ISOLATION, CHARACTERISATION AND ANTIMICROBIAL RESISTANCE OF *SALMONELLA* IN GAME MEAT AND FRESH PRODUCE IN SOUTH-WEST, NIGERIA

BY

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A PROJECT SUMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED SCIENCES, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE(B.Sc) IN BIOTECHNOLOGY.

AUGUST 2022

DECLARATION

I hereby declare that this project report written under the supervision of Dr. G. B. Akanni is a product of my research work information derived from various sources has been duly acknowledged in the text and a list of references provided. This research project report has not been previously presented anywhere for the award of any degree or certificate.

_____ _____

IBIKUNLE, JOY A. Date

CERTIFICATION

This is to certify that this research project titled "**ISOLATION, CHARACTERISATION AND ANTIMICROBIAL RESISTANCE OF *SALMONELLA* IN GAME MEAT AND FRESH PRODUCE IN SOUTH-WEST, NIGERIA"** was carried out by **IBIKUNLE, JOY ADEDOYIN** with matriculation number **19010104008**. This project meets the requirement governing the award of Bachelor of Science (B.Sc.) Degree in Biotechnology from the Department of Biological sciences of Mountain Top University, Ogun State, Nigeria and is approved for its contribution to knowledge and literacy presentation.

……. **…….**

DR. G.B AKANNI **DATE**

PROJECT SUPERVISOR

..... **……..**

DR. C.I AYOLABI **DATE**

HEAD OF DEPARTMENT

DEDICATION

This project is dedicated to God, for the strength, wisdom and ability to perform this project, to my parents Pastor (Mr and Mrs David Ibikunle), thank you for your support, financially, spiritually, emotionally and materially.

To my supervisor Dr Akanni for guiding me and helping me throughout this project.

I also dedicate this work to my spiritual father Dr. D.K. Olukoya, for the prayers and support all through this journey.

And to all my friends, thank you for making this project a success. May God continually bless you all.

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May God in His infinite mercies continue to bless you all, Amen!

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ABBREVIATIONS

AST	Antimicrobial Susceptibility Testing
BPW	Buffered Peptone Water
CLSI	Clinical and Laboratory Standards Institute
ESBL	Extended Spectrum Beta Lactamase
RVS	Rappaport Vassiliadis Soya broth
Spp.	Species
Subsp.	Subspecies
WHO	World Health Organization
XLD	Xylose lysine desoxycholate
HEA	Hektoen enteric agar
µg	Microgram
SPI	*Salmonella* Pathogenicity Island

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ABSTRACT

Salmonella is one of the most frequently isolated foodborne pathogens. *Salmonella* infections that involve invasive serotypes causes infections that are often life-threatening. Ready-to-eat (RTE) foods such as fresh produce and smoked game meat are vehicles of transmission of *Salmonella*. This study conducted a survey of street vended RTE game meat and fresh produce. A total of 55 game meat samples were obtained from markets in South-western parts of Nigeria, while a total of 60 samples of fresh produce were obtained from road-side vendors in various parts of Ogun state. Molecular typing of isolates was performed, using *Salmonella* spp. specific PCR, antimicrobial susceptibility and ESBL (Extended Spectrum Beta lactamase) testing using Kirk-Bauer disk diffusion method was carried out. The prevalence of *Salmonella* spp. in game meat was 89% while fresh produce samples had a low prevalence of 20%. Multidrug resistance was observed in 12 *Salmonella* isolates from fresh produce. It was concluded that game meat and fresh produce collected from South-western parts of Nigeria are contaminated with strains of pathogenic multidrug resistant *Salmonella* spp.

Keywords: **Antimicrobial resistance, ESBL, Fresh produce, Game meat, Multi drug resistance, Non-typhoidal *Salmonella***

CHAPTER 1

1.0 INTRODUCTION

1.1 Background to the study

In Nigeria, game animals such as apes, chimpanzees, gorillas, crocodiles, antelopes, grass cutters, and rabbits are typically referred to as "bush meat" and used in a variety of specialties.(Ojo 2019) Game animals include both wild and domesticated mammals and birds (Agbelusi, 2009). Although they are routinely hunted for food, they are not typically viewed as domestic animals.(Clem and Serge 2015). They are said to be healthier than other meats since they have less saturated fat (Hoffman and Cawthorn 2012). They contain few calories. Additionally, it is known that wild game meat contains high levels of eicosapentaenoic acid, an essential omega-3 fatty acid with a number of health benefits (Calogero *et.al*.,2020). Wild game meat is high in vitamins and minerals and free of the growth hormones used in conventional cattle and meat production because it is raised in a natural environment. (Macháčková *et al*,.2021).

Fresh food is frequently referred to as fruits and vegetables that have undergone little processing and have not been dried or cooked.(Jayarama et al., 2020). This definition excludes dried fruit and vegetables, potted or dried components, wild rice, nuts of any sort, including raw nuts, popcorn, dried lentils, seedlings, floral, yogurt, ground meat, butter, and shellfish. Consequently, it contains the following: tomatoes, apples, beets, bell peppers, broccoli, cabbage, carrots, cauliflower, cucumber, beans, okra, spinach, and sweet potatoes (Rickman *et al.,* 2007). It has been demonstrated that eating fresh produce lowers blood pressure, prevents chronic diseases, stroke, and some types of cancer, lowers the risk of ocular and digestive ailments, and has a favorable effect on blood, which can help regulate hunger. These often referred to fruits and vegetables are said to provide significant amounts of fiber, vitamins, and minerals. In fact, research suggests that eating a healthy diet high in fresh produce may help stave off cancer and other chronic illnesses. They also offer dietary fiber, which has been linked to a lower risk of obesity and heart disease (Van *et al.,* 2000).

1.2 Statement of problem

1.2.1 Game meat

A source of protein in the diet is game meat. Although ready-to-eat game meat is dried or smoked and sold in open markets, due to improper preservation and unclean preparation, it is susceptible to germ infection (Sofos 2014**).** The majority of bush animals are caught in traps, shot at by hunters, and in some cases, discovered dead in the bush and sold right away in the market without any professional meat inspection procedures performed on the carcass to check and ensure that their flesh and organs are free of disease and any food-borne pathogen or contaminant.(Ojo 2019). Furthermore, when burning bush meat, substances known as polycystic hydrocarbons and nitrosamines, which are frequently found in smoke, contaminate them. (Knower et al.,2014). These substances are referred to as carcinogenic. In reality, some academic research has discovered a statistically significant link between eating a lot of food that has been smoked and developing digestive tract cancer. (Ogada 2014). Meat from hunted wildlife is sold domestically through a variety of channels, including either directly to consumers or to nearby retailers.(Ahmadi et al., 2018). However, there are recognized methods that have allowed human infection like *Salmonella*, including inadequate personal hygiene, cross-contamination preparation, cuts and open wounds of these animals.(Todd 2020). Important human bacterial enteric infections called Non-typhoidal *Salmonella* (NTS), which will be the focus of this study, they are important bacterial enteric pathogens of humans frequently carried out asymptomatically in susceptible animals. The World Health Organization (WHO) has identified NTS as a significant global source of sickness and mortality. (Havelaar *et al.*, 2015). NTS continues to be a hazard to human health on a global scale, especially by inflicting food-borne illnesses, and the main reservoirs of many pathogenic serovars are animals raised for food, such as game meats and fresh produce (fresh fruits and vegetables). (Eng et al., 2015). *Salmonella* Enterica serotypes other than Typhi and Paratyphi A cause NTS infections in humans. Human infections typically present as a diarrheal sickness. Invasive NTS illness, however, frequently affects young children, the elderly, and people with impaired immune systems.(Gordon 2008).

1.2.2 Fresh produce

Fresh produce is frequently consumed raw and fresh; therefore, germs can often be discovered on its surface. (Balali *et al.*, 2020) If produce is not adequately washed or becomes contaminated after washing, it may potentially become diseased. Salmonellosis (food borne gastroenteritis) is responsible

for 1.2 million of the 9.4 million episodes of food-related infections recognized in the United States, with a cost of 3.6 billion yearly, 23,000 hospitalizations, and 500 fatalities. (CDC report, 2016). *Salmonella* enterica subsp. enterica, which is present in non-typhoidal animal products, is the cause of the majority of salmonellosis cases; however, over the years, a wide range of other fruits and vegetables (such as cucumber, carrot, mango, etc.) have also been linked to salmonellosis outbreaks around the world. (Loic *et al.*, 2018). Untreated water used to irrigate a farm or contaminated water in a tank used to wash vegetables after it has been harvested can spread pathogens like *Salmonella*. (Jung *et al.,* 2014). Produce can also become contaminated by other things, such as infected raw materials, or by individuals handling it with dirty hands (Alimi 2016). *Salmonella* contamination in fresh produce can happen during the growing, harvesting, processing, and transportation of the produce. *Salmonella* has been known to stick to and internalize fresh produce, making it a common source of bacteria. Agricultural farms may potentially receive contaminated waste and irrigation water. In some instances, direct contact between produce or seeds and polluted manure or animal wastes might result in crops or produce infection (Kilonzo *et al.,* 2018).

1.3 Significance of study

The goal of this study is to determine the prevalence of non-typhoidal *Salmonella* in fresh produce and game meat from the south-western states of Nigeria. The antibiotic resistance patterns of the non-typhoidal *Salmonella* was investigated. The findings of this study will inform the steps to be taken in educating the public on how to manage non-typhoidal *Salmonella* in fresh produce and game meat, including the necessary precautions to be taken when handling fresh produce and game meat.

1.4 Aims and objective of study

In this study, Non-typhoidal *Salmonella's* microbiological profile and characteristics in fresh produce and game meat from different marketplaces in Nigeria will be examined. The genes and antimicrobial resistance pattern of non-typhoidal *Salmonella* will be identified.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Salmonella

Salmonella enterica species are Gram-negative bacteria that affect both humans and animals and are responsible for several food- and water-borne diseases. They thus represent a major danger to the general public's health (Wiedemann *et al.,* 2015). Fresh produce infected with *Salmonella* has been related to food poisoning in an increasing number of instances in recent years. Numerous studies have shown that one important component of *Salmonella*'s pathogenesis is its capacity to pass through potential obstacles, requiring the intrusion of a wide variety of cells, and that the level of absorption may be affected by a variety of factors (Palmer and Slauch, 2017). The genus *Salmonella* consists of just two species: *S. bongori* and *S. enterica*, the latter of which has six subspecies: *enterica*, *salamae, arizonae, diarizonae, houtenae*, and *indica.* More than 1,500 serotypes of *S. enterica* subsp. enterica differ significantly in their host range and disease effects, which range from enteritis to typhoid fever, despite sharing a large amount of genetic similarity (Ohl and Miller, 2001). *Salmonella* enterica subsp. enterica is a serious economic and public health concern on a global scale. *Salmonella* serotypes vary in how well they can adapt to their hosts, which has an impact on how harmful they are. Serotypes that are adapted to humans, such as *S. Typhi* and *S. Paratyphi* A, B, and C, cause systemic typhoid fever. Animals are not harmed by these serotypes. These animals also suffer from severe systemic disorders brought on by *S. Gallinarum* and *S. Abortusovis*, which are specific to ovine and fowl, respectively. But *S. choleraesuis*, which mainly uses pigs as hosts, can also lead to a systemic illness that is fatal in humans. Serotypes like *S. Enteritidis* and *S. Typhimurium*, which are frequently found in the environment, can infect both humans and animals (Hoelzer *et al.,* 2011). Some of these induce chlorosis on plant leaves, which can occasionally be fatal (Gu *et al.,* 2013b).

When they consume contaminated food or water, mammals become ill. *Salmonella* infection in both animals and people depends on the bacteria's ability to survive the demanding conditions of the gastric tract before moving through the intestinal epithelium and, in the case of systemic infections, colonizing the mesenteric lymph nodes and internal organs. In order to infiltrate non-phagocytic cells and persist in the host environment, *Salmonella* has devised strategies to interact with host cells and trigger its own internalization (Rosselin *et al.,* 2012).

Salmonella commonly enters agricultural settings through animal feces. Directly contaminated by animal waste, plants, surface water used for irrigation, and pesticide or fertilizer diluent can all get contaminated. *Salmonella* can adapt to a wide range of environmental variables, including high temperatures and low pH, so it may survive outside of the host organism. *Salmonella* can attach to plant surfaces and stick there before actively infecting and populating the interiors of many plants (Gu *et al.,* 2011), thereby inhibiting the plant immune system (Schikora *et al.,* 2012). *Salmonella* from plants also continues to be virulent toward mammals (Schikora *et al.,* 2011).

2.1.1 History of *Salmonella*

Salmonella is one of the food-borne bacteria that is isolated the most frequently. It has a huge influence on worldwide public health, resulting in 93.8 million cases of food-borne illness and 155,000 fatalities annually. The genus *Salmonella* is named after renowned veterinary bacteriologist Daniel E. Salmon and is a member of the enterobacteriaceae bacterial family (1850-1914). *Salmonella* was first identified by Soholerin in 1839, and Eberth first isolated it in the mesenteric lymph nodes and spleen of a typhoid fever patient in 1880. In 1888, Salmon and Smith developed *Salmonella* from pigs that had passed away from hog cholera. A young man who had eaten raw meat from an infected cow died of gastroenteritis in 1888, and Gartner later identified the cause as the *Salmonella* bacteria. From a sick mouse, Loeffler identified *S. typhimurium* in 1892. Bacillus paratyphique was the name given to a bacterium that Achard and Bensaud discovered in 1896; however, Boycott (1911) recognized this organism as *S. schottmulleri.*

S. pullorum, the etiological agent of Pullorum disease, was discovered by Rettger in 1899 when the condition was still recognized as fatal septicemia of newborn chicks. Schottmuller established the existence of two distinct paratyphoid bacilli in 1900; they were later known as *S.* paratyphi A and B. *Salmonella* was officially given to the genus in 1934 in honor of American veterinary bacteriologist Daniel E. Salmon, a name proposed for it by Lignieres in 1900. Jordan isolated *S.* panama during an outbreak among American troops at the Panama Canal. Hormaeche and Peluffo allegedly isolated *S.* montevideo from a monkey in Uruguay in 1936. The Gram-negative bacterium *S.* var. arizona was initially discovered in an Arizona lizard carcass in 1939. In 1940, Bruner and Edwards described *S.* melagridis for the first time (Hafez 2013).

