Antibiotic resistance of *Salmonella* isolated from commercial chicken feeds in Dar es Salaam, Tanzania

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SUMMARY

Salmonella resistance to antimicrobials is rapidly growing worldwide. Antibiotics are commonly used in prevention of bacterial infections as well as treatment of infected chickens in Tanzania. A study on *Salmonella* was conducted in commercially produced chicken feeds from feed mills in Dar es Salaam, Tanzania, between May 2015 and June 2016 with the objective of estimating the prevalence of *Salmonella* contamination and their antibiotic susceptibility patterns. Feed samples were collected from a total of 384 randomly selected feed bags of different types from six feed mills. Cultural and biochemical tests were performed for identification of *Salmonella* in the samples. All isolates were subjected to eight commonly used antibiotics for sensitivity test using disc diffusion method. The overall prevalence of *Salmonella* in the study was 22.1%. Prevalence of *Salmonella* contamination was 22.2%, 39.1%, 14.7%, 0.0%, 25% and 42.9% of the samples from feed mills named A, B, C, D, E and F respectively. Significantly higher ($p = 0.001$) prevalence of *Salmonella* contamination was recorded in feed mill B. Although *Salmonella* isolates were less resistant to ciprofloxacin (14.1%), the resistance increased towards amikacin (63.4%), sulphamethoxazole/trimethoprim (85.9%), gentamicin (87.1%), kanamycin (88.2%), streptomycin (96.5%), amoxycillin (98.8%) and tetracycline (100.0%). Isolation of *Salmonella* from commercial chicken feeds in Dar es Salaam connotes the importance of hygienic processing and handling of feeds for effective control of *Salmonella* contamination in both humans and farms. The antibiogram pattern shows the presence of antibiotic resistant *Salmonella* species hence; suitable measures should be implemented to avoid indiscriminate use of antibiotics in chicken feed chain.
Keywords: *Salmonella*. Poultry mash, feed mills, antibiogram, antibiotic sensitivity.

INTRODUCTION

Antimicrobial resistance in *Salmonella* is rapidly growing worldwide from about 20% in 1990s (Kruger *et al.*, 2004) to the currently up to 100% reported cases (Akbar and Anal, 2013). Development of resistance is linked with indiscriminate use of antibiotics in animals for therapeutics, prophylaxis and growth promotion (McEwen and Fedorka-Cray, 2002; Kumari *et al.*, 2013) . Resistance in bacteria from animals is of great importance as it can spread to humans (Mølbak *et al.*, 1999; Fey *et al.*, 2000) and thus necessitating the use of more expensive drugs, making treatment less effective, and even leaving infections untreatable both in animals and humans (2005). A study by Lee *et al.* (2003) showed a rapid increase of the prevalence of resistance of *Salmonella* to one or more drugs from 0% in 1995 to 93.5% in 2001, but later on Taddele *et al.* (2012) indicated that all (100%) *Salmonella* tested were resistant to erythromycin with 86.7% of them showing resistance to nalidixic acid and more than 53% of the strains being up to 100% resistant to kanamycin and tetracycline. A study by Borah *et al.* (2013)

revealed that all *Salmonella* strains isolated from human, cattle and poultry were resistant to furazolidone whereas Whichard *et al.*, (2007) reported a concurrent resistance to Quinolones and Cephalosporins which are main drugs used in treatment of *Salmonella* in both humans and animals. Antibiotics are commonly used in prevention of bacterial infections as well as treatment of infected chickens in Tanzania (Mubito *et al.*, 2014). Because of the increase in antimicrobial resistance, regular testing of *Salmonella* sensitivity to antibiotics is important in providing and reviewing treatment guidelines.

MATERIALS AND METHODS

A cross-sectional study was carried out in Dar es Salaam to analyse the aspects of *Salmonella* contamination and its antibiogram in commercial chicken feeds. The region has a land area of 1,590.5 km^2 located at 6°48' South, 39°17' East (in the Eastern part of Tanzania) with a tropical hot and humid weather. Dar es Salaam is Tanzania's most important city for business with the highest number of chicken per household in the country (FAO, 2008; Tanzania National Bureau of Statistics, 2012).

A total of 384 feed samples were collected from six feed mills during the period of May 2015 and June 2016. Selection of feed bags was done using systematic sampling method and the interval of selection was determined according to the expected number of bags from the finished product bins ready for bagging. A sample of about 50 g was collected using a sterile zip-lock bag and transported to the laboratory for testing.

