



# Sulfonamides and $\beta$ -lactam antibiotic residues and human health risk assessment in commercial chicken meat sold in Nairobi City, Kenya

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## ABSTRACT

The use of antibiotic pharmaceuticals in chicken husbandry has risen steadily over time. Antibiotic residues in chicken meat poses risks to human health in addition to their contribution to the advancement of antibiotic resistance. Despite the increased use of antibiotics in chicken farming in Kenya, assessments of the residues and human exposure have not been conducted. In this study, the sulfonamides (SAs); sulfapyridine (SPD), sulfadiazine (SDZ) and sulfamethazine (SMZ) and the  $\beta$ -lactams ( $\beta$ Ls); ampicillin (AMP), penicillin G (PEG) and amoxicillin (AMX) were determined in three chicken meat types; ex-layers, broilers, and indigenous meat marketed in Nairobi City, Kenya. Residual SAs ranged from 0.1 to 154.4  $\mu\text{g kg}^{-1}$ , with SPD recording the highest concentration in ex-layers' chicken meat samples. A range of 19.7 to 309.0  $\mu\text{g kg}^{-1}$  of BLs was found, where the highest amount represented AMX in ex-layers. Mean AMX contents in all chicken types, and AMP in broilers were above the Maximum Residue Limits (MRLs). For SAs, only SPD mean content was above MRL in ex-layers. Human health risks from exposure to antibiotic-contaminated chicken meat was evaluated using % ADI. All tested  $\beta$ Ls were of no risk (<1% ADI) to human health. SPD and SDZ posed considerable risk (1–5% ADI) in some chicken meat, whereas SPD in ex-layers' chicken meat posed distinctive risk (>5% ADI) to children. Considering the co-occurrence of different types of antibiotics in same samples, obtained MRLs and % ADI (for some of the antibiotics) are indicative of potential human health risks. Information is valuable in provoking response from concerned agencies and fostering activities that advocate for judicious use of antibiotics.

## 1. Introduction

Since their introduction in the 1940s, the global market for veterinary antibiotic pharmaceuticals has significantly increased for their prophylactic and therapeutic purposes [1]. However, a significant portion is used for non-therapeutic purposes as feed supplements to enhance growth and productivity of animals [2], to meet the ever increasing demand for animal protein [3,4]. The global demand for meat has increased tremendously over the decades, largely driven by population growth and increased income per capita [3,5]. This demand is projected to increase by 14% by 2030 compared to the base period average of 2018–2020, and a 17.8% projected growth for poultry meat within the same period [4]. There is a global shift toward poultry meat consumption, especially in low income

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developing countries due to lower cost compared to other meats [4], which has led to increased use of antibiotics in poultry farming for both therapeutic and non-therapeutic applications to meet the demand. Inappropriate use of antibiotics as growth supplements in chicken farming may lead to the persistence of residues in meat. This can lead to toxic effects and allergic reactions to antibiotic drug agents including serum sickness, cutaneous reactions, severe allergic reactions and hypersensitivity resulting to anaphylaxis [6,7]. For example, the potential of allergic reaction upon consumption of meat products having penicillin residues was confirmed by Baynes et al. [8], that can appear as severe anaphylaxis and even skin rash. Furthermore, repeated exposure to residual antibiotics from animal products may aggravate the immune responses in persons with weak immune system, negatively impacting the intestinal gut flora [9, 10]. Antibiotic misuse can result in the development and spread of antibiotic-resistant bacteria (ARB), thereby reducing the efficacy of drugs used in human and animal therapy.

Antibiotic resistance is an issue of global health concern. Bacteria commonly found in poultry and livestock are frequently present in fresh meat products and may therefore serve as reservoirs for antibiotic resistant genes (ARGs) that could potentially be transferred to pathogenic organisms in humans [11,12]. Moreover, the handling of animal products containing antibiotic residues, ARB and ARGs poses a threat of spreading antibiotic resistance to humans. Also, in the digestive systems of antibiotics-treated animals [13], selection and multiplication of ARB takes place, and excreted antibiotics continue exerting selection pressure in the environment. The increase and dissemination of ARGs in contaminated environments is of particular concern to human health [14]. Kenya, like other global nations, has recognized the threat of the rising resistance to antimicrobials and responded by developing the National Policy [15], for the prevention and containment of antimicrobial resistance (AMR). One of the key objectives of this policy is to strengthen the knowledge and evidence base on AMR through surveillance and research. It is therefore important to determine residual antibiotics in animal products to assist in risk assessments and to help implement important control measures leading to reduced development and transfer of ARGs and other threats to human health [16].

