The test concluded once the negative control changed color, validating results ([20](#orodho)).

**2.7.1 Preparation of milk and Beef samples for HPLC**

The preparation of milk and beef samples for High-Performance Liquid Chromatography (HPLC) analysis followed a slightly modified procedure. Specifically, 8 mL of a 10% aqueous solution of acetic acid was added to 100 mL of raw milk and separately to 100 mL of lean beef juice. The mixture was then centrifuged at 3500 rpm for 10 minutes at 4°C**.** The resulting supernatant was carefully extracted using a disposable syringe to avoid disturbing the fat layer. It was then passed through a 0.45 µm nylon membrane filter. A 1 mL aliquot of the filtered extract was transferred into a 2 mL sterile vial**,** ready for the detection and quantification of antibiotic residues by HPLC.

**2.7.2 Quantification of the antimicrobial residues by HPLC**

Antimicrobial residue levels were quantified using an HPLC Jasco systemequipped with a C-18 column(octadecyl silyl, 100 × 4.6 mm, 5 µm). Detection was conducted at 214 nm and 355 nm**,** with 10 µL of the extract injected into the system. Elution was performed using an isocratic mobile phasecomposed of methanol, acetonitrile, and water (55:25:20v/v/v**)** at aflow rate of 1.2 mL/min**.** To prepare the standard, 1 mg of antimicrobial residue was weighed and dissolved in 50 mL of HPLC-grade water in a volumetric flask. A 5.0 mL aliquot was diluted further in 100 mL of HPLC-grade water and stored at 4°C, yielding a final concentration of 50 µg/mL**.**

**2.7.3 Bacterial isolation**

For bacteriological analysis, 25 g of beef sample and 1ml of milk sample was homogenized with 225 mL of lactose broth for 2 minutes and incubated at 37°C for 24 hours**.** A loopful of this broth culture was streaked onto Eosin Methylene Blue (EMB) agar and incubated for another 24 hours at 37°C. Suspected *E. coli* colonies (minimum of five) were subjected to biochemical identification using IMViC tests (Indole, Methyl Red, Voges-Proskauer, Citrate), Triple Sugar Iron Agar (TSIA)**,** Catalase test and Coagulase test.

**2.7.4 Antimicrobial susceptibility testing**

The disk diffusion method, was used for antimicrobial susceptibility testing. A lawn culture was prepared on Mueller-Hinton agar using overnight-grown bacteria in nutrient broth. Various Antibiotic discs including Tetracycline (30 µg)and Nitrofurantoin (30 µg) were aseptically placed on the agar, which was then incubated at 37°C for 16–18 hours. Zones of inhibition of the test samples(beef/milk) and their controls were interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2010)guidelines.

**2.7.5 DNA Extraction**

Whole genomic DNA from bacterial cultures was extracted using the boiling method**.** Bacterial cultures were transferred to a 1.5 mL Eppendorf tube and centrifuged at 14,000 g for 5 minutes. After discarding the supernatant, a second 1 mL culture was added, and the centrifugation was repeated. The resulting pellet was resuspended in 600 µL of sterile distilled water and further processed. After another centrifugation, 200 µL of sterile distilled water was added to the pellet, which was then boiled at 100°C for 10 minutes using a Labnet heating block (USA**)** and quickly cooled on ice for 5 minutes**.**

**2.7.6 Molecular Confirmation of bacterial spp. Using PCR**

Polymerase Chain Reaction (PCR) was used to confirm bacterial species by targeting the iroBand invA genes, with the latter serving as a control for *Salmonella enterica*. The 25 µL PCR mixture contained 5 µL of template DNA**,** 6.5 µL of sterile distilled water**,** 0.5 µL each of forward and reverse primers**,** and 12.5 µL of DreamTaq master mix (Thermo-Fisher Scientific, South Africa)**.** Amplification conditions for invA were as follows: denaturation at 95°C for 30 seconds**,** annealing at 58°C for 30 seconds**,** extension at 72°C for 1 minute**,** and afinal extension at 72°C for 5 minutes**.** The amplification conditions for the iroBgene were similar, with slight adjustments to the annealing temperature specific to the target species.