Salmonella isolation and identification

Isolation of *Salmonella* in feed samples was done at Central Veterinary Laboratory, Tanzania Veterinary Laboratory Agency (TVLA) according to the standard culture methods (ISO 6579:2002(E), 2002; OIE, 2012). A 25 g portion of feed sample was pre-enriched in 225 ml of buffered peptone water (Himedi, Mumbai, India) and incubated at 37°C for 24 hr. Then 0.1 ml of the pre-enrichment culture was added to 10 ml of Rappaport-Vassiliadis broth (Himedia, Mumbai, India) and incubated at 41.5°C for 24 hr. Loopful inoculums were subsequently streaked into Xylose Lysine Deoxychocolate Agar (XLD-Agar, Scharlau Chemie S.A., Barcelona Spain) and McConkey's agar (Himedia, Mumbai, India) and incubated at 37°C for 24 - 48 hr to obtain only single type of colonies. The isolates were identified as *Salmonella* species based on the colony appearance, Gram stain, triple-sugar-iron (TSI) reaction, indole reaction, methyl-red (MR) reaction, Voges-Proskauer (VP), and citrate utilisation according to Ewing (1973).

Antibiotic resistance test

Antibiotic resistance was tested in vitro using the disc diffusion method as described by Bauer et al. *(1966)*. The antimicrobial agents tested were gentamicin (10 µg), tetracycline (30 µg), amoxycillin (10 µg), kanamycin (30 µg), ciprofloxacin (5 µg), amikacin (30 µg), streptomycin (10 µg) and sulphamethoxazole/trimethoprim SXT (23.75/1.25 µg) (Oxoid Limited, Basingstoke, Uk). All (n=85) isolates obtained from the feed sample was tested for antibiotic sensitivity. A pure bacterial culture was inoculated into nutrient broth and incubated at 37ºC for 24 h. The broth

was homogenously streaked using sterile cotton swabs on Mueller-Hinton agar plates. Antibiotic discs were placed aseptically using sterile forceps on the surface of the inoculated plates. The plates were then inverted and incubated at 37°C for 24 h. After incubation, the plates were examined and the diameters of the zone of complete inhibition were measured to the nearest millimeter using a millimeter ruler and compared with a zone interpretation chart as described by the Clinical and Laboratory Standards Institute (2014).

Data Analysis

Data were analyzed using IBM SPSS Statistics version 20 computer program. The Pearson's chi-square (χ^2) test at a significance level of 5% was used to determine the prevalence of *Salmonella* contamination among different feeds, between batches and among feed mills (McHugh, 2013). The difference was considered statistically significant if the p-value was less than or equal to 0.05.

RESULTS

A total of 384 commercial chicken feed samples consisting of Broiler starter mash (n=121), Broiler grower mash (n=47), Broiler finisher mash (n=102) and Layers mash (n=114) samples (Table 1) from the selected feed mills in Dar es Salaam were tested for *Salmonella*. Out of the 384 samples, 85 tested positive (22.1%) for *Salmonella.* The prevalence of *Salmonella* was highest in samples from broiler starter (29.8%) followed by broiler finisher (19.6%), layers (18.4%) and grower (17.0%). The prevalence of *Salmonella* in commercial chicken feed was investigated in six feed mills in Dar es Salaam (Table 2). The results showed that 22.2% (n=45), 39.1% (n = 92), 14.7% (n = 75), 0.0% (n = 85), 25% (n = 13) and 42.9 (n = 35) of the samples from feed mills A, B, C, D, E and F respectively were positive for *Salmonella*

Table 1. *Salmonella* isolated from different feed types

Feed type	No. of samples tested	No. of *Salmonella* positive (%)	χ^2-value	P-value
Broiler Starter Mash	121	36 (29.8)	6.077	0.108
Broiler Grower Mash	47	8 (17.0)		
Broiler Finisher Mash	102	20 (19.6)		
Layers Mash	114	21 (18.4)		
Total	384	85 (22.1)		

Table 2. Distribution of *Salmonella* among feed mills

Feed mill	No. of samples tested	No. of *Salmonella* positive (%)	χ^2-value	p-value
A	45	10 (22.2)	50.976	0.001
B	92	36 (39.1)		
C	75	11(14.7)		
D	85	0 (0.0)		
E	52	13(25.0)		
F	35	15 (42.9)		
Total	384	85 (22.1)		

The isolation of *Salmonella* in feed also varied between the two batches sampled from each feed mill (Table 3). A total of 44 out of 186 samples (23.7%) in batch number 1 were positive for *Salmonella* while 41 out of 198 (20.7%) in batch number 2 were positive for *Salmonella*