2.1.2 Characteristics and serotypes of *Salmonella*

Salmonella are gram-negative, non-capsulated, medium-sized, straight, non-spore-forming rods that are (0.7 - 1.5 2.0 - 5.0 Mm). They can be easily stained with common dyes like methylene blue or carbol-fuchsin (Rahman *et al.,* 2018). They are facultatively anaerobic, therefore they may thrive in both aerobic and anaerobic conditions. They are chemoorganophilic because they have a respiratory and fermentative mode of metabolism. Peritrichous flagella are used by *Salmonella* bacteria to migrate (except *S. pullorum* and *S. gallinarum*). Fimbriae can also form on them.

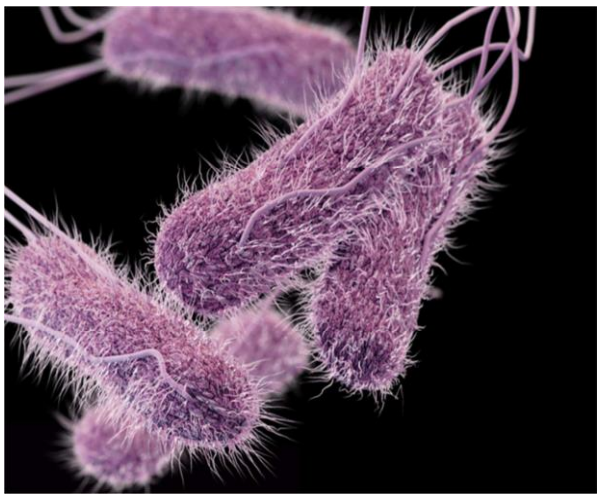

Figure 2.1: *Salmonella* bacteria with peritrichous flagella (Rahman *et al.,* 2018)

Typically, they release acid and sometimes gas as they ferment carbohydrates. Mannitol, arabinose, maltose, dulcitol, sorbitol, mucate, trehalose, xylose, and mannose are commonly fermented while lactose, sucrose, raffinose, salicin, and adonitol are not. *Salmonella* decarboxylates lysine, ornithine, and arginine but not glutamic acid. In contrast to *S.* paratyphi, which lacks the lysine decarboxylase enzyme, *S.* typhi is unique in that it does not synthesize ornithine decarboxylase. They may survive for several months in microenvironments including excrement, moist soil, and stream sediments, and they can withstand prolonged subfreezing in water (Chau 2010).

2.1.3 Epidemiology of *Salmonella*

Salmonella infections caused by invasive serotypes can be fatal, necessitating appropriate and effective antibiotic treatment. The growth of multi-drug resistant (MDR) *Salmonella* serotypes is significantly affecting the efficacy of antibiotic therapy, and a rise in the prevalence of MDR strains may increase the mortality rates related to *Salmonella* infections (Carrasco *et al.,* 2012). The worsening and

extension of symptoms in patients who develop MDR strains is proof that MDR *Salmonella* serotypes are more virulent than susceptible strains, according to epidemiological study (Eng *et al.,* 2015).

Non-typhoidal *Salmonella* is a major cause of bloodstream infections globally, and those who are malnourished, have HIV, or have malaria are more likely to have the infection. According to a review of the literature done by Trong et al. (2010) to determine the incidence of invasive non-typhoidal *Salmonella* (iNTS) in different age groups. Data were first classified by HIV and malaria prevalence before being extrapolated using population estimates from 2010. The case-fatality ratio (CFR) was decided upon by expert agreement. They estimated that there are 3.4 million instances of the iNTS disease per year, with a range of 2.1-6.5 million (overall incidence 49 cases [range 30–94] per 100,000 population). Incidence rates are highest in Africa (227 occurrences [range 152-341] per 100,000 persons), and babies, kids, and teenagers are the most affected (1.9 [range 1.3–2.9] million cases).

Incidence rates are highest in Africa (227 occurrences [range 152-341] per 100,000 persons), and babies, kids, and teenagers are the most affected (1.9 [range 1.3–2.9] million cases). The Institute for Health Metrics and Evaluation reports that in 2010, enteric NTS infection caused 4,847,000 lost disability-adjusted life years (70 disability-adjusted life years/100,000 people) and 81,300 diarrheal fatalities (1.2 deaths per/100,000 people). However, these estimates do not account for invasive NTS (iNTS) disease, which typically has little to do with diarrhea. According to a review of the literature on community-acquired bacteraemia in Africa, NTS was responsible for a significant portion of infections in some parts of the continent, including 88% in eastern Africa, 97% in southern africa, and 87% in western and central africa, compared to only 1% in northern africa. Furthermore, 29% of these infections were caused by *Salmonella* enterica. Furthermore, this study showed that the 2 most common serovars generating iNTS infections were *S.* enterica serovars Typhimurium and Enteritidis, which together accounted for 65.2% and 33.1%% of all NTS serotyped isolates. The iNTS disease seems to be more widespread in some regions of Africa than it is elsewhere in the world. Host risk factors appear to have a significant impact on the epidemiology of the iNTS disease in Africa, where the condition is closely associated to malaria, baby and child malnutrition, and adult HIV infection (Ao *et al.,* 2015). Estimating the number of illnesses and fatalities brought on by iNTS disease has been challenging due to a lack of population-based surveillance data on bloodstream infections, particularly in Africa. Due to the availability of high-quality country-level data on the two primary host risk factors for iNTS, namely HIV and malaria, it is possible to extrapolate from the scant

population-based iNTS surveillance data that are currently available for other regions (Kirk *et al.*, 2010).

In sub-Saharan Africa, the frequency of iNTS is highest in children and young adults. Africa's NTS sources and means of transmission are equally poorly known. Additionally, genomic research and combined human and animal studies support the idea that infected people may be a significant source of infection (Crump and Heyderman 2015).

In Africa, invasive bacterial illness is primarily caused by invasive NTS. The management of patients could be affected in various ways by this discovery. Antimicrobial agents appropriate for the management of iNTS must be included in recommendations for the empiric management of sepsis. Aside from the fact that aminoglycosides are inappropriate for treating intracellular infections like iNTS, African strains of iNTS are now frequently resistant to the usual first-line medications ampicillin, trimethoprim sulfamethoxazole, and chloramphenicol. Further raising concerns is the fact that in some regions of Asia, iNTS strains also exhibit fluoroquinolone and extended-spectrum cephalosporin resistance (Graham 2010). In populations at risk for infections, antimicrobial resistance appears to have contributed to the formation and spread of specific NTS serovars and strains. There is evidence that *Salmonella* Typhimurium ST313 has evolved multidrug resistance and adapted to immunosuppressed people, notably those with HIV, in sub-Saharan Africa. Controlling iNTS is likely to depend on preventing and treating host diseases that make people more susceptible to it. In some regions, declines in iNTS disease have been environmentally linked to decreases in malaria transmission. Successful antiretroviral medication therapy is expected to have comparable impacts on iNTS illness as decreases in HIV seroprevalence and decreases in the percentage of HIV-infected individuals with low CD4-positive T-lymphocyte counts (Lokken *et al.*,2016).

2.1.4 *Salmonella* pathogenesis

As the infection is virtually usually contracted through microbial ingestion, the pathogenesis of *Salmonella* is a multi-factorial process that varies with the serovars, dose, age, and immune condition of the host (Rahman *et al.*, 2018). *Salmonella* infections are characterized by a variety of complex virulence factors, such as the capacity to invade cells (aided by components like the surface polysaccharide O antigen, flagellar H antigen, and fimbriae), a full lipopolysaccharide coat, the capacity to replicate intracellularly, and the production of at least three toxins: Endotoxin (a lipopolysaccharide of the bacterial cell wall that can cause fever when released into the bloodstream

of the infected person), Enterotoxin (which inhibits epithelial cells' secretory response and causes fluid to accumulate in the intestinal lumen in *Salmonella* food poisoning), and Cytotoxin are some of the other toxins that can cause illness (Cause iron chelating structural damage to intestinal epithelial cells by inhibiting protein synthesis).

Iron from the host's iron-binding proteins is provided by iron chelating proteins (bacteria's siderophores and enterobactin), which is necessary for bacterial growth. Other virulence factors that are known to contribute to the development of disease include adhesion pilli, colicin and porin production, the capacity to withstand the fatal effects of serum complement, big plasmids, and others (30- 60 mega daltons) (Van *et al.*,2018).

2.1.5 Classification of *Salmonella* spp

Since *Salmonella's* taxonomic categorization has been in dispute for a while, it is presently based on the Kauffman-White scheme (1934), in which serological detection of somatic (O), flagella (H), and capsular (K) or (Vi) antigens is the main method for typing. The figure below illustrates the two main species of *Salmonella, Salmonella* enterica and *Salmonella* bongori, according to the most recent nomenclature:

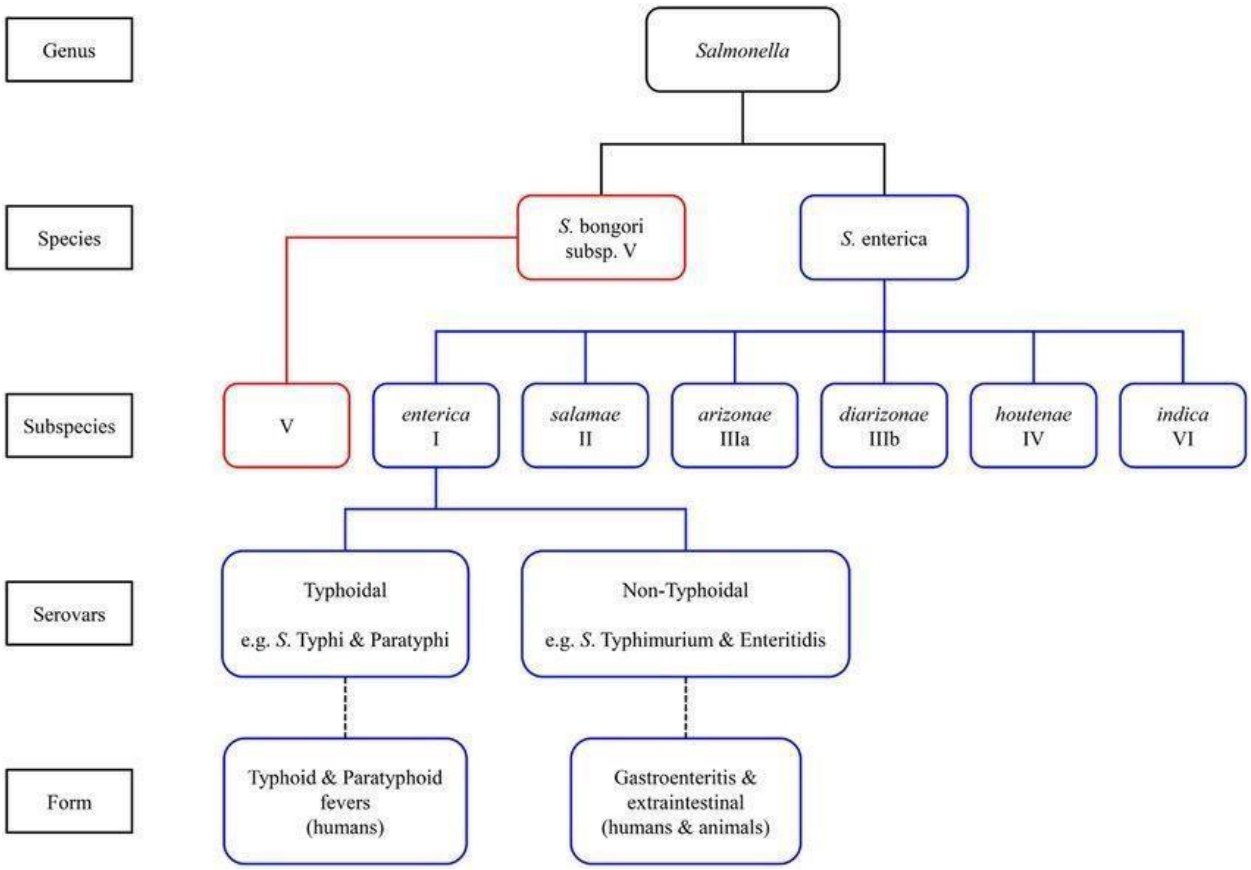

Figure 2.2: *Salmonella species* and subspecies Classification. Source: *Hurley et al.*, (2014)

2.1.6 Salmonellosis: Sources and mode of transmission

One of the most prevalent zoonotic diseases and a public health risk that is still present in some communities, particularly in developing nations and among those who lack good hygiene, is salmonellosis.

General Sources

The main human source of *Salmonella* is the gastrointestinal tract. People working on poultry farms and other farms with animals can mechanically spread *Salmonella* through their hands, feet, and clothing. This allows them to infect cattle, sheep, pigs, dogs, and horses on the same farm as well as birds.

Some serotypes are restricted to a certain animal reservoir, while many are capable of causing disease in humans through direct contact with diet (zoonosis). The importance of animal feces is greater than that of human feces, and it should be emphasized that poultry products and game animals are susceptible to contamination from this source (Thomas *et al.*,2020).

