SULFONAMIDES RESIDUES DETECTED IN BROILER MEAT FROM POULTRY FARMS IN PERI-URBAN, NAIROBI, KENYA

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ABSTRACT

Introduction: Poultry farmers mainly use sulfonamides to control diseases in their production systems in peri-urban, Nairobi. However, information on sulfonamides residues-level in broiler meat supplied in peri-urban Nairobi remains scanty yet the residues may be harmful to human.

Methods: This study determined sulfonamides residues in broiler meat from poultry farms in peri-urban, Nairobi. Ninety broiler thigh muscles were sampled from different farms in peri-urban Nairobi. Sulfadimidine, sulfachloropyridazine and sulfamethoxazole residues were extracted from the thigh muscles using acetonitrile/water mixture (1:1) and cleaned up on solid phase extraction cartridges. The extracted drug residues were analysed by High Performance Liquid Chromatography (HPLC) using a UV detector.

Results: Overall, sulfonamides were detected in 57.7% of the samples. The mean concentration of the sulfonamides in the samples was 0.064 ± 0.13 µg/g. The limit of detection for this analysis was 0.002µg/g with a mean recovery of between 85.4% and 95.5%. Sulfamethoxazole was detected in most samples (36.7%) while sulfachloropyridazine was the least detected (7.8%). Thirteen samples (14.4%) had sulfonamides levels exceeding the recommended Maximum Residue Limit (MRL) of 0.1µg/g. Samples with residues exceeding the MRL limit were linked to those from broilers slaughtered during drug administration (Chi square value 3.97, $p < 0.05$).

Conclusions: The study found that broiler meat produced in peri urban Nairobi contains sulfonamide residues in levels that may be harmful to human health. We recommend enforcement of the existing laws to mitigate harmful effects of the drug residues.

Keywords: sulfonamides, broiler meat, residues, peri-urban.

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INTRODUCTION

Commercial poultry farming is widely practiced worldwide and is a source of animal proteins in form of meat and eggs. Broiler farming is known to provide an alternative source of animal protein substituting red meat especially beef and mutton (Mehtabuddin et al., 2012). Broiler farming is a lucrative venture worldwide because of the quest for white-meat and the faster growth of broilers as compared to beef cattle and sheep. In Kenya, the poultry sector contributes about 55% to the livestock sector and 30% of the agricultural Gross Domestic Product (GDP) (Republic of Kenya, 2008). Peri-urban Nairobi has the highest number of chickens per household as compared to the other peri-urban areas such as Kisumu, Nakuru and Mombasa (Okello et al., 2010). Sanitary risks such as lack of biosecurity and poor hygiene practices have been reported to be a major problem for poultry farmers in peri urban Nairobi (Carron et al., 2018).

The sanitary risks mentioned above usually results in high prevalence of infectious diseases necessitating the indiscriminate usage of antimicrobial drugs for the treatment and management of bacterial as well as coccidian parasites. Indeed, a previous study on antimicrobial usage revealed that each year approximately 14,600kg of active antimicrobials are used in food animal production in Kenya with the use of tetracycline and sulfonamides-trimethoprim combination accounting for approximately 78% (Mitema et al., 2001). Sulfonamides are mostly used in the poultry production either for treatment and control of poultry diseases or for growth-promotion (Doyle, 2006). Sulfonamides are a class of broad spectrum antimicrobial agents active against most gram positive and gram negative bacteria as well as coccidian parasites (Löhren *et al.*, 2009; Mor *et al.*, 2012). Recently, we found that most poultry farmers in peri-urban Nairobi use sulfonamides to manage diseases caused by bacteria and coccidian parasites (Muthuma et al., 2016). The indiscriminate use of these drugs including failure to observe withdrawal periods after administering the antimicrobial drugs is known to result in detectable residues in chicken eggs and meat (Tajick and Shohreh, 2006).

