Molecular Characterization of Multidrug Resistant *Salmonella* Isolates From Food Animals and Animal Products in Tanzania

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SUMMARY

Food animals are major sources of human salmonellosis. Animals raised for food play an important role in transmission of antimicrobial resistant *Salmonella* strains to humans. The aim of this study was to determine the antimicrobial resistance profile, the occurrence of class 1 integrons and the resistance gene cassettes mobilized in the class 1 integrons of *Salmonella* isolates. A cross-sectional design was carried out in pastoral regions of Tanzania with large population of livestock. *Salmonella* isolates were recovered from 4.2% of the total of 1540 samples from apparently healthy animals and the animal products. The results showed that *Salmonella* isolates were detected in 5.2%, 3.7% and 3.8% of the swine, cattle and chicken, respectively. Sixty-one *Salmonella* isolates belonged to *Salmonella enterica* subsp. *enterica*. Predominant serotypes were *Salmonella* I 8,20:i:- (32.8%), *S.* Hadar (10.9%), *S.* Colindale (6.3%), *S.* Anatum (6.3%) and *S.* Heidelberg (6.3%). *S.* I 8,20:i:- isolates were widespread in different samples from different food animals. Of 64 *Salmonella* isolates, about 35.9% were resistant to at least one antimicrobial, whereas, 82.6% were multi-drug resistant (MDR) *Salmonella.* About 8.7% of the MDR *Salmonella* isolates were found to also carry integrons (*intI1)* and 100% of *intI1*-positive isolates contained resistance gene cassettes known as *aac(3)-Id-aadA7* showing high rate of MDR. The occurrence of clonal MDR *Salmonella* isolates in food animals and animal products from pastoral communities indicates the high significance of informal traditional sector as an important source of foodborne pathogens in the food chain and the entry of pathogens to the pastoralist communities.

Keywords: *Animal products, Antimicrobial resistance, Class 1 integrons, Food animals, MDR Salmonella, Pastoralists, Public health*

INTRODUCTION

Foodborne diseases caused by non-typhoidal *Salmonella* (NTS) represent an important public health problem worldwide. NTS serovars are among the most important foodborne bacterial pathogens with broad host range including food animals and humans (Scallan *et al*., 2011).

Previous studies have implicated food animals to be the major reservoirs of *Salmonella* (Li *et al*., 2013). The *Salmonella* serovars isolated from food animals have significant overlap with those causing illness in humans. Of particular importance as far as the food animals are concerned is the food chain which has been shown to play an important role in the transmission of *Salmonella* from food animals to humans (Thong and Modarressi, 2011).

The importance of the animal products in the dissemination of other important zoonotic disease pathogens, namely, *Mycobacterium bovis*, causing Bovine Tuberculosis and *Brucella* spp. causing Brucellosis was shown to be a key factor in pathogens transmission (Cleaveland *et al*., 2007).

In Tanzania, there is no official *Salmonella* surveillance data in place, however, there

are a few studies conducted that have estimated the prevalence of NTS ranging from 7.6-28% in humans (Mtove *et al*., 2010; Meremo *et al*., 2012), whereas, the serological and cultural prevalence of *Salmonella enterica* subspecies *enterica* serovar gallinarum (*Salmonella gallinarum*) were recorded to range from 2.6 - 28% (Mdegela *et al*., 2000) and the overall prevalence of NTS in livestock was found to be 2.3% (Otaru *et al*., 1990).

Majority of studies on Salmonellosis in Tanzania have largely addressed detection by isolation of *Salmonella*. Study conducted by Vaagland *et al*. (2004) has shown the exclusivity of *Salmonella* as a zoonotic pathogen of public health importance in the country for its incrimination in causing enteritidis meningitis in children.

Interestingly, another study conducted by Mtove *et al*. (2010) has shown an appealing scenario in which children with invasive NTS infection were more likely to also have malaria. With the increasing trend in consumption of food products of animal origin, there is a need for more focused studies that investigate an increased potential for exposure to *Salmonella* through the food chain and the public health implications.

The magnitude of antimicrobial resistance in *Salmonella* as a significant threat to public health is not well documented in Tanzania. The excessive use and the uncontrolled over-the counter sale of antimicrobials, particularly β-lactams, tetracyclines and fluoroquinolones in developing countries is highly likely to aggravate the magnitude of antimicrobial resistance (MacGowan and Macnaughton, 2013). The β-lactams and fluoroquinolones are reported to be important classes of antimicrobials used to treat complicated cases of salmonellosis in humans and veterinary medicine (Gonzalez-Sanz *et al*., 2009). The production of extended-spectrum β- lactamases has been shown as one of the main mechanisms of resistance to broad-spectrum β-lactams among the Enterobacteriaceae.

The β-lactamases genes have been detected worldwide in various serovars of NTS, located in plasmids or integrons, facilitating rapid transmission among *Salmonella* serovars (Cloeckaert and Schwarz, 2001).

Moreover, antimicrobials such as chloramphenicol, ampicillin, and trimethoprim-sulphamethoxazole were drugs of choice treatment of Salmonellosis for many years, until the recent years, where the antimicrobial resistance of *S. enterica* to commonly used antimicrobials has become a matter of concern worldwide (Hammad *et al*., 2011).

