PREVALENCE, CHARACTERISATION AND ANTIMICROBIAL RESISTANCE PROFILES OF *SALMONELLA* ISOLATES FROM HEALTHY BROILER AND INDIGENOUS FREE RANGE CHICKENS IN MOROGORO, TANZANIA

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ABSTRACT

The study was conducted to determine prevalence, biochemical profiles and antimicrobial susceptibility profiles of non typhoidal salmonella (NTS) in indigenous free range and broiler chickens in Morogoro Municipality. Between November 2019 and May 2020, a total of 384 cloaca swab samples 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). Antimicrobial sensitivity tests were done using Ampicillin, Cefaclor, Imipenem, Gentamycin, Ciprofloxacin, Sulfamethaxazole-Trimethoprim and Tetracycline antimicrobial discs. Out of 384 samples, 11 (2.9%) samples 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 and 3 isolates were from group D. Bigwa ward showed high prevalence (5.2%) of *Salmonella* than the other wards, broilers being the more prevalent in *Salmonella* than free range chickens. Antimicrobial susceptibility results showed variable level of sensitivity to majority of antimicrobial tested, however, variable level of resistance were also found with 7 isolates resistant to Ampicillin, 4 isolates resistant to Sulfamethoxazole-Trimethoprim and 3 isolates resistant to Tetracycline. Screening for resistant genes detected three isolates with Sulfamethaxole (*sulII*) resistant gene and none for Tetracycline and Ampicillin. This study revealed the presence of *Salmonella* carriers among chicken kept in Morogoro Municipal 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 chickens as indicated by faecal carriers found. Contamination or spread from rats that are natural carriers to poultry needs to be further investigated.

DECLARATION

I, Lidia Abiael Munuo, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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DEDICATION

This work is dedicated to my parents Mr and Mrs Abiael Munuo for their ever presented inspiration and love that rested the foundation of my education. May God bless them!

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
°C	Degree Celsius
µg	Microgram
µl	Microliter
µM	Micromole
BA	Blood Agar
BGA	Brilliant Green Agar
Bp	Base pair
CDC	Centres for Disease Control and Prevention
CLSI	Clinical and Laboratory Standards Institute
Conc.	Concentration
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine Tetra acetic Acid
HRS	Hours
L	Ladder
LIA	Lysine iron Agar
MCA	MacConkey Agar
MDR	Multiple Drug Resistance
Mg	Milligram
MR	Methyl Red
NA	Nutrient Agar
NC	Negative control
P- Value	Probability Value
PC	Positive control

PCR	Polymerase Chain Reaction
pH	Hydrogen iron concentration
PHE	Public Health England
QC	Quality Control
S/N	Serial Number
SGI	*Salmonella* Genomic Island
SPI	*Salmonella* Pathogenic Island
spp	Species
SUA	Sokoine University of Agriculture
TBE	Tris Borate EDTA
TSI	Triple Sugar Iron agar
UV	Ultraviolet rays
VP	Voges Proskauer

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Salmonella enterica are group of bacteria that cause enteric and systemic infection in animals and human worldwide (Abdi *et al.,* 2017). Apart from being a public health problem *salmonella* infections cause huge financial losses in the poultry industry worldwide (Alvarez-Fernandez *et al.,* 2011; Kimathi, 2016; Moutoutou *et al.,* 2017). Host specific *Salmonella* infections are known to cause systemic infection, Typhoid in human and *S.gallinarum* and *S. pullorum* disease in poultry (Kimathi, 2016). A wide range of Nontyphoidal Salmonella (NTS), are known to be harboured by poultry who transmit them to human beings as food borne diseases (Castiglioni-Tessari *et al.*, 2012; Umeh and Enwuru, 2014). In addition to being a foodborne, *Salmonella* infections are also acquired through direct or indirect animal contact in homes, farm environments or other public or private settings (Moutoutou *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; Moutoutou *et al.*, 2017) and it is estimated to cause about 3.7 billion dollars annual economic losses in the poultry industry worldwide (Nidaullah *et al.,* 2017).

Recently, several studies on NTS has been shown to contribute to the increased number of cases of bacteremia where *S. typhimurium* and *S. enteritidis* have been isolated (Muthumbi *et al*., 2015; Kimathi 2016). 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 these *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 of antimicrobials that are commonly used. Resistance to commonly used antibiotics for the treatment of *Salmonella* infection in animals and human has been studied and reported in many parts in the world (Mengistu *et al.,* 2014; Muthumbi *et al*., 2015; Manyi-Loh *et al.,* 2018). The use 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. interitidis* (Hamada *et al.,* 2003; Van *et al.,* 2007; Andino *et al.,* 2015).

Rapid changes in identification of *Salmonella* have raised questions about types of *Salmonella* reported. Invention of genotypic and molecular techniques like pulsed-field gel electrophoresis, Polymerase chain reaction (PCR), ribotyping and sequencing have been useful addition in 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 for prevalence determination (Castiglioni-Tessari *et al.,* 2012), despite the disadvantage of being unable to reveal genetic constitution and intra-serovars variations (Wise *et al.,* 2009). Different methods have been recommended for antimicrobial susceptibility testing of *Salmonella*, however, disc diffusion method remain to be the golden standard in Antimicrobial Resistance (AMR) testing (Mrope, 2017).

Most studies on the detection of *Salmonella* in chickens in Africa were carried out on specific areas and some on specific serovars. A study by Aragaw *et al.* (2010), in Ethiopia found 0.8% prevalence of *S. gallinarum* and *S. pullorum*, this was far lower than that of

Mdegela *et al.* (2000) who showed 0% and 2.6% prevalence of *S. gallinarum* in indigenous scavenging chickens and broilers respectively in Tanzania. A study by Wesonga *et al.* (2010) in Kenya found 12.5% prevalence of *S.typhimurium*. The information on prevalence of nontyphoidal 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 a lot of antimicrobials at different levels to tackle other diseases (Van *et al.,* 2007; Andino *et al.,* 2015; Boamah *et al.,* 2016). The effect of these in selecting antimicrobial resistant *Salmonella* is not precisely known. Thus this study is aiming at establishing prevalence, antimicrobial resistance profile and resistance gene determination in nontyphoidal Salmonella in Morogoro, Tanzania.

1.2 Problem Statement

Chickens have been reported to be the main source of nontyphoidal salmonellosis in human beings. *S. gallinarum* and *S. pullorum* are known to affect chickens, however, they have little or no effect to humans. Therefore information of the occurrence has little effect on human nontyphoidal Salmonella (NTS). However, the occurrences of *Salmonella* spp in chicken's production systems (indigenous free ranged and intensive farming (broilers) used in Tanzania is not precisely known. The chicken production systems are also known to use antimicrobials at different levels to tackle other diseases. The effect of these in selecting antimicrobial resistant *Salmonella* spp is not precisely known. Understanding of the prevalence and types of *Salmonella* and antimicrobial resistance patterns from chickens will lead to better recommendation for control of NTS from chickens and use for antimicrobial stewardship in the country.

1.3 Justification

About 90% of nontyphoidal *Salmonella* infections are food borne while the remaining percent are direct or indirect animal contact in homes, farm environments or other public or private settings (Kimathi, 2016). Chickens are usually convicts in occurrences of human salmonellosis, therefore, the detection of *Salmonella* species in chicken production chain particularly from the farm level is of great concern (Samanta *et al.,* 2014). Treatment to both human and animals has now become a challenge due to increased risk of antimicrobial resistance which pose threat to healthy life (Mwambete and Stephen, 2015; Britto *et al.,* 2018). Understanding the prevalence and types of *Salmonella* spp and antimicrobial resistance patterns from chickens will lead to better recommendation for control of NTS from chickens and the use of antimicrobials in the country.

1.4 Objectives

1.4.1 Overall objective

To determine prevalence, serotypes, antimicrobial resistance profiles and resistance genes of *Salmonella* spp isolated from indigenous free range and exotic (broilers) chickens in Morogoro Municipality.

1.4.2 Specific objectives

1. To identify and to determine prevalence of *Salmonella* spp among selected indigenous free range and exotic (broilers) chickens in Morogoro Municipality.
2. To determine serotypes from the isolated *Salmonella* spp.
3. To determine antimicrobial susceptibility profiles and association of resistance genes of *Salmonella* spp between indigenous free range and exotic (broilers) chickens.

1.5 Research Questions

1. To what extent are the indigenous free ranged and exotic chickens (broilers) contaminated with *Salmonella* species in Morogoro?
2. What are the antimicrobial resistance profiles and their associated genes in the isolated *Salmonella* spp?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Classification of *Salmonella* spp

Considerable literatures have been published on the classification and typing of *Salmonella* (Brenner *et al.,* 2000; Freitas *et al.,* 2010; Castiglioni-Tessari *et al.,* 2012). Genus *Salmonella* is composed of aerobic and facultative anaerobic, catalase positive, oxidase negative and gram negative rod bacteria that belong to family *Enterobacteriaceae* (Umeh and Enwuru, 2014). It contains two species which are *Salmonella enterica* and *Salmonella bongori* with over 2500 serovars (Freitas *et al.,* 2010). The advance in molecular techniques came out with classification of *Salmonella* spp into seven subspecies (I, II, IIIa, IIIb, IV, V and VI) (Brenner *et al.,* 2000).

