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Prevalence, characterization and antimicrobial resistance profiles of *Salmonella* isolates from healthy broiler and free-range chickens in Morogoro, Tanzania

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Abstract
Background: *Salmonella enterica* is a group of bacteria that cause enteric and systemic infection in animals and humans worldwide.
Objectives: The study was conducted to determine the prevalence, characterization, and antimicrobial susceptibility profiles of Non-Typhoidal Salmonella in indigenous free-range and broiler chickens in Morogoro Municipality.
Methodology: A cross-sectional study was conducted from November 2019 to May 2020 whereby 384 cloaca swab samples from health chickens from Magadu, Mzinga, and Bigwa wards were collected. Identification was done by standard bacteriological methods, serotyping, and genetically confirmed by PCR using *Salmonella* specific primers pair and *Salmonella* enterica primer pair (*invA* and *iroB* gene primers). A sensitivity test was done using Ampicillin, Tetracycline, Imipenem, Gentamycin, Ciprofloxacin, Sulfamethoxazole trimethoprim, and Cefaclor antimicrobial discs. The descriptive statistic method was used for analysis and paired t-test assuming unequal variance was used for comparing overall prevalence of *Salmonella* spp between free range and broiler chickens.
Results: Out of 384 samples, 11 (2.9%) samples were confirmed to be Salmonella of which 8(4%) were from broilers and 3(1.6%) were from free range chickens. Of the 11 isolates, 8 were from group B serotypes and 3 isolates were from group D serotypes. Susceptibility results showed a variable level of sensitivity to the majority of antibiotics tested; however, levels of resistance were also found in 7/11 isolates resistant to Ampicillin, 4/11 isolates resistant to sulfamethoxazole-trimethoprim, and 3/11 isolates resistant to tetracycline. Three isolates were found to harbor Sulfamethoxazole(*sulII*) resistant gene.
Conclusion: This study revealed the presence of *Salmonella* carrier among chickens kept in Morogoro with antimicrobial resistances from both free range and broilers chickens. The results underline the importance of the biosecurity measures in the production and processing of chicken for human consumption, similarly, improvement of management is recommended to stop transmission of *Salmonella* from natural carriers to chicken as indicated by fecal carriers found.

Keywords; Nontyphoidal *Salmonella,* antimicrobial resistance, chickens.

INTRODUCTION

Salmonella enterica is a group of bacteria that cause enteric and systemic infection in animals and humans worldwide (Abdi *et al.,* 2017). Apart from being public health problem Salmonella infections cause huge financial losses in the poultry industry worldwide (Alvarez-Fernandez *et al.,* 2011). Host-specific *Salmonella* infections are known to cause systemic infection, typhoid in people, and Gallinarum and Pullorum disease in poultry (Kimathi, 2016). A wide range of Nontyphoidal *Salmonella* (NTS), is known to be harbored by poultry that transmits them to human beings as food-borne diseases (Castiglioni-Tessari *et al.,* 2012; Umeh&Enwuru, 2014).

In addition to being foodborne, *Salmonella* infections are also acquired through direct or indirect animal contact in homes, farm environments, or other public/ private settings (Moutoutou

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et al., 2017). NTS is estimated to cause about 93.8 million cases of gastroenteritis and about 155 thousand deaths in humans, 80.3 million cases were estimated as a foodborne origin (Majowz *et al.*, 2010; Antunes *et al.,* 2016) and it is estimated to cause about 3.7 billion dollars annual economic losses in the poultry industry worldwide (Nidaullah *et al.,* 2017).

Recently, NTS has been shown to contribute to the increased cases of bacteremia where *S.typhimurium* and *S. enteritidis* have been isolated (Muthumbi *et al*., 2015). In Tanzania, about 12,055 cases of salmonellosis were reported in Njombe Region, under Health Management Information System data of 2016 (Ngogo *et al.,* 2020). Most of this Salmonella spp have been shown to possess virulence genes located in the *Salmonella* Pathogenicity Islands (SPI) (Zishiri *et al.,* 2016).

Several studies on NTS have also linked Antimicrobial Resistance (AMR) to the exposure to antibiotics that are commonly used in the area. Resistance to commonly used antibiotics for the treatment of *Salmonella* infection in animals and humans has been studied and reported in many parts of the world (Mengistu *et al.,* 2014; Muthumbi *et al*., 2015; Manyi-Loh *et al.,* 2018). The uses of these antibiotics as growth-promoting agents, prophylaxis, or therapeutics in animal farming have been linked to the development and spread of resistant bacteria in animals, including zoonotic pathogens such as *S. typhimurium*,*S. infantis* and *S. enteritidis* (Van *et al.,* 2007; Andino *et al.,* 2015).