Specific concern is for those strains of *Salmonella* that have acquired multi-drug resistance (MDR) against two or more antimicrobial agents (Lindsey *et al*., 2009). Several factors are involved in the development of MDR. Class 1 integrons is among the most common type of integrons identified in multi-drug resistant *Salmonella* known for the dissemination of resistance genes among pathogens in the microbial population (Thong and Modarressi, 2011). Therefore it is not surprising that there is an increasing interest among researchers to investigate and seek more knowledge on this factor.

The aim of this study was to determine the prevalence, antimicrobial susceptibility patterns, the phenotypic and genotypic relatedness of *Salmonella* isolates recovered from food producing animals and food of animal origin. In addition, we investigated the occurrence of class 1 integrons and resistance gene cassettes mobilized in the class 1 integrons.

MATERIALS AND METHODS

Study area and sample collections

Study was approved and carried out in three regions of Tanzania mainland, namely, Morogoro, Iringa and Arusha in accordance with Sokoine University of Agriculture institutional guidelines (Reference number: SUA/FVM/R.1/10). Morogoro and Iringa were selected largely because of their large populations of pastoral and agro-pastoral communities (Barabaig, Maasai and Sukuma) and large populations of cattle herds, all originating from different zones of the country. Arusha region was selected for the reasons that it is the home to Maasai and Barabaig pastoralists who constantly move with their cattle herds in search for water and good pastures.

Samples from live and slaughtered animals were collected to investigate the possibility of *Salmonella* species circulating in these animals as a result of the livestock interactions on the communal lands.

Sampling was conducted in households and slaughterhouses of cattle, swine, sheep, goats and poultry from February 2013 to March 2014. Sample size was calculated according to Charan and Biswas, (2013). Fecal (n = 136) and milk (n = 48) samples from cattle, feces from goats (n = 103) from the pastoral and agro-pastoral communities, as well as from non pastoral communities, and feces (n = 215) and milk (n = 238) from dairy cattle farms were collected.

Other samples from live animals included: swine feces (n = 473), chicken cloacal swabs (n = 48), chicken eggs (n = 50) and feces (n = 6), from farms with and without mixed farming. Samples from slaughtered animals were cattle carcass swabs (n = 181) and swine carcass swabs (n = 23). Environmental samples included the floor drag swabs of the slaughterhouses and slaughter slabs (n = 12). Also, one slaughterhouse pit latrine which is frequently used by workers was sampled. Briefly, a sterile cotton swab with a long hanging thread tied on a stick was submerged into the septic tank through its hole. The swab was kept in a sterile whirl-pak, kept in cool box and transported to the laboratory for processing. This sampling process of the slaughterhouse latrine was repeated at least twice a month for seven consecutive visits. All samples were kept in a cool box before transporting to Sokoine University of Agriculture for further processing.

Salmonella isolation and identification

Conventional methods of isolation were used as described previously (Gebreyes *et al*., 2004). These were used for isolation and identification of Salmonellae. Briefly, about 10g portion of each fecal and feed sample were pre-enriched in 90 ml of buffered peptone water (BPW; Becton Dickinson, Sparks, MD).

In addition, about 90 ml of BPW was added to each Whirl-Pak bag containing individual carcass and floor drag swabs, and both incubated at 37°C for 24 h. A 100 µl of each pre-enriched suspension following overnight incubation was added into 9.9 ml of Rappaport-Vassilliadis (RV) enrichment broth (Becton Dickinson, Sparks, MD) and incubated at 42°C for 24 h. Following overnight incubation at 42°C, a 10 µl of each of the enriched suspension was inoculated onto Xylose-lactose-Tergitol 4 (XLT-4) agar (Becton Dickinson, Sparks, MD) or Xylose-lysine deoxycholate (XLD) agar (Himedia, Mumbai, India) plates and incubated at 37°C for 24 h.

The incubation time was extended to 48 h in cases where colonies were doubtful. Three isolated presumptive *Salmonella* colonies were selected from each positive sample for biochemical tests. Each selected presumptive *Salmonella* colony was inoculated onto triple sugar iron (TSI) agar (Becton Dickinson, Sparks, MD) slants, Lysine iron agar (LIA) slants (Becton Dickinson, Sparks, MD) and urea broth (Becton Dickinson, Sparks, MD) and incubated at 37°C for 16-24 h.

Some atypical presumptive *Salmonella* isolates were observed after performing TSI, LIA and urea tests, as a result of biochemical indeterminacies, all presumptive *Salmonella* isolates were stored at -80°C until further testing using *invA* Polymerase Chain Reaction (PCR) and *16S rDNA* gene sequencing. Absence of *invA* gene suggests that the isolates are tentatively *Citrobacter* isolates until sequenced for *16S rRNA* gene.

Phenotypic characterization

Sixty-four *Salmonella* isolates were serogrouped by slide agglutination using commercially available *Salmonella* O polyvalent A-1 and vi antiserum (MiraVista Diagnostics, Indianapolis, IN) according to the recommendations of the manufacturer. The *Salmonella* isolates were serotyped at the Office International de Épizooties (OIÉ) Reference Laboratory for Salmonellosis of the Public Health Agency of Canada, Laboratory for Foodborne Zoonoses, Guelph, Ontario, Canada.

