

2022-06-13

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

Munuo, Lidia

AJOL

<https://dx.doi.org/10.4314/thrb.v23i1.6>

Provided with love from The Nelson Mandela African Institution of Science and Technology

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

Lidia A. Munuo^{1,*}, Abdul A. S. Katakweba², Beatus M. Lyimo³ and Amandus P. Muhairwa¹

¹College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3015, Morogoro, Tanzania.

²Institute of Pest Management, Sokoine University of Agriculture, P.O Box 3110, Morogoro, Tanzania

³School of Life Science and Bioengineering, Nelson Mandela African Institution of Science and Technology, P.O Box 447, Tengeru, Arusha, Tanzania.

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, Mzingu, 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(*sulIII*) 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

*Corresponding author, Lidia A. Munuo: mankalydia@gmail.com

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°C freezer for further analysis.

Molecular Detection of *Salmonella* spp (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 1.5µl 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: initial denaturation at 95°C for 1 minute, followed by 34 cycles of denaturation at 95°C for 30 seconds, 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-I* 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/ <i>Salmonella</i> genes	Gene	Sequence	Bp	References
Ampicillin	<i>pse-1</i>	F; CGCTTCCCGTTAACAAGTAC R; CTGGTTCATTTAGATAGCG	419	Zishiri et al .,2016
Tetracycline	<i>tet A</i>	F:GCTACATCCTGCTTGCCTTC R:CATAGATCGCCGTGAAGAGG	210	Zishiri et al .,2016
Sulfamethoxazole	<i>SulII</i>	F; CCTGTTTCGTCCGACACAGA R; GAAGCGCAGCCGCAATTCAT	667	Adesiji et al .,2014
<i>InvA</i>	<i>InvA</i>	139F;GTGAAATTATCGCCACGTTCCGGCAA 141R; TCATCGCACCGTCAAAGGAACC	284	Jamshindi et al .,2009
<i>iroB</i> gene	<i>iroB</i> 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. number	sampled/Total	Positives	Overall prevalence (%)	Alpha	P-value	Calculated/ Test statistic	Critical/ Tabulated t-value
	Broilers	Free range chicken		Broilers	Free range	0.05	0.45	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				

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.

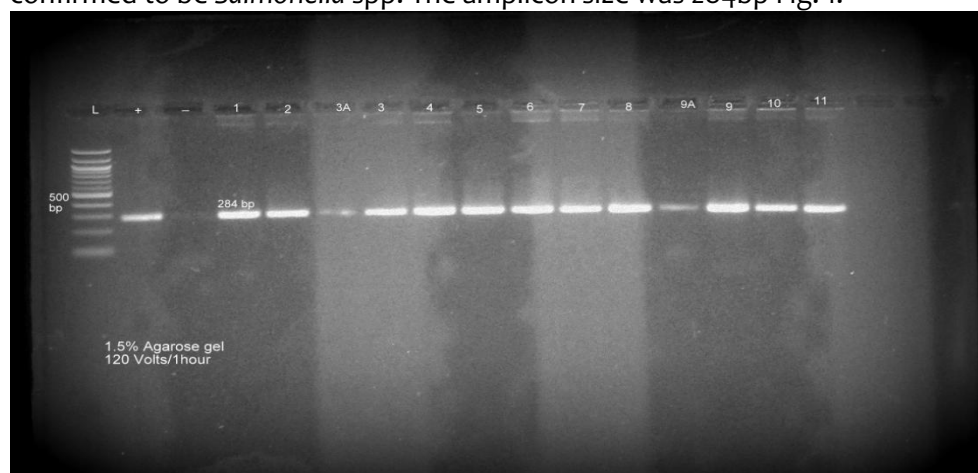


Figure 1: *Salmonella* gene detection, 284bp detected (*invA* gene detection)

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.