**Diagram 1: Primers for detecting antimicrobial-resistant genes**



**2.7.7 Screening of Antibiotic Resistance Genes Using PCR**

Polymerase Chain Reaction (PCR) was employed to detect antibiotic resistance genes in the bacterial isolates using genus-specific primers. The PCR reactions were prepared in a 12.5 µL volume, comprising 12.5 µL of DreamTaq master mix (Thermo-Fisher Scientific), 0.5 µL each of forward and reverse primers**,** 6.5 µL of steriledistilled water, and 5 µL of template DNA. In place of DNA**,** sterile distilled water was used as the negative control, while DNA extracted from a known pure culture of *Salmonella enterica* served as the positive control**.**

The amplification process was carried out using a BioRad thermocycler (Singapore**)**, following standardized thermal cycling conditions: an initial denaturation at 95°C for 3 minutes, followed by 30 cyclesofdenaturation at 95°C for 30 seconds**,** primer-specific annealing temperature for 30 seconds**,** andextension at 72°C for 1 minute**.** A final extension step at 72°C for 8 minutes concluded the amplification process ([21](#Naidoo),[22](#Samtiya)). This molecular approach provided a reliable method for confirming the presence of antibiotic resistance genes in the bacterial isolates, contributing to the broader surveillance of antimicrobial resistance in food products.

**2.7.8 Agarose Gel Electrophoresis**

To confirm the presence of bacteria harboring antibiotic resistance genes, the extracted DNA and amplified gene products from *Salmonella enterica* and other bacterial species were quantified. Following quantification, aliquots of the PCR products were subjected to agarose gel electrophoresis for separation and visualization of the amplified DNA fragments. This method provided a visual confirmation of the gene presence based on expected band sizes on the gel.

**2.8 Data Analysis**

All collected data were first subjected to exploratory data analysis (EDA) to identify trends, detect anomalies, and summarize the main characteristics of the dataset**.** Quantitative methods were employed to analyze bacterial isolate culture data. The prevalence of antimicrobial residues was calculated and expressed in percentages. The relationship between antimicrobial residues and bacterial resistance was assessed using logistic regression analysis, with p-values less than 0.05 considered statistically significant. To evaluate associations between antimicrobial resistancegenes and variables such as animal host and sample collection location, Fisher's exact test was applied. The Pearson correlation test was used to determine the strength and direction of the relationships among detected resistance genes. Additionally, binary logistic regression was employed to examine whether the location of the sample collection site significantly predicted the presence of resistance genes. All statistical analyses were conducted using SPSS software version 27 and Microsoft excel 2023([23)](#Meshack).

**2.9 Quality Assurance**

To maintain high data integrity and accuracy, daily quality control procedures were conducted and recorded. PremiTest ampoules were prepared in duplicate and tested alongside known positive and negative controls to validate the reliability of the assay. The principal investigator (PI) examined the prepared ampoules, while a second set of slides was independently evaluated by the research supervisor, ensuring inter-rater reliability.

**2.10 Ethics Approval and Consent to Participate**

Approval for the study was obtained from the Jomo Kenyatta University of Agriculture and Technology (JKUAT) Ethical Review Board (*JKU/ISERC/02317/1391*) and National Commission for Science Technology and Innovation (*NACOSTI/P/25/414753*). Since the research did not involve human participants, informed consent was not required. However, sample confidentiality was ensured by storing all biological specimens with coded identifiers. The anticipated benefit of this study is the advancement of knowledge on antimicrobial resistance, which is critical for informing policy on antibiotic usage and ultimately protecting public health.

**3. results**

**RESULTS AND SUMMARY OF FINDINGS**

* 1. Objective 1: **To determine the presence and type of antimicrobial residues in milk and beef found in Kabete community, Kenya**



***Figure 1: Antimicrobial Residues (µg/L or µg/kg) Detected in both milk and beef samples***

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| --- |
|  |
| Sample Type | N | % |
| Beef | None detected | **57** | 95.0% |
| Oxytetracycline (32 µg/kg) | 1 | 1.7% |
| Oxytetracycline (50 µg/kg) | 1 | 1.7% |
| Sulfamethazine (96 µg/kg) | 1 | 1.7% |
| Milk | Amoxicillin (35 µg/L) | 1 | 1.7% |
| Ampicillin (24 µg/L) | 1 | 1.7% |
| None detected | **55** | 91.7% |
| Penicillin G (13 µg/L) | 1 | 1.7% |
| Penicillin G (8.5 µg/L) | 1 | 1.7% |
| Sulfamethazine (25 µg/L) | 1 | 1.7% |