S. enterica subspecies *enterica* are known to cause infection to warm blooded animals including human and animals. Other *S. enterica* subspecies and *S. bongori* are more common in cold-blooded animals and the environment, with lower pathogenicity to humans and livestock (Brenner *et al.,* 2000; Jay *et al.,* 2003). Some serotypes are host specific, like *S. typhi* is associated in typhoid fever in human beings, while *S. pullorum* and *S. gallinarum* are responsible for bacillary white diarrhoea and fowl typhoid in poultry, respectively (Moutoutou *et al.,* 2017). *S. Choleraesuis* is responsible for pigs infection, *S. Abortusovis* is involved in abortions to sheep and *S. dublin* which cause infection to bovines (Grimont *et al.,* 2000). It is also thought that 80 out of 2500 serovars are known to be non-host specific and they are involved in animal and human salmonellosis (Zoonosis) (Freitas *et al.,* 2010). *S. typhimurium* and *S. enteritidis* are the most common serovars followed by *S. typhimurium* monophasic, *S. derby, S. infantis, S. agona, S. hadar, S. heidelberg* and *S. virchow* (Moutoutou *et al.,* 2017).

Salmonella serovars typing is based on the antigens found in bacterial cells, somatic (O), flagellar (H) and capsular (Vi). Capsular (Vi) antigen is associated with virulence, and is only expressed by serovars *typhi, paratyphi* C and *dublin* (Grimont *et al.,* 2000; Castiglioni-Tessari *et al.,* 2012). Somatic (O) and flagellar (H) antigens, are used to determine different serovars in each subspecies, which form a total of 2,610 serovars today, as recognized by Kauffman-White scheme (Castiglioni-Tessari *et al.,* 2012).

2.2 Prevalence of *Salmonella* in Chicken

Salmonella is an important cause of food borne diseases and resulted in the illness, mortality, and economic losses in human and poultry industry worldwide (Thung *et al.,* 2016). Several researches for determining the prevalence of *Salmonella* in chickens have been conducted in many countries worldwide. Different prevalence study conducted on chicken shows different prevalence based on the nature of the sample collected and also the analytical methods used.

The following were examples of the *Salmonella* prevalence studies conducted worldwide. In Cameroon there were a prevalence of 60% in chicken meat and the most prevalent serotypes were *S. enteritidis* and *S. hadar* (Wouafo *et al.,* 2010), Turkey there was a prevalence of 34% from chicken carcases where by *S. typhimurium, S. infantis, S. heidelberg* were the most predominant serovars (Yildirim *et al.,* 2011). In Bangladesh was 26.6% from cloaca swab, intestinal fluid, egg surface, hand wash of chicken handler and soil of chicken market, *S. enteritidis* and *S. typhimurium* were the prevalent serovars identified (Akond *et al.,* 2012), Southern Ethiopia had 16.7% from cloaca swabs, personnel hand wash and bedding no *Salmonella* serovars reported (Abdi *et al.,* 2017).

In India was 6.1% cloaca swabs no *Salmonella* serovars reported (Samanta *et al.,* 2014), Paraguay with 3.5% cloaca swabs no *Salmonella* serovars reported (Leotta *et al.,* 2010), Iran 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) and Egypt with 5.33% from chicken organ samples whereby *S. sinchem, S. typhimurium, S. gallinarum, S. enteritidis, S. virchow, S. kentucky, S. heidelberg, S. farsta,* and *S. hydra* were serovars identified (Helal *et al.,* 2019).

In Tanzania, studies on the isolation of *Salmonella* from commercial chicken (broilers and layers), chicken eggs and feeds has been conducted and showed prevalence of *Salmonella* spp. Study conducted by Mdegela *et al*. (2000), on prevalence of *S. gallinarum* in indigenous scavenging chicken and broilers where 912 cloaca swabs were collected and identified, 0% cultural prevalence on local scavenging chicken, and 2.6% cultural prevalence on broilers were obtained. Also a study conducted by Mrope (2017), in Morogoro on chicken eggs showed *Salmonella* prevalence of 36.7%, *Salmonella* serovars were not identified. Additionally, study conducted by Mdemu *et al.* (2016), in Ilala Dar es Salaam on the prevalence of *Salmonella* spp in commercial chicken feeds showed the overall prevalence of 29.1% where by *Salmonella* serovars were not identified. Information on the prevalence of *Salmonella* in broilers and indigenous range chicken at farm level is scarce.

Several studies done world-wide showed a number of death associated with foodborne diseases. Worldwide it has been estimated by World Health Organisation (WHO) that three million deaths are associated with *Salmonella* annually (Garedew-Kifelew *et al.,* 2014). Also the Centres for Disease Control and Prevention (CDC) estimates *Salmonella*

bacteria to cause about 1.35 million infections, 26,500 hospitalizations, and 420 deaths in the United States every year and food being the source for most of these illnesses (CDC, 2014). In Tanzania, about 12,055 (16.5%) cases of salmonellosis have been reported in Njombe region, under Health Management Information System data of 2016 (Ngogo *et al.,* 2020). The most prevalent *Salmonella* spp isolated in most research done includes *S.typhimurium, S. infantis, S.heidelberg, S. enteritidis, S.newport,* and *S. kentucky.*

2.3 Antimicrobial Resistance in *Salmonella* spp

Antimicrobial resistance is when a bacterium develops the ability to survive exposure to antimicrobial designed to kill them or stop their growth and it has been a global health challenge threatening the health of humans and animals (Balamurugan *et al.,* 2018). It occurs due to mutations in the DNA of the bacteria, or the acquisition of antimicrobial resistance genes from other bacterial species through horizontal gene transfer (Wingley, 2014). Studies have shown that antimicrobial resistance might been emerged in nature prior to human use of drugs because some organisms produce antibiotic compounds to acquire resistance as means to survive in the presence of their own products and competing species (Katakweba, 2014).

Usage of antimicrobial is associated with the growth stimulation especially in chicken production, increasing feed efficiency, weight gain, prevention and treatment of infections (Boamah *et al.,* 2016; Manyi-loh *et al.,* 2018). Health services to both animals and human in developing countries have been sub optimal with an increased tendency for animal owners to stock drugs and engage unskilled people like farmers themselves and animal attendants to treat their animals, also human tendency of taking medicine based on last history of disease without relying on medical diagnosis (Karimuribo *et al.,* 2005; Katakweba *et al.,* 2012). In Tanzania people have free access to antimicrobial from the

Agro veterinary shops without any prescriptions which is the common tendency as well in any other African country (Carlos, 2010; Tagoe and Attah, 2010; Katakweba *et al.,* 2012). Antimicrobial use in animal feeds and random use in humans and animals has created a selection pressure that favours increased bacterial resistance. Antimicrobial resistant *Salmonella* strains are now widespread due to selection from the use of antimicrobial (Wouafo *et al.,* 2010; Solghan *et al.,* 2010).

In developed countries, it has been reported that one of the sources of increased resistance in *Salmonella* is of zoonotic origin, whereby bacteria in the food-animal hosts attain resistance before further transmission to humans through the food chain (Threlfall, 2002), *S. kentucky* strains isolated from poultry have been identified as being resistant to antimicrobial such as Ampicillin, Tetracycline, and Streptomycin which are commonly used in both veterinary and human medicine (Li *et al.,* 2007). On the other hand, the emergence of multidrug-resistant non-typhoidal *Salmonella* strains such as *S. typhimurium* and *S. enteritidis* has also been reported with increasing frequency in African countries such as Mozambique and Ghana (Wilkens *et al.,* 1997; Mandomando *et al.,* 2009).

In recent years, strains of *Salmonella* resistant to antimicrobials such as Ciprofloxacin, Sulfisoxazole, Nalidixic acid, Ceftazidime, and Ampicillin have been spread worldwide with isolates resistant to quinolones being reported with increasing frequency in some African countries (Raufu *et al.,* 2014; Andoh *et al.,* 2017). The Study conducted in Morocco by Ziyate *et al.* (2016), showed that there was approximately 65.6% Salmonella resistances to at least one antimicrobial tested, *S. kentucky* showed high resistance level (25%) to different tested drugs followed by *S. typhimurium* (4.6%). In Tanzania also some of the studies showed *Salmonella* resistance to some of the antibiotics, example study

conducted by Mwambete and Stephen (2015), showed that there was high resistance rate of 59.1% to all isolated *Salmonella* spp against Co-trimoxazole (Sulfamethaxole-Trimethoprim).

2.4 *Salmonella* as a Zoonotic Infection

Salmonella is known to be the important pathogen in poultry industry and it is one of the major causes of foodborne gastroenteritis in human (Abdi *et al.,* 2017). Human salmonellosis is commonly caused by consumption of foods contaminated with *Salmonella*. *Salmonella* can enter the food through food processing, handling, preparation and distribution. The common *Salmonella* serotypes associated with human infections from food of animal origin includes *S. typhimurium, S. enteritidis, S. kentucky, S. newport and S. heidelberg* (Betancor *et al.,* 2010; Kimathi, 2016). These spp are associated with poultry products and other products of animal origin, and some of them are responsible for the zoonotic transmission of salmonellosis in the community (Kimathi, 2016).

2.5 Transmission of *Salmonella* Resistance Gene

The use of antibiotics, including use as growth promoters in chicken have created a selective pressure to bacteria to acquire a variety of genes, gene complexes and /or mutations that confer resistance to most of the antibiotics used to both animals and human beings (Michael *et al.,* 2006; Van *et al*., 2007). Poultry farmers may pick up resistant bacteria through handling chicken, feeds, pens and droppings, and they can transfer the bacteria to community members. Droppings that contain resistant bacteria may create a vast pool of resistance genes available for transfer to bacteria that can results in zoonotic diseases. Also farm wastes are spread on agricultural fields as fertilizers, and waste run-off can enter rivers, lakes, and ground water leading to spread of these resistant genes (Wright, 2010; Heuer *et al.,* 2011; Katakweba *et al.,* 2012; Athumani, 2017).