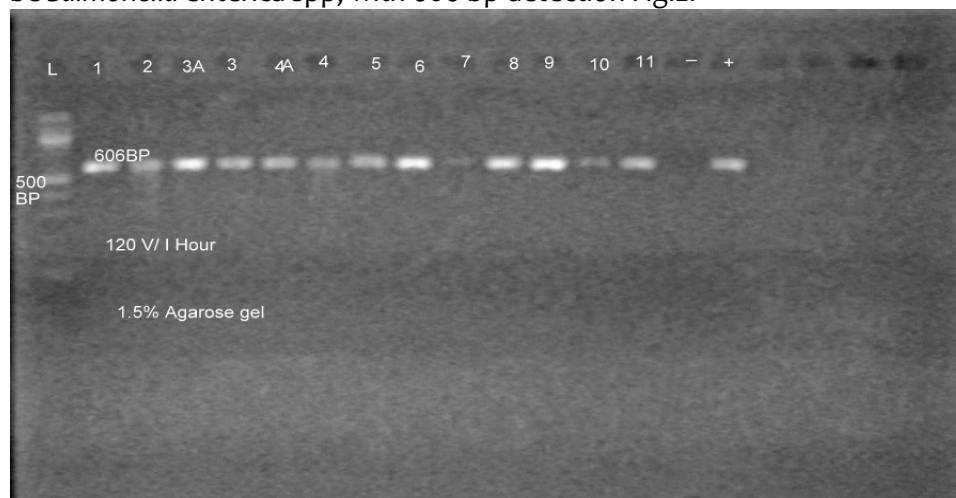


Figure 2: *Salmonella enterica* gene detection, 606bp detected (*iroB* gene detection)

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 sensitivity profiles			Broilers sensitivity profiles			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-I* gene), Tetracycline resistant gene (*tetA* gene), and Sulfamethoxazole Trimethoprim resistant gene (*sulIII* gene). The results showed no resistance genes for tetracycline and ampicillin detected while 3/11 isolates carried sulfamethoxazole resistance gene (*sulIII* gene) Fig. 4 below.

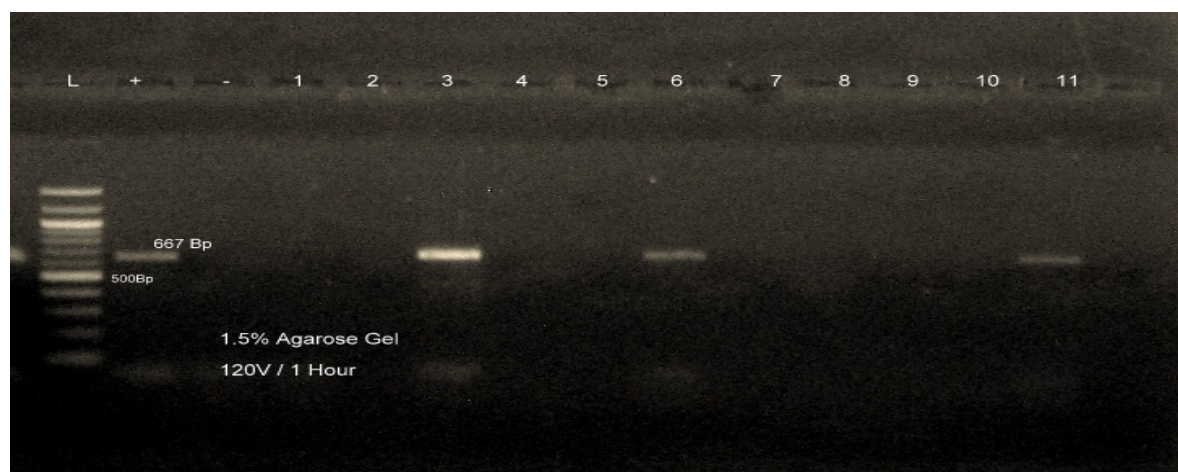


Figure 1: Sulfamethoxazole resistant gene amplification, 667 bp, L = Ladder, + = Positive control - = Negative, 1-11 = isolates (3, 6, 11 positive *SulIII* broilers isolates), 1, 2, 4, 5, 7, 8, 9, and 10= Negative *SulIII* gene

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 *SullI* 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 Alamet *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 (Akondet *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.

Acknowledgment: The authors acknowledge the owners and managers of the chicken farms for allowing this study to be conducted on their farms and the Nelson Mandela African Institution of Science and Technology for support during research work. Technical staff in the Department of Veterinary Medicine and Public health and Department of Microbiology, Parasitology, and Biotechnology at Sokoine University of Agriculture for their laboratory assistance. The authors also appreciate the contribution of each one who made this study possible.

