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Sulfonamide residues in commercial layer chicken eggs in Dar-es-Salaam, Tanzania

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ABSTRACT

Sulfonamides are used in poultry production for prevention and treatment of bacterial and protozoal infections. Misuse of these drugs or poor adherence to drug withdrawal period has resulted in reports of unacceptable residue levels in poultry products from many developing countries. This study investigated sulphonamide residues in chicken eggs sold in Dar-es-Salaam, which is the biggest consumer of poultry pruducts in Tanzania. A total of 96 eggs randomly collected from commercial layer chicken production farms in Dar-es-Salaam were analysed for sulfadiazine and sulfamethazine residues by use of High Performance Liquid Chromatography (HPLC). Extraction of residues from eggs was executed using liquid-liquid extraction in acetonitrile. The quantification of residues was achieved by using the UV detector at 265 nm and C_{18} column; (25 cm x 4.6 mm x12 µm). The mobile phase composed of 10 mM potassium di-hydrogen phosphate buffer. Results indicated that all analysed samples contained sulfadiazine while 59.4% contained sulfamethazine residues. Out of the 96 positive samples which were positive for sulfadiazine residues, 28 (29.2%) contained residues above the maximum residues limit (MRL). However, sulfamethazine residues detected in 54% of eggs were all below the MRL. These finding suggest that egg consumers in Dar-es-Salaam are at a risk of exposure to sulfonamide residues.

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INTRODUCTION

Sulfonamides are a group of synthetic antibiotics with broad spectrum effects against most Gram positive, Gram negative bacteria and protozoa (Löhren *et al.*, 2009; Mor *et al.*, 2012). They are frequently used in the poultry industry for therapeutic, prophylactic, or growth-promoting purposes (Doyle, 2006). Sulfonamides are also used to treat various types of infections in digestive and respiratory tracts (Furusawa and Hanabusa, 2002).

The widespread use of sulfonamides as a result of their availability and low cost has resulted in considerable increase in resistant bacteria strains for these compounds (Van den Bogaard *et al.*, 2001). Also the extensive application of this antimicrobial agent in chicken production have resulted in residues being detected in poultry products (eggs and meat) when adequate withdrawal periods have not been observed (Mor *et al.*, 2012; Sirdar *et al.*, 2012c). It is well established that consumption of animal products containing sulphonamide residues poses potential human health risks which include hypersensitivity or analphylactic shock (Reig and Toldrá, 2008), cancer (Pensabene *et al.*, 1997; Goetting *et al.*, 2011) and induction of bacterial resistance to the antimicrobials (Shao *et al.*, 2005; Mor *et al.*, 2012), amongst other risks.

Therefore to safeguard public health against threats posed by antimicrobial residues, the MRL for sulfonamides in poultry products have been established at 100 µg/kg by Codex Alimentarius commission and the EU (Sasanya *et al.*, 2005; Malik *et al.*, 2013). In developed countries, such as the USA, Japan and EU, regulations and strict monitoring measures on the use of antimicrobials have been established (Donoghue, 2003; Salehzadeh *et al.*, 2006; Passantino and Russo, 2008; Reig and Toldrá, 2008; Sirdar, 2010). But in developing countries such strict regulations are not in place or are not enforced (Sirdar *et al.*, 2012).

In Tanzania, legislations regarding antibiotic drug application in food producing animals as well as monitoring and control of their residues are not adequately enforced (Nonga *et al.*, 2009). Poor enforcement of these regulations may explain the reported high prevalence of antimicrobial residues in poultry products (Kurwijila *et al.*, 2006; Nonga *et al.*, 2009; Nonga *et al.*, 2010). However, all the latter studies employed qualitative assays which indicate presence of antimicrobials but could not establish whether the residues exceed the MRL. This study

therefore was designed to provide quantitative information on sulfonamide residues in eggs collected in Dar-es-Salaam, Tanzania.

MATERIALS AND METHODS

Study area

This study was conducted in Dar-es-Salaam which is the largest city in Tanzania and the major centre for commercial layer chicken production (Msami, 2008). Within the city, there are numerous fast food vendors along the streets, in Bars and Hotels whose menu is mainly comprised of chicken meat and eggs. The study included the three administrative districts in Dar-es-Salaam region namely Kinondoni, Ilala and Temeke.

Sample collection

The study farms were purposively selected based on keeping commercial layer chickens and consent to participate in this study. The study sites included Kitunda, Kipunguni, Chanika and Tabata/segerea in Ilala District; Kigamboni, Mbagala and Mtonikijichi in Temeke District, and Wazo Hill, Kiluvya and Kibamba in Kinondoni District. From each of the selected sites, 10 randomly selected egg producers were visited and two eggs were randomly collected from each farm and labelled. The egg samples were then transferred under cold condition to the Tanzania Food and Drug Administration Agency (TFDA) Laboratory in Dar-es-Salaam for analysis.