Most of these *Salmonella* spp are able to infect host depending on the genetic determinants known as virulence genes which is located in the *Salmonella* Pathogenicity Islands (SPI). SPIs are portions of DNA acquired from other microorganisms by horizontal gene transfer and they are not available in non-pathogenic strains (Zishiri *et al.*, 2016). Also most of these antimicrobial resistance genes in *Salmonella* are carried on mobile genetic elements such as plasmids, transposons, gene cassettes and genomic islands as a results these resistance genes are easily swapped among bacteria living in the same habitat like enterobacteriaceae habitat in the gastrointestinal tract of humans and animals (Michael *et al.*, 2006; Rychlik *et al.*, 2006). The trend of transmission of these *Salmonella* resistant genes and environmental components needs to be addressed in order to control the increasing spread of antimicrobial resistant infections.

2.6 Characterisation of *Salmonella* spp

2.6.1 Biochemical characterisation

Salmonella can be identified by different biochemical tests. Different sugars either in-cooperated in agar media such as Lysine Iron Agar (LIA) ,Triple Sugar Iron Agar (TSI) and Kligler Iron Agar (KIA) or single sugars such as glucose prepared have been recommended as biochemical tests for identification of *Salmonella* spp (Carter and Chengappa, 1991). Glucose, arabinose, maltose, mannose, mannitol, rhamnose, trehalose, xylose, Simmons citrate, lysine decarboxylation, ornithine decarboxylation and Dulcitol are fermented to produce acid and usually gas. (Barrow and Feltham, 1993; PHE, 2014). Also catalase and urease enzymes tests, Methyl red (MR), Voges Proskauer (VP) test, Indole and Simmons citrate agar (IMVIC) test are used, where all *Salmonella* spp are catalase positive., urease negative, Indole negative, Methyl red positive and Voges Proskauer negative and Citrate utilization positive/negative and motility tests are used

such as hanging drop method and growing in semi- solid motility medium (PHE, 2014; Mrope, 2017).

2.6.2 Serotyping of *Salmonella* spp

Typing of *Salmonella* is based on the antigens found in bacterial cells, somatic (O), flagellar (H) and capsular (Vi). Capsular (Vi) antigen is associated with virulence, and is only expressed by serovars Typhi, Paratyphi C and Dublin (Grimont *et al.,* 2000; Castiglioni-Tessari *et al.,* 2012). Somatic (O) and flagellar (H) antigens, are used to determine different serovars in each subspecies, which form a total of 2610 serovars today, as recognized by Kauffman-White scheme (Castiglioni Tessari *et al.,* 2012). The somatic and flagella antigens are tested against each specific antiserum, or they are tested against pools of antisera first and then tested against each of the specific antisera from the positive pools. The number of positive antisera is used in O and H antigen nomenclature (Mridha *et al.,* 2020).

2.6.3 Molecular characterisation

Different molecular techniques like pulsed-field electrophoresis, Polymerase chain reaction (PCR), phage typing, ribotyping, sequences and nucleic acid hybridization have been developed and used as alternative to conventional methods for *Salmonella* identification and differentiation (Christensen *et al.,* 1993; Lukinmaa *et al.,* 2004; Scaria *et al.,* 2008; Wise *et al.,* 2009). These methods are based on the amplification of DNA products of the target organism. They are recommended as a quick detection method compared to conventional methods which takes a long time approximation of 5-7 days (Shanmugasamy *et al.,* 2011). Extraction of DNA for amplification is done normally by either conventional methods such as boiling methods or by using commercial extraction kits and normally 5-10 suspected colonies of the bacterium is used for DNA extraction

(Rahn *et al.,* 1992; Jamshindi *et al.,* 2009). Polymerase Chain Reaction (PCR) specific primers are used to amplify DNA products of the suspected colonies using specific temperatures, time and cycles in a thermal cycler (Mrope, 2017). There after electrophoresis is run in stained agarose gel to enhance visibility of amplified DNA bands (Jamshindi *et al.*, 2009; Paiao *et al.*, 2013).

2.7 Diseases caused by *Salmonella* and their Economic Importance

Salmonelosis in human and other warm blooded animals is predominantly caused by *Salmonella enterica subspecies* I *(*Brenner *et al.,* 2000; Stevens *et al.,* 2009). Over 2600 serovars have been classified based on reactivity of antisera to somatic lipopolysaccharide (O) and flagellar (H) antigens. From a clinical perspective, these may be broadly grouped on the basis of host range and disease presentation (Brenner *et al.,* 2000). Broad host serovars such as *Typhimurium* and *Enteritidis* tend to produce acute but self-limiting enteritis in a wide range of hosts, whereas host-specific serovars are associated with severe systemic disease in healthy outbred adults of a single species that may not involve diarrhoea (e.g. *S. typhi* is associated in typhoid fever in human beings, while *S. pullorum* and *S.gallinarum* are responsible for bacillary white diarrhoea and fowl typhoid in poultry, respectively) (Moutoutou *et al.,* 2017). Host-restricted serovars are primarily associated with systemic disease in one host (e.g. Dublin in cattle, Choleraesuis in pigs), but may cause disease in a limited number of other species (Stevens *et al.,* 2009).

The social and economic impact of food-borne disease is significant. It imposes costs upon the public sector, on industry, in particular the wholesale and retail food industry, and very importantly upon the infected person and their family. The illness may result in admission to hospital and, in a small cases may results in death (Sockett and Roberts, 1991).

Public sector costs fall on the health sector which is directly involved in the care of patients and on public health and hospital laboratory and environmental health services responsible for investigating the illness (Sockett and Roberts, 1991). Costs to industry include the loss of productivity of those who are ill and those who may need to be off work to care for them and those who are prevented from working as a precautionary to stop the spread of infection. It also involves the loss of business, productivity and goodwill of industries or organizations implicated in an outbreak. Most importantly, it imposes costs upon the persons who are ill and those who care for them. These costs include those directly attributable to the illness, those associated with the lost opportunities to carry out normal daily activities and the pain, suffering and sometimes death which results from the illness (Sockett and Roberts, 1991; Stevens *et al.*, 2009).

2.8 Treatment of *Salmonella* Infection

The following are among the recommended treatments of infection due to *Salmonella* as recommended by World Health Organisation (WHO, 2018). These includes electrolyte replacement such as sodium, potassium and chloride ions, lost through vomiting and diarrhoea and rehydration. Also routine antimicrobial therapy is not recommended for mild or moderate cases in healthy individuals. This is because antimicrobials may not completely eliminate the bacteria and may select for resistant strains, which subsequently can lead to the drug becoming ineffective (Katakweba *et al.*, 2012). Health risk groups such as young children, the elderly and immunocompromised patients may need to receive antimicrobial therapy and they are administered if the infection spreads from the intestine to other body parts (Mrope, 2017). Due to the global increase of antimicrobial resistance, treatment guidelines are advised to be reviewed on a regular basis taking into account the resistance pattern of the bacteria based on the local surveillance system (WHO, 2018).

2.9 Control of *Salmonella* Infection

Control of salmonella infection requires measures at all stages of the food chain, from agricultural production, to processing, manufacturing and preparation of foods in both commercial establishments and at home (Hasan *et al.,* 2010). The contact between infants/ young children and pet animals that may be carrying *Salmonella* (such as cats, dogs, and turtles) needs careful supervision (WHO, 2018). Practicing good personal hygiene from chicken keeping such as wearing gloves during handing of chickens and to the preparation of foods which includes washing hands before and after handling of foods, proper storage of foods under cold chain and separation of foods types during preservation in the fridge to avoid cross contamination with food pathogens. Also thoroughly cooking of vulnerable foods and avoid eating raw or under cooked foods are another control measures (Shanmugasamy *et al*., 2011; Mrope, 2017). Discourage using antimicrobial to treat infections without testing their susceptibility because they can promote emergence and spread of drug resistance. Also discourage using of antibiotics as a growth promoters and for weight gain in animals (Mrope, 2017).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Morogoro Municipality within Morogoro Region and it is located about 200 km West of Dar es Salaam. It has 531.6 square kilometres of total land area and the population growth rate is 4.4% per annum, projecting a total population of 316, 603 (URT, 2013). The region lies between latitudes 5° 58’ and 10’ south of the equator and between longitude 35° 25’ and 38° 30’ East Greenwich. It have average temperature of 24° C. The minimum is 18° C in mountainous areas and has a maximum of 30° C in lowland areas. The variation in rainfall is between 500 mm in low areas and 2200 mm in the mountainous areas (URT, 2007). About 33% of the population is engaged in subsistence farming and livestock keeping (URT, 2013). The Municipal Council has one division, which is subdivided into 29 Administrative Wards. Three Wards namely Magadu, Mzinga and Bigwa (Fig. 1) were purposively selected as sampling areas based on accessibility of the area and availability of both indigenous and exotic chickens as study material.