In animal husbandry, antibiotics from various classes are used, such as sulfonamides (SAs), quinolones (QNs), tetracyclines (TCs), macrolides (MLs) and  $\beta$ -lactams ( $\beta$ Ls) [17]. A random check from Kenya's agro-veterinary outlets showed that antibiotics from these groups are used in animal production, and some study reports indicate that they are widely used by poultry farmers, particularly intensive small-scale and commercial farmers [18,19]. However, the use of antibiotics in animal medication is not well regulated and documented in Kenya, with the same study reporting that antibiotics are readily available to farmers, primarily from agro-vets stores and that farmers administer them without the aid of trained veterinary personnel [19]. Ordinarily, farmers are expected to obtain a trained veterinarian's service in the administration of drugs to animals, but that is not always the case. This raises the risk of misuse of medications and consumers are therefore inadvertently predisposed to health risks. There is also lack of data documenting antibiotic residues in poultry products in Kenya. Available information [18,20] on potential risks of residual antibiotic pharmaceuticals in animal products do not cite any supporting scientific data, which points to the importance of this research. This study therefore investigated selected sulfonamides and  $\beta$ -lactam antibiotics in chicken meat from point of sales in Nairobi City, Kenya, and assessed the risk of exposure to humans to improve food safety and highlight the need for suitable regulations in use of antibiotics in chicken farming. The

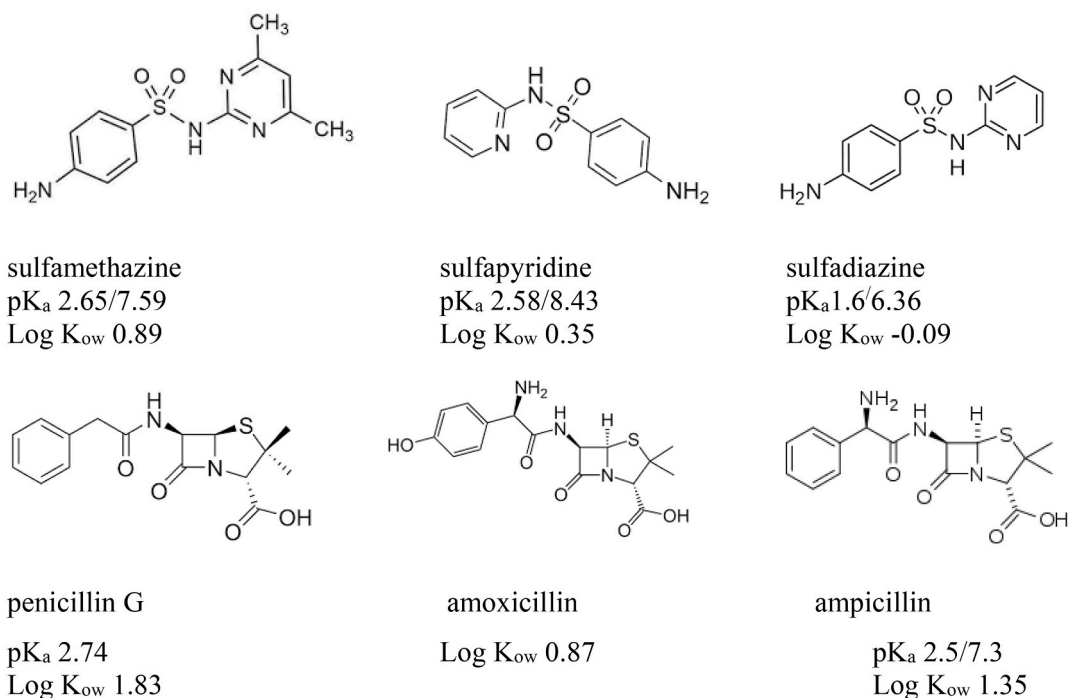


Fig. 1. Chemical structures and physiochemical characteristics of the investigated antibiotic compounds.