Serovar Heidelberg isolates were also phage typed after assigning the serotypes of the *Salmonella* isolates. Briefly, the somatic (O) antigens were determined by slide agglutination tests (Ewing, 1986) and the flagellar antigens were determined using a microplate agglutination technique (Shipp and Rowe, 1980).

In addition, the Grimont antigenic formulae were used to identify and assign the serotypes of the *Salmonella* isolates. Phage typing of *Salmonella* Heidelberg isolates was conducted as previously described (Demczuk *et al*., 2003).

Briefly, the plates were incubated and lytic patterns were read and recorded (Amavisit *et al*., 2001). *Salmonella* isolates that reacted with the phages but did not conform to any recognized phage type were designated atypical (AT).

Antimicrobials selected for testing based on their classes and in particular those which are drugs of choice in human and veterinary medicines (Gonzalez-Sanz *et al*., 2009; Gebreyes *et al*., 2004). Sixty-four *Salmonella* isolates were tested for antimicrobial susceptibility by a panel of 14 antimicrobials using the Kirby-Bauer disc diffusion method (Wayne, 2009).

The antimicrobial agents used and their respective disc potencies were as follows: ampicillin (Am; 10µg/ml), amoxicillin-clavulanic acid (Ax; 30µg/ml), amikacin (An; 30 µg/ml), ceftriaxone (Ce; 30µg/ml), cephalothin (Ch; 30µg/ml), chloramphenicol (Cl; 30µg/ml), ciprofloxacin (CIP; 5µg/ml), gentamicin (Gm; 10µg/ml), kanamycin (Km; 30µg/ml), streptomycin (S; 10µg/ml), trimethoprim (TMP; 5µg/ml), sulfisoxazole (Su; 250µg/ml), and tetracycline (Te; 30µg/ml).

Escherichia coli ATCC 25922, *Enterococcus fecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains. *Salmonella* isolates showing resistance to three or more antimicrobial agents were classified as multi-drug resistant (MDR) and those isolates with intermediate resistance profiles were considered susceptible (Wei *et al*., 2019).

Detection of *16S rDNA* and *invA* gene in *Salmonella* isolates

Sixty-four presumptively positive *Salmonella* isolates were tested for carriage of invasion (*invA*) gene by PCR. *Salmonella* isolates were inoculated onto tryptic soy agar (TSA) plates and incubated at 37°C for 16-24 h.
Genomic DNA was extracted using the Qiagen DNeasy tissue kit according to the manufacturer's instructions (Qiagen Ambion, Austin, TX, USA).
A set of forward primers (5'-TCGTCATTCCATTACCTACC-3') and reverse primers (5'-AAACGTTGAAAAACTGAGGA-3'), was used to amplify the *invA* gene under the following PCR conditions: hot start *Taq* activation at 94°C for 3 min, denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min, and amplification was done in

35 cycles. The reaction mixture was kept at 72°C for 10 min after the final cycle (Hoorfar *et al*., 2000). Furthermore, all 64 *Salmonella* isolates were prepared for *16S rDNA* sequencing. Primers used for amplification of the *16S rDNA* included 27F (5'-AGAGTTTGATYMTGGCTCAG-3') and 907R (5'-CCGTCAATTCMTTTGAGTTT-3') (Mao *et al*., 2012).

The PCR amplification conditions were initial denaturation at 95°C for 4 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min, and then the amplification cycle was repeated for a further 35 cycles and final extension was done at 72°C for 7 min. 10µl of the PCR product of each isolate tested were run on 1% agarose gel stained with 5 µl of 10-mg/ml ethidium bromide for 1 h at 120 V using 0.5X Tris borate-EDTA (TBE) as running buffer. A 1-kb Plus DNA ladder was used as a molecular size marker.

The PCR products generated for sequencing of *16S rDNA* gene were purified using ExoSAP-IT PCR clean-up method. Briefly, a 5 µl of each of the post-PCR reaction products and a 2 µl of ExoSAP-IT reagent (Miles Road, Cleveland, OH) were mixed together, followed by incubation at 37°C for 15 min and 80°C for 15 min. Following clean-up, a 10 µl of each purified PCR products were pre-mixed separately in the same tube with 5 µl of 5 pMol/µl of each of sequencing primers. The pre-mixing and the submission were done according to the organization guidelines (GENEWIZ, South Plainfield, NJ).

Detection of class 1 integron and resistance gene cassettes

The presence of class 1 integron and gene cassettes integrated between conserved segments of class 1 integrons were detected by polymerase chain reaction (PCR).

Primers used for amplification of the *intI1* included *IntI*-F (5'-GCCTTGCTGTTCTTCTACGG-3') and *intI1*-R (5'-GATGCCTGCTTGTTCTACGG-3') (Levesque, *et al*., 1995), while primers used for conserved segments included 5'CS (5'-GGCATCCAAGCAGCAAG-3') and 3'CS (5'-AAGCAGACTTGACCTGA-3') (Ploy, *et al*., 2000). The PCR conditions were hot Start *Taq* activation at 94°C for 5 min, followed by 25 cycles of 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s, and then a final extension step at 72^0C for 7 min (Lindstedt *et al*., 2003). About 10 µl of the PCR product of each isolate tested were run on 1% agarose gel stained with 5 µl of 10-mg/ml ethidium bromide for 1 h at 120 V using 0.5X Tris borate-EDTA (TBE) as running buffer. A 1-kb Plus DNA ladder was used as a molecular size marker.