Sulphonamide extraction

Sulphonamide residues were extracted from eggs using the liquid-liquid extraction method as previously described (Sasanya *et al.*, 2005; Mehtabuddin *et al.*, 2012). Five millilitres (mL) of pre-homogenized whole eggs, one mL potassium di-hydrogen phosphate and 20 mL of acetonitrile were homogenized for 20 seconds after vigorous mixing by vortexing. The

homogenate was centrifuged at 1750 (×g) for 15 minutes (using HettichRotofix 32A). The supernatant was transferred into a 25 mL flask, the pellet loosened and re-homogenized with 10 mL acetonitrile and the mixture was re-centrifuged. The second supernatant was added to the first and the mixture evaporated to dryness on a rotary evaporator (BUCHI rotavapor R-215), aided by heating bath (B-491) and vacuum controller (V-855) at 35ºC and 183 bar. Eight mL of n-hexane and one mL of mobile phase were then used to dissolve the residues. The dissolved residues were re-centrifuged at 1750 (×g) for 15 minutes after vigorous vortexing. The upper hexane layer was drawn off using Eppendorf micropipette and discarded. One gram of anhydrous NaCl was added to the lower aqueous layer and the mixture was vortexed and re-centrifuged. The aqueous layer was then filtered through disposable Whatman 0.45 µm nylon filters into amber coloured HPLC vials, which were transferred to HPLC auto sampler vial rack for HPLC analysis.

Sample analysis using HPLC

The standards and samples analysis was conducted using HPLC system (SHIMADZU, Japan) equipped with HPLC pump (LC-20AT), vacuum degasser (DGU-20A5), auto sampler (SIL-10AF), communicator bus model (CBM-20A), column oven (CTO-10AS VP) and diode array detector (SPD-M20A). The Supelcosil C-18 column; (25 cm x 4.6 mm x12 µm) was used for the separation of sulfadiazine and sulfamethazine. The mobile phase composed of 10 mM potassium di-hydrogen phosphate buffer (adjusted the pH to 3.05 with phosphoric acid) with 0.01% triethylamine and methanol. The flow rate of mobile phase was set at 1.2 mL/min. A 20 μL volume of sample was injected in the column at 35 ºC, and peaks were detected at 265 nm. The LC-run time was 13 minutes and the retention time for sulfadiazine and sulfamethazine were 5.514 and 8.848 minutes, respectively. A standard calibration curve was obtained by running sulfonamide standard solutions (in triplicate) on HPLC and then plotting peak areas against concentrations in ng/g. Sulfadiazine and sulfamethazine standard concentrations ranged from 50, 100, 200, 300, to400 ng/mL. The best fit of line was calculated by equation of line. Linearity was evaluated through the correlation coefficient. The quantification of residues in samples was carried out by using the standard curve calibration based on peak area toward concentration. The limits of detection as based on recoveries of spiked concentrations were 20

ng/g for sulfadiazine and 25 ng/g. for sulfamethazine. The percentage recoveries of sulfadiazine and sulfamethazine are indicated in Table 1.

Table 1: Average recovery of sulfadiazine and sulfamethazine at different spiking concentration levels of eggs

Spiked concentration (ng/mL)	Recovery (%)	
	Sulfadiazine	Sulfamethazine
50	65-70	60-70
100	78-85	75-82
300	86-92	80-89

RESULTS

The quantitative results of 96 analysed eggs indicated that all samples had detectable levels of sulfadiazine and 59.4% of the samples had detectable levels of sulfamethazine residues. Sulfadiazine residues ranged 22-230 ng/g (mean, 94.3 ng/g) while sulfamethazine residue ranged from 0.0 – 94 ng/g (mean, 28.8 ng/g). Out of the 96 eggs sampled, 28 (29.2%) contained sulfadiazine residues at levels (103 – 230 ng/g) exceeding the MRL of 100 ng/g set by Codex. None of the samples had sulfamethazine concentrations exceeding the MRL. The prevalence and levels of sulfadiazine and sulfamethazine residues in samples from all the locations surveyed are shown in Table 2and Table 3, respectively.

Table 2:Prevalence and levels of sulfadiazine residue in poultry eggs

Location	Number of samples	Samples with detectable levels (%)	Overall range (ng/g)	Samples exceeding MRL (%)	Range in samples exceeding MRL (ng/g)
Kitunda	9	9.4	29.5 - 135.4	3.1	128.6 - 135.4
Tabata	10	10.4	22.5 - 117.3	2.1	103.3 - 117.3
Kipunguni	9	9.4	26.1 - 156.1	2.1	131.2 - 156.1
Chanika	9	9.4	36.7 - 138.1	1.0	138.1
Kiluvya	9	9.4	83.4 - 151.4	3.1	133.2 - 151.4
Kibamba	10	10.4	38.6 - 131.3	1.0	131.3
Tegeta	10	10.4	68.9 - 130.4	2.1	118.2 - 130.4
Mbagala	10	10.4	92.2 - 151.7	8.3	117.6 - 151.7
MtoniKijichi	10	10.4	72.7 - 143.6	4.2	107.6 - 143.6
Kigamboni	10	10.4	24.3 - 230.2	2.1	131.9 - 230.2