study also finds its relevance to the National Action Plan [15] for the containment of AMR by strengthening the knowledge base.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Certified antibiotic reference standards (>98% purity) sulfamethazine (SMZ), sulfapyridine (SPD), sulfadiazine (SDZ), penicillin G (PEG), amoxicillin (AMX) and ampicillin (AMP) were purchased from Merck KGaA, Darmstadt, Germany, through their Kenyan agent company, Scientific Laboratory Supplies. The structures are given in Fig. 1. High-performance liquid chromatography (HPLC)-grade methanol (MeOH), acetonitrile (ACN), and formic acid (FA) were purchased from Precise Lab Africa (Nairobi, Kenya). Oasis hydrophilic-lipophilic balance cartridges (HLB, 60 mg, 3 mL), and Glass microfiber filters (0.45  $\mu\text{m}$  and 0.22  $\mu\text{m}$ ) were obtained from Waters Corporation (Milford, MA, USA). Analytical grade ammonium formate ( $\text{NH}_4\text{HCO}_2$ ) and acetic acid and all other reagents (analytical grade) were purchased from Precise Lab Africa. Standard stock solutions of antibiotics standards were prepared at  $1 \text{ g L}^{-1}$  in HPLC-grade methanol and ACN for SAs and  $\beta$ Ls, respectively and stored at  $-20^\circ\text{C}$ . Working solutions were prepared on a regular basis and stored at  $4^\circ\text{C}$  for SAs and  $-4^\circ\text{C}$  for BLs.

### 2.2. Sampling

A total of 36 chicken samples comprising of Broilers (22), Ex-layers (6) and Indigenous (8) (not including blank samples) were obtained randomly from six open markets; Burma, Gikomba, City Market, Highridge, Kangemi, Dagoreti, and two Supermarkets in Nairobi city, Kenya. Samples (indigenous) that served as blanks and spiking for recoveries (matrix matched) were obtained from rural Kitui County. Purchased samples were immediately packed in ziplock propylene bags, sealed, and kept in dry ice during transportation to the laboratory. All samples were homogenized and stored at  $-23^\circ\text{C}$  until extraction and analysis. Each sample was analyzed in triplicate for six antibiotics from SAs and  $\beta$ Ls antibiotic classes using Ultra-Performance Liquid Chromatography (UPLC).

### 2.3. Sample preparation and extraction

Sample preparation involved a solvent extraction step followed by clean-up using SPE cartridges. Chicken meat samples were cut into small pieces within  $1 \text{ cm}^3$  and homogenized three times (Armco Blender, ABL-355ECO). The solvent extraction procedure by Pugajeva et al. [21] was adopted with some changes. Briefly, homogenized meat samples (5 g) were transferred to 50 mL polypropylene centrifuge tubes. A 10 mL solution of 0.1% formic acid in acetonitrile was added and mixed for 20 min. 9 mL of the supernatant was placed into a centrifuge tube and frozen for 30 min at  $-23^\circ\text{C}$ . The sample was centrifuged at 3500 rpm for 15 min, and a 5 mL portion of the extract was evaporated to a volume of 300  $\mu\text{L}$  using a Concentrator (miVac DNA-23050-A00) at  $40^\circ\text{C}$ . Thereafter, the mixture was reconstituted to 10 mL using acidified LC water (0.1% FA). All extractions of the samples were done in triplicates. Oasis HLB cartridges 60 mg/3 mL were used for sample cleanup to minimize matrix interference using SPE vacuum manifold (MiliporeSigma™ Supelco™ Visiprep, Thermo Fisher Scientific). The cartridges were conditioned with 3 mL methanol and equilibrated with 3 mL acidified LC-water. The samples were passed through the cartridges at a flow rate of  $8 \text{ mL min}^{-1}$ , rinsed with 3 mL of 10% MeOH-LC water, and dried for 10 min in a stream of air. Finally, each cartridge was eluted with 3 mL 70:30 v/v MeOH: ACN. Prior to UPLC analysis, the eluate was evaporated using a rotary evaporator at  $40^\circ\text{C}$ , reconstituted to 1 mL with a 2:1 (v/v)  $\text{H}_2\text{O}$ -ACN solution containing 5 mM  $\text{NH}_4\text{HCO}_2$  and 0.01% acetic acid, filtered through a polyvinylidene difluoride (PVDF) membrane filter (0.22  $\mu\text{m}$ ), and stored in 1.5 mL amber vials at  $4^\circ\text{C}$ .

### 2.4. UPLC-PDA analysis

UPLC analysis was done with a Shimadzu UPLC (Prominence LC-2030 plus), coupled to a photodiode array (PDA) detector. The method was optimized for the analysis of the SAs and  $\beta$ Ls. The mobile phase consisted of an aqueous phase (A) 5 mM  $\text{NH}_4\text{HCO}_2$ , 0.01% acetic acid and 0.1% FA v/v, and an organic phase (B) of acetonitrile in a gradient elution program (v/v); 5% B for 1 min, changed to 20% B over the next 5 min, to 30% B in 4 min, to 40% B in 4 min to 80% B for 2 min and finally to 5% B in 4 min. The flow rate was  $0.6 \text{ mL min}^{-1}$  at a column temperature of  $35^\circ\text{C}$  and wavelength set at 254 nm.