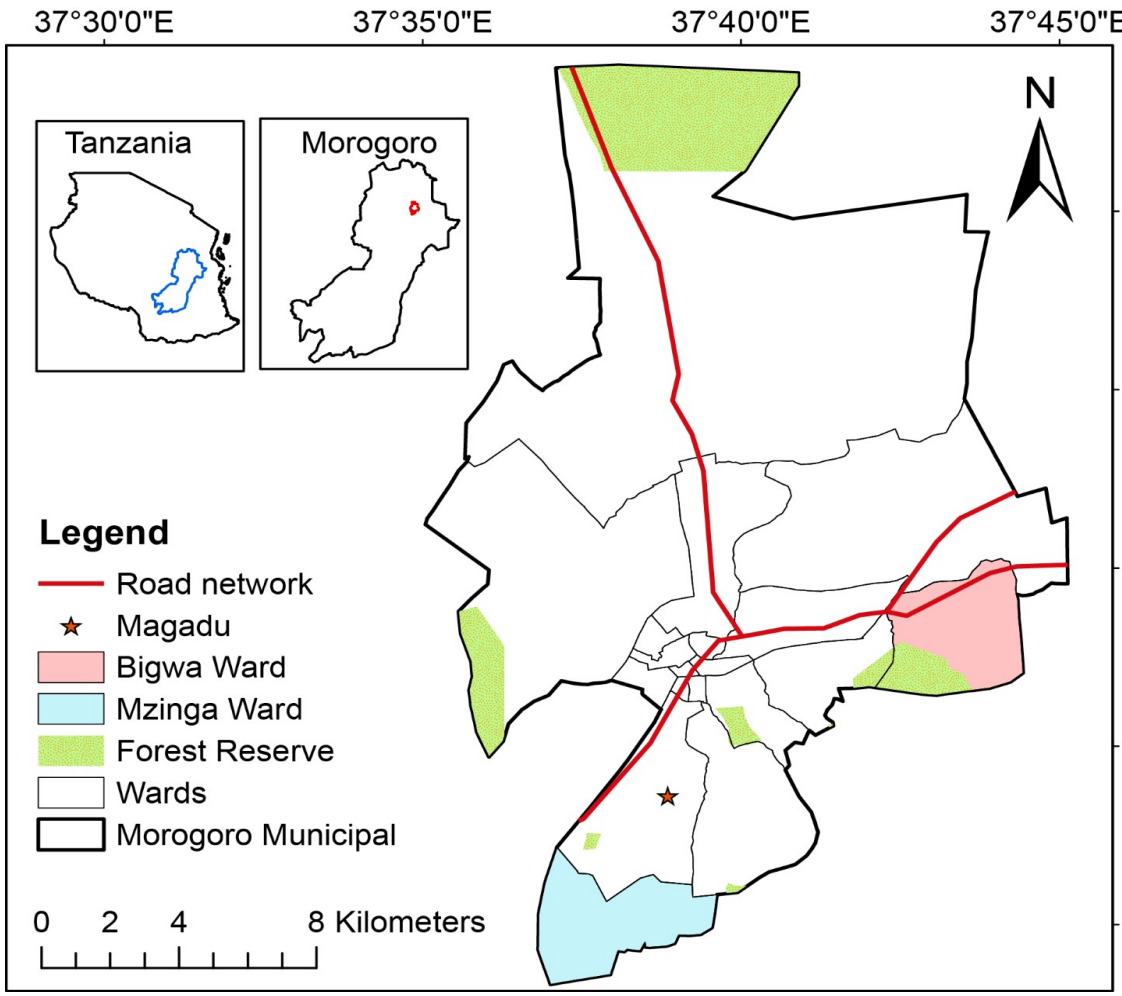

Figure 1: Map of Morogoro Municipality showing study sites. (Source; Drawn by Grite Nelson)

3.2 Study Design and Sampling Method

A cross-sectional study design was employed in this study (Setia, 2016). Multistage random sampling technique was used (Jain and Hausman, 2006). First stage involved selection of three wards, Magadu, Mzinga and Bigwa. Second stage involved the selection of streets. In each ward three streets were purposively selected based on accessibility and availability of chickens/or flocks, also the closeness of study site to the laboratory where analysis took place as samples were collected and processed on the same day to avoid missing of target organism and contamination. In Magadu ward, Kididimo, Falkland and Magadu streets were selected, In Mzinga ward, Konga, Kidangawa and Mtoni streets

were selected and in Bigwa ward, Barabarani, Stendi and Mgolole streets were selected. List of all chicken farms within the street were used as a sampling frame. Table of random numbers was used to select chicken farms to be used. Within each selected farm, samples was also collected randomly by assuming a plus sign in the centre of the chicken house where in each angle of the plus sign samples were randomly collected.

3.3 Sample Size Determination

The sample size was determined by simple random sampling formula by Thrusfield, (2005).

$$n=z^2pq/d^2$$

Whereby n= required sample size, Z= Z value for a given confidence level, p = expected prevalence q = (1 - p) and d = Allowable error of estimation

The confidence level was assumed 95% with an allowable error of 5%, and thus Z was 1.96. Prevalence of 50% was used in calculation, which resulted into n = 384 as sample size, whereby 198 broilers samples and 186 indigenous free range samples.

3.4 Sample Collection Method

A sterile swab was used to collect the faecal sample from the chicken cloaca. Cloaca swabs were used because it provides evidence of persistent intestinal colonization by microorganism such as *Salmonella* in individual chicken (Islam *et al.*, 2016). Moderate adults, mixed sexes indigenous chickens and 2-5 weeks exotic chickens cloaca swabs were collected. The collected swab were kept in a sterile tube containing 10ml of selenite faecal broth and kept in cool box with ice pack (4^oC), then samples were transported to the microbiology laboratory at the Department of Microbiology, Parasitology and Biotechnology at SUA for further analysis.

3.5 Isolation of *Salmonella* spp.

Isolation of *Salmonella* spp from cloaca swab samples was done by using conventional and standard microbiological protocols (Wallace *at al*., 2009; PHE, 2014). General, selective solid media and selective enrichment broth were used for primary isolation of *Salmonella.* All media were prepared aseptically and according to manufacturer's instructions. MacConkey agar (MCA) (Himedia LOT 0000338365) and Blood Agar (BA) (Himedia LOT 0000316699-India) were used as general media while Brilliant Green agar (BGA) (Himedia LOT 0000370098- India) was used as a selective media. Selenite faecal broth (Himedia Lot 0000364831- India) was used as selective enrichment broth. The agar plates were incubated at 37^0C for 16-24 hours before inoculation for sterility check-up and observation of the cultured sample (Wallace *at al*., 2009; PHE, 2014).

3.5.1 Inoculation and sub culture in solid culture media from (MCA, BA and BGA)

In the laboratory, samples taken into the sterile universal bottle containing 10ml sterile selenite broth media were incubated at 37^0C for 16-24 hours. From inoculated selenite faecal broth samples were sub cultured into basic solid media; BA and MCA, and BGA as a selective medium. All inoculated agar plates were incubated at 37°C for 24 hours and results were recorded (Wallace *et al.*, 2009; PHE, 2014).

3.6 Identification of Suspected *Salmonella* Colonies

Identification of suspected *Salmonella* colonies was done phenotypically from different media inoculated, Gram staining method, biochemical tests such as IMVIC (Methyl red (MR), Voges Proskauer (VP) test, Indole and Simmons citrate agar), Triple Sugar Iron agar (TSI), Lysine iron agar (LIA), catalase test, Motility, Glucose, Dulcitol and Maltose. *Salmonella* were genetically confirmed by PCR and identification of species was done by serotyping (PHE, 2014).

3.6.1 Morphological identification

Morphological identification of *Salmonella* isolates was done by using different culture media which included (MCA and BA) as a general media and selective media (BGA) .Identification was done based on similar morphological appearance of suspected colonies including colour, shape, smell, presence of red small colonies on BGA and formation of colour as indication of gas production like black centred colonies as production of hydrogen sulphide gas used. Growth and colony characteristics such as size, moistness, colour of colonies (including specific colours formed by *Salmonella* spp on specific media) were recorded (Hasan *et al.,* 2010; Islam *et al.,* 2016).

3.6.2 Microscopic identification

The suspected colonies were subsequently smeared on microscopic slides using sterile wire loop and saline then fixed and stained by using standard gram staining technique and were observed under microscope on 100x objective lens under immersion oil (Mrope, 2017; Hala, 2018).

3.6.3 Biochemical identification

Identification of *Salmonella* was done by inoculating presumptive colonies on TSI, LIA, IMVIC, Motility, Glucose, Dulcitol, and Maltose and incubated for 24 to 48 hours at 37°C. Colonies that produced alkaline slant with acid butt and hydrogen sulfide production on TSI, positive for lysine, use citrate as a sole source of carbon (positive), negative for Indole test, negative for VP, positive for MR test, positives for Glucose, Dulcitol, Maltose and motility media were considered to be *Salmonella* (Barrow and Feltham, 1993; Wallace *et al.,* 2009; Castiglioni-Tessari *et al.,* 2012).

3.6.4 Serotyping of suspected *Salmon*ella 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 were done according to National Health Laboratory Quality Assurance and Training Center, Standard Operating Procedure for Isolation and Identification of *Salmonella* spp (Appendix 3). The results were observed and recorded.

3.7 Molecular Techniques for Identification of *Salmonella* Species and Detection of *Salmonella* Resistance Genes

3.7.1 DNA extraction

Genomic DNA was extracted from the suspected *Salmonella* spp isolates by using Qiagen Kit (Lot.160049589 -Germany). Between 5-10 colonies from the pure culture plate were taken by using sterile wire loop and added into PowerBead Tube provided in the kit whereby extraction process was done following manufacturer instruction. *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 (Zishiri *et al.*, 2016). Nanodrop spectrophotometer (NanoVue plus machine) was used to check quality and concentration of DNA.

