# ANTIMICROBIAL RESISTANCE IN NON-TYPHOIDAL SALMONELLA RECOVERED FROM SELECTED FOOD ANIMALS IN KENYA

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# Antimicrobial Resistance in Non-Typhoidal Salmonella Recovered from Selected Food Animals in Kenya

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**A Thesis Submitted in partial fulfillment for the Degree in Master of Science in Infectious Diseases and Vaccinology in the Jomo Kenyatta University of Agriculture and Technology**

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## DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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## DEDICATION

This work is dedicated to my dad Stephen Thuku and my late mum Ann Muthoni who pushed me to be better than my best and who continuously provided the strength to believe in myself. Will never forget mum's happiness when I told her about this study and its benefits. Her reply kept me going even after her demise.

And to my brothers Sammy and Joel and my sister Damaris who have been the pinnacle of soundness and unfailingly offered me a reason to keep going on.

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## LIST OF ABBREVIATION

| | |
|---|---|
| **AMR** | Antimicrobial resistance |
| **ASM** | American Society of Microbiology |
| **ATCC** | American type culture collection |
| **CDC** | Centers for Disease Control |
| **CIA** | Critically Important Antibiotics |
| **CLSI** | Clinical and Laboratory Standards Institute |
| **CMR** | Centre for Microbiology Research |
| **DNA** | Deoxyribonucleic Acid |
| **FS** | Fully Susceptible |
| **GI** | Gastrointestinal |
| **HIV** | Human Immunodeficiency Virus |
| **iNTS** | invasive Non-Typhoidal Salmonella |
| **KEMRI** | Kenya Medical Research Institute |
| **MDR** | Multi-Drug Resistance |
| **MGE** | Mobile Genetic Elements |
| **NTS** | Non-Typhoidal Salmonella |
| **PCR** | Polymerase Chain Reaction |

| | |
|---|---|
| **PI** | Principal Investigator |
| **SERU** | Scientific and Ethics Review Unit |
| **SPP** | Species |
| **TSA** | Tryptic Soy Agar |
| **WHO** | World Health Organization |

## DEFINITION OF OPERATIONAL TERMS

| | |
|---|---|
| **Bacteremia** | Presence of bacteria in the blood |
| **Enterica** | Subspecies of Salmonella enterica |
| **Enterobacteriaceae** | A family of gram-negative, facultatively anaerobic, rod-shaped bacteria that do not form endospores |
| **Enterohepatic** | Circulation of bile salts and other secretions from the liver to the intestine, where they are reabsorbed into the blood and returned to the liver |
| **Enteroinvasive** | Bacteria that bind and penetrate the epithelial cells |
| **Extraintestinal** | Bacteria that occur outside the intestines |
| **Gastroenteritis** | Inflammation of the lining membrane of the stomach and the intestines |
| **Intracellularly** | Bacteria occurring inside the cell |
| **Microbiota** | Microscopic organism of a particular environment |
| **Pathovar** | Bacterial strain with similar characteristics differentiated at infrasubspecific level from other strains of the same species on the basis of distinctive pathogenicity to one or more hosts. |
| **Resistance** | Ability of microorganisms to get used to the effects of an antibiotic which they were once sensitive |
| **Salmonellosis** | A common bacterial infection caused by Salmonella infection |

| | |
|---|---|
| **Serovar** | A subdivision of a species distinguishable from other strains on the basis of antigenicity |
| **Susceptible** | Vulnerability of a microorganism to the effects of a particular antimicrobial drug |
| **Typhoidal** | Pertaining to typhoid fever |

## ABSTRACT

Invasive Non-Typhoidal *Salmonella* (iNTS) are a major cause of serious bloodstream infections with a case fatality of 20-25%. NTS infection is often invasive in Sub-Saharan Africa (iNTS) and is implicated as a major cause of hospitalization and deaths among infants and HIV adults due to its invasive nature. NTS are non-spore forming bacillus in the *Enterobacteriaceae* family and the infection caused by iNTS is known as Salmonellosis. Food animals have been known as the main sources of NTS but recently humans have been implicated as potential reservoirs of these organisms. Enormous spread of iNTS in food animals and the environment have led to rise in its prevalence thus becoming a major concern in public health and global food chain. Antibiotic resistance iNTS is linked to more severe disease outcomes including bloodstream infections and hospitalization as a result of MDR. Continuous surveillance of resistance genes and their transferability would enable policy makers to develop effective treatment strategies. In addition, understanding resistant phenotypes would form important background to guide health and veterinary professionals in health care. This was a laboratory based cross-sectional study whose major objective was to determine the antimicrobial resistance in Non-Typhoidal Salmonella recovered from selected food animals (Cattle, Chicken and pigs) in Kenya. Ethical clearance was granted by KEMRI/ Scientific and Ethics Review Unit, study number KEMRI/CMR/P00058/SERU 3522. Antimicrobial susceptibility profiles of 289 iNTS archived isolates were determined using the Kirby-Bauer antibiotic sensitivity technique. The following antimicrobials were used with their respective concentrations; Ampicillin (AMP, 10µg), Ciprofloxacin (CIP, 5µg), Sulfamethoxazole/Trimethoprim (SXT, 1.25/23.75µg), Amoxicillin-clavulanic acid (AMC, 20/10µg), Nalidixic acid (NAL, 30µg), Ceftazidime (CAZ, 30µg), Cefotaxime (CTX, 30µg), Ceftriaxone (CRO, 30µg), Chloramphenicol (C, 30µg), Gentamicin (GEN, 10µg), Tetracycline (TET, 30 µg) and Azithromycin (AZM, 10 µg). Conjugation assay determined the transferability of multidrug resistant phenotypes. According to the study findings, high rates of resistance were against Ciprofloxacin (58%), which is a flouroquinolone for *Salmonella*. Resistance to Ampicillin drug was 45.8% followed Sulfamethoxazole/Trimethoprim 39.6%, Ceftriaxone 30% and then Chloramphenicol 24.3% drugs. 61% of *S. enteritidis* isolates were observed to exhibit resistance phenotype and the most common resistance phenotype was Ampicillin, Amoxycillin, Sulfamethoxazole/Trimethoprim, Chloramphenicol. 53% of *S. typhimurium* iNTS isolates were similarly observed to exhibit resistance with the most common resistance phenotype being Ampicillin, Amoxycillin, Sulfamethoxazole/Trimethoprim, Gentamycin. In conclusion, there is moderately high level of resistance in iNTS species against first line drugs and high level of resistance against 3$^{rd}$ generation Cephalosporins and fluoroquinolones. The study recommends appropriate measures such as rational use of antibiotics to help preserve the potency of antimicrobials and to improve successful treatment outcomes and also Molecular work also ought to be carried out to detect the genes transferred and mapping of the resistant phenotypes among isolates.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of the study

Non-Typhoidal Salmonella (NTS), often invasive in Sub-Saharan Africa (iNTS), is a Gram negative, oxidase negative, non-spore forming anaerobic bacillus belonging to the *Enterobacteriaceae* family. The infection caused by iNTS is known as salmonellosis and is one of the most widely distributed foodborne infection in both adults and children. NTS is totally different from typhoidal *Salmonella. Salmonella enterica* serovar Typhi (*S. Typhi*) and other pathovars of *S. paratyphi* are the ones majorly known as typhoidal *Salmonella* serovars and are mostly restricted to human beings as their hosts. Any other Salmonella serovars falling outside these two groups are typically referred to as the Non-Typhoidal Salmonella and have the ability to interact with human hosts and non-human hosts (Singletary *et al*., 2016).

Infections caused by iNTS have a substantial burden globally estimated to be 3.4 million cases and 681,316 deaths annually, with almost 2/3 (63.7%) of all cases occurring in children under the age of five (Ao *et al*., 2015). African countries appear to be more prone to diseases caused by iNTS than in other parts of the world with death rates increasing every year due to the great challenge of access to reliable clean water and improved sanitation facilities. In Kenya, iNTS have been implicated as a major cause of hospitalization and deaths among young children and HIV adults due to its invasive nature (FAO/WHO, 2009).

In humans, salmonellosis infections are often associated with food animals and their products such as poultry, eggs, meat, as well as dairy products. The strains of iNTS (*S. typhimurium* and *S. enteritidis*) can be found naturally in the environment and in both domestic and wild animals including cats, dogs, amphibians, reptiles and rodents. The infections start as soon as there is ingestion of bacteria in contaminated food or water

and as few as $10^3$ bacteria may produce non-typhoidal gastroenteritis in exposed individuals.

Food animals are the most commonly known source of proteins in Kenya and this study determined the antimicrobial resistance profiles and the transferability of resistant phenotypes of iNTS using food animal (Cattle, Chicken and pigs) isolates and environmental samples from farms located in Kiambu, Kisumu, Kwale, Mombasa, and Nairobi County in Kenya.

iNTS studies carried out in Kenya have used clinical samples of admitted patients in referral hospitals. There has been limited information concerning antimicrobial profiles of NTS obtained from food animals and their surrounding environment. Kenya is among the many countries that lack high-quality and easily affordable diagnostic facilities leading to inadequate documentation of previous incidences (Kariuki *et al.*, 2015).

Results of this study provide critical and essential data to farmers, veterinary officers, health officials and also individuals consuming animal products. The data on resistance profiles could be used by policy makers to understand ways of containing Salmonellosis infection in Kenya.

### 1.2 Statement of the problem

Invasive Salmonellosis infection is a major public health problem in Kenya and in Sub Saharan Africa. The infection is caused by enteroinvasive bacteria belonging to the genus *Salmonella*. Currently, almost 30% of all deaths from foodborne diseases are in children under the age of 5 years (WHO, 2015). iNTS species can be disseminated to the environment through human and animal waste which in turn causes contamination of farm products (manure) due to lack of environmental barriers. Antimicrobial resistance is often enhanced by uncontrolled use of antibiotic agents for the prevention and treatment of various infections in humans as well as animals. A common scenario is addition of antibiotics to animal feeds as growth promoters or for feed efficiency. This has greatly enhanced the selection and transference of drug resistant strains of

*Salmonella* (Kariuki *et al*., 2006). Multi-drug resistance in iNTS has become a global concern. Community and healthcare associated outbreaks have been reported all over the world due to these resistant strains (Paulson & Zaoutis, 2015).

While diverse potential vehicles of transmission of iNTS exist, commercial dairy products, chicken meat and eggs have been identified as the most important food vehicles for these organisms. Due lack of high-quality and easily affordable diagnostic facilities, there has been inadequate documentation of previous incidences. The biological mechanisms that contribute to the invasive nature of iNTS are not clearly understood.

Another major problem is that there is scarce information concerning the comparison of antimicrobial resistance profiles of iNTS from food animal isolates and also from environmental sources. This research study was carried out to generate relevant data on antimicrobial susceptibility profiles of iNTS obtained from food animals' isolates and environmental sources from selected sites in Kenya. The study has documented important findings on the transferability of resistance phenotypes of iNTS isolates obtained from food animals and environmental sources from selected sites in Kenya.

### 1.3 Justification of the study

Salmonellosis infections present commonly with self-limiting gastroenteritis in humans and iNTS is a leading cause of bloodstream infection. iNTS is reported as the cause of suspected septicemia in 28 % of cases in Kenya. The incidence of iNTS disease is estimated at 7.1% for children and 15.6% for adults and an incidence rate of 21.3% among HIV-infected patients compared with 3.1% among HIV-uninfected patients (Tabu *et al*., 2012).

Extensive research has been documented concerning resistance among *Salmonella* strains to different classes of antibiotics all over the world (Paulson & Zaoutis, 2015). However, *Salmonella* have become more resistant even to Fluoroquinolones and extended spectrum Cephalosporins which are the most commonly used drugs for the

treatment of iNTS. Therefore, antibiotic susceptibility profiles and conjugation studies of NTS are warranted to ascertain if the resistant genes are possibly transferred across organisms. Results from conjugation assays provide critical and essential data to farmers, veterinary officers, health officials to understand the risks of transference of resistance phenotypes from pathogenic bacterial strains to the normal commensals in the body thus making the treatment of iNTS infection difficult coupled circulation of MDR resistant salmonellosis infections and prolonged hospital stays.