### 2.5. Method evaluation

The parameters for method validation included accuracy, linearity, precision, limit of quantification (LOQ), limit of detection (LOD) and selectivity. Extraction method was validated by use of fortified matrix-matched blank chicken meat samples at two levels, 5 and  $10 \mu\text{g kg}^{-1}$  followed by the determination of recoveries. Unspiked blank samples were used as control. The accuracy, expressed as percent recoveries was obtained by use of equation (1);

$$\text{Recovery (\%)} = \frac{A_S - A_B}{A_C} \times 100 \% \quad (1)$$

Where  $A_S$  is the amount of analyte recovered in spiked sample,  $A_B$  the amount of analyte in blank and  $A_C$  the actual amount spiked in

sample.

Instrumental performance was expressed as the method limit of detection (LOD) and limit of quantification (LOQ), which were calculated from standard deviation ( $\sigma$ ) of the response of the curve (based on  $\sigma$  of y-intercepts of regression lines) and the slope of the calibration curve ( $s$ ) of solvent matched standards for the range 0.1–20  $\mu\text{g L}^{-1}$  as  $3.3 \sigma/s$  and  $10 \sigma/s$ , respectively. The linearity of the UPLC method was determined from the correlation coefficient ( $R^2$ ) of five-point calibration curves (0–20  $\mu\text{g mL}^{-1}$ ) for all the test antibiotic compounds.

The selectivity was assessed by running 6 blank chicken meat extracts along with blank extracts spiked with all standard compounds. The possibility of matrix interference at the retention times of the analytes under consideration was determined by comparing blank and spiked samples. In evaluation of the precision of the method, three sets of three spiked samples at two levels, 5 and 10  $\mu\text{g kg}^{-1}$ , were analyzed in three separate occasions and the relative standard deviation (% RSD) of the concentrations determined were calculated.

## 2.6. Risk analysis

Risk assessment of the consumption of chicken meat contaminated with antibiotic residues was evaluated using Equation (2) as previously described by Juan et al. [22];

$$\text{EDI} = \frac{C \times \text{FIR}}{\text{BW}} \quad (2)$$

Where EDI is the estimated daily intake ( $\text{g person}^{-1} \text{day}^{-1}$ ); C the median antibiotic concentration in poultry ( $\text{g kg}^{-1}$ ); FIR is the food ingestion rate ( $\text{kg day}^{-1}$ ), while the typical body weight, BW, used was 20 kg for children and 70 kg for adults.

Based on the EDI, the ratio of the potential exposure to specific antibiotic residues and the level at which no adverse effects are expected, expressed as the % EDI to ADI ratio (% ADI), was calculated according to Equation (3) and compared to the acceptable daily intake (ADI,  $\mu\text{g kg}^{-1} \text{day}^{-1}$ ) [23,24]. ADI is an estimated amount of residue that can be ingested daily over a lifetime without any appreciable health risk, which is expressed on a body weight basis. ADI values used in the determination of the % EDI to ADI ratio were obtained from literature [25–27]. % ADI of <1%, 1–5% and above 5% was considered negligible, considerable and distinctive, respectively [23,28].

$$\% \text{ ADI} = \frac{\text{EDI}}{\text{ADI}} \times 100 \quad (3)$$

## 2.7. Data evaluation

Antibiotic residue data was evaluated for means, frequency and percentages. Statistical analysis was performed using Microsoft Excel 2010 (Apple Inc., USA), SPSS 16.0 (IBM corporation, USA) and Origin 10.5.106 (OriginLab corporation, USA) computer programs.

## 3. Results and discussion

### 3.1. Method performance

Samples preparation and UPLC-PDA methods were validated for the determination of SAs and BLs by assessment of linearity, LOD, LOQ, accuracy, sensitivity and precision. The linear regressions showed good linearity for all test antibiotics with high correlation