The problem with antimicrobial residues including sulfonamides in broiler meat is that they may cause adverse health effects such as allergy or analphylactic reactions (Reig and Toldrá, 2008) and cancer (Pensabene *et al.*, 1997; Goetting *et al.*, 2011) compromising food safety. The residues may also contribute to the emergence of resistance to the drugs by a range of bacteria (Shao *et al.*, 2005; Mor *et al.*, 2012). Subsequently, Codex Alimentarius Commission(CAC) and the European Union (EU) have set the Maximum Residue Level (MRL) for sulfonamides in poultry products at 0.1µg/g in order to protect public health against harmful effects of sulfonamides residues (Sasanya *et al.*, 2005; Malik *et al.*, 2013). Recently, it has been reported that sulfonamides class of antimicrobials are the most commonly used drug by poultry farmers in peri-urban, Nairobi for controlling diseases in broiler production systems (Muthuma *et al.,* 2016). Nonetheless, there is scanty information about current data on the levels of sulfonamides residues in broiler meat supplied in peri-urban Nairobi yet the residues may have serious harmful effects on human health especially if the levels exceed the recommended MRL. Unlike, the developed countries, such as the USA, Japan and EU, where regular strict surveillance measures for monitoring sulfonamides residues in poultry products have been done regularly to safeguard human health (Donoghue, 2003; Salehzadeh *et al.*, 2006; Passantino and Russo, 2008; Reig and Toldrá, 2008; Sirdar, 2010), the same is generally not the case in developing countries including Kenya (Sirdar *et al.*, 2012).

Therefore, this study was done to determine sulfonamides residues in broiler meat from poultry farmers in peri-urban, Nairobi. The current findings of this study may form a basis for recommending relevant food safety measures for mitigating the harmful health effects of the residues to humans.

MATERIALS AND METHODS

Study area and sample collection

The study area included Njiru and Karasani sub counties situated in peri-urban areas of Nairobi City, Kenya. Kasarani and Njiru are located towards North Eastern and Eastern side of Nairobi, respectively**.** These sub counties were purposely selected because they have high numbers of poultry farmers rearing broilers. Questionnaires were administered to the broiler farmers as described previously (Muthuma et al., 2016). Thereafter, a total of 90 broiler thigh samples were sampled between October and December 2014. The broiler-carcasses were serially numbered and one carcass sampled randomly from the batch. A thigh muscle was detached from each carcass sampled and then packed in a 40 micron polythene bag. Three broiler meat samples were collected per farm on three different occasions during the study period. The packed samples were labeled and transported, in cool boxes with ice parks, to the Laboratory at the Public Health, Pharmacology and Toxicology of the University of Nairobi. The samples were stored at -20^{0}C pending analysis.

Materials and Chemicals

The materials used were Solid Phase Extractor (SPE)-strata X C18 (Phenomenex, U.S.A), analytical column Luna 5 C18-100A (Phenomenex, U.S.A), Filter paper 0.45µm (Sigma Aldrich, Germany), syringe filter 0.2µm Acrodisc 13 (PALL, U.K) and Glass Pasteur pipette 150mm (Germany).

Sulfadimidine, sulfachloropyridazine and sulfamethoxazole standards were purchased from Sigma Aldrich, Germany. The other chemicals used for the residues analysis included Potassium dihydrogen phosphate (Sigma Aldrich, Germany), methanol HPLC grade (V.W.R Chemicals, France), Deionized water (Total, Kenya) and Acetonitrile HPLC grade (Pancreac, Spain).