Animal-derived foods, including bush meat, are a significant source of human *Salmonella* infections, particularly those that may get contaminated during handling by a sick person or a carrier person (Forshell and Wierup 2006).

Feces with *Salmonella* can contaminate pasture, plants, soil, food, and water. The environment, particularly moist soil, water, animal excrement in particular, blood-and-bone meals, and fecal particles, can support their survival for nine months or longer. Environmental contamination can operate as a source of illness for both humans and other animals. (Hoelzer *et al.*,2011).

Sources of microbial contamination in fresh produce

Salmonella species can come into contact with fresh food in a variety of ways and at any point in the manufacturing process, but the farm poses the greatest risk. There are a number of potential points of contamination in the farm-to-sale production, processing, and distribution of fruits and vegetables (fresh produce), including irrigation water, manure, handling by workers, and contact with contaminated surfaces, (FDA 2017). Which is further discussed below:

Soil contamination

The human intestinal bacteria in the soil are introduced to agricultural soils by animal waste and irrigation with sewage water. In Ghana, (Amoah *et al.*, 2007), found unacceptably high levels of fecal coliforms in lettuce grown in urban cities of Accra and Kumasi. Same strains of *Salmonella* present in edible parts of vegetables were documented.

Cross-contamination

Cross-contamination of ready-to-eat foods from hands or contaminated surfaces (fresh produce). Cleaning food preparation surfaces and washing hands have been found to help minimize *Salmonella* contamination, according to other studies (Mugampoza *et al.*, 2013).

Fertilizers

Fresh food can get infected at any step, including during growth, harvesting, and processing. Fresh food can become contaminated prior to harvest either directly or indirectly by (wild) animals, insects, water, soil, soiled equipment, and human handling. The two most important concerns, however, are fecal irrigation water contamination and the use of manure or compost as fertilizer in crop-growing areas

(Ibenyassine, 2007). Globally, animal dung is utilized as a plant fertilizer, especially in areas where extensive animal production and arable cultivation coexist (van Pelt, 2008). The likelihood that fresh fruit grown in infected manure-enriched soils will be contaminated will largely depend on the pathogen's ability to survive in manure and manure-adjusted soils. The conditions for enteric pathogens to survive are deemed unfavorable once they have been expelled from the animal intestine (Unc *et al.*, 2004). Manure allows for the longer-term survival of pathogens like *Salmonella* enterica (up to months) (Franz, 2008).

2.1.6.1 Transmission routes of *Salmonella*

Most *Salmonella* serovars transmit from host to host largely through the fecal-oral pathway. Some *S.* Typhi patients develop into asymptomatic carriers of the human host-adapted pathogen *Salmonella* enterica serovar Typhi. These people contaminate water or food sources by excreting enormous amounts of the bacteria in their feces, which spreads the infection. The carrier condition, which causes outbreaks of food-borne illness, has also been described in livestock animals. Controlling disease outbreaks requires the detection and treatment of carriers (Gopinath *et al.*,2012).

Despite being regularly found in surface waterways, particularly those utilized for recreation, irrigation, or as a source of drinking water, *Salmonella* was rarely documented in water-borne outbreaks in industrialized countries. It has been demonstrated that a frequent source of crop contamination in outbreaks of *Salmonella* related to crops is irrigation water contamination (Levantesi *et al.*,2012).

2.2 Virulence factors, antibiotic resistance and susceptibility of *salmonella*

The following are the virulence factors of *Salmonella*, which are currently poorly understood variables in how *Salmonella* successfully infects hosts as diverse as humans, animals, or plants.

Colonization

There are several steps involved in a *Salmonella* infection, including adherence to the host's surfaces, attachment, and creation of bacterial components that aid in invasion, early multiplication, and the capacity to overcome or avoid human defenses (Andino and Hanning 2015).

Adhesion to Host Surfaces

Adhesion to tissues is one of the first essential steps in *Salmonella* successfully colonizing a host. In the adhesion process, there are two distinct steps: a loose attachment that depends on bacterial factors and is reversible after an initial adhesion that is reversible (Cevallos-Cevallos *et al.*, 2012).

Fimbrial structures

Fimbriae are proteinaceous surface appendages that are 0.5–10 nm long and 2–8 nm wide. At their distal end, fimbriae contain a protein that binds with its host receptor, mediating bacterial adherence to inert or host surfaces.

Non-fimbrial adhesins

According to their secretion mechanism, two different kinds of non-fimbrial adhesins have been identified in *Salmonella*: BapA and SiiE are both produced by Type-1 secretion systems, whereas ShdA, MisL, and SadA are autotransporters, also referred to as Type-V secretion systems.

The two biggest *Salmonella* proteins, BapA (386 kDa) and SiiE (595 kDa), have a lot of bacterial Immunoglobulin-like domains in common. These two proteins' genes are highly conserved across *Salmonella* serotypes (Biswas *et al.*, 2011; Suez *et al.*, 2013).

Other structures

The primary role of the bacterial components flagella and LPS is not to mediate adherence. Flagella provide chemotaxis and motility and activate the innate immune system of the host. Most Gram-negative bacteria have a significant amount of LPS in their outer membrane, which shields the bacteria from harmful substances like bile salts and antibiotics. The lipid A, which is part of the bacterial membrane, the core oligosaccharide, and the O-antigen, which is the most periphery moiety, make up LPS. Additionally, it causes septic shock in animal hosts and, as flagella, it activates the innate immune system (Tan and Kagan, 2014).

Invasion

Salmonella has evolved many strategies in animals to cause its own internalization in various cell types in order to endure, proliferate, and disseminate throughout the host (Rosselin *et al.*, 2012).

T3SS-1 dependent mechanism

The structural elements of the secretory apparatus, chaperones, regulators, and several effectors involved in mammalian host invasion are encoded on the SPI-1 island. The SPI-1 genes are expressed when *Salmonella* enters the intestinal environment, enabling the T3SS-1 to assemble at the bacterial surface. Following a bacterial-host cell interaction, the T3SS-1 translocates at least 15 proteins encoded by the SPI-1, SPI-5 pathogenicity islands, and pro-phages into host cells. *Salmonella* cell invasion has been demonstrated to require SopE, SopE2, SopB, SipA, SipC, and SptP among these effectors. Inducing actin recruitment and polymerization at the entrance point, caused by the cooperative action of SopE, SopE2, SopB, SipA, and SipC, causes "ruffles" to develop on the membrane surface. These ruffles protrude from the surface of the cell and internalize the bacteria in a vacuole within the host cell. The host cell can resume its normal morphology after ruffle formation because the endocytic vacuole closes and the cellular cytoskeleton recovers to its initial condition. This restoration is made possible by the effector SptP rearranging the actin cytoskeleton. Only mammalian cells have been used to study the "Trigger mechanism" that leads to T3SS-1 invasion (Velge *et al.*, 2012).

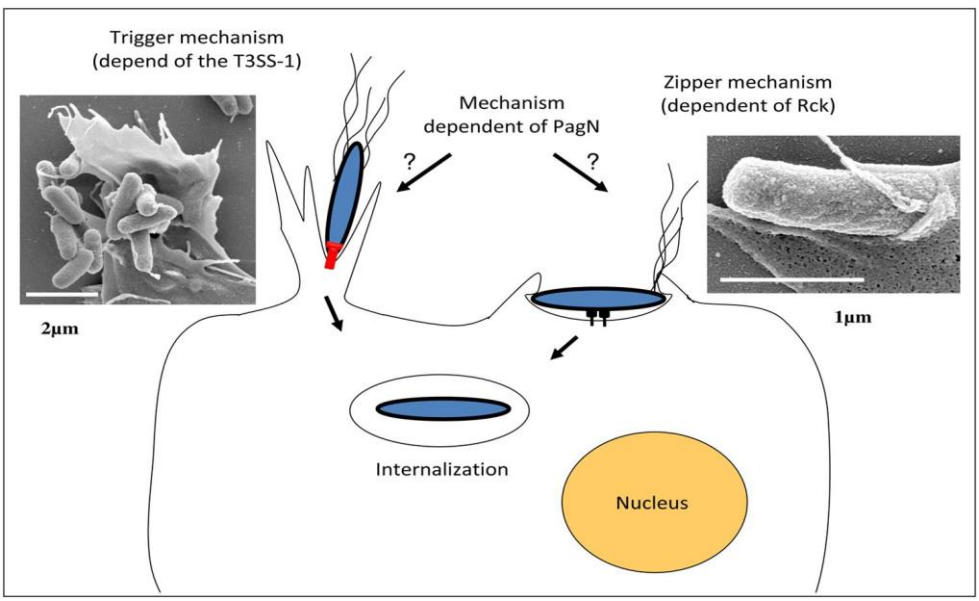

Figure 2.3: Models of *Salmonella* invasion mechanisms.

T3SS-1 independent mechanisms

Rck and PagN, two *Salmonella* invasins, have been identified thus far. Furthermore, research has shown that *Salmonella's* invasion mechanisms are not limited to the PagN, Rck, and T3SS-1. *Salmonella* mutants lacking the T3SS-1, Rck, or PagN were nonetheless capable of invading several animal cell lines (Rosselin *et al.*, 2012).

Salmonella's role in invading animal cells has been thoroughly documented in vitro and proven using a variety of techniques. have demonstrated that rck deletion in *S.* enteritidis reduces animal epithelial cell invasion by more than a factor of two without affecting the bacteria's adhesion to the cells. Additionally, research utilizing Rck-coated latex beads and an initially non-invasive E. coli strain that overexpresses Rck has demonstrated that Rck alone can cause cell invasion in a receptor-dependent way. The G113-V159 peptide is the smallest portion of Rck needed to trigger invasion. When Rck interacts with its receptor, which is expressed on the animal cell membrane, a signaling cascade involving cellular proteins that support local actin buildup and weak, tightly adherent membrane extensions results. This mechanism, known as a "Zipper," has only been explored in animal cells (Figure 2.3).

Multiplication

Salmonella can proliferate once it has been ingested into tissue. *Salmonella's* capacity to colonize plants may be an efficient means of survival and reproduction since it establishes a connection between the pathogen's release into the environment through animal feces and the recontamination of herbivorous and omnivorous hosts.

Intracellular multiplication within animal cells

Through its T3SS-1, as well as through its Rck and PagN invasins, *Salmonella* can enter host cells. The intracellular behavior of *Salmonella* internalized in animal or plant cells via the invasin-mediated

pathways has not, however, been studied, in contrast to the T3SS-mediated entry mechanism.

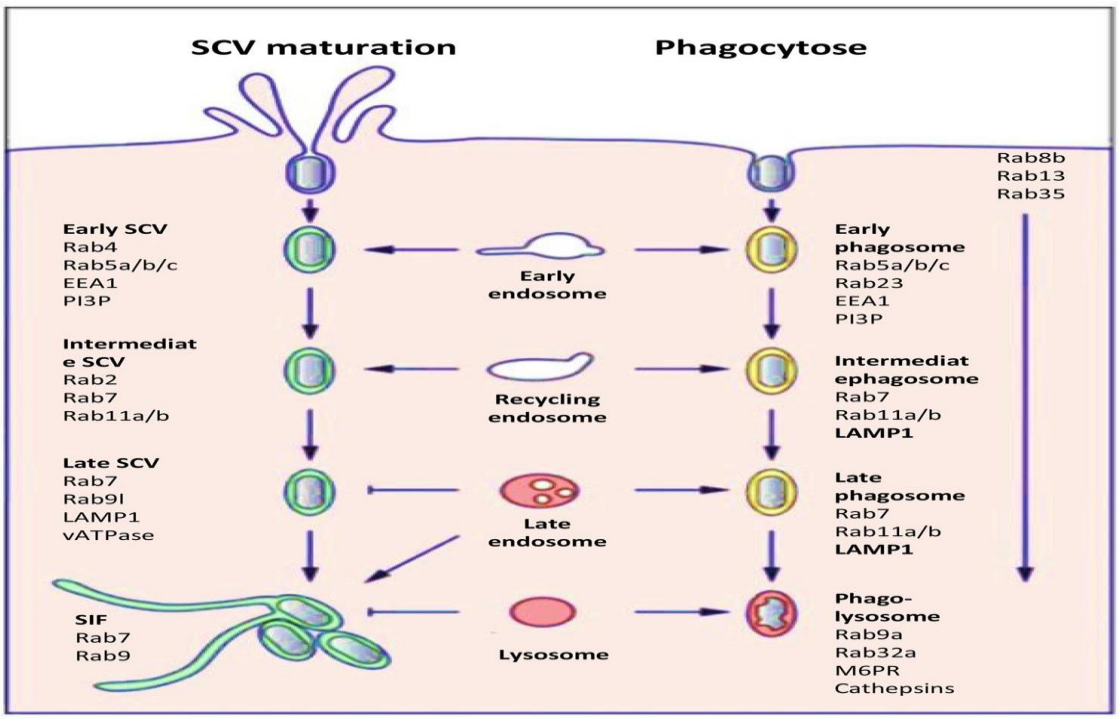

Figure 2.4. Host cell markers present on the scv (left) or on a phagosome (right).

Heterogeneity of *Salmonella* behavior within animal cells

Salmonella populations within animal host cells are varied, according to studies of the bacterial invasion process. In SCV, the bulk of bacteria develop into replicative compartments.