Gene cassettes sequencing

The PCR products generated from sequencing of *16S rDNA* and variable regions of gene cassettes of class 1 integrons were purified using ExoSAP-IT PCR clean-up method. Briefly, a 5 µl of each of the post- PCR reaction products and a 2 µl of ExoSAP-IT reagent (Miles Road, Cleveland, OH) were mixed together, followed by incubation at 37°C for 15 min and 80°C for 15 min. Following clean-up, a 10 µl of each purified PCR products were pre-mixed separately in the same tube with 5 µl of 5 pMol/µl of each of sequencing primers. The pre-mixing and the submission were done according to the organization guidelines (GENEWIZ, South Plainfield, NJ).

Salmonella whole genome sequencing (WGS)

Genomic DNA of the *Salmonella* isolates (n = 64) was extracted using the QIAamp DNA Mini Protocol (Valencia, CA). Extraction of genomic DNA was performed with the fully automated Qiagen QIAcube and samples were sequenced at the US Food and Drug Administration (FDA) using Illumina MiSeq (Hampton, VA) based on published methods (Hoffmann *et al*., 2015). Fastq sequence reads from the sequences were deposited at the National Center for Biotechnology Information (NCBI)

website under the genome trakr: OSU-ICOPHAI project with BioProject ID: 275961 and the accession: PRJNA275961.

SeqSero genotype-based serotyping

WGS data of *Salmonella* isolates was utilized to determine the inconclusive serotype of the dominating *Salmonella* isolates which was previously identified as *Salmonella* ser I 8,20:i:- by Kauffmann-White serotyping scheme (Brenner *et al*., 2000). To determine the serotypes of the *Salmonella* isolates, we submitted the downloaded pair of fastq format files for each isolate into SeqSero website (http://www.denglab.info/SeqSero) by selecting reads (pair-end) in the option menu (Zhang *et al*., 2015).

Detection of *Salmonella* acquired resistance genes

A web-based method, ResFinder was used to detect the acquired resistance genes from the *Salmonella* whole genome data. Briefly, the paired fastq format files were downloaded for each *Salmonella* isolate using http://www.ebi.ac.uk/ link.
Two fastq files of each *Salmonella* isolate were submitted online to the Center for Genomic Epidemiology website (http://www.genomicepidemiology.org/) [Zankari *et al*., 2012]. Under a drop-down list we selected *Salmonella* as targeted organism, the type of the reads were Illumina-paired end reads with 85% threshold ID, 80% length. The files of the results were received through the email contact provided.

Salmonella 16S rDNA and gene cassettes sequence data analysis

Salmonella reverse sequence data were converted to match the complement DNA forward sequences using the online reverse complement software available online at www.bioinformatics.org/sms/rev_comp.html. Both forward and reverse complement sequences of each isolates were aligned using ClustalW2 software available at www.ebi.ac.uk/clustalw and trimmed to obtain the consensus DNA sequences.
The consensus nucleotide sequences of *16S rDNA* were chimera checked using online DECIPHER software (Wright *et al*., 2012). None of the nucleotide sequences of *16S rDNA* deciphered chimeras. The consensus DNA sequences were compared with the best-matching sequences available on the NCBI databases using the GenBank BLASTN available at http://blast.ncbi.nlm.nih.gov/Blast.cgi.

Data analysis

Data were handled using Microsoft excel 2007 (Ms Corp., Redmond, WA, USA) and statistical analysis was conducted with the MedCalc® statistical software package version 12.7.1.0 (8400 Oostende, Belgium). Prevalence of *Salmonella* and antimicrobial resistance profiles were analyzed at the animal and sample levels. A value of $P \leq 0.05$ was considered significant.

RESULTS

Salmonella prevalence and the serotypes

Salmonella isolates were detected from 64 of 1540 apparently healthy animals, animal products, floor swabs, and sewage samples indicating 4.2% prevalence. The results showed that *Salmonella* isolates were detected in 5.2% (26 of 496) of the swine; 3.7% (30 of 818) of cattle and 3.8% (4 of 104) chicken specimens. Twenty five-percent (3 of 12) of slaughterhouse floor swabs and 14.3% (1 of 7) sewage samples were also found to be positive for *Salmonella*. *Salmonella* isolates were recovered from dairy cattle feces and milk and other samples as shown in Table 1. *Salmonella* recovery from swine, cattle and different sample types was not significantly different between any of the groups: swine versus cattle group (P = 0.245), Zebu cattle versus dairy cattle (P = 0.27), dairy cattle feces versus dairy cattle milk (P = 0.365) and Zebu cattle feces versus dressed cattle carcasses (P = 0.902).

Out of the 64 isolates, 61 belonged to *S. enterica* subspp. *enterica* and three were *S. enterica* subspp. *salamae*.