Extraction and clean-up of Sulfonamides Residues

Sulfonamides residues were extracted from the broiler meat and cleaned-up using a method described previously with slight modifications (Alawi et al., 2014). Briefly, the frozen meat samples were thawed overnight, cut into small pieces and 10g of the cut pieces homogenized in 10ml of deionized water for 5 minutes using a stomacher (Stomacher 400 Lab Blender, Seaward, UK). Thereafter, 1 ml of acetonitrile was added into 0.5 g of the homogenate in a test-tube, and the mixture kept at room temperature for 10 minutes followed by vortexing for 2 minutes. The vortexed mixture was centrifuged at 5,000 rpm for 10 minutes and the initial supernatant recovered in a test tube. The remaining sediment was re-extracted with 1ml of deionized water/acetonitrile (1:1), centrifuged as outlined above and the second supernatant recovered. The initial extract was pooled with the second extract and then evaporated in a sand bath at 45^0C followed by dissolution of the residue in 2ml of 0.01M KH_2PO_4 (PH 3.9). The dissolved residue was cleaned-up using SPE column fitted with C18 cartridge column (Strata C18-E). Sulfonamide residues were eluted from the column using 2ml of methanol. The recovered eluent was evaporated in sand bath at 45^0C, reconstituted with 500µl of acetonitrile/water (1:1) and then filtered with disposable syringe filter 0.2µm Acrodisc into High Performance Liquid Chromatography (HPLC) auto sampler vials for subsequent analysis by HPLC (Shimadzu, Japan).

Validation of the High Performance Liquid Chromatography method

To obtain reliable data, the HPLC assay was validated by determining linearity, selectivity, accuracy, Limit of Detection (LOD), precision and Limit of Quantification (LOQ). Linearity was determined by constructing standard curves for each sulfonamide analysed using concentrations of 0.08, 0.2, 0.6, 1.0, 2.0, 4.0, 6.0 and 8.0µg/g prepared from 100 µg/g of each sulfonamide standard. Linearity was eventually obtained by regression analysis of the peak area against analyte concentrations by obtaining the linear regression coefficient (R^2). The absence of interfering peaks, which is an indicator of assay selectivity, was determined using sulfadimidine, sulfachloropyridine and sulfamethoxazole standards each at 4µg/g. The mean recovery of the assay was obtained by spiking residues-free broiler meat with each of the sulfonamide standards at concentrations of 0.1, 2.0, 6.0, 8.0 and 10.0µg/g. The precision was also determined using the standards at concentration of 0.10µg/g. A linear equation was used to determine the recoveries after residues extraction from the spiked samples. The LOD and LOQ were determined using a method described previously by Ravisankar and Devala (2013).

Analysis of meat samples for Sulfonamides residues

The sulfonamides residues extracted from the broiler meat samples were analysed using HPLC equipped with column oven, SPD-20A variable wavelength UV-Vis detector and reverse phase C-18 150mm x2.4mm x 4.0m column (Shimadzu, Japan). Twenty microliters of each sample and sulfonamides standards were injected into Auto-sampler of HPLC. Thereafter, the analysis was done at 30^{o}C using 0.01M potassium dihydrogen phosphate buffer and acetonitrile at 70:30 v/v as mobile phase with flow rate of 1.0 ml/min. Standard curves were derived from the peak areas and then used to calculate the quantities of sulfonamide residues in the samples.

Data Analysis

Proportions of samples with sulfonamides residues were determined using Microsoft Excel. A chi-square was done to determine the association between failure to observe drug withdrawal period and level of sulfonamides residues in broiler meat above the acceptable MRL using Insta+v3.36.

RESULTS

Results of HPLC assay validation

The precision of the assay was determined by obtaining the mean recoveries in the samples spiked with sulfonamides standards at 0.1µg/g. The mean recoveries for the drugs were 98.1%, 85.4% and 94.1% for sulfadimidine, sulfachloropyridazine and sulfamethoxazole respectively (Table 1). The linear calibration curves for sulfadimidine, sulfachloropyridazine and sulfamethoxazole revealed regression coefficient (R^2) of 0.9973, 0.9974 and 0.9970 respectively. These values were more than the acceptable limit of 0.99 indicating that the linear calibration lines were suitable for quantifying the sulfonamide residues in broiler meat (Fig 1 A-C). The limits of detection and limits of quantifications for the assay were 0.001µg/g and 0.003µg/g respectively. The assay sufficiently separated the three sulfonamides yielding chromatograms with retention times of 5.560 minutes, 7.130 minutes and 8.425 minutes for sulfonamidine, sulfachloropyridazine and sulfamethoxazole respectively. All the peaks for the three sulfonamides did not have interfering peaks indicating that the assay was selective (Fig 2).