3.7.2 PCR for amplification of suspected *Salmonella* DNA

Amplification of DNA 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. The PCR was run in a total volume of 25µl with the initial concentration of 10µM of primers. The PCR reaction mixture comprised of 1.5µl of

suspected isolates DNA template, and 23.5µl of prepared Master mix reaction made by adding required 1µl of primers, 12.5µl One Taq w/standard buffer 2x concentrate (new England, BioLabs) PCR Master Mix, and 9µl Nuclease free water. The PCR mixture were run for DNA amplification in Thermal Cycler (Agilent Technologies (Sure cycler 8800) Malaysia). PCR machine was used using the amplification conditions of 34 cycles, initial denaturation at 95°C for 1 minute, denaturation at 95°C for 30 seconds, annealing temperature at 58°C for 30 seconds, extension at 72°C for 30 seconds, final extension at 72°C for 5 minutes, and holding time at 4°C with the expected amplicon size of 284 bp and 606 bp for *invA* and *iroB* respectively (Jamshindi *et al.*, 2009; Zishiri *et al.*, 2016).

3.7.3 Detection of antimicrobial resistance genes

Polymerase Chain Reaction (PCR) was used to detect resistance genes associated with antibiotic resistant *Salmonella* spp. Three different resistance genes were detected by using specific primers as shown in the Table 1. Genomic DNA was extracted (as per section 3.7.1). Amplification of resistance genes was carried out using specific primer (Table 1). The total reaction volume for each PCR were 25µL and total number of cycles for each PCR was 34. The following conditions was used in each: 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) detection was carried out 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. Sulfamethaxazole Trimethoprim resistant gene (*sulII* gene) detection was carried out 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 (Rahn *et al.,* 1992; Jamshindi *et al.,* 2009; Adesiji *et al.,* 2014; Zishiri *et al.,* 2016).

3.7.4 Agarose gel electrophoresis and visualization of PCR DNA products

The amplified DNA products from PCR were analysed by 1.5% Agarose gel electrophoresis prepared by dissolving 1.5g of Agarose in 100 ml of 1X Tris - Borate EDTA (TBE) buffer heated to boil by using microwave oven. The agarose gel was then cooled down to 45°C where 5µl of ethidium bromide (DNA stain) was added to stain the gel and then poured into gel casting tray fixed with a combs for solidification. 5µl of PCR product mixed with 3µl of Gel loading dye purple (6X) (New England, BioLabs) were loaded into agarose gel well. Positive DNA template and non-template were also loaded as positive and negative control. A 100 bp DNA ladder (New England, BioLabs) was used as a marker for PCR products. A current of 120 V was applied to each gel for 60 minutes using consort EV 243 electrophoresis system. The agarose gel was visualized under UV trans-illuminator (Uvitec) and the picture was taken for analysis (Jamshindi *et al.*, 2009; Shanmugasamy *et al.*, 2011).

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;GTGAAATTATCGCCACGTTCG GGCAA 141 R; TCATCGCACCGTCAAAGGAACC	284	Jamshindi *et al.*, 2009
iroB gene	*iroB* gene	F:TGC GTA TTC TGT TTG TCG GTCC	606	Zishiri *et al.*, 2016

R:TAC GTT CCC ACC ATT CTT CCC

3.8 Antimicrobial Susceptibility Testing of *Salmonella* spp

Antimicrobial susceptibility test was done by using disc diffusion method in accordance to Clinical and Laboratory Standards Institute for susceptibility testing (Liofilchem, 2017; CLSI, 2018). Antimicrobial tested were from different classes of antibiotics and were among those commonly used in both human and animals. It included beta lactams antibiotics (Ampicillin (AMP 25µg), Cefaclor (CF 30µg) and Imipenem (IMI 10µg)) Aminoglycosides (Gentamycin (Gn 10µg)), Fluoroquinolones (Ciprofloxacin (CIP 5µg)), Sulfamethaxazole-Trimethoprim (SXT 25µg) and Tetracycline (TE 30µg).

Salmonella isolates were sub cultured on Nutrient Agar (NA) and incubated at 37°C for 24 hours. The inoculum of isolates was prepared by taking one pure colony from distinct colonies on NA using sterile wire loop and mixed in 200µl sterile normal saline solution. Turbidity of bacterial suspension was adjusted to 0.5 Standard McFarland solution. Muller Hinton agar media Oxoid (LOT 2114571) was used and was prepared according to manufactures instructions. The suspension of each isolate and the positive control (*E. coli* ATCC 25922) were spread on individual dried surface of Muller Hinton agar plate using sterile swabs. Selected antimicrobial 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. Antimicrobial profiles were determined based on zones of inhibition shown by each drugs. 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). The chart (Table 2) was used as quality control (QC) for test procedures to determine results where inhibition zones of *Salmonella* spp were interpreted by comparing with those provided by the chart and recorded as Sensitive (S/≥mm), Intermediate (I/≤mm), and Resistant (R/<).

Table 2: Antimicrobial susceptibility profile interpretation chart for *Salmonella* spp

Antibiotics	Code	Conc	S/ ≥ mm	I/mm	R/≤ mm
Ampicillin	AMP	10µg	17	14-16	13
Tetracycline	TE	30µg	15	12-14	11
Imipenem	IMI	10µg	23	20-22	19
Gentamycin	GN	10µg	15	13-14	12
Ciprofloxacin	CIP	5µg	21	16-20	15
Sulfamethaxazole Trimethoprim	SXT	25µg	16	11-15	10
Cefaclor	CF	30µg	18	15-17	14

S=Sensitive I=Intermediate R=Resistant Conc=Concentration

Source (Liofilchem, 2017; CLSI, 2018)

3.9 Ethical Considerations

The permission to carry out this study was granted by the Morogoro Municipal Livestock Officer with Ref: No.AB.210/249/01/12 while ethical approval for the study was given by the Ethical Committees of Sokoine University of Agriculture, Tanzania with reference No SUA/DPRTC/R/186 approved on 29th January 2020. Voluntary participation of each chicken farmer was obtained after informed about the study purposes.

3.10 Data Analysis

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

CHAPTER FOUR

4.0 RESULTS

4.1 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 and biochemical tests of *Salmonella* were used in primary identification of *Salmonella* as recorded in Table 3 and Table 4. Percentage prevalence of *Salmonella* spp in broilers and indigenous free range chickens are as shown in Table 5.

Table 3: Cultural and morphological growth characteristic results of *Salmonella* spp.

Culture media	**BA**	**MCA**	**BGA**	**Motility**	**TSI**	**LIA**
Colony characteristics	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

4.2 Biochemical characteristics

Different biochemical tests were done and the results obtained were summarized in Table 4.

Table 4: Results of biochemical characteristics of *Salmonella* spp

	Biochemical Reaction							
Tests	Indole	MR	VP	Glucose	Dulcitol	Maltose	Citrate	Catalase

								utilisation	
Overall reaction	-	+	-	+	+, -	+	+, -		+

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

4.3 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 (Appendix 1).

Table 5: Prevalence of *Salmonella* spp among selected wards within Morogoro Municipality

Wards	**No. sampled/Total number**		**Positives/ Prevalence per wards**	**Overall prevalence (%)**		**Alpha**	**P-value**	**Calculated/ Test statistic**	**Critical/ Tabulated t-value**
	Broilers	**Indigenous 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**					

4.4 Molecular Detection of *Salmonella* spp

4.4.1 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. 2.

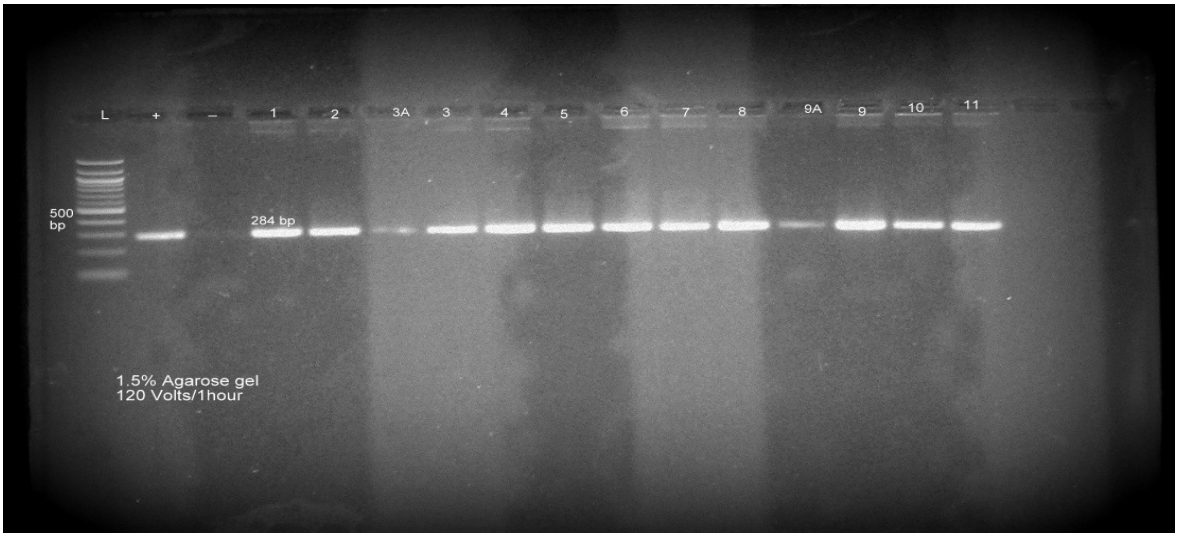

Figure 2: ***Salmonella* gene detection, 284bp *(invA* gene detection)**

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. 3.