***Table 1: Shows types of samples collected and Antimicrobial Residues (µg/L or µg/kg) Detected.***

* 1. **Objective 2: To identify antimicrobial-resistant bacteria isolated in milk and beef found in Kabete**



***Figure 2: Shows the frequency of Isolated antimicrobial-resistant Bacterial Species in sample types***

***Table 2: shows frequency of Isolated antimicrobial resistant Bacterial Species in sample types***

|  |  |  |
| --- | --- | --- |
| Sample Type | N | % |
| Beef | None | 57 | 95.0% |
| Staphylococcus aureus | 2 | 3.3% |
| Staphylococcus aureus (MRSA) | 1 | 1.7% |
| Milk | Escherichia coli | 4 | 6.7% |
| None | 54 | 90.0% |
| Staphylococcus aureus | 2 | 3.3% |



***Figure 3:******antimicrobial resistance in the isolated bacteria***

**key:** *Amp= Ampicillin, Tet****=*** *Tetracycline, Sul= Sulfonamides, Gen= Gentamicin, Cip= Ciprofloxacin, R = Resistant****,*** *S* ***=*** *Susceptible and**N/A = Not Applicable*

It can be noted that most samples had no bacterial isolation and therefore antibiotic resistance could not be obtained. Ampicillin resistance was the most frequently observed with some bacteria showing multidrug resistance which is clinically significant.



***Figure 4: Shows antibiotic resistance profile (Amp, Tet, Sul, Gen, Cip) in sample types of beef and milk.***

**Key:**  *blaZ=Beta-lactam resistance gene, blaZ, mecA= Beta-lactam resistance and methicillin resistance, blaCTX-M, tetA, sul1= Resistance to cephalosporins (blaCTX-M), tetracycline (tetA), and sulfonamides (sul1), blaTEM= Resistance gene often associated with penicillin and cephalosporins, blaTEM, tetA= Combined resistance to beta-lactams (blaTEM) and tetracycline (tetA), blaTEM, sul1=Combined resistance to beta-lactams (blaTEM) and sulfonamides (sul1), blaTEM, tetA, sul1= Combined resistance to beta-lactams (blaTEM), tetracycline (tetA), and sulfonamides (sul1), None= No resistance genes detected and N/A= Not Applicable (likely because no bacteria were isolated).*

***Table 4: Shows antibiotic resistance profile (Amp, Tet, Sul, Gen, Cip) in sample types of beef and milk.***

|  |
| --- |
|  |
| Sample Type | N | % |
| Beef | Amp-R, Tet-S, Sul- S, Gen-S, Cip-S | 2 | 3.3% |
| Amp-R,Gen-R,Cip-R | 1 | 1.7% |
| N/A | 57 | 95.0% |
| Milk | Amp-R, Tet-R, Sul- R, Gen-R, Cip-R | 1 | 1.7% |
| Amp-R, Tet-R, Sul- R, Gen-S, Cip-S | 3 | 5.0% |
| Amp-R, Tet-R, Sul- S, Gen-S, Cip-S | 3 | 5.0% |
| Amp-R, Tet-S, Sul- R, Gen-S, Cip-S | 1 | 1.7% |
| Amp-R, Tet-S, Sul- S, Gen-S, Cip-S | 2 | 3.3% |
| Amp-S, Tet-R, Sul- R, Gen-S, Cip-S | 1 | 1.7% |
| N/A | 49 | 81.7% |

 **key:** *Amp= Ampicillin, Tet****=*** *Tetracycline, Sul= Sulfonamides, Gen= Gentamicin, Cip= Ciprofloxacin, R = Resistant****,*** *S* ***=*** *Susceptible and**N/A = Not Applicable*.