Table 1. The percent (%) mean recoveries of the modified HPLC assay obtained using broiler meat spiked with the sulfonamides

Sulfonamide	% Mean Recovery ± SD
1. Sulfadimidine	98.1 ± 3.3
2. Sulfachloropyridazine	85.4 ± 1.3
3. Sulfamethoxazole	94.5 ± 4.3

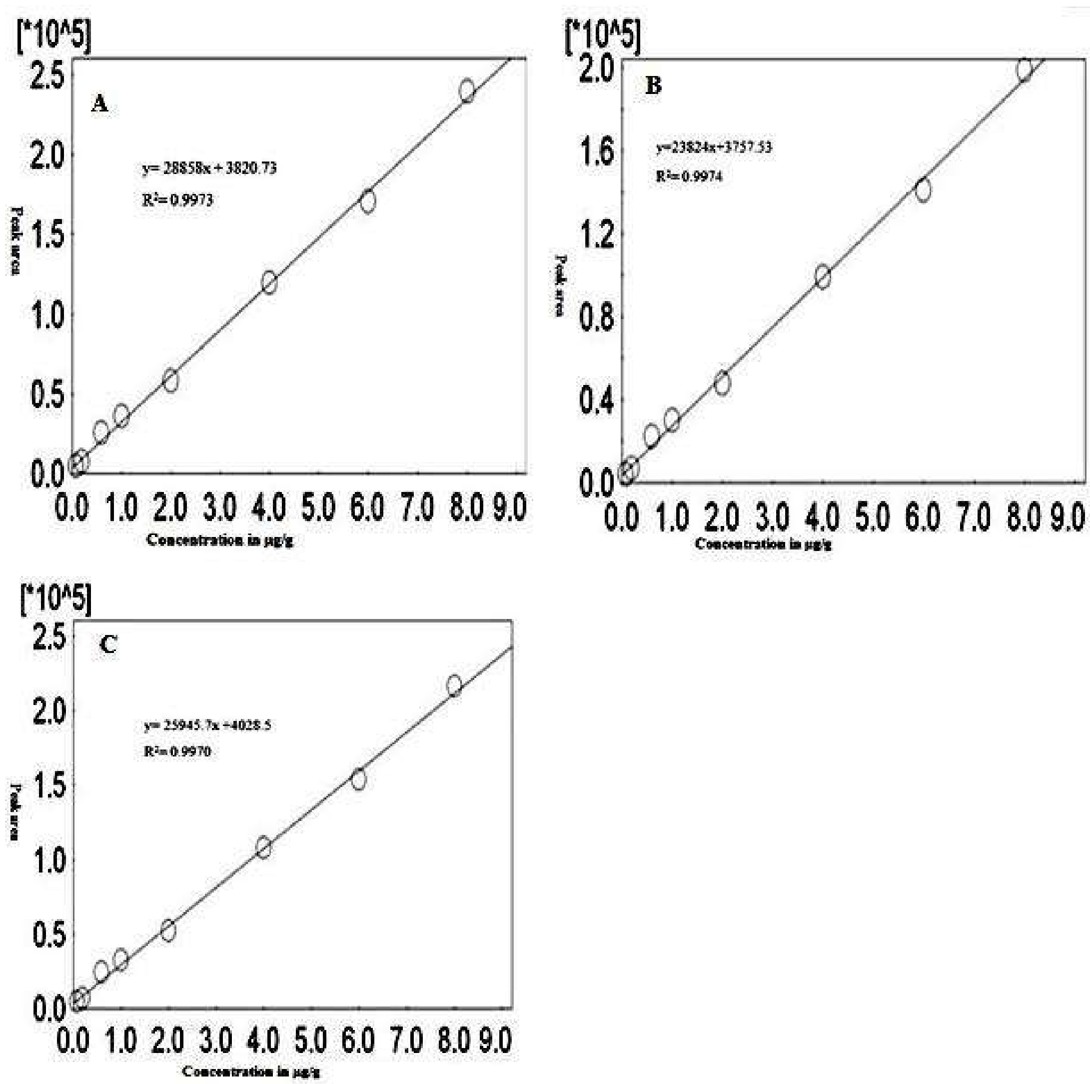

Figure 1. Linear calibration curves generated after analysis by HPLC using sulfonamides standards. A. Sulfadimidine; B. Sulfachloropyridazine; C. Sulfamethoxazole.