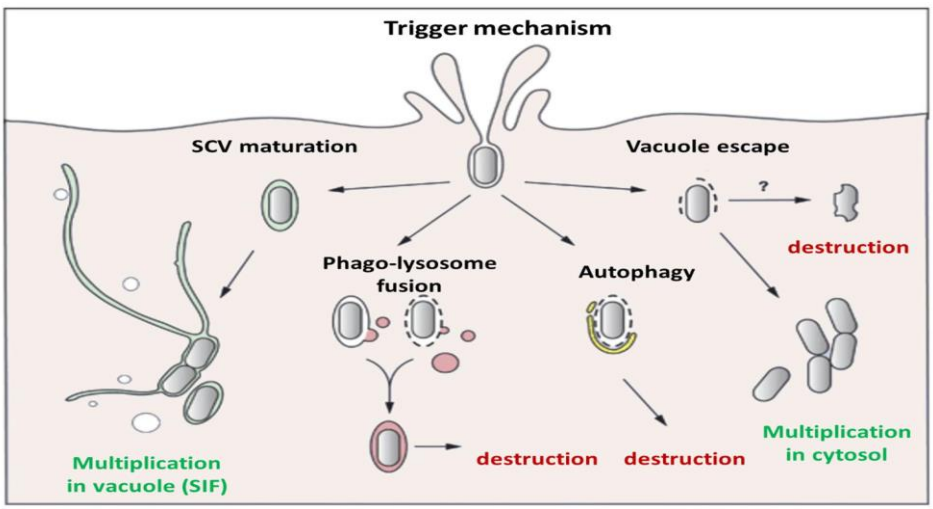

Figure 2.5. Different behaviors of internalized *Salmonella*.

Host Defenses

The ways in which plants and animals perceive and react to invasive organisms vary significantly. They do, however, share some characteristics. When pathogens enter an organism, both in the animal and plant worlds, a fast innate immune response is triggered to stop the pathogen from spreading. Both membrane-bound and intracellular receptors that are germline-encoded are necessary for this response. This initial line of defense in animals is followed by an adaptive response, in which genes producing immunological receptors and antibodies are subjected to somatic rearrangements that enable the detection of extremely specific pathogen epitopes. Production of soluble substances like cytokines and chemokines causes specialized immune cells to activate and move. Last but not least, an immunological memory is created during adaptive response, enabling the creation of an improved response in the event of a repeat encounter with the same pathogen. Since plants lack specialized immune cells, their defense depends on the afflicted cell's capacity to identify the pathogen and trigger the necessary reaction. The general plant immune system has been modeled as a zigzag. There are two different types of systemic resistance that can develop in plants when they come into touch with pathogenic or non-pathogenic helpful bacteria. The plant develops systemic acquired resistance (SAR), which defends it from a variety of infections, when it becomes infected with pathogenic bacteria or fungi. The host plant interacts with soil rhizobacteria or mycorrhizal fungi to produce ISR, the second kind of systemic resistance. There is a wealth of literature on both the animal detection of *Salmonella* and the induced immune response (Broz *et al.,* 2012; Ruby *et al.,* 2012).

Receptors of the innate immune response

Extracellular receptors.

Pathogens in the extracellular environment are detected by membrane-embedded receptors, which are present in both animals and plants. They can identify conserved themes in the architecture of bacteria, viruses, and fungi. The C-type lectin receptors and the Toll-like receptors are the two major classes of these receptors in mammals (TLR). There are two types of extracellular receptors in plants. The extracellular domain of a receptor-like kinase (RLK), which can be an LRR, a lectin, or a LysM domain, a transmembrane domain, and an intracellular kinase domain are all included. The cytoplasmic portion is absent from the receptor-like proteins (RLP), which instead have an extracellular LRR and a transmembrane domain (Wigley, 2013).

Intracellular receptors.

Bacteria create effector proteins that are transported into the host cell's cytosol via the T3SS-1 or T3SS-2 machinery in order to enter and survive in the host cell. Cytosolic receptors in plant and animal cells can recognize these effectors (Man *et al.*, 2014).

Suppression of innate immune response by *Salmonella*

Innate immune receptors and their ligands may interact in animals in one of two ways. The first is the transcriptional activation of multiple genes involved in inflammation, such as IL-6, iNOS, or TNF, as a result of the activation of the MAPKs cascade or the important transcription factor NF-B. The maturation of pro-inflammatory cytokines like IL-1 or IL-18 as well as the cell death process known as pyroptosis are the results of the second process, which is the building of multiproteic scaffoldings called inflammasomes. Pro-caspase 1 is recruited and activated during this process.

Table 2.1: *Salmonella* effectors which inhibit immune signaling pathways

Effector	**Translocated by**	**Inhibit**	**Reference**
AvrA	T3SS-1	NF-kB	Collier-Hyams *et al.,* (2002)
		MAPK	Wu *et al.,* (2012)
SseL	T3SS-2	NF-kB	Le Negrate et al. (2008)
SseK	T3SS-2	NF-kB	Li *et al.,* (2013)
SspHI	T3SS-1	NF-kB	Haraga and Miller (2003)
SpvC	T3SS-1	MAPK	Mazurkiewicz *et al.,* (2008)
SptP	T3SS-1	Syk	Choi *et al.,* (2013)

Salmonella's capacity to survive outside of its hosts is a crucial characteristic that allows this bacterium to occasionally contaminate fresh produce and thus cause outbreaks of food-borne illness. In the past ten years, research has focused heavily on how human enteric pathogens can use plants as alternative hosts. It has become obvious that *Salmonella* not only passively thrives on or within plants but also actively infects them. These interactions, however, have not been thoroughly defined, in contrast to

Salmonella's interactions with animals or animal cells. It has been determined that certain characteristics are shared, such as the utilization of the T3SS or how animals and plants recognize this disease (Wiederman *et al.,* 2015).

2.3 Antibiotic resistance and susceptibility of *salmonella*

The rise of microorganism's resistant to clinically significant medications is a global issue caused by antibiotic resistance, demanding novel therapeutic approaches (Levy and Marshall 2004). Antibiotic-resistant bacteria can infect people with illnesses that are fatal and seriously endanger their health and wellbeing. Antibiotic-resistant bacteria are thought to be responsible for 2 million illnesses and 23,000 fatalities per year in the United States. The U.S. economy loses $35 billion in productivity as a result of these disorders, adding $20 billion to healthcare costs (Agrawal *et al.,* 2016).

For the safety of the public's health, antibiotic resistance in foodborne organisms like *Salmonella* is a key concern. To target them in the animal food supply, more attention is needed. *Salmonella* is challenging to eradicate from its reservoir hosts, and food animals frequently act as the pathogen's reservoirs. The majority of foodborne infections, hospitalizations, and fatalities are brought on by non-typhoidal *Salmonella.* At least 100,000 of these illnesses are caused by antibiotic-resistant *Salmonella,* including those that are resistant to clinically significant medications like ceftriaxone (36,000 illnesses/year) and ciprofloxacin (33,000 illnesses/year). It is linked to more than 1,200,000 illnesses annually (Divet *et al.*,2018).

One of the main issues with worldwide public health is *almonella* infections. *Salmonella spp.* multiresistance and antibiotic resistance have significantly increased during the past ten years, particularly in developing nations where the treatment of humans and animals with antibiotics has expanded and become more indiscriminate (Gordana *et al.,* 2012).

In order to estimate the incidence of *Salmonella* spp. and look into the distribution of serovars and antibiotic resistance of the recovered isolates, 300 retail RTE food products were gathered from 15 Chinese province capitals between 2015.6-2016.6. *Salmonella* spp. were found in seven (2.3%) of the food samples overall. likewise, the MPN concentrations varied from 0.3 to >110 MPN/g. Eight serovars and eight MLST patterns were found among the 26 recovered *Salmonella* isolates, with *Salmonella* enterica serovar Derby ST40 being the most common. All isolated *Salmonella* serovars

have been connected to human illnesses in the past. Thirty percent of the isolates were multidrug resistant, and eighty percent were resistant to at least one class of antibiotics. Tetracycline resistance was highest in isolates (70.0%), followed by tet(A) (60.0%) and tet(B) (10.0%). Instances of *Salmonella* spp. considering the high rates of antibiotic resistance found in the isolates found in RTE meals could pose hazards to human health (Xiaojuan *et al.*, 2022).

Another major hazard to human and animal health is antimicrobial resistance (Roca *et al.*,2015). Its risks are mostly related to the inability to effectively treat patients who are afflicted with diseases resistant to antibiotics and to the significant risk of transmission of such germs. The misuse of antibiotics, particularly their overuse in therapeutic treatments and usage as growth promoters in animal production systems, is a factor in the emergence of this resistance. This is a major problem because a lot of the antibiotic-resistant *Salmonella* have been found in contaminated foods of animal origin, putting human health at risk and driving up healthcare costs. Additionally, there is a risk associated with the use of generic antimicrobials in veterinary and human medicine due to the isolation of certain bacterial strains from animal products used in food that produce -lactamases and/or AmpC -lactamases. Antibiotic resistance in *Salmonella* strains and virulent clones can complicate the management of human infections, make disease control challenging, and pose a serious threat to public health globally. The World Health Organization has designated *Salmonella* as a "priority pathogen" as a result, and it seeks to direct and encourage the development of new medications to combat it (Castro *et al.*, 2020).

Biochemical mechanisms, such as enzymatic inactivation, blocking antibiotic access to the target site, and active efflux pumps, are two key categories of resistance mechanisms that this pathogen has displayed. Additionally, there are genetic pathways including mutation, horizontal gene transfer, and vertical gene transfer. Inappropriate prescription and dispensing practices, poor hygiene practices (external or behavioral factors), and the presence of mobile genetic elements in the organisms, such as plasmid DNA, transposons, and integrons, are some of the factors that have been identified to contribute to the emergence and spread of antibiotic-resistant *Salmonella* (Kabiru and Samuel 2017).

2.3.1 Causes of antimicrobial resistance

When bacteria and other germs adapt, they lose their susceptibility to medical treatment, which is known as antimicrobial resistance. While the incorrect use of antibiotics receives most of the attention, there is mounting evidence that the quality of the medications is also a significant role. Resistance may develop in response to medications that contain less of the active component. Antimicrobial resistance can be addressed by many strategies, such as ensuring that all people have access to reasonably priced medications, properly managing the use of currently available antimicrobial treatments, and funding the discovery of novel therapies (Micheal *et al.*,2014).

2.3.2 Prevalence and antimicrobial resistance patterns of *Salmonella*

According to Miranda (2009), The MICs for 10 antimicrobials were calculated for 93 *Salmonella* strains that were isolated from foods. 35.3% of meat, 30.3% of cheese, 21.8% of vegetables, 17.3% of pork, and 15.1% of beef samples contained *salmonella. Salmonella* isolates showed high resistance to ampicillin (66.7% of isolates), tetracycline (61.3%), and chloramphenicol (64.5%) but low resistance to cefotaxime (0%), gentamicin (3.2%), and kanamycin (4.3%). In contrast to other foods that were evaluated, isolates from meat and vegetables had higher levels of quinolone resistance. With the exception of fish, all meals tested contained high concentrations of multiresistant strains, ranging from 100% of pig samples to 47.1% of vegetable samples. Eight *Salmonella* serovars were discovered in a survey done by Listari et al. (2009) on food samples; the most common ones were Kentucky, Hadar, and Enteritidis. Amikacin, ceftriaxone, and ciprofloxacin were effective against all *Salmonella* isolates, although quinolones (7.1%) and extended-spectrum cephalosporins (45.2%) had lower susceptibilities. 52.4% of the *Salmonella* isolates shown resistance to two or more antimicrobials. These samples contained *Salmonella* Kentucky isolates that were sensitive to 11 of the tested antibiotics. Like in the majority of impoverished nations, Nigerian vegetable producers heavily rely on wastewater for irrigation and untreated manure for soil augmentation. *Salmonella* is one of the most significant foodborne diseases in the world, and one of the main ways it is transmitted to people is through fresh vegetables. *Salmonella*, resistance and virulence genes, and the diversity of isolates were genotyped in 440 samples of vegetables and environmental samples (irrigation water and manure-treated soil). Five irrigation fields in the states of Kano and the Plateau provided the samples, which were then cultivated using the selective isolation with previous enrichment method. The Microbact 24E (Oxoid, UK) identification kit and traditional biochemical techniques were used to identify and

characterize presumed isolates. *Salmonella*'s capacity for virulence was further established by the Polymerase Chain Reaction (PCR) amplification of virulence genes (invasive A and enterotoxin). The genetic variety of confirmed isolates was demonstrated by enterobacterial repetitive intergenic consensus (ERIC) fingerprinting PCR. The susceptibilities of confirmed isolates to eight widely used antimicrobial agents were assessed. 61 (13.9%) of the samples tested positive for *salmonella*. *Salmonella* typhi (7.7%), *Salmonella* paratyphi (2.0%), and *Salmonella* typhimurium (4.1%) were among the serotypes present. S. typhi was most frequently found in vegetables and had the highest rate of isolation. *Salmonella*e exhibit simultaneous resistance to all tested antibiotics. The *Salmonella* bacteria from the various samples had striking similarities and were closely connected genetically, according to their fingerprinting patterns.

2.4 ESBL and multidrug resistance

Multidrug-resistant Enterobacterales (MDRE) pose a growing risk to the world's health by increasing morbidity and death as well as the cost of healthcare. Different -lactamases (such as ESBLs and carbapenemases) are frequently responsible for multidrug resistance, sometimes in conjunction with other resistance mechanisms (e.g., porin loss, efflux) (Janina *et al.,* 2021).