Rapid changes in the identification of *Salmonella* have raised questions about the types of *Salmonella* reported. The invention of genotypic and molecular techniques like pulsed-field electrophoresis, Polymerase chain reaction (PCR), ribotyping, and sequences have been useful addition in the epidemiological tracing of *Salmonella* infection (Christensen *et al.,* 1993; Lukinmaa *et al.,* 2004; Scaria *et al.,* 2008; Wise *et al.,* 2009). However, serotyping continues to be an important epidemiological tool for the identification of *Salmonella* serovars and making it possible to determine the prevalence (Castiglioni-Tessari *et al.,* 2012), despite the disadvantage of being unable to reveal genetic constitution and intra-serovars variations (Wise *et al.,* 2009). Similarly, different methods have been recommended for antimicrobial susceptibility testing of *Salmonella*, however, the disc diffusion method is a common one used worldwide by the Clinical and Laboratory Standards Institute (Mrope, 2017).

Most studies on the detection of *Salmonella* in chicken in Africa were carried out in specific areas and some on specific serovars like studies of Aragaw *et al.* (2010); Mdegela *et al.* (2000); &Wesonga *et al.* (2010). The information on the prevalence of non-typhoidal *Salmonella* among chickens in Morogoro is scarce and salmonellosis status from the farm level needs to be determined for its proper control and management. The chicken production systems are also known to use antimicrobials at different levels to tackle other diseases (Andino *et al.,* 2015; Boamah *et al.,* 2016). The effect of these in selecting antibiotic-resistant *Salmonella* is not precisely known. Thus, this study is aiming at establishing prevalence, antimicrobial resistance profile, and resistance gene determination in non-typhoidal *Salmonella* spp in Morogoro, Tanzania.

MATERIAL AND METHODS

Study Area

The study was conducted in Morogoro Municipality, in Morogoro Region between October 2019 and May 2020. The Municipal Council has one division, which is subdivided into 29 Administrative Wards. About 33% of the population is engaged in subsistence farming and livestock keeping (URT, 2013). Three wards of Magadu, Mzinga, and Bigwa were purposively selected as sampling areas based on the accessibility of the area and the availability of both chickens as study materials.

Study design and sample collection method

A cross-sectional study design was employed whereby a multistage random sampling technique was used. A total of 384 cloaca swab samples were collected from healthy free range and broilers chickens using sterile swabs. The swabs were taken in a sterile tube containing 10ml of selenite faecal broth and kept in a cooler box with an ice pack (4°C), then transported to Microbiology

Laboratory at the Department of Microbiology, Parasitology, and Biotechnology at Sokoine University of Agriculture (SUA) for further analysis.

Isolation and Identification of *Salmonella* spp.
Isolation of *Salmonella* spp from cloaca swab samples was done by using conventional and standard microbiological protocols as described by Wallace *et al*., (2009), PHE, (2014), using MacConkey agar (MCA), Blood Agar (BA), Brilliant Green agar (BGA) and Selenite Faecal Broth all from Himedia, India. All media were prepared aseptically and according to the manufacturer's instructions. Suspected *Salmonella* colonies were identified phenotypically from different media inoculated and by using the Gram stain method, biochemical tests (Triple Sugar Iron, Lysine iron agar, Simmons citrate agar, Motility, Glucose, Dulcitol, Maltose, Indole, Methyl red, Voges Proskauer test, and catalase test (IMVC)), serotyping and genetically confirmed by PCR.

Serotyping of suspected *Salmonella* isolates
Suspected *Salmonella* isolates were further confirmed by slide agglutination method using commercial *Salmonella*-specific polyvalent O (A-S) antisera, *Salmonella* O Group B antisera, and *Salmonella* O Group D antisera. Once the polyvalent group O was positive for agglutination, the isolates were tested in antisera against O groups B and D. Serotyping was done according to National Health Laboratory Quality Assurance and Training Centre, Standard Operating Procedure for Isolation and Identification of *Salmonella* spp.

DNA extraction of Salmonella isolates
Genomic DNA was extracted from the suspected Salmonella spp isolates by using Qiagen Kit (Germany). In brief 5-10 colonies from the pure culture plate were taken by using a sterile wire loop and added into the tube provided in the kit and the extraction process was done following manufacturer instructions. S. typhimurium (ATCC NO 14028) was also extracted and used as a positive control. 100µl of DNA was eluted in 1.5ml Eppendorf tube and stored in -20^{o}C freezer for further analysis.