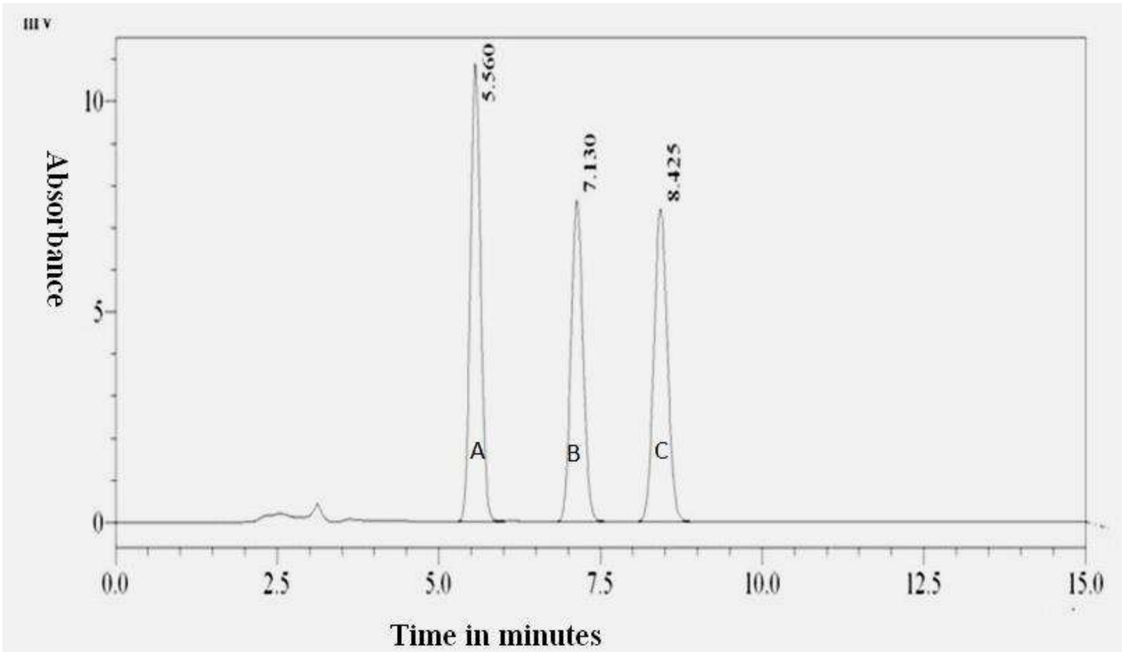

Figure 2. The chromatogram of (A) sulfadimidine, (B) sulfachloropyridazine and (C) sulfamethoxazole standards with their retention times shown on corresponding peaks.

Sulfonamides residues detected in broiler meat

Most broiler farmers in peri-urban Nairobi have been reported to misuse sulfonamides during treatment of diseases caused by bacteria and coccidian parasites (Muthuma et al., 2016). Therefore, we hypothesized that the misuse may lead to sulfonamide residues in broiler meat intended for human consumption. To confirm this hypothesis, the validated HPLC assay was used for the detection and quantification of sulfadimidine, sulfachloropyridazine and sulfamethoxazole residues in broiler meat sampled from poultry farms in peri-urban Nairobi. Overall, the sulfonamide residues were detected in 52(57.7%) broiler meat samples while the other 38 (42.3%) samples did not contain the sulfonamide residues. In particular, 33 (36.7) samples had sulfamethoxazole residue while sulfadimidine was detected in 22 (24.4%) samples. Sulfachloropyridazine residue was detected in 7 (7.8%) meat samples only (Table 2). Five meat samples contained both sulfamethoxazole and sulfadimidine residues while 4 samples contained sulfachloropyridazine and sulfadimidine residues. Only one sample contained sulfachloropyridazine and sulfamethoxazole residues in the broiler meat samples analysed.