Extended Spectrum Beta-lactamase is an enzyme that some bacterial strains contain (Nkechukwu *et al.,*2014). With the exception of cephamycins and carbapenems, ESBLs are enzymes that can hydrolyze penicillin, cephalosporin, and oxyiminino-lactam compounds (such as cefuroxime, third generation cephalosporin, and aztreonam). The majority of ESBLs are Ambler class "A" -lactamases, and -lactamase inhibitors block their activity (Clavulanate, sulbactam and tazobactam). Since ESBLs are plasmid-mediated, they are quite contagious across Enterobacteriaceae family members. This potential makes -lactam resistance and resistance to other commonly used antibiotics, such as quinolones and aminoglycosides, more likely to spread (Kocagoz *et al.,* 2006).

A class of enzymes known as extended-spectrum beta-lactamases (ESBLs) can break down antibiotics, particularly third-generation cephalosporins and aztreonams, however they are blocked by clavulanic acid. These enzymes can be mediated by chromosomal or plasmid mechanisms, although the plasmid that is more usually seen in Enterobacteriaceae is where they are mostly reported (Rozwandowicz *et al.,* 2018).

ESBLs are ongoing changes that alter the arrangement of amino acids close to the active site of these -lactamases. As a result, new enzymes with expanded substrate profiles are created. Everywhere in the

world, ESBLs are common, and significant amounts of E. coli and Klebsiella pneumoniae strains contain them as well. Three (3) groups of extended spectrum beta-lactamases (ESBLs), Temoniera (TEM), Sulphydryl group (SHV), and CTX-M, having 183, 134, and 103 variations each, have been identified. According to Paterson et al. (2005), TEM and SHV are the predominant kinds in the majority of countries among the variants. In Nigeria, where multi-drug-resistant (MDR) *Salmonella* strains are among the most frequent causes of bacteremia in children, antibiotic resistance, particularly to the most frequently used antimicrobials like Chloramphenicol, Ampicillin, and Cotrimoxazole in humans and in animal production systems, is of critical concern. There is evidence of *Salmonella* serotypes that are less susceptible to fluoroquinolones in humans. Nigeria is one of the African nations where the MDR and decreased susceptibility to Ciprofloxacine linked with NTS have been described. Antimicrobial resistance in the food chain is viewed as a significant public health concern. This is due to the considerable waste in the hunt for new medications and uncertainty since the current medications will not be effective against the serovars (Okpa *et al.*, 2020).

CHAPTER THREE

3.0 METHOD AND METHODOLOGY

3.1. Study area

The bush meat used in this study (n = 55) was purchased from marketplaces in the neighboring states of Ogun (6.99800N, 3.47370E), Lagos (6.52440N, 3.37920E), Ondo (7010'N, 5005'E), Oyo (7.85257N, 3.93125E) and Osun (7.629209N, 4.187218E). The fresh produce samples were gathered in Ogun State's Ilesan, Abeokuta, Sango, Ota, Ijebu-ode, Sagamu, and Mowe because of their proximity to the city of Lagos and their dense populations.

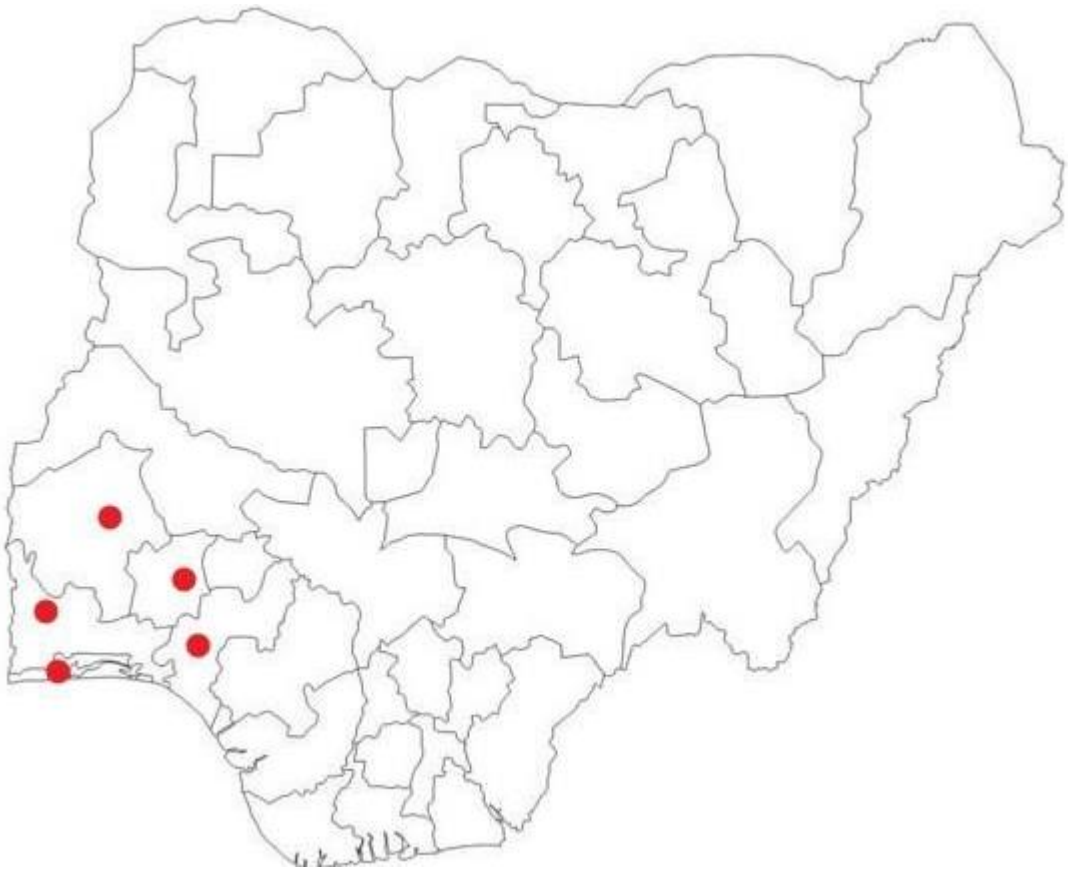

Figure 3.1: Map showing the location of South West (Ogun, Lagos, Osun and Ondo) in Nigeria

Table 3.1: Game meat samples and location

LOCATION	GAME-MEAT	NUMBER OF SAMPLES
Lagos State	Pangolin Bird Deer Bush dog Grasscutter Etu Wild Cat Atika Agbonrin Antelope Monkey Rabbit Porcupine	25
Ogun State	Antelope Grasscutter Rabbit Bush rat Igala Hedgehog Guinea fowl Alligator	12
Ondo State	Civet Cat Rabbit Antelope Grasscutter Grasscutter Guinea Fowl	9
Osun State	Hare Sese Antelope	5
Oyo State	Aparo Eta Esii Tuku Guinea Fowl	4
Total		55

3.1.1 Sample collection

From Ogun, Lagos, Ondo, Osun and Oyo states, smoked game meat was gathered. While fresh produce was gotten from various markets in Ogun state (Ilesan, Abeokuta, Sango, Ota, Ijebu-ode, Sagamu and Mowe). The purchased samples were delivered to the lab, where they were immediately tested, and then maintained in sterile food-grade bags with ice packs in cooling boxes.

3.2 Reagent and equipment used

Reagents: Xylose lysine desoxycholate (XLD agar), Rappaport-Vassiliadis Soya Peptone broth (RVS), Hektoen Enteric Agar (HEA), 20% Glycerol, Brain Heart Infusion Broth (BHI), Buffer Peptone Water, Distilled water, 1% Buffered peptone water (BPW), 0.1% BPW.

For molecular identification:

Agarose, 1x TAE buffer, master mix, specific primers, Nuclease free water, Ethidium Bromide.

3.2.1 Materials

Petri dish, glass spreader, inoculating loop, cotton wool, 70% ethanol, latex hand gloves, beaker, wash brush, markers, measuring cylinder, conical flask, test tubes, test tubes racks, Duran (scotch bottles), Eppendorf tubes, cork borer, sterile tips, micropipette, distilled water, paper tape, thin foil, weighing balance stomacher bags, wash bottles, glass pipettes, PCR tubes, Hot plate stirrer, Autoclave, Vortex mixer, Water distiller, Water bath (set at 80°C), Incubator (37°C), Bunsen burner, Centrifuge, Heating block, Thermal cycler, Gel electrophoresis tanks, Gel documentation system.

3.3 Preparation of culture media

Different selective and differential medium were utilized for enhancement during the isolation and identification of *Salmonella* isolates.

3.3.1 Buffer peptone water (BPW)

Peptone water is a microbiological growth medium made of sodium chloride and peptic digest of animal tissue. The medium is rich in tryptophan and has a pH of 7.20.2 at 25 °C. Peptone water can be used as a main enrichment medium for the growth of bacteria and is also a nonselective broth medium.

Preparation

1. The dehydrated medium (1g) was dissolved in the appropriate volume (1 litre) of distilled water to make up 0.1% peptone water based on manufacturers instruction's instructions in a conical flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminum foil.
2. The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15mins.
3. It was then dispensed by pipetting into various test tubes for serial dilution.

3.3.2 Brain heart infusion broth (BHI)

Microorganisms can develop on a growth media called brain heart infusion (BHI). It is a nutrient-rich medium that can be used to cultivate a wide range of discerning organisms. BHI is widely utilized in both clinical and research settings to cultivate a wide range of microorganisms. Numerous picky species, such as certain bacteria, yeasts, and fungi, thrive on BHI.

Preparation

1. 37 grams of the dehydrated medium was dissolved in 1000 ml of distilled water based on manufacturer's instructions in a conical flask and was mixed thoroughly. The conical flask is then closed using a foil cork (made up of cotton wool wrapped in aluminum foil).
2. The mixture was stirred for a while using the magnetic stirrer to completely dissolve the powder.
3. 5ml of the media was then dispensed into various test tubes and covered with foil cork and was then sterilized by autoclaving at 121^0C for 15minutes.

3.3.3 Rappaport vassiliadis soya peptone broth (RVS BROTH)

For the isolation of *Salmonella* species, Rappaport Vassiliadis Soya Peptone Broth is utilized as an enrichment growing medium. It enhances *Salmonella*e because, in comparison to other gut bacteria, they can multiply at substantially lower pH levels and higher temperatures and are better able to withstand the high osmotic pressure in the medium. The pH of RVS broth is 5.2 or such.

Preparation

1. 26.6g of the medium was dissolved in 1000ml of distilled water based on manufacturer's instructions in a conical flask and was mixed thoroughly. The conical flask is then closed using a foil cork (made up of cotton wool wrapped in aluminum foil).
2. The mixture was stirred for a while using the magnetic stirrer to completely dissolve the powder.
3. 9ml of the media was then dispensed into various test tubes and covered with foil cork and was then sterilized by autoclaving at 121^0C for 15minutes.

3.3.4 Xylose lysine deoxycholate agar (XLD)

A specific medium for the isolation of *Salmonella* and Shigella spp. from clinical specimens and food samples is Xylose Lysine Deoxycholate (XLD) Agar. It is advised to test foods, dairy products, and water using XLD Agar, which is a component of the USP microbiological limit test for determining if *Salmonella* is present or absent in specimens.

Preparation

1. Suspend 55 grams of dehydrated medium in 1000 ml purified or distilled water.
2. Heat with frequent agitation until the medium boils. Note: DO NOT AUTOCLAVE.
3. Transfer immediately to a water bath at 50°C.
4. After cooling, pour into sterile Petri plates.

3.3.5 Hektoen enteric agar (HEA)

A selective and differentiating medium called Hektoen Enteric Agar was created to isolate and distinguish *Salmonella* and Shigella from other Enterobacteriaceae species.

Preparation

1. Suspend 72.66 grams in 1000 ml purified/ distilled water.
2. Heat to boiling to dissolve the medium completely. Note: DO NOT AUTOCLAVE.
3. Cool to 45-50°C.
4. Mix well and pour into sterile Petri plates.

3.3.6 Mueller-hinton agar

Mueller-Hinton Most antimicrobial susceptibility tests are conducted using agar (AST). It has been chosen as the standard medium for the Bauer-Kirby method by the Clinical & Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing, respectively. (EUCAST).

Preparation

1. 38g of dehydrated medium was dissolved in the appropriate volume of distilled water based on manufacturer's instructions (1 litre) in a conical flask and was mixed thoroughly. The conical flask is then closed using a foil cork (made up of cotton wool wrapped in aluminum foil).
2. The mixture was stirred for a while using the hot plate magnetic stirrer to completely dissolve the powder.

The mixture was then sterilized by autoclaving at 121°C for 15 minutes.

3.4 Sample preparation

A conical flask was filled with 225 ml of 0.1% peptone water (enrichment broth) and 25 g of each game meat using a sterile blade. The samples were homogenized in a stomacher for 2 minutes. then kept at 37°C for 24 hours. *Salmonella* will use this as its major enrichment.

3.4.1 Secondary Enrichment

The secondary enrichment media was inoculated with the BPW primary enrichment that had been incubated.

RVS was employed as a secondary enrichment. It contains a particular nutrient that *Salmonella* species need to flourish.

In test tubes containing 9 ml of RVS, one milliliter of the primary enrichment was pipetted using a micro-pipette with a 1000 l setting. For correct identification, the test tubes were labeled, and it was incubated at 42°C for 18 to 24 hours.

3.4.2 Sub-culturing

To purify the isolated bacterial colonies, sub-culturing is used to transform them from a mixed culture to a fresh, single culture. The bacterial isolates that underwent sub-culturing were those that could be distinguished by their colony morphology, shape, color, elevation, and other physical traits.

Using the streaking method, a loopful of the isolate was taken using the inoculating loop and streaked onto the new XLD petri dish. The plates were then inverted and incubated at 37°C for 18–24 hours. The inoculating loop was heated using the Bunsen burner and allowed to cool before being taken from the incubated RVS test tube.