Molecular Detection of Salmonellaspp (Salmonella Specific PCR)
DNA amplification for the invA gene and iroB gene was carried out using Salmonella Specific primer pair and Salmonella enterica serovars enterica primer pair (Table 1) obtained from (Inqaba Africa).PCR reaction was performed using Agilent Technologies (Sure cycler 8800)PCR machine.

The PCR reaction was performed in a total volume of 25µl that included 1.5µlDNA template,12.5µl One Taq w/standard buffer 2x concentrate (New England, BioLabs) PCR Master Mix, 1µlof each primer, and 9µl Nuclease free water. The following PCR running conditions were used: initial denaturation at 95˚C for 1 minute, followed by 34 cycles of denaturation at 95˚C for 30seconds, annealing at 58˚C for 30 seconds, extension at 72˚C for 30 seconds, and final extension at 72˚C for 5 minutes (Zishiri et al., 2016, Jamshindi et al., 2009). Gel electrophoresis (1.5 % agarose) using a consort EV 243 electrophoresis system was used to analyse the PCR products and 100 bp DNA ladder (New England, BioLabs) was used as a size standard. The agarose gel was visualized under UV trans-illuminator (Uvitec) and the picture was taken using camera.

Antibiotics Susceptibility Testing of *Salmonella*spp
Disc diffusion method was used to determine antimicrobial susceptibility of the *Salmonella* spp by the Clinical and Laboratory Standards Institute for susceptibility testing (Liofilchem, 2017; CLSI 2018). In this study the following antibiotics were tested Ampicillin (AMP 25µg), cefaclor (CF 30µg), Imipenem (IMI 10µg), Gentamycin (Gn 10µg), Ciprofloxacin (CIP 5µg), Sulfamethoxazole-

Trimethoprim (SXT 25µg) and Tetracycline (TE 30µg).In brief, the test was conducted by preparing the inoculum of each isolate, and the control (*E. coli* ATCC 25922) and turbidity of bacterial suspension were adjusted to 0.5 Standard McFarland solution.

Muller Hinton agar media (Oxoid) was used and was prepared according to the manufacturers' instructions. The suspension of each isolate was spread on a dried Muller Hinton agar plate using a sterile swab. Selected antibiotic discs (Liofilchem-Italy) were then applied to the surface of the inoculated plates using sterile forceps. The plates were then incubated at 37°C for 18-24 hours. Antibiotic profiles were determined based on zones of inhibition shown by each drug. Zones of inhibitions were measured using a ruler and recorded as diameter in mm and interpreted as Sensitive (S), Resistant (R), and Intermediate (I) (Liofilchem, 2017; CLSI, 2018).

Detection of Antibiotic Resistance Genes

PCR was used to detect resistance genes from extracted Salmonella genomic DNA. Three different resistance genes were detected by using specific primers as shown in Table 1. The genes include the Ampicillin resistant gene (pse-l gene), Tetracycline resistant gene (tetA gene), and Sulfamethoxazole Trimethoprim resistant gene (sulII gene).PCR reaction was performed using Agilent Technologies (Sure cycler 8800) PCR machine. The PCR reaction was performed in 34 cycles with a total volume of 25µl that included 1.5µl DNA template,12.5µl One Taq w/standard buffer 2x concentrate (New England, BioLabs) PCR Master Mix, 1µl of each primer, and 9µl Nuclease free water.

The following PCR running conditions were used: Ampicillin resistant gene (pse-1 gene) with initial denaturation at 94 °C for 12 min, denaturation at 94 °C for 1min, annealing at 57 °C for 30 seconds, and extension at 72 °C for 5 min. Tetracycline resistant gene (tetA gene)with initial denaturation at 94 °C for 5 min, denaturation at 94 °C for 25 seconds, annealing at 55 °C for 30 seconds, extension at 72 °C for 50 seconds, and a final cycle at 72 °C for 5 min. Sulfamethoxazole Trimethoprim resistant gene (sulII gene) with initial denaturation at 94 °C for 5 min, denaturation at 94 °C for 25 seconds, annealing at 52 °C for 30 seconds, extension at 72 °C for 50 seconds, and a final cycle at 72 °C for 5 min (Adesijiet al., 2014; Zishiriet al., 2016). Gel electrophoresis (1.5 % agarose) was used to analyse the PCR products and 100 bp DNA ladder (New England, BioLabs) was used as a size standard. The agarose gel was visualized under UV trans-illuminator (Uvitec) and the picture was taken using camera.