**Table 1**  
Method validation parameters for the determination of SAs and BLs.

Antibiotics	Spiked amounts( $\mu\text{g kg}^{-1}$ )	Mean Recoveries (%) (n = 6)	Linear Equations	$R^2$	LOD LOQ		Intra-day RSD (%) (n = 6)
					$\mu\text{g kg}^{-1}$		
<b>Sulfonamides</b>							
Sulfadiazine	5	116.3 $\pm$ 2.88	$y = 10512x + 194677$	0.9998	0.16	0.50	0.21
	10	110.0 $\pm$ 3.76					0.67
Sulfapyridine	5	104.0 $\pm$ 0.02	$y = 13552x + 139238$	0.9990	0.44	1.46	0.27
	10	102.0 $\pm$ 0.36					0.47
Sulfamethazine	5	102.4 $\pm$ 1.18	$y = 52209x + 130803$	0.9934	0.20	0.67	0.55
	10	106.0 $\pm$ 6.62					0.10
<b><math>\beta</math>-Lactams</b>							
Penicillin G	5	99.6 $\pm$ 2.63	$y = 20946x + 188429$	0.9990	0.16	0.54	0.01
	10	102.0 $\pm$ 2.85					0.20
Amoxicillin	5	94.5 $\pm$ 0.98	$y = 21770x + 170025$	0.9937	0.31	1.05	3.26
	10	98.1 $\pm$ 3.67					0.04
Ampicillin	5	91.4 $\pm$ 0.88	$y = 7316.5x + 202747$	0.9902	0.27	0.91	1.20
	10	89.3 $\pm$ 7.09					0.27

coefficient,  $R^2$ , values ranging from 0.9902 to 0.9998 (Table 1). This indicated the suitability of the calibrations for the quantification of antibiotic residues. Mean recoveries ranged from 102 to 116.3% for SAs and 89.3–102% for  $\beta$ Ls. There were no detectable antibiotic residues in the indigenous chicken samples taken from Kitui County that served as quality control samples for LOD and LOQ testing. Method LOD values ranged from 0.16 to 0.44 and 0.16–0.27  $\mu\text{g kg}^{-1}$  for SAs and  $\beta$ Ls, respectively. The LOQ values ranged from 0.50 to 1.46 and 0.54–1.05  $\mu\text{g kg}^{-1}$  for SAs and  $\beta$ Ls, respectively. The absence of any signal at the same retention time as the antibiotics indicated that there were no matrix interferences that could have resulted in a false positive signal, resulting in chromatograms that were suitable for sample analysis. The intraday precision (% RSD) for SAs ranged from 0.10 to 0.67%, while those for  $\beta$ Ls ranged from 0.01 to 3.26%.

### 3.2. Antibiotics in chicken meat samples

From the sample size of 36, 72.2% tested positive for antibiotic residues (Fig. 2). According to the results, at least one of the tested antibiotic was found in 72.2% of the samples. In addition, the samples were found to be multi-contaminated by different antibiotics. According to chicken types, 77.3, 66.7 and 62.5% of the broilers, ex-layers and indigenous chicken, respectively, were contaminated with antibiotic residues. Varying levels of prevalence of antibiotics in chicken meat have been reported [29–33], including a high detection frequency of 77.5% of antibiotic residues in chicken meat from a total of 80 samples [9]. Antibiotic residues are detected in animal products mainly due to their indiscriminate and unregulated use, and non-observance of the required withdrawal period. When used inappropriately, antibiotic residues can result in accumulation of ARB and ARGs in poultry tissues such as the liver, kidney, and muscles [34–37]. Antibiotics are known to permeate animal tissues, affecting individuals who inadvertently consume them, ranging from potential carcinogenicity to allergies, reproductive problems, mutagenic, anaphylactic shocks, teratogenic, and digestive disorders [9,38].

#### 3.2.1. Sulfonamides in chicken meat

The quantified amounts of SAs (SDZ, SPD, and SMZ) varied in the different chicken meat samples, but were generally lower than  $\beta$ Ls. All the three sulfonamides were detected in the three types of chicken samples in the order  $\text{SDZ} < \text{SMZ} < \text{SPD}$  in ex-layers and  $\text{SMZ} < \text{SDZ} < \text{SPD}$  for both broilers and indigenous chicken samples (Table 2). Therefore, SPD showed the highest mean values of 101.39, 56.61 and 39.91  $\mu\text{g kg}^{-1}$  in ex-layers, broiler and indigenous chicken meat samples, respectively, followed by SMZ in ex-layers (39.34  $\mu\text{g kg}^{-1}$ ), and SDZ in broilers (35.24  $\mu\text{g kg}^{-1}$ ) and indigenous chicken meat (42.01  $\mu\text{g kg}^{-1}$ ), respectively. Jammoul et al. [9], reported SDZ content of 17.3  $\mu\text{g kg}^{-1}$  in chicken meat, whereas in a study conducted in Tanzania by Mubito et al. [39] on chicken meat and eggs, SDZ and SPD concentrations ranged from 22 to 230 and 0.0–94  $\mu\text{g kg}^{-1}$ , respectively. Previously reported amount of SDZ ranged from 7 to 300  $\mu\text{g kg}^{-1}$  and 4–800  $\mu\text{g kg}^{-1}$  for SMZ [39–43], and up to 1640  $\mu\text{g kg}^{-1}$  for sulfonamides in general [40,43–45] in different edible chicken tissues. Therefore, amounts obtained in this study are within reported values.