The broiler samples with detectable levels of the sulfonamides residues were analyzed further to establish whether the levels were above the recommended MRL. Overall, 13 (14.4%) samples had sulfonamides residues above the acceptable MRL (0.1µg/g), recommended by the CAC and the EU. Of these samples, 12 (13.3%) had sulfamethoxazole residues above MRL while 1 (1.1%) sample only had sulfachloropyridazine residues above the recommended level. On the other hand, all the sulfadimidine residues detected in the meat samples were below the acceptable MRL (Table 2). The detection of sulfonamides residues in broiler meat in levels above the acceptable MRL appeared to be associated with failure to observe sulfonamides withdrawal period (Chi square value 3.97, $p < 0.05$). In particular, the risk of broiler meat containing the residues above the recommended MRL was six times more when the broiler farmers failed to adhere to the sulfonamides withdrawal period during treatment (Odds Ratio = 6.0). Out of the 90 samples analyzed, 10 (11.1%) samples contained residues comprising of sulfamethoxazole/sulfadimidine, sulfamethoxazole/sulfachloropyridazine or sulfachloropyridazine/sulfadimidine combinations. However, most of the 10 samples contained residues below the recommended MRL with only 3 (30%) samples having residues combinations above the MRL.

Table 2. Sulfonamides residues detected in broiler meat produced in peri-urban Nairobi

	Sulfamethoxazole	Sulfadimidine	Sulfachloropyridazine
1. Number (%) of samples with detectable drugs	33(36.7)	22 (24.7)	7(7.8)
2. Overall range (µg/g)	0.023 - 0.456	0.004 – 0.069	0.003 – 0.325
3. Number (%) of samples with levels > MRL	12(13.3)	0(0)	1(1.1)
4. Overall range (µg/g)	0.148 – 0.456	N/A	N/A

N/A; no range for 0 value/single values

DISCUSSION

Sulfonamides are widely used in the poultry production systems either for management of poultry diseases or for growth-promotion (Doyle, 2006). Recently, it has been reported that poultry farmers in peri-urban Nairobi indiscriminately use sulfonamides for the treatment and management of poultry diseases (Muthuma et al., 2016). Inadequate biosecurity and poor hygiene practices has been reported in poultry farms in peri-urban Nairobi (Carron et al., 2018) and this is believed to be responsible for indiscriminate use of sulfonamides for managing bacterial and parasitic coccidian diseases, which are prevalent in the peri-urban farms (Muthuma *et al.,* 2016). The indiscriminate use of the veterinary drugs including failure to observe withdrawal periods is known to result in harmful detectable residues in eggs and meat of chicken (Tajick and Shohreh, 2006). Therefore, this study determined residues of sulfonamides in broiler meat from poultry farms in peri-urban, Nairobi by using HPLC method.

Several analytical methods including HPLC have been used for analysis of sulfonamide residues in poultry meat and eggs (Wang et al., 2007). Similarly to the previous reports, the HPLC assay used in this study yielded linear calibration curves with regression coefficient (R^2) greater than the acceptable limit of 0.99 indicating that the linear calibration could be used for the quantification of sulfonamide residues in broiler meat. The limits of detection and limits of quantifications for this assay were 0.001µg/g and 0.003µg/g respectively well below the recommended MRL of 0.100 µg/g for sulfonamides confirming the suitability of using the method for regulatory purposes.