Table 1.Primer sets used

Drugs/*Salmonella* genes	Gene	Sequence	Bp	References
Ampicillin	*pse-1*	F; CGCTTCCCGTTAACAAGTAC R; CTGGTTCATTTCAGATAGCG	419	Zishiri *et al*.,2016
Tetracycline	*tet A*	F:GCTACATCCTGCTTGCCTTC R:CATAGATCGCCGTGAAGAGG	210	Zishiri *et al*.,2016
Sulfamethoxazole	*SulII*	F; CCTGTTTCGTCCGACACAGA R ;GAAGCGCAGCCGCAATTCAT	667	Adesiji *et al*.,2014
InvA	*InvA*	139F;GTGAAATTATCGCCACGTTCGGGCAA 141R; TCATCGCACCGTCAAAGGAACC	284	Jamshindi *et al*.,2009
iroB gene	*iroB gene*	F:TGC GTA TTC TGT TTG TCG GTCC R:TAC GTT CCC ACC ATT CTT CCC	606	Zishiri *et al*.,2016

Data Analysis

All the data were entered into a Microsoft Excel spreadsheet whereby the descriptive statistic method was used for analysis. A paired t-test assuming unequal variance was used for comparing overall prevalence of *Salmonella* spp between free range and broiler chickens.

RESULTS

Isolation and Identification of *Salmonella* spp

Results found that 11 isolates of *Salmonella* spp were recovered from 384 collected cloaca swab samples from Magadu, Mzinga, and Bigwa Wards. Cultural and morphological growth characteristics of *Salmonella* were used in the primary identification of *Salmonella* as recorded in Table 2. Percentage prevalence of *Salmonella* spp in broilers and free-range chickens were determined in Table 4.

Table 2: Cultural and morphological growth characteristic results of *Salmonella* spp.

Culture media	BA	MCA	BGA	Motility	TSI	LIA
Colony characteristic	Greyish/whitish, non-haemolytic and medium size colonies	Pale, colourless, smooth, transparent, raised colonies	Red colonies with bright red background	Motile	Yellow butt, blackening, gas formation	Purple butt, blackening, gas formation

Biochemical Test Results

Different biochemical tests were done and the results obtained were summarized in Table 3.

Table 3:**Results of biochemical characteristics of *Salmonella*spp**

	Biochemical Reaction							
	Indole test	MR Test	VP Test	Glucose	Dulcitol	Maltose	Citrate utilisation test	Catalase test
Overall reaction	-	+	-	+	+, -	+	+, -	+

+ = positive reaction, - = negative reaction, +, - = some positive and some negative

Serotyping Results

All the *Salmonella* isolates (11/11) were confirmed positive by serotyping using polyvalent O (A-S) antisera. 8/11 isolates were under serogroup B and 3/11 isolates were under serogroup D. The most prevalent serogroup identified in this study was serogroup B.

Table 4: Prevalence of *Salmonella* spp among selected wards within Morogoro Municipality

Wards	No. sampled/Total number		Positives	Overall prevalence (%)		Alpha	P-value	Calculated/ Test statistic	Critical/ Tabulated t-value
	Broilers	Free range chicken		Broilers	Free range	0.05	0.45	0.86	3.18
Magadu	65/136	71/136	3/136(2.2%)	4.04	1.61				
Mzinga	83/133	50/133	2/133(1.5%)						
Bigwa	50/115	65/115	6/115(5.2%)						
TOTAL	**198/384**	**186/384**	**11/384(2.9%)**	**2.9**					

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Molecular Detection of *Salmonella*spp

For the *invA* gene detection, results showed that all eleven (11/11) samples were genetically confirmed to be *Salmonella* spp. The amplicon size was 284bp Fig. 1.

