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Assessment of Veterinary Antibiotic use and Occurrence of Veterinary Antibiotics in Livestock Manure from Farms in Rongai Sub-County, Kenya

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Abstract

Veterinary antibiotics are commonly used in livestock rearing to prevent diseases and stimulate growth. The release of antibiotics into the environment has become a significant environmental and public health concern. This research evaluated antibiotic use, livestock treatment, manure utilization, livestock waste treatment methods and antibiotic residues in livestock manure. Questionnaires were administered to 170 farmers rearing both cattle and poultry. Subsequently, 28 livestock manure samples from 15 cattle and 13 poultry rearing farms were collected from various farms to assess concentrations of tetracyclines (Tetracycline, Oxytetracycline) and sulfonamides (Sulfadiazine, Sulfamethoxazole) residues. Residues analysis was done using High Performance Liquid Chromatography-Diode Array Detector (HPLC-DAD). Veterinarians were the most preferred in treating both cattle and poultry in farms. Tetracyclines and sulfonamides were the most consumed class of antibiotics among both poultry and cattle rearing farmers. Compost manure and Biogas were the most preferred use of animal waste within farms. Antibiotic presence in samples was detected in 80% and 93% of cattle and poultry manure respectively. Maximum antibiotic concentrations of 16.24 and 15.18 (mg/kg) were recorded in poultry and cattle manure, respectively. There was a statistically significant difference in antibiotic concentrations in poultry and cattle manure (P<0.05). The results of this research are important in monitoring rising concerns about veterinary antibiotics on environmental and public health.

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Introduction

Antibiotic spread levels in the environment have increasingly become a worldwide concern, this is linked to increased development and spread of resistant bacterial strains, that have the potential to infect humans and livestock becoming difficult

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to manage.[1] Globally, they are used for treatment in human therapy and veterinary medicine. For livestock treatment, antibiotics are mainly administered by three different routes, through injection, introduction in feed and drinking water. Successful use of antibiotics in livestock rearing for metaphylaxis, prophylaxis and in some instances for growth promotion, with the aim of producing healthy livestock.[2] Most pharmaceutical drugs get excreted from organisms in their original form, or as metabolites that may still exhibit activity, with approximately 30% and 90% of the initial dose given excreted.[3] Hence, livestock manure used for fertilization of agricultural land may be contaminated with antibiotics.

The total annual use of antibiotics has increased from 100000 to 200000 tons globally,[4] with a large number of veterinary drugs being consumed annually in different countries. Globally, antibiotic consumption is estimated at 420 tons in the United Kingdom, 14,000 tons in the United States[5] and 84,240 tons in China.[6] According to Van-Boeckel,[7] the average global scale of antibiotics consumption per annum is 45mg/kg, 148mg/kg and 172mg/kg for cattle, chicken and swine respectively. However, consumption patterns vary across regions attributed to variations in farming types, agricultural advancement, availability of antibiotics, pests and diseases, and climatic conditions.[7]

