Phenotypic and Genotypic Characterization of *Salmonella* Isolates from Chickens and Humans in South Sudan

By

SHEREEN AHMED MOHAMED SAAD

A thesis submitted to the University of Zambia in fulfilment of the requirements for the award of the degree of Doctor of Philosophy in Microbiology

The University of Zambia

School of Veterinary Medicine

Lusaka, ZAMBIA

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DECLARATION

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Shereen Ahmed Mohammed Saad Date

APPROVAL

This thesis of Shereen Ahmed has been approved as fulfilling for the award of the Doctor of Philosophy in Microbiology by the University of Zambia.

Prof. Bernard Hang'ombe

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Supervisor	Signature	Date
……	….……	……..
Internal Examiner	Signature	Date
…….	……	……..
Internal Examiner	Signature	Date
……	…….	…….
External Examiner	Signature	Date
……	…….	…….
Chairperson	Signature	Date

(Board of Examiners)

ABSTRACT

Salmonellae are known to be among the most important food-borne pathogens of public health significance. A Cross sectional study was conducted in Wau, Municipality Western Bahr El-Ghazal State, South Sudan, to assess the phenotypic and genotypic characteristic of *Salmonella* isolates from chickens and humans. The fecal samples were randomly collected from 147 chicken keepers and 270 chicken's cloacal swabs from selected household levels. The samples were cultured on Xylose Lysine Deoxycholate Agar for the isolation of *Salmonella* and further confirmed using a series of biochemical tests and PCR.

Following confirmation of *Salmonella,* the phenotypic and genotypic characterization was done. Phenotypic characterization was done through serology, biotyping and antimicrobial susceptibility profiles while the genetic diversity was determined through molecular typing methods that included the detection of virulence genes by PCR and whole genome sequencing of targeted genes. The data was analyzed using SPSS-23 software, for descriptive statistics to obtain frequencies of *Salmonella* occurrence while the Bioinformatics specialist software was used for determining antimicrobial resistance gene (ARG) and virulence gene distribution.

The phenotypic results revealed that, 9 (3.3%) out of the 270 chicken faecal samples were found to be positive for *Salmonella,* while 11 isolates (7.5%) out of 147 human samples were obtained. The identified species for both, humans and chicken included *Salmonella aberdeen* (4), *Salmonella enteritidis* (3), *Salmonella uganda* (1), *Salmonella typhimurium* (6), *Salmonella* serovar *montevideo* (1) and non-typhi (5). On antibiotic susceptibility, seven isolates from humans showed 100% sensitivity to co-trimoxazole, chloramphenicol, streptomycin, nalidixic acid, cefotaxime and gentamicin. However, some isolates showed resistance to ciprofloxacin 1 (9.1%), tetracycline 1 (9.1%), and ampicillin 2 (18.2%). All the chicken isolates were susceptible to chloramphenicol, cefotaxime, streptomycin, sulfamethoxazole/trimethoprim, nalidixic acid and gentamicin.

Eight isolates (*Salmonella sentfenberg* (1), *Salmonella enteritidis* (1), *Salmonella typhimurium* (1) and *Salmonella typhi* (5)) were chosen for further characterization using whole genome sequencing. Following WGS analysis, ARG were found for sulfonamides 8 (100%), tetracycline 7 (87%), erythromycin 4 (50%), β-lactamases 6 (75%), erythromycin 4 (50%), streptomycin 4 (50%) and chloramphenicol 2 (25%). The gene *sul*1 and *sul*2 (8 isolates) was the most frequently detected followed by the gene *tet(D)* and *tet(A)* (7 isolates), and *blaCTX-M-15* (6 isolates). The ARG against streptomycin were very diverse and included genes *aac(3), aph(3)* and *aph(6).* The ARG against β-lactamases included *blaCTX-M-15, blaTEM-1, blaOXA-10, blaOXA-1,* and *blaTEM-1.* The *dfrA19* was the only resistance gene found for trimethoprim. All the chosen eight *Salmonella* serotypes were negative for the presence of *spiA, pagC, msgA, sipB, ivpan,* and *spvB* virulence genes while they were positive for *iroB, sinH, iroC* and *cdtB* virulence genes.

Thirty mobile genetic elements were identified of which the majority belonged to the insertion sequence, miniature inverted repeat, unit transposon and integrative conjugative element. Human isolates showed twenty-four (80%) mobile genetic elements compared to chicken which had only six (20%) mobile genetic elements only. The identified mobile genetic elements were found to be associated with antibiotic resistance of *aac(6)-Iaa* and *blaCTX-M-15* Beta-lactamases genes. This study has demonstrated the diversity of *Salmonella* isolated from humans and animals in South Sudan requiring efforts for a conclusive one health intervention.

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DEDICATION

This work is dedicated to my beloved late mother, Madeline Peter, who inspired me always to look higher. May her soul rest in peace.

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LIST OF ABBREVIATIONS AND ACRONYMS

ARG	Antimicrobial Resistance Gen
AMR	Antimicrobial Resistance
API	Lightweight Python
ARRA	Amplified Ribosomal Restriction Analysis
ART	Antiretroviral Therapy
ATP	Adenosine Triphosphate
BGA	Brilliant Green Agar
BHI	Brain Heart Infusion
DNA	Deoxyribonucleic Acid
DNA RAPD	DNA Random Amplified Polymorphism
EMCs	Metagenomic Technique Based on Enriched Mixed Cultures
ESBL	Extended spectrum beta-lactamases
FBD	Food-Borne Diseases (FBD)
FDA	Food And Drug Administration
FT	Fowl Typhoid
H	Flagellum Antigen
HCPH	Hamilton County Public Health (HCPH)
HGT	Horizontal Gene Transfer
ICE	Integrative Conjugative Elements
IMGE	Mobile Genetic Elements
In	Integrons
invA	Invasion Protein A
*inv*NTS	Invasive Non-Typhoid *Salmonella*
IS	Insertion Sequences
ISPAH	Intervet Shering-Plough Animal Health
ISR	Internal Spacer Region
KWS	Kauffmann White Scheme
LIA	Lysine-Iron Slant Agar
MAAIF	Ministry of Agriculture Animal Industry and Fisheries

MAPKs	Mitogen-Activated Protein Kinases Signaling
MARF	Ministry of Animal Resources and Fisheries
MDR	Multi Drug Resistance
MGE	Mobile Genetic Elements
MLST ST	Multi-Locus Sequence Typing Sub-Typing
MLVA	Multi-Locus Variable-Number Tandem-Repeats Analysis
MOH	Ministry of Health
NININ	National Nanotechnology Infrastructure Network
NTS	Non Typhoidal *Salmonella*
O	Somatic Antigen
PAI	Pathogenicity Islands
PCR	Polymerase Chang Reaction
PCR ERIC	PCR Enterobacteria Respective Intergenic Consensus
PCR-RFLP	PCR-restriction fragment length polymorphism
PD	Pullorum Disease
PFGE	pulsed-field gel electrophoresis
PFGE	Pulsed-Field Gel Electrophoresis
RFLP	Restriction Fragment Length Polymorphism
RISA	Internal Spacer Analysis
RM	Restriction-Modification
RNA	Ribonucleic Acid
rRNA	Ribosomal RNA
SIM	Sulfur Indole Motility Agar
SISTR platform	*Salmonella* in Silico Typing Resource
SNPs	single nucleotide polymorphisms
SPSS	Statistics Package for Service Solution
T3SS	III Secretion System
TF	Typhoid Fever
Tn	Transposons
TSI	Triple Sugar Iron Slant Agar
UBG	University of Bahr El Ghazal

WBGS	Western Bahr El- Ghazal State
WGS	Whole Genome Sequencing
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate Agar

CHAPTER ONE

INTRODUCTION

1.1 Background

Worldwide, *Salmonellae* are known to be among the most important food-borne pathogens of public health significance transmitted through consumption of contaminated food (Domınguez et al., 2002). Animals and animal products are the frequent vehicles of these bacterial species to humans. Human Salmonellosis is most often caused by the consumption of contaminated foods such as raw and processed meat products, including chicken, beef, and pork (Berends et al., 1996).

Surveillance and monitoring of animal products provide epidemiological data important for preventing foodborne Salmonellosis. The genuses *Salmonella* are Gram-negative aerobic or facultative aerobic bacteria belonging to the family *Enterobacteriaceae.* There are many different *Salmonella* serotypes (Domınguez et al., 2002). Serotypes *Salmonella typhimurium* and *Salmonella enteritidis* are the most common serotypes reported in the world. The host range of the bacterium is wide and includes humans, chickens, pork, fish and cattle (Mbuko et al., 2009). This has resulted into *Salmonella* being classified as host-specific and non-host specific. Host specific *salmonella* include Some serovars like *Salmonella typhi* (*S. typhi*, the pathogen of typhoid), *Salmonella paratyphi* (*S. paratyphi*, the pathogen of paratyphoid), as well as *S. gallinarum* (chickens) and *S. dublin* (cattle). The non-host specific includes *S. enteritidis* and *typhimurium* (Mubita et al.,2020).

The non-host specific group of serotypes exhibits a broad host range and generally tend to colonize the intestinal tract and invade enterocytes of the intestinal mucosa, manifesting gastroenteritis, but in contrast fail to disseminate beyond the lymph nodes, unless in the event that the host has an underlying immune defect (Kingsley et al., 2013). The group consists of serotypes such as *S. typhimurium* and *S. enteritidis* (Rotger et al., 1999), and is perceived as a major cause of both gastroenteritis and septicemia syndromes in humans (Roudier et al., 1990) and animals (Baumler et al., 2013). For instance, *Salmonella typhimurium* and *S. enteritidis* serotypes can cause disease

in broad range of highly susceptible animal hosts such as poultry, cattle, rodents, pet hedgehogs as well as humans (Anderson et al., 2017).

Salmonellae are widespread in nature, and can be detected in many cold and warm-blooded animals across the world. In numerous countries today, they are the most important bacterial diarrhea-causing pathogens in humans, as they are mostly transmitted from animals to humans through consumption of foods of animal origin such as chicken and eggs (WHO, 2009). *Salmonella* is classified as a zoonosis as they cause salmonellosis in both animals and humans where they cause significant morbidity and mortality on a global scale (Ejeh et al., 2014).

The *typhoid* form of *Salmonella typhoid*-like diseases is mainly triggered by the serovars *S. typhi, S. paratyphi* A, B and C The disease can be transmitted from human to human (Hendriksen et al., 2004). The pathogens are ingested orally and transmitted via blood. The infectious dose is low (10^2 - 10^3colony-forming units (cfu)/ml). After a short incubation period (a few days up to 3 weeks) severe, cyclic general infection occurs accompanied by diarrhea, high temperature and possible damage to the intestines, heart, liver, kidneys and gallbladder. Particularly in the case of patients with gallstones, the pathogens may be excreted over long periods (Lamas et al., 2018).

In many cases, antibiotics are used in the control of salmonellosis. The worldwide overuse or misuse of antimicrobials in different fields, such as human medicine, veterinary medicine and agriculture, and as prophylactic supplements or growth-promoting agents in feed has contributed to development of antimicrobial resistance (Lamas et al., 2018). Animals have been implicated as important sources of *Salmonella*-contaminated food products that are responsible for human salmonellosis and in the United States approximately 40,000 cases of Salmonellosis are reported resulting in 600 deaths. Globally, worldwide health concern has been the occurrence of Antimicrobial-resistant strains of various pathogenic bacteria (Carramiñana et al., 2004).

The number of *Salmonella* strains with single- and multidrug resistance has been increasing. Some researchers have suggested that the increased use of specific anti antimicrobial agent in food animals selects for bacteria resistant to antimicrobial used in humans, and these resistant bacteria might spread via the food chain to humans and cause human infections, leading to the banning of

these antimicrobial. The most significant animal in many cases is chicken. Therefore, in any country, it is important to undertake deep understanding of *Salmonella* from different settings. In this study, the phenotypic and genetic characteristics of *Salmonella* found in chickens and humans from South Sudan was studied to provide a measured result on the extent of implication of chickens to human salmonellosis.

Antimicrobial resistance (AMR) and particularly multidrug resistance (MDR) is becoming very common among various *salmonella* serotypes that have been isolated from humans and chicken world over (Zhao et al., 2003). The extent of AMR varies from region to region and is usually influenced by the abuse of antibiotics in humans and animals (Arumugaswamy et al., 1995). Reports of cases of *Salmonella* isolates being resistant to important antibiotics have been reported dating back to the 1960s during which resistance was reported to have been limited to one antibiotic (Parveen et al., 2007). However, from the 1970s onwards, there has been an increase in the number of *Salmonella* isolates that have shown resistance to various clinically significant antibiotics and this has been exacerbated by the recovery of such isolates in foods of animal origin (Doumith et al., 2016). This is a growing public health concern as human Salmonellosis caused by resistant strains of *Salmonella* may be difficult to treat (Doumith et al., 2016).

Since the mid-1970s, there has been an increasing trend of *Salmonella* isolates exhibiting MDR phenotypes worldwide (Baowei et al., 2011). The MDR exhibited by some *Salmonella* isolates and other pathogens are obtained from extrachromosomal genes that may impart resistance to an entire class of antimicrobials (Kumar et al., 2009). More recently, most of the resistance genes have been associated with large transferable plasmids and other DNA mobile elements, such as transposons and integrons (Kariuki et al., 2000; Parveen et al., 2007). Moreover, MDR seems to be more serious in some serotypes compared to others (Kayode et al., 2010). Therefore, there is a need for continuous monitoring of human and animal *Salmonella* isolates that exhibit resistance to most antimicrobials on a global scale (Porwollik et al., 2004).

Many people live together with their chickens in the same living spaces, sharing the same utensils with the chickens and then come into contact with their droppings (feces), which constitute a focal source of *Salmonella* infection. Hand-washing maybe less appreciated and this could be a major

vehicle that perpetuates transmission of infectious diseases such as Salmonellosis. Poor Veterinary services in rural areas, especially during rainy season, due to poor road infrastructure may contribute to the increase of *Salmonella* spread between chicken houses and infection in human. In addition, the unregulated use of antimicrobials by unqualified veterinary practitioners and uneducated owners, may also contribute to the development of antimicrobial resistance (AMR) (Fadlalla et al., 2012).

In recent years, a shift in *Salmonella* serotypes related to chicken and chicken production has been reported in diverse geographical regions, being particularly associated with the spread of certain well-adapted strains (Pearce et al., 2005). Moreover, AMR in non-Typhoidal *Salmonella* is considered one of the major public health threats related to food-animal production, including the poultry production chain and poultry meat, which is an additional concern in the management of Salmonellosis (Nierop et al., 2005). In addition, the circulation of the same multidrug-resistant *Salmonella* strains and identical mobile genetic elements encoding AMR resistance genes from poultry to humans highlights this scenario (Nierop et al., 2005). Due to the importance of *Salmonella* in the clinical and public health setting, there has been a significant effort to deepen the knowledge about pathogenic determinants of this bacterium in animals and humans.

In South Sudan, the prevalence of salmonellosis or *Salmonella* strains circulating among chickens and humans is unknown, and this complicates the control of the disease. In addition, no molecular study has been conducted to understand the transmission patterns of *Salmonella* between indigenous chickens and chicken keepers or to assess the magnitude and patterns of AMR associated with *Salmonella* strains in the country. Therefore, monitoring the emergence of resistant bacterial strains in foods destined for human consumption is a risk management option that can prevent the development and spread of antimicrobial resistance in microorganisms.

1.2 Statement of the Problem

South Sudan is a new country with under-developed infrastructure like health and diagnostic facilities. Chickens may be implicated in the contamination of foods with *Salmonella,* owing to contaminated feeding troughs and environment from which chickens survive. Chickens are reared by almost every household as they are a cheap source of proteins in South Sudan. Both Muslim

and Christian communities keep chickens as an important source of income. They are also a source of income for poor households who need to sell to earn a living. Furthermore, chickens are allowed to scavenge for food in order to minimize feeding costs. This predisposes the birds to various pathogens and this may be considered a source of infection (WHO, 2009).

1.3 Justification of the Study

In South Sudan, there is paucity of data for understanding the transmission of salmonellosis from chickens to humans and vice versa. The observation poses a public health threat and contributes to socioeconomic hardships, especially in low-income communities. One way of addressing these problems is through the promotion of the use of the One Health concept and application of genomic epidemiology. This approach can provide a clear understanding of the evolutionary origin of the *Salmonella* strains circulating in the country (Wierup et al., 1988). It can also provide an understanding on the flow of resistomes amongst chickens, humans and between chicken and humans. The data from this study will inform authorities on how to control salmonellosis and limit the flow of resistomes, as well as provide an accurate genetic picture of the *Salmonella* strains prevailing in South Sudan from chickens and humans.

1.4 Research Questions:

1) What *Salmonella* strains are found in chicken and humans in South Sudan?
2) What are the phenotypic and genotypic characteristics of *Salmonella* found in chicken and humans in South Sudan?
3) Are the isolates susceptible to the drugs of choice in the treatment of salmonellosis?
4) What are the mobile genetic elements harbouring AMR and virulence genes in *Salmonella* circulating among chickens and humans?

1.5 General objective:

To characterize *Salmonella* isolated from chicken and humans in South Sudan

1.5.1 Specific objectives

1. To isolate *Salmonella* from chicken and humans in South Sudan.

2. To determine the phenotypic and genetic diversity of circulating *Salmonella* among chickens and humans.
3. To determine the antimicrobial susceptibility patterns of circulating *Salmonella* among chickens and humans.
4. To identify mobile genetic elements harbouring AMR and virulence genes in *Salmonella* circulating among chickens and humans.

CHAPTER TWO

LITERATURE REVIEW

2.1 Classification of *Salmonella*

The genus *Salmonella* are Gram-negative, facultative anaerobic rod-shaped bacilli that belong to the family *Enterobacteriaceae* (Domınguez et al., 2002). This genus, which is estimated to have diverged from *Escherichia coli* (*E. coli*) approximately100 to150 million years ago, has adapted to colonies many different niches. For example, *Salmonella* can be found as both a commensal and pathogen in warm and cold-blooded animals and is capable of surviving freely in the environment.

Various methods for the classification and nomenclature of the *Salmonella* species, subspecies, subgenera and serotypes have been proposed over the years (Kariuki et al., 2000; Kagirita et al., 2017). The six *Salmonella* subspecies includes: *enterica* (serotype I), *salamae* (serotype II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), and *indica* (VI). The former serotype V was *bongori,* which is now considered its own species.

The serotype or serovar, is a classification of *Salmonella* into subspecies based on antigens that the organism presents. It is based on the Kauffman-White classification scheme that differentiates serological varieties from each other. Serotypes are usually put into subspecies groups after the genus and species, with the serotypes/serovars capitalized, but not italicized: An example is *Salmonella enterica* serovar *typhimurium*. More modern approaches for typing and subtyping *Salmonella* include DNA-based methods such as pulsed field gel electrophoresis, multiple-loci variable number tandem repeat (VNTR) analysis, multilocus sequence typing, and multiplex-PCR-based (Baratto et al., 2012).

Salmonella is further subdivided into six sub species that comprise above 2,600 serotypes. *S. enterica* subspecies are originate globally in all warm-blooded animals and in the environment whereas *S. bongori* is limited to cold-blooded animals, predominantly reptiles (Hendriksen et al., 2004).

Salmonella species are non-spore-forming, predominantly motile enterobacteria with cell diameters between 0.7 and 1.5 µm, and lengths from 2 to 5 µm. The bacteria have peritrichous flagella all around the cell body (Rasooly et al., 2008). They are chemotrophs, obtaining their energy from oxidation and reduction reactions using organic sources. They are also facultative aerobes, capable of generating ATP with oxygen (aerobically) when it is available, or when oxygen is not available, using other electron acceptors or fermentation (anaerobically) (Kariuki et al., 2000).

2.2 History of *Salmonella* Infection

In humans, *Salmonella* was first isolated from a sample in 1884 by bacteriologist called Georg Gaffky and the pathogen was later identified as *Salmonella enterica* subspecies *enterica* serotype *typhi* (Gossner et al., 2016). Whereas, in animals, *Salmonella* pathogen was first isolated from porcine intestine which manifested classical swine fever by Euzéby et al., (1999). The genus name *Salmonella* was derived from the last name of the veterinary scientist, Dr. Daniel Elmer Salmon, who was the administrator of the United States Department of Agriculture (USDA) research program, although a number of scientists were involved towards the research of *Salmonella* pathogen (Kass et al., 1987; Marineli et al., 2013). *Salmonella* was formerly called "*Bacillus choleraesuis*" and this name changed to *Salmonella choleraesuis* in 1900 by Lignnieres (Mubita et al., 2020). *Salmonella* species are relatively close to their counterparts Enterobacteriaceae (*Escherichia, Yersinia* and *Shigella*) in terms of the composition of guanine (G) and cytosine (C) content rated at about 52% (Hermesch et al., 2008).

Salmonella is a facultative intracellular pathogen; non-sporing, oxidase negative, catalase positive, non-capsulated; Gram-negative, straight rod-shaped bacteria (Barlow et al., 2002; Mathews et al., 2011) and are approximately 3 x 0.6 µm in size (Montville et al., 2008). *Salmonella* is flagellated and predominantly motile (Fàbrega et al., 2013), with an exception of the non-motile mutants *S. gallinarum* and *S. pullorum* (Schofield et al., 1945). The taxonomy of *Salmonella* is perceived to be complex and subject to evolving (Brenner et al., 2000; Ryan et al., 2017), such that it has taken several concepts to determine the taxonomy and nomenclature of the bacteria. Notable characteristics were based on clinical symptoms caused by the *Salmonella* strains (Grimont et al., 2000). Further, surface antigenic specificities (Stokes et al., 1957; Kauffmann et al., 1960) and

biochemical tests including the ability to ferment substrates (Williams et al., 2005) were adopted while DNA relatedness has transformed the classification (Crosa et al., 1973).

Therefore, the genus *Salmonella* is currently divided into two validly published species called *Salmonella enterica* (six subspecies) and *Salmonella bongori* one subspecies (Reeves et al., 1989; Michael et al., 2001;Grimont et al., 2007) based on their 16S rRNA nucleotide sequence analysis (Christensen et al., 1998; Grimont et al., 2000). *Salmonella bongori* was previously known as *Salmonella* subspecies "V" (Reeves et al., 1989). However, a new and third species "*Salmonella subterranean"* was proposed and subsequently approved by the judicial commission on March 18, 2005 (Shelobolina et al., 2004). *Salmonella subterranean* is still written within parentheses, due to the reason that some researchers claim that the species should not belong to the *Salmonella* genus but rather to *Escherichia* genus.

Salmonella enterica is the type species and most diversified group represented by six phylogenetic subspecies namely, *enterica, salamae, arizonae, diarizonae, houtenae* and *indica* (Grimont et al., 2007; Su et al., 2007). These subspecies can also be identified by Roman numerals in the same order; I, II, IIIa, IIIb, IV, and VI (Rotger et al., 1999; Grimont et al., 2000; Popoff et al., 2003). On the other hand, (Su et al., 2007) attempted to name *S. enterica* subspecies I, as *S. enterica* subsp. *enterica* (subspecies I), *S. enterica* subsp. *aalamae* (subspecies II), *S. enterica* subsp. *arizonae* (subspecies IIIa), *S. enterica* subsp. *diarizonae* (subspecies IIIb), *S. enterica* subsp *hountanae* (subspecies IV) and *S. enterica* subsp *indica* (subspecies VI. *Salmonella enterica* subspecies possesses the highest diversity of serotypes.

Salmonella enterica are considered the most important zoonotic pathogenic subspecies of the genus *Salmonella* in human and veterinary medicine worldwide and comprises more than 2,500 serotypes (Kingsley et al., 2000; Popoff et al., 2003; Eswarappa et al., 2008; Fookes et al., 2011; Feng et al., 2012; Evangelopoulou et al., 2014). *Salmonella* subspecies I, consists of the largest group responsible of causing disease in humans and animals, and of this group only about 150 serotypes are clinically significant as human or animal pathogens (Guiney et al., 2011; Roer et al., 2016). These serotypes are responsible for 99% of all clinical isolates in humans (Bell et al., 2002; Control et al., 2008; Crum et al., 2008; Pui et al., 2011) and animals (Li et al., 1995; Porwollik et

al., 2004; Hernandez et al., 2012). Whereas, *Salmonella* serotypes belonging to subspecies II, IIIa, IIIb, IV and VI are associated with cold-blooded animals and the environment but occasionally cause disease in humans (Lamb et al., 1984).

Salmonella bongori was first isolated in 1966 from a lizard in the city of Bongor, Chad, upon which it derived its name bongori (Minor et al., 1969). *Salmonella bongori* is associated with infections with ectotherms (cold-blooded animals) such as reptiles (Rahman et al., 2019), but has also been isolated from endotherms such as humans and birds in Italy (Foti et al., 2009). *S. bongori* and other subspecies belonging to *S. enterica* subspecies II, IIIa, IIIb, IV, and VI are commonly isolated from cold-blooded animals (Nataro et al., 2011).

Salmonella infections can be regarded as two types; the first is primarily of importance for public health which may cause illness in man (Dallap et al., 2010; Jean et al., 2011; Rui-hong et al., 2016; Chong et al., 2017) and animals (Dallap et al., 2010; Jean et al., 2011). The second type is caused by the serotypes *S. pullorum* and *S. gallinarum*, which cause severe disease in poultry but rare in human (Johnson et al., 2018). However, the principal agents of salmonellosis were classified into two groups; *typhoid* pathogens and non-*typhoid Salmonella* (NTS) by Feasey et al., (2013). Although these two groups are genetically related, they are different in the way they evoke diseases in humans (Okpa et al., 2022). Typhoid pathogens are responsible for enteric fever (*typhoid*), while gastroenteritis (Kupz et al., 2014) and invasive non-*typhoidal* Salmonellosis (iNTS) is characterized by bacteriemia (Guiney et al., 2011) or extra-intestinal infection caused by NTS pathogens (Rodríguez et al., 20014**)**.

2.3.1 Human Salmonellosis

Although most human salmonellosis cases are foodborne and waterborne infections can also be acquired through direct or indirect animal contact in homes, veterinary clinics, zoological gardens, farm environments or other public, professional or private settings (Abunna et al., 2017), and poor sanitation (Brandwagt et al., 2018). Human salmonellosis comprises three major disease forms: enteric fever, non-typhoid gastroenteritis, and non-typhoid invasive (bacteremia) disease (Darby et al., 2008; Guiney et al., 2011), in addition to a carrier state (Eng et al., 2015). Bacteremia is a condition whereby the *Salmonella* pathogens enter the bloodstream after invading the wall of the

intestinal tract (Eng et al., 2015). In 2010, the National Nanotechnology Infrastructure Network (NININ) hypotheses indicated that *Salmonella* is able to kill up to 30% of patients who get it, if they are not treated (Kaiser et al., 2014).