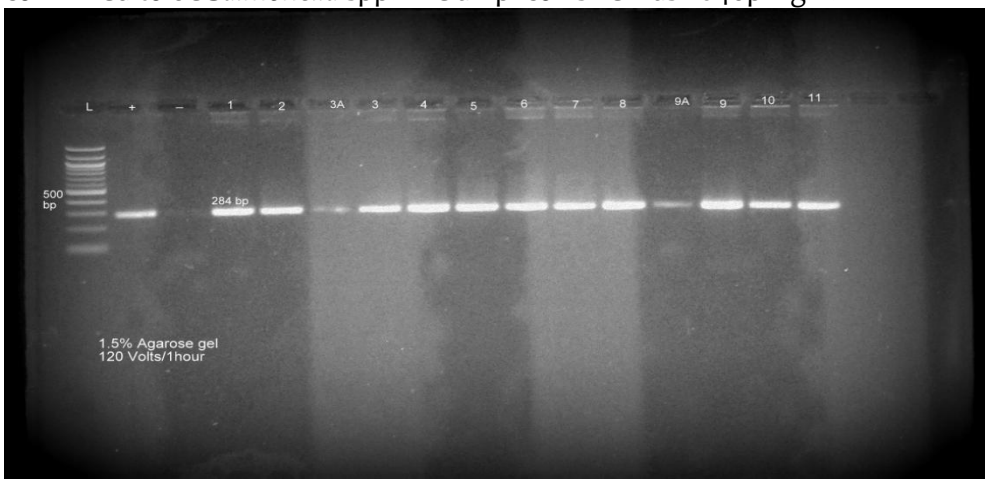

Figure 1: *Salmonella* gene detection, 284bp detected (*invA* gene detection)
L = Ladder, + = Positive control - = Negative control, Lane 1, 4, 5, = Free range positive isolate and Lane 2,3,6,7,8,9,10,11 = Broilers positive isolates

For the *iroB* gene detection, the results showed that all eleven (11/11) samples were confirmed to be *Salmonella* enterica spp, with 606 bp detection Fig.2.

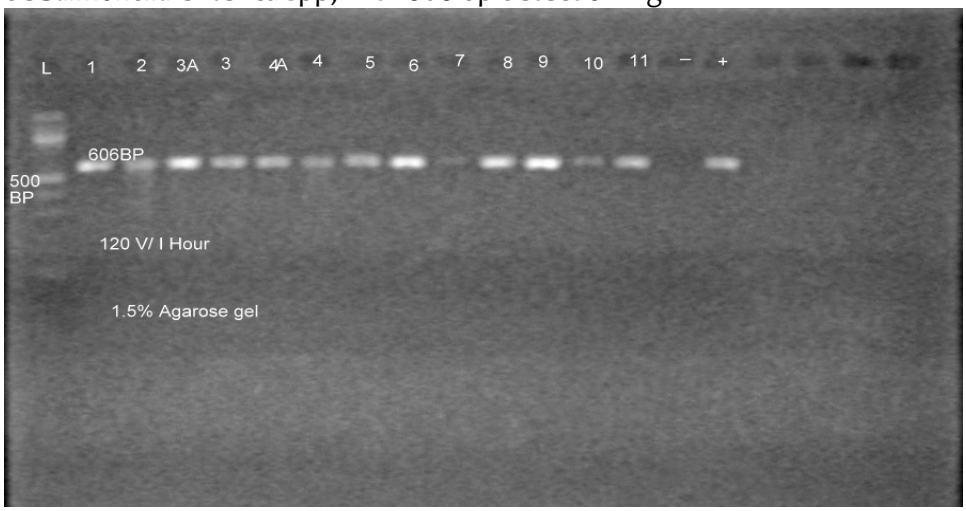

Figure 2: *Salmonella* enterica gene detection, 606bp detected (*iroB* gene detection)
L = Ladder, Lane 1, 4, 5, = Free range positive isolate and Lane 2,3,6,7,8,9,10,11 = Broilers positive isolates, - = Negative control, + = Positive control

Antimicrobial Susceptibility Test

Seven antibiotics were tested and the results obtained were as indicated in **Table 5** and **Appendix 1**.

Antibiotics	Free range profiles		sensitivity	Broilers profiles		sensitivity	Overall sensitivity profiles		
	R	S	I	R	S	I	R	S	I
Ampicillin	2/3	1/3	0	5/8	3/8	0	7/11	4/11	0

Gentamycin	0	3/3	0	0	8/8	0	0	11/11	0
Tetracycline	1/3	2/3	0	2/8	6/8	0	3/11	8/11	0
Sulfamethoxazole Trimethoprim	0	3/3	0	4/8	4/8	0	4/11	7/11	0
Imipenem	0	2/3	1/3	0	7/8	1/8	0	9/11	2/11
Ciprofloxacin	0	3/3	0	0	8/8	0	0	11/11	0
Cefaclor	0	3/3	0	0	5/8	3/8	0	8/11	3/11

Table 5: Antimicrobial susceptibility results from the isolated *Salmonella* spp
R = Resistance, S = Susceptible, I = Intermediate

Detection of *Salmonella* resistance gene by PCR

Three different resistance genes were detected by using specific primers as shown in Table 1. The genes include the ampicillin resistant gene (pse-l gene), Tetracycline resistant gene (*tetA* gene), and Sulfamethoxazole Trimethoprim resistant gene (*sulII* gene). The results showed no resistance genes for tetracycline and ampicillin detected while 3/11 isolates carried sulfamethoxazole resistance gene (*sulII*gene) Fig. 4 below.