The predominant serovars were *Salmonella* ser. I 8,20:i:- (32.8%, 21/64), *S. enterica* subspp. *enterica* serovar Hadar (*Salmonella* Hadar) (10.9%, 7/64), *Salmonella* Colindale (6.3%, 4/64), *Salmonella* Anatum (6.3%, 4/64) and *Salmonella* Heidelberg (6.3%, 4/64). Interestingly, *Salmonella* serovar I 8,20:i:- isolates were widespread in different samples from different food animals and products, including the milk from a mastitic cow

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Table 1. Prevalence of *Salmonella* species by sample source and animal host species

Source of samples		Number of samples (%)	Prevalence (%)
Animal feces	Cattle	351 (22.8)	17 (4.8)
	Swine	473 (30.7)	26 (5.5)
	Poultry	6 (0.4)	3 (50)
	Small ruminants	103 (6.7)	0 (0)
		933 (60.6)	46 (4.9)
Carcass swabs	Cattle carcass	181 (11.8)	6 (3.3)
	Swine carcass	23 (1.5)	0 (0)
	Cloacal swabs	48 (3.1)	1 (2.1)
		252 (16.4)	7 (2.8)
Milk	Dairy cattle	238 (15.5)	7 (2.9)
	Zebu cattle	48 (3.1)	0 (0)
		286 (18.6)	7 (2.4)
Poultry	Chicken eggs	50 (3.2)	0 (0)
		50 (3.2)	0 (0)
Environment	Abattoir floor swabs	12 (0.8)	3 (25)
	Abattoir sewage	7 (0.5)	1 (14.3)
		19 (1.2)	4 (21.1)
	Total	1540 (100)	64 (4.2)

Antimicrobial resistance phenotyping

According to the disc diffusion, the most common antimicrobial resistance was to tetracycline (25%), followed by sulfisoxazole (21.9%), trimethoprim (17.2%), amoxicillin-clavulanic acid (15.6%), cephalothin (14.1%), ampicillin (14.1%), streptomycin (6.2%), ciprofloxacin (3.1%) and chloramphenicol (1.6%). No antimicrobial resistance was found to amikacin, gentamycin and ceftriaxone, however, intermediate resistance was found to ceftiofur (1.6%) and kanamycin (1.6%) (Table 2).

Of the 64 *Salmonella* isolates resistant to one or more antimicrobials, 20.8% (5 of 64), 6.3% (4 of 64), and 1.6% (1 of 64) of the isolates were recovered from the dressed cattle swabs, cattle feces, and milk samples, respectively. In addition, 18.8% (12 of 64) and 3.1% (2 of 64) of the isolates were recovered from swine feces and chicken samples, respectively. Sixty-three percent (40 of 64) of the *Salmonella* isolates were pansusceptible, 37.5% (24/64) of the isolates were resistant to at least one antimicrobial, and 29.7% (19/64) were multi-drug resistant (MDR) *Salmonella.* Of the 19 MDR *Salmonella* isolates, 47.4% (9 of 19), 42.1% (8 of 19), 10.5% (2 of 19) of the isolates were recovered from the swine, bovine and chicken samples, respectively. The frequency of antimicrobial resistance of *Salmonella* spp. recovered from different animal species and sample types was not significantly different between any of the three groups, namely swine versus cattle ($P = 0.477$), Zebu cattle versus dairy cattle ($P = 0.338$) and dairy cattle feces versus dairy cattle milk ($P = 0.767$). However, the frequency of antimicrobial resistance of *Salmonella* spp. was significantly different between Zebu cattle feces and dressed carcasses ($P = 0.05$).

Table 2. Biochemical tests, *invA* PCR, and antimicrobial susceptibility testing (AST) results

Biochemical tests and results				*InvA* PCR	Antimicrobials	*Salmonella* isolates		
Urease test	TSI	LIA	H_2S			Res [n (%)]	Intermed [n (%)]	Pans [n (%)]
-	+	+	+	+	Ampicillin (Am)	9 (14.1)	2 (3.1)	53 (82.8)
-	+	+	+	+	Chloramphenicol (Cl)	1 (1.6)	-	63 (98.4)
-	+	+	+	+	Streptomycin (S)	4 (6.2)	6 (9.4)	54 (84.4)
-	+	+	+	+	Trimethoprim (TMP)	11 (17.2)	-	53 (82.8)
-	+	+	+	+	Sulfisoxazole (Su)	14 (21.9)	-	50 (78.1)
-	+	+	+	+	Tetracycline (Te)	16 (25)	-	48 (75)
-	+	+	+	+	Amoxicillin-clavulanic acid (Ax)	10 (15.6)	3 (4.7)	51 (79.7)
-	+	+	+	+	Cephalothin (Cp)	9 (14.1)	7 (10.9)	48 (75)
-	+	+	+	+	Ceftriaxone (Ce)	-	-	64 (100)
-	+	+	+	+	Ciprofloxacin (CIP)	2 (3.1)	-	62 (96.9)
-	+	+	+	+	Kanamycin(Km)	-	1 (1.6)	63 (98.4)
-	+	+	+	+	Amikacin (An)	-	-	64 (100)
-	+	+	+	+	Gentamycin (Gm)	-	-	64 (100)
-	+	+	+	+	Ceftiofur (XLN)	-	1 (1.6)	63 (98.4)

Abbreviations: TSI, Triple sugar iron agar test; LIA, Lysine iron agar test; H_2S; Res, Resistant; Intermed, Intermediate; Pans, Pansusceptible; Hydrogen sulfide gas; An, Amikacin; Ax, Amoxacillin-clavulanic acid; Am, Ampicillin; Cp, Cephalothin; XLN, Ceftiofur; Ce, Ceftriaxone; Cl, Chloramphenicol; CIP, Ciprofloxacin; Gm, Gentamycin; Km, Kanamycin; S, Streptomycin; Su, Sulfisoxasole; Te, Tetracycline; TMP, Trimethoprim.