Overall, 52 (57.7%) of broiler meat samples analyzed in this study had sulfonamides residues similarly to the other previously reported studies in Tanzania (Nonga et al., 2010; Mubito et al., 2014). To the best of our knowledge, this is the first study to detect sulfonamides residues in broiler meat in Kenya since the last three decades. The only other recent study that detected sulfonamides residues in eggs is that reported by Azegele (2010). This study detected a higher percentage of sulfonamides residue in eggs contrary to our finding for chicken meat. In Pakistan, 42.1% of poultry meat samples analyzed had sulfonamides residues (Mehtabuddin., et ., 2012), a value lower than that reported in the current study. Muthuma et al. (2016) has reported the indiscriminate use of sulfonamides for the treatment and management of poultry diseases in peri-urban Nairobi; a finding that may explain the high occurrence of sulfonamides residues in poultry meat. Indeed, the high occurrence of sulfonamides residue in poultry products have previously reported in many developing countries including Uganda, Kenya, Sudan and Malaysia (; Sasanya *et al.*, 2005; Stolker *et al.*, 2007; Sirdar, 2010; Sirdar *et al.*, 2012c; Malik *et al.*, 2013). In Kenya, this finding can be explained by the wide spread use of sulfonamides in poultry production systems (Mitema *et al.*, 2001). The sulfonamides tested in this study are not strictly regulated in Kenya by government agencies and therefore are readily available for use by farmers in the poultry production system. If this is not addressed, sulfonamides are likely to be misused continuously resulting in the presence of the drug residues in poultry products.

Of the samples with sulfonamides residues above the acceptable MRL (0.1µg/g), most had sulfamethoxazole residues with one sample only having sulfachloropyridazine residues above the recommended level. This finding has significant public health implication because sulfamethoxazole is also widely used in combination with trimethoprim in Kenya to manage secondary bacterial infections in patients infected with human immunodeficiency virus (HIV) (Polyak et al., 2016). Exposure to sulfamethoxazole residues after consumption of contaminated poultry meat is critical because the same drug is also used in human medicine and this can potentially result in bacterial resistance to the drugs.

Previously Mubito et al (2014) suggested that failure to observe antimicrobial withdrawal after administration is responsible for high occurrence of residues in poultry products. This claim appears to have been corroborated by the current study in which the risk of broiler meat having the residues above the recommended MRL was higher (Odds Ratio = 6.0) when the broiler farmers did not adhere to the recommended sulfonamides withdrawal period during treatment. In the developed countries, such as the USA, Japan and EU, the use of sulfonamides in poultry production have been banned in order to safeguard human health (Donoghue, 2003; Salehzadeh *et al.*, 2006; Passantino and Russo, 2008; Reig and Toldrá, 2008; Sirdar, 2010). This is contrary to many developing countries including Kenya where sulfonamides are still widely used and even misused during poultry production (Mubito et al., 2014; Muthuma et al., 2016). The inadequate enforcement of legislations and regulations for the use of veterinary drugs as well as weak systems for monitoring residues in food products in Kenya may have also contributed to the presence of sulfonamides residue in poultry meat. In fact, other researchers have advocated for enforcement of the laws by regulatory agencies in developing countries in order to safeguard the consumer from exposure to the harmful drug residues in food ((Mubito et al., 2014; Sattar et al., 2014). Therefore, we also suggest creation of awareness of poultry farmers in Kenya with regard to strict adherence to the laws and regulations detailing judicious use of veterinary drugs including antimicrobials.

Ten (11.1%) broiler meat samples analyzed in this study had combinations of residues comprising of sulfamethoxazole/sulfadimidine, sulfamethoxazole/sulfachloropyridazine or sulfachloropyridazine/sulfadimidine. Apart from the sulfonamides detected in the current study, it is possible that broiler meat produced in peri-urban Nairobi may also contain other veterinary drug residues. Indeed, a recent study has reported the use of tetracyclines and amprolium hydrochloride by the poultry farmers in peri-urban Nairobi (Muthuma et al., 2016). Nevertheless, more elaborate large scale studies are required to confirm the claim above and unravel the extent of the problem not only in peri-urban Nairobi but also in other parts of the country.

CONCLUSION

This study has demonstrated that broiler meat produced by poultry farmers in peri-urban Nairobi contain sulfonamides residues above the recommended MRL indicating that the consumers may be at high risk of exposure to dangerous levels of the drug residues in poultry meat. We also conclude that failure to observe the drug withdrawal period by the farmers may have contributed to the presence of the drug residues in broiler meat; nonetheless, a further study is still required to give more elaborate data. Therefore, we recommend enforcement of laws and biosecurity measures that discourage extensive use of antimicrobial drugs in the poultry production systems in Kenya and other developing countries.

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