2.2.1.1 The Typhoid Fever in Humans

Salmonella typhi (ST) is a highly human adapted pathogen which causes enteric fever commonly known as typhoid fever (TF), a serious public health global infection which is responsible for life threatening infections in humans only (Su et al., 2007; Roy et al., 2016; Chong et al., 2017). Other human restricted *Salmonella* serovars *S. paratyphi* A, B and C which cause paratyphoid fever and collectively including ST are known as typhoidal *Salmonella* (TS) (Connor et al., 2005; Raffatellu et al., 2008; Crump et al., 2010). *Salmonella paratyphi* A exhibits symptoms similar to TF, but often less severe (Fookes et al., 2011).

One of the first reported human outbreaks happened in 1899, when British troops in South Africa were decimated by typhoid (Hardy et al., 2000). Of those troops about 13,000 deaths were due to the disease. Since then, typhoid continued to be a serious public health problem in mid-nineteenth-century in England, but with the introduction of piped and filtered water supply in most urban areas, its prominence as a cause of death had diminished although more than eight thousand cases were still recorded in England and Wales in 1913 (Hardy et al., 2004). In rare and extreme cases, this highly contagious bacterium had been intentionally used as a terrorist tool in 1984; where salad bars in Oregon were contaminated with the bacteria (Wright et al., 1997). In the year 1907, about 3,000 New Yorkers in the United States (US) had been infected by *Salmonella typhi* and Mary Mallon a "healthy carrier" and a cook at the time was probably the main source for the outbreak (de Oliveira et al., 2019). During that period, immunization against *Salmonella typhi* was not yet ensued not until about 1911, while on the other hand antibiotic treatment had not developed until 1948 (Regan et al., 2009). The risk of further transmission of ST increases in the event that a chronic carrier is involved in food handling activities (Brandwagt et al., 2018).

2.2.1.2 Non-Typhoid Salmonellosis of Human

Non-*typhoidal* salmonellosis infections are caused by all serotypes of *Salmonella* except *S. typhi* and *S. paratyphi* A, B and C (Taramasso et al., 2016). Non-typhoid *Salmonella* subspecies *Enterica*, are the most important causative pathogens of foodborne diseases (FBD), followed by other pathogens that cause localized infections such as parasitic helminthes (Havelaar et al., 2015). NTS strains found in Africa, are also capable of causing invasive disease such as bacteremia, unlike those strains found in developed countries which are only responsible for diarrheal diseases (Crump et al., 2015).

2.2.1.3 Human Invasive Non-Typhoid Salmonellosis

Globally, the disease due to invasive non-typhoid *Salmonella* (invNTS) in humans had an annual incidence of 1.02 per 100,000 populations, as reported by an international bacteremia surveillance study (Laupland et al., 2010). On the other hand, recent studies on the global estimates of invNTS disease, was between 2.1 to 6.5 million cases with an overall incidence rate of 49 cases per 100,000 populations per annum (Ao et al., 2015). While another study indicated global invNTS to be approximately 3.4 million illnesses and about 681 deaths (Ao et al., 2015). In San Diego alone, five cases of Salmonellosis were found positive with invasive *S. dublin,* following drinking of raw cow milk, and of these one died (Joshua et al., 1983; Fierer et al., 1983). In the US, the New York City Department of Health and the New York State Department of Health investigated the largest nosocomial outbreak of *S. enteritidis* to have occurred in 1987, in which 404 of the 965 patients (42 percent) at one hospital were affected, and 9 patients died. Further investigation revealed that *S. enteritidis* with the same phage type were found in patients, epidemiologically implicating raw eggs, and the ovary of a hen from the farm corporation that supplied the implicated eggs (Telzak et al., 1990).

In African countries NTS pathogens can cause a significant burden of life-threatening invasive infection (Feasey et al., 2012), characterized by mortality rate of about 20%, even when appropriate therapeutic antimicrobial agents are used (Barbara et al., 2016). Infants, young children and young adults are the most affected with the incidence risk of 227 cases per 100,000 populations (Ao et al., 2015). The disease has preference for immune-compromised patients (MacLennan et al., 2010), in particular the HIV patients are highly susceptible to invasive NTS

but tend to resist typhoid fever (Schreiber et al., 2011). Against this background, all immune-compromised patients suspected to be infected with any *Salmonella* serotype, need to be placed on an effective antimicrobial therapy before the disease develops into life-threatening systemic infection (Layton et al., 2007). In addition to immunological status, genetic background of the host also plays a role among the predisposing factors to invNTS disease (Gordon et al., 2011).

Surprisingly, invNTS Salmonellosis is less frequently reported in Asia than Africa (Khan et al., 2010), suggesting that certain areas of Africa are more predisposed to invNTS diseases than in other regions of the world (Jelsbak et al., 2012). In contrast, the incidence of invasive salmonellosis in developed countries is less, although a number of outbreaks have been reported in some countries such as Italy (Huedo et al., 2016), New-Zealand (Bloomfield et al., 2017; Bloomfield et al., 2021). In sub-Saharan Africa alone, an annual incidence risk of invNTS among young children of less than 5 years of age was in the range of 175–388 per 100, 000 cases (Sigaúque et al., 2009). The cases of invNTS were high 1800–9000 per 100 000 persons, in a study involving non-antiretroviral therapy (ART) treated HIV-prevalent cohorts (Feasey et al., 2012). In Africa, the disease is endemic in the rural than urban areas of sub-Saharan Africa countries where the effect of causing bloodstream infections is more pronounced (Morpeth et al., 2009).

2.2.2 Chickens Salmonellosis

Salmonella pathogens can cause disease in all animals and poultry, characterized by septicemia, acute enteritis (subacute) and chronic enteritis (Lin et al., 2002; Ayachi et al, 2015). *Salmonella* pathogens are highly adaptive and potentially pathogenic to domestic animals in particular young, pregnant and lactating animals which tend to be the most susceptible to a wide range of severe infections (Kemal et al., 2014). Several outbreaks of classical salmonellosis have been reported worldwide.

2.2.2.1 Fowl Typhoid

Fowl typhoid (FT) is an avian host specific Salmonellosis caused by *Salmonella gallinarum* (Chadfield et al., 2003; Rajagopal et al., 2013) and is of worldwide concern (Nagaraja et al., 1984; Chadfield et al., 2003; Sannat et al., 2017). The disease was first reported in England in 1888 by Poppe et al., (2000) and it affects birds of all ages (Lutful et al., 2010; Batista et al., 2015). The

disease was first described as “fatal septicemia” or “white diarrhea” by Poppe et al., (2000). Data on the outbreaks of FT is available for many countries; for instance in Korea, FT was reported in commercial broilers, baeksemi (a mixed breed of male meat-type breeder and female commercial layer), commercial layers, native chickens, and broiler breeders and the incidence rate was 47.7, 28.4, 17.2, 5.1, and 1.3%, respectively (Kang et al., 2010).

In South Eastern Nigeria an outbreak of FT affected 11,000 laying birds in Undi region of which the total mortality was 25% (Ezema et al., 2009). While in Japan, a flock of 400 Japanese quail (Coturnix coturnix japonica) at 91 days were affected with FT (Sousa et al., 2014). In the year 2012, two FT outbreaks were reported in August and October out of which 34,000 and 4,000 were reported dead respectively, in Northern Ireland, UK (Barrow et al., 2012). In Raigarh India, Chhattisgarh, an outbreak of FT was reported in adult layer birds of about 7-8 months at a Government Poultry Farm (Shoaib et al., 2017). In India several FT outbreaks were reported in the year 2002 (Kumar et al., 2009; Barrow et al., 2012). In Romania, 71 cases of FT outbreak in pheasants were reported, of which 22 died and 49 were slaughtered for laboratory investigation (Arbune et al., 2018). Several other workers have reported FT outbreaks (Lucas et al., 1955).

2.2.2.2 Pullorum Disease in Chicken

pullorum disease (PD) is another important fowl infection primarily of young chickens and turkeys (Lutful et al., 2010). The disease which was first discovered by Rettinger et al., (2019). It is considered by many bacteriologist as a variant of *S. gallinarum* which can be differentiated by chemical reactions (Kwon et al., 2000; Yang et al., 2019). Fowl typhoid and pullorum are two distinct diseases that specifically affect avian species (Barrow et al., 2012). Several outbreaks due to *S. pullorum* have been reported worldwide (Michael et al., 2001; Haider et al., 2013). In Zambia, ten outbreaks due to *S. gallinarum* and *S. pullorum* were reported on poultry farms involving day old broiler chickens and 5 to 18 months old layers’ chickens (Sato et al., 1997).

2.2.2.3 Paratyphoid Infections in Chicken

There are numerous types of *Salmonellae* that are not host-adapted that can produce paratyphoid infections. Various species of birds including chicken are infected by these *Salmonella* (Barrow et al., 2012). Through contamination and improper handling of chicken products, typhoid infections

have serious implications for public health. Among the most prevalent *Salmonella* infections in chicken are those caused *by S enterica typhimurium, S enterica enteritidis, S enterica Kentucky,* and *S enterica Heidelberg*. Different serotypes have varying levels of pathogenicity (Michael et al., 2001).

Geographical differences greatly affect which species are more prevalent. Usually, infection spreads horizontally via infected rodents, contaminated environments, or infected birds (Barrow et al., 2012). Most serotypes are primarily transmitted to offspring from infected breeders through fecal contamination of the eggshell, with the exception of *S. enterica enteritidis* and *S. enterica arizonae* (Barrow et al., 2012). Through transovarial transmission, *S. enterica enteritidis* and *S enterica arizonae* can infect the inside of the egg. Infected birds continue to be carriers (Michael et al., 2001).

Young birds exhibit clinical symptoms of paratyphoid illness, but clinical disease is typically absent in mature chickens (Kang et al., 2010). Most typically, the first few weeks of life are when young birds die. Despite the fact that certain clinical symptoms are not unique. Infection with *S. enterica* lead to depression, low growth and frailty dehydration and diarrhea, decreased feed consumption, and decreased egg production in hens are all possible effects of S. *enteritidis* (Yang et al., 2019; Kwon et al., 2000).

Young birds may have enlarged livers with localized necrosis, unabsorbed yolk sacs, enteritis with mucosal necrosis, and cecal cores, among other abnormalities. Sometimes the eye or synovial tissues are the site of an infection. On the other hand, there might not be any lesions if there is an abrupt death from septicemia (Kang et al., 2010). A diagnosis requires the isolation, identification, and serotyping of the causative agent. Serology is not very trustworthy.

Salmonella serotype *S. enterica enteritidis*, a Paratyphoid, is a serious threat to food safety, especially for the egg-laying business (Kwon et al., 2000). Transmission from breeders, contaminated settings, sick rats, and contaminated feed are a few potential causes in commercial layers. Breeders' pathogen are primarily transmitted through eggshell contamination, while

transovarial transmission is also possible, unlike with other Paratyphoid *Salmonella* (Yang et al., 2019).

2.3 Adaptation of *Salmonella* Serotypes in the Host

The genus *Salmonella* constitutes many pathogenic serotypes that are highly adaptive to vertebrates and are host specific (Evangelopoulou et al., 2014). Most serotypes are capable of causing diseases in multiple hosts but some are highly adapted to a single-host species (Bäumler et al., 2013). This phenomenon greatly applies to *Salmonella enterica* subspecies by virtue of their broad host range and their degree of host adaptation (Eswarappa et al., 2008). *Salmonella enterica* subspecies I have the largest diverse group of serotypes that have evolved to survive in a wide range of environments and across several hosts (Foley et al., 2013) and 99% of human and animal infection are caused by this group (Achtman et al., 2012). The diversity of *S. enterica* subspecies I is attributed to mutational evolution, as well as intra and inter species recombination that is potentially influenced by restriction-modification (RM) systems (Roer et al., 2016). *Salmonella enterica* subspecies can conveniently be divided into three groups namely; host-restricted serotypes, host-adapted serotypes and broad host-range (non-host adapted) (Rotger et al., 1999).

2.3.1 Host Restricted *Salmonella* Serotypes in Human

This group of *Salmonella* contains few serotypes which are exclusively limited to few susceptible host ranges for transmission of the disease (Bäumler et al., 2013). These serotypes manifest a systemic disease syndrome, which results into dissemination of pathogens beyond the lymph nodes in immune competent hosts, and they display a decreased intestinal involvement to a point where they are no longer associated with gastroenteritis (Kingsley et al., 2013). However, host restricted serotypes tend to cause higher mortality rates than those with a broad host range. The infection due to host restricted serotypes tends to persist in the tissue long after the clinical symptoms (Barrow et al., 2012). typhoid fever (TF) is a typical example of the disease caused by *S. typhi* a highly adapted serotype in human (Selander et al., 1990; Crump et al., 2015). Other serotypes include; *S. paratyphi* A, B and C (Srikantiah et al., 2004) and *S. Sendai* (Bäumler et al., 2013) which are capable of causing acute infections called paratyphoid fever in humans and have animal reservoirs (Germ et al., 2019). *Salmonella paratyphi* B can also cause sporadic gastroenteritis and less frequently paratyphoid fever in humans (Benenson et al., 2001).

2.3.2 Host Restricted *Salmonella* Serotypes in Chicken

Chicken hosts are associated with a serotype which produces the most prevalent infection and is of the highest commercial implication is *S. gallinarum* and its biotype *S. pullorum*. *S. gallinarum* produces fowl typhoid which may be supplemented by high mortality in chicken of all ages, whereas the *S. pullorum* biotype usually produces significant mortality only in young chickens (Barrow et al., 2012; More et al., 2017). Both biotypes infect other avian genera (Sannat et al., 2017).

2.3.3 Non-Host Adaptable *Salmonella* Serotypes

This group of serotypes exhibits a broad host range and generally tend to colonize the intestinal tract and invade enterocytes of the intestinal mucosa, manifesting gastroenteritis, but in contrast fail to disseminate beyond the lymph nodes, unless in the event that the host has an underlying immune defect (Kingsley et al., 2013). The group consists of serotypes such as *S. typhimurium* and *S. enteritidis* (Rotger et al., 1999), and is perceived as a major cause of both gastroenteritis and septicemia syndromes in humans (Roudier et al., 1990) and animals (Kelterborn et al., 1967). For instance, *Salmonella typhimurium* and *S. enteritidis* serotypes can cause disease in broad range of highly susceptible animal hosts such as poultry, cattle, rodents, pet hedgehogs as well as humans (Nakamura et al., 1987; Anderson et al., 2017). Further, these serotypes have been implicated in food-borne Salmonellosis worldwide, and have been detected in most species of domestic and wild animals used as food animals for human consumption (Gantois et al., 2009).

Other non-host adapted *Salmonella* serotypes that have been associated in food-borne diseases (FBD) include; *Salmonella Infantis, S. Heidelberg, S. Bovis-morbificans* and *S. Agona* (Lax et al., 1995). However, reported death due to zoonotic *Salmonella* serotypes are few less than 0.5% of cases of human illness (Miller et al., 1995).

However, a variable number of common broad-host-range serotypes carry virulence plasmids which encode *spv* genes (Libby et al., 1997). Therefore, these are potential serotypes capable of causing systemic disease in a wide range of animals including humans and can also manifest diverse clinical symptoms, from asymptomatic infection to serious typhoid-like syndromes (Nakamura et al., 1985; Uzzau et al., 2000).

2.4 Transmission of *Salmonella*

The transmission of *Salmonella* is varied from host to host. The dynamics of transmission may be influenced by other factors as outlined in Figure 2.1. The main source of infection is the contaminated feed through and water which lead to humans and chickens' infection.

2.4.1 Transmission of *Salmonella* in Humans

Salmonella is spread by the faecal-oral route and can be transmitted by food and water, and by direct animal contact, and rarely from person-to-person. Humans usually become infected by eating foods contaminated with faeces from an infected animal. As a result, implicated foods are often of animal origin such as beef, poultry, milk, and eggs (Vila et al., 2021). *Salmonella* pathogens are maintained in animal reservoirs, and infection in humans' follows ingestion of contaminated animal products or ingestion of farm produce contaminated with infected animal faeces, or in close contact with infected animals (Morse et al., 1978; Hendriksen et al., 2004; Soltan et al., 2016).

A variety of foods especially of animal origin such as poultry, eggs and dairy products are the most common vehicles of salmonellosis (Pui et al., 2011; Knegt et al., 2015) and can disseminate *Salmonella* pathogens through shedding in faeces. Chickens are the main culprits, since they do not often show signs of the disease, as a result the entire flock can become colonized unnoticed and tend to shed the pathogens through their faeces over a period of time (Clavijo et al., 2006; Penha et al., 2019), and consequently the environment become contaminated with *Salmonella* pathogens. Although, human foodborne salmonellosis is largely attributed to animal reservoirs (Knegt et al., 2015), the transmission of the disease will largely depend on the animal host susceptible to the type of *Salmonella* serotype (Baumler et al., 2013). The transmission of *Salmonella* can lead to different types of Salmonellosis.

2.4.1.1 Human Typhoid Salmonellosis

Human typhoid infection are mainly related to exposure to human faeces a major mode of transmission involving person to person, usually via the faecal-oral route, or contamination of drinking water or food by carriers whose hands are soiled with excreta pathogens (WHO, 2009), and there are known animal reservoirs for these pathogens (Giannella et al., 1996). Other potential

vehicles include; sea food (Heinitz et al., 2000) and houseflies (Greenberg et al., 1970). Transmission of typhoid infection is dependent on several factors such as infective dose and immune-status of the host (Pees et al., 2013).

2.4.1.2 Human Non-Typhoid Salmonellosis

Currently, the predominant route of infection is through foodborne transmission from animals to humans and is common among the highly-industrialized countries (Morpeth et al., 2009). A large number of food-producing animals are the principal reservoirs of many pathogenic *Salmonella* serotypes in regard to human infections (Humphrey et al., 2000). Cattle, swine and poultry are the prime sources of *Salmonella* infections (Uzzau et al., 2000). In 1968, foodborne outbreak of gastroenteritis was reported in New Jersey, USA involving a family dinner thanks giving day at which turkey was served to 18 persons with a 95% attack rate of illness and 14% case-fatality rate (Janeway et al., 1971).

On the other hand, eggs have been prominently implicated as source of *S. enteritidis* a cause of human illness for several decades (Gantois et al., 2009). However, profession practice can also be a source of *Salmonella* infection, for instance, an outbreak of *S. typhimurium* associated with a veterinary clinic was reported involving one cat, two veterinary technicians, four persons associated with clinic patients, and a nurse not linked to the clinic (Herrera et al., 2012). In 2012, an outbreak of *S. infantis* was reported involving a total of 49 individuals, 47 from 20 states in USA and two from Canada, and the pathogen was traced to a food production facility (Imanishi et al., 2014).

Several other studies also have indicated that ingestion of farm plant produce such as raw vegetables (lettuce), contaminated with animal waste can bring about salmonellosis infection (Mahon et al., 1997). Other potential routes for transmission of NTS pathogens includes the shedding of bacteria in animal excreta which can be transmitted directly to humans through contact (Ramos et al., 2017), or by indirect route through contamination of the environment (Julia et al., 2000). Transmissions of *Salmonella* to humans through contaminated food processing plants are of great importance (Bangtrakulnonth et al., 2004). Equally, fomites and mechanical vectors (insects and flies) are also known to spread *Salmonella* (Pui et al., 2011). In the period 2006 to

2011, foods of animal origin were linked to most of the foodborne diseases in USA (Gieraltowski et al., 2016).

2.4.2 Transmission of *Salmonella* in Chickens

Transmission in chicken usually occurs directly from infected birds, contaminated environments, or infected rodents (Figure 2.1). Chickens might have *Salmonella* germs in their droppings and on their bodies (feathers, feet, and beaks), even when they appear healthy and clean (Heres et al., 2004). The germs can get on cages, feed and water equipment, litter, plants, and soil in the area where the chicken live and roam. *Salmonella* also can get on the hands, shoes, and clothes of humans' who handle or care for chicken.

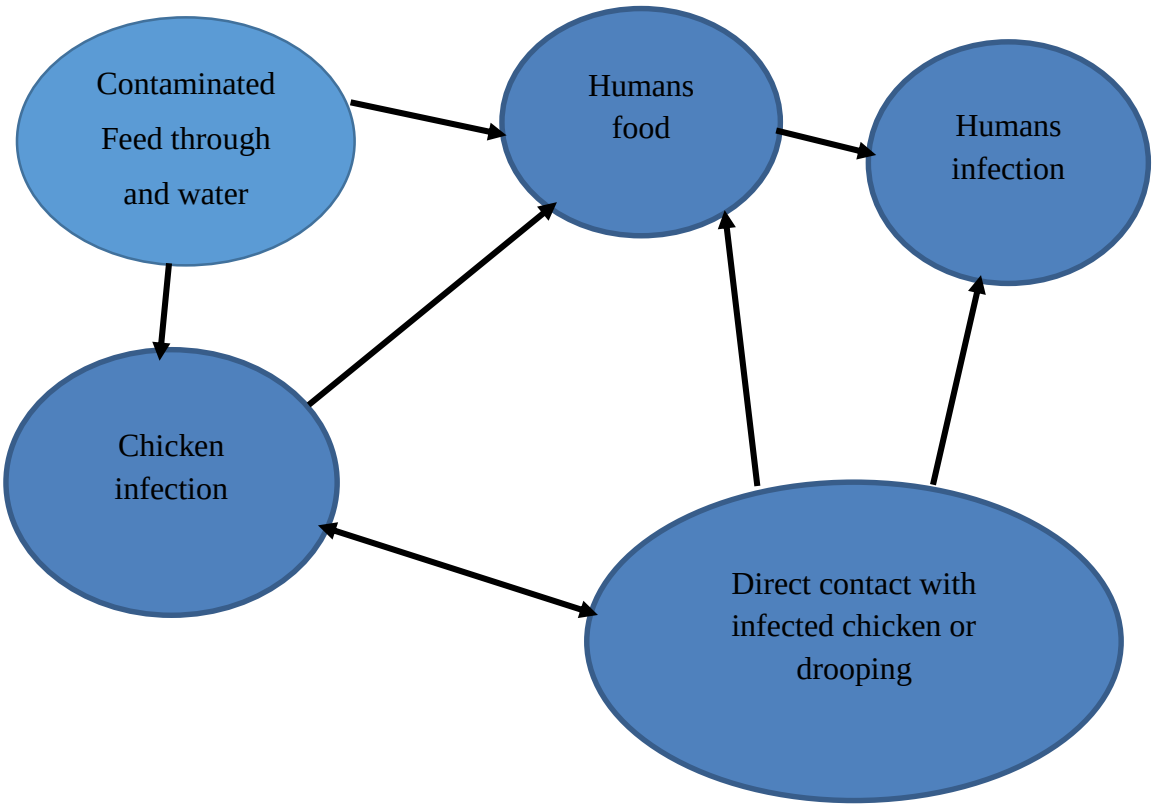

Figure 2.1: Possible ways of *Salmonella* Transmission

Horizontal transmission is the common type of *Salmonella* transmission which is normally due to faecal contamination of egg shell during the laying process. Further to contamination as a result of a single source of faecal contamination, other routes may amplify *Salmonella* and these may include: insects via eggs and larva from generation to another (Wierup et al., 1988). Dogs and cats can also play major roles as reservoir and by roaming around chicken pens where they can easily transmit *Salmonella* because dogs and cats are asymptomatic carriers of *Salmonellae* which has been attributed to the indiscriminate feeding habits of these species, tending to ingest feed regardless of freshness or contamination. Shedding of *Salmonella* by dogs and cats has been linked to illness in humans living in the same household with the shedding pet (Crump et al., 2015). Rodents and wild birds may serve as reservoirs and mechanical transmitters of *Salmonella* to chicken (Pearce et al., 2005).

2.4.2 Vertical Transmission of *Salmonella* in Chickens

The other mode of transmission is the vertical route that is as a result of insemination. The uterus may be infected and then transfer the *Salmonella* to the egg. The contaminated egg will lead to production of infected eggs and chicks (Heres et al., 2004). In the nature, both carnivorism and shedding of pathogens through faecal contamination of the environment are common routes of spreading *Salmonella* pathogens among wild birds (Tizard et al., 2004). Figure 2.1, shows various transmission routes. Since, *Salmonella* pathogens frequently colonizes the intestinal tract of food producing animals such as chicken, this could result into the contamination of the avian reproductive tract and subsequently the egg during the passage to the outside world (Stevens et al., 2009). Animal feeds have been linked to source of Salmonellosis (Jones et al., 1982).

2.5 Mechanism of *Salmonella* Pathogenicity

After *Salmonella* bacteria are ingested, they pass through the stomach and colonize the small and large intestine. There, the bacteria invade the intestinal mucosa and proliferate. The bacteria can invade the lymphoid tissues of the gastrointestinal tract and spread to the bloodstream. Dissemination to the bloodstream depends on host factors such as host health status (level of immunity) and virulence of the *Salmonella* strain and occurs in less than 5% of infections (Kagirita et al., 2017). If the infection spreads to the bloodstream, any organ can become infected (e.g., liver, gallbladder, bones, or meninges or large intestines). Illness usually lasts 4–7 days. If the infection

spreads to the bloodstream and distant organs, the illness increases in duration and severity and will usually include signs and symptoms related to the organ affected (Babu et al., 2004). A small proportion of humans' and animals infected with *Salmonella* develop reactive arthritis as a long-term sequel of the infection.

The mechanisms of infection differ between typhoidal and nontyphoidal serotypes, owing to their different targets in the body and the different symptoms that they cause. Both groups must enter by crossing the barrier created by the intestinal cell wall, but once they have passed this barrier, they use different strategies to cause infection (Hendriksen et al., 2004). Nontyphoidal serotypes preferentially enter M cells on the intestinal wall by bacterial-mediated endocytosis, a process associated with intestinal inflammation and diarrhoea. They are also able to disrupt tight junctions between the cells of the intestinal wall, impairing the cells' ability to stop the flow of ions, water, and immune cells into and out of the intestine. The combination of the inflammation caused by bacterial-mediated endocytosis and the disruption of tight junctions is thought to contribute significantly to the induction of diarrhea (Alikhan et al., 2018).

Salmonellae are also able to breach the intestinal barrier via phagocytosis and trafficking by CD18-positive immune cells, which may be a mechanism key to typhoidal *Salmonella* infection (Hendriksen et al., 2004). This is thought to be a stealthy way of passing the intestinal barrier, and may, therefore, contribute to the fact that lower numbers of typhoidal *Salmonella* are required for infection than nontyphoidal *Salmonella*. *Salmonella* cells are able to enter macrophages via macropinocytosis (Heres et al., 2004).