The fact that the *S*. Heidelberg isolates are one of the most frequently encountered phage types among human sporadic cases and in outbreak cases, the phage typing indicated that three *S*. Heidelberg isolates belonged to the phage type 19 and one *S*. Heidelberg isolate did not conform to any recognized phage type and was considered atypical (Supporting Table 1).

Polymerase Chain Reaction (PCR) of targeted genes

About 8.3% (2/24) of the resistant *Salmonella* isolates were found to also carry integrons (*intI1*) and 100% (2/2) of *intI1*-positive isolates contained resistance gene cassettes known as *aac(3)-Id-aadA7* of size 1500 bp showing high rate of MDR. In addition, three of 17 (17.6 %) of the resistant *Citrobacter* isolates amplified *intI1* gene and 100% (3/3) of *intI1*-positive isolates contained resistance gene cassettes known as *dfrA1-orfC*, *dfrA7* and *dfrA15* of size 1250 bp, 800 bp and 700 bp, respectively.

SeqSero genotype-based serotyping and acquired resistance genes Analysis

Kauffmann-White serotyping scheme identified the inconclusive serotype of dominant *Salmonella* isolates as *Salmonella* ser I 8,20:i:-.

SeqSero genotype-based serotyping later identified the serotype as *Salmonella* Kentucky. Further, the web-based method, ResFinder for detection of acquired antimicrobial resistance genes from the whole-genome data identified *strB*, *sul2*, *dfrA*; *sul2, dfrA; strB, sul2*, *tet*(A), *dfrA14*; and *dfrA14* from *Salmonella* isolates with SuTeTMP resistance phenotypes.

Interestingly, *Salmonella* isolate (S16783) (*S*. Manchester) and S16683 (I 8, 20: i :-), both with pansusceptible resistance phenotypes were observed to contain resistance genotypes such as *aadA1*, *blaTEM-1B*, *blaTEM-1A* and *sul2*.

DISCUSSION

While other previous studies in Tanzania have also reported high prevalence of Non-typhoidal *Salmonella* (7.6 - 29%) in humans (Mtove *et al*., 2010; Meremo *et al*., 2012) and 2.3 - 37.3% in livestock (Otaru *et al*., 1990), in this study, the *Salmonella* floor prevalence (25%) was higher than any other sources from which samples were collected.

The prevalence of *Salmonella* in the cattle carcasses (3.3%) was higher than in the cattle feces (2.3%). A higher prevalence of *Salmonella* spp. on the carcasses than in the feces is a clear indication of failure to realize proper preventive measures in producing safe meat (pork, beef, mutton) and meat products for public consumption (Carrasco *et al*., 2012).

The major source of contamination of the slaughterhouse floor and cattle carcasses could be attributed to poor handling of fecal matters during evisceration, bacterial load on the animal skin, the slaughterhouse personnel and the equipment used during the slaughter process (Teklu and Negussie, 2011).

Strict preventive measures need to be instituted to limit possible contamination of the slaughterhouse floor and the dressed carcasses by foodborne pathogens (Kich *et al*., 2011). The individual animal-level prevalence of *Salmonella* between the key food animals varied from 3.7% in cattle, 3.8% in poultry and 5.2% in swine, is higher than the prevalence reported by Otaru *et al*. (1990) possibly because of the isolation and identification protocol used in the current study which allows high recovery of *Salmonella* (Gebreyes *et al*., 2004).

The overall prevalence of Non-typhoidal *Salmonella* from this study was reported to be 4.2% similar to the prevalence (4.25%) reported by other study conducted in febrile children admitted to a referral hospital in Tanzania (Christopher *et al*., 2013). In another similar study conducted in apparently healthy livestock in Southern Tanzania, the overall prevalence of *Salmonella* was reported to be 2.3% (Otaru *et al*., 1990). In contrast to the current study, the higher *Salmonella* prevalence reported elsewhere in Tanzania were from the symptomatic children and HIV/AIDS patients admitted to referral hospitals, whereas, relatively low prevalence (2.1-2.6%) of *Salmonella* was also reported from diarrheic children aged less than five years admitted to major hospitals in Tanzania (Oketcho *et al*., 2012).

Previous study by Bywater *et al*. (2004) reported persistence of *Salmonella* infections in food animals in the subclinical stage and thus they are often clinically asymptomatic carriers (Haley *et al*., 2015).

Some of the serovars isolated in the current study were also reported in outbreaks scenarios, including *S*. Heidelberg, S. Hadar and *S*. Anatum (Jackson *et al*., 2013), *S*. Virchow and *S*. Infantis (Chironna *et al*., 2014). In Africa, *S*. Heidelberg is also one of the important serovars in terms of public health and is frequently involved in human and animal salmonellosis, and often exhibits MDR patterns (Hoffman *et al*., 2014).

However, in the current study, of four Heidelberg isolates, one isolate was phenotypically pan-susceptible and the other three isolates were resistant to at least two antimicrobials, namely, R-type AxTe, AmAxCp and AmAxCpSu. *S*. Typhimurium and *S*. Enteritidis reported before in livestock and human were not recovered in this study (Allard *et al*., 2013).