### 1.4 Research Questions

1. What are the antimicrobial susceptibility profiles of NTS isolates obtained from food animals?
2. What is the transferability potential of resistance determinants in iNTS isolates obtained from food animals?

### 1.5 Objectives

#### 1.5.1 Broad objective

To determine the antimicrobial resistance in Non-Typhoidal Salmonella recovered selected food animals (Cattle, Chicken and Pigs) in Kenya (Kiambu, Kisumu, Kwale, Mombasa and Nairobi).

#### 1.5.2 Specific objectives

1. To determine antimicrobial susceptibility profiles of NTS isolates recovered from food animals (Cattle, Chicken and Pigs) and environmental sources from selected sites in Kenya.
2. To determine transferability potential of resistance determinants in NTS isolates recovered from food animals (Cattle, Chicken and Pigs) and environmental sources from selected sites in Kenya.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Introduction

Non-Typhoidal Salmonella species are important commensal microorganisms with farm animals being a major reservoir. However, these organisms have been known as important cause of infection in both humans and animals. These foodborne pathogens cause gastroenteritis, bacteremia and subsequent focal infection and also invasive diseases in immunocompromised individuals. Invasive *Salmonella* infections are problematic, even in modestly compromised hosts, as a result of persistence in deep or endovascular sites. This chapter documents important literature about the infection caused by iNTS organisms, nomenclature, epidemiology and disease burden of iNTS. The pathogenesis of these organisms in various hosts is also discussed in this chapter and other relevant subtopics related to iNTS.

## 2.2 Salmonellosis Infection in Humans

Salmonellosis in both humans and animals continues to be a major distressing health problem worldwide and the incidence in low income countries could be even much higher than expected. *Salmonella* organisms are widely dispersed in the environment, and in animals they can be found in various parts including the gastrointestinal tracts of domesticated and wild mammals, reptiles, birds, and insects (Jennifer *et al.*, 2016). Apart from human and animal hosts, iNTS are widely spread in the environment and this wide distribution has led to their increasing prevalence in the global food chain (Crump & Heyderman, 2015).

Salmonellosis is said to be an infection from contaminated food products of animal origin like dairy products, eggs, meat, poultry, and pork, however, other foods, including green vegetables contaminated by manure, have been implicated in its transmission (Eguale, 2018). Person-to-person transmission can also occur through the fecal-oral

route. Human cases also occur where individuals have contact with infected animals, including pets. However, the infected animals often do not show signs of disease (WHO, 2018).

Salmonellosis infection outcomes differ by serotype (Jones *et al*., 2008). *Salmonella* serotypes Enteritidis and Typhimurium are known to have a broad host range. They are both commensals and pathogens that cause a broad spectrum of diseases in man and animals and are known as invasive Non-Typhoidal Salmonella with protean manifestations in humans, acute gastroenteritis, bacteremia, and extraintestinal localized infections involving many organs. Their virulence and adaptability have resulted in an enormous medical, public health and economic impact worldwide (Chun *et al*., 2018).

Salmonellosis infections in both developed and developing countries leads to economic losses with animal and human illnesses, being the second most often reported zoonotic disease and the most important bacterial food-borne disease in industrialized countries (Himel *et al*., 2012). The *Salmonella* bacteria colonize the digestive tract of reptiles, birds, and mammals, including humans, causing gastroenteritis and other kinds of infections in humans (Stevenson *et al*., 2017). Wild birds are also sentinels for *Salmonella* and allows for the spread of antimicrobial resistance in the environment (Skov *et al*., 2008).

In the United States, *Salmonella* enterica causes more than 1 million foodborne illnesses each year (Scallan *et al*., 2011). However, foodborne outbreaks caused by *Salmonella* have decreased in some serotypes but have remained a concern for several others. The pattern for salmonellosis is typical with majority of outbreaks being associated with consumption of contaminated poultry products in developed countries (Jeanjean *et al*., 2014).

In sub-Saharan Africa, emergence of NTS as a causative for invasive bloodstream infection is common among adults with HIV and children with malaria and malnutrition (Mogasale *et al*., 2014). Infected children and adults present with a non-specific febrile

illness making it difficult for presumptive diagnosis and treatment especially in rural settings. The case fatality from iNTS in African adults and children is 22-25% (Mary *et al*., 2009).

In Kenya, invasive salmonellosis infections have been ranked second only to pneumococcal pneumonia and are a major cause of morbidity and mortality in children under 5 years (Berkley *et al*., 2005). Luckily, the government of Kenya introduced the pneumococcal vaccine in 2011 and thus, pneumococcal cases are likely to drop down leaving NTS as major cause of morbidity and high mortality in children (O'Donnell *et al*., 2012).

A recent study carried out in western Kenya shows that, most stool samples from children are contaminated with NTS compared to *Shigella.* A comparison between children who died and those who survived showed that samples taken from children who died contained NTS and those who survived were nearly seven times more likely to have NTS in their stool. Other factors associated with death included being malnourished, having oral thrush (a fungal infection of the mouth), having previously sought hospital care for diarrhea, and being dehydrated (O'Reilly *et al*., 2012).

### 2.2.1 Nomenclature of NTS

The nomenclature and classification of *Salmonella* species are somehow difficult and confusing, but DNA hybridization studies classifies *Salmonella* of medical importance organisms as a single species, known as *Salmonella cholerasuis,* which has 2,500 different serotypes, or serovars (Gal-Mor *et al*., 2014). The species name *S. choleraesuis* is confusing, due to the existence of another *Salmonella* serotype Choleraesuis, that is associated with bacteremia and therefore the name "*Salmonella enterica*" has been proposed as a replacement for the name *"Salmonella choleraesuis."* (Gordon *et al*., 2010). While an alternative nomenclature describes the genus as a single species, *Salmonella choleraesuis*, the Judicial Commission of the International Committee on the Systematics of the Prokaryotes supports the two-species

designation (Tindall *et al*., 2005). It is fortunate that clinical diagnosis that leads to decision-making is based on the host, severity of illness, and antimicrobial susceptibilities, and not specific serotype involved (Grimont & François-Xavier, 2007).

*Salmonella* was named after a scientist Daniel E. Salmon, a veterinary surgeon who first isolated (what was called at the time) "*Bacillus choleraesuis*" from porcine intestines in 1884 (Salmon & Smith, 1885). Later, that name was changed in 1900 to "Salmonella choleraesuis" by Lignieres (Nomenclature, 1934). Today the *Salmonella* genus is split into just 2 species: *Salmonella enterica* and *Salmonella bongori*, with *S. enterica* being split into 6 additional subspecies.

The terms "serovars" and "serotypes" are generally considered to be synonymous. The World Health Organization (WHO)/Institut Pasteur use the term "serovar," while the Centers for Disease Control (CDC) and the American Society of Microbiology (ASM) originally used the word "serotype" but have steadily changed it to "serovar" in order to maintain international consistency (Ryan *et al*., 2017). Generally, *Salmonella* organisms are motile, Gram-negative, facultative anaerobic bacilli which rarely ferment lactose and are classified according to antigenic surface antigens: polysaccharide O (somatic) antigens, H (flagella) antigens and VI (capsular) antigens (Nataro *et al*., 2011).

### 2.2.2 Serological Classification of NTS

Serotyping is a common serological procedure used in the laboratory to separate strains of microorganisms into different groups based on their antigenic composition. The conventional serotyping or antigenic classification of *Salmonella* was introduced years back and was traditionally founded upon antibody reaction of surface antigens: somatic O antigens and flagella H antigens (Agasan *et al*., 2002).

The different variations of O antigens found in NTS, is as a result of difference in the sugar units, the covalent bonds found between sugar units and O antigen linkage subunits. The O antigen can be defined as a heat stable polysaccharide usually found on the outermost surface of the lipopolysaccharide and is composed of 5-6 sugar units.

(D'Aoust & Maurer, 2007). Identification of O antigen done in two parts; The first step is to test the isolate using O grouping sera by slide agglutination. The other test is carried out to identify the group using specific antisera that react with individual antigens. The O antigen is used to determine the group in which the *Salmonella* isolate belongs to whereas the H antigen determines the serovar.

H antigens are composed of flagellin subunits which are a filamentous part of the bacterial flagella (Nataro *et al*., 2011). *Salmonella* serovars express either one type of H antigen, that is, monophasic, or two types of H antigen, that is, diphasic. *Salmonella* is unique amongst enteric bacteria in this regard. Both phases may be detected in a culture as a whole but individual cells of diphasic isolates only express H antigen from one phase at a time. Tube agglutination or slide agglutination tests are performed to determine the H antigens. *Salmonella* isolates are tested firstly with H typing antisera which recognize multiple antigens and then with H single factor which identify specific antigens (Feasey *et al*., 2012).

### 2.2.3 Epidemiology and Disease burden of NTS globally

Currently, almost 30% of all deaths from foodborne diseases are in children under the age of 5 years (WHO, 2015). In Kenya, *Salmonella* enterica serotype Typhimurium and *Salmonella* enterica serotype Enteritidis have been implicated as the main serotypes causing iNTS in children. The prevalence of iNTS disease 45% in children under 2 years of age, 7.1% for children and 15.6% for older persons and an incidence rate of 21.3% among HIV-infected patients compared with 3.1% among HIV-uninfected patients (Tabu *et al*., 2012).

The burden of iNTS disease has been highly reported in parts of sub-Saharan Africa (Crump & Heyderman, 2015). Scarcity of data stems due to difficulty in accurate diagnosis of the disease, as it highly requires laboratories capable of microbiology and trained technicians, both of which are challenging to maintain in resource-limited settings. It is estimated that the global burden of iNTS disease is 3.4 million cases and

681,316 deaths annually, with almost 2/3 (63.7%) of all cases occurring in children under the age of five (Ao *et al*., 2015) while in Sub-Saharan Africa and Europe the incidence of iNTS disease is to be 227 and 102 cases of iNTS per 100,000 population, respectively. NTS is reported as the cause of suspected septicemia in 28 % of cases in Kenya.

Figure 2.1 shows the unpredictably high incidence of iNTS originating from Europe. Investigations reveal that cases were driven from Russia, the Ukraine, and Estonia, which had considerably higher incidences than Western European countries (Figure 2.1). Notably, the Americas and Southeast Asia show considerably lower incidences of iNTS disease (23 and 21 cases/100,000 population, respectively). The Middle East and North Africa were jointly seems to have almost negligible incidence of 0.8 cases/100,000 population (Ao *et al*., 2015).

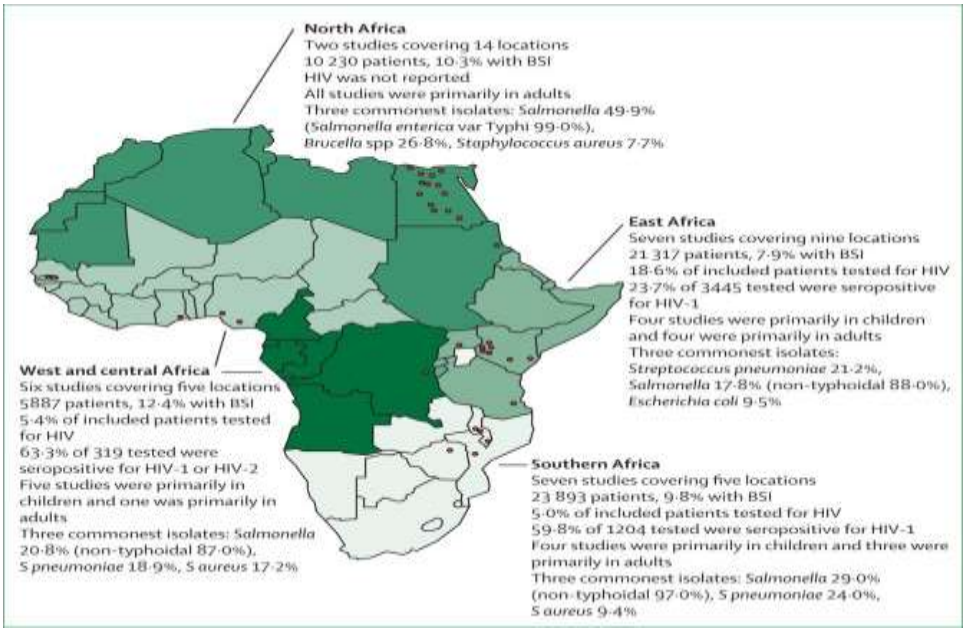


**Figure 2.1: Map of Africa showing results of a meta-analysis of studies investigating the cause of bloodstream infection in febrile adults and children in Africa Source:** (Reddy et al., 2010)**.**

A study carried out by WHO, (2015) and Kirk *et al.* (2015) reveal that iNTS disease is more prevalent in Africa in comparison to other parts of the world. However, while these modeled data are helpful in understanding the geographical variation of the disease; the sources of data are sparse, resulting in the use of predicted incidences within some age groups.

iNTS disease is highly seasonal with high infection peaks being experienced at the commencement of rainy seasons and infection rate tend to slow down once the wet season is over. This happens to both adults and children coinciding with increased incidences of malaria and malnutrition. iNTS disease has also been present in epidemics that last several years and are caused by sequential single serotypes among adults and children. These epidemics have been linked to the emergence of resistance to commonly used antimicrobial drugs ( Gordon *et al*., 2010).