The determined amounts of SAs could be due to excessive use in chicken medication. The widespread use of sulfonamides for coccidiosis treatment and prophylactic purposes is thought to be the explanation for their increasing occurrence in chicken products [39]. Also, the widespread use of pharmaceuticals, including SAs, in chicken medication may be due to the easy access by farmers, which explains the presence of residual SAs antibiotics in chicken meat.

#### 3.2.2. $\beta$ -lactams in chicken meat

Table 3 shows the amount of quantified  $\beta$ Ls in chicken meat samples. AMX was the only antibiotic found in ex-layers' meat samples, at a concentration of 124.06  $\mu\text{g kg}^{-1}$ . PEG was not found in any of the samples. Broiler meat samples, on the other hand, had AMX and AMP at a mean value of 113.36 and 102.48  $\mu\text{g kg}^{-1}$ , respectively. AMX was the sole  $\beta$ L antibiotic found in the indigenous chicken

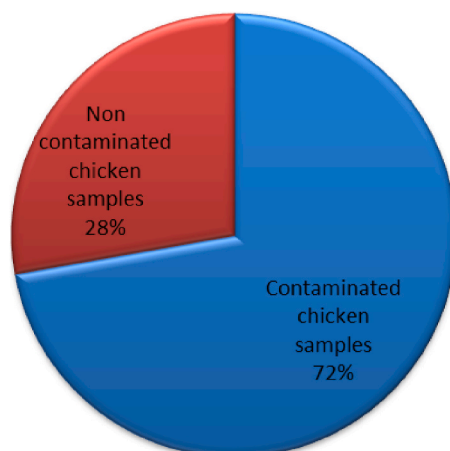


Fig. 2. Frequency of occurrence of antibiotic residues in chicken meat samples.

**Table 2**  
Sulfonamides antibiotic residues in chicken meat.

Chicken Samples (n = 36)	Concentration ( $\mu\text{g kg}^{-1}$ )	SDZ	SPD	SMZ
Ex-layers	mean	38.85		39.34
	minimum	0.14	48.40	–
	maximum	77.56	154.38	39.34
	n positive	2	101.38	1
	% Positive	5.26	5.26	2.63
Broilers	mean	35.24	56.61	13.47
	minimum	12.78	12.94	0.8
	maximum	78.12	97.12	46.52
	n positive	9	11	12
	% Positive	23.68	28.95	31.58
Indigenous	mean	27.78	39.91	4.89
	minimum	7.92	1.91	0.46
	maximum	42.01	69.93	12.14
	n positive	3	5	5
	% Positive	7.89	13.16	13.16

**Table 3**  
 $\beta$ -lactams antibiotic residues in chicken meat.

Chicken Samples (n = 36)	Concentration ( $\mu\text{g kg}^{-1}$ )	PEG	AMX	AMP
Ex-layers	mean	n.d	124.06	n.d
	minimum	n.d	21.88	n.d
	maximum	n.d	309.03	n.d
	n positive	n.d	4	n.d
	% Positive	n.d	10.53	n.d
Broilers	mean	n.d	113.36	102.48
	minimum	n.d	19.67	n.d
	maximum	n.d	229.70	102.48
	n positive	n.d	17	1
	% Positive	n.d	44.73	2.63
Indigenous	mean	n.d	145.85	n.d
	minimum	n.d	49.63	n.d
	maximum	n.d	215.50	n.d
	n positive	n.d	5	n.d
	% Positive	n.d	13.16	n.d

n.d-not detected.

samples, at a mean value of  $145.85 \mu\text{g kg}^{-1}$ . This value is lower than  $444.3 \text{ mg kg}^{-1}$  AMX found in 33 indigenous chickens in Bangladesh [46]. Jammoul & Darra [9], reported that only three out of 80 samples (37.5% frequency) tested for AMX were positive, a frequency lower than that observed in this study where 44.7% broiler samples tested positive for the compound. 26% of broiler chicken breast meat samples (n = 77) tested positive for BLs in a study by Baazize-Ammi et al. [47] in Algeria, an occurrence frequency that was lower than that obtained in the current study (Table 3).