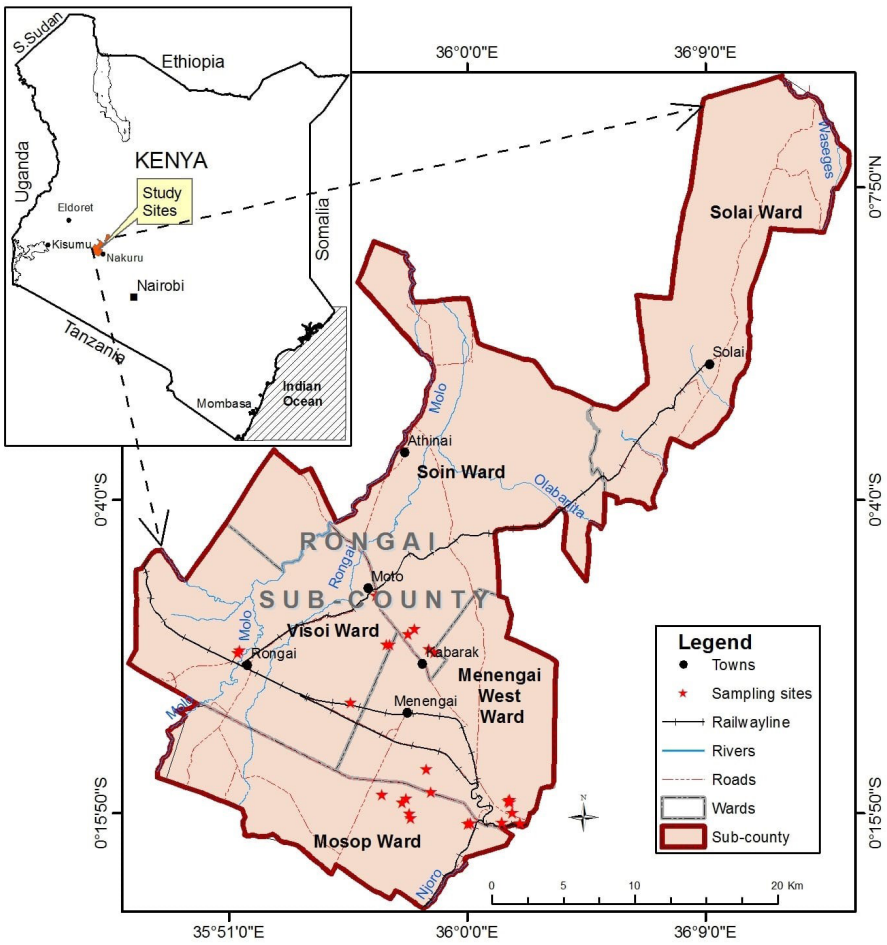

Fig. 1: Map of Rongai Sub-County showing the sampling sites. (Data source: Regional Centre for Mapping Resources for Development)

Residues of antibiotics used in livestock treatment can leach into the soil, potentially contaminating it, these compounds have the potential to infiltrate to surface water, groundwater and the food chain. Veterinary antibiotics from livestock are excreted through animal feces and urine, and end up in the environment through the application of livestock manure. The movement of antibiotics in the environment is affected by different processes such as adsorption/desorption, transport process and degradation process acting on these antibiotics.[8,9,10] Due to the increased use of antibiotics and poor management of animal manure in farms in Kenya, assessment of antibiotics concentrations in soils and manure amended soils is necessary. The objective of the study was to determine antibiotic use, livestock treatment, livestock waste management and antibiotic residues in livestock manure. These results could provide useful information for assessing impacts and potential risks presented by antibiotics in soils and ensure proper handling of antibiotic containing manure.

Materials and Methods

Study Area

The study sites were located in Rongai sub -county within Nakuru County, Kenya, Located between 0° 10' 23.99" N and 35° 51' 49.75" E. It has a total of 5 administrative units namely Menengai west, Rongai, Visoi, Mosop and Solai. It is located at an altitude of between 1650m and 1850m above sea level (a.s.l) covering an area of 1049.1 square Kilometers and temperature range of between 17°C and 29°C annually. The area receives an annual rainfall that ranges between 600-1000 mm.[11] It has a total population of 199,004 with 31% of households actively involved in cattle rearing, poultry rearing and farming.[12] The study area was selected due to the high number of farms rearing livestock and farming activities in the area and the potential to adversely impact human and environmental health.

Study Design

The study used a cross-sectional study design. It entailed conducting interviews to assess antibiotic use, livestock treatment and the management of livestock waste in farms. It also used an ecological survey and laboratory analysis to assess antibiotic levels in animal manure.

Sampling Procedure

A total of 170 structured interviews were carried out across the 5 administrative units in Rongai sub-county, assessing the type of livestock reared and farm practices. Questionnaires for this study were prepared by the researchers and pretested before they were administered. Information from the interviews were used to purposively select (Figure 1) poultry and cattle farms to be sampled. For inclusion into the study, farms had to be involved in either cattle or poultry rearing and managed animal waste on site. Sampling was done between (January and March 2023). Sampling sites were located and georeferenced. Samples collected from farms were packaged in polythene bags, labeled and stored for transportation to the laboratory. A total of 28 manure samples were collected from farms representing 15 and 13 cattle and poultry manure samples respectively. Three sub-samples were obtained from manure heaps at each designated sampling site within the farms and a composite sample made to be analyzed for the presence of tetracycline, oxytetracycline, sulfadiazine and sulfamethoxazole antibiotic. Composite samples were then oven dried, ground and one gram of the sample was extracted successively. Sample extraction of target antibiotics was conducted using a QuEChERS extraction procedure.[13]

Data Collection

Information on the treatment of cattle and poultry in farms, antibiotic use and use of livestock waste in farms were obtained by use of a questionnaire. Questionnaires were administered to farm owners and farmhands who were involved in livestock rearing. Informed consent was sought from farmers participating in the study. Since the study included human respondents, it was ethically approved by the National Commission for Science, Technology and Innovation in Kenya (License no NACOSTI/P/22/20346).