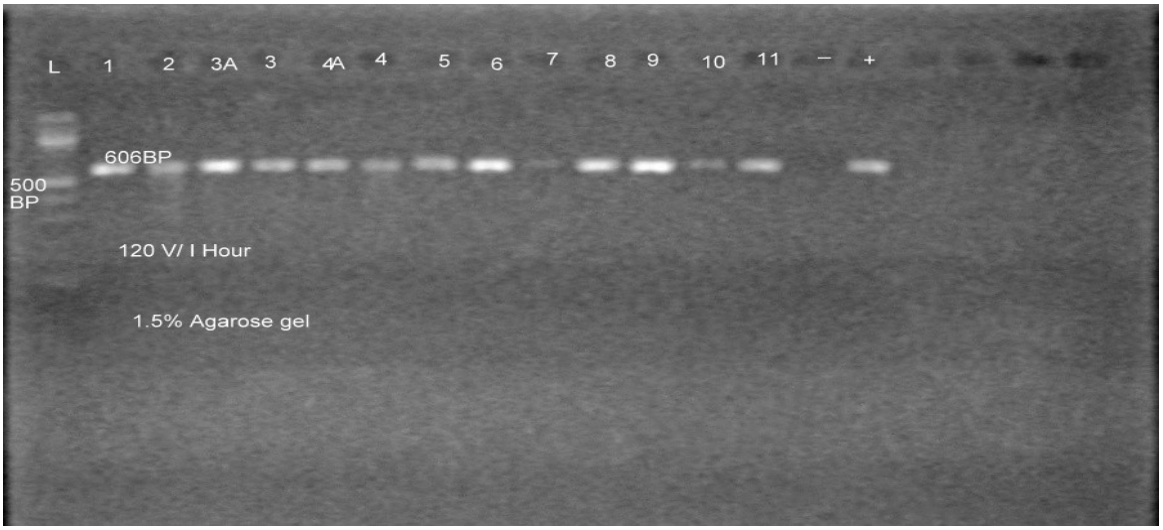

Figure 3: ***Salmonella* enterica gene detection, 606bp detected (*iroB* gene detection)**

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

4.5 Antimicrobial Susceptibility Testing

Seven antibiotics were tested and the results obtained are as indicated in the Table 6 and Appendix 2.

Table 6: Antimicrobial susceptibility results from the isolated *Salmonella* spp

Antimicrobial	Indigenous chickens sensitivity profiles			Exotic (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
Sulfamethaxazole 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

R = Resistance, S = Susceptible, I = Intermediate

Table 7: Multiple Drug Resistance (MDR) patterns from the isolated *Salmonella* spp

Antimicrobial	Indigenous free range chicken	Exotic(broilers)	Overall profile	MDR
Ampicillin	2/3	5/8	7/11	
Tetracycline	0	2/8	3/11	
Sulfamethaxazole-trimethoprim	0	2/8	4/11	

4.5.1 Detection of *Salmonella* resistance gene by PCR

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

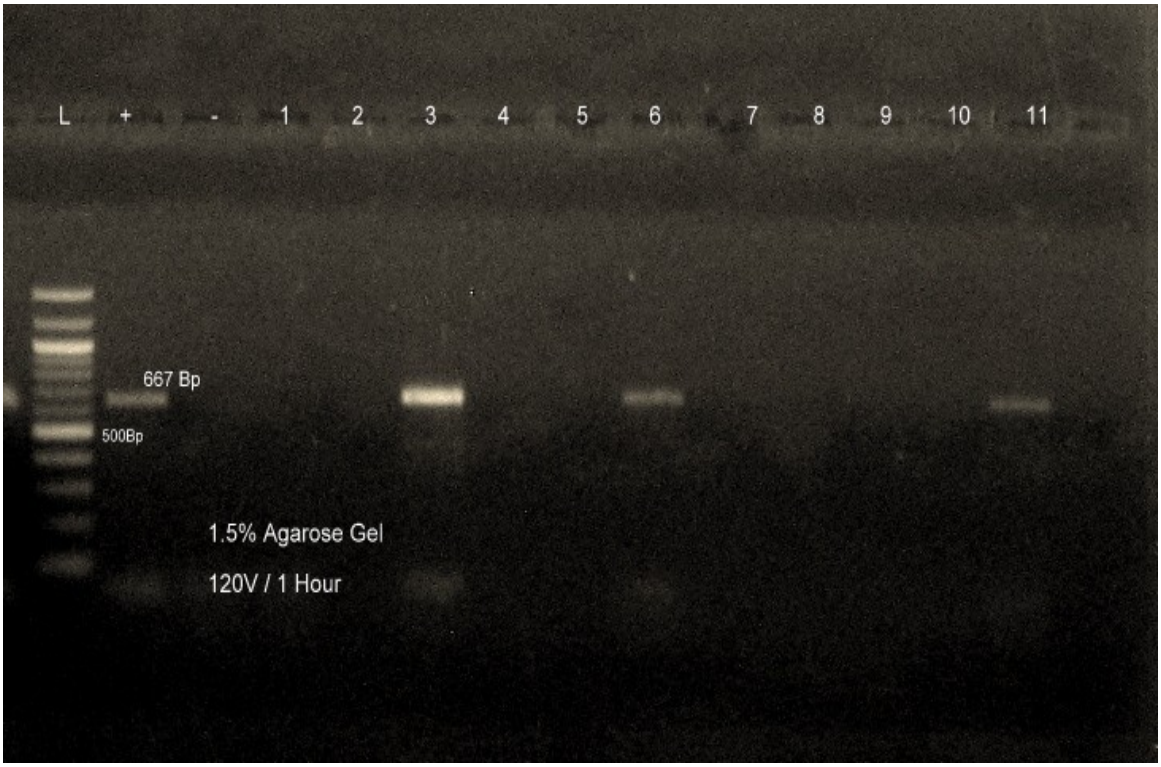

Figure 4: Sulfamethoxazole resistant gene amplification

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

CHAPTER FIVE

5.0 DISCUSSION

Overall the present study shows presence of *Salmonella* species in the gastrointestinal tract of healthy indigenous free range and broiler chickens in Morogoro Municipality. About 3% of the chickens were found to carry *Salmonella* whereas prevalence in broiler was 4% and 1.6% in indigenous free range chickens. However, statistically there were no significant difference between the two groups ($P \geq 0.05$). 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. -trimethoprim (co-trimoxazole) and 3 isolates resistant to tetracycline. Variable level of sensitivity to majority of antibiotics tested were

found; however, level of resistance were also found with 7 isolates resistant to Ampicillin, 4 isolates resistant to Sulfamethoxazole Screening for resistant genes detected *SulII* with 667 bp amplification.

The current study found 2.9% prevalence of *Salmonella* isolates from cloaca swabs. This indicates that healthy indigenous and exotic chickens were the carriers of the *Salmonella*. The found prevalence were 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 prevalence were high as compared to 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 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 the 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; Paião *et al.,* 2013), the current study sampled on 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 matrix such as chicken feed sample, hand swab of the chicken handler and chicken drinking water are encouraged (Akond *et al.,* 2012; Abdi *et al.,* 2017). The current study sampled only on faecal swab sample from chicken cloaca.

The current study found that serogroup B and serogroup D were the most common isolates 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) as the most isolates from chicken cloaca and carcases. However, these findings differ in the ratio of serogroup B to D in that their findings showed that there were more D serotype isolate than B while the current study showed more B serotype that D. The B serogroup were the most common serotype involved in animals and humans salmonellosis 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 prevalence in food chain (Liljebjelke *et al*., 2005).

Comparison of the isolation rate between broiler and free range chicken showed higher prevalence in broiler (4%) than free range chicken (1.6%) however, the difference ($P \geq 0.05$) was not statistically significant. These findings are in line with those of Kindu and Addis (2013), who found prevalence of *Salmonella* infection to be higher in indoor chickens (42.7%) than free ranging (40.8%) but without any statistical significant. Presumably free range chicken are 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)

are more likely to carry *Salmonella* than indigenous chickens, this is due to the reason that chicken kept indoor have lower immunity to diseases and poor management experienced by the chicken owners exposes them to various source of *Salmonella* contamination (Kindu and Addis, 2013). Also the factors like crowdness, chicken attendants and sources of feeds in broilers might be the source of contamination (Mdemu *et al*., 2016). Broader studies are recommended to compare the two systems for a sound conclusion regarding the variation observed.

Antimicrobial sensitivity results showed that *Salmonella* isolates had high sensitivity to majority of the antimicrobial tested. This means that most of the isolated *Salmonella* were sensitive to the antimicrobial tested. These findings are in line with the findings by Mrope (2017), where sensitivity to 100% in Ciprofloxacin, Imipenem and Sulfamethaxazole, Gentamycin 91% and Cefaclor 82% were found. Also Naik *et al.* (2015) from India, 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 study of Al-Ledani *et al.* (2014) in Iraq, and Ziba *et al.* (2020) in Zambia, which showed resistance to Ciprofloxacin and Gentamycin up to 60.5% and 31.6% respectively. This could be due to different serovars obtained in some areas, different sources of antimicrobials, chicken management systems, use and over use of antimicrobials and also geographical variation. Generally the common antimicrobial used it sensitivity profile can be used to treat *Salmonella* spp found in the study area as shown in the results.