MRL:Maximum residue limits

Table 3: Prevalence and levels of sulfamethazine residue in poultry eggs

Location	Number of samples	Samples with detectable levels (%)	Overall range (ng/g)	Samples exceeding MRL (%)	Range in samples exceeding MRL (ng/g)
Kitunda	9	7.2	0.0 – 64.1	-	-
Tabata	10	8.3	0.0 – 88.9	-	-
Kipunguni	9	2.1	0.0 - 56.2	-	-
Chanika	9	5.2	0.0 – 51.5	-	-
Kiluvya	9	3.1	0.0 – 43.6	-	-
Kibamba	10	6.3	0.0 – 77.7	-	-
Tegeta	10	2.1	0.0 - 28.8	-	-
Mbagala	10	9.4	0.0 – 94.3	-	-
MtoniKijichi	10	6.3	0.0 – 64.3	-	-
Kigamboni	10	9.4	0.0 – 91.4	-	-

MRL:Maximum residue limits

DISCUSSION

In this study, 96 eggs randomly collected from layers production farms in Dar-es-Salaam were examined for sulfonamide residues by using the HPLC. Results show that all the analysed eggs contained sulfadiazine residues and 59.4% contained sulfamethazine residues. Widespread use of sulfonamides for treatment of coccidiosis and prevention of infectious diseases is the likely reason for the high prevalence of sulfonamides in livestock and poultry products in Tanzania (Nonga et al., 2009; Nonga et al., 2010; Mubito et al., submitted). High prevalence of sulfonamide residues was also reported in other countries including Uganda, Kenya, Sudan and Malaysia and other developing countries (Mitema *et al.*, 2001; Sasanya *et al.*, 2005; Stolker *et al.*, 2007; Sirdar, 2010; Sirdar *et al.*, 2012c; Malik *et al.*, 2013). Since there are no any cheaper alternatives for sulphonamides, it is likely that residues of these compounds will continue to be found on poultry products because of their popularity (Pensabene *et al.*, 1997;Mor *et al.*, 2012). In view of the public health concerns associated with sulfonamides, there is need for regulatory authorities to intervene by tightening the law enforcement, regular screening program and penalize whoever found selling eggs or other foods of animal origin which contains antibiotic residues exceeding the regulatory limit.

A considerably large proportion of eggs (29.2%) contained sulfadiazine levels which exceeded the MRL. This is higher than what was reported for other developing countries such as Pakistan (10%) and Uganda (5%). Based on responses obtained using questionnaire survey on chicken

farmers in Dar-es-Salaam from where the egg samples were collected for analysis (Mubito *et al.*, 2014, submitted), failure to observe withdrawal periods may be behind the observed high prevalence of residues exceeding the MRL. All egg producers in Dar-es-Salaam admitted not to observe the drug withdrawal period for fear of incurring investment losses. This was verified by the observed highest proportion of eggs containing sulfadiazine levels above the MRL in Mbagala and Kigamboni. At the time egg samples were being collected for this study, sulfonamides were being used to contain an outbreak of coccidiosis in these areas. Another possible reason for presence of eggs containing antibiotics above MRL may be the incorrect drug administration. Due to inadequacy in the enforcement of good veterinary practices, most farmers in Dar-es-Salaam admitted not to apply antibiotics based on manufacturer's instructions or prescription by Veterinarians. Instead, they relied on instructions issued by drug vendors, most of whom are not qualified to do so. Other farmers relied on past experience to administer antibiotics, although drug formulations keep changing from time to time. These malpractices are likely to lead to overdose or overuse all culminating into residues above MRL.

Residues exceeding MRL were detected for sulfadiazine but not sulfamethazine. This was unexpected because both formulations are used in Dar es Salaam. The difference in chemical structure between the two sulfonamides and the consequent rate of elimination from the body may explain the differences. It was previously reported that sulfamethazine (sulfadimidine) is ten times more soluble in the free and five times more soluble in the acetylated form than sulfadiazine (Volini *et al.*, 1945). Therefore, it is rapidly absorbed into digestive tracts of hens and most rapidly excreted from plasma and tissues than sulfadiazine (Furusawa, 2002; Tansakul *et al.*, 2007). The rapid elimination may explain the comparatively lower residual levels of sulfamethazine detected in eggs.

CONCLUSION

Results from this study clearly demonstrate that egg consumers in Dar-es-Salaam are at high risk of exposure to high and non-tolerable drug residues in eggs. Overcoming drug residues in poultry products will need the involvement of consumers, the producers and the regulatory bodies. The consumers need to be aware of the public health consequences of drug residues in

food and receive assurance on the safety from regulatory authorities and producers. Producers must be aware of legal and public health consequences of the product they produce and strive to use good management practices and good veterinary practices. The regulatory authorities should enforce the laws enacted to safeguard the safety of consumers and if possible come up with new laws/regulations to plug the gaps. Furthermore, they must also address the factors that compel producers to disregard the existing laws.

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