Typhoidal serotypes can use this to achieve dissemination throughout the body via the mononuclear phagocyte system, a network of connective tissue that contains immune cells, and surrounds tissue associated with the immune system throughout the body. Much of the success of *Salmonella* in causing infection is attributed to two type III secretion systems which function at different times during an infection. One is required for the invasion of nonphagocytic cells, colonization of the intestine, and induction of intestinal inflammatory responses and diarrhea.

The other is important for survival in macrophages and establishment of systemic disease. These systems contain many genes which must work co-operatively to achieve infection. The AvrA protein toxin injected by the SPI1 type III secretion system of *S. typhimurium* works to inhibit the innate immune system by virtue of its serine/threonine acetyl transferase activity, and requires binding to eukaryotic target cell phytic acid (Alikhan et al., 2018).

Salmonellosis is known to be able to cause back pain or spondylosis. It can manifest as five clinical patterns: gastrointestinal tract infection, enteric fever, bacteremia, local infection, and the chronic reservoir state (CDC, 2013). The initial symptoms are nonspecific fever, weakness, and myalgia among others. In the bacteremia state, it can spread to any parts of the body and this induces localized infection or it forms abscesses (Hendriksen et al., 2004). The forms of localized *Salmonella* infections are arthritis, urinary tract infection, infection of the central nervous system, bone infection, soft tissue infection. Infection may remain in the latent form for a long time, and when the function of reticular endothelial cells is deteriorated, it may become activated and consequently, it may secondarily induce spreading infection in the bone several months or several years after acute salmonellosis (CDC, 2013; Crump et al., 2015).

Pathogenicity islands are present in both positive and gram-negative bacteria and are linked to human, animal and plant pathogens, though they have been detected in non-pathogenic bacteria as well (Amanda et al., 2019). A typical pathogenicity islands (PAI) is a unique DNA region found in the genome of pathogenic bacteria but absent from non-pathogenic bacterium of the same species (Schmidt et al., 2004). Pathogenicity Island is usually large and unstable components of the chromosome (Lee et al., 2000) and contains about 10-200 kb clusters of genes associated with virulence, survival, extra-intestinal and fitness of bacteria (Amanda et al., 2019).

2.6 Diagnosis of Salmonellosis

Multiple diseases can cause fever, diarrhoea, and abdominal cramps. Therefore, salmonellosis cannot be diagnosed on the basis of symptoms alone (CDC, 2013). Diagnosing Salmonellosis requires testing clinical specimens such as stool or blood from an infected person to distinguish it from other illnesses that can cause diarrhea, fever, and abdominal cramps. The bacterium is usually isolated in the laboratory from the patient's stool or other clinical materials and is identified by

using a series of biochemical tests. Once *Salmonella* is identified in the specimen, additional testing can be done to further characterize the *Salmonella* (Wierup et al., 1988). Subtyping, serotyping, pulsed field gel electrophoresis, and molecular tests are used for definitive identification of *Salmonella*. Antimicrobial susceptibility testing of *Salmonella* isolates is an important adjunct to diagnostic testing of sick animals. Both provide insights into the epidemiology of the patient's infection. Antimicrobial susceptibility testing also provides valuable information in the treatment of the patient, if use of antibiotics is deemed appropriate (CDC, 2013).

2.6.1 Culture and Isolation

The samples should be inoculated in a pre-enrichment culture non-selective liquid medium of buffered peptone water, and incubated at 37ºC for 24 hours. Aliquots from pre-enrichment can be inoculated into selective enrichment liquid media at a ratio of 1/10 in Rappaport-Vassiliadis broth and at 1/10 in Selenite-Cysteine broth. A loopful of each broth should be streaked on plates of Xylose Dextrose Agar (XLD). The temperature and the period of incubation need to be standardized at 37ºC for 24 hours, respectively, for both chicken droppings and stool samples. The suspected colonies of *Salmonella* from each plate should be collected for presumptive identification by biochemical tests (Love et al., 2008).

The following tests can be employed to aid the identification of *Salmonella* species: oxidase, hydrogen sulphide, urease, indole, fermentation of glucose, sucrose, mannitol, and lactose. Colonies with a biochemical profile of *Salmonella* should be further confirmed through serological testing by the use of polyvalent serum against O and H *Salmonella* antigens. The colonies that agglutinate during a period of one to two minutes are considered as positive for *Salmonella*, and should be preserved in nutrient agar for a short time and then preserved with glycerol at -40ºC, if required for further analysis (Love et al., 2008).

2.6.2 Molecular Identification of *Salmonella* Strains

The once widely used conventional diagnostic methods for discriminating bacteria by biochemical and serological characteristics is no longer favoured because of its inaccuracy and now various molecular techniques are preferred to identify the phylogenic and fingerprinting of the bacteria (Wattiau et al., 2011). Molecular methods include polymerase chain reaction PCR related assays,

in situ hybridization, microbiome profiling, and molecular typing of pathogens (Cai et al., 2016). However, of these methods, (PCR) and DNA sequencing have been widely used to identify infectious pathogens (Cai et al., 2016). Other molecular methods are in use for the detection of genes such as multiplex PCR (Fitzgerald et al., 2007), quantitative PCR (Piknova et al., 2005) and real time PCR (Cai et al., 2016). PCR-ribotyping (Sanchez et al., 1993), PCR-enterobacteria intergenic consensus (PCR-ERIC) methods (Kumar et al., 2009) and microarray analysis PCR (Rasooly et al., 2008), are among the emerging technologies for the diagnosis of bacterial infections.

2.6.2.1 Char**acterization of *Salmonella* Strains by PCR-Invasion Protein *A* gene**

Salmonella invasion *invA gene is* found in all the *Salmonella* serotypes (Lostroh et al., 2001) and is located in the *invA* locus (Rahn et al., 1992). Invasion protein A gene is highly conserved across pathogenic bacteria, as a member of a set of several inner membrane proteins that form type 111 secretion system (T3SS) and it plays a key role in the functioning of the T3SS (Calayag et al., 2017). The *inv*A gene is the first gene of an operon consisting of at least two additional invasion genes, involved in the earliest steps of the invasion of intestinal epithelium cells by the facultative intracellular pathogen *Salmonella* spp (Galán et al., 1996).

Identification of *Salmonella* genus using the conventional techniques, is not only time consuming but the results may also be not very accurate. To overcome these potential problems, the molecular approach for organism recognition, known as polymerase chain reaction (PCR), has been used for characterisation of *Salmonella* spp. in various biological samples. Invasion protein A gene is now widely used in PCR assays as an international standard for identification and characterization of genus *Salmonella* (Arnold et al., 2004). Chromosomal invasion protein A (*inv*A) is a specific PCR target gene used to detect *Salmonella* species from different samples (Jamshidi et al., 2009), and has also been used to provide prevalence rates of *Salmonella* spp in contaminated broiler carcasses and as such has been considered as an appropriate alternative to conventional culture method (Ezzat et al., 2014). In our literature review, distribution of *inv*A gene detected by PCR methods.

The *inv*A gene was used in this study because of its specific sensitivity and wide application in identification of a *Salmonella* strains resulting in 100% PCR amplification of the *invA* gene

product (de Oliveira et al., 2003). From the literature reviews, the PCR amplified *invA* gene products of *Salmonella* strains were sequenced to understand the genetic links between the isolates from different sources and serotypes (Ainslieet al., 2018).

2.6.2.2 Molecular Characterization of *Salmonella* Serotypes

Several PCR based molecular methods have been developed for easy identification of *Salmonella* genus and serotypes isolated from various biological samples by probing its DNA (Rodriguez et al., 2014). For example, the PCR-restriction fragment length polymorphism (PCR-RFLP) was used to differentiate two biotypes namely *S. gallinarum* and *S. pullorum* (Cheraghchi et al., 2014). While the multiplex PCR can discriminate strains into serotypes with easy, it has its own limitations of failing to resolve the differences between variants of the same serovar due to antigenic alterations or subtle point mutation in H1/H2 antigens responsible for loss of flagella expression (Hong et al., 2008). Among these protocols, some utilize serotype-specific primers to identify *Salmonella enterica* species and its serotype samples (Kim et al., 2006).

Several other methods have been developed for rapid identification of *Salmonella* serotypes based on pulsed-field gel electrophoresis (PFGE). This is useful for fingerprinting strains in outbreaks situations (Liebana et al., 2003). Restriction fragment length polymorphism (RFLP) (Paiva et al., 2009) and multiple genetic fingerprinting (Liebana et al., 2003), may be used to differentiate *Salmonella* species. Multi-locus variable-number tandem-repeats analysis (MLVA) has become a valuable molecular subtyping technique for *Salmonella*, particularly for highly homogenic serotypes such as *Salmonella Enteritidis* (Muvhali et al., 2017).

2.6.3 Characterization of *Salmonella* Virulence Plasmids

The defined genomic islands on *Salmonella* pan-genome (Fookes et al., 2011; Hayward et al., 2013) referred to as *Salmonella* pathogenicity islands (*SPIs) (*Elder et al., 2016) and range from *SPI*-1 to *SPI*-23 for *Salmonella*. In the chromosome of *Salmonella enterica* only five *SPI-1* to *SPI*-5 are considered major *SPIs,* while *Salmonella bongori* harbours only 4 of them *(SPI-1, SPI-3* to *SPI*-5*)* without *SPI-2* (Porwollik et al., 2004). However, most attention has been paid to *SPI-1* and *SPI*-2 (Elder et al., 2016), which are critical for invasion of nonphagocytic cells in the intestinal tract, and for survival in macrophages and systemic spread, respectively (Hansen et al., 2001;

Giacomodonato et al., 2007). The *SPI-1* and *SPI-2* each encode a type III secretion system (T3SS) for virulence proteins that have a defined role during the intestinal and systemic phases of Salmonellosis (Giacomodonato et al., 2007).

These virulence proteins manipulates host signal transduction pathways and cellular processes to the advantage of the pathogens (Ramos et al., 2017), and their expression is activated by a transcription factor referred to as *HilA*, also encoded on *SPI-1* (Murray et al., 2000). These virulence genes are associated with the intestinal infection located on the *Salmonella* pathogenicity Island 1 and 2 (*SPI-1* and *SPI-2*) (Sırıken et al., 2013). The *SPI-1* encodes a first T3SS which expresses several invasion genes such as *inv*A (Murray et al., 2000).

For example, *SPI-1* has been detected in all *Salmonella* species (*S. enterica* subspecies and *S. bongori*) (Ochman et al., 1996; Hensel et al., 1997), and could have been passed on to all lineage ancestral to *Salmonella* serotypes (Li et al., 1995).

The *inv*A gene is a unique gene which encode proteins that are required for entry of the bacteria into epithelial cells. Most of the virulence genes are encoded within *Salmonella* pathogenicity islands (SPIs) on the chromosome as units of large cassettes. These are clusters of chromosomal virulence genes found only within the genus *Salmonella*. The accessory virulence genes called *Salmonella* plasmid virulence A, B and C (*spv*ABC) genes were selected on the basis that they are indicative of being potential to facilitate extra-intestinal salmonellosis in humans and animals (Hang'ombe, et al., 2008).

Among the PCR target genes used to detect *Salmonella* species includes *spvA, spvB* and *spvC* (Jamshidi et al., 2009; Shagufta et al., 2017). The role of *spv*A is to promote the macrophage phase avoiding destruction by neutrophils (Gebreyes et al., 2009), and but also the carriage of *spv*A together with the MDR phenotype enables the bacterium to be of clinical importance when compared with MDR strains without the *spvA* gene or part of the spv operon (Zhang et al., 2010). The function of *spv*B encodes an enzyme ADP-ribosylates which are modifies actin cytotoxin required for systemic survival (Mohler et al., 2008), and it is responsible for the *spv* virulence

phenotype (Lesnick et al., 2001**),** while *spv*C has phosphothreonine lyase activity which inhibit mitogen-activated protein kinases signaling (MAPKs) (Guiney et al., 2011).

In the same manner lack of *spv*C gene may be indicative of partial virulence of *Salmonella* strains (Guiney et al., 1995). The function of *spv*D is not well defined. *Salmonella* serotypes in possession of *spv* genes are reported to have the propensity of causing serious *Salmonella* infections in patients receiving immunosuppressive therapy such as HIV patients with low levels of CD4 T cells, a strong indication that they cannot fight the disease (Guiney et al., 1995; Guiney et al., 2011).

2.6.4 Sequencing of *Salmonella* Strains

Bacterial genomes are diversified structures composed of genes present in every strain of the same species (core genome), and genes may be present in some but not all strains of a species will have the same accessory genes (Wiesner et al., 2009). The molecular assays target unique gene sequences associated with the pathogens (Mortimer et al., 2004). In this case, polymerase chain reaction (PCR)-based methods have been used for both insertional and repetitive deoxyribonucleic acid sequences analysis (Boceska et al., 2017).

Whole Genome Sequencing (WGS) is a modern molecular epidemiology approach that uses WGS assemblies from pure cultures to study and compare foodborne outbreaks. It's a metagenomic technique based on enriched mixed cultures (EMCs) of complex matrices for early pathogen discovery (McClelland et al., 2001). WGS, produce higher resolution *Salmonella* phylogenetic trees that could distinguish isolates collected from humans, animals, and the environment. Furthermore, WGS is a valuable method for AMR prediction, plasmid replicon discovery, and virulence gene detection (McDermott et al., 2016).

All species (bacteria, plants, and mammals) have a unique genetic code, or genome, made up of nucleotide bases (A, T, C, and G). You can identify an organism's unique DNA fingerprint, or pattern, if you determine the sequence of its bases (Rahn et al., 1992). Sequencing is the process of determining the order of bases. Whole genome sequencing is a laboratory approach that establishes the order of bases in an organism's genome in a single operation. All organisms (bacteria, vegetable, mammal) have a unique genetic code, or genome, that is composed of

nucleotide bases (A, T, C, and G). If you know the sequence of the bases in an organism, you have identified its unique DNA fingerprint, or pattern. Determining the order of bases is called sequencing. Whole genome sequencing is a laboratory procedure that determines the order of bases in the genome of an organism in one process (Rahn et al., 1992).

Traditional serology and the Kauffmann White Scheme (KWS) were the gold standard for *Salmonella* serotyping until recently. Whole Genome Sequencing (WGS) has recently emerged as a viable option in this field (Ibrahim et al., 2018). Serotype information remains a critical component of food safety and public health efforts to decrease salmonellosis. Simultaneously, recent improvements in WGS have enhanced the ability to do advanced pathogen characterization, as well as trace back studies to establish the source of foodborne disease during outbreaks. In silico data analysis methods have been used to accomplish serovar prediction based on WGS. The tools that have been developed include *Salmonella in silico* Typing Resource (SISTR), SeqSero, and *in silico* 7-gene MLST ST (Multilocus Sequence Typing Sub-Typing) which is generated using the SISTR platform (Ibrahim et al., 2018).

Public health experts worldwide are working hard to verify these tools as a replacement for existing monitoring methods in order to provide a more powerful strategy for molecular epidemiology in support of public health investigations. According to Yachison et al., (2017) study that included 1,041 *Salmonella* isolates obtained from a laboratory between 1999 and 2017. The isolates were important for public health since they all came from food, feed and environmental swabs. They were all serotyped using both standard serology and WGS, as well as an in silico serovar prediction method called SeqSero. About 899 (86.4%) of the 1,041 *Salmonella* isolates were more identical. In 80 isolates (7.7%), SeqSero assignments deviated from standard serological testing, while 62 isolates had no serotype prediction. When compared to the traditional KWS serotyping, the study was an excellent example of using WGS and SeqSero as a data analysis tool for predicting *Salmonella* serotypes, which can provide numerous advantages.

On the Luminex platform, molecular tests for serotyping bacterial isolates are frequently designed to detect serotypes based on DNA markers inside genes responsible for O and H antigen production (Vanegas and Joys, 1995; Fitzgerald et al., 2007). By combining PCR with a multiplexed bead-

based detection method, the Luminex xMAP technology detects nucleic acids. By hybridizing with antigen-specific-DNA probes, the antigen encoded by the PCR fragment is detected. Although it is relatively quick and inexpensive when looking at specific or common serotypes, one present limitation of this technology is that molecular serotyping does not identify all serotype antigens and concentrates primarily on the most frequent serotypes reported for human clinical specimens. (McQuiston et al., 2004, 2011).

In just one sequencing test, next generation sequencing technology delivers a wealth of information regarding bacteria's species, serovar, virulence, pathogenicity, antibiotic resistance, and subtype. WGS can yield high-quality sequence data in public health laboratories, allowing for better identification of clinical strains and better assessment of virulence and antimicrobial-resistance genes (Wu et al., 2021). WGS has already transformed genomics, allowing researchers to analyze gene expression by sequencing RNA and evaluating host-pathogen relationships (Oakeson et al., 2018). WGS can also be used to pinpoint the source of a disease within a community. It is critical for detecting mutations and understanding the genetics of *Salmonella* and other microbes. It can also assess strain evolution during an outbreak and detect contextual data on genetic interconnectedness (Gilchrist et al., 2015). WGS is widely used to locate outbreak clusters and quickly infer phylogenies from sequencing reads (Ferrari et al., 2017).

WGS is increasingly employed as an alternate technology for acquiring quick and accurate serotype information (Gymoese et al., 2017). WGS can be used as the exclusive method for routine serotyping of *Salmonella* isolates. This method allows for the quick identification of *Salmonella* serotypes as well as the detection of a variety of single nucleotide polymorphisms (SNPs) inside the genome. These SNPs can be used to analyze the epidemiology of an outbreak, linking human instances of illness to the source of contamination and distinguishing outbreak-related from unrelated random clinical cases (Lienau et al., 2011). WGS can give researchers and physicians more information on antibiotic resistance markers and virulence factors, allowing them to better understand serotypes and swiftly identify and study outbreaks while giving trace-back and trace-forward information (Inns et al., 2015; Taylor et al., 2015).

For surveillance and foodborne outbreak purposes, WGS is increasingly replacing conventional molecular subtyping approaches (Bakker et al., 2011; Leekitcharoenphon et al., 2014). It allows for high-resolution molecular subtyping and provides useful additional information for further characterization of developing clones based on genetic differences and evolutionary research (Bale et al., 2016; Bekal et al., 2016). This information is crucial for acquiring clonal information in outbreak investigations during any outbreak response. Several studies have demonstrated that WGS-based typing offers greater discriminatory power than conventional molecular typing approaches for *Salmonella* (Koser et al., 2012; Deng et al., 2015). Next-generation sequencing is currently transforming public health microbiology, allowing for faster serotype determination utilizing WGS data. SeqSero (www.denglab.info/SeqSero) is a brand-new web-based tool for detecting *Salmonella* serotypes using whole-genome sequencing data (Zhang et al., 2019).

2.7 Treatment of Salmonellosis

Early treatment is essential for septicemic salmonellosis, but there is controversy regarding the use of antimicrobial agents for intestinal salmonellosis (Fadlalla et al., 2012). Oral antibiotics may be ineffective and may deleteriously alter the intestinal microflora, thereby interfering with competitive antagonism and prolonging shedding of the organism. There is also concern that antibiotic-resistant strains of *Salmonellae* selected by oral antibiotics may subsequently infect humans (Basbas et al., 2021). By suppressing antibiotic-sensitive components of the normal flora, antibiotics may also promote transfer of antibiotic resistance from resistant strains of *E coli* to *Salmonella*. Use of chemotherapeutic antibiotics for growth stimulation has been banned in many countries for this reason.

Broad-spectrum antibiotics administered systemically are indicated for treatment of septicemia (Fadlalla et al., 2012). Initial antimicrobial therapy should be based on knowledge of the drug resistance pattern of the organisms previously found in the area. Nosocomial infections may involve highly drug-resistant organisms. Trimethoprim-sulfonamide combinations may be effective. Alternatives are ampicillin, fluoroquinolones, or third-generation cephalosporins (Basbas et al., 2021). Resistance to ampicillin, trimethoprim, sulfonamide, tetracyclines, and aminoglycosides is generally plasmid mediated and transfers readily between different bacteria. Resistance to quinolones is mutational, but random mutations may be selected by antibiotic use

and may be transferred by bacteriophages. Treatment should be continued daily for up to six days.

If oral medication is chosen, it should be given in drinking water and not mixed into solid feed, because affected animals are thirsty due to dehydration and their appetite is generally poor (Fadlalla et al., 2012). Fluid therapy to correct acid-base imbalance and dehydration may be necessary. Calves, adult cattle, and horses need large quantities of fluids. Antibiotics such as ampicillin or cephalosporins lead to lysis of the bacteria with release of endotoxin or flunixin meglumine may be used to reduce the effects of endotoxemia.

The intestinal form is difficult to treat effectively in all species. Although clinical cure may be achieved, bacteriologic cure is difficult, either because the organisms become established in the biliary system and are intermittently shed into the intestinal lumen, or because the animals are reinfected from the environment at a time when their normal gut flora, which is inhibitory to colonization by pathogens, is depleted by antibiotic therapy (Kalra et al., 2003). A concern with antimicrobial therapy is that it may increase the risk of creating carrier animals. In people and other animal species, antimicrobial therapy prolongs the period after clinical recovery during which the pathogen can be retrieved from the gastro intestinal tract GIT tract.

2.7.1 Commonly Used Antibiotics for Treatment of Human Salmonellosis

Treatment of *Salmonella* gastroenteritis may not be essential, but for the treatment of patients with invasive infections (Angulo et al., 2000; Zanini et al., 2014). The clinical condition of a patient will usually dictate the choice of the antibiotics to be prescribed from a range of the traditional first line antibiotics that include; ampicillin, amoxicillin or trimethoprim/sulfamethoxazole (Diseases, 2006; Jackson et al., 2016) and chloramphenicol (Eng et al., 2015). Of these antibiotics, chloramphenicol, ampicillin and co-trimoxazole were considered drugs of choice to treat typhoid fever (Kalra et al., 2003). On the other hand, patients at increased risk of invasive disease, including immune-compromised patients or infants of less than 3 months of age may be recommended to be prescribed antibiotics to eliminate the bacteria (American Academy of Pediatrics, 2006).

However, the advent of antimicrobial resistant pathogens towards traditional antibiotics prompted a renewed effort to counter the effect by introduction of new drugs such as extended-spectrum cephalosporins; ceftriaxone, cefotaxime, and fluoroquinolones to treat MDR *S. Typhi* strains (Sood et al., 1999). Among these antibiotic agents, fluoroquinolones and third-generation cephalosporins are the drugs of choice for invasive *Salmonella* infections in humans (Angulo et al., 2000). On the other hand, ciprofloxacin is used against NTS infections only in situations showing signs of severe gastroenteritis (Pui et al., 2011), but also can be a first-line drug of choice for treatment of typhoid fever (Ammar et al., 2016).

Azithromycin is effective in the management of uncomplicated TF (Harish et al., 2011). All these antimicrobial agents are now considered primary antimicrobial treatment options and any resistance against them may complicate treatment (Tate et al., 2017). Empirical treatment of TF can be backed by other measures such as oral or intravenous hydration, lukewarm bath, sponging, appropriate nutrition and blood transfusion (Kalra et al., 2003).

2.7.2 Antimicrobial Agents for Treatment of Animal Salmonellosis

It was not until 1950, that American farmers were excited about the news that a laboratory in New York had discovered that the use of antibiotics in livestock feed improved animal's growth and reduced the production cost than the use of conventional feed supplements (Chen et al., 2013). This was undoubtedly considered as one of the major breakthroughs in the history of veterinary medicine, since the use of antibiotics in animal husbandry and veterinary medicine has resulted in healthier and more productive farm animals, consequently ensuring the welfare and health of humans as well (Economou et al., 2015).

The exact amount of antibiotics used in the United States is hard to quantify, but estimated between 15 and 17 million pounds of antibiotics are administered to livestock every year (Mohiuddin et al., 2019; Edraki et al., 2021). On another hand, usage of antibiotics in animals is estimated at 18 percent of the 22.7 million kilograms of antibiotics produced in the United States per year (Edraki et al., 2021). In the European Union, use of antibiotics as growth promoters in animal feeds has been allowed in the member states during the last 50 years (Koluman et al., 2013). In 1951, the use of antibiotics as animal additives without veterinary prescription was approved in the United

States by Food and Drug Administration (FDA) (Melendez et al., 2010). However, only antibiotics that are not absorbed in the digestive tract are authorized as growth promoters (Koluman et al., 2013).

The demand for antibiotics for use in livestock industry is high such that manufacture of these products is now measured in terms of thousands of tons per year to combat both infections and pathogens that have been exposed to unprecedented levels of antimicrobial agents (Muurinen et al., 2022). Therefore, over consumption of antibiotics in animal food production has contributed greatly to the fueling of antibiotic resistance which has become a global crisis. It is observed that the current use of antibiotics in food animals outstretches that of human consumption (Özbek et al., 2021), and this should be a source of public health concern. On a global scale, antibiotic consumption is more pronounced with four countries; China, US, Brazil and India accounting for almost 50% of global usage (da Silva et al., 2020).

In the US alone it is estimated that 80 percent of all antibiotics used are given to food animals, mainly for growth promotion (Özbek et al., 2021). The global average annual consumption of antimicrobials per kilogram of animal produced was estimated at 45 $mg \cdot kg^{-1}$, 148 $mg \cdot kg^{-1}$, and 172 $mg \cdot kg^{-1}$ for cattle, chicken, and pigs, respectively (Pires et al., 2021). Antibiotic consumption in livestock worldwide is likely to raise by 67% between 2010 and 2030, in particular in countries that have high demand for animal protein for human consumption such as Brazil, Russia, India, China, and South Africa, due to the growing number of animals raised for food production (Pires et al., 2021).

According to da Silva et al., (2020), of the 27 classes of antibiotics currently in use, only seven classes are used exclusively for agriculture purposes. About 60% of antibiotics used in agriculture comprise tetracycline, penicillin and macrolides (da Silva et al., 2020) which at the same time are recommended for human use. The consequence of antibiotic use in animals of food origin has been down played for a long time by the animal industry. However, these major concerns about the steep rise in the use of antibiotics in animal farming around the world have recently been recognized by the United Nations (UN) Food and Agriculture Organization (FAO) and a great effort is being paid to control the situation (Srivastava et al., 2017).

2.7.3 Antimicrobial Resistance in *Salmonella*

The mechanisms to resist antibacterial substances has evolved in bacteria over a period of several billion years (Saliu et al., 2017). According to Economou et al., (2015), the continued use and misuse of antibiotics in farm animal settings as growth promoters or as nonspecific means of infection prevention and treatment has boosted antibiotic consumption and resistance among bacteria in the animal habitat. Subsequently, this reservoir of resistance can be transmitted directly or indirectly to humans through food supply (Figure 2.3) and direct or indirect contact (Varma et al., 2006; Economou et al., 2015; Mubita et al., 2020). In the long run use of antibiotics in animal husbandry such as feed additives have contributed to the development of resistant bacteria to drugs used to treat infections (Dallal et al., 2010). As a result, of the widespread antimicrobial resistance (AMR) in the 21st century has brought about public health crisis by reducing the effectiveness of prevention and treatment of the overwhelming range of infections caused by various pathogens such as bacteria, parasites, viruses and fungi (Prestinaci et al., 2015).