### 2.2.4 Pathogenesis of iNTS in immunocompetent hosts

NTS have been noted to be analogous to typhoidal strains in evading the gut mucosal immune response to cause systemic disease in that is prone to infect human beings (Feasey *et al*., 2012). To gain selective access and exploit the gut mucosal the NTS gain an advantage over the normal flora of the gut microbiota in the inflamed gut lumen (Stecher *et al*., 2007).

The correlation between prevalence of iNTS and malaria has been found to be significant and various mechanisms are being proposed in recent studies from malaria endemic regions. iNTS in a healthy individual has been rarely reported from Kenya but increased reports of iNTS incidence in immunocompetent hosts from developing countries have been reported in relation with the high prevalence of malaria in regions that provide an ecological niche for it (Elantamilan *et al*., 2016).

In most cases, Salmonellosis infections present as bacteremia and pleuropulmonary involvement occurs during chronic illnesses such as diabetes, malignancies and HIV (Kashif *et al*., 2014 ). Clear understanding of immunity mechanisms that protect against

fatal infection from invasive strains of NTS is highly required to enable researchers answer the recent bothering question of why invasive NTS disease is endemic in Africa. Such data will guide pharmaceutical firms for developing an effective vaccine against NTS (Hohmann, 2001).

### 2.2.5 Pathogenesis of iNTS in HIV-positive adults

Three key immunological defects have been described that could contribute to the invasive pathogenesis of NTS in HIV-infected adults in Africa. An important defect is identified in the gut mucosa. The gastrointestinal tract is a site of early responses, and reduced expression of genes is implicated in the maintenance of the epithelial barrier to fight infection (Raffatellu *et al*., 2008).

Bacterial translocation across the gut mucosa and systemic dissemination of salmonellae is common (Parry *et al*., 2015). Loss of gut mucosal interleukin 17 cells in HIV infection is probably a key mechanism by which salmonellae disseminate from the gut to cause invasive disease in these patients. Loss of neutrophil chemo-attraction could also explain the apparent absence of enteritis and diarrheal disease during HIV-associated iNTS (Gordon *et al*., 2002).

Excessive production of cytokines during intracellular infection could lead to persistence and recurrence of iNTS. Persistence and intracellular replication are some of the major pathogenic competencies that cause invasive disease in *Salmonella* (Raffatellu *et al*., 2008).

### 2.2.6 Clinical presentation of NTS

The clinical presentation of NTS infection is not very specific between both children and adults. Thus, the disease recognition and management pose a challenge, mostly in areas that are not well equipped with laboratory diagnostic facilities of bloodstream infections (Mandomando *et al*., 2009). iNTS presents as a febrile illness with frequent respiratory symptoms with diarrhea not being a prominent feature. However, clinicians are often

faced with a febrile patient without an obvious clinical focus of infection (Brent *et al*., 2006).

Typically, the incubation period of NTS is 6–72 hours while atypical illness has been documented to last even 14 days after exposure (Jennifer *et al.,* 2017). Manifestation of the illness is commonly as acute diarrhea accompanied by abdominal pain, fever, and sometimes vomiting. The illness can last 4–7 days, and recovery without treatment is common. Approximately 5% of people develop bacteremia or focal infection (such as meningitis or osteomyelitis). Salmonellosis infection outcomes differ by serotype (Jones *et al*., 2008) and health status of the human host (Shu-Kee *et al.,* 2015). Infections with some serotypes, including Dublin and Choleraesuis, are more likely to result in invasive infections. Death rates are higher with invasive infections among infants, older adults, and people with immunosuppressive conditions (including HIV) and malignant neoplasms. Antibiotic-resistant organisms of NTS have been associated with a higher risk of bloodstream infection and hospitalization (O'Donnel *et al*., 2014).

Gastroenteritis is the most common clinical presentation of NTS infection and is usually self-limiting in immunocompetent individuals as the infection becomes confined to the terminal ileum and colon (Gordon *et al*., 2010). In contrast with NTS, typhoidal salmonella bacteria gain access to underlying lymphoid tissues and multiply intracellularly within mononuclear phagocytes, after passing the intestinal mucosa (Gordon, 2011). Infection quickly becomes systemic with spreading of the pathogen from the intestine to the mesenteric lymph nodes, liver, spleen, bone marrow, and gallbladder. Secondary infection of typhoidal organisms to the small bowel can occur via secretion in the bile through the enterohepatic cycle (Gordon *et al*., 2008). Lack of robust intestinal inflammation and the absence of neutrophil transmigration could be aiding the invasion of typhoidal serovars into the deeper tissues of the gut and its dissemination to systemic sites (House *et al*., 2001).

A useful clinical feature to predict NTS has been Splenomegaly especially among adults in areas with high HIV prevalence and lack of diarrhea in iNTS among immunosuppressed patients has been described in many settings. Patients with iNTS also often display underlying conditions such as anemia, malnutrition, and advanced HIV disease (Schwarz *et al*., 2010).

Children often present with nonspecific symptoms with lower respiratory tract infection. This circumstance, leads to health care workers commencing antimicrobial treatment that is inappropriate for iNTS especially when antimicrobial resistance among *Salmonella* enterica is common (Schwarz *et al*., 2010). In addition, febrile presentations are often not identified as bloodstream infection by pediatric guidelines, resulting in delayed or missed antimicrobial treatment (Nadjm *et al*., 2012).

### 2.2.7 Clinical features of NTS disease in Sub-Saharan Africa

Febrile systemic illness is typically the most frequent clinical presentation of iNTS disease resembling enteric fever; but diarrhea is often absent while some clinical features are usually non-specific and diverse (Hohmann, 2001) . A study of bacteremia carried out in Malawi showed that in adults, presence of high fever and combination with splenomegaly indicated an invasive disease (Peters *et al*., 2004).

HIV infection, malnutrition and malaria (severe malarial anemia) are the major risk factors for iNTS disease (Graham *et al*., 2000). Sickle-cell anemia is also an important risk factor in African children. Genomic typing of index and recurrent strains suggests that recrudescence is more common than reinfection as a cause of recurrences (Okoro *et al*., 2012 ). In young children, the problem is majorly the overlapping presentations of malaria infection and pneumonia, and pediatric practices for the diagnosis in low-income settings leads to inadequate identification of iNTS disease (Nadjm *et al*., 2010).

### 2.2.8 Food Animal Sources and Environmental Risk Factors of iNTS

Apparent transmission between human is not commonly considered as a risk factor for NTS (Gordon *et al*., 2008). Food animal products such as eggs, meat and dairy products have been indicated as vehicles for transmission because NTS disease colonizes most of them (Crump *et al*., 2015). The isolation of NTS from cattle, goats, sheep, and pigs in African slaughterhouses shows that critical control points from farm to fork ought to be implemented in both low income and industrialized countries to improve food safety and much more control the transmission of NTS disease (Schlundt *et al*., 2004).

There are sudden high peaks of NTS disease during rainy seasons in both adults and children explaining that environmental risk factors are important (Soko *et al*., 2005 ). Drinking water sources are increasingly contaminated with fecal organisms at the onset of rainy seasons and that could be in line with increased risks of waterborne NTS. Regular treating of water at home, serious protection of water sources and other water treatment strategies at home are measures that may greatly reduce the risk of diarrhea (Clasen *et al*., 2007).

NTS species require relatively simple nutritional requirements and can survive for long periods of time in the environment. Their growth and survival are influenced by a number of factors such as temperature, pH, water activity and the presence of preservatives (FDA, 2013).

### 2.2.9 Antimicrobial Resistance of NTS

Antimicrobial resistance occurs naturally and this phenomenon is often enhanced by uncontrolled use of antibiotic agents for the prevention and treatment of various infections in humans as well as animals. A common scenario is addition of antibiotics to animal feeds as growth promoters or for feed efficiency. This has greatly enhanced the selection and transference of drug resistant strains of *Salmonella* (Kariuki *et al*., 2006). There are several studies confirming the use of antibiotics in the food of animals as the source for multidrug resistant *Salmonella* serovars.

Multi-drug resistance in NTS is becoming a global concern. Fluoroquinolones and extended spectrum cephalosporins are the most commonly used drugs for the treatment of NTS (Kariuki & Onsare, 2015). However, different researchers show that there is resistance among *Salmonella* strains to different classes of antibiotics leaving behind just a few treatment options (Paulson & Zaoutis, 2015). Community and healthcare associated outbreaks have been reported all over the world due to these resistant strains.

MDR is mainly associated with mobile genetic elements like integrons, plasmids and transposons that encode multiple resistance genes and is of particular concern due to the increasing global travel and globalization (Mary *et al*., 2009). An increasing trend in the prevalence of antimicrobial resistance has been observed in iNTS over decades. The MDR phenotypes of *Salmonella* serovars began to appear in the in the United Kingdom (Murdoch *et al*., 1998) with resistance to five antimicrobial agents; ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline. However, between 1998 and 2005 there was a steady decline from 32% to 22% (Adachi *et al*., 2005) and in 2011 20% (CDC, 2013). Since 2009, iNTS isolates have displayed resistance to extended-spectrum Cephalosporins and Carbapenems (Jure *et al*., 2014; Irfan *et al*., 2015). Some NTS isolates have shown resistance to most Aminoglycosides, Trimethoprim-Sulfamethoxazole and Azithromycin (Irfan *et al*., 2015).

Development of resistance to quinolones (Nalidixic-acid) and fluoroquinolones such as ciprofloxacin is another important resistance trend among NTS isolates. Although Nalidixic acid is not used for treatment, development of resistance to this drug is of clinical importance since it may be associated with reduced clinical effectiveness of Fluoroquinolone treatments (Stevenson *et al*., 2017). Antimicrobial treatment is reserved only in invasive infections, in immunosuppressed and in extremes of ages as antimicrobials can prolong the illness and excretion in Non-typhoidal Salmonellosis (Gal-Mor *et al*., 2014; Schultz & Theobald, 2008).

Antimicrobial resistance in NTS is associated with an increased frequency of bloodstream infection and hospitalization among patients. Among the subset of patients with the most common serotype, *Salmonella typhimurium*, the association between resistance, bloodstream infection, and hospitalization is particularly strong (Jay *et al*., 2005). One of the most severe complications of salmonellosis is bloodstream infection that potentially results in sepsis, endocarditis, meningitis, septic metastases, and death (Hohmann, 2001 ). The rise in antimicrobial-resistant infection leads to enormous increase in the risk of bloodstream infection and thus the human-health consequences of increasing resistance may be substantial (Jay *et al*., 2005).

In Thailand, there is a common drug known as Enrofloxacin that is highly used as a veterinary Fluoroquinolone. It is used in animals in the chicken, pigs and other sea foods in various industries. Ceftiofur, a third-generation cephalosporin, is also used extensively in swine for treatment and prevention of disease and as a growth promoter. Should this be compared with previous susceptibility patterns (Hakanen *et al*., 2001 ), NTS infections in humans are currently more resistant to quinolones and Cephalosporins in Thailand (Wanla *et al*., 2007).