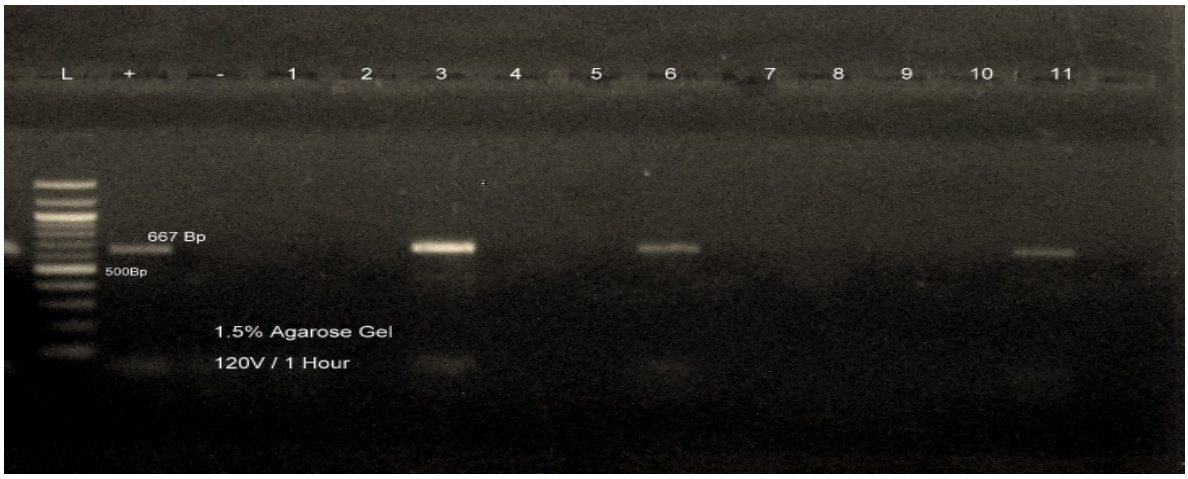

Figure 1: Sulfamethoxazole resistant gene amplification, 667 bp, L = Ladder, + = Positive control - = Negative, 1-11 = isolates (3, 6, 11 positive *SulII* broilers isolates), 1, 2, 4, 5, 7, 8, 9, and 10= Negative *SulII* gene

DISCUSSION

Overall, the present study shows the presence of *Salmonella* species in the gastrointestinal tract of healthy indigenous free-range and broiler chickens in Morogoro Municipality. About 3% of the chicken were found to carry *Salmonella* and prevalence was found at 4% in broiler chickens and 1.6% in indigenous free-range chickens. However, statistically, there were no significant differences between the two groups (P>0.05). Serotyping confirmed 11 isolates as positive with polyvalent O sera (A-S) and of these 8 isolates were from group B while 3 isolates were from group D. PCR results confirmed eleven (11) isolates as a *Salmonella* spp using both *invA* and *iroB* gene primer. A variable level of sensitivity to the majority of antibiotics tested was found; however, a level of resistance was also found with 7 isolates resistant to Ampicillin, 4 isolates resistant to sulfamethoxazole-

trimethoprim (co-trimoxazole), and 3 isolates resistant to tetracycline. Screening for resistant genes detected *SulII* with 667bp amplification.

The current study found that serogroup B and serogroup D were the most isolated from the cloaca of chicken. These results support those of Al Mamun *et al*., (2017) and Mridha *et al*., (2020), who found serogroup B (O: 4, 5, 27) and serogroup D (O: 9, 46) in the most isolates from chicken cloaca and carcasses. However, these findings differ in the ratio of serogroup B to D in that their findings showed that there was more D serotype isolate than B while the current study showed more B serotype than D serotype. The B serogroup was the most common serotype involved in animals and human salmonellosis was frequently isolated before the outbreak of *S. enteritidis* (Oliveira *et al*., 2006). Generally, these serogroups (B and D) contain serovars that can infect a wide variety of animal hosts and they are widely distributed in the environment hence increasing their prevalence in the food chain (Liljebjelke *et al*., 2005).