According to the CDC, (2013), depressingly. by the 1990s there were more exasperating reports underscoring the worsening situation of antimicrobial resistance to a range of medically important antimicrobial agents worldwide and the trend was envisaged to rise (Tripathi et al., 2017). Countries such as the United States of America (Lee et al., 1994), Greece (Tassios et al., 1997), Thailand (Hoge et al., 1998) and Turkey (Yildirmak et al., 1998), have highlighted the emergence of MDR. The recent surveillance data from 22 countries by the World Health Organization (WHO) revealed widespread and in certain cases high levels of antibiotic resistance against common bacterial infection across the globe (Khadim et al., 2020).

The most recent estimates of global antibiotic resistance, indicate that *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* are the most encountered resistant bacteria associated with both hospital and community-acquired infections (Freire et al., 2014), and including *Streptococcus pneumonia* and *Salmonella* spp (Khadim et al., 2020). The emergence of antimicrobial resistance strains among *Salmonella* have been linked to antibiotic use in livestock worldwide and has been a source of great concern (Kalra et al., 2003). As a consequence, millions of people are now exposed worldwide to the challenges of emergence of antimicrobial resistance

pathogens and their effect is more felt in developing countries (Saeed et al., 2015; Saeed et al., 2020).

A retrospective study by Wallinga et al., (2002), indicated that the use of antibiotics in animal industry contributes greatly to the prevalence of multidrug-resistant infections in humans. Antibiotics in animal feeds increase antibiotic resistance in *Salmonella* by promoting transfer of resistance factors (Giannella et al., 1996). However, there are several alternative ways that confer antimicrobial resistance phenotype to pathogens and notable among these are the acquisition of resistance genes via horizontal gene transfer and selection of resistant variants in the population (Poppe et al., 2006; McEwan et al., 2015). Traditionally, antibiotic resistance was associated with failed treatment of clinical conditions, but now worldwide propagation of antibiotic resistance is also considered to be linked to environmental reservoirs that are connected to anthropogenic activities such as animal husbandry, agronomic practices and wastewater treatment (Waglechner et al., 2017).

Antimicrobial resistance can be attributed to other different factors such as the type of the serotype and antibiotic used (Su et al., 2004). Figure 2.3 presents a potential public health concern involving the use of antibiotics for treatment or prevention of animal diseases as well as being used as growth promoters in animal feed in veterinary medicine (McEwen et al., 2002; Poppe et al., 2006), which subsequently result into the development of MDR (Sugiyama et al., 2016). Multidrug resistance phenotype is by nature a complex matter, which affects different bacteria belonging to the family of *Enterobacteriaceae* such as *Salmonella*, *Shigella* and *Escherichia coli* (Threlfall et al., 2005) and also some none enteric bacteria.

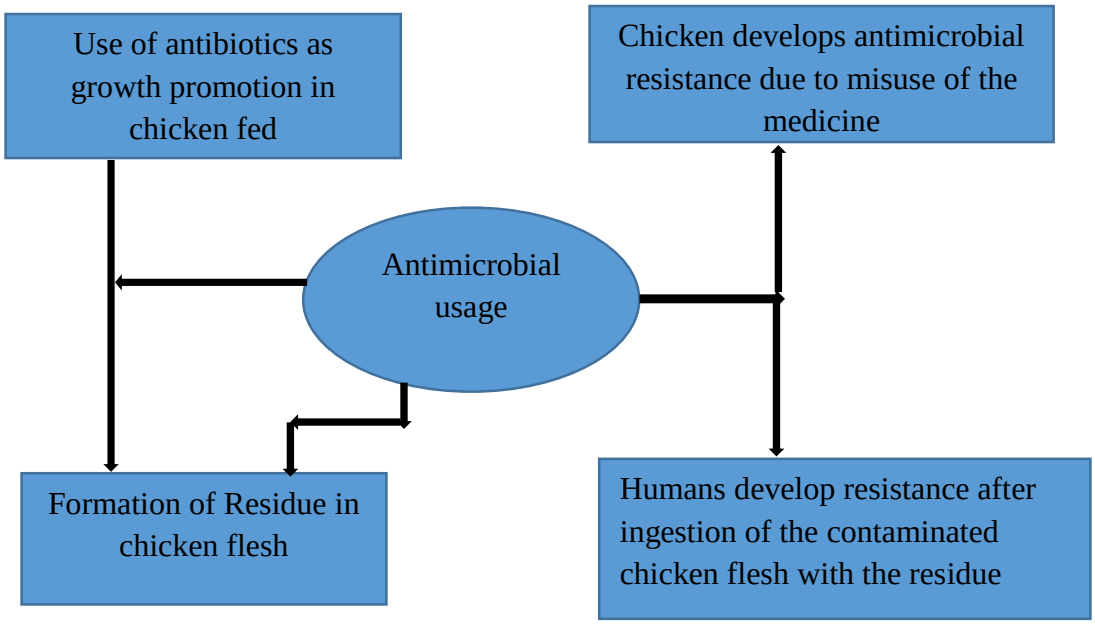

Figure 2.3 The Cycle of Antibiotic Use in Food Supplies

Therefore, antibiotic resistance profiles in humans can be an indication of the most likely used antibiotics in a particular area (Brien et al., 1982). According to the report by O'Neil et al., (2018), the Department of Health, United Kingdom, on the review of antimicrobial resistance worldwide, indicates that the scenario is worrisome and likely to endanger the effectiveness of treating a number of diseases in humans, since now there are deaths of about 700,000 people due to antimicrobial resistance pathogens a year worldwide. For instance, MDR *S. typhimurium* ST313, has caused epidemics in several African countries, and has propelled the use of expensive antibiotics in countries with the poorest health services in the world (Feasey et al., 2013).

2.7.3.1 Antimicrobial Resistance of *Salmonella* Infection in Humans

Worldwide, there has been an increase of public health concern over the occurrence of antibiotic-resistant strains of pathogenic bacteria, including *Salmonella*, which can be acquired through consumption of contaminated food (Mcwatters et al., 2002; Busani et al., 2005). This development has a negative impact on the efficacy of the drugs, which is characterized by delays to eliminate the pathogens and followed by higher morbidity and mortality as a consequence (Varma et al., 2005). The development of drug resistance in organisms, is primarily driven by non-prudent overuse of antimicrobial agents in animals of food origin (Threlfall et al., 2005; Tinega et al., 2016;

Kalule et al., 2019). Subsequently, this reservoir of resistance can be transmitted directly or indirectly to humans through the food chain and direct or indirect contact (Economou et al., 2015).

Countries in Africa and Asia have frequently of isolated multi drug resistance (MDR) *S. typhi* (Ochi et al., 2015; Ochi et al., 2016). While, in 1972, the first record of chloramphenicol–resistant *S. typhi* was isolated from an outbreak due to typhoid in India (Harish et al., 2011). Since 1989, strains of MDR *S. typhi* have emerged in these continents that are resistant to ampicillin, chloramphenicol, and trimethoprim/sulfamethoxazole and have been responsible for several outbreaks (Threlfall et al., 1997). Globally *S. typhi* strains have affected 21 million people with 220000 deaths each year (Saeed et al., 2015; Saeed et al., 2020). Several strains of *S. typhi* have become resistance to chloramphenicol, ampicillin, and co-trimoxazole due to possession of plasmids encoding antimicrobial resistance (Kalra et al., 2003).

Multi drug resistance against *S. typhi* involving aminoglycoside (gentamycin and amikacin) and cotrimoxazole have been reported in the Indian subcontinent (Chandane et al., 2017). While, in Shache county in the Xinjiang Province, China, an outbreak of typhoid fever in the year 2010 which was caused by MDR *S. typhi* strain that contained the resistance (R) type to ampicillin, chloramphenicol, streptomycin, trimethoprim/sulfamethoxazole and tetracycline pattern and was associated with the carriage of a large conjugative plasmid which increases the virulence of *S. typhi* (Yang et al., 2017). In Karachi-Pakistan, out of 209 food handlers who concerted to participate in the study, 19 (9.1%) yielded *S. typhi* isolates, of which 77.7% and 11.1% were resistant to ampicillin and cotrimoxazole respectively (Siddiqui et al., 2015).

According to Leekitcharoenphon et al., (2016), the world is facing an epidemic of a clone of MDR bacterium named H58 causing typhoid fever, rolling across Africa and Asia that is likely to increase the cost of treatment of infection and also lead to more complications. According to (Dougan et al., 2014), haplotype H58 strain is well adapted, coupled with the ability to resist antimicrobial treatment than other *S. typhi* strains. Arising from this background, fluoroquinolone became the first-line drug of choice for treatment of MDR *Salmonella typhoid* strains (Qamar et al., 2014). However, antimicrobial resistance to these drugs is widespread in South Asia and also are occasionally reported in sub-Saharan Africa (Chau et al., 2007). Further More, increasing

number of multi-resistant strains of *S. typhi* are now exhibiting decreased susceptibility to ciprofloxacin, with concomitant treatment failures (Threlfall et al., 2005).

Following the first emergence of MDR *S. typhimurium* D104 strains in 1990, a number of NTS strains have developed MDR phenotype and this phenomenon has increased in many countries (Helms et al., 2005). Therefore, the emergence of multi drug resistance (MDR) strains of *Salmonella* poses a serious public health problem (Stanaway et al., 2019; Medalla et al., 2021), and has become a global problem (Lin et al., 2002; Su et al., 2004). Abuse of antibiotics in humans increase antibiotic resistance in *Salmonella* by promoting transfer of R factors (Cardinale et al., 2005). Similarly, over use of antimicrobial agents in food animals has caused concern regarding the impact they cause on human health (Qutaishat et al., 2003; Varma et al., 2006).

In the United States (USA), antibiotic-resistant *Salmonella* have been isolated from various food products, and have been the causative agent in several food-borne disease outbreaks (Laufer et al., 2015). In Greece, between 2011 and 2012, *Salmonella* strains isolated from fecal samples of patients with gastroenteritis revealed antimicrobial resistance rates to ampicillin (9.3%), amoxicillin/clavulanic acid (4%), ticarcillin (10%), piperacillin (10%), chloramphenicol (2%) tetracycline (15.3%), cotrimoxazole (8.7%), nalidixic acid (13.3%), ciprofloxacin (1.3%), and norfloxacin (1.3%) (Maraki et al., 2014). Based on longitudinal data on NTS isolates from two States in Australia, over a thirty-seven year period (1979 to 2015), revealed that of 58,830 bacterial isolates from patients, 17% of them were non-susceptible to at least one antibiotic agent, 4.9% were non-susceptible to ciprofloxicin, and 0.6% were non-susceptible to cefotaxime (Williamson et al., 2018).

Over the last decade, antibiotic resistance and particularly multiple resistance (to four or more drugs) has increased dramatically in *Salmonella* isolated from infected patients in Western Europe and North America (Threlfall et al., 2000; Threlfall et al., 2003). However, the prevalence of invasive NTS and MDR serotypes in human Salmonellosis tend to vary in distributions from one country to another (Kiratisin et al., 2008), and the incidence rate and case fatality of each infection also varies (Peter et al., 2015). The increase has been due to the epidemic spread of multidrug-

resistant strains such as *S. typhimurium* ST313 in several African countries, and has driven the use of expensive antimicrobial drugs in the poorest health services in the world (Feasey et al., 2012).

Similarly, *S. typhimurium* DT104 strains are commonly resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT resistance type) according to Threlfall et al., (1997) and Poppe et al., (1998). Humans also acquire these pathogens through the food chain (Wall et al., 2010). The increase of a penta-resistant sub-type of typhimurium brought about great concern in the United Kingdom, Europe, and North America, when this was observed in the 1990s (Davis et al., 2003). Multi-resistant *S. typhimurium* definitive type 104 (DT104) emerged from an unknown location and was disseminated globally during the 1980s and 1990s (Davis et al., 2003). In Zambia, 45 strains of NTS serotypes were isolated from 2990 samples of stool, blood and cerebral spinal fluid (CSF) from children less than two years at The University Teaching Hospital, Lusaka, Zambia, and revealed MDR profile (Dube et al., 1983).

2.7.3.2 Antimicrobial Resistance of Salmonellosis in Chickens

Increases in antimicrobial resistance to *S. gallinarum* in chickens has become a worldwide problem. In Nigeria, antimicrobial resistance pattern of 28 *S. gallinarum* isolates from commercial poultry were amoxicillin-clavulanic acid (35.7%), sulphamethoxazole (42.9%), ciprofloxacin (14.3%), chloramphenicol (35.7%), ceftazidime (78.6%), ceftriaxone (82.1%), gentamycin (0.0%), streptomycin (39.3%) and ampicillin (92.9%) Akeem et al., 2017; Musa et al., (2017).

In another investigation conducted in the State of Nasarawa, Nigeria, the study revealed antimicrobial resistance of *S. gallinarum* isolates from free-range chickens as follow; ciprofloxacin (81.7%), gentamicin (76.1%), ampicillin (6S.8%), chloramphenicol and cotrimoxazole (66.2%), tetracycline (58.1%), while neomycin, nalidixic acid, colistin, oxytetracycline, norfloxacin, kanamycin and amoxicillin had 43.9%, 42.2%, 35.5%, 33.4%, 30.1%, 24.4% and 12.9% resistances respectively (Salihu et al., 2014). In South Korea, most *S. gallinurum* isolates (99/105; 94.3%) were resistant to nalidixic acid and resistant/intermediately resistant to fluoroquinolones, and 63.8% (67/105) of the isolates were resistant to three or more antimicrobials (Seo et al., 2019). In Bangladesh, antimicrobial susceptibility testing of *S. gallinarum* strains isolated from commercial layers, revealed that 54.5% of the isolated *Salmonella enterica* serovars were highly

sensitive to ciprofloxacin, whereas the 81.8% isolates were resistant to amoxycillin, doxycycline, kanamycin, gentamycin, and tetracycline (Rahman et al., 2016).

In Brazil, antimicrobial susceptibility of 50 *S. gallinarum* and *S. pullorum* isolates, from outbreaks that occurred between 1987 to 1991 and 2006 to 2013 revealed the following: nalidixic acid (58%), ciprofloxacin (63%), enrofloxacin (67%), tetracycline (92%), fosfomycin (96%) and sulphamethoxazole (96%), while *S. pullorum* isolates (Carvalho et al., 2013; Penha et al., 2016) showed the following susceptibility rates: nalidixic acid (65%), ciprofloxacin (71%), Enrofloxacin(94%) and tetracycline (94%). All isolates were susceptible to β-lactams tested, even though, resistance to quinolones and fluoroquinolones increased over time (Filho et al., 2016). In Raigarh India, Chhattisgarh, susceptibility test of *S. gallinarum* revealed that they were intermediate sensitive to amoxycillin, amoxyclav, gentamicin and ciprofloxacin and resistant to most of the antibiotics including chloramphenicol, ampicillin, ceftazidime, cefexime, cefepime, azithromycin, nalidixic, tetracycline, oxytetracycline, and streptomycin (Sannat et al., 2017).

Since early 1990s a multi-resistant strain of *S. typhimurium* definitive phage type (DT) 104, displaying resistance up to six commonly used antimicrobials, with about 15% of isolates also exhibiting decreased susceptibility to ciprofloxacin (Threlfall et al., 2002). These strains were resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclines (ACSSuT resistance type (Threlfall et al., 1994). Multi-resistant *S. typhimurium* definitive phage type (DT) 104, have become widely distributed in cattle in the UK since the early 1990s (Rushdy et al., 1998). During the period 2012-2013, NTS strains isolated from livestock and food samples around Kolkata, India was resistant to nalidixic acid (24.5%), ampicillin (17.0%), amoxicillin (17.0%), streptomycin (15.1%), tetracycline (11.3%), chloramphenicol (9.4%), co-ciprofloxacin (5.7%) and only one isolate was resistant to both fluoroquinolones and third generation cephalosporins (Sudhanthiramani et al., 2016). The problem of NTS antimicrobial resistance is real and has been reported in several countries (Eng et al.,2015). In Nigeria, the antimicrobial resistance pattern of *S. enteritidis* isolates from commercial poultry were amoxicillin-clavulanic acid (100.0%), sulphamethoxazole (66.7%), ciprofloxacin (33.3%), chloramphenicoal (66.7%), ceftazidime (67.7%), ceftriazone (100.0%), gentamycin (0.0%), streptomycin (100.0%) and ampicillin (100.0%) (Agada et al., 2020).

In Eastern China, a total of 163 *Salmonella* sp. isolates representing 15 serotypes were recovered from faecal samples of domestic animals (chicken, duck, goose and pig) during 2008-2009 and were tested for antimicrobial susceptibilities; resistance was most often observed to carbenicillin (65.4%), followed by nalidixic acid (48.8%), tetracycline (46.9%), sulfafurazole (45.7%), ampicillin (43.2%), streptomycin (38.3%) and trimethoprim/sulfamethoxazole (33.3%) (Pan et al., 2010). *Salmonella* sp. isolates from chicken sources displayed the highest rate of resistance being resistant to at least one antimicrobial (100%) followed by those recovered from pig (93.4%), goose (90.7%) and duck (80%) (Pan et al., 2010). In another study, in China, of the 178 *S. enteritidis* isolates, 73% were resistant to ampicillin, 33.1% to amoxicillin/clavulanic acid, 66.3% to tetracycline, and 65.3% to doxycycline, whereas all of these isolates were susceptible to the other drugs used in the study (Lu et al., 2011).

2.7.8 Causes of Antimicrobial Resistance

The high increase of antimicrobial resistance (AMR) worldwide, and over a long period have subsequently had a negative toll on the use of traditional antibiotics, and the newer antimicrobial agents for treatment of *Salmonella* infections (Lin et al., 2002), in the sense that there are now fewer drugs to be considered as treatment options. The factors that facilitate the occurrence and spread of antimicrobial resistance in bacteria population are complex as though the most singled out is the use of antimicrobial agents in food animals, particularly in animal feed (Lin et al., 2002). Similarly, the use of antibiotics to treat a variety of bacterial infections in humans and animals have also contributed to the development of numerous mechanisms that render bacteria resistant to some and in certain cases to nearly all antibiotics (Abdellatif et al., 2018; karim et al., 2019).

Feather More, other studies have shown that there are several mechanisms through which *Salmonella* develops antimicrobial resistance such as the production of enzymes that degrade cell permeability to antibiotics, activation of antimicrobial efflux pumps and production of β-lactamase to degrade the chemical structure of antimicrobial agents (Foley et al., 2008). In principle antimicrobial resistance evolves around the mobile genetic resistance elements or complex mechanisms at play, which from time to time are overcome by the discovery of new (Mayer et al.,

1988; Waglechner et al., 2017) and effective drugs. Below is an attempt to highlight a few biochemical and genetic aspects of antibiotic resistance mechanisms in bacteria.

2.7.9 Antimicrobial Resistance due to Extended Spectrum β-lactamases (ESBLs)

According to the Centers for Disease Control and Prevention (Bottichio et al., 2016), extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae carry a broad-spectrum beta-lactamase enzyme that enables them to become resistant to a wide variety of penicillin and cephalosporin antibiotics. These ESBLs constitutes a family of enzymes produced by Gram negative bacteria, that are capable of offering resistance to some of the most widely used antibiotics worldwide (Reuland et al., 2014; Liakopoulos et al., 2016). Therefore, Gram-negative pathogens are particularly worrisome, because they are becoming resistant to nearly all drugs that would be considered for the treatment (Bottichio et al., 2016).

Food-producing animals are the primary reservoir of zoonotic pathogens, and in recent years the presence of ESBL producers among *Escherichia coli* (*E. coli*) and *Salmonella* strains has increased (Carattoli et al., 2008). European countries, a study by Saliu et al., (2017), revealed that the highest prevalence of ESBL-producers was in chickens and chicken's products, with *CTX-M-1, TEM-52* and *SHV-12* being the most common ESBL-types in poultry. Further, the study showed that, *E. coli* and *Salmonella* spp. are the common bacteria in poultry, which carry ESBL-genes (Saliu et al., 2017).

In Brazil, 98 *Salmonella* spp. strains were obtained from the cutting rooms of four different broiler processing and exporting plants, and 45% of these strains were positive for ESBL activity (Ziech et al., 2016). In another study, in Brazil, 630 *Salmonella* recovered from both clinical and non-human (food, poultry, and environment) sources from 2009 to 2014 were tested for β-lactamases genes by polymerase chain reaction. Of these, 46 displayed ESBL phenotype of which $bla_{CTX\text{-}M\text{-}8}$ and $bla_{CTX\text{-}M\text{-}2}$ genes were detected at frequencies of 47% and 41%, respectively (Fernandes et al., 2017).

In Korea, 20 of 1,279 NTS strains isolated from food animals and humans produced *CTX-M*-type extended-spectrum β-lactamase. Further, all strains expressed *CTX-M-15*, except two which co-

expressed *CTX-M*-14 and *TEM*-1 (Tamang et al., 2011). In China, of 890 *Salmonella* isolates from retail chicken carcasses screened for ESBLs-production phenotype via the double-disk synergy test method, 96 (10.8%, n = 890) were ESBLs-producing *Salmonella* (Qiao et al., 2017). The study by Taneja et al., (2014), characterized occurrence of ESBL phenotypes in 53.4% of NTS isolated over a period of nine years from human gastroenteritis cases in India. In this study, they documented the ESBL and *AmpC* co-production and the occurrence of a *Salmonella enterica* serovar *Senftenberg* carrying blaCTX-M-15 and blaCMY-2 resistance genes.

In Phatthalung Province, Thailand, 69 *Salmonella enterica* isolates were recovered from animal feces and meat samples (swine = 40; chicken=29) at small-scale rural farms. Of these, 21 ampicillin-resistant *S. enterica* isolates (20 from swine and 1 from chicken feces) tested positive for ESBL phenotype by a disc diffusion method with cefpodoxime 10 mg (Lertworapreecha et al., 2016).

Extended Spectrum β-lactamases (ESBLs) confer bacterial resistance to very important antimicrobial agents. Livestock are recognized as important reservoir for the zoonotic food-borne transmission of resistant bacteria (Carattoli et al., 2008), while migratory wild birds can serve as mechanical and/or biological vectors for enterobactericeae harboring ESBLs that would be resistant to cephalosporins (Mohsin et al., 2017). However, different investigators have shown that *Salmonella* is capable of producing a variety of ESLBLs enzymes and the majority being CTX-M types (Su et al., 2005) and AmpC β-lactamases (CMY-2 type) (Allen et al., 2002) which could hydrolyze cephalosporins and cephamycins respectively. This being said, ESBLs are perceived as a group of plasmid-mediated, diverse in nature, complex and fast evolving enzymes that are contributing to a major therapeutic challenge today in the treatment of hospitalized and community-based patients (Verdet et al., 2000; Ktari et al., 2009; Rawat et al., 2010).

ESBLs have generally contributed to the increase of resistance to commonly used antibiotics, such as ampicillin, cotrimoxazole, gentamicin, erythromycin, tetracycline, and third-generation cephalosporins (Livermore et al., 1995; Vahaboglu et al., 1995; Feasey et al., 2015), fluoroquinolones and trimethoprim-sulfamethoxazole (Talan et al., 2016; Jeon et al., 2019; Hasan et al., 2021).

However, *Salmonella* resistance to cephalosporins in isolates from domestic animal have also been reported in different countries of the world such as USA (Eaves et al., 2004), Netherlands (Liakopoulos et al., 2016) and China from food producing animals and fish (Wang et al., 2011). Others include *Salmonella* strains from humans in India (Sabharwal et al., 2010).

2.8 Mobile Genetic Elements of Antimicrobial Resistance (MGE-AMR)

A unique major risk to human health is antimicrobial resistance (AMR) (Johansson et al., 2021). Bacteria can acquire AMR through horizontal gene transfer (HGT), which typically involves mobile genetic components, or through changes in the genome (MGEs) (Johansson et al., 2021). MGEs are distinct DNA segments that can migrate between and/or within bacterial cells. Based on their traits and genetic makeup, they are divided into categories. Here, the term "integrating MGEs" refers to components that can integrate into the host DNA (iMGEs) (Brandis et al., 2018).

2.8.1 Type of Mobile Genetic Elements

A mobile genetic element (MGE) (Table 2.1) is a form of DNA that may move inside the genome. They consist of:

1. Insertion sequences (IS) (Table 2.1) and transposons (Tn) are discrete DNA segments that are able to move themselves (and associated resistance genes) almost randomly to new locations in the same or different DNA molecules within a single cell (Silva et al., 2012). Other elements, such as integrons (In), use site-specific recombination to move resistance genes between defined sites (Silva et al., 2012). As these types of MGE are often present in multiple copies in different locations in a genome. They can also facilitate homologous recombination (exchange of sequences between identical or related segments) (Brandis et al., 2018). Intercellular mechanisms of genetic exchange include conjugation/mobilization (mediated by plasmids and integrative conjugative elements [ICE]), transduction (mediated by bacteriophages), and transformation (uptake of extracellular DNA). Interactions between the various types of MGE underpin the rapid evolution of diverse multi-resistant pathogens in the face of antimicrobial chemotherapy (Johansson et al., 2021).

2. Plasmids and integrative and conjugative elements (Table 2.1) are also agents of gene transfer because they can move genes between cells by conjugation as well as integrate them (Johansson et al., 2021). Conjugation/mobilization (mediated by plasmids and integrative conjugative elements [ICE]), transduction (mediated by bacteriophages), and transformation are examples of intercellular methods of genetic exchange (uptake of extracellular DNA). The rapid growth of numerous multi-resistant infections in the face of antibiotic treatment is supported by interactions between the different forms of MGE (Silva et al., 2012).

Transposons (Tn) (Table 2.1) and insertion sequences (IS) are distinct DNA segments that have the ability to randomly transfer to new positions inside the same or other DNA molecules within a single cell, along with any associated resistance genes. Site-specific recombination is used by other elements, such as integrons (In), to transfer resistance genes between predetermined sites (Brandis et al., 2018). These kinds of MGE can promote homologous recombination since they are frequently found in many copies throughout a genome (exchange of sequences between identical or related segments) (Brandis et al., 2018).