After the amount of time needed for the development to necessitate subculturing, the plates were examined. To create a pure culture, the isolated bacterial colonies underwent subculturing. Colonies that could be distinguished by their color (black) were put onto brand-new petri plates with HEA agar. The inoculating loop, which is heated with a Bunsen burner and allowed to cool for about 7 seconds before taking the loop from the original mixed culture, was used to take a loopful of the preferred isolates and streak them onto the new petri dish.

3.3 Preservation of isolates

Salmonella spp. were isolated in pure culture and incubated on Hektoen enteric agar (HEA) for 18 to 24 hours in a sterile environment before being inoculated into 5 ml of BHI broth. Following incubation, 750 l of the inoculum was put to a sterile Eppendorf tube that also contained 750 l of sterile 20% sterile glycerol (duplicated), which acts as a cryoprotectant, and it was kept at -40C.

3.4 DNA extraction

1. The isolates were centrifuged for 5 minutes at 10,000RPM, then the supernatant was decanted.
2. The Eppendorf tube was filled with 1ml of sterile distilled water, vortexed, and centrifuged at 10,000 RPM for 5 minutes, the supernatant was discarded, and the process was repeated.
3. After which, 200 µl of sterile nuclease-free water was pipetted into the Eppendorf tube containing the pellets, vortexed, and then placed in the heating block to boil at 100◦C for 15 minutes, the solution was then placed in ice to cool, and the contents of the Eppendorf tube were centrifuged for 5 minutes at 14,000RPM.
4. A new set of Eppendorf tubes were labelled with the corresponding codes of the isolates and 150μl supernatant containing the extracted DNA was then transferred into the new Eppendorf tubes and stored in the freezer at -20^{o}C for further analysis.

3.5 Polymerase chain reaction

The components of the PCR used to identify *Salmonella* spp. are listed in table 3.2 below.

1. The PCR cocktail was made and then transferred to a PCR tube before being placed in the thermocycler.
2. Initial denaturation was performed at 95°C for 5 minutes, followed by 35 cycles of 95°C for 2 minutes, 42°C for 30 s, and 72°C for 4 minutes, followed by a final elongation step at 72°C for 10 minutes.
3. There were also negative control reactions provided. The template DNA was replaced with sterile water for the negative controls.
4. Electrophoresis was used to confirm the PCR products, and a Gel Documentation system was used to visualize them under UV light.

Table 3.2: PCR reaction component used for *Salmonella* spp., amplification

No.	**Reagent**	**Initial concentration**	**Final concentration**	**Volume/rxn**
1.	Master Mix	5x	1x	2µl
2.	Forward Primer	20µm	0.4µm	0.2µl
3.	Reverse Primer	20µm	0.4µm	0.2µl
4.	dH_2O			5.6µl
5.	DNA			2µl
6.	Total			10µl

Table 3.3: *Salmonella* primer sequence

Primer	Target gene	Target	PCR product size (bp)	Sequences	Reference
STM4057-f	STM4057	*Salmonella* subspecies I	137	5’ -GGTGG CCTCG	Kim et al. (2006a)

	ATGAT TCCCG-3’
STM4057-r	5’ -CCCAC TTGTA GCGAG CGCCG-3’

Table 3.4: Protocol for thermal cycler

Analysis	**Step**	**Temperature**	**Time**
1x	Initial denaturation	95℃	5 min
35x	Denaturation	95℃	2 min
	Annealing	42℃	30 sec
	Polymerization	72℃	4 min
1x	Final polymerization	72℃	10 min
1x	Hold	4℃	∞

Table 3.5: PCR reaction components used for 16sRNA amplification

Reagent	**REACTION**
Mastermix	2 µl
16sRNAf	0.125 µl
16sRNAr	0.125 µl
H20	5.75 µl
DNA	2 µl
Total	10

3.6 Agarose gel electrophoresis

1. Dry Agarose powder was used to make the agarose, and 1.8g of it was dissolved in 100ml of 1x TAE buffer. The mixture was heated until a clear solution was obtained.
2. A micropipette was used to add 3 µl of ethidium bromide to the mixture. It was then spun, allowed to cool and then transferred to the gel tray which results in a slab with the combs in

place, that create wells in the slab. It was then allowed to solidify before carefully removing the combs.

3. The gel tray containing the slab was placed into the gel tank and 1x TAE buffer was added to the gel tank. 3 µl of DNA ladder was added to the first well, and 4 µl of amplicon (one sample per well) was pipetted into each well that was produced.
4. The tank was attached to the power pack and the gel was ran at 100 volts for 45 minutes. The resulting gel was inspected using the gel documentation system.

3.7 ESBL

Isolates were examined for the ESBL phenotype. On the same plate, screening and confirmatory tests were run concurrently. Briefly, Mueller-Hinton agar was used to plate each test isolate (Oxoid, UK). A 0.5 McFarland suspension of each was made in peptone broth (MAST, UK) after an overnight incubation at 37 °C. Then, this suspension was swabbed onto a Mueller-Hinton agar plate that was balanced on cations (MAST, UK), and allowed to dry fully. The plate was covered with antibiotic discs from a D52C ESBL detection kit (MAST, UK), and the apparatus was then incubated at 37 °C for 18 hours. Cefoperazone (30 g), Ceftazidime (30 g), and clavulanic acid (10 g) were placed on discs. Inhibition zone diameters were measured and evaluated following incubation. For any of the antibiotics, a zone difference of 5 mm between the single and combination disks was regarded as positive for ESBL formation.

3.7.1 DNA extraction

ESBL-producing donor isolates and transconjugants both had their DNA extracted using the techniques previously mentioned. 10 l of each extract were combined with 2 l of 6X gel loading dye for electrophoresis, which was carried out on 0.7% agarose gel stained with 1 g/ml ethidium bromide. In 1X Tris-acetate-EDTA (TAE) buffer, electrophoresis was performed at 10 V/cm for 1 hour, and the results were seen under UV transillumination. The resulting plasmid DNA samples were applied to further PCRs.

3.7.2 PCR for ESBL Genes

In 25 l reaction mixes including 25 units/ml of Taq DNA polymerase, 200 M of each of dATP, dGTP, dTTP, and dCTP, 0.2 M of each primer, 1.5 mM MgCl2, and 5 l of plasmid or whole DNA template, PCR amplifications of the blaTEM, blaSHV, and blaCTX-M genes were carried out. The following

thermal cycling profile was used for the amplifications: initial denaturation for 15 min at 95 °C, 35 cycles of amplification consisting of 30 s at 94 °C, 1 min at the correct annealing temperature for the particular primer, 1 min at 72 °C for primer extension, and 10 min at 72 °C for the final extension with a soaking step at 4 °C. Reagent concentrations were changed to use just half the primer concentration from the prior reaction and 1.0 l of the prior product as the template in cases where reamplifications of the prior PCR product were required. Table 7 displays the employed primers and the annealing temperatures that correlate to them. 10 l of each PCR product were produced and ran through electrophoresis according to the previous instructions.

Table 3.6: Sequences, annealing temperatures and expected product sizes of primer sequences targeting the specified ESBL genes (Daniel *et al.,* 2016)

Gene	Primer	Annealing temp. (℃)	Expected product size (bp)
bla$_{TEM}$	f: 5′-AAA CGC TGG TGA AAG TA-3′ r: 5′-AGC GAT CTG TCT AT-3′	45	720
bla$_{SHV}$	f: 5′-ATG CGT TAT ATT CGC CTG TG-3′ r: 5′-TGC TTT GTT ATT CGG GCC AA-3′	60	726
bla$_{CTX-M}$	f: 5′-GAC GAT GTC ACT GGC TGA GC-3′ r: 5′-AGC CGC CGA CGC TAA TAC A-3′	55	499

3.8 Antibiotic susceptibility test

The Kirby-Bauer standard disk diffusion technique and Clinical Laboratory Standards Institute (CLSI) guidelines were followed when conducting the antibiotic susceptibility test. Each strain underwent the disc diffusion test on Mueller-Hinton agar (Oxoid, England). Test tubes containing Brain Heart Infusion broth (OXOID, England) were prepared and autoclaved. 5ml of BHI was

injected with *Salmonella* isolates, and the mixture was then incubated at 37°C for 24 hours. Mueller Hinton agar was made, autoclaved, then added to sterile Petri plates and given time to set.

The turbidity of each isolation culture was compared to 0.5 McFarland standards (if necessary, adjusted by adding sterile saline into tubes until culture was more turbid). Isolates were inoculated onto Mueller-Hinton agar using swab sticks, and the inoculated plates were left at room temperature for 30 minutes to allow drying. Antibiotic-impregnated discs (Celtek Diagnostic, Belgium Inc.) were distributed over the surface of Muller-Hinton agar cultures and incubated for 20 hours at 37^0C. *Salmonella* isolates were tested using the disk diffusion method for susceptibility to the following 12 antibiotics: chloramphenicol (30 μg), cefotaxime (30 μg), gentamicin (10 μg), cefuroxime (300μg), cotrimoxazole (30 μg), tetracycline (30 μg) cefoperazone (30 μg), meropenem (300 μg), vancomycin (10 μg), ciprofloxacin(30 μg), amikacin (5 μg), ceftriaxone sulbactam (45 μg), utilizing the disk diffusion technique in accordance with the Clinical Laboratory Standards Institute's guidelines (CLSI, 2020). According to an established interpretative chart (CLSI, 2020), the diameters of the zones of inhibition were measured to the closest millimeter and categorized as resistant, intermediate, or susceptible.

3.9 Precautions

- Personal protective equipment, such as a covered shoe, a nasal cover, gloves, and a lab coat, were observed.
- At every stage of the project, aseptic practices were observed.
- There was no cross-contamination of the samples.
- Ensured that the samples were appropriately labeled at all times.
- To avoid killing the organism of interest, the inoculating loop was allowed to cool before making contact with the organism for sub-culturing.
- Ensured that the petri-dish was incubated inverted.
- Ensured proper timing, most especially during autoclaving.

3.10 Data Analysis

All data were entered into Microsoft Excel 2016 which was used to calculate the mean and standard deviation of microbial counts as well for descriptive analysis of data such as graphs, bar charts etc.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

A total of 55 samples from game meat and 60 samples from fresh produce were analyzed in this study. The prevalence of *Salmonella* spp in game meat was 89% while fresh produce samples had a low prevalence of 20% respectively (Fig. 4.1). Grasscutter showed higher prevalence of *Salmonella* spp. while porcupine, quail and alligator showed zero prevalence of *Salmonella* in this study (Fig. 4.2).

Table 4.1: Prevalence of *Salmonella* spp. in Game meat and Fresh Produce in South-west, Nigeria. (n=115)

Sources	Number of samples	Number of positive samples	Prevalence
Fresh produce	60	12	20
Game meat	55	45	89