Data Analysis

Descriptive statistics, including mean and standard error, were computed for all datasets. Data management was conducted using Sigma Plot 14.0. Analysis of variance was employed to assess variation in antibiotic concentration across livestock manure samples obtained from all sampling sites. All analysis were conducted at a confidence level of 95%.

Results

Antibiotic use Among Livestock Rearing Farmers

A total of 170 farmers were assessed in the study with 68 farmers rearing poultry and 102 farmers rearing cattle. Tetracyclines, sulfonamides, penicillin and macrolides were reported to be the most consumed class of antibiotics in 60%,8%, 20% and 12% of the cattle rearing farms. However, among poultry rearing farms tetracyclines, sulfonamides, quinolones and other classes were the most commonly used classes in 35%,40%,20%,5% of poultry farms in this study (Figure 2).

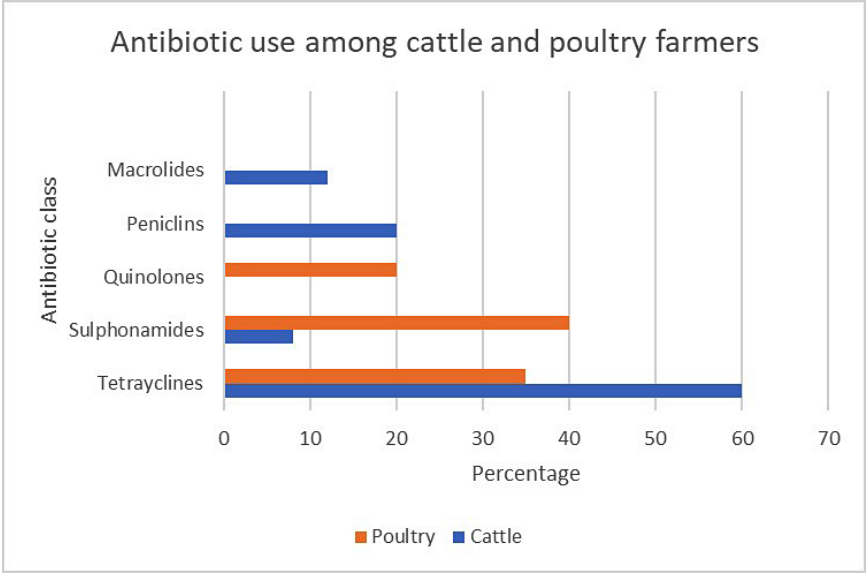

Fig. 2: Antibiotic use among cattle and poultry farmers

Treatment of Cattle and Poultry

The findings indicated that among the 170 farmers, 65% primarily relied on veterinarians to treat their livestock. 85% of cattle farmers consulted a veterinarian, while 10% and 5% of the farmers relied on getting prescriptions from agrovets and self-knowledge respectively. In contrast, among poultry farmers 11% consulted a veterinarian, while 71 % and 18% of farmers relied on getting prescriptions from agrovets and self-knowledge.

Livestock Waste use in Farms

In the study, all farmers utilized livestock waste, with 92% using waste as compost, 7% used as biogas units, and 1% incorporated it in feed, predominantly sourced from poultry droppings that were dried and properly sieved. The slurry generated from biogas units was subsequently utilized as manure in farm settings.

Antibiotic Residues in Cattle Manure

Results showed that 80% of the samples analyzed presented detectable levels of antibiotic residues in manure. Antibiotic concentration of the tetracycline group was higher ranging from 1.0123 mg/kg and 15.178mg/kg, compared to 0.1209 mg/kg and 3.9409 mg/kg within the sulfonamide group. Table 1 outlines the mean and maximum value concentrations of antibiotic residues in cattle manure.

Table 1: Mean and maximum concentrations of antibiotics in cattle manure.

Antibiotic	Mean±SE (mg/kg)	Maximum value (mg/kg)
Tetracycline	2.43±1.14	15.18
Oxytetracycline	0.64±0.27	3.29
Sulfadiazine	0.09±0.05	0.75
Sulfamethoxazole	0.67±0.34	3.94

Antibiotic Residues in Poultry Manure

Analysis of poultry manure samples revealed the presence of antibiotic residues in 93% of samples. The tetracycline group exhibited higher antibiotic concentrations compared to the sulfonamide group. Tetracycline concentrations ranged from 1.059 mg/kg to 16.24 mg/kg, while sulfonamide concentrations were between 0.3755 mg/kg and 4.9553 mg/kg. Table 2 outlines the mean and maximum value concentrations of antibiotic residues in poultry manure.