Table 3. Distribution of *Salmonella* between batches in each feed mill

Feed mill	Batch no.	No. of samples tested	No. of *Salmonella* positive (%)	χ^2-value	p-value
A	1	23	5 (21.7)	0.407	0.524
	2	22	5 (22.7)		
B	1	24	9 (37.5)	0.036	0.849
	2	68	27 (39.7)		
C	1	32	6(18.8)	0.744	0.389
	2	43	5 (11.6)		
D	1	42	0 (0.0)		
	2	43	0 (0.0)		
E	1	32	12(37.5)	6.933	0.008
	2	20	1 (5.0)		
F	1	26	12 (46.2)	0.449	0.503
	2	9	3 (33.3)		
Total		384	85 (22.1)		

Antibiogram of the isolates are presented in Table 4. The highest resistance was recorded in Tetracycline (100.0%) followed by amoxycillin (98.8%), streptomycin (96.5%), kanamycin (88.2%), gentamicin (87.1%), sulphamethoxazole/trimethoprim (85.9%), amikacin (63.4%) and ciprofloxacin (14.1%).

Table 4. Antibiotic resistance pattern of *Salmonella* isolates from feed mills

Feed mill	Total No. of isolates	No. of *resistant isolates*							
		S	Ac	Am	Cf	Tc	K	G	SXT
A	10	10	10	8	2	10	8	8	8
B	36	35	36	23	6	36	32	31	32
C	11	11	11	6	0	11	11	11	10
E	13	12	13	9	1	13	12	11	11
F	15	14	14	9	3	15	12	13	12
Total	85	82 (96.5)	84 (98.8)	55 (63.4)	12 (14.1)	85 (100.0)	75 (88.2)	74 (87.1)	73 (85.9)

S = streptomycin (10 µg); Ac = amoxycillin (10 µg); Am = amikacin (30 µg); Cf = ciprofloxacin (5µg); Tc = tetracycline (30 µg); K = kanamycin (30 µg); G = gentamicin (10 µg); SXT = sulphamethoxazole/trimethoprim SXT (23.75/1.25 µg)

DISCUSSION

The present study demonstrated that the prevalence of *Salmonella* in feeds in Dar es

Salaam (22.1%) agrees with, but is slightly less than, the previously reported prevalence of 29.4% *Salmonella* contamination in feed mills in Ilala District, Tanzania (Mdemu *et al.,* 2016). Although some studies have reported a higher prevalence (Chowdhury *et al.,* 2011), the present data shows that the levels of contamination in this study are higher compared to some of the reported data (Tavechio *et al.,* 2002; Molla *et al.,* 2010; Nourmohamadi and Shokrollahi, 2014). Cabarkapa *et al.* (2009) and Čabarkapa *et al.* (2009) reported that no *Salmonella* was detected in a study on feeds in Serbia whereas the prevalence of 0% to 5.3% *Salmonella* contamination in poultry feeds has been reported from different states in the European Union (Ekesbo, 2013). The prevalence of *Salmonella* in commercial chicken feeds reported here connotes the importance of hygienic processing and handling of feeds for effective control of *Salmonella* contamination in both humans and farms.

Although broiler starter mash in this study showed a slightly higher prevalence than other feed types, statistical analysis of the data revealed that there was no significant difference (χ^2 = 6.077, P = 0.108) on the prevalence of *Salmonella* contamination among broiler starter mash, broiler grower mash, broiler finisher mash and layers mash. A study by Younus *et al.* (2009) showed a similar trend but with lower prevalence of *Salmonella enteritidis* and *Salmonella typhimurium* in chicken feeds in Pakistan. The results of this study therefore confirms that *Salmonella* contamination do not vary depending on the type of feed.

Statistical analysis of the data indicated that there was significant difference (χ^2 = 50.976, P = 0.001) on the prevalence of *Salmonella* contamination among feed mills A, B, C, D, E and F (Table 2). The differences recorded in this study are in agreement with the previous data (Mdemu *et al.,* 2016) indicating that some of the feed mills in the region are more likely to formulate *Salmonella* contaminated feeds than others therefore releasing feeds with different levels of *Salmonella* contamination in the market. Davies and Wray (1997) reported different levels of *Salmonella* contamination in ten feed mills in Great Britain suggesting that feeds can be contaminated at the feed mill. The reasons of these differences are beyond the scope of this study and therefore it is recommended that further studies be undertaken.