$\beta$ Ls in chicken meat have been confirmed in previous studies;  $1.43\text{--}3.41 \mu\text{g kg}^{-1}$  AMX and  $0.51\text{--}0.53 \mu\text{g kg}^{-1}$  AMP [48] in Korea;  $522.9 \text{ mg kg}^{-1}$  (broiler meat) AMX in Bangladesh [46],  $16.92$  to  $152.62 \mu\text{g kg}^{-1}$  (liver) and  $45.38$  to  $60.55 \mu\text{g kg}^{-1}$  (breast muscle) AMX [49] in Bangladesh;  $5.20$ ,  $17.45$ , and  $7.33 \mu\text{g kg}^{-1}$  of AMX in chicken muscle, liver, and kidney, respectively [50], in China; and  $52.7$  and  $5.08 \mu\text{g kg}^{-1}$  maximum content of AMP and PEG, respectively [51], in Nigeria. As noted for sulfonamides, this may be attributed to the unregulated use of antibiotics and failure to adhere to the specified withdrawal period by farmers.

The quantified antibiotic residues (mean values) were compared with the recommend Maximum Residue Limits (MRLs) by European Union [52], (Table 4). SAs and  $\beta$ Ls have MRLs of 100 and  $50 \mu\text{g kg}^{-1}$ , respectively. The mean residue contents of SDZ and SMZ

**Table 4**  
Comparison of mean quantified antibiotic residue levels with MRLs.

Antibiotic compounds		Ex-layers	Broilers	Indigenous	MRL <sup>a</sup>
		$(\mu\text{g kg}^{-1})$			
Sulfonamides	Sulfadiazine	38.845	35.24	27.78	100
	Sulfapyridine	101.38	56.61	39.91	100
	Sulfamethazine	39.34	13.47	4.89	100
$\beta$ -Lactams	Penicillin G	n.d	n.d	n.d	50
	Amoxicillin	124.06	113.36	145.85	50
	Ampicillin	n.d	102.48	n.d	50

<sup>a</sup> (European Union, 2009).

were below the MRLs for all chicken types. For SPD, ex-layers had mean value above the MRL, while broilers and indigenous chicken samples had amounts below MRL. For  $\beta$ LS, all the three chicken sample types had mean residue values of AMX above the MRL and that of AMP in broilers. It is a common observation from different studies on antibiotic residues in chicken meat exceeding the maximum regulatory levels [40–44,51].

In this study, 17, 19 and 14 chicken samples tested positive for SMZ, SPD, and SDZ, respectively, out of 36 samples tested for SAs. Only one sample had SPD above the MRL. This concurs with study by Mehtabuddin et al. [44] that reported that 13 of 30 chicken samples tested positive for SAs, with 7 samples being above MRLs, hence a potential health risk for human consumption. Fowl typhoid, *coryza pullorum* and *coccidiosis* are all treated with SAs. SAs are quickly transported and absorbed by the chicken's body, accumulating in the chicken's edible tissues.

$\beta$ LS, TCs, MLs, SAs and aminoglycosides (AGs) were all tested in a study in Nepal [53], whereby six of the 66 samples tested exceeded the MRLs. A study conducted in Lebanon reported that three chicken samples were above the recommended MRL for AMX, none exceeded MRL for AMP, and four chicken samples exceeded MRL for PEG [9]. In the current investigation, 25 chicken samples tested positive for AMX, with 21 samples exceeding the MRL, out of the 36 chicken samples tested for  $\beta$ LS (AMX, AMP, and PEG). The affordability of the medications makes them widely available to farmers who administer them without the assistance of trained veterinary officers, which could explain the high amounts observed. This in addition to the lack of compliance with the specified withdrawal period.

### 3.3. Risk analysis associated with the antibiotic residues

The % EDI to ADI ratio in adults (70 kg BW) as well as in children (20 kg BW) was used to estimate the human health risk associated with the ingestion of chicken meat containing residual antibiotics. ADI is a standard criterion for assessing the safety of chemical contaminants in animal edible tissues.

The % ADI were <1% for adults for all SAs in broilers and indigenous chicken meat and for SDZ and SMZ in ex-layers' chicken meat (Table 5). For children, % ADI of <1% was noted for SMZ in ex-layers and broiler chicken meat, hence posed negligible risk. % ADI for SPD in ex-layers for adults; SPD and SDZ in broilers' chicken meat, SDZ in ex-layers' chicken meat, and SPD in indigenous chicken meat for children was between 1 and 5%, indicating a considerable risk to these groups. % ADI for SPD in ex-layers' chicken meat was >5% suggesting distinct risk to children.