Table 2.1: Mobile Genetic Elements of Antimicrobial Resistance AMR

Mechanisms of transfer	Mechanisms of incorporation`	Mobile elements	Reference
Conjugation	1 – Autonomous replication	Plasmids (1)	(Johansson et al., 2021)
Transformation	2 – Transposition	Transposons (2)	(Johansson et al., 2021)
Transduction	3 – Site-specific recombination	Insertion sequence	(Johansson et al., 2021)

2.9 Risk Factors of Salmonellosis

According to Gras et al., (2014), several case-control studies have investigated risk factors for human salmonellosis while others have used *Salmonella* subtyping to attribute human infections to different food and animal reservoirs. Further their study, confirmed that most human cases (90%) were attributable to layers/eggs and pigs, and of these layers/eggs and broilers were the most likely reservoirs of salmonellosis in adults, in urban areas, and in spring/summer, whereas pigs and cattle were the most likely reservoirs of Salmonellosis in children, in rural areas, and in autumn/winter (Mughini -Gras et al., 2014).

At household levels, parameters with the greatest influence risk were identified as pork handling practice, followed by prevalence in pork sold in the central markets (Dang et al., 2017). While, the risk of salmonellosis per 100 g serving of ground beef ranged increases dependent on the type of cooking and the fat content (Abdunaser et al., 2009). During consumption of pork, the risk for *Salmonella* contamination increases because of inappropriate temperature conditions during storage, and handling of the product (Sadeleer et al., 2009). According to the Norwegian Scientific Committee for Food Safety (NSCFS) by Pérez et al., (2015), the temperatures used during the feed production are sufficient to eliminate *Salmonella,* but high concentrations of *Salmonella* in feed ingredients, may result in the production of *Salmonella*-contaminated feed (Pérez et al., 2015).

Salmonellosis may be acquired by association with reptiles, mainly affects young children and results in a higher incidence of hospitalisation and invasive disease than other *Salmonella* infections (Mermin et al., 2004). Further, Health Centers, such as the hospitals, or clinics or any other place designated for patients to receive medical services, or diagnostic laboratories for infectious diseases are potential areas to contract hospital-acquired infections in particular for children (Morpeth et al., 2009). Ownership of backyard flocks can increase poultry associated *Salmonella* infections and outbreaks, most notably in urban areas where zoonotic diseases from farm animals were once considered rare (Guard et al., 2015).

Not using a chopping board for raw meat only and consuming raw/undercooked meat were risk factors for infection with *Salmonellas* originating from pigs, cattle and broilers (Mughini-Gra et al., 2014). Direct animal contact with domestic animals such as pig and cattle have been associated with human infections (Hoelzer et al., 2011; Cummings et al., 2012; Gras et al., 2014; Stipetic et al., 2016), and also petty animals and visits to the zoo is a risk factor in particular for the children (Morpeth et al., 2009).

Floods during rainy seasons are risk factors due to their potential to contaminate the environment as well as the sources of drinking water with fecal organisms (Morpeth et al., 2009). Occupational exposure to animals and/or NTS colonization among chicken farmers (Trung et al., 2017). Risks

factors can also be associated with highly dynamic population of animals as well as food animals as reservoirs of pathogens across boundaries (Stipetic et al., 2016).

Significant associations of host risk factors were observed with exposure to consumption of antimicrobial agents (Ruddat et al., 2014), immunocompromised patients (Gordon et al., 2008), malaria and anemia (MacLennan et al., 2013), malnutrition (Rosanova et al., 2002), HIV infection (Tennant et al., 2010; Levine et al., 2011), gastric acid suppression, sickle cell disease (Hand et al., 1978), and schistosomiasis (Hsiao et al., 2016). Children under the age of Three years are particularly at risk for invasive iNTS disease (Morpeth et al., 2009; Crump et al., 2010). Children was also being particularly vulnerable to gastroenteritis because of the immaturity of their intestinal barrier, enteric nervous system, and immune response to pathogens (Cremon et al., 2014).

Other risk factors include; wild birds or animals having access to animal feed storage facilities, practicing of mixed farming (dairy herds, beef herds and calf herds), sourcing of animals such as cattle from unclean herds, confinement of animals in relatively small area, and having a high population of feral cats around the farm area (McCrea et al., 2006).

2.9.1 Public Health Importance of Salmonellosis

Salmonella spp. have historically been a significant global public health issue. *Salmonella* is extremely pervasive and persistent in the environment, which makes it more challenging to stop its spread (Buzby et al., 2009). *Salmonella* spp. can even be fatal to humans and chicken (Mubita et al., 2020). In addition, a significant obstacle to effectively treating a *Salmonella* infection is the development of antibiotic resistance in *Salmonella* (Mubita et al., 2020). A good way to stop the emergence of antibiotic resistance in the chicken industry is to restrict the use of antibiotics in feed (Lin et al., 2002).

Salmonellosis due NTS pathogens, has brought about significant morbidity and mortality rate worldwide (Cardoso et al., 2006). *Salmonella* pathogens are associated with foodborne and waterborne diseases through faecal contamination and are the most problematic diseases in terms of control worldwide coupled with high endemicity (Bean et al., 1990; Hennessy et al., 1996;

Brenner et al., 2000; Threlfall et al., 2002; Finstad et al., 2012). Food handlers play an important role in the transmission of food-borne diseases (Ogah et al., 2015). In 2010, Hamilton County Public Health (HCPH), confirmed the existence of food-borne outbreak following the consumption of pooled pork prepared in a private home and sold at the church festival (Bugarel et al., 2017). Therefore, most Salmonellosis infections are acquired by consuming contaminated food and food products, especially food from animal origins (Buzby et al., 2009).

2.9.2 Unsafe Contaminated Foods Rendering Food Dangerous (Food safety)

infection with *Salmonella* can resulted from variety of contaminated food items including: chicken, turkey, beef, pork, eggs, fruits, sprouts, other vegetables, prepared items like frozen pot pies, chicken nuggets, and dinners with filled chicken (Ehuwa et al., 2021). Chicken, ground turkey, ground beef, raw tuna, mushrooms, onions, peaches, papayas, sliced fruits, cashew brie, and tahini have all been implicated in recent *Salmonella* outbreaks that have infected people in numerous states. *Salmonella* germs can also be transmitted by contaminated food, drink, the outdoors, other people, and pets (Thames et al., 2020).

2.10 Control and Prevention of Salmonellosis in Animals

Animal feeds must be treated to kill *Salmonella* before distribution to prevent spread of infection. Food animal flocks and herds should be routinely tested for *Salmonella* and those found to be positive are sent for special slaughter followed by heat treatment of the chicken meat (Wierup et al., 1988). The major reservoirs for human *Salmonella* infection are chicken and livestock, and reducing *Salmonella* harbored in these animals can substantially reduce infections in humans.

Basic food safety precautions are key in controlling Salmonellosis. These measures include protecting processed foods from contamination and cooking and refrigerating foods adequately to prevent survival and growth of *Salmonella*. Food workers and consumers should be educated about the need to cook meats, poultry, and eggs thoroughly and the need to wash hands and utensils with soap and water immediately after they have been in contact with raw meat, poultry, or eggs. Pasteurization of milk, hygienic slaughter practices, and thorough cooking of those foods can reduce the risk of becoming ill (Jevšnik et al., 2008).

Some serotypes of *Salmonella* are more resistant to antibiotics. Some of these antibiotics are the same or similar to those used in humans and have major implications for treatment of human infections, when antibiotics are deemed necessary. Judicious use of antibiotics among both humans and animals is imperative in the control of this pathogen (Wierup et al., 1988). Finally, subtyping of isolates and integrating enteric disease surveillance programs are critical to *Salmonella* control efforts. By examining results of *Salmonella* sub typing from food animals, environmental samples, and humans, public health officials can draw conclusions about sources of human infection and focus on control efforts accordingly.

Good control measures at the farm level are likely to yield into lower occurrence of *Salmonella* infections, a reduction of cross-contamination of carcasses processed at the slaughterhouse and subsequently, a reduction in human salmonellosis (Davis et al., 2003; Andres et al., 2015). For instance, use of biosecurity measures in pig farms can help to control important pig diseases as well as reducing the within-herd prevalence of *Salmonella* (Andres et al., 2015). In the slaughter process, Good Health Practice is essential to ensure the prevention or minimization of contamination with *Salmonella* (Besser et al., 2018). Organic farmers should consider control of rodents, wild birds and flies as an integral part of hygiene measures to prevent transfer of food-borne pathogens as well as disinfection of boots/clothes and equipment for farm workers and visitors (Meerburg et al., 2007).

According to the report from Consultation et al., (1994), a recommendation in poultry farming, should be to consider vaccination of birds as an additional measure to reduce *Salmonella* infection within the flock. Further, systems also need to be developed that will produce *Salmonella*-free elite parents, grandparent, and great grandparents' flocks (Consultation et al., 1994). In rare cases live attenuated *Salmonella* vaccines haave successfully been used in pigs, cattle, and chickens to evoke cell-mediate immune response and was proven to have protected animals against both systemic disease and intestinal colonization (Bashahun et al., 2017).

Accordingly, Intervet Shering-Plough Animal Health (ISPAH) has produced a vaccine for the vaccination of fowl typhoid with a special *S. gallinarum* (9R strain) in poultry industries in several countries and have conferred protection (Noordhuizen et al., 2012). Other control measures could

include; control of rodents, birds and feral cat density around the farm, proper management of waste such as effluent, vaccination of animals to mitigate the spread and severity of the infection (McGuirk et al., 2003). Poultry industry requires stringent measures to control the spread of *Salmonella* through key areas such as trade of live parent stock and grandparent flocks within and between countries (Bengtson et al., 1994). Monitoring the trade of contaminated feed products can also reduce the spread of *Salmonella* (Wierup et al., 1988; Sundström et all., 2014). Further, reduction of organisms that are discharged into the environment such as waste effluent is another important measure that can be employed to curb the spread of *Salmonella* (Devi et al., 1991; Harrington et al.,1991).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Wau County, Western Bahr el Ghazal state, South Sudan (Figure 3.1). The state share international borders with Sudan to the North and Central African Republic to the West with coordinates of 8.6452°N, 25.2838°E and 626.9 meters above sea level. The climate is tropical with annual rainfall ranges between 400 -1600 mm and a temperature. Wau County is located on the western bank of Jur River and it is comprised of five Payams namely Bagari, Besselia, Kpaile, Wau North and Wau South. Wau County has been chosen because most households keep chickens besides Ducks, gout sheep and cattle. It has a human population of 151,320 and about 29,614 households according to the fifth Sudan population census (Anonymous, 2008).

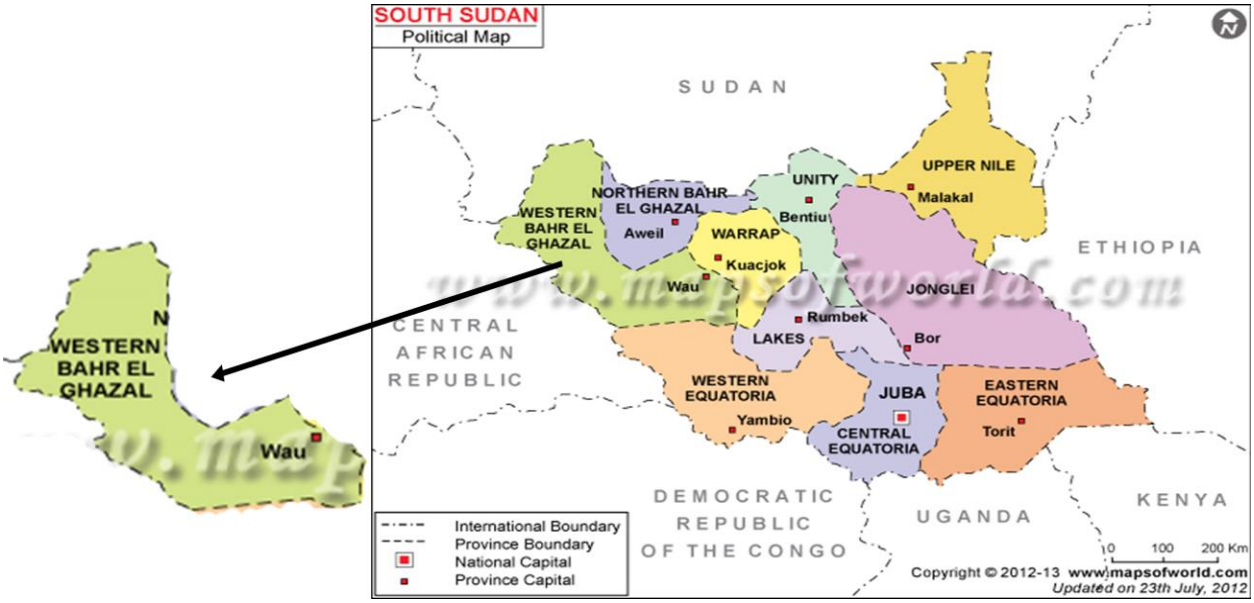

Figure: 3.1 Study Area Western Bahr el Ghazal State (South Sudan Google Map)

3.2 Study Design

The study design conducted was a cross-sectional survey study design that do targeted local live chicken and chicken keepers/handlers at household level in Wau County. Of the Five Payams of Wau County, two was randomly selected for the study. The State Ministry of Animal Resources and Fisheries had provided lists of the households keeping chicken in the selected Payams and Bomas.

3.3 Study Population

The study population was local live chickens and chicken keepers in Wau County, Western Bahr el Ghazal State, South Sudan.

3.3.1 Inclusion Criteria

All households with chickens within the selected area were randomly selected and included in the study.

3.3.2 Exclusion Criteria

Households that are not keeping chickens within the selected study area were excluded.

3.4 Sample Size Determination

3.4.1 Sample Size Determination: Human Samples

For chicken keepers, the sample size was calculated by using survey formula (Thrushfield 2013). The estimated prevalence of 41.5% was determined from a study by (El Siddig.et al.,2015).

$$n = \frac{Z\alpha^2 \rho Q}{L^2}$$

Where Z=1.96, α2 =95, p= 41.5% Q=1-41.5%, L2 =5% and n =**180** samples

3.4.2 Sample Size Determination: Chicken Samples

The estimated prevalence of 18.2% for chicken samples was obtained from a study by (El Hussein et al.,2010).

For chicken sample, the same survey formula by (Thrushfield 2013) was used as above.

$$n = \frac{Z\alpha^2 \rho Q}{L^2}$$

Where Z=1.96, α2 =95, p= 18.2%, Q=1-18.2%, L2 =5% and n **240** = samples

3.5 Fecal Sample Collection and Transportation

Fecal samples were collected from the local live chicken and detailed information on the date of collection, age, sex, and breed of the chicken was recorded prior to sample collection. The samples were collected with sterile wooden swabs, from cloaca was transferred into 10ml sterile universal containers containing 5ml Cary Blair Transport Medium and immediately placed into a cooler box containing ice packs. The samples were then transported and stored in a refrigerator at 4ºC. The samples were then dispatched to the laboratory for analysis at Microbiology Laboratory in the School of Veterinary Medicine, University of Zambia.

Before collecting human stool samples, consent was sought from community gatekeepers such as chiefs, opinion leaders and elders. Detail information on the participant's name, sex, age, occupation and the date of collection were recorded. Stool samples were carefully collected into sterile 10ml plastic universal containers with spoons, to which 5ml Cary Blair Transport Medium has been added. The samples were then transported and stored in a refrigerator at 4ºC before being shipped to the University of Zambia for processing.

For shipment of samples from South Sudan to Zambia, the World Health Organization (WHO) and the International Air Transport Association (IATA) guidelines and regulations, which cover the transportation of biological and dangerous goods, was strictly followed. Before shipping the samples, export and import permits were sought from the South Sudan and Zambian governments, respectively.

3.6 Laboratory Assays

3.6.1 Isolation of *Salmonella* from Stool Specimens

The samples were inoculated in a pre-enrichment culture non-selective liquid medium of buffered peptone water, and incubated at 37ºC for 24 hours. Aliquots from pre-enrichment were inoculated

into selective enrichment liquid media at a ratio of 1/10 in Rappaport-Vassiliadis broth and at1/10 in Selenite-Cysteine broth. A loopful of each broth was streaked on plates of Xylose Dextrose Agar (XLD, HiMedia, India). The temperature and the period of incubation were standardized at 37ºC for 24 hours, respectively, for both chicken droppings and human stool samples. The suspected colonies of *Salmonella* from each plate were collected for presumptive identification by biochemical tests that included oxidase, hydrogen sulphide (H_2S), urease, indole, fermentation of glucose, sucrose, mannitol, and lactose. The media that were utilized for presumptive identification were Urease, Triple Sugar Iron slant agar (TSI), Lysine-Iron slant agar (LIA), Sulphur Indole Motility agar (SIM), and the Citrate test. All the tests were incubated at 37ºC for 24 hours.

3.6.2 Serological Typing of *Salmonella* Isolates

Salmonella strains were differentiated into serotypes by serotyping analysis according to the method described by Su and Chiu (2007) and Mubita et al. (2020). All isolates of *Salmonella* were referred to Deltamune (Pty) Laboratory in South Africa, SANAS Accredited Veterinary Laboratory, for confirmation and serotyping. The Characterization was done using the method described in the Microbiological Manual and serotyping (10.2:1995 CCFRA) based on White-Kauffman-Le Minor Scheme (WHO Collaborating Centre) by Issenhuth et al., (2014). The scheme discriminated serotypes on the basis of their somatic (O), flagella (H) and capsular (Vi) antigens present on the surface of *Salmonella* (Brenner et al., 2000).

The *Salmonella* isolates were characterized into sero groups based on the presence of distinctive "O" antigenic factor, using O polyvalent antiserum and specific monovalent antiserum for A-S group antigen and GROUP BC1, C2, D1, E and G respectively in accordance with the manufacture's protocol for identification of surface antigens and their differentiation into serogroups. Briefly, a drop of the appropriate antiserum was placed onto a clean microscopic slide. A single colony of overnight culture on nutrient agar was picked and emulsified in the antiserum drop to obtain a thoroughly mixed suspension. The slide was gently rocked forward and backwards/side wards for 1 minute. Agglutination or clumping between 1-10 seconds was considered a positive reaction.

3.6.3 Biotyping of *Salmonella*

The isolated *Salmonella* strains were tested for sugar utilization using phenol red broth (HiMedia, India) prepared based on the manufacture instruction containing sugars and alcohol, dissacharides (maltose, trehalose, melibiose and cellobiose), hexoses (glucose, mannose and galactose), pentoses (arabinose, xylose, and rabitol), polyhydric alcohol (inositol, adonitol, mannitol, dulcitol and salicin) and trisaccharides (raffinose). To a tube of phenol red broth, containing either one percent (w/v) of sugar or alcohol the isolate was inoculated using a sterile straight wire. The broth tubes were then incubated at 37±0.5ºC for 48hours and the results were recorded positive if the production of acid was inducing a change in the phenol red indicator, from pink to yellow.

3.6.4 Molecular Confirmation of *Salmonella*

The final confirmation of *Salmonella* was done through PCR. The suspected *Salmonella* isolates were cultured in Brain Heart Infusion agar (HiMedia, India) for 24h at 37ºC. A loopful of the colonies was emulsified into 0.1 mL of distilled water and heated at 95ºC for 10min for genomic DNA extraction. After which, centrifugation at 15,000*xg* for 5 min was preformed and the supernatant used as DNA sample. The supernatant containing DNA was subsequently stored at −20ºC awaiting further analysis.

The modified PCR based on the method of Rahn et al., (1992) was used to confirm the isolate through the amplification of the *Salmonella inv*A gene. The *inv*A gene was amplified by PCR with primers indicated in Table 3.1. With this system, a DNA fragment of 284 bp was amplified in samples containing the *Salmonella*. The corresponding DNA fragment were not amplified in a sample containing *E. coli* used as a negative control.

3.6.5 Detection of Extended Spectrum Beta-Lactamases (ESBLs) in *Salmonella* Strain

The Detection of extended spectrum cephalosprinase producing isolates was accomplished using freshly prepared MacConkey Agar (HIMEDIA) containing 2 mg/l of cefotaxime (Sigma-Aldrinch, Munich, Germany) according to the method described by Mubita et al., (2020).

3.6.6 Determination of Antimicrobial Susceptibility Patterns of *Salmonella* Isolates

The antibiotic susceptibility profiling of the *Salmonella* isolates was determined using the Kirby-Bauer disc diffusion method based on the Clinical Laboratory Standard Institute (CLSI) guidelines (Weinstein et al., 2020). The antibiotic discs (Oxoid, UK) tested were ampicillin (10 µg), sulfamethoxazole/trimethoprim (1.25/23.75 µg), streptomycin (300 µg), ciprofloxacin (5 µg), cefotaxime (30 ug), tetracycline (30 µg), gentamicin (10 µg), nalidixic acid (30 µg) and chloramphenicol (30 ug) (Fadlalla et al., 2012**)**. Interpretation of susceptibility patterns on other anti- microbial disks was done using guidelines laid down in the CLSI, which provides break points corresponding to zone of inhibition diameter. Quality control Standard laboratory procedures were strictly adhered to so as to avoid contamination. *E. coli* ATCC 25922 was used as a quality control organism.

3.6.6.1 Determination of Multiple Antimicrobial Resistances Indexing MARI

The multiple antibiotic resistance index was calculated as follows; a/b, where 'a' represents the number of antibiotics to which the particular isolate was resistant and 'b' the number of antibiotics to which the isolate was exposed. MARI values greater than 0.2 were considered significant indicating that the strains could have originated from sources where antibiotics were often used (Afshan et al., 2021). While MARI value less than 0.2 suggested that strains originate from animal sources which were less frequently exposed to antibiotics or never at all (Aromolaran et al., 2022).

3.6.7 Determination of the Genetic Diversity of *Salmonella* Isolates

The genetic diversity of strains or genotypes circulating among chickens and humans was determined through characterization of isolates. This was done using molecular typing methods detailed below.

3.6.7.1 Detection of Virulence Genes

The detection of virulence in *Salmonella* isolates was done through the presence of 7 **(**Table 3.1**)** virulence genes (*InvA, spiA, pagC, msgA, sipB, spaN,* and *spvB*). These were detected using PCR forward and reverse primers adapted from Vlisidou et al., (2019). The primer sets are indicated into Table 3.1.

Table 3.1: Primer Sets used in the Study

Virulence Gene	Primer Set	Reference
InvA	F- 5'-GTGAAATTATCGCCACGTTCGGGCAA-3' R- 5' – TCATCGCACCGTCAAAGGGAACC-3'	(Rahn et al., 1992)
spiA	F-5-CCAGGGGTCGTTAGTGTATTGCGTGAGATG-3 R-5CGCGTAACAAAGAACCCGTAGTGATGGATT-3	(Severet al., 2019)
pagC	F-5CGCCTTTTCCGTGGGGTATGC-3 R-5GAAGCCGTTTATTTTTGTAGAGGAGATGTT-3	(Severet al., 2019)
msgA	F-5GCCAGGCGCACGCGAAATCATCC-3 R-5GCGACCAGCCACATATCAGCCTCTTCAAAC-3	(Severet al., 2019)
sipB	F-5GGACGCCGCCCGGGAAAAACTCTC-3 R-5ACACTCCCGTCGCCGCCTTCACAA-3	(Severet al., 2019)
span	F-5AAAAGCCGTGGAATCCGTTAGTGAAGT-3 R-5CAGCGCTGGGGATTACCGTTTTG-3	(Severet al., 20199)
spvB	F- 5' – ATGTTGATACTAAATGGTTTTTCA-3' R- 5' – CTATGAGTTGAGTACCCTCATGTT-3'	(Huang et al., 2005)

The virulence gene results were analyzed visually and an isolate was considered positive by containing the virulence gene of interest if it produced an amplicon of the expected sizes judged by the molecular markers.

3.6.7.2 Sequencing of Selected Genes from *Salmonella* Isolates

Sequencing of the selected genes was done after PCR. The PCR products was purified from the gel using a commercial kit as prescribed by the manufacturers.

3.6.7.2.1 Brilliant Dye Reaction

The purified DNA products were subjected to sequencing using the brilliant dye terminator ver.3.1 (NimaGen, Nijmegen, The Netherlands). The brilliant dye reaction was performed using a reaction mixture containing 1µl of brilliant dye, 3.5µl of the 5x sequencing buffer, 0.16 µM of each of the primers (forward and reverse, same primer sequences used in amplification above), 12.18µl of nuclease-free water and 3.0µl of the DNA template. The total reaction volume was 20µl. The PCR conditions for this reaction were as follows: initial denaturation at 96°C for 1 minute, 35 cycles of 10 seconds at 96°C, 5 seconds at 50°C and 2 min at 60°C with no final extension period. The final hold temperature was set at 4°C.

3.6.7.2.2 Ethanol Precipitation

After the big dye reaction, 2µl of 3M sodium acetate, 2µl of 125mM EDTA and 90µl of 100% ethanol were added to the sample. This was then vortexed and incubated in the dark for 10 minutes at ambient room temperature. After incubation, the mixture was then centrifuged at 15,000 rpm for 20 minutes and the supernatant was discarded gently. Thereafter, 200µl of 70% ethanol was added. This was then centrifuged at 15,000 rpm for 10 minutes and the supernatant was discarded gently. The samples covered in aluminium foil were then dried in the vacuum dryer for 10 minutes making sure that all the ethanol was completely dry. After drying, 20µl of HiDi formamide was added and vortexed. This was then denatured in a thermocycler (Applied Biosystems, AB, Japan) at 95°C for 5 minutes and the final hold temperature was at 4°C. The purified DNA was then sequenced by using a sequencing machine (3130 Genetic Analyzer). The resulting sequences was aligned by using the multi-sequence alignment CLUSTALX program.

3.6.7.3 Whole-Genome Sequencing Data Analysis

Whole genome sequence (WGS) data was analysed using a custom-made pipeline. FastQC was firstly used on the raw sequence data for quality control (https://github.com/s-andrews/FastQC/) and this was then followed by trimming and barcode removing using SICKLE (https://github.com/najoshi/sickle). SPADES was used for de novo assembly (Rodrigues et al., 2020) and polishing of the assembled sequences was done using PILON (Walker et al., 2014). Assessment of the polished contigs was then done using Quast (Gurevich et al.,2013) and reference guided assembly of contigs into a final consensus sequence was performed using Ragtag (Alonge et al., 2021). Assessment of functionality and variations in specific sequences and housekeeping genes was conducted using MLST (https://github.com/tseemann/mlst). Abricate (https://github.com/tseemann/abricate) was used in the construction of a dendogram, identification of AMR, resistance genes and annotation. Roary was used for pangenome analysis (Allali et al., 2017).

3.6.7.3.1 Mobile Genetic Elements MGE for Antimicrobial Resistance AMR

Mobile genetic elements were detected using the methods outlined below.