***Table 5: shows different resistance genes detected in sample types of beef and milk.***

|  |
| --- |
| **Resistance genes detected in beef and milk samples** |
| Sample Type | N | % |
| Beef | *blaZ* | 2 | 3.3% |
| *blaZ,mecA* | 1 | 1.7% |
| None | 57 | 95.0% |
| *Milk* | *blaCTX-M, tetA, sul1* | 2 | 3.3% |
| *blaTEM* | 1 | 1.7% |
| *blaTEM, sul1* | 1 | 1.7% |
| *blaTEM, tetA* | 2 | 3.3% |
| *blaTEM, tetA, sul1* | 2 | 3.3% |
| *blaZ* | 2 | 3.3% |
| None | 49 | 81.6% |
| *tetA, sul1* | 1 | 1.7% |

**Key:**  *blaZ=Beta-lactam resistance gene, blaZ, mecA= Beta-lactam resistance and methicillin resistance, blaCTX-M, tetA, sul1= Resistance to cephalosporins (blaCTX-M), tetracycline (tetA), and sulfonamides (sul1), blaTEM= Resistance gene often associated with penicillin and cephalosporins, blaTEM, tetA= Combined resistance to beta-lactams (blaTEM) and tetracycline (tetA), blaTEM, sul1=Combined resistance to beta-lactams (blaTEM) and sulfonamides (sul1), blaTEM, tetA, sul1= Combined resistance to beta-lactams (blaTEM), tetracycline (tetA), and sulfonamides (sul1), None= No resistance genes detected and N/A= Not Applicable (likely because no bacteria were isolated).*

**4. DISCUSSION**

**4.1 Occurrence of Antimicrobial Residues in Milk and Beef**
Most milk and beef samples from Kabete had no detectable antibiotic residues, consistent with previous reports in Kenya showing low residue prevalence (4-8%). Only 5% of beef and 8.3% of milk samples tested positive, with oxytetracycline detected in beef and β-lactams (penicillin G and amoxicillin) in milk, reflecting common veterinary drug use. Although within regulatory limits, their presence is notable due to potential hypersensitivity reactions and microbial resistance risks. Similar findings were reported by ([25](#WHO14)), who found β-lactam residues in 7% of Nairobi milk samples. Residues suggest non-adherence to drug withdrawal periods or extra-label use ([18)](#lander). The detection of multiple residue types highlights the range of antibiotics used, raising concerns over unregulated usage. While most samples were residue-free, occasional contamination underlines the need for improved farmer education and regulatory monitoring. Low but persistent residue levels align with regional trends and warrant attention due to cumulative public health risks.

**4.2 Antimicrobial-Resistant Bacterial Contamination and Patterns**
Resistant bacteria were isolated from some milk and beef samples. In beef, *S. aureus*, including MRSA, was found in 5% of samples; in milk, 7.5% harbored bacteria, *E. coli* (6.6%) and *S. aureus* (3.3%). *E. coli* contamination likely stemmed from poor hygiene during milking or storage. The 6.6% contamination rate is much lower than the 66% observed in Kibera ([24](#Brown)), suggesting better handling in Kabete. Resistant *E. coli* in milk showed widespread ampicillin resistance, confirming the antibiotic’s reduced efficacy due to prolonged use in animals and humans. Though MRSA was detected in only 1.7% of beef samples, its presence is concerning given its zoonotic risk. Similar low MRSA levels have been reported in other regions. One milk isolate was resistant to all five antibiotic classes tested. Many isolates showed resistance to two or more antibiotics, especially ampicillin and tetracyclines. These findings are consistent with global data on MDR in foodborne bacteria driven by agricultural antibiotic use. Ampicillin resistance dominance aligns with the extensive use of β-lactams in livestock. Co-resistance patterns likely stem from genetic linkages, such as integrons ([22](#Samtiya)). Statistically, milk was significantly more likely to harbor bacteria than beef (χ², p < 0.05), suggesting it offers a more favorable environment for bacterial growth or undergoes less effective post-harvest handling.

**4.3 Distribution of Antimicrobial Resistance Genes**
Molecular analysis revealed several resistance genes. In beef, blaZ was detected in 3.3% of samples, and one isolate carried both blaZ and mecA, confirming the presence of MRSA. In milk, which had higher bacterial contamination, a wider array of genes was detected. BlaZ appeared in 3.3%, while blaTEM-a plasmid-mediated β-lactamase-was found alone or in combination. Notably, 3.3% of milk isolates carried the extended-spectrum β-lactamase gene blaCTX-M along with tetA and sul1, indicating resistance to three antibiotic classes. The global spread of CTX-M enzymes in foodborne *E. coli* is well documented.