Table 2: Mean and maximum value concentrations of antibiotics in poultry manure.

Antibiotic	Mean±SE (mg/kg)	Maximum value (mg/kg)
Tetracycline	1.17±1.17	15.23
Oxytetracycline	3.37±1.40	16.24
Sulfadiazine	0.96±0.43	3.97
Sulfamethoxazole	0.16±0.57	4.96

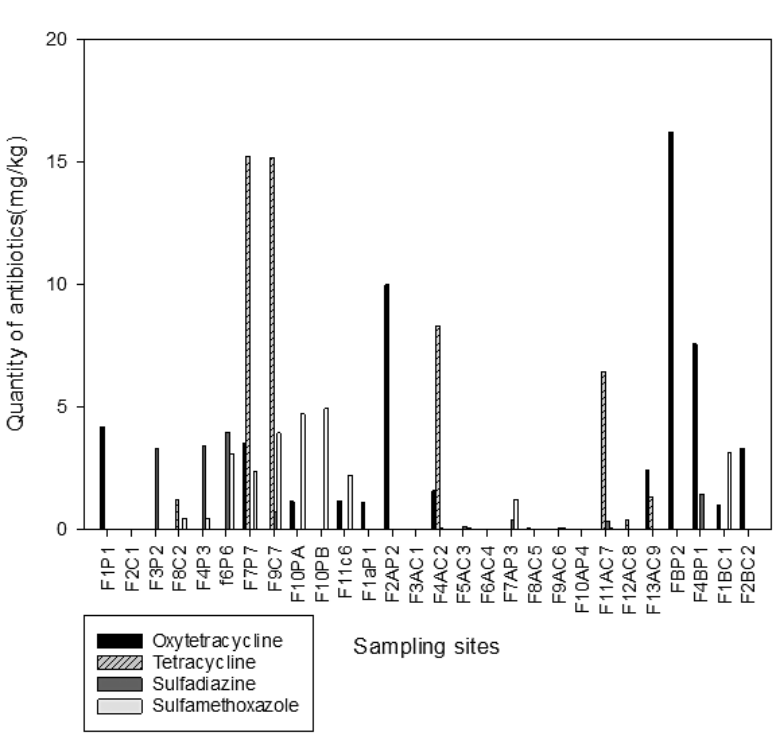

Fig. 3: Antibiotics in livestock manure.

Normality tests for the data in this study were performed using the Shapiro-Wilk test. The findings indicated that the data exhibited a normal distribution ($P>0.05$). Statistical analysis using Analysis of Variance (ANOVA) showed significant differences in antibiotic concentration between poultry and cattle manure samples collected in the study area. Figure 3 shows the distribution of antibiotic concentration in various manure samples.

Antibiotic Residues in Manure from Compost and Biogas Units.

Analysis showed antibiotic concentrations in slurry from biogas units were lower compared to those from compost heaps. Antibiotic concentration in biogas slurry samples fell between 0.06 mg/kg and 6.44 mg/kg. Table 3 presents antibiotic levels identified in manure from compost and biogas units.

Table 3: Antibiotic concentration in manure from compost and biogas units

Group of Antibiotic	Compound	Quantity (mg/kg) (Biogas)	Quantity (mg/kg) (Compost)
Tetracycline	Oxytetracycline	Nd	0.06-16.24
	Tetracycline	6.44	13.16-15.23
Sulfonamide	Sulfadiazine	0.34-0.38	0.01-3.97
	Sulfamethoxazole	0.06-1.21	0.04-4.96

Discussion

Antibiotic utilization patterns observed among cattle rearing farmers in this study align closely to those reported in various comparable studies. Tetracycline and penicillins were found to be the most consumed class of veterinary antibiotics in cattle rearing farms in Pennsylvania.[14] A study by Muloi[15] within central highlands in Kenya, found tetracycline and penicillins as the most frequently used class of antibiotics. Similar studies by Redding[16] and Alhaji[17] found tetracycline, beta-lactam/aminoglycoside, trimethoprim-sulfonamides, beta-lactamase, aminoglycosides and macrolides as among the most consumed class of antimicrobials. In Tanzania, studies assessing antimicrobial use among cattle and poultry rearing farmers in Dar es Salaam found tetracycline, sulfonamides, penicillins and macrolides as the most consumed class of antimicrobials.[18,19]

In poultry farming, findings mirrored studies investigating antibiotic use among poultry farmers in specific regions of Nigeria and Kenya. These studies identified sulfonamides and tetracyclines as the predominant class of veterinary drugs.[20,21] A study by Kariuki[22] found tetracycline and sulfonamide antibiotics as the most consumed class of drugs among poultry rearing farmers in Kiambu county. According to Conde-Cid[23] tetracyclines and sulphonamide class of antibiotics are commonly used due to their wide spectrum in the treatment of various livestock diseases.