In this study small level of resistances were found to Ampicillin, Sulfamethoxazole-Trimethoprim and Tetracycline. These findings are consistent with those of Bacci *et al.* (2012), in Italy Kagambega *et al.* (2013), in Burkina Faso, Abdi *et al.* (2017), in Southern

Ethiopia and Moe *et al.* (2017), in Yangon, Myanmar who found most of *Salmonella* isolates to be resistant to Ampicillin, Tetracycline and Sulfamethaxazole.

These antimicrobials 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 for 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 and they are frequent used in chicken (Katakweba *et al.*, 2012; Wigley, 2014). It is also possible to hypothesize that indigenous 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 small sample size, caution must be applied in interpretation, because no evidence of antimicrobial use was established. Apparently, 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, Sulfamethaxazole Trimethoprim and Tetracycline. These findings mirror those of previous studies by Kagambega *et al.* (2013), in Burkina Faso, Mengistu *et al.* (2014), in Ethiopia and Abdi *et al.* (2017), in Southern Ethiopia which found that resistance to Ampicillin, Sulfamethaxazole Trimethoprim and Tetracycline were the common MDR phenotypes. This study was unable to demonstrate resistance to Ciprofloxacin, Gentamycin, Imipenem and Cefaclor that was shown by Adesiji *et al.*

(2014), in India and Ziyate *et al.* (2016), in Morocco. 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 (*S.typhimurium*), further work is required to establish if rodents are exposed to antimicrobials in addition to the type of bacteria found in the guts.

The antimicrobial resistance genes results found that, three isolates in broilers contain Sulfamethoxazole (*sulII*) resistance gene and was unable to show presence of resistance gene for the Ampicillin (*pse-1*) and Tetracycline (*tetA*). These results agree with those of Bacci *et al.* in Italy (2012), who found *pse-1* gene absent to all the isolates from chicken carcasses (skin swabs) and low percent of *sulII* gene while Zishiri *et al.* in South Africa (2016), found high percentage of *sulII* genes from the chicken meat. However, phenotypic results showed resistance to Ampicillin, Tetracycline and Sulfamethaxazole but only Sulfamethaxazole was carried resistant gene. This is because 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, *tet* A gene was not found despite the fact that they are widely distributed in *Salmonella* strains circulating in animals and was found on plasmids as well as on the chromosome (Frech and Schwarz, 2000; Pezzella *et al.,* 2004). According to Katakweba *et al.* (2018), in Tanzania the *sulII* is the most common gene encoding sulphonamides resistances. Sulfamethaxazole, Tetracycline and Ampicillin were the most commonly used antimicrobials in the study area hence the possibility of detecting these genes was high (Katakweba *et al.*, 2018).

On the figure number four, some of the band (Lane 6 and 11) are the positive for Sulfamethoxazole resistant gene but it is faint. One cause of faint bands in gel

electrophoresis is insufficient amplification of the sample during PCR so this might be the reason for the faint band (Pezzella *et al.,* 2004).

Finally the number of limitations need to be considered. First this study sampled only chicken cloaca swabs, multiple sampling source such as hands swab of the chicken handler, feeds and chicken drinking water could have created a nice ground for broad prevalence establishment and antimicrobial susceptibility. Second, sample size used was small, larger sample size is encouraged. Third, in this study only cross sectional study design was used, cross sectional prospective longitudinal study could help to have the variable number of samples at different period of time. Fourth, in this study moderate adults, mixed sexes indigenous chickens and 2-5 weeks exotic chickens cloaca swabs were collected, sampling of chickens at a different age groups could have given the best prevalence determination. Fifths, this study could not tell the specific serovars obtained as it just show the serogroups and this is due to lack of specific kits for serotyping.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This dissertation investigated the occurrence of *Salmonella* in exotic (broiler) and indigenous chickens with the aim of establishing prevalence, serotype and antimicrobial resistances. It is now possible to state that healthy broiler and free range chicken are the carriers of *Salmonella* spp especially serotype group B and D. Broiler chicken had high prevalence of *Salmonella* compared to free range chicken but was not statistical significant. AMR and MDR emerged as forecast of selection to antimicrobial used. Evidence from this study suggests that rodent exposure (as a primary host of *Salmonella*

typhimurium), 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 faecal carriers found. This work contributes to the existing knowledge of salmonellosis in chickens, highlighting on non-host specific *Salmonella*, which cannot cause disease in chickens but pose public health risks and has added AMR risk to people and animals.

6.2 Recommendations

i. This research has thrown up a number of questions that need further investigation like sampling of rats as a source of *S. typhimurium.*

ii. Further studies on the specific serovars identification is recommended, either by using high technology sciences like sequences, matrix assisted laser desorption ionisation time of flight equipment or by using wide range serotyping.

iii. Studies like this should also be considered to be extended in other regions.

iv. Chicken management practice should adhere to biosecurity measures to prevent *Salmonella.*

v. Public health sectors are recommended to conduct various training or seminars in order to educate health stakeholders such as veterinary officers, catering officers, and farm workers about safe handling of chicken and other risk factors which contribute to acquire salmonellosis.

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APPENDICES

Appendix 1: Serotyping test

Sample no.	*Polyvalent A-S*	*GRP 4(B)*	*GRP 9(D)*
102	POSITIVE	POSITIVE	NEGATIVE
118	POSITIVE	NEGATIVE	POSITIVE
166	POSITIVE	POSITIVE	NEGATIVE
121	POSITIVE	POSITIVE	NEGATIVE
199	POSITIVE	POSITIVE	NEGATIVE
291	POSITIVE	POSITIVE	NEGATIVE
301	POSITIVE	POSITIVE	NEGATIVE
302	POSITIVE	POSITIVE	NEGATIVE
308	POSITIVE	NEGATIVE	POSITIVE
372	POSITIVE	NEGATIVE	POSITIVE
353	POSITIVE	POSITIVE	NEGATIVE
S.G-PC	POSITIVE	NEGATIVE	POSITIVE
S.T-PC	POSITIVE	POSITIVE	NEGATIVE

Appendix 2: Antimicrobial susceptibility results profiles based on zones of inhibition (mm)

Source	***No***	***AMP 10 µg***		***GENT 10 µg***		***TE30 µg***		***STX25 µg***		***IMI10 µg***		***CIP5 µg***		***CF30 µg***	
		S ≥ 17		**S ≥ 15**		**S ≥ 15**		**S ≥ 16**		**S ≥ 23**		**S ≥ 21**		**S ≥ 18**	
		I:14-16		**I:13-14**		**I:12-14**		**I:11-15**		**I:20-22**		**I:16-20**		**I:15-17**	
		R ≤ 13		**R ≤ 12**		**R ≤ 11**		**R ≤ 10**		**R ≤ 19**		**R ≤ 15**		**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

Appendix 3: Laboratory standard operating procedure for isolation and Identification of *Salmonella* spp extracted from National Health Laboratory Quality Assurance and Training Center.

1. Principle

Salmonellae are motile Gram-negative rods belonging to the family Enterobacteriaceae. They are characterized by O, H and Vi antigens. There are numerous serotypes, with different host specificity. They are causative agents of gastroenteritis. Although most infections are self-limiting, advance stages including systemic infections and death can occur. Hence, isolation and identification of these causative agents in clinical material is essential.

Strains of Salmonella are categorized as typhoidal and nontyphoidal. Strains of nontyphoidal Salmonella usually cause an intestinal infection (accompanied by diarrhea, fever, and abdominal cramps) that often lasts 1 week or longer. Less commonly, nontyphoidal Salmonella can cause extraintestinal infections (e.g., bacteremia, urinary tract infection, or osteomyelitis), especially in immunocompromised persons. Persons of all ages are affected; the incidence is highest in infants and young children. Salmonellosis is transmitted by direct contact with animal, through food of animal origin, by nonanimal foods, by water, and occasionally by human contact.

Typhoid fever, caused by *Salmonella serotype Typhi*, is a serious bloodstream infection common in the developing world. Typhoid fever typically presents with a sustained debilitating fever and headache. Adults characteristically present without diarrhea. Illness is milder in young children, where it may manifest as nonspecific fever. Humans are the only reservoir for *S. Typhi*; healthy carriers have been noted. Typhoid fever has a low infectious dose (<103) and a long, highly variable incubation period (1 to 6 weeks). It is transmitted through person-to-person contact or fecally contaminated food and water. Fatal complications of typhoid most commonly occur in the second or third week of illness. In developing countries, typhoid fever is frequently diagnosed solely on clinical grounds; however, isolation of the causative agent is necessary for a definitive diagnosis and for the performance of antimicrobial susceptibility testing.

Salmonella Typhi is most frequently isolated from blood cultures than from fecal specimens. Blood cultures are positive for 80% of typhoid patients during the first week

of fever but show decreasing results afterwards. Fecal cultures are positive in approximately half the cases during the first week of fever, but the largest number of positive cultures occurs during the second and third weeks of disease. Bone marrow cultures are frequently positive; organisms can also be isolated from duodenal aspirates, rose spots, and infrequently from urine cultures.

A syndrome similar to typhoid fever is caused by "paratyphoidal" serotypes of Salmonella. The paratyphoid serotypes (*Salmonella serotype Paratyphi A, S. Paratyphi B, and S. Paratyphi C*) are isolated much less frequently than *Salmonella Typhi*.