Drug susceptibility to Nalidixic acid has been shown to correlate well with reduced susceptibility to ciprofloxacin. Inappropriate use of cephalosporin drug in treatment of pig farming, have led to an alarming increase in ceftriaxone resistance in *S. Choleraesuis* (Wanla , *et al*., 2007). Major revisions and interventions in the current policies for use of antimicrobial drugs in food animals in most countries are warranted (Kariuki *et al*., 2006).

Cases of NTS and antimicrobial resistance to commonly used conventional drugs are on the rise especially in the HIV immunosuppressed individuals. Over the years, systemic bacterial infections caused by iNTS have been treated with antimicrobials. However, *Salmonella* have become more resistant to antibiotics and thus, the potential for new antibiotics are not encouraging (Gordon, 2011). Increasing antibiotic resistance is alarming remembering that there is no NTS vaccine available. The development of an

effective vaccine for systemic *Salmonella* infections remains an important global health priority (Crump & Mintz's, 2010)

### 2.2.10 NTS Vaccine Prospect

Effective iNTS disease vaccines might differ inherently from the vaccines produced against *S. typhi* infections. Several studies carried out in Africa showed that naturally acquired antibodies against NTS correspond with a reduced risk of iNTS disease (Gordon, 2011). There are several vaccine candidates targeting *S. typhimurium* and *S. Dublin* that are still under development and are thought to provide protection against both serovars (MacLennan *et al*., 2008). The current status of iNTS vaccine considerations has been described in a recent review (Tennant *et al*., 2016), several potential iNTS vaccines are under development, including live-attenuated, subunit-based, and recombinant antigen-based substances. Both humoral and cellular immunities are likely required to achieve full protection against iNTS disease (Mastroeni & Rossi, 2016).

Live-attenuated vaccines provide both types of immune response; however, they may pose a risk for immunocompromised individuals. Inactivated iNTS vaccines may induce humoral immunity only and suppress NTS during the acute phase of infection, but are likely not to achieve systematic clearance in infected individuals (Mastroeni & Rossi, 2016).

Previously, when animals were considered to be the only reservoir of NTS organisms, the implementation of hygiene and safety measures along a regulated and appropriate food value chain was thought to be sufficient for the reduction of iNTS transmission. However, with the speculation that humans may be a growing alternative reservoir for *S. typhimurium* resistant strain 313 (ST313), the development and deployment of iNTS disease vaccines appear to be a more viable solution (Kariuki *et al*., 2006). However, iNTS disease vaccines would not only require considerable funding to progress existing vaccine candidates, but also will require parallel vaccine deployment strategies to

identify appropriate target age groups, schedules, formulations, and potential vaccine adjuvants (Mastroeni & Rossi, 2016).

### 2.3 Antimicrobial Resistance dynamics

The usage of antimicrobials in animal production is likely to contribute to the selection, spread, and maintenance of AMR bacteria in farms. Resistance is acquired through uncontrolled use of antibiotics in human medicine and also animal production (figure 2.2) either during treatment or use of growth promoters that contain traces of antibiotics (Enne, 2010). The resistant bacteria, AMR determinants, or the antimicrobials themselves may be disseminated to the environment through farm waste, and may reach humans as a result of direct contact with animals, the consumption of contaminated foods of animal origin, water, and vegetables (Da Costa *et al*., 2013).

Antimicrobials used in the treatment of animals are very similar to those used in human medicine and therefore resistance against antimicrobials of importance for human medicine is of the utmost concern (WHO, 2016). The high rise in mobility of humans and increase in industrial food production as well as absence of environmental barriers between existing living communities leads to enormous spread of antimicrobial resistance worldwide (Acar and Röstel, 2014). This study subjected the NTS isolates to commonly used antibiotics to determine the profiles of antimicrobial resistance among food animal isolates.

High quality antimicrobials used in animal production are expected to go high due to increased demand, especially in emerging economies (Van Boeckel *et al*., 2015). Whenever antimicrobial drugs are in use, bacteria inevitably acquire resistance mechanisms through spontaneuos mutation or by acqusition of genes from other bacteria. The later may develop through conjugation (Figure 2.2), transduction or transformation. Horizontal gene transfer between bacteria is usually critical to the dissemination of resistance within a population of mixed bacteria (Barbosa & Levy, 2013). This process takes place in the presence of antimicrobial drugs (Enne, 2010).

This study determined the transferability of resistance genes through the process of conjugation.

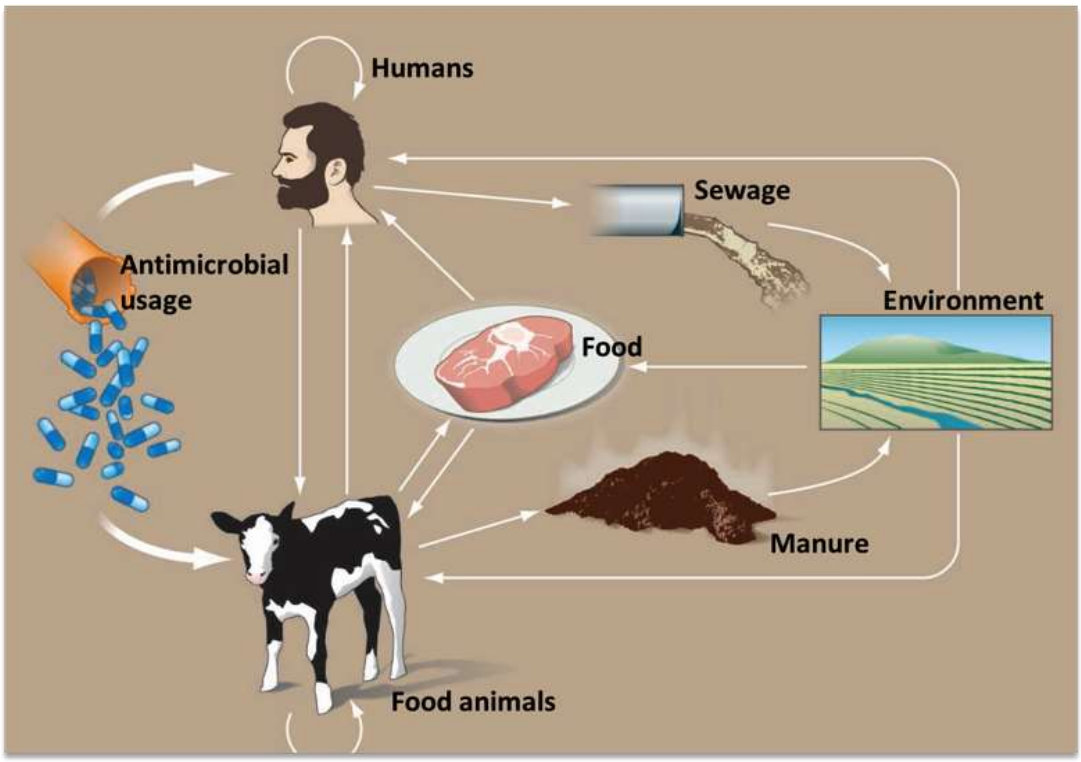


**Figure 2.2: The antimicrobial transmission dynamic- A one Health Approach**

Source: (Woolhouse *et al*., 2015)

## 2.4 Effects of Antimicrobial Resistance

Antimicrobials have been of great benefits in the field of infectious disease and medicine in general. Most drugs prove to be effective in killing microbes right from their development but with time resistance sets in. Microbes start adapting to whatever pressure thrown to them leading to emergence of resistance. High quality antimicrobials are then enrolled and microbes still adapt to them. As a result, circulation of antimicrobial drugs in the ecosystem becomes the fate of antimicrobials.

Conjugation assay carried out in this study showed clearly that the fate of MDR resistance genes is transferability within bacterial species and at the end the resistant NTS strains are disseminated via environmental pathways. Research has shown that the environment is a major factor in harboring resistance genes (Liu *et al*., 2018). The number of resistance genes present in the natural environment is still far from being estimated and thus more studies ought to be carried out in this particular field in order to clearly understand the process of acquisition of these genes by human pathogens (Da Costa *et al*.,2013).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Study site

This study was carried out at Centre for Microbiology Research, Kenya Medical Research Institute, a biomedical research laboratory located in Nairobi County. Samples were obtained from farms located in Kiambu, Kisumu, Kwale, Mombasa, and Nairobi counties (Figure 3.1).

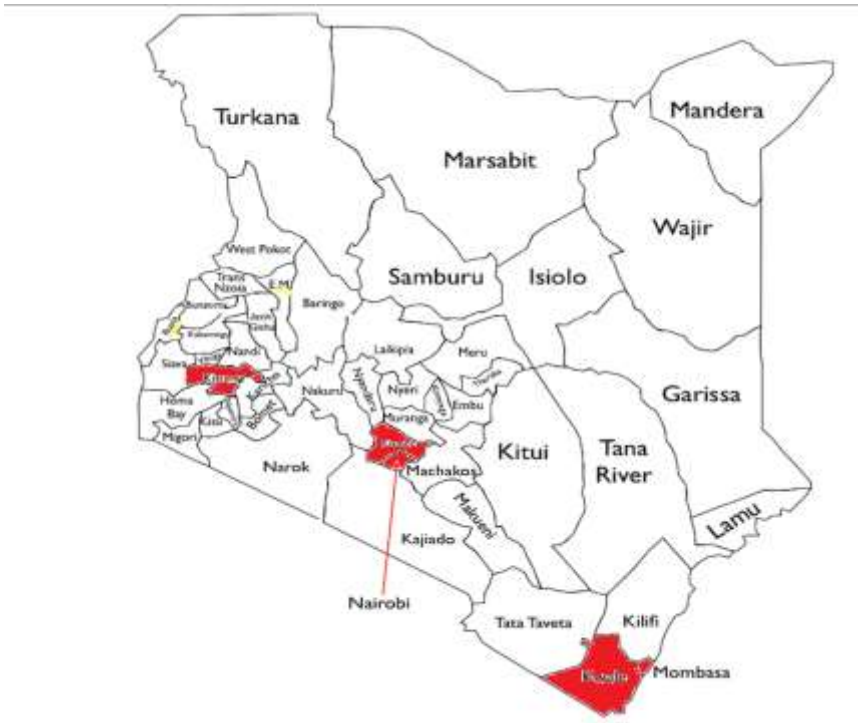


**Figure 3.1: Map of Kenya showing all the counties highlighting the research study sites.**

Adapted from Geocurrents maps

### 3.2 Study design

This was a laboratory based cross-sectional study to determine the antimicrobial profiles and resistance genes transferability of NTS isolates.

### 3.3 Study population

The study involved archived isolates of *S. enterica* serovars *Enteritidis* and *Typhimurium* at the enteric bacteriology laboratories, Center for Microbiology Research, KEMRI. The isolates had been archived for a period of one year and a few months since data collection began in May 2016 and went up to August 2017.

#### 3.3.1 Inclusion criteria

i. *S. enterica* serovars *Enteritidis* and *Typhimurium* isolates that were well preserved at -80 °C freezer, in well labeled leak proof vials.
ii. Uncontaminated cultures.

#### 3.3.2 Exclusion criteria

i. Isolates that were not well labeled to indicate the name, date of collection and source of origin.
ii. Samples that were obtained from counties not selected for this study (Kisumu, Mombasa, Kwale, Nairobi, and Kiambu).

### 3.4 Sample Size Determination of archived isolates

The sample size was determined using the (Fisher *et al.,* 1998) formulae.

$$n = \frac{Z^2PQ}{d^2}$$

Where;

n = Estimated sample size

Z = 1.96 at 95% confidence level

P = prevalence,

Q = 1-pd,  Degree of accuracy which is 0.05 at 95% confidence level.

Therefore;

$$n = 1.96^2 \times \frac{(0.25)\,(0.75)}{0.05^2}$$

$$n = 289.$$

### 3.5 Sampling technique

Systematic sampling technique was used to obtain the sampling fraction from the total population of archived samples in the freezer box at the CMR laboratories, KEMRI. The first sample was picked at random to ensure probability in the sampling aspect. The sampling fraction was calculated by dividing the sample size to the total number of the population. The fraction figure was rounded off to the nearest ten.