Considering the  $\beta$ LS, all values of the % ADI were < 1% for all the antibiotics for adults and children alike, hence posed no human health risk.

Allergic reactions, long-term toxic effects from low-dose antibiotic exposure, and the development of antibiotic-resistant microorganisms in medicated animals are possible human concerns linked to antibiotic residues [6,54]. Microbiological impacts may be some of the most serious health risks for humans, and antibiotic residues found in edible tissues may cause bacterial resistance in consumers, which is one of the leading causes of therapeutic failure in such people [55]. As a result, food containing antibiotic residues may cause serious health issues, particularly in children. Dietary exposure to SAs pose potential hazards to human health. These include hazard for urinary system, cause allergic reactions (after binding with protein in human body), alteration of intestinal flora and reaction of hemopoietic system. Acetylated sulfonamide metabolin is not easily dissolved and precipitate in urine, especially in aciduria, which causes damage to kidney. SAs residues in meat may exert undesirable effect on normal flora in intestinal tract that can cause drug sensitivity to sulfonamides [56–59]. Allergic reactions have been reported in people after consuming meat containing penicillin [60,61]. Amoxicillin-clavulanate and penicillin can cause hepatitis (mainly cholestatic) [57,61,62]. As reported in a review by Arsène et al. [62], exposure to antibiotics in food (including SAs and  $\beta$ LS) can cause adverse effects including mutagenicity, reproductive disorders, teratogenic effects and carcinogenicity.

The development and dissemination of drug resistance, not residues in animal food material, is a key issue with SAs. Drug resistance to SAs is caused by mutations in the dihydropteroate synthase gene, which result in enzymes with structural alterations and decreased affinity for this antibiotic family [63]. Horizontal gene transfer in integrons, plasmids, and transposons can spread drug resistance genes between bacteria strains or genera. Multiple SAs resistance genes have been reported in bacteria, and drug resistance in one antibiotic group can cause cross-resistance in another group [64,65]. Based on the present study, it is reasonable to conclude that the potential hazards associated with the consumption of edible chicken tissues contaminated with antibiotic residues is of concern in Nairobi City.

## 4. Conclusion

The tested chicken meat samples contained varying amounts of antibiotics. With an average amount of 124.06  $\mu\text{g kg}^{-1}$ , AMX was found to be the sole antibiotic detectable in ex-layers' meat samples, whereas broiler meat samples had AMX and AMP mean content of 113.36  $\mu\text{g kg}^{-1}$  and 102.48  $\mu\text{g kg}^{-1}$ , respectively. SAs mean contents increased in the order SDZ < SMZ < SPD in ex-layers' chicken meat; SMZ < SDZ < SPD in the broiler and indigenous meat samples. SPD mean content in ex-layers was above the MRL. The average amounts of the AMX and AMP in the three types of chicken meat samples were higher than the MRLs. From the human health risk assessment, only SPD in ex-layers' chicken meat posed distinctive risk to children. For all types of chicken meat tested, AMX and AMP had % ADI <1%. Presence of SPD and SDZ posed considerable risk to both children and adults in ex-layers' chicken meat. Though not all of the antibiotic residues posed distinctive risk to consumers, some had content above MRLs, and noting the co-occurrence of antibiotics residues in same samples, their consumption is of concern due to associated health effects and antibiotic resistance. Indigenous chicken meat (locally known as Kienyeji) contained antibiotic residues, which is contrary to the popular belief that

**Table 5**  
Risk (% ADI) associated with antibiotic residues in chicken meat.

Antibiotics		% ADI					
		Ex-layers		Broilers		Indigenous	
		Adults	Children	Adults	Children	Adults	Children
SAs	SDZ	0.388	1.357	0.391	1.367	0.210	0.735
	SPD	1.544	5.403	0.971	3.399	0.699	2.448
	SMZ	0.197	0.688	0.233	0.814	0.061	0.212
βLs	PEG	n.d	n.d	n.d	n.d	n.d	n.d
	AMX	0.155	0.541	0.115	0.402	0.108	0.377
	AMP	n.d	n.d	0.051	0.179	n.d	n.d

indigenous chicken meat is safe compared to other chicken meat types. This study provides a basis for the support of continuous monitoring of antibiotic residues in meat and also provides the much needed data for addressing the concerns of antibiotics residues in food of animal origin.

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### Author contribution statement

Fredrick Odundo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Anastasiah Ngigi: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Martin Magu: Conceived and designed experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

### Data availability statement

Data will be made available on request.

### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: None reports was provided by None. None reports a relationship with None that includes: None has patent None pending to None. None.

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