2. Materials

a. Culture media: MacConkey Agar with CV and salt (MAC), Sheep Blood Agar (SBA), Xylose Lysine Deoxycholate (XLD), Selenite Broth (SEL).
b. Biochemical identification media: Triple Sugar Iron (TSI), Sulfide Indole Motility (SIM), Lysine Iron Agar (LIA), Urea.
c. Salmonella somatic antisera: Polyvalent O, O:2 (A), O:4 (B), O:7 (C1), O:8 (C2C3), O:9 (D1).
d. Antisera to determine H (flagellar) antigens for *Salmonella Typhi* and *Salmonella Paratyphi* A, B, and C.

3. Specimen

Whole stool, swab prepared fromm whole stool, or rectal swab in Cary Blair medium
Referred isolates, preferably 18 – 24 hour growth in Tryptic Soy Agar (TSA) or Heart Infusion Agar (HIA) slants (screw-capped).
Safety precautions: Observe strict safety practices in handling samples and cultures due to low infectious dose of *S. typhi*.

4. Quality Control (QC)

Refer to QC form. Record results on QC form.

5. Procedure

A. <u>Primary Inoculation:</u>

1. Inoculate samples on MAC and XLD plates and SEL broth
2. For referred isolates, subculture to a fresh SBA and MAC plate.

3. Incubate plates overnight at 35 ± 1°C. Note: Maximal recovery of Salmonella from fecal specimen is obtained by using an enrrichment broth (e.g., SEL), although isolation from acutely ill persons is usually possible by direct plating of specimens. Subculture SEL to XLD after 16 – 18 hours incubation; incubate plate overnight at 35 ± 1°C.

B. Culture Examination

1. Examine plates for characteristic colonies of Salmonella.
2. Select one of each type of suspect colony from the plates.
3. Inoculate onto biochemical screening media (TSI, SIM, LIA, Urea slant). For each colony type, use a single colony to inoculate all media.
4. Incubate tubes (with cap loosened) and plate overnight at at 35 ± 1°C.

C. Identification

1. Check growth on SBA and MAC for purity and colonial morphology. Repeat test if mixed.
2. Perform oxidase test on colonies from SBA. All Salmonella are oxidase negative.
3. Read and interpret reactions on biochemical screening media.
4. Observe for reactions typical of Salmonella. Consult table of reactions below.
5. Perform serotyping when the screening biochemical tests fit Use growth on SBA for serotyping. Proceed as follows:
   - Test for agglutination with Salmonella polyvalent O.
   - Always include an autoagglutination control.
   - If the isolate agglutinates with Salmonella polyvalent O, perform serotyping with group specific O antisera (2, 4, 7, 8, and 9).
6. Confirm identification using complete biochemical tests
7. Perform suceptiblity testing on all confirmed Salmonella isolates
8. Record all observations on the culture worksheet.

Typical Colonial morphology on Primary Isolation Media:

MAC: Transparent or colorless opaque; 2-3 mm

XLD: Red (with or without black centers), or yellow with black centers; 1-2 mm

HE: Blue or green with or without black centers) or yellow with black centers

DCA: Colorless colonies 2-3 mm

SBA: Most Enterobacteriaceae are indistinguishable on SBA.

Reactions of Salmonella in screening tests:

Screening Medium	Salmonella Typhi	Salmonella Paratyphi A	Nontyphoidal Salmonella or Salmonella Paratyphi B or C
TSI	K/A, H_2S+	K/A, gas+, H_2S negative	K/A, gas+, H_2S+
KIA	K/A, H_2S+	K/A, gas+, H_2S negative	K/A, gas+, H_2S+
LIA	K/K, H_2S+	K/A, gas+, H_2S negative	K/K, H_2S+
Hydrogen sulfide	Weak	negative	positive
Urea	Negative	negative	negative
Motility	positive	positive	positive
Indole	Negative	negative	negative

For TSI/KIA: K = alkaline (red); A = acid (yellow); H_2S+ = black H_2S produced
For LIA: K = alkaline (purple); A = acid (yellow); H_2S+ = black H_2S produced

- An alkaline reaction (purple) in the butt of the medium indicates that lysine was decarboxylated.
- An acid reaction (yellow) in the butt of the medium indicates that lysine was not decarboxylated

S. Typhi characteristically produces an alkaline slant, an acid butt (TSI/KIA) with a small amount of blackening of the medium (H_2S+) at the site of the stab on the slant and in the stab line; no gas is produced. Confirm isolate by slide agglutination with group O:9 (D) and Vi antisera.

Serotyping:

The purpose of Serotyping is to determine which of the >2500 Salmonella serovars a specific isolate belongs. This is necessary to facilitate public health surveillance for Salmonella infection and to aid in the recognition of outbreaks.

Salmonella isolates are serotyped based on the antigenic properties of their O (somatic) antigens, H (flagellar) antigens, and Vi (capsular) antigen. These antigens are detected using slide agglutination with commercially produced antisera, the O and Vi antigens using a suspension of growth from an agar plate

while the H antigens using suspension of broth culture. The serotype is deduced from the specific pattern of agglutination reactions using the Kauffman White classification scheme. O serogroup determination is adequate for confirmation of isolates biochemically identified as Salmonella.

Determination of O antigens: First test the isolate in polyvalent O antisera using slide agglutination method. Once the polyvalent group O is positive for agglutination, test the isolate in antisera against O groups 2, 4, 7, 8, 9.

H antigens: Salmonellae commonly express two different flagellar antigens although specific serotype such as Typhi and Enteritidis possess only one flagellar antigen. The two flagellar antigens are referred to as phase 1 and phase 2. Individual flagellar antigens can be composed of multiple antigenic factors.
Determination of H antigens: Refer to antisera product insert.

The Vi antigen (heat-labile) is useful for the identification of S. Typhi. It is also occasional detected in Salmonella ser. Dublin, S. Paratyphi C, and some Citrobacter strains, so its detection does not constitute definitive evidence of Salmonella ser. Typhi.

Determination of Vi antigen: If S. Typhi is suspected, test the culture (live, unheated) in group O:9 (D) antiserum and Vi antiserum on a slide. The Vi antigen can mask the O antigens, blocking their reactivity with the O grouping antiserum. If only the Vi antiserum is positive, prepare a heavy suspension of the isolate in a tube containing 1-2 ml NSS. Place the tube in boiling water for 15 minutes to remove the capsule. Allow to cool and re-test the heated suspension in Vi and group O: 9 (D) antiserum. After heating, Salmonella ser Typhi isolates will be negative in the Vi antiserum but positive in group O: 9 (D) antiserum. Expression of the Vi antigen is variable and but tends to occur more frequently in freshly isolated cultures than in cultures that have been subcultured.

Identification Problems: Several potential problems may prevent accurate serotype determination.

- The strain may express the Vi antigen, which can block the binding of antibodies against the O antigens
- The strain may be rough, i.e., fails to make complete O antigens. Rough strains have a tendency to weakly agglutinate in multiple O grouping antisera.
- The strain may be mucoid and not agglutinate in any O antisera.
- Isolates can be nonmotile and not express any flagellar antigens.
- Salmonella Paratyphi A may be overlooked because it is not routinely screened with group O:2 (A) antiserum, or because it is H_2S negative and lysine negative.
- Salmonella Paratyphi C may express the Vi antigen.
- Salmonella Choleraesusis and Salmonella Paratyphi C have the same antigenic formula but can be differentiated biochemically (API 20E).
- Citrobacter and E. coli strains may possess O, H, or Vi antigens that are related to those of Salmonella; biochemical identification (API 20E) may be necessary to confirm that an isolate is Salmonella.

Antimicrobial Susceptibility testing (AST)

AST is not recommended for uncomplicated Salmonella gastroenteritis, and routine susceptibility testing of fecal isolates is not warranted for treatment purposes. However, determination of antimicrobial resistance patterns is valuable for surveillance purposes and should be performed to monitor the development and spread of antimicrobial resistance among Salmonella isolates.

Treatment with the appropriate antimicrobial agent can be crucial for immunocompromised patients and patients with invasive Salmonella (e.g. blood) and typhoidal infections. Susceptibilities of these isolates should be reported as soon as possible.

6. Interpretation and Reporting of Results

a. A preliminary report of *Salmonella spp.* may be issued when an isolate shows typical reactions in the biochemical screening media and is positive with Salmonella polyvalent O antisera.

b. An isolate is confirmed as Salmonella when the specific O serogroup (2, 4, 7, 8, 9) has been determined and biochemical identification has been completed (i.e., API 20E for this lab)

c. Report confirmed Salmonella isolates by group (O2, O:4, O:7, O:8, O:9).

d. Report to the serotype level if biochemically and serogically confirmed.

"Salmonella Paratyphi A"

"Salmonella Paratyphi B"

"Salmonella Paratyphi C"

"Salmonella Typhi"

e. Report isolates that are serologically A, B, C, or D but are not biochemically/ serologically serotype Paratyphi A, B, C, or Typhi:

"Salmonella group O:2 (A) – not Paratyphi A"

"Salmonella group O:4 (B) – not Paratyphi B"

"Salmonella group O:7 (C) – not Paratyphi C"

"Salmonella group O:9 (D) – not Typhi"

Source:

a. Manual of Clinical Microbiology. American Society for Microbiology (ASM), Washington D.C., USA. 9th edition, 2007.

b. Clinical Microbiology Procedures Handbook. American Society for Microbiology. Washington D.C., USA, 2nd edition, 2007.

c. Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World. U.S. Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, U.S.A, and World Health Organization (WHO) Geneva Switzerland. 2003

d. Antigenic Formulae of the Salmonella Serovars. WHO Collaborating Centre for Reference and Research on Salmonella and Institute Pasteur. Paris, France. 2007.