### 3.6 Sample Collection

A total of 289 NTS archived samples were analyzed. The samples were collected from food animals isolates for an ongoing study, 'Ecology and epidemiology of antimicrobial-resistant foodborne pathogens (*E. coli*, *Salmonella* spp. and *Campylobacter* spp.) in selected sites in five counties in Kenya: Investigating farmer knowledge and attitudes towards antimicrobial use and resistance and public health significance, SERU 3205'.

### 3.7 Sample processing

Processing of samples was done under high sterility levels to avoid contamination of the isolates. Streaking technique was used to inoculate samples on well labeled MacConkey Agar plates and all isolates were subjected to this reviving step at 37 °C overnight incubation. Macroscopic examination was carried out to distinguish *Salmonella* colonies for their morphological characteristics before sub-culture.

Sterile loops were used to pick distinct colonies for sub-culture on well labeled Tryptic Soy Agar (TSA) plates followed by incubation at 37 °C overnight. On TSA agar, pure and distinct colonies were observed and were processed for inoculation on Muller Hinton Agar, incubated at 37 °C overnight. Purification on Mueller-Hinton was to process pure colonies of *Salmonella* for use in serotyping technique. Serotyping was carried out using *Salmonella* antisera and samples determined as *S. typhimurium* or *S. enteritidis* were processed for bio-banking at $^{-}$80 °C.

### 3.8 Laboratory based methods

#### 3.8.1 Serotyping

Pure colonies of *Salmonella* isolates were obtained by inoculation on MacConkey media using streaking method. Distinct colonies obtained from the overnight incubation were then sub-cultured on TSA media followed by purification on Muller Hinton Agar and incubated overnight at 37 °C.

Serotyping technique was performed according to Kauffmann–White classification scheme (Appendix III). A pure colony was emulsified in a drop of normal saline on a glass slide using a sterile wire loop followed by addition of a drop of *Salmonella* antisera. This procedure involved firstly, polyvalent O (somatic) antisera to aid in the initial sero-grouping to ascertain that colonies were *Salmonella* species. Secondly,

Polyvalent H (flagella) was used to identify the H antigens using flagella H antigens i ($1^{st}$ phase) and flagella antigen 1 and 2 ($2^{nd}$ phase).

*Salmonella* monovalent O antisera tested were O:1,2; O:4,5; O:9; O:12; O:1; O:6,7; O:6,8 while the *Salmonella* monovalent H antisera tested were g,m; g,p; i; 1,2; 1,7; 1,5. Commercially bought normal saline was used in this process together with sterile loops and clean uncontaminated glass slides. Figure 3.2 shows results of agglutination test on a glass slide.

Through the serotyping technique, isolates classified as *Salmonella enteritidis* or *Salmonella typhimurium* were stocked for use in the determination of antimicrobial susceptibility profiles of NTS and conjugation assays of MDR strains.

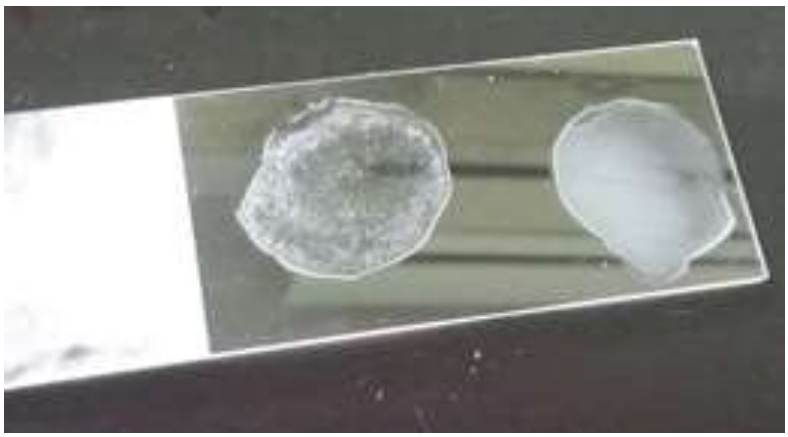

**Figure 3.2: Salmonella agglutination test showing a positive test (left) and a negative test (right).**

### 3.8.2 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of the pure isolates was performed according to the Kirby-Bauer disc diffusion method (Bauer *et al*., 1966). A sterile loop was used to touch the top of well distinct colonies then transferred to a tube of normal saline. The colony

was emulsified at the side on the inside of the tube to avoid clumping of the cells. The inoculum standard was adjusted to a 0.5 McFarland standard which is equal to approximately $10^6$ CFU/mL.

A sterile cotton swab was dipped into the inoculum then rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab. The swab was streaked over the entire surface of the Mueller Hinton agar plate. Disks were dispensed to the agar surface using Oxoid disk dispenser and sterile forceps. Plates were incubated on inverted position at 37 ℃ for 18 hours in ambient air. Figure 3.3 shows zones of inhibition that were measured using calipers.

**Figure 3.3: Plate showing antimicrobial disks with their respective zones of inhibition**

The following antimicrobials were used with their respective concentrations; Ampicillin (AMP, 10µg), Ciprofloxacin (CIP, 5µg), Sulfamethoxazole/Trimethoprim (SXT, 1.25/23.75µg), Amoxicillin-clavulanic acid (AMC, 20/10µg), Nalidixic acid (NAL, 30µg), Ceftazidime (CAZ, 30µg), Cefotaxime (CTX, 30µg), Ceftriaxone (CRO, 30µg),

Chloramphenicol (C, 30µg), Gentamicin (GEN, 10µg), Tetracycline (TET, 30 µg) and Azithromycin (AZM, 10 µg) (Table 3.1).

**Table 3.1: Antimicrobials used and their respective drug category.**

| **PLATE A** | | **PLATE B** | |
|---|---|---|---|
| **Antimicrobial** | Class | Antimicrobial | Class |
| **Ampicillin (AMP)** | Penicillin | Tetracycline | Tetracycline |
| | | Gentamicin | Aminoglycoside |
| | | Ciprofloxacin (CIP) | Fluoroquinolone |
| **Cefotaxime (CTX)** | 3rd Generation Cephalosporin | Nalidixic Acid (NAL) | quinolone |
| **Ceftriaxone (CRO)** | 3rd Generation Cephalosporin | Chloramphenicol (CHL) | Chloramphenicol |
| **Ceftazidime (CAZ)** | 3rd Generation Cephalosporin | Sulfamethoxazole/Trimethoprim (SXT) | Folate and dihydofolatebiosynthesis inhibitor |
| **Azithromycin** | Macrolides | | |
| **Amoxicillin/Clavulanic acid (AMC)** | β-lactam/β-lactamase inhibitor combination | | |

All the antimicrobials used for the study were obtained from Oxoid Ltd (UK). The cultures were examined for zones of inhibition which were measured using calipers. Interpretation of the results were recorded as sensitive, intermediate or resistant based on the national committee for Clinical and Laboratory Standards Institute criteria (CLSI, 2017), 27th Edition. The standard reference strain *Escherichia* coli (ATCC-25922) was used to assure testing performance of the potency of antibiotic discs and the quality of the media. Intermediate resistant strains were included in the resistant category.

Multidrug resistance (MDR) was defined as non-susceptibility to ≥3 classes of antibiotics (Akullian *et al*., 2018).

### 3.8.3 Conjugation Assay to Determine Transferability of Resistance determinants

Bacterial conjugation was carried out to determine the horizontal transfer of resistance phenotypes from the donor cells bearing conjugative plasmids to recipient cells. Out of all the 289 samples analyzed, 56 samples were MDR and they were subjected to conjugation assays using Sodium Azide-resistant strain *E. coli* J53 as the recipient strain. *S. enteritidis* and *S. typhimurium* were the donor strains.

Pure cultures of donor and recipient strains were suspended in separate tubes of tryptic soy broth and incubated at 37 ℃ overnight. Another tryptic soy broth was prepared and dispensed in sterile falcon tubes to which donor and recipient strains were added in the ratio of 300µl:100µl and incubated at 37 ℃ overnight. The tubes with transconjugants were mixed carefully then 100µl was dispensed on three set of plates (MacConkey containing Ampicillin drug, MacConkey containing Sodium Azide and MacConkey containing both Ampicillin and Sodium Azide) then the plates were incubated at 37 ℃ overnight. Antimicrobial susceptibility tests were carried out on transconjugants to determine the transferability of resistant phenotypes (Kang *et al*., 2005).

## 3.9 Data Analysis

Data was entered and analyzed using the statistical package for social sciences (SPSS 21.0, IBM SPSS, New York, USA). Chi square test (Fisher's exact) was used and the antibiotic susceptibility patterns presented in form of tables. Percentages of all MDRs phenotypes were calculated using frequency distribution. Results were presented in tabular and graphical formats.

### 3.10 Dissemination of findings

This study results were presented before a scientific panel in Jomo Kenyatta University of Agriculture and Technology. These results were also published with IISTE Journal of Biology Agriculture and Healthcare **Vol**-9, **Issue No** 2 (2019) of JBAH. The following are the URLs for the Issue and published article: **ISSN (Paper)** 2224-3208 and **ISSN (Online)** 2225-093X. **DOI**: 10.7176/JBAH/9-2-06. (Appendix IV).

### 3.11 Ethical Consideration

Institutional approval was granted by the School of Biomedical Sciences at Jomo Kenyatta University of Agriculture and Technology (JKUAT). Scientific and Ethics clearance to carry out the study was granted by KEMRI Scientific and Ethics Review Unit (SERU) study number KEMRI/CMR/P00058/SERU 3522. Scientific and Ethical approval for the use of archived bacterial isolates in Kenya was granted by KEMRI Scientific and Ethics Review Unit (SERU) for an approved on-going study, *'Ecology and epidemiology of antimicrobial-resistant foodborne pathogens (E. coli, Salmonella spp. and Campylobacter spp.) in selected sites in five counties in Kenya: Investigating farmer knowledge and attitudes towards antimicrobial use and resistance and public health significance, (SERU/CMR/P0036/3205) by Kariuki et al, 2016'*.(Appendix II)

### 3.12 Expected outputs of research

The results of this study provide baseline data for future studies of transmission of Multi-Drug Resistant NTS strains in Kenya. The evidence-based results can guide in management of NTS in humans and farm animals. This will greatly reduce the trend of antimicrobial resistance by ensuring that only effective antimicrobial drugs are prescribed in the treatment of humans and farmers are advised on the best remedies for increase in production other than boosters containing traces of antibiotics. Once the prevalence of antimicrobial resistance (AMR) reduces, there will be a great reduction in the cost of hospitalization and increased production in terms of growing the economy. Governmental authorities should also be alert in detecting any behavioral changes of

Salmonella towards antibiotics with a positive view of controlling the use of drugs in humans and farm animals.

# CHAPTER FOUR

# RESULTS

A total of 289 NTS samples were analyzed 136 (46%; 95% C.I: 40.3%-51.8%) were isolated from chicken (rectal swabs), 45 (16%; 95% C.I: 11.2%-20.3%) were isolated from cows (rectal swabs), 28 (10%; 95% C.I: 5.7%-12.3%) were isolated from pigs (rectal swabs) and environmental (food animals effluent) isolates were 80 (28%; 95% C.I: 22.8%-33.2%) (Figure 4.1).