The prevalence of 2.9% *Salmonella* cloacal carriers found was low compared to other studies elsewhere in the world including Iran with 5.8% from cloaca swabs, serovar typhimurium, and serovar enteritidis as the prevalent ones (Jafari *et al*., 2007), Kenya 3.6 % from faecal samples, serovar typhimurium, and serovar enteritidis as the prevalent ones (Nyabundi *et al*., 2017), Brazil with 25% from cloaca swabs, *S. typhimurium* and *S. enteritidis* as prevalent serovars (Paião*et al*., 2013), Wesonga *et al*. (2010), in Kenya and Alam*et al*. (2020), in Bangladesh, 12.5% (*S. typhimurium*) and 35% (*S. typhimurium*) prevalence of *Salmonella* in chicken cloaca swabs respectively. These prevalences were high as compared to the prevalence of the present study possibly because of the analysis method used whereby pre-enriched multiplex polymerase chain reaction (m-PCR) assay was used and it is a specific and rapid alternative method for *Salmonella* spp identification (Paião *et al*., 2013) as compared to this study which employed culture-based technique (colony isolation) then confirmed by PCR.

Further studies are recommended to compare different methods in the given systems. Also, sampling of chicken at different ages pose the possibility of finding contamination rate based on ages as newly hatched chicks were very vulnerable to infection with *Salmonella* than the older chicken (Sterzo *et al*., 2005), the current study sampled moderate adults' chickens and not on chicks, so this should be taken into consideration while studying this prevalence. The prevalence variations may also be due to several management factors such as hygiene, sanitation, and biosecurity of the farms. For the better prevalence establishment, different sample matrices such as chicken feed samples, hand swabs of the chicken handler, and chicken drinking water are encouraged (Akond*et al*., 2012; Abdi *et al*., 2017). The current study sampled only a faecal swab sample from the chicken cloaca.

A comparison of the isolation rate between broiler and free-range chicken showed a higher prevalence in the broiler (4%) than in free-range chicken (1.6%) however, the difference (P>0.05) was not statistically significant. These findings are in line with those of Kindu and Addis (2013), who found the prevalence of *Salmonella* infection to be higher in indoor chickens (42.7%) than in free-ranging (40.8%) but without any statistically significant. Presumably free-range chicken is at higher risk of bacterial contamination due to direct contact with the transmitting vectors such as rodents, insects, and other animals (Liljebjelke *et al*., 2005). This study showed that intensively managed chicken (broilers) is more likely to carry *Salmonella* than freely range chickens (indigenous), this is due to the reason that chicken kept indoors have lower immunity to diseases and poor management experienced by the chicken owners exposes them to the various source of *Salmonella* contamination (Kindu & Addis, 2013). Broader studies are recommended to compare the two systems for a sound conclusion regarding the variation observed.

Antibiotic sensitivity results showed that *Salmonella* isolates had high sensitivity to the majority of the antibiotic tested. These findings are in line with the findings by Mrope (2017), who found sensitivity to 100% in Ciprofloxacin, Imipenem and Sulfamethoxazole, Gentamycin at 91% and Cefaclor at 82%. Also, Naik *et al*. (2015), found high sensitivity profile in Ciprofloxacin while 96.87%

and 96.87% were sensitive to Gentamicin and Imipenem respectively. These findings contradict some of the studies done worldwide including a study by Al-Ledeni *et al*. (2014), and Ziba *et al*. (2020), which showed resistance to Ciprofloxacin and Gentamycin up to 60.5% and 31.6% respectively

In this study, a high level of resistance was found to Ampicillin, Sulfamethoxazole-Trimethoprim, and Tetracycline. These findings are consistent with those of Bacci *et al*. (2012); Kagambega *et al*. (2013); and Moe *et al*. (2017), who found that most *Salmonella* isolates are resistant to Ampicillin, Tetracycline, and Sulfamethoxazole. These antibiotics are widely used to treat bacterial infections in both people and animals and they are highly prescribed in Tanzanian hospitals to treat a variety of bacterial infections (Murutu, 2016). Mubito *et al*. (2014), found that these are the most used drugs in poultry production in Tanzania, and they are widely used as therapy, prophylaxis, or growth promotion. The presence of resistance to these antibiotics might be related to selection pressure due to antibiotic usage, or due to the occurrence of resistant clonal strains that were successfully disseminated within populations (Katakweba *et al*., 2012; Wigley, 2014). It is also possible to hypothesize that free-range chickens can be exposed to drug residues due to improper disposal from the environment and thus aid in selection pressure (Wesonga *et al*., 2010; Kissinga *et al*., 2018). However, with a small sample size, caution must be applied in interpretation, because no evidence of antimicrobial use was established. There is little use of antimicrobials in free-range chicken which were also shown to carry resistant isolates.