The highly dominant serovars recovered from this study included *Salmonella* I 8,20:i:- (from all the studied animal species) followed by *S*. Hadar (from swine, bovine and the slaughterhouse floor). The

study conducted by Jackson *et al*. (2013) has reported that *S*. Enteritidis, *S*. Heidelberg and *S*. Hadar formed 80% of all outbreaks attributed to eggs and poultry. In this study, *S*. Cerro was also recovered from the dairy cattle but this study could not be certain if this serovar is adapted and persisted in the dairy cattle as reported in the United States (Haley *et al*., 2015). The other common serovars in the current study include but not limited to *S*. Kentucky, *S*. Colindale, *S*. Uganda, *S*. Karamoja and *S*. Weltevreden (Supporting Table 1).

Antimicrobial resistance poses a serious threat to public health and it continues to increase and is becoming one of the most devastating events ever recorded in human history (MacGowan and Macnaughton 2013).

Occurrence of antimicrobial resistance in the human-animal-ecosystem interface is an evolutionary response that is highly linked to the strong selective pressure. This occurs as a result of exposure to antimicrobial agents such antibiotic and non-antibiotic agents (Kolar *et al*., 2001). Antimicrobial resistance in animals and environmental isolates is of public health concern because of the risk of transfer of antimicrobial resistance isolates or the resistance determinants to consumers through the food chain (Baquero *et al*., 2008).

Despite the dwindling rate in their effectiveness against the infectious agents, use of antimicrobials has saved countless lives (Davies and Davies, 2010). Over the years, resistance to all classes of antimicrobials has emerged, and this has led to emergence of antimicrobial resistant microbes, which are becoming a serious menace to the contemporary world (Zhang *et al*, 2006).

The magnitude of antimicrobial resistance in Tanzania is not well researched, although some studies have been conducted in humans than in animal subjects (Mshana *et al*., 2013).

While the investigation of the risk factors for occurrence and persistence of antimicrobial resistance was outside the scope of this study. However (Komba *et al*., 2014) highlighted the problem of the over-the-counter sale of medicines as a source of misuse of antimicrobials. This is also considered to be among the reasons for the fading off of antimicrobials activities in treating common disease conditions (Rodriguez-Rojas *et al*., 2013).

Detection of *sul2* from *Salmonella* I 8,20:i:- (*S*. Kentucky) and *aadA1*, *blaTEM-1B*, *blaTEM-1A* from *S*. Manchester with susceptible phenotypes is an important findings in the current study. Detection of such genes is essential to understanding the risk of carriage of antimicrobial resistance genes by *Salmonella* isolates with susceptible when antimicrobial resistance is not phenotypically expressed (Zankari *et al*., 2012).

Other resistance genes such as *aac(3)-Id-aadA7*, *dfrA1*, *dfrA14*, *strB*, *sul2* and *tet* (*A*) were detected from *S*. Kentucky. The *aac(3)-Id–aadA7* is an integron-borne gene, containing only a single cassette array of 1500 bp, which is known to be transmitted by the class 1 integron-mediated MDR *S*. Kentucky.

Detection of class 1 integrons in Tanzania was also reported from *E*. *coli* and *S*. *enterica* subsp. *arizonae* from a new flock of lesser flamingoes imported from Tanzania to Hiroshima Zoological Park, Japan. In contrast to dihydrofolate reductase (*dfrA7*) reported from the flock of lesser flamingoes, in this study, the DNA-sequencing results of the inserted gene cassette in class 1 integrons identified gene cassette harboring aminoglycoside acetyltransferase [*aac(3)-Id*] and aminoglycoside adenyltransferase (*aadA7*) genes (Sato *et al*, 2009). Detection of integron-borne *aac(3)-Id-aadA7* gene is not only reported in *S*. Kentucky (Doublet *et al*., 2008) but also reported elsewhere in *S*. Newport (Doublet *et al*., 2004) and *Vibrio fluvialis* (Ahmed *et al*., 2004).

Frequency of the antimicrobial resistance in this study was shown to vary with *Salmonella* serotype. Although *S*. Kentucky *(S.* I 8,20:i) was shown to be a highly occurring serotype (33%), it was also found to be the most resistant serotype in the current study, with 57% (12 of 21) of its strains resistant to at least one antimicrobial.

In addition, *S*. Kentucky is reported in the current study to be highly MDR with R-types including TeSuTMP and ClAxCpTeSuTMP respectively.

Previous studies in Tanzania have also detected *S*. Kentucky, but none of these studies conducted in Tanzania and elsewhere in East Africa reported the *S*. Kentucky in large proportions (33%) as depicted in the current study (Mhongole *et al*., 2017). While this study cannot confirm if *S*. Kentucky is adapted to food animals, but provides evidence that *S*. Kentucky had higher presence in food animals for the period when this study was conducted.

To our knowledge, there is no study has reported recovery of the *S*. Kentucky from the mastitic cow milk. Although it is not uncommon to isolate the co-existence of *Salmonella* serotypes with other mastitis causing organisms (Holschbach and Peek, 2018). In this study we have isolated *S*. Kentucky from a mastitic dairy cow and other sources (Supporting Table 1). .