Conflict of interest: Authors do not have any conflict of interest and all had equal contributions to the Manuscript.

References

- Abdi, R. D., Mengstie, F., Beyi, A. F., Beyene, T., Waktole, H., Mammo, B., Ayana, D. and Abunna, F. (2017). Determination of the sources and antimicrobial resistance patterns of *Salmonella* isolated from the poultry industry in Southern Ethiopia. *BioMed Central Infectious Diseases* 17(352): 1 – 12
- Adesiji, Y. O., Deekshit, V. K. and Karunasagar, I. (2014). Antimicrobial-resistant genes associated with *Salmonella* spp. isolated from human, poultry, and seafood sources. *Food Science and Nutrition* 2(4): 436 – 442.
- Akond, M. A., Shirin, M., Alam, S., Hassan, S. M. R., Rahman, M. and Hoq, M. (2012). Frequency of drug resistant *Salmonella* spp. Isolated from poultry samples in Bangladesh. *Stamford. Journal of Microbiology* 2: 15–19
- Alam, S. B., Mahmud, M., Akter, R., Hasan, M., Sobur, A., Nazir, K. N. H., Rahman, M. (2020). Molecular Detection of Multidrug Resistant *Salmonella* Species Isolated from Broiler Farm in Bangladesh. *Pathogens*, 9(3), 201, 1-12pp
- Al- Ledeni, A. A., Khudor, M. H. and Oufi, N. M. (2014). Isolation and Identification of *Salmonella* spp. from poultry farms by using different techniques and characterization of their antimicrobial susceptibilities. *Basic Journal of Veterinary Research* 1(1): 246-259.
- Al Mamun Md. A, Kabir S. M. L, Islam Md. M, Lubna M, Islam S.K S, Akhter A. H. M T and Hossain Md. M. (2017). Molecular identification and characterization of *Salmonella* species isolated from poultry value chains of Gazipur and Tangail districts of Bangladesh. *African Journal of Microbiology Research*, 11(11), 474–481.
- Andino, A. and Hanning, I. (2015). *Salmonella enterica*: Survival, Colonization, and virulence differences among serovars. *The Scientific World Journal* 520179: 16pp.
- Antunes P, Mourão J, Campos J and Peixe L. (2016). Salmonellosis: the role of poultry meat. *Clinical Microbiology and Infection*; 22: 110–121