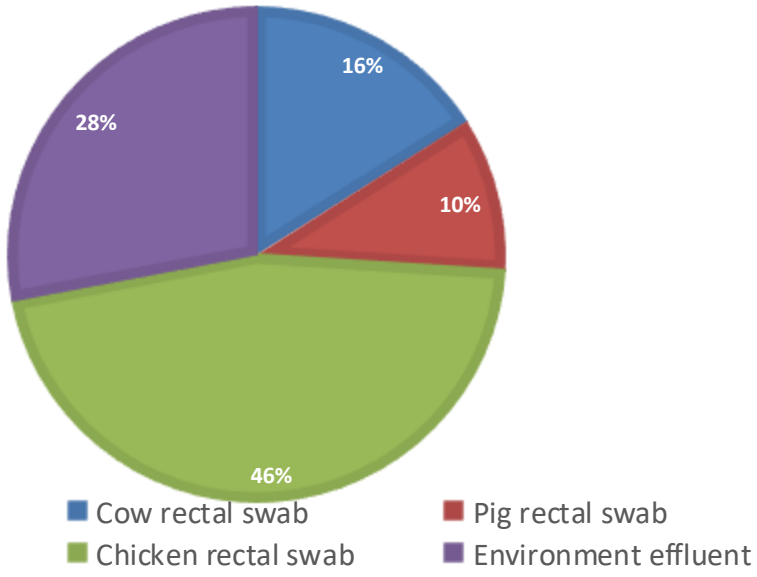


**Figure 4.1: A pie chart showing the percentages of all the food animal and environmental isolates collected during the study.**

## 4.1 Antimicrobial susceptibility profiles of NTS isolates

Invasive Non-Typhoidal Salmonella (iNTS) isolates showed varying levels of resistance against commonly used antimicrobial agents that are critically important during treatment of iNTS infections. This study showed an important resistance trend in

Ciprofloxacin (58%), which is a flouroquinolone for *Salmonella*. Resistance to Ampicillin drug was 45.8% followed Sulfamethoxazole/Trimethoprim 39.6%, Ceftriaxone 30% and then Chloramphenicol 24.3% (Figure 4.2).

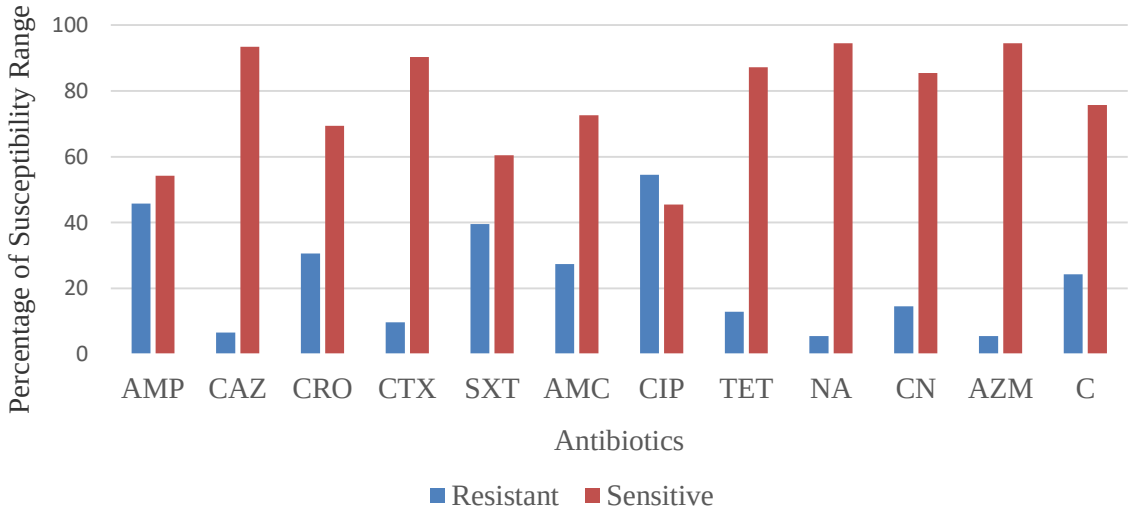


**Figure 4.2: Resistance Pattern of iNTS isolates**

**AMP**- Ampicillin; **CAZ**-Ceftazidime**; CRO**-Ceftriaxone; **CTX**-Cefotaxime; **SXT**-Sulfamethoxazole/Trimethoprim; **AMC**-Amoxicillin-clavulanic; **CIP**-Ciprofloxacin; **TET**- tetracycline; **NA**- Nalidixic Acid; **CN**-Gentamicin; **AZM**- Azithromycin; **C**-Chloramphenicol.

*S. tyhimurium* species were commonly revived from the study population in compared to *S. enteritidis* species. The table 4.1 shows the resistance profiles of the two different species (*S. tyhimurium* and *S. enteritidis*) against commonly used antimicrobial drugs. *S. tyhimurium* exhibited high resistance to Ciprofloxacin, Ampicillin, Ceftriaxone and Sulfamethoxazole/Trimethoprim compared to *S. enteritidis* species.

**Table 4.1: Resistance profiles of individual iNTS species.**

| | | Resistance (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SPP** | *N* | AMP | CAZ | CRO | CTX | SXT | AMC | CIP | TET | NA | CN | AZM | C |
| ***STM*** | 289 | 47.1 | 5.4 | 31.5 | 10.7 | 37.9 | 26.7 | 50.0 | 13.1 | 6.3 | 13.6 | 5.8 | 22.8 |
| ***SE*** | 289 | 42.6 | 9.7 | 28 | 2.4 | 43.9 | 29.3 | 8.0 | 12.2 | 0.0 | 17.1 | 4.9 | 28 |

KEY: **n**-Estimated sample size; **AMP**- Ampicillin; **CAZ**-Ceftazidime**; CRO**-Ceftriaxone; **CTX**-Cefotaxime; **SXT**-Sulfamethoxazole/Trimethoprim; **AMC**-Amoxicillin-clavulanic; **CIP**-Ciprofloxacin; **TET**- tetracycline; **NA**- Nalidixic Acid; **CN**-Gentamicin; **AZM**- Azithromycin; **C**- Chloramphenicol.

75% of the *S. typhimurium* isolates exhibited resistance to at least one antimicrobial whereas 8% were fully susceptible. 89% of *S. enteritidis* isolates were resistance to at least one antimicrobial whereas 14.5% were fully susceptible. *S. typhimurium* isolates (55%) exhibited Multidrug resistance. Compared to (68%) of *S. enteritidis* isolates that were Multidrug resistant (Figure 4.3).

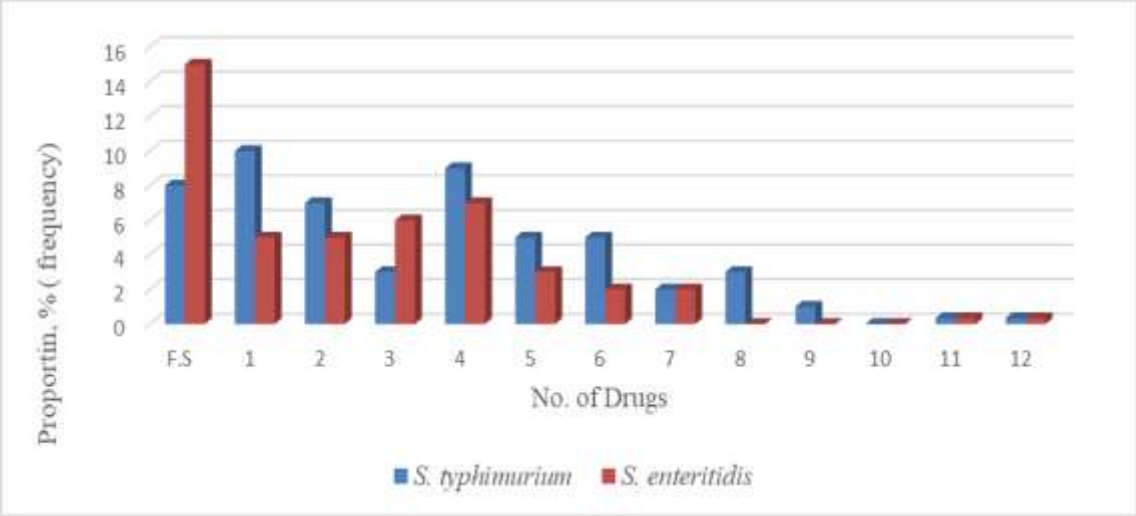


**Figure 4.3: Antimicrobial susceptibility patterns among NTS isolates**

**KEY: F.S -** Fully Susceptible

### 4.2 Transferability of resistance phenotypes in iNTS isolates

Conjugation assay was performed on the 56 iNTS isolates confirmed to be Multidrug resistant (MDR), where MDRs were defined as strains resistant to three or more classes of antibiotics. *E. coli* J53 isolate, which is resistant to Sodium Azide was used as the recipient cells for this experiment. Out of 18 *S. enteritidis* isolates, 61% were observed to exhibit resistance phenotype transferability as evidenced by conjugation assays (Table 4.2). The most common resistance phenotype transferred among the *S. enteritidis* iNTS isolates assayed was Ampicillin, Amoxycillin, Sulfamethoxazole/Trimethoprim, Chloramphenicol.

**Table 4.2: Antibiotic resistance phenotypes transferred from MDR Salmonella enteritidis to recipient E. coli J53 strain**

| Isolate | Identity | Donor Profiles | Transconjugants | Transconjugants profile |
|---|---|---|---|---|
| **SI 111** | *SE* | Amp,Amc,Sxt,Azm,Tet,C,Cn | Pos | Amp,Amc,Sxt,Azm,Cn |
| **SI 2146** | *SE* | Amp,Amc,Sxt,Tet,C | Pos | Amp,Amc,Sxt,Tet,C, |
| **SI 209** | *SE* | Amp,Amc,Sxt,Tet,C | Pos | Amp,Amc,Sxt, |
| **SI 98** | *SE* | Amp,Amc,Sxt,Tet | Pos | Amp,Amc,Sxt, |
| **SI 412** | *SE* | Amp,Amc,Sxt,C,Cn | Pos | Amp,Amc,Sxt,C |
| **SI 833** | *SE* | Amc,Tet,Azm,C | Pos | Amp,Tet,Azm |
| **SI 85** | *SE* | Amp,Amc,Sxt,Tet | Pos | Amp,Amc,Sxt |
| **SI 853** | *SE* | Amp,Amc,Sxt,Tet,C | Pos | Amp,Amc,Sxt,C |
| **SI 398** | *SE* | Amp,Amc,Sxt,Tet,Na,C | Pos | Amp,Amc,Sxt,Tet,C, |
| **SI 661** | *SE* | Amc,Amp,Sxt,C | Pos | Amp,Amc,Sxt,Tet,C, |
| **SI 2409** | *SE* | Amp,Amc,Sxt,Tet,C | Pos | Amp,Sxt,Tet,C |
| **SI 468** | *SE* | Amp,Sxt,Tet,C | Neg | - |
| **SI 4438** | *SE* | Amp,Amc,Sxt,Tet,C | Neg | - |
| **SI 1484** | *SE* | Amp,Sxt,C | Neg | - |
| **SI 4835** | *SE* | Amp,Amc,Sxt,Na,C | Neg | - |
| **SI 2521** | *SE* | Amp,Amc,Sxt,Tet,C | Neg | - |
| **SI 2054** | *SE* | Amp,Amc,Sxt,Cip,Azm | Neg | - |
| **SI 2172** | *SE* | Amp,Amc,Sxt,Azm | Neg | - |

KEY: **AMP**- Ampicillin; **CAZ**-Ceftazidime**; CIP-**Ciprofloxacin; **CRO**-Ceftriaxone; **CTX**-Cefotaxime; **SXT**-Sulfamethoxazole/Trimethoprim; **AMC**-Amoxicillin-clavulanic; **TET**- tetracycline; **AZM**-Azithromycin; **NA**-Nalidixic Acid; **CN**-Gentamicin; **C**- Chloramphenicol. **Neg**- negative and **Pos**-positive. **SE**-*Salmonella enteritidis*.

Out of 38 *S. typhimurium* iNTS isolates, 53% were similarly observed to exhibit resistance phenotype transferability as evidenced by conjugation assays (Table 4.3). The most common resistance phenotype transferred in the case of *S. typhimurium* iNTS isolates was Ampicillin, Amoxycillin, Sulfamethoxazole/Trimethoprim, Gentamycin.