Although *S*. Kentucky co-existed with other bacteria in the mastitic milk, the current study could not validate if the isolation of the *S*. Kentucky from the mastitic milk is a determinant for the condition in the dairy cow sampled as previously reported (Junaidu *et al*., 2011).

In summary, this study showed the occurrence of public health important MDR *Salmonella* isolates from different sources and locations.

This study clearly demonstrated the presence of epidemiologically important *Salmonella* serovars, including Heidelberg, Hadar, Infantis, Anatum, Virchow and Kentucky, which are frequently implicated in foodborne disease outbreak scenarios worldwide. Occurrence of clonal MDR *Salmonella* isolates in food animals and animal products indicates the potential public health risk associated with food animals as a source of MDR foodborne pathogens. However, this concern is more valid among communities where consumption of unpasteurized milk and undercooked or uncooked meat is a common is a practice. Therefore, we strongly recommend instituting sensitization to discourage consumption of unpasteurized milk and raw meat as well as improvement of hygienic practices throughout the food production chains as among the measures to limit transmission of MDR pathogens.

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SUPPORTING INFORMATION

Supporting Table 1. *Salmonella* isolates from different locations, animal species and sample types from March 2013 to March 2014.

Resistance types	Serovars	Phage type	Source (Number of isolates)	Sample (Number of isolates)	Year of isolation
Pans	S. Amager		Swine (1)	Feces (1)	2013
	S. Anatum		Bovine (3)	Feces (3)	2013
	S. Colindale		Bovine (2)	Feces (2)	2013
			Swine (1)	Feces (1)	2013
	S. Hadar		Bovine (2)	Feces (2)	2013
			Swine (2)	Feces (2)	2013
			Abattoir (1)	Floor swab (1)	2013
	S. Heidelberg	19	Swine (1)	Feces (1)	2013
	S. Ilala		Chicken (1)	Feces (1)	2013
	S. Infantis		Swine (1)	Feces (1)	2014
	S. Isangi		Abattoir (1)	Floor swab (1)	2013
	S. Karamoja		Bovine (1)	Carcass swab (1)	2013
			Abattoir (1)	Floor swab (1)	2013
	S. Leoben		Abattoir (1)	Sewage (1)	2013
	S. Manchester		Bovine (2)	Feces (2)	2013
	S. Roan		Bovine (1)	Feces (1)	2013
	S. Uganda		Swine (2)	Feces (2)	2013
	S. Virchow		Swine (2)	Feces (2)	2013
	S. Weltevreden		Swine (1)	Feces (1)	2013
	S. serovar 6,7:-:1,7		Bovine (1)	Feces (1)	2013
	S. serovar I 8,20:i:-		Swine (3)	Feces (3)	2013
			Bovine (6)	Milk (6)	2013
	S. serovar II 9,12:1,w:e,n,x		Chicken (1)	Feces (1)	2013
	S. serovar II 4,12,[27]:e,n,x:1,[5],7		Bovine (2)	Feces (2)	2013

Supporting Table 1. *Salmonella* isolates from different locations, animal species and sample types from March 2013 to March 2014 continued...

Resistance types	Serovars	Phage type	Source (Number of isolates)	Sample (Number of isolates)	Year of isolation
Ax	S. Hadar		Swine (1)	Feces (1)	2013
Cp	S. serovar I 8,20:i:-		Bovine (1)	Milk (1)	2013
Su	S. Cerro		Bovine (1)	Feces (1)	2013
AxTe	S. Heidelberg	19	Swine (1)	Feces (1)	2013
STe	S. Anatum		Swine (1)	Feces (1)	2013
AmAxCp	S. Ahuza		Bovine (1)	Carcass swab (1)	2013
	S. Colindale		Bovine (1)	Feces (1)	2013
	S. Heidelberg	Atypical	Bovine (1)	Carcass swab (1)	2013
	S. serovar I 6,7:-:-		Bovine (1)	Carcass swab (1)	2013
	S. serovar I 8,20:i:-		Bovine (1)	Carcass swab (1)	2013
AmAxTe	S. serovar I 8,20:i:-		Bovine (1)	Carcass swab (1)	2013
AmCpTe	S. serovar I:8,20:i:-		Swine (1)	Feces (1)	2014
SuTeTMP	S. serovar I:8,20:i:-		Chicken (1) Swine (6)	Feces (1) Feces (6)	2013 2013
AmAxCpSu	S. Heidelberg	19	Swine (1)	Feces (1)	2013
AmSSuTe	S. Hadar		Swine (1)	Feces (1)	2013
CipSuTeTMP	S. Kentucky*		Bovine (2)	Feces (2)	2013
ClAxCpSuTeTMP	S. serovar I 8,20:i:-		Chicken (1)	Cloacal swab (1)	2014

Abbreviations: Pans, pansusceptible; S. serovar I:8,20:i:- was further typed as *S.* Kentucky; **S.* Kentucky isolates carried *aac(3)-Id-aadA7* gene cassettes of size 1500 bp.; Ax, amoxacillin-clavulanic acid; Am, ampicillin; Cl, chloramphenicol; CIP, Ciprofloxacin; An, amikacin; Gm, gentamycin; Km, kanamycin; S, streptomycin; Su, sulfisoxasole; TMP, trimethoprim; Te, tetracycline; XLN, ceftiofur; Ce, ceftriaxone; Cp, cephalothin