The distribution of *Salmonella* in contaminated feeds can be sporadic and uneven (Jones, 2011) such that the levels of contamination might differ between batches. The data presented in this study shows the distribution of *Salmonella* contamination between feeds produced in two manufacturing orders (batches) of each feed mill (Table 3). Statistical analysis of the data indicated that there was a significant difference between batches in feed mill E (χ^2 = 6.933, P = 0.008) where *Salmonella* was isolated from only 5% of the samples in batch number 2 as compared to 37.5% in batch number 2. The difference in the levels of contamination between batches may also indicate inconsistency in the operation of the feed mills hence suggesting that periodic testing of *Salmonella* in feed mills should be conducted frequently (by batch) to provide for a higher degree of assurance.

All *Salmonella* isolates were tested for antibiotic resistance by disc diffusion method against the commonly used commercial antibiotics (gentamicin, tetracycline, amoxycillin, kanamycin, ciprofloxacin, amikacin, streptomycin and

sulphamethoxazole/trimethoprim). Of all the tested antibiotics, ciprofloxacin showed the lowest resistance (14.1%). Although several authors have reported that *Salmonella* is 0% resistant to ciprofloxacin (Ranju *et al.,* 1998; Mijovic, 2012), studies show that resistance is rapidly increasing with *Salmonella* isolates from humans, animals and environment worldwide. The level of resistance to ciprofloxacin recorded here is lower than the resistance of 26.3% and 19.2% recorded by Borah *et al.* (2013) and Mushtaq, (2006) respectively, but is slightly higher than the resistance of 13.66% and 4.2% reported by Choudhary *et al.,* (2013) and Nagshetty *et al.,* (2010) respectively. In the review of antimicrobial resistance in four countries (Zambia, Democratic Republic of Congo, Mozambique and Tanzania), Mshana *et al.* (2013) reported a rapid increase in ciprofloxacin resistance in the Democratic Republic of Congo from 0.0% in 2009 to 15.4% in 2012, and recently Wasihun *et al.* (2015) have reported 50% and 12.5% resistance of *S. paratyphi* and *S. typhi* respectively in Ethiopia. The evidence of rapid increase on resistance of *Salmonella* to ciprofloxacin poses a threat to the public health and prompts a careful and appropriate use of antibiotics. The results of the present study, however, differs with the recent report by Katani *et al.* (2015) which showed that *Salmonella* isolates from house crows in Tanzania were 0% resistant (100% sensitive) to ciprofloxacin and the findings by Mwambete and Stephen (2015) on chicken droppings in Tanzania which indicates that 59% *Salmonella* were resistant to ciprofloxacin , but is similar to the report by Meremo *et al.* (2012) which showed 8% resistance of ciprofloxacin on Non-Typhoidal *Salmonella* from humans in Tanzania. More surveillance of *Salmonella* resistance to ciprofloxacin is therefore required in the fight against resistance.

Salmonella isolates are shown to be more resistant to both Amikacin (63.4%) and Gentamicin (87.1%). The results partially agrees with Över *et al.* (2001) who reported 62.5% and 100% S*almonella* resistance to amikacin and gentamicin respectively in isolates from hospitals in Turkey, and closely related to the recent study in slaughtered swine in Philippines which showed that all *Salmonella* isolates (100%) were resistant to both amikacin and gentamicin (Ng and Rivera, 2014). A study by Wasfy *et al.* (2000) show different scenario in Egypt as *Salmonella* isolated from hospital specimens were about 0.1% and 20% resistant to amikacin and gentamicin respectively. A similar trend was observed by Obi *et al.* (2004) who reported respectively 0.0% and 3.4% *Salmonella* resistance to amikacin and gentamicin on isolates from water in South Africa.

In a study on isolates from hospital samples in Tanzania, Blomberg *et al.* (2007) reported an average of 30% *Salmonella* resistance to gentamicin but Meremo et al. (2012) reported a higher resistance of 61.5% in *Salmonella* from hospital samples in Tanzania. The study by Mwambete and Stephen, (2015) shows a similar trend with the present study but with lower levels of resistance (31.7% and 46.7%) for amikacin and gentamicin respectively. It appears that the resistance of *Salmonella* against both amikacin and gentamicin has rapidly increased with time to the current levels rendering the antibiotics less effective against *Salmonella*.

Salmonella isolates shows to be much more resistant to amoxycillin (98.8%) and is comparable to the 100% resistance reported by Katani *et al.* (2015). The resistance of *Salmonella* to

sulphamethoxazole/trimethoprim (85.9%), streptomycin (96.5%), and tetracycline (100.0%) agrees with Dione (2010) showing that *Salmonella* isolates are resistant to these antibiotics and therefore cannot be effective in the treatment of infections by these bacteria. It is therefore recommended that measures should be implemented to avoid indiscriminate use of antibiotics in the feed chain. Furthermore, sensitivity testing of commonly used antibiotics should be done frequently to monitor the development of microbial resistance.

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