Other combinations included blaTEM/tetA, blaTEM/sul1, and tetA/sul1. The co-occurrence of tetA and sul1 likely reflects genetic linkage, consistent with findings by ([25](#WHO14)). Genotypic profiles matched phenotypic patterns: ampicillin-resistant isolates carried blaZ or blaTEM, and tetracycline or sulfonamide resistance aligned with tetA or sul1. Some isolates carried multiple resistance genes, even if not phenotypically resistant to all antibiotics-posing a risk of persistent resistance via gene co-selection. The gene diversity in milk likely reflects its higher contamination and exposure. These results support the study’s framework: phenotypic resistance is underpinned by specific genetic mechanisms. Continuous molecular surveillance is essential for tracking resistance genes in food sources.

**4.4 Implications for Public Health and Livestock Management**
The co-occurrence of residues, resistant bacteria, and resistance genes in milk and beef has major implications for public health and livestock practices. Consumers of raw or undercooked products risk exposure to resistant bacteria such as ESBL-producing *E. coli* and MRSA. This supports the One Health model, where animal antibiotic misuse contributes to human AMR. Ingesting low-level residues can also alter gut microbiota, enabling resistance gene transfer ([22](#Samtiya)). These findings justify stronger food safety measures, such as milk pasteurization, thorough meat cooking, and regular residue and resistance testing.

From a livestock perspective, although few samples had residues, even isolated violations highlight the need for strict enforcement of withdrawal periods. Kenya has relevant policies, but gaps remain in implementation. Farmers must be educated on adherence to withdrawal periods and prudent antibiotic use. Multi-drug resistant bacteria in livestock signal poor stewardship. Improved husbandry, vaccination, and avoidance of non-therapeutic antibiotic use are essential ([25)](#WHO14). Farmer training on dosage, administration routes, and drug choices could reduce misuse.

Routine surveillance for residues and resistant bacteria across the value chain-from farms to markets-will help detect emerging risks like MRSA or ESBLs. This integrated approach is vital to AMR control ([26)](#WHO2018). Our findings, though limited to Kabete, reflect the broader farm-to-fork nature of AMR transmission. The presence of genes like blaCTX-M and mecA in foodborne bacteria raises concerns about horizontal gene transfer to human pathogens, reinforcing the urgency of cross-sectoral antimicrobial stewardship.

**5. Conclusion**

This study demonstrated that milk and beef marketed in Kabete largely comply with antimicrobial residue standards, yet a small but important proportion contained antibiotic residues and harbored antimicrobial-resistant bacteria. The co-detection of drug residues, multi-drug resistant *E. coli*, and MRSA - along with corresponding resistance genes, highlights the interplay between antibiotic usage in food animals and the emergence of resistance. These findings underscore the need for sustained surveillance and concerted One Health interventions to ensure food safety and protect public health.

**6. RECOMMENDATION**

Strengthen regulatory enforcement to ensure farmers and traders strictly observe antibiotic withdrawal times before milk and meat enter the market. Implement targeted training programs on prudent antibiotic use for livestock keepers emphasizing alternatives like vaccination and proper dosing to reduce misuse of veterinary drugs. Establish regular screening of dairy and meat products for antibiotic residues and resistant bacteria, coupled with trace-back mechanisms to identify and rectify on-farm practices that lead to contamination.

Invest in better milking, slaughter, and distribution hygiene including promoting pasteurization of milk and proper meat handling to lower the risk of contamination with pathogenic and resistant microbes. Enhance integrated surveillance systems that track antimicrobial use and resistance across humans, animals, and food, informing evidence-based policies and early interventions in both the health and agriculture sectors.

**Consent**

Written informed consent was obtained from each milk and beef seller in Kabete sub county during the study period (3rd January, 2025 to 23rd May 2025). All authors declare that ‘written informed consent was obtained from the study participants. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal."

**Ethical approval**

Approval for the study was obtained from the Jomo Kenyatta University of Agriculture and Technology (JKUAT) Ethical Review Board(*JKU/ISERC/02317/1391*) and National Commission for Science Technology and Innovation(*NACOSTI/P/25/414753*). Since the research did not involve human participants, informed consent was not required. However, sample confidentiality was ensured by storing all biological specimens with coded identifiers. The anticipated benefit of this study is the advancement of knowledge on antimicrobial resistance, which is critical for informing policy on antibiotic usage and ultimately protecting public health.

**Abbreviations**

ABF: Antibiotic Free; AMR: Antimicrobial Resistance; FDA: Food and Drug Administration; MRL: Maximum residue limit; NCCLS: National Committee for Clinical Laboratory Standards; WHO: World Health Organization.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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