- Aragaw, K., Terefe, L. and Aberal, M. (2010). Prevalence of *Salmonella* Infection in Intensive Poultry Farms in Hawassa and Isolation of *Salmonella* species from sick and dead chickens. *Ethiopian Veterinary Journal* 14(2): 115 – 124.
- Alvarez-Fernandez, E., Calleja, C. A., Garcia-Fernandez, C. and Capita, R. (2011). Prevalence and antimicrobial resistance of *Salmonella* serotypes isolated from poultry in Spain: Comparison between 1993 and 2006. *International Journal of Food Microbiology* 153: 281–287.
- Bacci, C., Boni, E., Alpighiani, I., Lanzoni, E., Bonardi, S. and Brindani, F. (2012). Phenotypic and genotypic features of antimicrobial resistance in *Salmonella enterica* isolated from chicken meat and chicken and quail carcasses. *International Journal of Food Microbiology* 160(1): 16 – 23.
- Boamah, V. E., Agyare, C., Odoi, H. and Dalsgaard, A. (2016). Practices and factors influencing the use of antibiotics in selected poultry farms in Ghana. *Journal of Antimicrobial Agents* 2(2): 1 – 8.
- Castiglioni-Tessari, E. N., IbaKanashiro, A. M. Z., Stoppa, G. F., L., R., De Castro, A. G. M and Cardoso, P. A. L. S. (2012). Important Aspects of *Salmonella* in the Poultry Industry and in Public Health. *Salmonella - A Dangerous Foodborne Pathogen*. (Edited by Barakat, S. M. M), University of Campus Step, China. pp. 181 – 207.
- Clinical and Laboratory Standard Institute (CLSI) (2018). Performance standards for antimicrobial Susceptibility Testing. 28th Edition. CLSI supplement M100. Wayne, PA. 296pp.
- Christensen, J. P., Olsen, J. E., & Bisgaard, M. (1993). Ribotypes of *Salmonella enterica* serovar Gallinarum biovars Gallinarum and Pullorum. *Avian Pathology*, 22(4), 725–738.
- Frech, G., and S. Schwarz. (2000). Molecular analysis of tetracycline resistance in *Salmonella enterica* subsp. *enterica* serovars Typhimurium, Enteritidis, Dublin, Cholerasesuis, Hadar and Saintpaul: construction and application of specific gene probes. *Journal of Applied Microbiology*. 89:633-641.
- Jafari, R. A., Ghorbanpour, M. and Jaideri, A. (2007). An investigation into *Salmonella* infection status in backyard chickens in Iran. *International Journal of Poultry Science* 6(3): 227 – 229.
- Kagambèga, A., T. Lienemann, L. Aulu, A.S. Traoré, N. Barro, A. Siitonen, and K. Haukka. (2013). Prevalence and characterization of *Salmonella enterica* from the feces of cattle, poultry, swine and hedgehogs in Burkina Faso and their comparison to human *Salmonella* isolates. *Biomed Central Microbiology*. 13:253–260.
- Katakweba, A. A. S., Mtambo, M. M. A., Olsen, J. E. and Muhairwa, A. P. (2012). Awareness of human health risks associated with the use of antibiotics among livestock keepers and factors that contribute to selection of antimicrobial resistance bacteria within livestock in Tanzania. *Livestock Research for Rural Development* 24(10): 1 – 14.
- Katakweba, A. A. S., Muhairwa, A. P., Lupindu, A. M., Damborg, P., Rosenkrantz, J. T., Minga, U. M., Mtambo, M. M. A. and Olsen, J. E. (2018). First Report on a Randomized Investigation of Antimicrobial Resistance in Fecal Indicator Bacteria from Livestock, Poultry, and Humans in Tanzania. *Microbial drug resistance* Volume 24, (3) 260-268.
- Kimathi, R. K. (2016). Detection of *Salmonella* species in Kenya using 16s rRNA PCR coupled to high resolution melting point assay. Dissertation for Award of MSc Degree at University of Nairobi, Kenya, 94pp.
- Kindu A, Addis M. (2013). A survey on *Salmonella* infection among chicken flocks in Jimma town. Ethiopia. *World Applied Sciences Journal*; 21 (10):1415–20.
- Kissinga H.D, Mwombeki F, Said K, Katakweba A.A.S ,Nonga H. E and Muhairwa A. P (2018). Antibiotic susceptibilities of indicator bacteria *Escherichia coli* and *Enterococci* spp. isolated from ducks in Morogoro Municipality, Tanzania. *BioMed Central, Res Notes* 11:87 pp 1-6.