**Table 4.3: Antibiotic resistance phenotypes transferred from MDR S. typhimurium to recipient E. coli J53 strain.**

| Isolate | Identity | Donor Profiles | Transconjugant | Transconjugants profile |
|---|---|---|---|---|
| **SI 26** | *STM* | Amp,Amc,Sxt,Azm,Caz,Cro, | Pos | Amp,Amc,Sxt,Azm, |
| **SI 24** | *STM* | Amp,Amc,Sxt,Azm,Te,C,Cn | Pos | Amp,Amc,Sxt,Azm,C,Cn |
| **SI 182** | *STM* | Amp,Amc,Sxt,Cn,Cip | Pos | Amp,Amc,Sxt,Cn |
| **SI 137** | *STM* | Amp,Amc,Sxt,Cn | Pos | Amp,Amc,Sxt,Cn |
| **SI 016** | *STM* | Amp,Sxt,Cn,C,Cip | Pos | Amp,Sxt,Cn |
| **SI 1615** | *STM* | Amp,Sxt,Amc,Azm | Pos | Amp,Amc,Sxt, |
| **SI 046** | *STM* | Amp,Amc,Sxt,Cip,Azm | Pos | Amp,Amc,Sxt,Azm, |
| **SI 72** | *STM* | Amp,Amc,Sxt,Cn | Pos | Amp,Amc,Sxt,Cn |
| **SI 1451** | *STM* | Amp,Amc,Sxt,Cn | Pos | Amp,Amc,Sxt,Cn |
| **SI 1309** | *STM* | Amp,Amc,Sxt | Pos | Amp,Amc,Sxt, |
| **SI 1909** | *STM* | Amp,Amc,Sxt | Pos | Amp,Amc,Sxt, |
| **SI 1650** | *STM* | Amp,Amc,Cn | Pos | Amp,Amc,Cn |
| **SI 2140** | *STM* | Amp, Sxt,Na,C | Pos | Amp,Sxt,C |
| **SI 122** | *STM* | Amp,Sxt,C | Pos | Amp,Sxt,C |
| **SI 1680** | *STM* | Amp,Amc.Sxt,Na,Cn | Pos | Amp,Sxt,Na,Cn, |
| **SI 180** | *STM* | Amp,Amc,Sxt | Pos | Amp,Amc,Sxt, |
| **SI 1994** | *STM* | Amp,Amc,Sxt,C | Pos | Amp,Amc,Sxt,C |
| **SI 2767** | *STM* | Amp,Amc,Sxt,Tet,Na,C | Pos | Amp,Amc,Sxt,Tet,C, |
| **SI 3042** | *STM* | Amp,Caz,Cip,Amc,Tet,C | Pos | Amp,Amc,Tet,C, |
| **SI 223** | *STM* | Amp,Amc,Sxt,Cn | Pos | Amp,Amc,Sxt,Cn |
| **SI 301** | *STM* | Amp,Amc.Sxt,Cn | Neg | - |
| **SI 3400** | *STM* | Amp,Sxt,Amc,Cn | Neg | - |
| **SI 2805** | *STM* | Amp,Amc,Sxt,Tet,Na,C | Neg | - |
| **SI 3355** | *STM* | Amc,Amp,Sxt | Neg | - |
| **SI 3372** | *STM* | Amp,Amc,Sxt,Tet,Cn | Neg | - |
| **SI 3934** | *STM* | Amp,Cip,Cn | Neg | - |
| **SI 3899** | *STM* | Amp,Sxt,Azm,C | Neg | - |
| **SI 1927** | *STM* | Amp,Amc,Cn,Azm | Neg | - |
| **SI 1936** | *STM* | Amp,Amc,Cn,Azm,C | Neg | - |
| **SI 3751** | *STM* | Amp,Cip,Sxt,Cn | Neg | - |
| **SI 3909** | *STM* | Amp,Amc,Sxt,Na,C | Neg | - |
| **SI 1277** | *STM* | Amp,Amc,Sxt,C | Neg | - |
| **SI 113** | *STM* | Amp,Sxt,Azm,C | Neg | - |
| **SI 4233** | *STM* | Amp,Amc,Sxt,Te,C | Neg | - |
| **SI 3470** | *STM* | Amc,Amp,Sxt,Azm | Neg | - |
| **SI 3954** | *STM* | Sxt,Amc,Caz,Cip,Cro | Neg | - |
| **SI 2128** | *STM* | Amp,Amc,Sxt,C | Neg | - |
| **SI 4244** | *STM* | Amp,Amc,Sxt,Tet,C | Neg | |

KEY: **AMP**- Ampicillin; **CAZ**-Ceftazidime**; CIP**-Ciprofloxacin; **CRO**-Ceftriaxone; **CTX**-Cefotaxime; **SXT**-Sulfamethoxazole/Trimethoprim; **AMC**-Amoxicillin-clavulanic; **TET**- tetracycline; **AZM**-Azithromycin; **NA**-Nalixidic Acid; **CN**-Gentamicin; **C**- Chloramphenicol. **Neg**- negative and **Pos**-positive, **STM**-*Salmonella typhimurium*

# CHAPTER FIVE

# DISCUSSION, CONCLUSION AND RECOMMENDATIONS

## 5.1 Discussion

The prevalence of Non-Typhoidal Salmonella isolates obtained from food animals and environmental sources was 25% (95% C.I; 20.0%-30.0%). The prevalence was similar to a previous published study by Ifeanyi *et al*., (2017). The prevalence falls within the prevalence in Sub-Saharan Africa between 20%-25% which is associated with the concurrent endemicity of malaria, a potential risk factor of infection (Feasey *et al*., 2012). A recent study carried out in Uganda reported a prevalence of 21% (Terence *et al*., 2017). This was high compared to the prevalence reported in Ethiopia 14.6% (Eguale, 2018), but much lower compared to a study in USA that reported Salmonella prevalence to be 38.8% (Alali *et al*., 2010). Another study carried out in Bangladesh reported a prevalence of 18% (Himel *et al*., 2012).

### 5.1.1 Antimicrobial susceptibility profiles of iNTS isolates

In early years, antibiotic resistance of iNTS could only be reported in low percentages with isolates having high resistance to Ampicillin and Chloramphenicol. But over the years, resistance to Sulfamethoxazole/Trimethoprim started to emerge (Lunguya *et al*., 2005). The results of this study show that in Kenya, iNTS resistance of food animal isolates to Fluoroquinolones should be a major concern because this drug is mainly used in the treatment of Salmonellosis infections (Figure 4.2). Ampicillin, Sulfamethoxazole/ Trimethoprim and Chloramphenicol drugs are is still in the front line with an increased resistance profile.

This study revealed an important trend in the development of MDR among isolates of both *Salmonella typhimurium* and *Salmonella enteritidis*. The spectrum of resistance in iNTS isolates used in this study extended further to include Ceftazidime, Ceftriaxone,

Cefotaxime and Nalidixic acid drugs with the resistance levels agreeing with rates found in a study carried out in Thailand by Toni *et al*., (2018).

Majority of the farmers in the selected counties (Kisumu, Mombasa, Kwale, Nairobi, and Kiambu) are known to practice intensive but small-scale livestock production contributing to production of animal proteins (meat, milk and eggs) in the country. These findings should therefore be of concern to the farmers and stakeholders because resistant bacteria from the supplied animal products can potentially spread to other parts of the country.

### 5.1.2 Transferability potential of resistance phenotypes in iNTS isolates

A high percentage proportion (55%) of the transconjugants expressed successful acquisition of resistance phenotypes from donor cells. The high proportion of strains exhibiting resistance gene transferability involving antibiotics such as Ampicillin, Sulfamethoxazole/Trimethoprim and Amoxicillin-clavulanic as evidenced in the conjugation assays could be as a result of increased use of Penicillin, folate-pathway inhibitors and Beta lactamase inhibitors in farm during production (Kariuki *et al.*, 2016). The observed high proportion to tetracycline resistance phenotype transferability would be due to acquisition of genes that prevent effective binding or production of tetracycline-inactivating enzymes (Jana & Timothy, 2018).

Conjugation accelerates the spread of resistance genes in Gram-negative bacteria and could be responsible for emergence of new antimicrobial resistance strains (Leungtongkam *et al*., 2018). This study denotes that the transfer of plasmid through horizontal gene transfer can take place even in environments that would otherwise prevent bacterial reproduction and thus Non-pathogenic bacteria such as *E. coli* in humans can acquire resistance genes through horizontal gene transfer from other bacteria. Antimicrobial resistance in iNTS infection could still be associated with prolonged hospital stays and high cost of treatment (WHO, 2018).

Antibiotic resistance might continue persisting in the environment due to waste produced by livestock and this could cause a wide spread public health concern affecting humans and food supply as well (Christy *et al*., 2018). iNTS infection in humans may spread systemically to other organs through the blood stream resulting in invasive salmonellosis which is much predominant in Sub-Saharan African countries (John *et al*., 2015).

Multidrug resistance in iNTS species with significant co-morbidities including immunocompromised immunity, use of immunosuppressive medication and malnutrition play a significant role in severe complications of *Salmonellosis* infection (Chun *et al*., 2018). While Conjugation assays and Antimicrobial tests are conducted in-vitro, the results might not be the exact presentation in vivo. Nevertheless, affords invaluable insights into in vivo by creating an impression of how these processes work inside the human body.

iNTS infections were known to occur mainly through the ingestion of contaminated food products, poultry, eggs, dairy products and meat but recent studies shows that human beings are also a possible reservoir of MDR iNTS. The infection may spread systemically to other organs through the blood stream resulting in invasive salmonellosis which is more prominent in Sub-Saharan Africa. Individuals at extremes of ages, immunocompromised patients and people using immunosuppressive medications are at risk of invasive salmonellosis and severe complications from invasive *Salmonella* infection (Chun *et al*., 2018).

**5.2 Study Limitations.**

The study sites where the archived samples were collected involved only five counties in Kenya, a country that has forty-seven counties and thus could not be an exact presentation of NTS in the whole country. However, the counties selected are some of the places that practice intensive but small-scale livestock production with their market both locally and internationally.

This study used secondary data which in a way limited the ability to compare the greatest contributing factor of resistant genes between food animals and environmental isolates. This objective could otherwise be tackled by collecting and analyzing of primary data. Molecular work also ought to be carried out to detect the genes transferred and mapping of the resistant phenotypes among isolates.

While Conjugation assays and Antimicrobial tests are conducted in-vitro, the results might not be the exact presentation in-vivo. However, the laboratory tests are conducted under monitored conditions to understand how the processes work inside the human body. The study was limited to *Salmonella typhimurium* and *Salmonella enteritidis*, the two NTS serovars implicated as the major cause of invasive salmonellosis in humans in Kenya whereas there quite a number of NTS serotypes. However these two organisms are considered as the major serotypes found among food animals in Kenya.

CMR laboratory is well equiped with modern state of the art equipment. However, there is insufficient space big enough to allow processing of many samples in a day and thus a lot of time was required for this study.

### 5.3 Conclusion

In conclusion, this study established antimicrobial susceptibility profiles among NTS isolates obtained from food animals and environmental sources and moderately high level of resistance in iNTS species against first line drugs and high level of resistance against 3$^{rd}$ generation Cephalosporins and Fluoroquinolones were recorded.

The conjugation assay carried out determined that transferability potential of resistance determinants in iNTS isolates pose a great risk especially in the future of antimicrobials which are currently the available treatment options for *Salmonella* infections. Horizontal gene transfer of resistant phenotypes therefore is feared to cause greater risk in the treatment of enteric infections because MDR genes could easily be transferred from pathogenic strains to non-pathogenic normal microorganisms in the body.

Results of this study shows food animals could be traced as significant reservoir of antimicrobial-resistant iNTS. It is therefore important that there be strict control on the use of antibiotics in animal production to prevent the emergence of antibiotic resistance in iNTS.

### 5.4 Recommendations

According to this study, there is evident resistance of NTS isolates to commonly used antimicrobials. It is therefore recommended that there be continuous surveillance of antimicrobial resistance profiles among common foodborne pathogens such as NTS in order to inform policy on ways to contain it.

Health education to farmers on the risks of antimicrobial resistance should be emphasized during seminars and farmers association awareness day. This would reduce the unnecessary antimicrobial use in the treatment of food animals, proper hygiene practices and public awareness of the risks associated with antimicrobial resistance to mitigate risk factors of antimicrobial resistance.

Microbiological laboratories should actively monitor prevailing mutants of NTS to institute effective management strategies especially to the vulnerable populations. Monitoring of MDR strains will reduce the spread of NTS resistant phenotypes. Appropriate measures such as rational use of antibiotics should be implemented to help preserve the potency of antimicrobials to improve successful treatment outcomes. Molecular work also ought to be carried out to detect the genes transferred and mapping of the resistant phenotypes among isolates.