Another important finding, though to a small proportion, is the presence of Multiple Drug Resistance (MDR) isolates. Out of the 11 isolates, 3 were found to be resistant to Ampicillin, Sulfamethoxazole Trimethoprim, and Tetracycline. These findings are a mirror to those of previous studies by Kagambega *et al*. (2013); and Mengistu *et al*. (2014), which found that resistance to Ampicillin, Sulfamethoxazole Trimethoprim, and Tetracycline were the common MDR phenotypes. This study was unable to demonstrate resistance to Ciprofloxacin, Gentamycin, Imipenem and Cefaclor as shown by Adesiji *et al*. (2014), and Ziyate *et al*. (2016). It is difficult to explain this result, but it may be related to geographical variation and the types of serovars isolated. Being rodent-borne bacteria, further work is required to establish if a rodent is exposed to antimicrobial in addition to the type of bacteria found in the guts.

The antimicrobial resistance gene results found that 3 isolates in broilers contain Sulfamethoxazole (*sulII*) resistance gene and were unable to show the presence of the resistance gene for the Ampicillin (pse-1) and Tetracycline (*tetA*). These results agree with those of Bacci *et al*. (2012), who found the pse-1 gene absent in all the isolates from chicken carcasses (skin swabs) and a low percentage of sulII gene while Zishiri *et al*. (2016), found a high percentage of sulII genes from the chicken meat. However, phenotypic results showed resistance to Ampicillin, Tetracycline, and Sulfamethoxazole but only Sulfamethoxazole carried the resistant gene. This is because the phenotype of most isolates is influenced by specific and non-specific resistance mechanisms such as lower membrane permeability and a high active efflux (Bacci *et al*., 2012). Surprisingly, *the tetA* gene was not found even though they are widely distributed in *Salmonella* strains circulating in animals and were found on plasmids as well as on the chromosome (Frech & Schwarz, 2000; Pezzella *et al*., 2004). According to Katakweba *et al*. (2018), *sulII* is the most common gene encoding sulphonamides resistances. Sulfamethoxazole, Tetracycline, and Ampicillin were the most commonly used antimicrobials in the study area hence the possibility of detecting these genes was high.

To conclude, the presence of *Salmonella* in this study suggests that rodent exposure, public health risk contamination of meat, and proper cooking (if not done) are the possible source of transmissions of *Salmonella* from natural carriers to chicken as indicated by fecal carriers found. This work contributes to the existing knowledge of salmonellosis in chickens, highlighting non-host specific *Salmonella* which cannot cause disease in chickens but pose public health risks and has added to their AMR risks to people and animals. Finally, the number of limitations need to be

considered. First, this study sampled only chicken cloaca swabs, multiple sampling sources such as hands swab of the chicken handler, feeds and chicken drinking water could have created a nice ground for the broad prevalence establishment and antimicrobial susceptibility. Second, the sample size used was small, a larger sample size is encouraged. Third, in this study, only a cross-sectional study design was used, a cross-sectional prospective longitudinal study could help to have a variable number of samples at different periods of time.

Ethical Issues: The permission to carry out this study was granted by the Morogoro Municipal Livestock Officer while ethical approval for the study was given by the Ethical Committees of Sokoine University of Agriculture, Tanzania with reference No. SUA IDPRTCIR1186 was approved on 29th January 2020. Voluntary participation of each chicken farmer was obtained after being informed about the study's purposes.

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Appendix 1: Antimicrobial susceptibility results profiles based on zones of inhibition (mm)

Source	No	AMP 10 µg S ≥ 17 I:14-16 R ≤ 13		GENT10 µg S ≥ 15 I:13-14 R ≤ 12		TE30 µg S ≥ 15 I:12-14 R ≤ 11		STX25 µg S ≥ 16 I:11-15 R ≤ 10		IMI10 µg S ≥ 23 I:20-22 R ≤ 19		CIP5µg S ≥ 21 I:16-20 R ≤ 15		CF30 µg S ≥ 18 I:15-17 R ≤ 14	
Free range	118	0	R	25	S	22	S	22	S	29	S	35	S	19	S
Broiler	301	0	R	24	S	22	S	18	S	34	S	35	S	17	I
Broiler	353	23	S	26	S	10	R	30	S	52	S	51	S	18	S
Free range	121	19	S	25	S	10	R	20	S	51	S	46	S	19	S
Free range	102	0	R	30	S	32	S	27	S	21	I	35	S	20	S
Broiler	372	0	R	27	S	34	S	22	S	50	S	39	S	15	I
Broiler	166	0	R	26	S	24	S	0	R	40	S	35	S	21	S
Broiler	291	31	S	22	S	21	S	30	S	45	S	45	S	20	S
Broiler	199	32	S	26	S	18	S	9	R	38	S	35	S	18	S
Broiler	308	0	R	28	S	16	S	0	R	21	I	34	S	22	S
Broiler	302	0	R	18	S	11	R	0	R	46	S	35	S	15	I