- Liljebjelke, K. A., Hofacre, C. L., Liu, T., White, D. G., Ayers, S., Young, S., & Maurer, J. J. (2005). Vertical and horizontal transmission of *Salmonella* within integrated broiler production system. *Food borne pathogen diseases*, 2(1), 90–102.
- Liofilchem - Antibiotic Disc Interpretative Criteria and Quality Control - Rev.13 / 20.11.2017. Downloaded on 15th January 2020.
- Lukinmaa S, Nakari UM, Eklund M, Siitonen A (2004). Application of molecular genetic methods in diagnostics and epidemiology of food-borne bacterial pathogens. *Acta Pathologica, Microbiologica, et Immunologica Scandinavica (APMIS)* 112:908–29.
- Majowicz, S. E., Musto, J., Scallan, E., Angulo, F. J., Kirk, M., O'Brien, S. J. and Hoekstra, R. M. (2010). The global burden of non-typhoidal *Salmonella* Gastroenteritis. *Clinical Infectious Diseases* 50(6): 882–889.
- Manyi-Loh, C., Mamphweli, S., Meyer, E. and Okoh, A. (2018). Antibiotic use in agriculture and its consequential resistance in environmental sources: Potential public health implications. *Molecule* 23(795): 1 – 48.
- Mengistu, G., Mulugeta, G., Lema, T. and Aseffa, A. (2014). Prevalence and Antimicrobial Susceptibility Patterns of *Salmonella* serovars and *Shigella* species. *Journal of Microbiology Biochemistry Technology* 32: 1 – 8.
- Moe, A.Z., Paulsen, P., Pichpol, D., Fries, R., Irsigler, H., Baumann, M. P. O. and Oo, K. N. (2017). Prevalence and Antimicrobial Resistance of *Salmonella* Isolates from Chicken Carcasses in Retail Markets in Yangon, Myanmar. *Journal of Food Protection*, 80(6): 947-951.
- Mouttotou, N., Ahmad, S., Kamran, Z. and Koutoulis, K. C. (2017). Prevalence, risks and antimicrobial resistance of *Salmonella* in poultry production chain. *Current Topics in Salmonella and Salmonellosis*. Current Topics in Salmonella and Salmonellosis University of Campus Step, China. 20pp
- Mdegela, R. H., Msoffe, P. L. M., Waihenya, R. W., Kasanga, J. C., Mtambo, M. M. A., Minga, U. M. and Olsen, J. E. (2000). Comparative pathogenesis of experimental infection with *Salmonella* Gallinarum in local and commercial chickens. *Journal of Tropical Animal Health and Production* 34: 195 – 204.
- Mrope, S. A. (2017). Isolation, identification and antibiogram of *Salmonella* enterica from chicken eggs collected from selected wards in Morogoro Municipality, Tanzania. Dissertation for Award of MSc Degree at Sokoine University of Agriculture, Morogoro, Tanzania, 84pp.
- Mubito, E. P., Shahada, F., Kimanya, M. E. and Buza, J. J. (2014). Antimicrobial use in the poultry industry in Dar es Salaam, Tanzania and public health implications. *American Journal of Research Communication* 2: 51–63.
- Muthumbi, E., Morpeth, S. C., Ooko, M., Mwanuzi, A., Mwarumba, S., Mturi, N., Etyang, A. O., Berkley, J. A. and Williams, T. N. K. (2015). Invasive Salmonellosis in Kilifi, Kenya. *Clinical Infection Disease* 61(4): 290 – 301.
- Murutu, R. (2016). Prevalence of antibiotic resistant *Salmonella* isolated from beef in Arusha region, Northern Tanzania. *American Journal of Research Communication* 4(7): 1 – 16.
- Mridha D, Uddin MN, Alam B, Akhter AHMT, Islam SKS, Islam MS, Khan MSR, Kabir SML (2020). Identification and characterization of *Salmonella* spp. from samples of broiler farms in selected districts of Bangladesh, *Veterinary World*, 13(2): 275-283.
- Naik VK, Shakya S, Patyal A, Gade NE, Bhoomika (2015). Isolation and molecular characterization of *Salmonella* spp. from chevon and chicken meat collected from different districts of Chhattisgarh, India, *Veterinary World* 8(6): 702-706.
- Nidaullah, H., Abirami, N., Shamila-Syuhada, A. K., Chuah, L.-O., Nurul, H., Tan, T. P., Rusul, G. (2017). Prevalence of *Salmonella* in poultry processing environments in wet markets in Penang and Perlis, Malaysia. *Veterinary World*, 10(3), 286–292.
- Ngogo, F. A., Joachim, A., Abade, A. M., Rumisha, S. F., Mizinduko, M. M. and Majigo, M. V. (2020). Factors Associated with *Salmonella* Infection in Patients with Gastrointestinal