3.6.7.3.2 AMR and MGEs in silico Prediction

Using ResFinder, AMR genes were predicted, and the gene with the best coverage and sequence identity was kept after overlapping genes were eliminated (Johansson et al., 2021). PlasmidFinder was used to forecast plasmids (Ehuwa et al., 2021). MGE sequences stored in fasta format with related metadata stored in json format make up the database that MobileElementFinder includes. In addition to lightweight Python API used by MobileElementFinder to communicate with the database, the database is delivered with tools for validation and maintenance. This enables the database to be integrated into other workflows or applications (Johansson et al., 2021).

3.6.7.3.3 Identification of the Mobile Elements

Firstly, all the duplicates from the current study sequences were removed using htc stream (Brandis et al., 2018) and the raw sequences were then trimmed using trim galo (Johansson et al., 2021). Information from the reference was extracted using bwa (Ehuwa et al., 2021) and thereafter, the clean samples were also indexed against the reference using bwa. The output files, sam files, were then converted into bam files using mge finder (Ehuwa et al., 2021). The bam files were then used to assemble to obtain contigs. Therefore, deno assembly of all the contigs was performed using SPADES (Johansson et al., 2021). The assembled contigs were annotated using Prokka (Johansson et al., 2021). The output from Prokka was used as an input for the mobile genetic element finder pipeline. Finally, the outputs from Prokka were uploaded on Centre for Genomic Epidemiology in order to identify the mobile genetic elements; resistance genes, virulence genes, transposons and plasmids.

3.6.7.3.4 Classification of Mobile Elements Associated with AMR

Each resistance gene was classified as either being iMGE-associated, carried by an MGE or having an unknown association (Ehuwa et al., 2021). The AMR was considered associated if it was located within 31 kb of an iMGE. The threshold corresponds to the longest ComTn (Tn*6108*) from *S. enterica* in the database and is intended to reflect which genes have the potential to be mobilized by surrounding iMGEs (Johansson et al., 2021).

3.7 Ethical Consideration

Ethics approval was sought from the University of Zambia Biomedical Research and Ethics Committee (UNZABREC) **REF. No. 1110-2020 (page 136)**. Additional ethics clearance and the Material Transfer Agreement (MTA) were obtained from the National Health Research Authority (NHRA) in Zambia, Ministry of Health (MOH) and the Ministry of Animal Resources and Fishers (MARF) in South Sudan.

CHAPTER FOUR

RESULTS

4.1 Demographic Characteristics of Chicken

Of the 270-chicken sampled, (45.6%) were aged between 1-59 days, 60-119 days (34.0%) and few number aged greater than 120 days (20.4%). There were more hens (77.8%) compared to cocks (22.2%) of which their feeds were supplemented mostly with tetracycline (39.2%) followed by salt + multivitamins (31,4%) and salt (18.2%). However, 61.1% were not fed on feed additives (Table 4.1). All the birds were on free range. The larger number of chickens were kept with ducks (43.3%).

Table 4.1: Demographic Characteristics of Chicken

Variables		Percentage	Frequency	Total
Sex	Hen	77.8	210	
	Cocks	22.2	60	270
Age	>1-59day	45.6	123	
	60-119day	34.0	92	
	>120day	20.4	55	270
Feed Additives	None	11.2	30	
	Salt	18.2	49	
	Tetracycline	39.2	106	
	Salt with Multivitamin	31,4	85	270
Others animals	None	29.3	79	
	Other Bird (ducks)	43.3	117	
	Dog and Cat	11.1	30	
	Sheep, goat and Donkey	16.3	44	

4.2 Demographic Characteristic of Chicken keepers

A total of 147 (Table 4.3) chicken keepers participated in the study, with more females (76.2%) compared to males (23.8%). The largest number of chicken keepers were aged less than18 years (60.5%), followed by 19-35 years (15.0%), 36-54 years (13.6%) and a few were aged above 55 years. The participants were in close contact with chicken mostly through feeding (81.6%), followed by cleaning (10.2%) and least via cooking (8.2%). There were more chicken keepers with formal education at secondary level (59.9%) and primary level (20.4%) compared to those with no education (7.4%). There were more participants who engaged were keeping chicken for more than 18 months (66.7%) followed by 13-18 months (22.4%), compared to those in the same activity for 7-12 months (4.8%). Regarding the flock size, 40.8% of the chicken's keepers had a flock size of less than 20 chickens while the least number of them, 9.6% kept more than 60 chickens as shown in Table 4.2.

Table: 4.2: Demographic Characteristic of Chicken Keepers

Variables		Percentage % of total	Frequency	Total
Sex	Male	23.8	35	
	Female	76.2	112	
Formal Education	None	7.4	11	
	Primary	20.4	30	
	Secondary	59.9	88	
	University	12.3	18	
Contact with chicken	Feeding	81.6	120	
	Cleaning	10.2	15	
	Cooking	8.2	12	
Duration of exposure	<6 Month	6.1	9	
	7-12 Months	4.8	7	
	13-18 Months	22.4	33	
	>18 Months	66.7	98	
Flock size	<20	40.8	60	
	21-40	37.4	55	
	41-60	12.3	18	
	>61	9.6	14	
Age group	<18	60.5	89	
	19-35	15.0	22	
	36-54	13.6	20	
	>55	10.9	16	
Total				147

4.3 Isolation and Identification of *Salmonella*

A total of 417 fecal samples were microbiologically processed for *Salmonella*, of which 270 (64.7%) were from chicken droppings (cloaca swabs), while 147 (35.3%) were human stool specimens. *Salmonella* was isolated from 9 (3.3%) out of the 270 chicken's droppings. Out of the 147 humans' stool collected, 11 (7.5%) samples were positive (Table 4.3).

Table: 4.3 Isolation and Identification of *Salmonella*

Host	Number of samples	Positive (%)	Negative (%)
Humans	147 (35.3%)	11 (7.5%)	136 (92.5%)
Chicken	270 (64.7%)	09 (3.3%)	261 (96.7%)
Total	417	20	397

4.4 Prevalence of *Salmonella* in Chicken Keepers and Chicken per Sampling Area

The chicken keepers' stool and chicken swab samples were collected from eight selected areas of Wau municipality, Western Bahr el Ghazal state, South Sudan that included; Kousti, Nazreath, Hai Fahal, Hai Dinka, Zugolona, Sikka Hadid, Lokoloko and Jebel Kheir. The overall prevalence of *Salmonella* in chicken keepers was 11 (7.5%) out of 147 participants (Table 4.4) while a prevalence of 3.3% *Salmonella* was obtained from 270 chickens sampled. Both Kousti and Nazareth had higher infections of 5 (18.5%) and 3 (20.0%) respectively for chicken keepers. However, chicken sampled from Kousti and Lokoloko had higher prevalences of 3 (6.0%) and 3 (3.75%) respectively. Hai Fahall, Hai Dinka, Zugolona and Sikka Hadid were free of the infection as shown in Table 4.4.

Table:4.4: Prevalence of *Salmonella* in Chicken Keepers and Chicken Per Sampling Area

Sampling Areas	Prevalence among chicken keepers (%)	Total chicken sampled	Prevalence among chicken (%)	Total chicken sampled
Kousti	5 (18.5%)	27	3 (6.0%)	50
Nazareth	3 (20.0%)	15	1(2.6%)	39
Lokoloko	2 (5.4%)	37	3 (3.75%	80
Hai Denka	0 (0.0%)	15	0 (0.0%)	22
Sikka Hadid	0 (0.0%)	15	0 (0.0%)	07
Jebel Kheir	1(5.6%)	18	2 (6.0%)	33
Hai Fahal	0 (0.0%)	10	0 (0.0%)	22
Zugolona	0 (0.0%)	10	0 (0.0%)	17
Total	%	147	270	**%**

4.5 Distribution of *Salmonella* Serotypes Isolated from Humans and Chicken

Four isolates of *Salmonella aberdeen* (Figure 4.5) were obtained from this study. Of the four, three of them were isolated from human host while one was isolated from chicken. Moreover, five Non *typeable Salmonella* were from human's source. However, *Salmonella* type *uganda* and *montevideo* were only isolated from chicken host as shown in Figure 4.5.

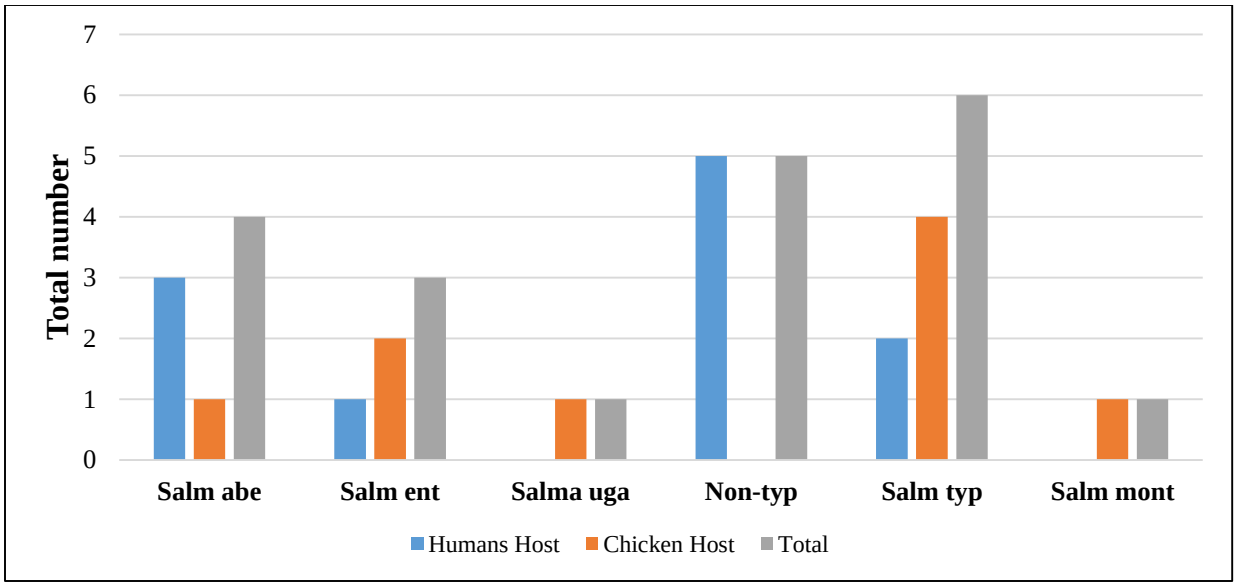

Figure 4.5: Distribution of *Salmonella* Serotype Isolated from Humans and Chicken

4.6 Characterization of *Salmonella* Sero-fermentative Groups

All *Salmonella* serotypes (Table 4.6) without exception utilized trehalose, melibiose, galactose, glucose, mannitol and dulcitol to produce acid (P = 0.01, 95% Cl). In contrast, all strains were exclusively negative for utilization of rabitol and raffinose. On the other hand, variable fermentation reactions were observed in the following substrates; arabinose, cellobiose, inositol and salicin as indicated in Table 4.6 below. The majority of the strains in this study complied with classic utilization of sugar substrates that include; positive to dulcitol, melibiose, glucose and arabinose. Negative reaction was indicated for salicin, mannitol, cellobiose and raffinose^{-}. *Salmonella* strain were biochemically grouped into two (I and II) fermentative profiles, based on their ability to utilize the four (cellobiose, arabinose inositol and salicin) substrates. All strains belonging to the fermentative group (*S aberdeen* and *S enteritidis*) were negative for utilization of cellobiose, arabinose, inositol and salicin as source of energy.

Table 4.6: Characterization of *Salmonella* Serotype Based on Fermentative Activity variable fermentative activity of sugars and polyhydric alcohol

Dulcitol	Raffinose	Rabitol	Arabinose	Inositol	Cellobiose inositol	Salicin	Trehalose	Melibiose	Galactose	Glucose	Mannitol
+	-	-	+	-	+	+	+	+	+	+	+
+	-	-	+	-	+	+	+	+	+	+	+
+	-	-	+	+	+	+	+	+	+	+	+
+	-	-	-	+	+	-	+	+	+	+	+
+	-	-	+	+	+	-	+	+	+	+	+
+	-	-	+	+	+	+	+	+	+	+	+
+	-	-	+	-	+	+	+	+	+	+	+
+	-	-	+	+	+	+	+	+	+	+	+
+	-	-	-	-	-	+	+	+	+	+	+
+	-	-	-	+	-	-	+	+	+	+	+
+	-	-	-	+	+	-	+	+	+	+	+
+	-	-	-	+	+	+	+	+	+	+	+
+	-	-	+	+	+	+	+	+	+	+	+
+	-	-	+	+	+	+	+	+	+	+	+
+	-	-	+	+	+	-	+	+	+	+	+
+	-	-	-	+	+	+	+	+	+	+	+
+	-	-	-	-	+	+	+	+	+	+	+
+	-	-	+	+	+	+	+	+	+	+	+
+	-	-	+	-	-	+	+	+	+	+	+
+	-	-	+	-	-	+	+	+	+	+	+

(+ = Positive; - = Negative)

4.7 Antimicrobial Susceptibility Pattern of *Salmonella* Isolates from Humans and chicken

Of the 11 isolates obtained from the 147 human samples, 7 isolates showed 100% susceptibility to the sulfamethoxazole/ trimethoprim, chloramphenicol, streptomycin, nalidixic acid, cefotaxime and gentamicin (Figure 4.7). However, some isolates showed resistance to ciprofloxacin 1 (9.1%), tetracycline 1 (9.1%), and ampicillin 2 (18.2%). All the chicken isolates (100%) were susceptible to chloramphenicol, cefotaxime, streptomycin, sulfamethoxazole/trimethoprim, nalidixic acid and gentamicin (Figure 4.7). The isolates were resistant to ampicillin (11.1%) and tetracycline (22.2%).

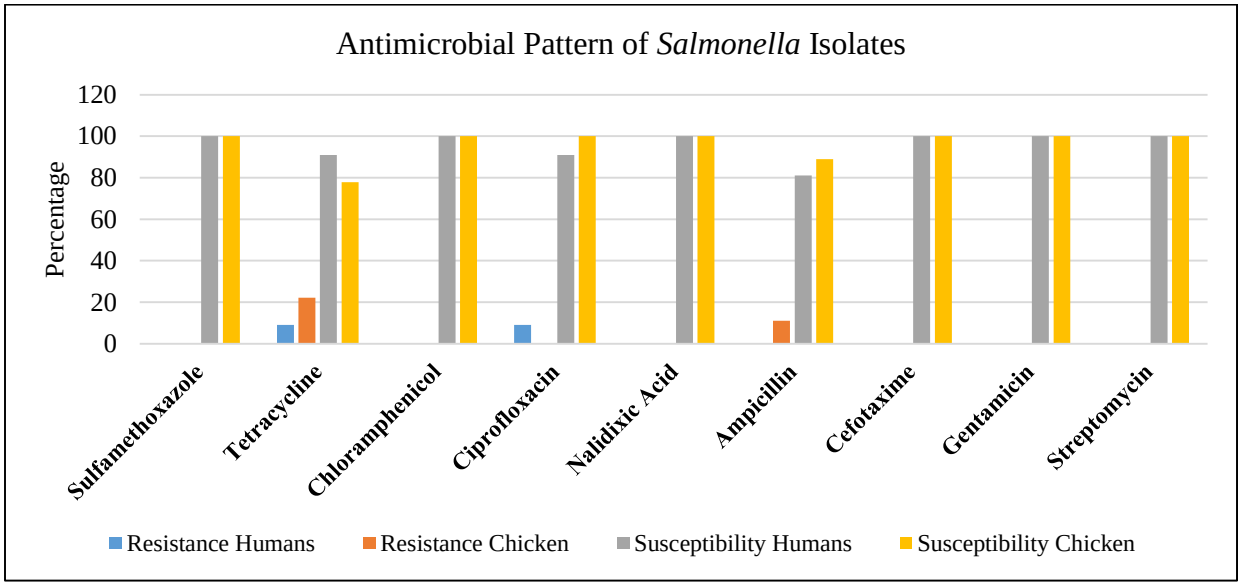

Figure 4.7: Antimicrobial Susceptibility Pattern of *Salmonella* Isolates from Humans and chicken

4.8 Antimicrobial Resistance Profiles of *Salmonella* Isolates

Antimicrobial resistance profile of 20 *Salmonella* strain were grouped into three (I-III) different MDR resistotype profiles (Table 4.8). This resistance was determined based on the ability of *Salmonella* strains to induce resistance to a single or group of antibiotics. Resistotype group I was represented by 2 (10%) *Salmonella* strains isolated from humans and chicken. Of the 7 *Salmonella* strains, 4 (20%) were from human's source, while 3 (15%) were from the chicken source. *Salmonella* strains isolated from chicken source were represented in group II (15%) of the Three MDR resistotype (I and II). The only *S. enteritidis* strain from the humans and chicken was resistant to two antimicrobial ampicillin and tetracycline.

Table 4.8: Antimicrobial Resistance Profile of *Salmonella* Strain by Serotype and Host

Strain No	Serotype	Resistotype	Group	Host
3	*enteritidis*	Amp, Te, Cip,Gn	I	Humans/chickens
1	*Salmonella typhimurium*	Cip,Gen,Te	II	Humans/chickens
3	Non-*typhable*	Amp, Cip	III	Humans

4.9 Characterization of *Salmonella* Strains Based on (ESBL) Resistance Profile

All the 20 *Salmonella* strains isolated in this study were none extended broad-spectrum beta-lactamases (ESBL) phenotypes. This proves their susceptibility to third generation cephalosporin (ceftriaxone, ceftazidime and cefotaxime). This was confirmed by the absence of bacteria growth on MacConkey agar containing 2 mg/l of cefotaxime.

4.10 Genotypic Characterization of Antimicrobial Resistance from WGS

Of the Eight *Salmonella* isolates that were screened in this study, the highest rate of antimicrobial resistance genes (ARG) was found for sulfonamides 8 (100%), tetracycline 7 (87%), erythromycin 4 (50.0%), β-lactamases 6 (75%), streptomycin 4 (50.0%) and the least was for chloramphenicol 2 (25%). The ARG gene coding sulfonamide *sul1* and *sul2* (in 8 isolates 100%) was the most frequently occurring among the isolates, followed by gene *tet*(D) (7 isolates), *tet(A)* (6 isolates), *blaCTX-M-15* (4 isolates). The ARG against streptomycin was very diverse, including *aac*(3), *aph*(3) *and aph*(6) genes .The ARG against β-lactamases included *blaCTX-M-15, blaTEM-1, blaOXA-10, blaOXA-1,* and *blaTEM-1*. *dfrA19 gen* was the only resistance gen found for trimethoprim. The ARG of *sul1, sul2* were for resistance to sulfonamides. The ARG of *tet(A)* and *tet(D)* were for resistance to tetracycline as shown in Table 4.10.

Table 4.10: Genotypic Characterization of Antimicrobial Resistance Genes

Class	n isolates (Percentage)	Genes Name
Sulfonamide	8 (100)	*Su1l* and *Sul 2*
Tetracycline	7 (87.5)	*tet(A)* and *tet(D)*
Beta Lactam	6 (75)	*blaCTX-M-15, blaTEM-1, blaOXA-10, blaOXA-1, and blaTEM-1*
Streptomycin	4 (50.0)	*aac(3), aph(3) and aph(6)*
Trimethoprim	5 (62.5)	*dfrA19*
Erythromycin	4 (50.0)	*ere(A)*
Chloramphenicol	2 (25)	*catB3*

4.11 Characterization of *Salmonella* Isolates by Antimicrobial Resistance and Virulence Genes

Antimicrobial resistance varied with *Salmonella* serotypes, with *Salmonella enterica* serovar *Enteritidis* (*Salmonella* ser. *enteritidis*) isolates being highly resistant to Sulfonamides; *Salmonella* ser (Table 4.11). *Salmonella typhimurium* was resistant to streptomycin, erythromycin, tetracycline and sulfonamides and *Salmonella* ser. *senftenberg* was resistant to sulfonamides, tetracycline and chloramphenicol. *Salmonella* ser. *typhimurium* isolates presented a more diverse were for ARG than *Salmonella* ser. *enteritidis* and *Salmonella* ser. *senftenberg*. Our data showed that Five isolates of *Salmonella* ser. *typhi* and *Salmonella* ser. *senftenberg* contained ARG resistant to greater than Five antimicrobials. In addition, almost all *Salmonella* isolates carried ARG resistantnce to Four antimicrobials.

Table 4.11: Characterization of *Salmonella* Isolate Antimicrobial and Virulence Genes

ID/No	Source	Serovar	Virulence genes	Antimicrobials Genes
2	Humans	*typhi*	*iroB, sinH, iroC and cdtB*	*sul2, tet (A), aac (6')-IIc, dfrA19, tet(D), blaCTX-M-15, ere(A), sul1* and *catB3*
4	Humans	*senftenberg*	*sinH, iroB and iroC*	*sul2, aac (6')-IIc, arr, dfrA19, tet(D), ere(A) and sul1*
7	Humans	*typhi*	Non	*sul1, dfrA19, aac (6')-IIc, tet (D) and aph (3'')-Ib*
8	Humans	*typhi*	Non	*Aph (6)-Id, sul2 and blaTEM-1*
	Humans	*typhi*	Non	*tet(A), sul2, cmlA5, blaOXA-10, tet(A), catB3, blaOXA-1*
9	Humans	*typhi*	Non	*sul2, tet(A), dfrA19, blaCTX-M-15, ere (A) and tet(D)*
22	Chicken	*enteritidis*	Non	*Aph (3')-Ia, dfrA19, tet(D), blaCTX-M-15, blaOXA-1 and sul1*
21	Chicken	*typhimurium*	Non	*blaTEM-1, ere(A), sul1, sul2, tet(A), tet(D)*

4.12 Characterization of *Salmonella* Serovar by Virulence Genes

The genes *iroB* and *iroC*, related to iron uptake and defense against oxidative stress, were found in *Salmonella* (*typhi*) and (*senftenberg*) strains respectively; all of them were isolated from the humans. However, the cytolethal distending toxin related gene *cdtB* was found only in two strains of *Salmonella typhi* from human isolates. The virulence gene *sinH* intimin-like protein *sinH* was detected in both *typhi* and *senftenberg* Serovar. While *Salmonella typhimurium* and *Salmonella enteritidis* isolated from chicken source didn't carry any resistance genes. However, all the *Salmonella* resistance genes found in this study were non-host specific suggesting that they could be transmitted from humans to chicken and vis versa as shown in Table (4.12).

Table 4.12: Characterization of *Salmonella* Serovar by Virulence Genes

ID/No	Source	Serovar	Virulence Genes
2	Humans	*typhi*	*iroB, sinH, iroC* and *cdtB*
4	Humans	*senftenberg*	*sinH, iroB* and *iroC*

4.13 Summary of the Identified Mobile Genetic Elements (MGEs)

Thirty mobile genetic elements (Table 4.13) were identified of which the majority were belonged to Insertion sequence (with total number of 15) followed by Miniature Inverted Repeat (with total number of 6) and the least was Unit transposon and Integrative Conjugative Element (with total number of 1 and 2 respectively). Human isolates had much higher 24(80%) mobile genetic elements comparing to chicken which had six elements only with a percentage of 20% as shown in Table 4.13.

Table 4.13: Summary of the Mobile Genetic Elements

Name	Type	Human	Percentage %	Chicken	Percentage %
Tn	Unit transposon	1	3.3	0	0.0
SPI-7	Integrative Conjugative Element	2	6.7	0	0.0
IS	Insertion sequence	15	50	4	13.3
IS630	Miniature Inverted Repeat	6	20	2	6.7
Total		24	80%	6	20%

4.14 Classification of the Mobile Genetic Elements

Transpose mobile genetic elements (Table 4.14) was the only type that was found in isolates from human and chickens. The subtype Insertion sequence was the predominant in human and chicken isolate However, Unit transposon or Integrative Conjugative Element subtype was only associated with humans isolate as shown in Table 4.14 below.

Table 4.14: Classification of the Mobile Genetic Elements

S/N	Name	Type	Frequency	Symbol
1.	*IS605*	Insertion sequence	6	*IS*
2.	*IS3*	Insertion sequence	9	*IS*
3.	*IS630*	Miniature Inverted Repeat	10	*IS*
4.	*Tn6024*	Unit transposon	1	*Tn*
5.	*IS4*	Insertion sequence	1	*IS*
6.	*IS3*	Insertion sequence		*IS*
7.	*IS110*	Insertion sequence	1	*IS*
8.	*SPI-7*	Integrative Conjugative Element	2	*IS*
Total			30	

4.15 Associations of Mobile Genetic Elements and Antimicrobial Resistance

Mobile genetic elements (Table 4.15) were associated with antibiotic resistance of *aac(6')-Iaa* and blaCTX-M-15 genes with percentage of (56.3%) and (35.3%) respectively. Beta-lactamases resistance genes tended to be associated with iMGEs more frequently in isolates from human than

chickens (43.8%). Humans isolates had a higher frequency 3 (75%) of streptomycin resistance genes linked with MGEs than chicken isolates 1 (25%) as shown in Table 4.15.

Table 4.15: Associations of Mobile Genetic Elements and Antimicrobial Resistance

Name	Associated AMR Gen	Drug Name	Family	Virulence Gens
IS605	*aac(6')-Iaa*	Streptomycin	*Aminoglycoside*	*nlpI*
IS3	*aac(6')-Iaa*	Streptomycin	*Aminoglycoside*	*nlpI*
IS630	*aac(6')-Iaa, blaCTX-M-15*	Streptomycin, cefotaxime, ampicillin and amoxicillin	*Aminoglycoside and Beta-lactamases*	*nlpI*
Tn6024	*blaCTX-M-15*	cefotaxime, ampicillin and amoxicillin	*Beta-lactamases*	*nlpI*
IS4	*blaCTX-M-15*	cefotaxime, ampicillin and amoxicillin	*Beta-lactamases*	*nlpI*
IS3	*blaCTX-M-15*	cefotaxime, ampicillin and amoxicillin	*Beta-lactamases*	*nlpI*
IS110	*blaCTX-M-15*	cefotaxime, ampicillin and amoxicillin	*Beta-lactamases*	*nlpI*
SPI-7	*blaCTX-M-15*	cefotaxime, ampicillin and amoxicillin	*Beta-lactamases*	*nlpI*

CHAPTER FIVE

DISCUSSION

5.1 *Salmonella* Isolation in The Study Area

Salmonella is an important cause of morbidity and mortality in human and animal population and has thus emerged as a significant and growing public health and economic problem worldwide (Wang et al., 2017). The overall prevalence of *Salmonella* in this study was 7.5% and 3.3% for chicken keepers and chicken respectively. This was lower when compared to a similar study conducted in Khartoum, Sudan where 70.1% chicken handlers and 18.1% chicken were found with *Salmonella* (El Hussein et al., 2010).