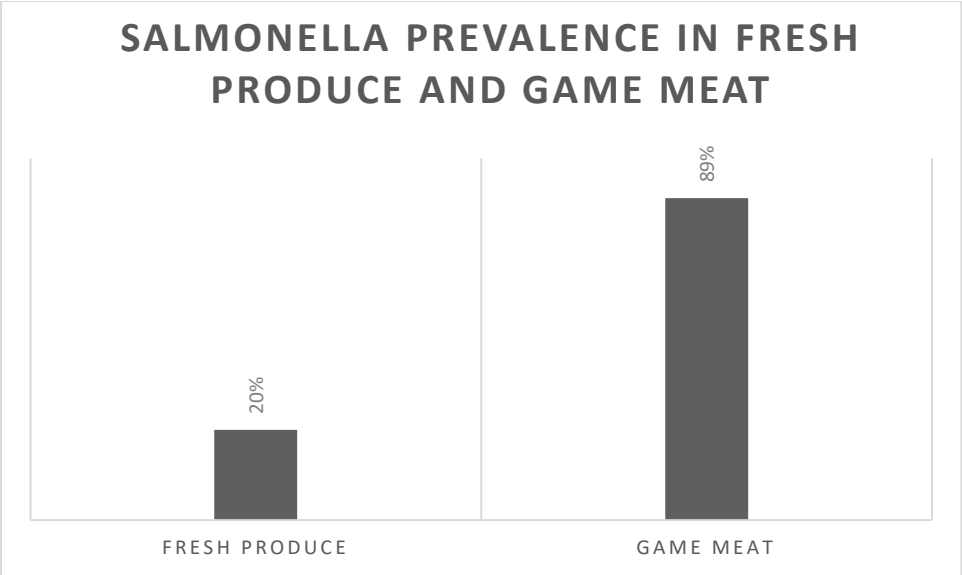

Fig. 4.1: Prevalence of *Salmonella* in fresh produce and game meat

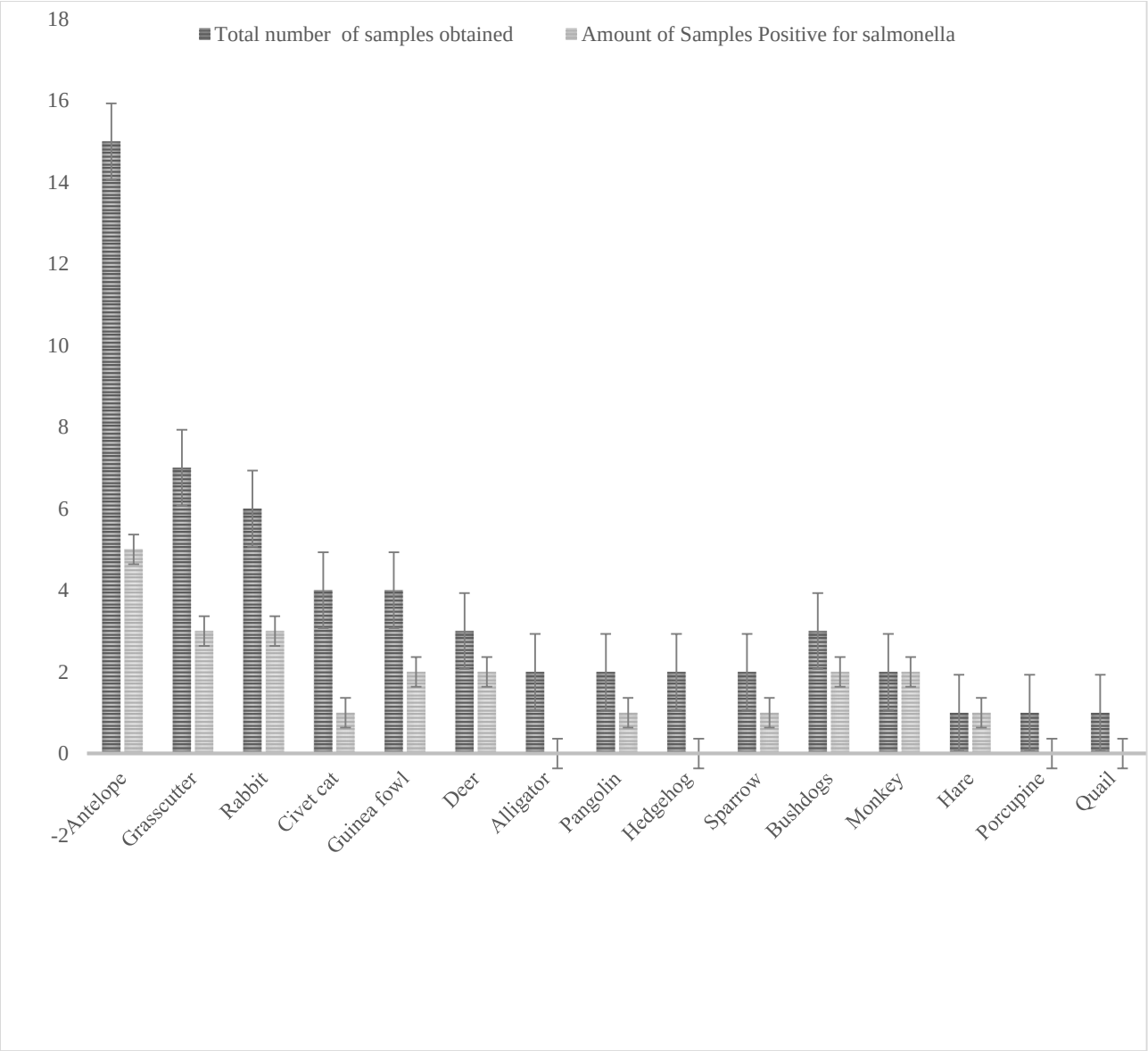

Fig. 4.2: Charts of microbial analysis showing the prevalence of *Salmonella* spp in the bushmeat samples.

4.1.1: Prevalence of *Salmonella* spp. in Fresh produce in South-west, Nigeria.

Prevalence of *Salmonella* spp was significantly high in carrot in this study while there was no record of *Salmonella* in pineapple and cabbage.

Table 4.2: Prevalence of *Salmonella* spp. in Fresh produce in South-west, Nigeria. (n=60)

Fresh Produce	Number of samples	Number of positive samples	Prevalence
Cucumber	6	1	16
Carrot	12	7	58
Watermelon	8	1	13
Pineapple	8	0	0
Pawpaw	10	2	20
Lettuce	6	1	16
Cabbage	10	0	0

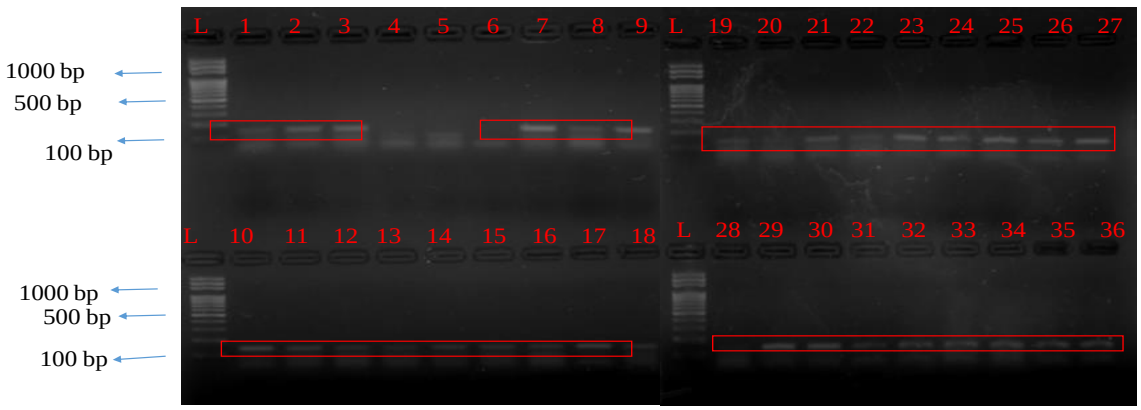

Fig. 4.3: Illustrative agarose gel electrophoresis image of a Simplex PCR assay of bushmeat samples for detecting the *Salmonella enterica* subsp. I (SSI) gene (137 bp). Lane L: molecular weight marker 100 bp (Bio-Mark). Lane 1: positive control strain (*Salmonella Typhimurium* CCM 7205). Lanes 2-36: *Salmonella* spp. strain isolated from bushmeat

Extended spectrum bectalactamase molecular result

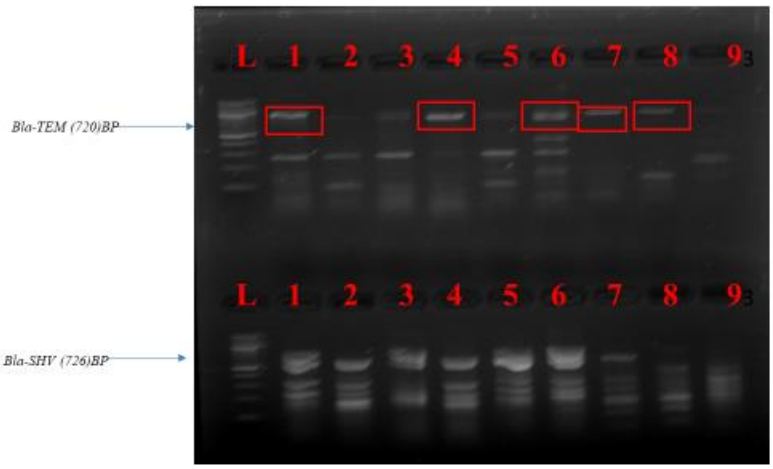

Fig 4.4: Illustrative agarose gel electrophoresis image of a simplex PCR assay of ESBL for detecting *Salmonella enterica* subsp. I (SSI) gene (137 bp). Lane L: molecular weight marker 100 bp (Bio-Mark). Lane 1,4,6,7,8(*Bla-TEM* [720BP]) and Lane 1-7 (*Bla-SHV* [726]BP).

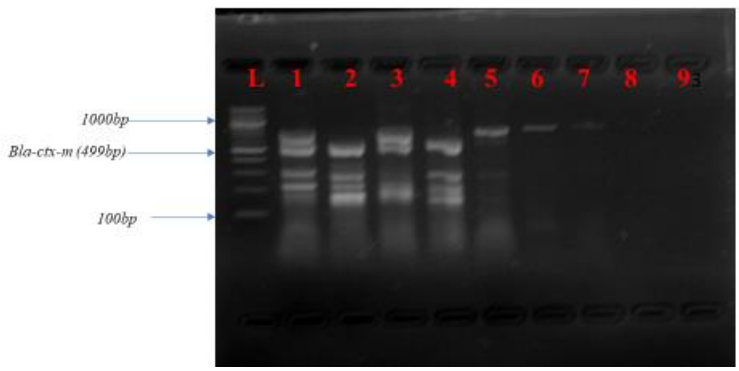

Fig 4.5: Illustrative agarose gel electrophoresis image of a simplex PCR assay of ESBL for detecting *Salmonella enterica* subsp. I (SSI) gene (137 bp). Lane L: molecular weight marker 100 bp (Bio-Mark). Lane 1-4(*Bla-ctx-m* [499bp])

Table 4.3: Antimicrobial Susceptibility Pattern of *Salmonella* spp Isolates from game meat and fresh produce samples in South-western, Nigeria

Antibiotics	Disc Code	Antibiotic Disc Content (µg/disc)	Number of *Salmonella* Isolates (n =12)		
			R	I	S
Chloramphenicol	CHL	30	7	2	3
Cefuroxime	CRX	300	4	-	3
Gentamicin	GEN	10	9	-	2
Cotrimoxazole	COT	30	7	5	-
Tetracycline	TET	30	8	-	-
Cefoperazone	CPZ	30	9	4	1
Cefotaxime	CTX	30	12	3	2
Ceftriaxone	CTR	45	6	3	3
Meropenem	MEM	300	4	-	10
Vancomycin	VAN	10	5	3	12
Ciprofloxacin	CIP	30	5	2	9
Amikacin	AMK	5	7	5	12

Key: AUG: Chloramphenicol (30 μg), CRX: Cefuroxime (300 μg), GEN: Gentamicin (10 μg), COT: Cotrimoxazole (30 μg), TET: Tetracycline (30 μg),CPZ: Cefoperazone (30 μg), CTX: Cefotaxime (30 μg), CTR: Ceftriaxone (45 μg), MEM: Meropenem (300 μg), VAN: Vancomycin (10 μg), CIP: Ciprofloxacin (30 μg), AMK: Amikacin (5 μg).

Multidrug resistance by the 12 isolates to all the antibiotics used in this study was observed. The importance of this resistance is highly significant because these antibiotics are commonly used nowadays. The majority of the *Salmonella* spp. strains from fresh produce samples showed different resistances to several antibiotics. Among the 12 *Salmonella* spp. strains tested, all strains showed resistance to two or more antibiotics. High levels of resistance were observed for Cefotaxime 100% (12/12), Cefoperazone 75% (9/12), Gentamicin 75% (9/12), Tetracycline 66.6% (8/12), Cotrimoxazole 58.3% (7/12), Chloramphenicol 58.3% (7/12), Ceftriaxone 50% (6/12) and Cefuroxime 33.3% (4/12).

There was a high susceptibility of the *Salmonella* strains to Meropenem 83.3% (10/12), Ciprofloxacin 75% (9/12), Vancomycin and Amikacin 100% (12/12) (Table. 4.3)

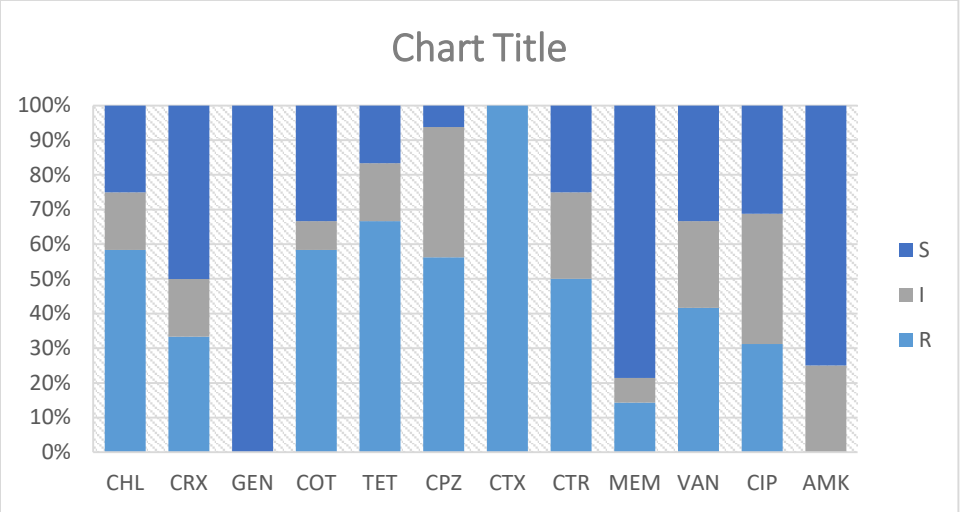

Fig. 4.6: Illustration showing the antibiotic resistance, susceptibility and intermediate of *Salmonella* spp.

Table 4.4: *Salmonella* isolates and multidrug resistance pattern in fresh produce

Salmonella Isolates	Multidrug resistance patterns											
MSIE1S1	CHL	CRX	GEN	COT*	+	CPZ*	CTX	CTR	+	+	CIP	+
MBIC2S3	CHL	CRX	GEN	COT	+	CPZ	CTX	CTR	MEM	+	+	+
MNIU1S1	+	+	GEN	+	TET	CPZ	CTX	+	+	+	+	+
MTIC1S2	CHL*	CRX	GEN	COT	TET	+	CTX	CTR*	+	+	+	+
MBIC2S2	CHL	CRX*	GEN	COT	TET	CPZ	CTX	+	+	+	CIP	+
MDIW1S1	CHL	CRX*	GEN	COT	TET	CPZ	CTX	CTR	+	+	+	+
MAIP1S1	CHL	+	GEN	COT*	TET*	CPZ	CTX	CTR*	+	+	+	+
MNA2S1	CHL	+	GEN	COT	TET	CPZ*	CTX	CTR	+	+	CIP	+
MSIC2S1	+	CRX*	+	+	TET	CPZ	CTX	CTR	+	+	+	+
MSIC2S2	CHL	CRX*	+	COT	TET	CPZ	CTX	+	MEM	+	+	+
MMIC2S1	CHL*	CRX	+	COT	TET	CPZ	CTX	CTR	+	+	+	+
MT1C1S1	+	CRX	+	+	+	CPZ	CTX	CTR*	+	+	+	+

Key- Resistant: CHL, CRX, GEN, COT, TET, CPZ, CTX, CTR.