- Complaints Seeking Health Care at Regional Hospital in Southern Highland of Tanzania. *BioMed Central Infection Disease* 20(135): 1 – 8.
- Nyabundi, D., Onkoba, N., Kimathi, R., Nyachio, A., Juma, G., Kinyanjui, P. and Kamau, J. (2017). Molecular characterization and antimicrobial resistance profiles of *Salmonella* isolated from faecal matter of domestic animals and animal products in Nairobi. *Tropical Diseases, Travel Medicine and Vaccines* 32: 1 – 7.
- Oliveira, GH, Berchieri Jr, A, &Montassier, HJ. (2006). Chicken serologic response to *Salmonella enterica* serotype Typhimurium assessed by Elisa. *Brazilian Journal of Poultry Science*, 8(1), 51-54
- Paião, F. G., Arisitides, L. G. A., Murate, L. S., Vilas-Bôas, G. T., Vilas-Boas, L. A. and Shimokomaki, M. (2013). Detection of *Salmonella spp*, *Salmonella Enteritidis* and Typhimurium in naturally infected broiler chickens by a multiplex PCR-based assay. *Brazilian Journal of Microbiology* 44(1): 37–42.
- Pezzella, C, Ricci, A, DiGiannatale, E, Luzzi, I, Carattoli, A. (2004). Tetracycline and streptomycin resistance genes, transposons, and plasmids in *Salmonella enterica* isolates from animals in Italy. *Antimicrobial agents and chemotherapy*, 48(3), 903–908.
- Public Health England (2014). Detection of *Salmonella spp*. microbiology services food, water and environmental microbiology standard method. *Food Investigation Survey Folio* 13(13): 1 – 26.
- Scaria, J., Palaniappan, R. U. M., Chiu, D., Phan, J. A., Ponnala, L., McDonough, P. Y. F., (2008). Microarray for molecular typing of *Salmonella enterica* serovars. *Molecular and Cellular Probes*, 22(4), 238–243.
- Sterzo, E. V, Paiva, J. B., Penha F. R. and Berchieri, J. A. (2005). Time required to protect the intestinal tract of chicks against *Salmonella enterica* serovar enteritidis using competitive exclusion. *Review Brazilian journal of Poultry Science* 7(2): 1 – 7.
- Umeh, S. I. and Enwuru, C. P. (2014). Antimicrobial Resistance Profile of *Salmonella* Isolates from Livestock. *Open Journal of Medical Microbiology* 4: 242 – 248.
- United Republic of Tanzania (URT) (2013). Population and Housing Census 2012. Population Distribution by Administrative Areas. 264pp
- Van, T. T., Moutafis, G., Istivan, T., Tran, T. L. and Coloe, P. J. (2007). Detection of *Salmonella spp*. in Retail Raw Food Samples from Vietnam and Characterization of Their Antimicrobial resistance. *Applied and Environmental Microbiology* 73: 6885–6890
- Wallace, H., Andrews, A. and Jacobson, T. H. (2009). *Bacteriological Analytical Manual*. (5th Edition), Food and Drug Administration, New Hampshire. 21pp.
- Wesonga, S. M., Muluvi, G. M., Okemo, P. O. and Kariuki, S. (2010). Antibiotic resistant *Salmonella* and *Escherichia coli* isolated from indigenous *Gallus domesticus* in Nairobi, Kenya. *East African Medicine Journal* 87(5): 205–10.
- Wigley P., (2014) .*Salmonella enterica* in the chicken: how it has helped our understanding of immunology in a non-biomedical model species. *Frontiers in Immunology*. 5:482.pp 1-7
- Wise, M. G., Siragusa, G. R., Plumblee, J., Healy, M., Cray, P. J. and Seal, B. S. (2009). Predicting *Salmonella enterica* serotypes by repetitive sequence-based PCR. *Journal of Microbiological Methods*, 76(1), 18–24.
- Ziba M. W, Bowa B, Romantini R, Marzio V. D, Marfoggia C, Antoci S, Muuka G, Scacchia M, Mattioli M and Pomilio F., (2020). Occurrence and antimicrobial resistance of *Salmonella spp*. in broiler chicken neck skin from slaughterhouses in Zambia. *Journal of Veterinary Medicine and Animal Health* Vol. 12(2), pp. 85-90
- Zishiri, O. T., Mkhize, N. and Mukaratirwa, S. (2016). Prevalence of virulence and antimicrobial resistance genes in *Salmonella spp*. isolated from commercial chickens and human clinical isolates from South Africa and Brazil. *Onderstepoort Journal of Veterinary Research* 83(1): a1067.11pp.

Ziyate, N., Karraouan, B., Kadiri, A., Darkaoui, S., Soulaymani, A. and Bouchrif, B. (2016). Prevalence and antimicrobial resistance of *Salmonella* isolates in Moroccan laying hens farms. The Journal of Applied Poultry Research 25: 539 – 546.

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	CIP5µ g 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	