This study advises that the invention and development of newer strategies to manage infectious diseases other than antimicrobial therapies to be encouraged and funded. More government resources should be allocated to development of vaccines against these infectious pathogens. For example, NTS vaccine is a promising, but an underdeveloped field, could yield great results as well as save the economy the production losses it undergoes when patients take longer periods in hospitals due to failure of antibiotics.

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# APPENDICES

## Appendix I: Ethical approval

The procedures followed during this study were in accordance with the ethical standards of the KEMRI Scientific and Ethics Review Unit (attached form) which acknowledged receipt of the revised study protocol.

**KENYA MEDICAL RESEARCH INSTITUTE**

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KEMRI/RES/7/3/1 September 1, 2017

TO: MBURU SERAH NYAMBURA
(PRINCIPAL INVESTIGATOR)

THROUGH: THE DIRECTOR, CMR,
NAIROBI.

Forwarded
06/09/2017

Dear Sir,

RE: KEMRI/SERU/CMR/P00058/3522 (*RESUMISSION OF INITIAL SUBMISSION*): MOLECULAR CHARACTERIZATION AND ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF NON-TYPHOIDAL SALMONELLA ISOLATES OBTAINED FROM FOOD ANIMALS IN SELECTED SITES IN KENYA.

Reference is made to your letter dated 29th August 2017. KEMRI/Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study protocol on August 31st 2017.

This is to inform you that the Committee notes that the issues raised during the 265th Committee C meeting of the KEMRI Scientific Ethics Review Unit (SERU) held on **July 27, 2017**, have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day **September 1, 2017** for a period of one year. Please note that authorization to conduct this study will automatically expire on **31st August 2018.** If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **31st August 2018**

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

**DR. MERCY KARIMI NJERU,**
**ACTING HEAD,**
**KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT.**

**In Search of Better Health**

## Appendix II: Authorization to use archived samples and metadata

Authorization to use archived samples for an approved on-going study, *'Ecology and epidemiology of antimicrobial-resistant foodborne pathogens (E. coli, Salmonella spp. and Campylobacter spp.) in selected sites in five counties in Kenya: Investigating farmer knowledge and attitudes towards antimicrobial use and resistance and public health significance, (SERU/CMR/P0036/3205) by Kariuki et al, 2016'* was sought from CMR, KEMRI through the laboratory In-Charge Dr. Robert Onsare.

KENYA MEDICAL RESEARCH INSTITUTE

Centre for Microbiology Research, P.O. Box 19464 - 00202, NAIROBI - Kenya,
Tel: (254) (020) 2720794, 2720038, Nairobi. Website: www.kemri.org

Tuesday, 1 Aug 2017.

To:
Mburu Serah Nyambura,
Principal Investigator

Through,
The Director,
Centre for Microbiology Research,
Nairobi

**Re: Authorization to use of study isolates and metadata**
**Title of protocol: SERU 3205: Ecology and epidemiology of antimicrobial-resistant foodborne pathogens (*E. coli, Salmonella spp* and *Campylobacter* spp) in selected sites in five Counties in Kenya: *Investigating farmer knowledge and attitudes towards antimicrobial use and resistance and public health significance.***

I hereby authorise use of isolates and metadata from the above study for the proposed study *'Molecular Characterization and Antimicrobial Susceptibility Profiles of Non-typhoidal Salmonella isolates obtained from Food Animals in Selected sites in Kenya' upon approval by SERU.* Protocol SERU 3205 sought permission for use and storage of all newly collected samples, whilst at the same time permitting the continued use of the samples colected and stored for further characterization using more recent and advanced technologies that may not ge available locally at the time.

Yours Sincerely,

Dr. Robert S. Onsare
Co- Principal Investigator

*In Search of Better Health*

**Appendix III: Kauffmann–White classification for Salmonella**

| **Serovar** | **"O" antigens** | **Phase 1 "H" antigens** | **Phase 2 "H" antigens** |
|---|---|---|---|
| *S*. Paratyphi B | 1,4,5,12 | b | 1,2 |
| *S*. Paratyphi B var. Odense | 1,4,12 | b | 1,2 |
| *S*. Java | 1,4,5,12 | b | (1,2) |
| *S*. Limete | 1,4,12,27 | b | 1,5 |
| *S*. Typhimurium | 1,4,5,12 | i | 1,2 |
| *S*. Agama | 4,12 | i | 1,6 |
| *S*. Abortus-equi | 4,12 | no phase 1 antigen | e,n,x |
| *S*. Abortus-ovis | 4,12 | c | 1,6 |
| *S*. Agona | 4,12 | f,g,s | no phase 2 antigen |
| *S*. Brandenburg | 4,12 | l,v | e,n,$z_{15}$ |
| *S*. Bredeney | 1,4,12,27 | l,v | 1,7 |
| *S*. Derby | 1,4,5,12 | f,g | no phase 2 antigen |
| *S*. Heidelberg | 1,4,5,12 | r | 1,2 |
| *S*. Saintpaul | 1,4,5,12 | e,h | 1,2 |
| *S*. Salinatis | 4,12 | d,e,h | d,e,n,$z_{15}$ |
| *S*. Stanley | 4,5,12 | d | 1,2 |
| *S*. Paratyphi C | 6,7, | c | 1,5 |
| *S*. Choleraesuis | 6,7 | c | 1,5 |
| *S*. Decatur | 6,7 | c | 1,5 |
| *S*. Typhisuis | 6,7 | c | 1,5 |
| *S*. Bareilly | 6,7 | y | 1,5 |
| *S*. Infantis | 6,7 | r | 1,5 |
| *S*. Menston | 6,7 | g,s,t | no phase 2 antigen |
| *S*. Montevideo | 6,7 | g,m,s | no phase 2 antigen |
| *S*. Oranienburg | 6,7 | m,t | no phase 2 antigen |
| *S*. Thompson | 6,7 | k | 1,5 |
| *S*. Bovismorbificans | 6,8 | r | 1,5 |
| *S*. Newport | 6,8 | e,h | 1,2 |
| *S*. Typhi | 9,12,Vi | d | no phase 2 antigen |
| *S*. Ndolo | 9,12 | d | 1,5 |
| *S*. Dublin | 1,9,12 | g,p | no phase 2 antigen |
| *S*. Enteritidis | 1,9,12 | g,m | no phase 2 antigen |
| *S*. Gallinarum | 1,9,12 | no phase 1 antigen | no phase 2 antigen |
| *S*. Pullorum | (1),9,12 | no phase 1 antigen | no phase 2 antigen |
| *S*. Panama | 1,9,12 | l,v | 1,5 |
| *S*. Miami | 1,9,12 | a | 1,5 |
| *S*. Sendai | 1,9,12 | a | 1,5 |
| *S*. Anatum | 3,10 | e,h | 1,6 |

| | | | |
|---|---|---|---|
| *S*. Give | 3,10 | l,v | 1,7 |
| *S*. London | 3,10 | l,v | 1,6 |
| *S*. Meleagridis | 3,10 | e,h | l,w |
| *S*. Cambridge | 3,15 | e,h | l,w |
| *S*. Newington | 3,15 | e,h | 1,6 |
| *S*. Minneapolis | (3),(15),34 | e,h | 1,6 |
| *S*. Senftenberg | 1,3,19 | g,s,t | no phase 2 antigen |
| *S*. Simsbury | 1,3,19 | no phase 1 antigen | $z_{27}$ |
| *S*. Aberdeen | 11 | i | 1,2 |
| *S*. Cubana | 1,13,23 | $z_{29}$ | no phase 2 antigen |
| *S*. Poona | 13,22 | z | 1,6 |
| *S*. Heves | 6,14,24 | d | 1,5 |
| *S*. Onderstepoort | 1,6,14,25 | e,h | 1,5 |
| *S*. Brazil | 16 | a | 1,5 |
| *S*. Hvittingfoss | 16 | b | e,n,x |
| *S*. Kirkee | 17 | b | 1,2 |
| *S*. Adelaide | 35 | f,g | no phase 2 antigen |
| *S*. Locarno | 57 | $z_{29}$ | $z_{42}$ |

## Appendix IV: Published Article

The findings of this study were published with IISTE Journal of Biology Agriculture and Healthcare with important findings to add in the field of scientific research.

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# Antimicrobial Susceptibility Profiles of Non-Typhoidal Salmonella Isolates Obtained from Food Animals in Selected Sites in Kenya

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**Abstract**
The organisms *Salmonella* are known to be facultative intracellular pathogens that can survive in the host macrophages. The Strains of Non-Typhoidal Salmonella (NTS) are a major cause of serious bloodstream infections with a case fatality of 20-25%. In most cases, the clinical presentations include: gastroenteritis, bacteremia, focal infection and enteric fever. In other continents of the world, NTS are associated with other clinical presentations but not majorly diarrhea. This study aims to determine the antimicrobial susceptibility profiles and resistance genes transferability of Non Typhoidal Salmonella isolates obtained from food animals (Cattle, Chicken and Pigs) and the environment in selected sites in Kenya at the Center for Microbiology Research (CMR) Institute. During this study, some of the laboratory methods that were carried out included antimicrobial susceptibility testing using the disk diffusion technique for all commonly used antimicrobials and Conjugation Assay to determine Transferable Resistance determinants. This study yielded relevant findings concerning the increasing rate of Salmonellosis in this country. The study first of all agrees with other studies that there is a high rate of NTS in food animals and this has a great connection with the increased population of these organisms in the environment. The high rate in continuous usage of antibiotics for treatment of infections in food animals and also to increase products production especially in chicken has led to the rise of resistance cases to commonly used antibiotics. Conjugation studies carried out revealed that resistant genes transferability from one bacterial species to the other is common. A total of 289 isolates used in this study, A total of 289 NTS samples were analyzed. Among the 289 samples, 134 (46%; 95% C.I: 40.3%-51.8%) were chicken isolates, 45 (16%; 95% C.I: 11.2%-20.3%) were cow isolates, 27 (9%; 95% C.I: 5.7%-12.3%) were pig isolates, 3 (1%; 95% C.I: -0.15%-2.15%) were isolates from goats and environmental isolates were 80 (28%; 95% C.I: 22.8%-33.2. The highest levels of resistance were against Ampicillin 42.7% had the highest resistance level followed by Sulfamethoxazole/Trimethoprim 38.2% and then Chloramphemicol 22.2%. The proportion of isolates resistant against Gentamicin was 9.4%. Environmental isolates showed a higher resistance profile against Ceftazidime 2.4%, Ceftriaxone 2.4%, Sulfamethoxazole/Trimethoprim 40.2% and Chloramphenicol 25.6% compared to food animal isolates. %). It is of importance that there be strict control on the use of antibiotics in animal production to prevent the emergence of antibiotic resistance in Non-Typhoidal Salmonella.

**Keywords:** Invasive Non-Typhoidal Salmonella, antimicrobial resistance, conjugation, resistance genes transferability, MDRs
**DOI**: 10.7176/JBAH/9-2-06

## 1. Introduction

The Genus *Salmonella* is a gram negative, non-spore forming anaerobic bacillus belonging to the Enterobacteriaceae family. The infection caused by Salmonella is known as Salmonellosis and is one of the most widely distributed foodborne infection in both adults and children. NTS is totally different from typhoidal Salmonella

*Salmonella* infections are known to cause gastroenteritis in most high-income countries' and bacteremia in low income countries (Seung-Joo, et al., 2012). The most susceptible group with disseminated NTS infections is HIV-infected individuals majorly in Africa and Asia (Reddy , et al., 2010). Other groups susceptible to NTS infections include patients with immune deficiencies and young children in sub-Saharan Africa (Gordon, et al., 2002 ). Infections caused by NTS, in particular *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) are a major cause of invasive bacterial disease, which typically manifest as bacteremia and meningitis, among African children, with Case-fatality rates exceeding 20% and 50% for NTS bacteremia and meningitis, respectively ( Gordon , et al., 2008).

A study carried out in Malawi, shows that NTS is the commonest cause of bacteremia, a hospital-based study. Here in Kenya, the estimated minimum incidence of NTS bacteremia is 175 per 100,000 among children younger than 5 years of age (James , et al., 2005) .