The first investigation on the prevalence of salmonellosis in chicken in South Sudan was in Malakal region by Soliman & Khan et al., (1959). In that study the investigation did not involve chicken keepers. The present study was aimed at investigating the prevalence of salmonellosis in chickens and chicken keepers in South Sudan, considering that the first study carried out in this new country involved only the chicken host. In 1943, *Salmonella gallinarum* in chicken was isolated for the first time in the nation (Yagoub et al., 1987). Additionally, the organism was also isolated from chickens in Malakal, Southern Sudan (Soliman & Khan, 1959). The study was conducted in Malakal region of South Sudan, where the prevalence of *Salmonella* in chicken was investigated (Soliman & Khan, 1959). The study considered a large sample size (996) that was pooled and hence increasing the chances of isolating *Salmonella*. However, higher prevalence rates have been reported by Cardinale et al., (2005) in some developing countries like Thailand (72%), Ethiopia (68.2%), Argentina (51.2%) and Korea (25.9%).

The prevalence obtained during the present study from chicken was 3.3% which is lower than 3.89% reported by Yagoub et al., (1987) and higher than the 1.13% reported by Soliman & Khan, (1959). It is notable that both Khan (1970) and Yagoub et al., (1987) managed to isolate S. *uganda*, that was also detected during the current study. *Salmonella uganda* could be domiciled in this part of the world where Uganda is a neighboring country.

In this study, twenty isolates of *Salmonella* were isolated from chicken droppings and humans stool samples. The serotype identified included. S. *enteritidis* (3 isolates), S. *aberdeen* (4), non typhable (5), *S. uganda* (1), *Salmonella* serovar *montevideo* (1) and serotype S. *typhimurium* (6). Of these isolates, 11 and 9 *Salmonella* were isolated from humans and chickens respectively. In the previous study by Soliman & Khan et al., (1959), *Salmonella gallinerum* serotypes were identified in chickens. In this study, 20 serotypes were documented for the first time, with S. *Salmonella typhimurium* being the abundant serotype observed in chickens. All the isolates identified are non-host adaptable. As they exhibited a broad host range and generally tend to colonize the intestinal tract and invade enterocytes of the intestinal mucosa, manifesting gastroenteritis, but in contrast fail to disseminate beyond the lymph nodes, unless in the event that the host has an underlying immune defect (Kingsley et al., 2013).

The non-host specific group consists of serotypes such as *S. typhimurium* and *S. enteritidis* (Rotger et al., 1999), and is perceived as a major cause of both gastroenteritis and septicemia syndromes in humans (Roudier et al., 1990) and animals (Kelterborn et al., 1967). For instance, *Salmonella typhimurium* and *S. enteritidis* serotypes can cause disease in a broad range of highly susceptible animal hosts such as poultry, cattle, rodents, pet hedgehogs as well as humans (Nakamura et al., 1987; Anderson et al., 2017). Further, these serotypes have been implicated in food-borne Salmonellosis worldwide, and have been detected in most species of domestic and wild animals used as food animals for human consumption (Gantois et al., 2009).

The current study obtained a salmonellosis prevalence of 7.5% in chicken keepers out of 147 stool samples (Table 4.1) and 3.3% chicken Salmonellosis in 270 chickens sampled. The prevalence of *Salmonella* was higher among chicken keepers than in chickens which was in agreement with a studies conducted by El Hussein et al., (2010) in Khartoum, Sudan. However, chicken keeper's salmonellosis prevalence was much lower than that reported by El Hussein et al., (2010) who obtained a prevalence of 19 (70.4%) out of 27 chicken handlers sampled (fecal sample) in selected restaurants in Khartoum. This could be attributed to the fact that the chicken handlers might have been in direct contact with chicken and related products during slaughtering and meal preparation. This, could have greatly increased their risks of exposure to the *Salmonella* infections than those engaged in domesticating chicken in particular (El Hussein et al., 2010).

The prevalence of *Salmonella* infection in chicken in the current study was higher than that reported by Yagoub et al., (1987) and Khan et al., (1970) in Sudan as 3.4% and 1.1% respectively. These studies were conducted more than 35 years ago of which the isolation methods used at that time may have had no enough power to detect all the *Salmonella*. Consequently, this makes their studies not suitable for comparisons with the current prevalence. However, El Hussein et al., (2010) obtained a prevalence of 18.1% that was greatly higher than in the present study.

In addition, higher *Salmonella* prevalence (19.2%) were reported in chicken carcasses in South Africa (Nierop et al., 2005). In Colombia the prevalence of *Salmonella* was found to be 27% (Donado et al 2012). However, in other countries *Salmonella* has also been reported such as Portugal (60%) (Antunes et al., 2003), Belgium (36%) (Uyttendaele et al., 1998), Australia (43.3%) (Pointon et al., 2008), and Spain (35.8%) (Dominguez et al 2002).

In Latin America, the reported *Salmonella* prevalence in chicken was 20% in Argentina (Jimenez et al., 2002) and 42% in Brazil (Fuzihara et al., 2000). In Southeast Asia, relatively higher *Salmonella* prevalence were reported for retail chickens in Vietnam (53.3%) (Van et al., 2007), China (52.2%) (Yang et al., 2011), and Thailand (57%) (Padungtod et al., 2006). The differences in *Salmonella* prevalence among these studies could be attributed to differences in sampling schemes or design, sample type (whole chicken versus chicken parts and chilled versus frozen chickens).

Moreover, other studies in Spain reported higher prevalence of chicken Salmonellosis greater than 60% (Arumugaswamy et al., 1995; Arvanitidou et al., 1998; Carramiñana et al., 2004), while the UK reported 25-29% (Patterson et al., 2001;Jørgensen et al., 2002). The variation in reporting Salmonellosis could be as a result of systems of production such as commercial systems due to chicken crowding in a house may report higher prevalence compared to free range.

5.2 Characteristics of the Isolated *Salmonella*

Salmonella isolated in this study had similar characteristics as other *Salmonella*. They were motile, gas producing, rod-shaped (bacillus) and Gram-negative bacteria (Kariuki et al., 2000). All *Salmonella* isolates were divided into (enterica) serovars using slide agglutination. The major serovars in order of the isolation rate were *Salmonella aberdeen* (4), *Salmonella enteritidis* (3), *Salmonella uganda* (1), *Salmonella typhimurium* (6), *Salmonella Montevideo* (1) and those that could not be typed (5).

5.2.1 Bio typing

Salmonella species may be differentiated in biotypes, where their various biochemical activities can be used to segregate them. Biotyping provides a means of separating strains of a species based on differences in selected biochemical tests (Anderson et al., 1977). In this study, the biotype group was based on sugar fermentation. In the current study most strains, displayed reactions to inositol, trehalose, melibiose, galactose, glucose, mannitol, dulcitol and glycerol through the production of acid. The absence or presence of some of these phenotypic activities may have little impact on the bacteria's capacity to persist and proliferate in the host population (Duguid et al.,1975).

In other studies, very few strains were incapable of producing flagella, gas from glucose, or trehalose fermentation in peptone water, and the lack of these three capabilities may be destructive (Jane et al., 2009). The biotyping of these isolates was based on the fermentation of inositol, Mannitol and arabinose (Jane et al., 2009). All the isolates that fermented trehalose, melibiose, galactose, glucose, mannitol and dulcitol were found to be negative for raffinose and rabitol (Hoszowski et al., 2001).

The proportion of isolates of various types in a randomly collected series may be significantly influenced by chance events that allow or prevent the spread of specific strains at specific periods and locations (Rabsch et al., 2002). As a result, it is impossible to determine the survival values of the various typing characters by looking at their frequency distribution (Rabsch et al., 2002). However, it is reasonable to assume that properties that are absent from a large number of strains

are unlikely to have a high survival value and that those that are absent from a smaller number of strains may be crucial for the persistence of the bacteria in the environment (Anderson et al 1977).

In the current setting, biotyping is rarely used as techniques have been developed that enhance the epidemiological power such as PCR and WGS (Wong et al., 2008). Biotyping generally is laborious and usually has to be used with other techniques to increase the power of identifying bacterial relationships (Rabsch et al., 2002). Previously this method was routinely used in most disease investigations and provided insight into routes of transmission, reservoirs of infection and mechanisms of persistent infections (Paiva et al., 2009).

5.2.2 Antimicrobial Susceptibility Patterns of Circulating *Salmonella* among Chickens and Humans

The Kirby-Bauer disc diffusion method was used to determine the phenotypic profiles of the isolates against antibiotics. In the present study, the antimicrobial susceptibility profiles of both chicken keepers and chicken *Salmonella* isolates were investigated. The patterns were varied from susceptible, moderately resistant and resistant to the tested antimicrobials. *Salmonella* isolates from humans were resistant to at least one or more antimicrobials. However, this was contrary to the results reported by Fadlalla et al., (2012) in a similar study conducted in Sudan where 81 (93.1% n=119) isolates exhibited the same resistant pattern. However, in the present study *tnteritidis* 1 (9.1%), *typhimurium* 1 (9.1%) and non-*typable Salmonella* 2 (18.9%) of the chicken keepers were highly resistant to tetracycline, ciprofloxacin and ampicillin respectively. This resistance pattern was greatly lower than that reported by Fadlalla et al., (2012). Where resistance patterns to ampicillin 29 (33.3%), nalidixic acid 28 (32.2%) and tetracycline 52 (59.8%) were obtained. Furthermore, de Oliveira et al., (2005) in Brazil reported higher resistance to tetracycline (11.8%) and sulfamethoxazole + trimethoprim (88.2%).

The same isolates were susceptible 11 (100%) to cotrimoxazole, chloramphenicol, cefotaxime, streptomycin, nalidixic acid and gentamicin. This was in agreement with the results of Fadlalla et al., (2012) except for ciprofloxacin which exhibited moderate resistance to some isolate 1 (9.1%). However, de Oliveira et al., (2005) reported 88.2% isolates being resistant to sulphonamides in a study conducted in Brazil which was comparable to our findings. There were four (30%) isolates

that showed multidrug resistance to more than one antimicrobial which was contrary to the findings of Fadlalla et al., (2012) who reported 41 (47.1%).

The cross resistance obtained among the isolates could reflect a prevalence of two resistance genes carried on plasmids as reported by Davidson et al., (1982). These could be transmitted via food chain from animals and their products to humans (Singer et al., 2003). Moreover, the continued usage of ampicillin and nalidixic acid as treatment remedies could have brought resistance. Besides, ampicillin is not suitable against gram negative bacteria such as *Salmonella* which may lead to intrinsic resistance.

Despite the plastic anemia effects caused by use of chloramphenicol, in combination with ampicillin were drugs of choice in the treatment of human salmonellosis (de Oliveira et al., 2005). Thus, it has been prescribed in human and food producing animals since 1970s' (de Oliveira et al., 2005) in Brazil. Currently the use of chloramphenicol in animals' food is outlawed (Guidi et al.,2015) and hence the non-observing of chloramphenicol resistance in poultry isolates. This observation could also be due to use of different antimicrobials in an unmonitored way contributing to antimicrobial resistance variation in *Salmonella* serotypes (Watkins et al., 2020).

The current study has also indicated that 7 (63.6%) isolates were 100% susceptible to sulfamethoxazole / trimethoprim, chloramphenicol, cefotaxime, streptomycin, nalidixic acid and gentamicin. This was in agreement with the results of Fadalall and colleagues (Fadlalla et al., 2012) except for some isolate that exhibited moderate resistance to ciprofloxacin 1 (9.1%). Furthermore, the results of the current study are also in congruent with a study conducted in Brazil were 88.2% of *Salmonella* isolates showed resistance to sulphonamides (Oliveira et al., 2005).

This resistance of *Salmonella* isolates to most of the antimicrobials could be due to a presence of resistance genes that are carried on the bacterial plasmids which can be acquired by consumption of contaminated animal products by humans (Davidson et al., 1982). Continuous use of antibiotics such as ampicillin and nalidixic acid as treatment remedies may also be the reason for development of resistance (Hur et al., 2012). Generally, these serovars showed low resistance to tetracycline

and high resistance to ciprofloxacin and ampicillin which are the antibiotics commonly used in South Sudan.

According to the current study, there was no relationship between phenotypic and genotypic resistance. The isolates from chicken and human sources differed in their genotypic resistance, and in many instances, the presence of resistance genes was not consistent with resistance to the relevant antimicrobials. This may have been resulted of gene expression (Ma et al., 2017).

The most fundamental level at which the genotype results in the phenotype is gene expression. The genotype is the genetic information contained in DNA, whereas the phenotype is the interpretation of that information. Such phenotypes are frequently manifested by the creation of proteins that regulate the structure and growth of the organism or function as enzymes catalyzing particular metabolic processes (Crump et al., 2015).

5.3 Genotyping of *Salmonella* Isolate

Salmonella is an important foodborne pathogen, causing food poisoning and human infections, as well as critically threatening food safety and public health (McClelland et al., 2001). *Salmonella* typing is essential for bacterial identification, infection tracing, epidemiological investigation, monitoring and surveillance of disease (McClelland et al., 2001). Whole genome sequencing is a high-resolution molecular typing method based on the whole genome for accurate bacterial tracing. In this study, eight isolates were chosen on the basis of their antibiograms and sample sources for further characterization using whole genome sequencing (WGS) and bioinformatics analysis (n=8). Those selected included eight species. Of those six were from human sources, while two were from chicken sources. The *typhi* serovar was isolated from both humans and chickens.

5.3.1 Genetic Diversity of Circulating *Salmonella* Among Chickens and Humans

Salmonellosis in humans has been a health problem in both developed and non-developed countries (Yachison et al., 2017). *Non-typhoidal Salmonella* caused by other species of *Salmonella* is different from *S. Typhi* a major cause of secondary bacteremia associated with gastritis (Wang et al., 2013). In the present study *S. enteritidis* and *S. typhimurium* were commonly isolated in

chicken while non typeable isolates of *Salmonella* were the most dominant serovars in humans sourced sample.

5.3.2 Antimicrobial Resistance Genes

Our study revealed that, ARG *blaTEM-1* was found in both one human serovar *typhi* and one chicken-sourced *Salmonella* ser *typhimurium*. All isolates from *Salmonella* serotypes *typhimurium* and *senftenberg* did not have ARG against quinolone, despite previous research showing an increase in quinolone resistance in *Salmonella* isolates linked to contaminated egg products in Europe during a 5-year survey from 2000 to 2004 (Meakins et al., 2008). In addition, among *Salmonella* ser. *typhimurium* and *Salmonella* ser. *senftenberg* isolates, chicken-sourced isolates comprised less diverse ARG than humans sourced isolates.

This could have resulted from continued use of antibiotics in humans without proper prescription by the pharmacist leading to the development of resistance. Also, the use of antimicrobials as feed additives in chicken feed may lead to development of antimicrobial resistance. In this study, there were 5 *Salmonella* isolates with ARG against 5 antibiotics from both chicken and human's sources; none of them was *Salmonella* ser. *enteritidis*. Furthermore, among the isolates studied, many carried ARG against 4 antimicrobials, particularly the isolates from *Salmonella* ser. *typhimurium*. This is a major concern as S *typhimurium* is a non-host adaptable specie. There is a high chance of this specie establishing itself in poultry and human.

According to our findings, resistance to chloramphenicol was associated with *Salmonella* ser. *typhi* (2 isolates) from human's source only and sulfonamides with *Salmonella* ser. *senftenberg* isolates from human's source.

Comparing the antimicrobial genotypic profiles and the phenotypic antimicrobial susceptibility of the strains from chickens and humans revealed that, strains exhibited phenotypic resistance against beta lactam, tetracycline and ciprofloxacin. This was proven by the genotypic suggesting a strong correlation with the resistance genes found. It is important to prove the correlation between the genotypic and phenotypic resistances. It has practical implications for developing prevention and control strategies for antimicrobial resistant bacteria.

5.3.3 Virulence Genes

Concerning the virulence genes, most of the strains studied had no virulence genes, only two from human's source serovar *typhi* had four gens, *iroB, sinH, iroC* and *cdtB* while serovar *senftenberg*, had three genes, *iroB, sinH* and *iroC*. The rest of the isolates did not carry any of the virulence genes. Although some genes such as *spv*A, *spvB, spvC, and spv*D are important transcriptional regulators (Hang'ombe et al., 2008) and mainly detected in *Salmonella*, in this study, these genes were not detected in all the isolates analyzed. The detected genes have virulence activity centered on disease manifestation. The role of *spv*A is to promote the macrophage phase avoiding destruction by neutrophils (Clements et al., 2021).

The function of *spv*B encodes an enzyme ADP-ribosylates which modifies actin cytotoxin required for systemic survival (Bomjan et al., 2018). While *spv*C has phosphothreonine lyase activity which inhibit mitogen-activated protein kinases signaling (MAPKs) (Guiney et al., 2011). However, the function of *spv*D is not well defined, while *iroB* and *iroC* gene functions are specifically recognized as outliers for *Salmonella* spp. outbreaks as they are involved in responsibility of the synthesis and transport of enterobactin, a siderophore produced by *Salmonella* spp. and is essential for iron uptake inside the host (Caza et al., 2008). Virulence genes *sinH* and *cdtB* produce proteins that carry the bacteria into the host cell (Caza et al., 2008). In this study, all these virulence genes were detected, reinforcing the understanding that *Salmonella* may contribute to disease causation in South Sudan.

5.4 Mobile Genetic Elements of Antimicrobial Resistance

In the current study, four major mobile genetics subtype were identified namely; insertion sequence (IS), Integrative Conjugative Elements (ICEs), Integrative Mobilizable Elements (IMEs) and Unit transposons (Tns). Among the smallest types of iMGEs is an insertion sequence (IS). They frequently contain a transposase gene. Larger iMGEs with conjugation capabilities include Integrative Conjugative Elements (ICEs), Cis-Mobilizable Elements (CIMEs), and Integrative Mobilizable Elements (IMEs). Either they can conjugate on their own or additional elements must conjugate in order for them to co-mobilize (Johansson et al., 2021). These elements contain numerous more genes and MGEs. Unit transposons (Tns) often carry a transposase gene. The

resolvase genes, accessory genes, and/or extra iMGEs are frequently present in them (Ehuwa et al., 2021).

MGEs and antimicrobial resistance are related (Johansson et al., 2021). AMR genes were categorized as being connected with integrating mobile genetic elements iMGEs if they were either carried by iMGEs or were found within 31 kb of them. In contrast to isolates from chicken, isolates from humans had a higher overall number of iMGEs linked to AMR genes (Brandis et al., 2018). This was particularly evident for b-lactam resistance genes, where 80% of the genes in human isolates were found close to iMGEs compared to 20% in chicken isolates.

5.5 Significance of the Isolated *Salmonella* to Public Health

The isolated *Salmonella* in this study is non host adaptable. This implies that all the *Salmonella* detected and identified in this study have the potential to cause disease. The most significant isolates were S. *typhi* and S *enteritis* which have been documented to cause illness (Yang et al., 2011).

The antimicrobial resistance pattern observed with the chicken isolates is of concern considering that the isolates from chicken were non-host adaptable. Probably due to the disorganized and widespread use of antimicrobials in chicken and human, the level of antimicrobial resistance in NTS in South Sudan is extraordinary. There is therefore a critical need to establish an investigation of resistance in *Salmonella* in South Sudan to assist in recommendations on the use of antimicrobials in both human and chicken. The genetic relationship between NTS isolated from human's stool and chicken dropping and their high prevalence of resistance to routine antimicrobials including ciprofloxacin suggest that resistant NTS could be an important emerging public health threat in South Sudan, e.g., as cause of infections that may need investigation. Sources and ways of transmission of antimicrobial resistance and AMR among NTS should be continuously monitored, e.g., in national microbiological and epidemiological surveillance programs.

The extensive use of antibiotics in chicken feed has made a substantive contribution to the emergence and spread of antibiotic resistance (Carramiñana et al., 2004). *Salmonella is* among the

most common food-borne pathogens associated with antibiotic resistance (Fadlalla et al., 2012). This study has described the genomic epidemiology of antibiotic-resistant *Salmonella* isolated from chicken and chicken keepers and has detailed the use of essential tools for microbial determination, epidemiological investigation, and possible outbreak tracing.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

1. The present study revealed overall prevalence of *Salmonella* as 7.5% and 3.3% for chicken keepers (humans) and chicken, respectively.

2. The phenotypic and genetic diversity of circulating *Salmonella* among chickens and humans was found to involve *Salmonella typhimurium* and *Salmonella enteritidis* which are the serious disease-causing enteric bacteria.

3. The phenotypic antimicrobial profiles demonstrated three antimicrobials resistance namely, penicillin, tetracycline and ciprofloxacin in chicken and humans isolate.

4. The genotypic characterization of *Salmonella* antimicrobial resistance revealed high rates of ARG against streptomycin, tetracycline, erythromycin, sulfonamides, and chloramphenicol

5. *Salmonella* ser. *typhimurium* isolates contained more ARG and a higher level of resistant than *Salmonella* ser. *enteritidis* and *Salmonella* ser. *senftenberg* isolates.

6. *Salmonella* antimicrobial-resistant isolates found in humans and chicken highlight the critical need for consumer and worker/farmer education to reduce/eradicate *Salmonella* in chicken production.

7. The study has generated important information to help future investigations on the one health epidemiology of *Salmonella* in South Sudan.

6.2 Recommendation

1. To effectively reduce *Salmonella* contamination in chicken, the surveillance-and-intervention strategies must include investigation and identification of management factors that affect the presence of *Salmonella*.

2. To minimize antimicrobial resistance, a national surveillance program should be established for monitoring MDR pattern in food-borne bacterial pathogens.

3. There is need for continuous *Salmonella* monitoring, investigation of virulence evolution to enhance the One Health Initiative, which is a collaborative, multisectoral, and transdisciplinary approach to achieving optimal health outcomes by recognizing the interconnection between animals, plants, people, and their shared environment.

4. Introduction and adoption of more uniform reporting of all isolates, regardless of whether the isolation was carried out in a hospital, public health laboratory, or other official laboratory, in order to strengthen *Salmonella* surveillance throughout the South Sudan.

5. All serotype, of *Salmonella* isolates from humans and chickens, or other sources (such as food or feed) should be reported to the relevant local public health department, which should then routinely report to the state public health department for intervention.

6. Salmonellosis is one of the many food-borne illnesses that affect people, and as such management should improve diagnosis to avoid similar illnesses.

7. Epidemiologists, veterinarians, sanitarians and laboratory staff, and other staff from local, state, and federal agencies should be educated on the significance of *Salmonella* in public health.

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APPENDICES

Appendix i: Ethical Approval

UNIVERSITY OF ZAMBIA
BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
Fax: + 260-1-250753
Federal Assurance No. FWA00000338

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia
E-mail: unzarec@unza.zm
IRB00001131 of IORG0000774

7th September, 2020.

Your REF. No. 1110-2020.

Dr. Shereen Ahmed Mohamed Saad,
University of Zambia,
Department of Paraclinical Studies,
PO BOX 50110,
Lusaka.

Dear Dr. Saad,

RE: "PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF SALMONELLA ISOLATED FROM CHICKENS AND HUMANS IN SOUTH SUDAN" (REF. NO. 1110-2020)

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee on 7th September, 2020. The proposal is **approved**. The approval is based on the following documents that were submitted for review:

a) **Study proposal**
b) **Questionnaires**
c) **Participant Consent Form**

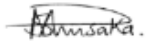
APPROVAL NUMBER : REF. 1110-2020

This number should be used on all correspondence, consent forms and documents as appropriate.

- **APPROVAL DATE : 7th September 2020**
- **TYPE OF APPROVAL : Standard**
- **EXPIRATION DATE OF APPROVAL : 6th September 2021**
  After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the UNZABREC Offices should be submitted one month before the expiration date for continuing review.
- **SERIOUS ADVERSE EVENT REPORTING**: All SAEs and any other serious challenges/problems having to do with participant welfare, participant safety and study integrity must be reported to UNZABREC within 3 working days using standard forms obtainable from UNZABREC.
- **MODIFICATIONS**: Prior UNZABREC approval using standard forms obtainable from the UNZABREC Offices is required before implementing any changes in the Protocol (including changes in the consent documents).

- **TERMINATION OF STUDY**: On termination of a study, a report must be submitted to the UNZABREC using standard forms obtainable from the UNZABREC Offices.
- **NHRA:** You are advised to obtain final study clearance and approval to conduct research in Zambia from the National Health Research Authority (NHRA) before commencing the research project.
- **QUESTIONS**: Please contact the UNZABREC on Telephone No.256067 or by e-mail on unzarec@unza.zm.
- **OTHER**: Please be reminded to send in copies of your research findings/results for our records. You're also required to submit electronic copies of your publications in peer-reviewed journals that may emanate from this study. Use the online portal: unza.rhinno.net for further submissions.

Yours sincerely,



Sody Mweetwa Munsaka, BSc., MSc., PhD
CHAIRPERSON
Tel: +260977925304
E-mail: s.munsaka@unza.zm

Appendix: ii

Permission to Undergo Field Research from Ministry of Health South Sudan

The Republic Of South Sudan
Wau State
Ministry Of Health
Director General Office

Date: October 8th 2019

TO WHOM IT MAY CONCERN

Subject: Permission to undergo field research

Mrs. **Shereen Ahmed Mohammed Saad** (Reg. No: **2018256926**) is a post-graduate student **(Doctor of Philosophy in Microbiology)** at the University of Zambia, School of Veterinary Medicine. Her approved Research Topic is: **"Phenotypic and Genotypic Characterization of Salmonella serotypes in Chickens and Human in South Sudan."**
As a part of her fulfilment of the Research Program, she has to undergo field research on the above Topic.

She will be carrying some consumables for her research, including swabs, gloves cottons wool, face masks, among others for her research in South Sudan. These are noninfectious materials.

Kindly, accord her any necessary assistance during her travel and research and work to facilitate the timely completion of the task.

Regards!

Akile Atanasio Yambio
Dir/ HRD & Training
SMOH/WS – Wau

Cc:
- Mayor/ Wau Municipality
- National Security/ Wau
- CID/ Wau
- Juba International Air-Port
- Wau International Air-Port
- File

Appendix: iii

Permission to Undergo Field Research from Ministry of Animal Resource and Fisheries

The Republic of South Sudan
Wau State –Wau
Ministry of Agriculture, Forestry, Animal Resources & Fisheries
Director General
Directorate of Animal Resources and Fisheries

Date: 12th September 2019

TO WHOM IT MAY CONCERN

Subject: Permission to Undergo Field Research for student/Miss Sherren Ahmed Mohamed Saad

This is to certify that, the above mentioned subject is a research student registered for a PhD of Veterinary Preventive Medicine at Zambia University, Zambia. Therefore, she has been given approval for data collection from chicken and their keepers within Wau Municipality.

Kindly provide her with all necessary assistance.