Results of this study showed farmers were likely to consult a veterinarian in treating their cattle. This was similar to studies conducted by Caudell[24] and Nthambi[25] that found veterinarians as the most preferred choice in treating cattle within farms in Northern Tanzania. This preference is primarily attributed to the substantial value of cattle and the heightened attention to their management. High reliance on veterinarians in the treatment of cattle in this study reduced the chances of self-diagnosing and administration of wrong doses of antibiotics by farmers. Among poultry farmers results were consistent with studies conducted in specific regions of Ghana and Kenya, that found prescriptions from agro-veterinary shops and self-prescription as the most preferred mode of treatment.[26,22]

According to Zhu[27] composting and conversion of waste into bioenergy are the main forms of manure management within farms. Incorporating sustainability has led to the adoption of cleaner production methodologies in livestock production necessitated as a measure of mitigating negative environmental effects from wastes generated and production systems and to offer an alternative to energy production by creating sustainable energy sources and resource use in farms.[28,29]

Antibiotic residues in most of the manure samples in this study were above detectable levels. However, values recorded in the study were lower than the maximum values observed in different studies that reported levels of above 100mg kg.[30,31,32,33] The average global scale of antibiotics consumption per annum of animal produced is 45 mg/kg^{-1} for cattle, 148 mg/kg^{-1} for chicken and 172 mg/kg^{-1} for pigs.[7] This outlines that poultry is administered with higher levels of veterinary antibiotics compared to cattle.

Veterinary antibiotic levels in manure from biogas units were lower compared to compost implying that biogas units were more effective in degrading

antibiotics. A similar study found anaerobic digestion units efficient in the elimination of sulfonamide residues in animal manure.[34] Oxytetracycline removal efficiency in anaerobic digestion was found to be at 60% after 64 days yielding a half-life of 56 days.[35,36] Anaerobic digestion is considered effective in the removal of antibiotics although its potential to eliminate or remove antibiotics is linked to the concentration, class of antibiotics, time, temperature and reactor type.[37] Results in this study were similar to a study by Akyol[38] assessing degradation rates of antibiotics in biogas units. According to Arum Widyasari-Mehta39 uptake of manure treatment processes such as anaerobic digestion is increasingly on the rise due to their ability to reduce organic matter pollution, pathogens and biogas production.

A study in Morogoro Tanzania recorded tetracycline levels of 1.573 mg/kg in poultry manure and 0.329 mg/kg in cattle manure.[40] Conde-Cid[23] found lower levels of tetracycline in cattle manure at 0.9 mg/kg in Galacia, Spain. Similar study by Ji[41] found higher tetracycline levels of 12.01 mg/kg and 10.31 mg/kg in manure samples from cattle and poultry farms in Shanghai, China. According to Zhao,[42] oxytetracycline levels in livestock manure in eight provinces in China reached concentrations of 1.55mg/kg in manure composting preparations. Results in studies assessing oxytetracycline levels in poultry manure in China, Iran and Egypt recorded concentrations of 4.57mg/kg,13.77mg/kg and 0.0013 mg/kg respectively.[43,44,45] Similar studies by[23,41] recorded oxytetracycline levels of 35mg/kg and 21.36 mg/kg in cattle manure and 21.96 mg/kg in poultry manure in Spain and China respectively. Oxytetracycline levels of up to 21.36 mg/kg and 21.96mg/kg were recorded in cattle manure and poultry manure respectively from farms in Shanghai, China.[41] An experimental study recorded oxytetracycline residues of <0.012 mg/kg in poultry droppings from poultry treated with 80mg/kg of oxytetracycline for 14 days.[46] Results in this study were lower compared to findings in other studies that recorded tetracycline concentrations of between 0.047-13.77 mg/kg in cattle manure in Malaysia and 78.516 mg/kg in poultry in Iran[47,44]