Regards,

Edward Lado
Director General
Directorate of Animal
Resource and Fisheries
Wau State

- ✓ Mayor/Wau Municipality
- ✓ National Security/Wau
- ✓ CID/Wau
- ✓ Wau International Airport
- ✓ Juba International Airport

Appendix iv: Consent Form

(WAU MUNICIPALITY), WESTERN BAHR EL- GHAZAL STATE, SOUTH SUDAN

Dear Sir, Madam

I am **Shereen Ahmed Mohammed Saad** a PhD student from Zambia University, who is investigating the Characterization of *Salmonella* in Chicken and Chicken keepers at house hold level in WAU MUNICIPALITY, WESTERN BAHR EL- GHAZAL STATE, SOUTH SUDAN. I would like to learn about the magnitude of the diseases **Phenotypic and Genotypic Characterization of *Salmonella* Isolated from Chickens and Humans in South Sudan** in the area and I do appreciate your cooperation to contribute a sample(s) to this study. I want to assure you that the sample and the information you provide will be confidential and your name will not appear any were. The participation is voluntary; however, I do appreciate your participation as your sample and the information you give are very important to this investigation. Thank you again.

If you agree please put your thumb print here …….

Or sign here ……

Appendix v: Sample Checklist

(WAU COUNTY), WESTERN BAHR EL- GHAZAL STATE, SOUTH SUDAN

Sample Check List

Payams …… Bomas ……

Date / Jun / 2019

Area code …… House number …… GIS……...

Human Sample Check List

Identification number	Type of contact with chicken	Duration	Abdominal crumbling/fever	Age	Sex	Time of sample collection

Comment

……

……

……

Chicken Sample Check List

Identification number	Use of feed supplement /anti-microbial /multivitamin	Age	Sex	Warty diarrhea/emaciation	Other animal at home	Time of sample collection

Comment

……

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Participant Question ……..

……

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……

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Thank you for cooperation

Hindawi
International Journal of Microbiology
Volume 2022, Article ID 8570081, 8 pages
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Research Article

Antimicrobial Susceptibility of *Salmonella* Isolated from Chickens and Humans in Wau, South Sudan

Shereen Saad,[1] **Ambros Jubara**,[1] **Charles Wani**,[1] **Musso Munyeme**,[2] **Alfateh Taha**,[1] **Steward Mudenda**,[3] **Mildred Zulu**,[4] **Wizaso Mwasinga**,[2] **Mulemba Samutela**,[5] **Rabecca Tembo**,[4] **Walter Muleya**,[6] **Geoffrey Kwenda**,[3] **and Bernard Mudenda Hang'ombe**[7]

[1]*Department of Clinical Studies, University of Bahr El Ghazal, College of Veterinary Science, Wau, Sudan*
[2]*Department of Disease Control, School of Veterinary Medicine, University of Zambia, Lusaka, P.O. Box 32379, Zambia*
[3]*Department of Pharmacy, School of Health Sciences, University of Zambia, Lusaka, P.O. Box 50110, Zambia*
[4]*Department of Pathology and Microbiology, School of Medicine, University of Zambia, Lusaka, P.O. Box 50110, Zambia*
[5]*Department of Biomedical Sciences, School of Health Sciences, University of Zambia, Lusaka, Zambia*
[6]*Department of Biomedical, School of Veterinary Medicine, University of Zambia, Lusaka, P.O. Box 32379, Zambia*
[7]*Department of Paraclinical Studies, School of Veterinary Medicine University of Zambia, Lusaka, Zambia*

Correspondence should be addressed to Shereen Saad; saadshereen01@gmail.com

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Academic Editor: Giuseppe Comi

Background. *Salmonella* infections are a public health problem across the globe. In South Sudan, there is little information regarding the prevalence and antibiotic resistance patterns of *Salmonella*. Therefore, this study assessed the prevalence and antimicrobial susceptibility of *Salmonella* isolates from chickens and humans in South Sudan. Fecal samples were collected and cultured on Xylose Lysine Deoxycholate Agar for the isolation of *Salmonella* and confirmed using biochemical tests and PCR through the amplification of the *invA* gene. A total of 417 fecal samples were examined, of which 270 (64.7%) were chicken cloacal swabs while 147 (35.3%) were humans' stool specimens. *Results*. Eleven (11) *Salmonella* isolates were isolated from humans while nine were from chickens. All 11 isolates from humans were susceptible to sulfamethoxazole-trimethoprim, chloramphenicol, streptomycin, cefotaxime, nalidixic acid, and gentamicin. However, 4 (36.7%) isolates showed resistance to ciprofloxacin, 2 (18.9%) to ampicillin, and 1 (9.1%) to tetracycline. All chicken isolates were susceptible to chloramphenicol, streptomycin, sulfamethoxazole-trimethoprim, ciprofloxacin, cefotaxime, nalidixic acid, and gentamicin but showed resistance to tetracycline 2 (22.2%) and ampicillin 1 (11.1%). *Conclusion*. Antimicrobial resistant isolates were isolated in both chickens and humans. Further, MDR isolates were found in both chicken and human samples, and this is a public health concern. This, therefore, calls for concerted efforts to educate producers and consumers on public health, food safety, food hygiene in food production, and enhancement of surveillance programmes on zoonotic bacteria and antimicrobial susceptibility.

1. Introduction

Worldwide, *Salmonella* has been listed among the most important food-borne pathogen that is transmitted through the consumption of contaminated food [1]. It causes approximately 1.4 million cases of disease and about 20,000 hospital cases and over 500 deaths every year [2]. A growing number of human Salmonellosis cases have been associated with the consumption of contaminated food of poultry origin, such as chicken and chicken products [3]. Besides, chicken products have also been reported to play a major role in the spreading of antimicrobial-resistant zoonotic bacterial pathogens [4] even though the hygienic standards for chicken production are quite high and usually vary from place to place [5, 6]. The problem of AMR is still rising.

Antimicrobial resistance (AMR) and particularly multidrug resistance (MDR) is becoming very common among various *Salmonella* serotypes that have been isolated from

Table 1: Isolation of *Salmonella* from fecal samples.

Host	Total tested *n* (%)	Positive *n* (%)	Negative *n* (%)
Human	147 (35.3)	11 (7.5)	136 (92.5%)
Chicken	270 (64.7)	9 (3.3)	291 (96.7%)

humans and chickens world over [7]. The extent of AMR varies from region to region and is usually influenced by the abuse of antibiotics in humans and animals [8]. Reports of cases of *Salmonella* isolates being resistant to important antibiotics have been reported dating back to the 1960s during which resistance was reported to have been limited to one antibiotic [9]. However, from the 1970s onwards, there has been an increase in the number of *Salmonella* isolates that have shown resistance to various clinically significant antibiotics, and this has been exacerbated by the recovery of such isolates in foods of animal origin [10]. This is a growing public health concern as human Salmonellosis caused by resistant strains of *Salmonella* may be difficult to be treated [10]. Since the mid-1970s, there has been an increasing trend of *Salmonella* isolates exhibiting MDR phenotypes worldwide [11]. The MDR exhibited by some *Salmonella* isolates and other pathogens is obtained from extrachromosomal genes that may impart resistance to an entire class of antimicrobials [12]. More recently, most of the resistance genes have been associated with large transferable plasmids and other DNA mobile elements, such as transposons and integrons [9, 13]. Moreover, MDR seems to be more serious in some serotypes compared to other serotypes [14, 15]. Therefore, there is a need for continuous monitoring of human and animal *Salmonella* isolates that exhibit resistance to most antimicrobials on a global scale [16].

In South Sudan, a young country where everything is still in its infancy, there is limited information regarding *Salmonella* species which was confirmed by PCR amplification of the invA gene. Therefore, this study assessed the prevalence and antimicrobial susceptibility of *Salmonella* isolated from humans and chickens in Wau, Western Bahr el Ghazal state, South Sudan, to inform control strategies.

2. Results

2.1. Isolation of Salmonella from Fecal Samples. The overall prevalence of *Salmonella* in the study was 4.8% (20/417), as shown in Table 1. The prevalence was relatively higher in humans, about 7.5% (11/147) compared to 3.3% (9/270) in chickens (Table 1). The *Salmonella* was further confirmed by PCR using the *invA* gene.

The prevalence of *Salmonella* in humans and chickens based on areas where samples were collected is shown in Table 2. The highest prevalence among human samples was from Baggari (9.4%) followed by Busuri (4.8%) (Table 2). For chicken samples, Baggari showed the highest prevalence of 4.5.% while Busuri payam showed a relatively lower prevalence of 1.7% (Table 2).

2.2. Detection of Extended Spectrum Beta-Lactamases (ESBLs) in Salmonella Strain. All the 20 *Salmonella* isolated from chickens and chicken keepers (humans) did not show any sign of growth on MacConkey agar supplemented with 2 mg/l of cefotaxime implying that all the 20 isolates were susceptible to cefotaxime.

2.3. Salmonella Serotypes Isolated from Human and Chickens. The *Salmonella* isolated from humans and chicken belonged to five serotypes, namely, *Salmonella* Aberdeen, Enteritidis, Uganda, Montevideo, and Typhimurium (Table 3). *Salmonella* Typhimurium was the most detected serotype (Table 3). Some nontypeable isolates were detected in humans while *Salmonella* Uganda and *Salmonella* serovar Montevideo were found in chickens only (Table 3).

2.4. Antimicrobial Susceptibility Patterns of Salmonella Isolates from Humans and Chickens. Of the 11 isolates obtained from the 147 human samples, seven isolates showed 100% susceptibility to the following drugs sulfamethoxazole/trimethoprim, chloramphenicol, streptomycin, nalidixic acid, cefotaxime, and gentamicin (Figure 1). However, some isolates showed resistance to ciprofloxacin 1 (9.1%), tetracycline 1 (9.1%), and ampicillin 2 (18.2%) (Figure 2). All the chicken isolates (100%) were susceptible to chloramphenicol, cefotaxime, streptomycin, sulfamethoxazole/trimethoprim, nalidixic acid, and gentamicin (Figure 1). The isolates were resistant to ampicillin (11.1%) and tetracycline (22.2%) (Figure 2).

2.5. Antimicrobial Resistance Profiles of Salmonella Isolates. Antimicrobial resistance profiles of the 20 *Salmonella* isolates were divided into three different MDR resistotype profiles (Table 4). This resistance was based on resistance to at least three different antimicrobials [17]. Resistotype group 1, resistance to ampicillin, tetracycline, ciprofloxacin, and gentamicin, was represented by 3 (15%) isolates. Resistotype group 2, resistance to ampicillin, gentamicin, and tetracycline, was detected in only 1 (5%) isolates, specifically *Salmonella* Typhimurium isolated from humans and chickens. However, resistotype group 3, resistance to ampicillin and ciprofloxacin, was found in three (15%) isolates. *Salmonella* strains isolated from the chicken source were represented in two groups of the three MDR resistotypes 1 and 2. The *S.* Enteritidis strain from the humans and chickens was resistant to two antimicrobials, namely, ampicillin and tetracycline.

3. Discussion

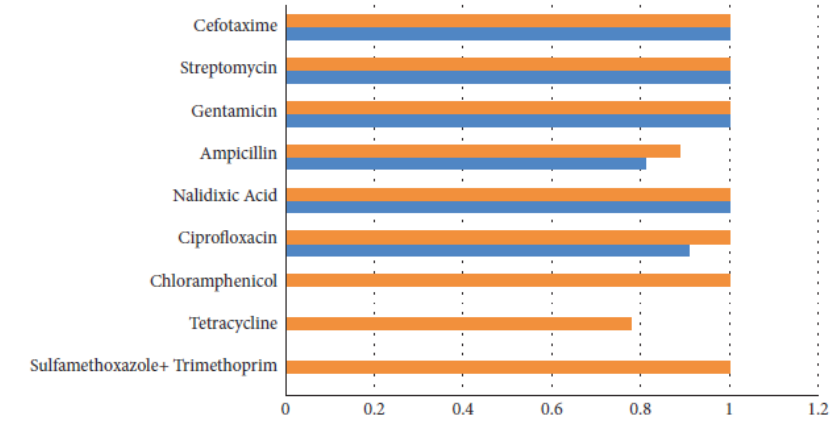
The present study investigated *Salmonella* in chickens and humans in Wau town of South Sudan. A previous study focused on *Salmonella* from chickens with prevalence of 1.1% [18]. Recently in 2017, Shereen Saad and collegues (unpublished work) investigated the prevalence of *Salmonella* and antimicrobial resistance in humans and chickens. However, the previous study did not involve molecular confirmation; as such, the study could not identify the serotypes circulating in the study area. The present study thus utilized molecular techniques to identify the different

Table 2: Prevalence of *Salmonella* in humans and chicken per sampling area.

Sampling area	Humans (*n*/total)	Prevalence among humans (%)	Chickens (*n*/total)	Prevalence among chickens (%)
Baggari	85	8 (9.4%)	157	7 (4.5%)
Busuri	62	3 (4.8%)	113	2 (1.7%)

Table 3: *Salmonella* serotype isolated from humans and chickens.

S/N	Isolate	Humans host	Chicken host	Total
1	*Salmonella* Aberdeen	3	1	4
2	*Salmonella* Enteritidis	1	2	3
3	*Salmonella* Uganda		1	1
4	Nontypeable	5		5
5	*Salmonella* Typhimurium	2	4	6
6	*Salmonella* serovar Montevideo		1	1
Total		11	09	20



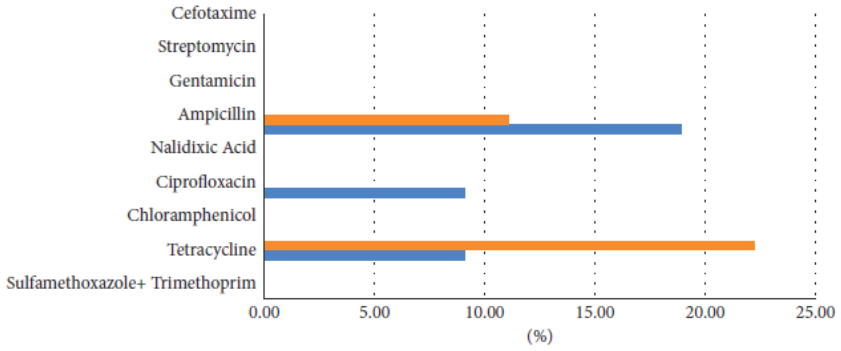
	Sulfamethoxazole + Trimethoprim	Tetracycline	Chloramphenicol	Ciprofloxacin	Nalidixic Acid	Ampicillin	Gentamicin	Streptomycin	Cefotaxime
Chicken	100%	77.80%	100%	100%	100%	88.90%	100%	100%	100%
Humans	0	0.00%	0.00%	90.90%	100%	81.10%	100%	100%	100%

Figure 1: Antimicrobial susceptibility profiles of *Salmonella* isolates from humans and chickens.

serotypes of *Salmonella* circulating present in both chicken keepers and chickens reared in Baggari and Busari regions of South Sudan.

Salmonella is an important cause of morbidity and mortality in human and animals and has thus emerged as a significant and growing public health and economic problem worldwide [19]. The overall prevalence of *Salmonella* in this study was 7.5% and 3.3% for humans and chicken, respectively. Human isolates showed its higher prevalence compared to chicken isolates. This difference may have resulted from the bigger volume of human sample (stool) in comparison to the chicken droopings. This was lower when compared to a similar study conducted in Khartoum, Sudan, where the prevalence was 70.1% in chicken handlers and 18.1% in chickens [20]. The difference in the prevalence was found in the current study and the previous study might be due to a larger sample size (996) that involved pooling of samples in the previous study. However, the higher prevalence of 68.2%, 72%, 25.9%, and 51.2% have been reported in Ethiopia, Thailand, Korea, and Argentina, respectively [21].

Salmonella isolated from chickens and chickens keepers were screened to determine AMR patterns. Three (3, 27.2%) isolates from chicken keepers were resistant to more than one antibiotic. This was not in agreement with the findings obtained by Fadlalla et al., where the resistance of *Salmonella*



	Sulfamethoxazole + Trimethoprim	Tetracycline	Chloramphenicol	Ciprofloxacin	Nalidixic Acid	Ampicillin	Gentamicin	Streptomycin	Cefotaxime
Chicken	0.00%	22.20%	0.00%	0.00%	0.00%	11.10%	0.00%	0.00%	0.00%
Humans	0.00%	9.10%	0.00%	9.10%	0.00%	18.90%	0.00%	0.00%	0.00%

Figure 2: Continued Antimicrobial resistance profiles of *Salmonella* isolates from humans and chickens.

Table 4: Antimicrobial resistance profiles of *Salmonella* isolates.

No. of strains	Serotype	Resistotype	Group No	Host
3	Enteritidis	Amp, Te, Cip, Gn	1	Humans/chickens
1	*Salmonella* Typhimurium	Cip, Gen, Te	11	Humans/chickens
3	Nontypeable	Amp, Cip	111	Humans

was observed in 81 human samples (93.1%) [22]. The present study further indicated that 1 (9.1%) of the human *Salmonella* isolates was highly resistant (>0.3) to tetracycline and ciprofloxacin while 2 (18.9%) were resistant to ampicillin. However, another study conducted in Sudan reported higher resistance patterns, including resistance to ampicillin 29 (33.3%), nalidixic acid 28 (32.2%), and tetracycline 52 (59.8%) compared to the ones reported in the present study [22]. Furthermore, another study conducted in Sudan also reported higher resistance profiles of *Salmonella* isolates to tetracycline 11.8% and sulfamethoxazole/trimethoprim 88.2% [20].

The current study has also indicated that 7 (63.6%) isolates were 100% susceptible to sulfamethoxazole/trimethoprim, chloramphenicol, cefotaxime, streptomycin, nalidixic acid, and gentamicin. This was in agreement with the results of Fadalall et al.'s study [22] except for some isolates that exhibited moderate resistance (>0.22) to ciprofloxacin 1 (9.1%). Furthermore, the results of the current study are also congruent with a study conducted in Brazil, where 88.2% of *Salmonella* isolates showed resistance to sulphonamides [23]. This resistance of *Salmonella* isolates to most of the antimicrobials could be due to the presence of resistance genes that are carried on the bacterial plasmids which can be acquired by the consumption of contaminated animal products by humans [24]. Continuous use of antibiotics such as ampicillin and nalidixic acid as treatment remedies may also be the reason for development of resistance [5]. Chloramphenicol combined with ampicillin has been widely used for the treatment of human salmonellosis despite this combination being known for causing aplastic anaemia. For example, it has been a drug of choice in Brazil since 1970s [23].

Salmonellosis in humans has been a health problem in both developed and undeveloped countries with nontyphoidal *Salmonella* (NTS) caused by other species of *Salmonella* different from *S. typhi* being a major cause of secondary bacteremia associated with gastritis [19]. In the present study, *S. Enteritidis* and *S. Typhimurium* were commonly isolated in chicken keepers while nontypeable isolates of *Salmonella* were the most dominant serovars. Generally, these serovars showed low resistance to tetracycline and high resistance to ciprofloxacin and ampicillin which are the antibiotics commonly used in South Sudan.

4. Conclusions

Antimicrobial resistant isolates were isolated in both chickens and humans. Further, MDR isolates were found in both chicken and human samples which is a public health concern. This, therefore, calls for concerted efforts to educate consumers on public health, food safety, food hygiene in food production, and enhancement of surveillance programmes on zoonotic bacteria and antimicrobial susceptibility.

5. Materials and Methods

5.1. Study Site. The study was conducted in the Western Bahr El Ghazal State, which shares boundaries with Sudan to the North and Central African Republic to the West with coordinates of 8.6452°N, 25.2838°E, and 626.9 meters above the sea level (Figure 3, study area map). The climate is tropical with an annual rainfall ranging between 400 and 1600 mm and temperature of 23.8°C–40°C. Of the five payams of Wau County, two payams, namely, Baggari and Bussuri were randomly selected for the study.

5.2. Study Design. The study design employed was a cross-sectional survey design targeting local live chickens and humans at the household level in Wau County, Western Bahr el Ghazal State, South Sudan. Sample collection was carried out between September and December 2019.

5.3. Sample Size and Sampling Technique. Using simple random sampling, a total of 270 cloacal swabs and 147 fecal samples were collected from local live chickens and humans, respectively. The samples were collected with sterile wooden swabs from the chicken cloacal, transferred into 10 ml sterile universal containers containing the 5 ml Cary Blair Transport Medium (Himedia), and immediately placed into a cool box containing ice packs. The samples were then transported and stored in a refrigerator at 4°C till analysis.

Before collecting human stool samples, consent was sought from community gatekeepers such as chiefs, opinion leaders, and elders. Additional consent was collected from human participants as a requirement for ideal sample collection. Stool samples (147) were carefully collected into sterile 10 ml plastic universal containers with spoons to which 5 ml Cary Blair Transport Medium has been added. The samples were then transported and stored in a refrigerator at 4°C until analysis.

5.4. Isolations and Identifications of Salmonella Species from the Fecal Sample. The samples were inoculated and incubated at 37°C in preenriched, nonselective buffered peptone water for 24 hours. An aliquot from peptone water (1 ml) was cultured in Rappaport-Vassiliadis broth in the ration of 1 to 10 for each. From the broth, a loopful was innoculated on Xylose Dextrose Agar (XLD, Oxoid, UK), The temperature and the period of incubation was done at 37°C for 24 hours for both chicken droppings and human stool samples [25, 26]. The suspected colonies of *Salmonella* from each plate were collected for presumptive identification by biochemical tests that included oxidase, hydrogen sulphide (H_2S), urease, indole, and fermentation of glucose, sucrose, mannitol, and lactose [27]. Furthermore, *Salmonella* was confirmed using PCR targeting the *invA*, as previously described [28].

5.5. Serological Typing of Salmonella Isolates. The *Salmonella* isolates were characterized into sero groups based on the presence of distinctive "O" antigenic factor, using O polyvalent antiserum and specific monovalent antiserum for A-S group antigen and GROUP BC1, C2, D1, E, and G, respectively, in accordance to the manufacture's protocol for the identification of surface antigens and their differentiation into serogroups. Briefly, a drop of the appropriate antiserum was placed onto a clean microscopic slide. A single colony of overnight culture on nutrient agar was picked and emulsified in the antiserum drop to obtain a thoroughly mixed suspension. The slide was gently rocked forward and backwards/side wards for 1 minute. Agglutination or clumping between 1 and 10 seconds was considered as a positive reaction.

5.6. Serotyping of Salmonella Strains. Salmonella strains were differentiated into serotypes by serotyping analysis according to the method described by [28, 29]. All isolates of *Salmonella* was referred to Deltamune (Pty) Laboratory, a South African SANAS Accredited Veterinary Laboratory, for confirmation and serotyping. Characterization was done using the method described in the Microbiological Manual, and serotyping (10.2 : 1995 CCFRA) was done based on White-Kauffman-Le Minor Scheme (WHO Collaborating Center) [30]. The scheme discriminated serotypes on the basis of their somatic (O), flagella (H), and capsular (Vi) antigens present on the surface of *Salmonella* [31].

5.7. DNA Extraction for Salmonella Confirmation. The bacteria were cultured on nutrient agar for 24 hrs at 37°C and DNA extraction was performed by the boiling method for 10 min and centrifugation at 5000 ×g for 5 min. The supernatant was then used for the DNA amplification using *Salmonella*-invA gene specific primers, namely, S139 (5′GTG AAA TTA TCG CCA CGT TCG GGC AA -3′ and S141 (5′ TCA TCG CAC CGT CAAAGG AAC C -3′), as described previously [32]. The reaction volume was 25 μl with 1 μl of the DNA template. The following PCR conditions were used: 94°C for 60 sec of the initial denaturation followed by 30 cycles of 60 sec at 94°C, 30 sec at 56°C, 30 sec at 72°C, 2 min and a final extension step of 10 min at 72°C. The amplified PCR products were then visualised on 1.5% agarose gel stained with ethidium bromide and visualised by UV illumination alongside a 100 bp DNA ladder.

5.8. Detection of Extended Spectrum Beta-Lactamases (ESBLs) in the Salmonella Strain. Detection of extended spectrum cephalosprinase production isolate was accomplished using freshly prepared MacConkey Agar (HIMEDIA) containing 2 mg/l of cefotaxime (Sigma-Aldrich, Munich, Germany) according to the method described in [28].

5.9. Determination of Antimicrobial Susceptibility Patterns of Salmonella Isolates. The antibiotic susceptibility profiling of the *Salmonella* isolates was determined using the Kirby–Bauer disc diffusion method based on the Clinical

Figure 3: Study area highlighted by X (Google Map).

Laboratory Standard Institute (CLSI) guidelines [33]. The antibiotic discs (Oxoid, UK) included ampicillin, sulfamethoxazole/trimethoprim, streptomycin, ciprofloxacin, cefotaxime, tetracycline, gentamicin, nalidixic acid, and chloramphenicol. Using CLSI guidelines that provide ranges for zone of inhibition, the AST on all isolates were read and grouped into Susceptible (S), Intermediate (I), and Resistance (R), and for quality control purpose, *E. coli* ATCC 25922 was used.

5.10. Determination of Multiple Antimicrobial Resistances Indexing MARI. The multiple antibiotic resistance index was calculated as follows: a/b, where "a" represents the number of antibiotics to which the particular isolate was resistant and "b" the number of antibiotics to which the isolate was exposed. MARI values >0.2 are considered significant indicating that the strains could have originated from sources where antibiotics are often used [34]. While MARI value <0.2 suggests the strains originate from animal sources which are less frequent exposed to antibiotics or never at all [35].

5.11. Data Analysis. Data analysis was done using Statistical Package for Social Sciences (SPSS) version 22.

Data Availability

All data used for the study are available upon request from the corresponding author.

Ethical Approval

The authors obtained ethical approval from the University of Zambia Biomedical Research Ethics Committee (UNZABREC) (REF. No. 1110–2020). Additional ethical approval was thought from National Health Research Authority (NHRA), Zambia.

Consent

Consent was obtained from all study participants in the study.

Conflicts of Interest

The authors declare there are no conflicts of interest in the study.

Authors' Contributions

Conceptualization was done by S. S. and B. H; methodology was developed by S. S. A. J, B. H., and J. K.; formal analysis was performed by S. S. M. S., M. M., W. M, and B. H.; review and editing were performed by S. S. B. H., C. W., S. M, and A. J.; visualization was done S. S. B. H., J. K., R. T, A.T and M. Z; and supervision was done by B. H., J. K., and A. J. All authors have read and agreed to the published version of the manuscript.

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Supplementary Materials

Supplementary material 1: the table shows the sample culture results as obtained from the XLD media. Supplementary material 2: the table shows results of subjecting *Salmonella* isolates to different sugars (Biotyping).

Supplementary material 3: the table shows the result obtained from PCR method targeting the *Salmonella InvA* gene. Supplementary material 4: the table provides the inhibition zones and their interpretation results (Antimicrobial Susceptibility Test results). (*Supplementary Materials*)

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