Sulfadiazine residues have previously been identified with maximum concentrations of upto 91mg/kg in poultry feces.[48] A study by Hou[49] found sulfadiazine concentrations ranging from 4.5 to 18.7mg/kg in animal manure samples in Northern China. Results from a study assessing the presence of antibiotics in calve manure in the Netherlands found concentrations ranging between 0.001 mg/kg and 0.065mg/kg of sulfadiazine in manure samples.[50] Conde-Cid[23] found sulfadiazine residue concentrations of 0.6 mg/kg in poultry manure within farms in Galacia, Spain. Sulfamethoxazole concentrations in this study were higher compared to results in similar studies. A study Ajibola[51] assessing sulfamethoxazole concentrations found levels of upto 0.0127 mg/kg in manure within poultry rearing farms. Results in a study assessing sulfonamide antibiotics in animal manure found concentrations of 0.415mg/kg and 0.022 mg/kg in cattle manure and poultry manure respectively within farms in Morogoro, Tanzania.[40]

Antibiotic concentrations in poultry manure in this study had higher levels of detection and concentration compared to cattle manure, these results were similar to various studies assessing antibiotic residues in livestock manure.[52,53,54] that is largely attributed to high doses of veterinary antibiotics that are frequently administered to poultry compared to cattle.[55,7] Guo[13] found lower concentrations of antibiotic residues in cattle manure compared to poultry within farms in China, attributed to the difference in the amount of dose administered and different metabolic characteristics among livestock. Low levels of antibiotic use in cattle rearing can be linked to strict levels of surveillance in milk compared to poultry products. This was also observed in a similar study that linked variability in dosage administered to livestock to increasing growth levels and disease prevention in different animal species.[21] Different degradation rates in livestock manure in the study area, use of poor manure management methods and continuous heaping of manure piles do not guarantee sufficient degradation of antibiotic residues. These conditions are likely to contribute to the different levels of antibiotics detected in manure.

Tetracycline antibiotics recorded in this study were higher compared to sulfonamides this could be attributed to high levels of administration, wide-scale use in the treatment of livestock and low degradation

rates in livestock manure during preparation in farms. Results in this study showed higher concentrations compared to similar studies. This could outline high use of veterinary antibiotics in the study area and lower degradation rate of antibiotics in manure.

Antibiotic concentrations in livestock manure in different studies have been found to have a large variation that can be linked to sampling time, source of manure and the region of sampling.[52] Application of animal manure in soils within the study area presents a risk posed by antibiotic contamination, since the trigger value of 100 µg/kg in soils was exceeded in most of the animal manure samples.[56] In this study, the total concentration of antibiotics in poultry manure was higher compared to cattle manure. This can be associated with the different treatments administered to poultry as compared to cattle. However, there is a lack of data outlining the exact quantities administered, frequency of application and dose administered to livestock.

Conclusion

Results from the study showed that most farmers relied on a veterinarian to treat their livestock. Compost manure was the most preferred use of livestock waste in farms. Antibiotic residues in the study recorded levels ranging from 0.06-16.24 mg/kg for oxytetracycline, 13.16-15.23 mg/kg for tetracycline, 0.01-3.97 mg/kg for sulfadiazine and 0.04-4.96 mg/kg for sulfamethoxazole in manure. Antibiotic residues in poultry manure were higher compared to cattle manure while tetracyclines recorded higher levels compared to sulfonamides in the study. Biogas units and composting were noted to be efficient in reducing antibiotic residues in manure. Consequently, utilization of livestock manure from this study within farms posed a significant human and environmental health risk.

Recommendations

Awareness campaigns on antibiotic use and proper manure management among farmers are imperative to address the antimicrobial resistance crisis. Such initiatives aim to foster improved veterinary antibiotic practices within farms and mitigate risks to human health and soil ecosystems. Farmers should adopt biogas utilization and enhance the composting process for animal manure within their farms. These methods have the potential to significantly reduce or eliminate antibiotics before the introduction of manure into farming systems. Furthermore, there is a need to formulate and enforce standards for monitoring antibiotic levels in the environment. This regulatory oversight is essential for safeguarding public health and environmental integrity.

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Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

The manuscript incorporates all datasets produced or examined throughout this research study.

Ethics Statement

The study did not involve an experiment on humans and animals.

Authors' Contributions

M.J.W, W.N.M and G.M.O were involved in the conceptualization and writing of the original draft. All authors have read and agreed to the published version of the manuscript.

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