Intermediate resistance: **CHL*, CRX*, COT*, TET*, CPZ*, CTR*,**

Susceptible: +

Although there was multiple resistance, VAN and AMK were the most effective antibiotics particularly VAN. The disk contents used were the same as those described in the CLSI 2020 document.

Plate 4.1: ESBL disk diffusion test performed on Mueller-Hinton Agar

4.2 Discussion

This study revealed that MDR resistant *Salmonella* is present in Game meat and Fresh produce purchased from South-western parts of Nigeria. Game meat and fresh produce are now recognized as major vehicle of food-borne disease outbreaks. This study reveals the presence of *Salmonella* spp in game meat and fresh produce with prevalence of 89% and 20% respectively.

All *Salmonella* Isolates identified from the fresh produce were multidrug resistant (MDR). The rise of MDR *Salmonella* isolates raises the possibility that they originated in regions where antibiotics are frequently overused or utilized to treat ailments and increase animal growth, and where feces are employed as organic manure for the cultivation of fresh produce. Multiple resistance by the 12 isolates to all the antibiotics means the isolates can be termed as Multi-Drug Resistant (MDR) because resistance to two or more antibiotics tested was exhibited. This is disturbing for the general public's health because it reduces the first-line antibiotics' ability to treat non-typhoidal salmonellosis and it makes the selection of antibiotics for treatment of these conditions more challenging.

Detection level of ESBL-producing strains in the isolates tested exhibited 42% prevalence (n = 5). It is important that subsequent work carried out on ESBL-producing strains should take ampC *β*-lactamase detection as well as influence from other types of β-lactamases that may be present into account. (Smet *et al.,* 2010). This is because as more and more isolates begin to concomitantly produce these enzymes, interactions between them will make antibiograms of the isolates rather difficult to interpret. (Oduro *et al.,* 2016). In the majority of cases, ESBL-producing bacteria in the hospital could transfer the phenotype by conjugation. Interpretative reading of the inhibition-zone chart suggests that other types of β-lactamases such as inhibitor-resistant TEMs (IRTs) and ampCs might have been present amongst the isolates. (Al-Taie, 2019).

The development of multi-drug resistance in the serotypes of *Salmonella* has a significant impact on the antibiotic treatment of *Salmonella* infections. Infections that involve the invasive serotypes are often life threatening and require effective antibiotic treatment. (Karon et al.2007).

The emergence of MDR *Salmonella* has also resulted in the increased severity of bacterial infections in humans and animals. Epidemiological studies show that MDR *Salmonella* strains cause more severe or prolonged syndromes than susceptible strains, implying that the MDR strains are more virulent than the susceptible ones. Data show that patients infected with MDR *Salmonella* strains are more ill and

septic at the onset of the disease, and the illness is typically accompanied by high fever, enlargement of the spleen and liver, and abdominal swelling. (Zaki *et al.*, 2011)

CHAPTER 5

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Salmonella infection remains a distressing public health concern worldwide. The genetic make-up of the *Salmonella* strains permits their adaptation in various environments, including human, animal and non-animal hosts. This increases the difficulty in eliminating the bacteria. Moreover, the emergence of MDR *Salmonella* strains poses a great challenge in terms of effective treatment of the infections caused by these strains. Several preventive measures have been proposed to stop the spread of *Salmonella* infection, and the restriction of indiscriminate use of antibiotics in food animals is by far one of the most effective measures. (Ruvalcaba *et al.,* 2022). Based on the findings of this study and the deductions derived from there, it could be concluded that game meat and fresh produce collected from South-western parts of Nigeria are contaminated with strains of pathogenic *Salmonella* spp, although the frequency of contamination was found to be low. In this study, carrots were considered as the major risk factor associated with the occurrence of *Salmonella* for fresh produce while Antelope is the major risk factor for game meat. In addition, this study has demonstrated that the samples collected may contribute to the prevalence of *Salmonella* in the South-western parts of Nigeria.

5.2 Recommendations

In light of the findings of the study, the following recommendations are made. Considering the low infective dose of this organism and the fact that these game meat or fresh produce can be consumed raw without any form of cooking, the general public need to be educated on the risk involved in the consumption of contaminated fruits and vegetables. The consumption of game meat should be reduced and should be heated or grilled before consumption. To prevent cross-contamination, fruits and vegetables should be stored separately from raw animal products. In order to prevent contamination of fresh fruits and vegetables from Farm to Fork, proper precautions must be followed. Antibiotic resistance can be prevented but not stopped because it is a normal process of bacterial evolution, hence antibiotic use must be managed and used carefully in order to prevent the spread of antibiotic resistance among *Salmonella* serovars. Due to this, we require fresh antibiotics to fight off bacteria that are resistant, as well as fresh diagnostic procedures to track the development of resistance. In order to dramatically slow the development and spread of illnesses that are

resistant to antibiotics, it may be necessary to change how antibiotics are used. Antibiotic stewardship is the process of only choosing and using the best antibiotics correctly and safely when they are needed to treat illness (Dafale *et al.*, 2016). By adhering to fundamental and basic hygienic principles when preparing and storing food, consumers can contribute to the prevention of infections and the slowing down of the spread of antibiotic resistance because infections are avoided if they can, reducing the need for antibiotics in the first place.

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APPENDIX

APPENDIX 1: Description of positive *Salmonella* isolates in game meat

Isolate coding	Description
A1L1S1	Epe fish market, Lagos State. Antelope
A1L1S2	Epe fish market, Lagos State. Antelope
A2L1S1	Epe fish market, Lagos State. Antelope
A2L1S2	Epe fish market, Lagos State. Antelope
A2L1S3	Epe fish market, Lagos State. Antelope
A2L1S4	Epe fish market, Lagos State. Antelope
AN01S1	Oja-oba market, Ondo State. Antelope
AN01S2	Oja-oba market, Ondo State. Antelope
AN01S3	Oja-oba market, Ondo State. Antelope
AN01S4	Oja-oba market, Ondo State. Antelope
AN01S5	Oja-oba market, Ondo State. Antelope
AN01S6	Oja-oba market, Ondo State. Antelope
AN01S7	Oja-oba market, Ondo State. Antelope
AN01S8	Oja-oba market, Ondo State. Antelope
B1L1S1	Epe fish market, Lagos State. Bush dog
B2L1S1	Epe fish market, Lagos State. Bush dog
B2L2S1	Oluwo market, Lagos State. Bush dog
BL1S1	Festac, Lagos State. Bird
BL1S2	Oluwo market, Lagos State. Bush dog
CC0101S2	Oja-oba market, Ondo State. Civet Cat
CC01S1	Oja-oba market, Ondo. Civet cat
D1L1S2	Epe fish market, Lagos State. Deer
D2L1S1	Epe fish market, Lagos State. Deer
D2L1S2	Epe fish market, Lagos State. Deer
DL1S1	Epe fish market, Lagos State. Deer

DL1S2	Oluwo market, Lagos state. Deer
G1L1S2	Oluwo market, Lagos State. Grasscutter
G3L1S1	Epe fish market, Lagos State. Grass cutter
G3L1S2	Epe fish market, Lagos State. Grasscutter
G4L1S1	Epe fish market, Lagos State. Grass cutter
GL1S1	Oluwo market, Lagos State. Grasscutter
GL1S3	Oluwo market, Lagos State. Grasscutter
GR01S1	Oja-oba market, Ondo. Grasscutter
GR01S2	Oja-oba market, Ondo. Grasscutter
GRS1S1	Sango Ota, Ogun State. Grasscutter
GUA1S1	Abeokuta, Ogun State. Guinea Fowl
IGS1S1	Sango Ota market, Ogun State. Igala
M1S3	Epe fish market, Lagos State. Monkey
M1S4	Epe fish market, Lagos State. Monkey
M1S5	Epe fish market, Lagos State. Monkey
P1L1S1	Epe fish market, Lagos State. Porcupine
P1L1S2	Epe fish market, Lagos State. Porcupine
PA2L1S1	Festac, Lagos State. Pangolin
RA01S1	Oja-oba market, Ondo. Rabbit
RA01S10	Oja-oba market, Ondo. Rabbit
RA01S11	Oja-oba market, Ondo. Rabbit
RA01S114	Oja-oba market, Ondo. Rabbit
RA01S12	Oja-oba market, Ondo. Rabbit
RA01S13	Oja-oba market, Ondo. Rabbit
RA01S15	Oja-oba market, Ondo. Rabbit
RA01S2	Oja-oba market, Ondo State. Rabbit
RA01S3	Oja-oba market, Ondo. Rabbit
RA01S4	Oja-oba market, Ondo State. Rabbit

RA01S5	Oja-oba market, Ondo State. Rabbit
RA01S6	Oja-oba market, Ondo State. Rabbit
RA01S7	Oja-oba market, Ondo. Rabbit
RA01S8	Oja-oba market, Ondo. Rabbit
RA01S9	Oja-oba market, Ondo. Rabbit
SL1S2	Festac, Lagos State. Sparrow

APPENDIX 2: Description of *Salmonella* positive isolates in Fresh produce

ID NO	**CODE**	**DESCRIPTION**
03	MSIE1S1	Ilesan, Lettuce
08	MBIC2S3	Abeokuta, Carrot
12	MNIU1S1	Sango, Cucumber
14	MTIC1S2	Ota, Carrot
22	MBIC2S2	Abeokuta, Carrot
24	MDIW1S1	Ijebu ode, watermelon
28	MAIP1S1	Sagamu, Pawpaw
31	MAIP1S1	Sagamu, Pawpaw
38	MSIC2S1	Ilesan, Carrot
41	MSIC2S2	Ilesan, Carrot
53	MMIC2S1	Mowe, Carrot
59	MTIC1S1	Ota, Carrot

A total of 60 samples of fresh produce were tested and 12 of them tested positive for *Salmonella* as presumptive *Salmonella* colonies.

APPENDIX 3: Fresh produce samples distribution

Sample	**Number of samples**
Cucumber	6
Carrot	12
Watermelon	8
Pineapple	8
Pawpaw	10
Cabbage	6
Lettuce	10
Total	**60**

APPENDIX 4: A graph showing bush meat samples gotten from various locations in Nigeria

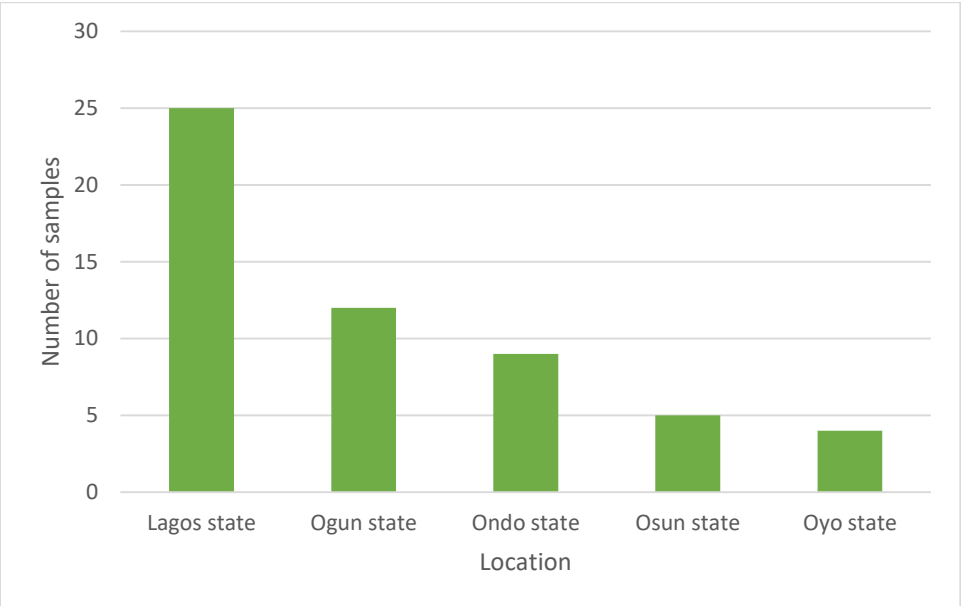

Plate 4.2: Disc diffusion test performed on Mueller-Hinton Agar

The diameters of the zones of inhibition formed on Mueller-Hinton Agar from the disk diffusion technique performed according to the Clinical Laboratory Standards Institute (CLSI) standard was measured and categorized as resistant, intermediate, or susceptible according to the established interpretative chart from CLSI 2020

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APPENDIX 5: Diameter of zone of inhibition around antimicrobial agents to nearest millimeter

***Salmonella* Isolate(s)**	**CHL**	**CRX**	**GEN**	**COT**	**TET**	**CPZ**	**CTX**	**CTR**	**MEM**	**VAN**	**CIP**	**AMK**
MSIE1S1	11	24	12	15	17	16	20	17	-	-	18	-
MBIC2S3	11	23	10	10	17	14	19	19	19	-	-	-
MNIU1S1	19	14	11	16	10	13	18	24	-	-	-	-
MTIC1S2	13	25	12	9	8	23	17	20	-	-	-	-
MBIC2S2	10	15	10	10	11	15	20	23	-	-	20	-
MDIW1S1	12	14	11	8	9	12	19	18	-	-	-	-
MAIP1S1	10	22	12	14	14	14	22	22	-	-	-	-
MNA2S1	9	16	10	10	9	21	18	19	-	-	20	-
MSIC2S1	18	15	9	18	9	11	19	17	-	-	-	-
MSIC2S2	12	17	17	7	10	12	22	23	17	-	-	-
MMIC2S1	13	21	17	8	8	16	21	21	-	-	-	-
MTIC1S1	25	23	15	20	16	13	17	18	-	-	-	-

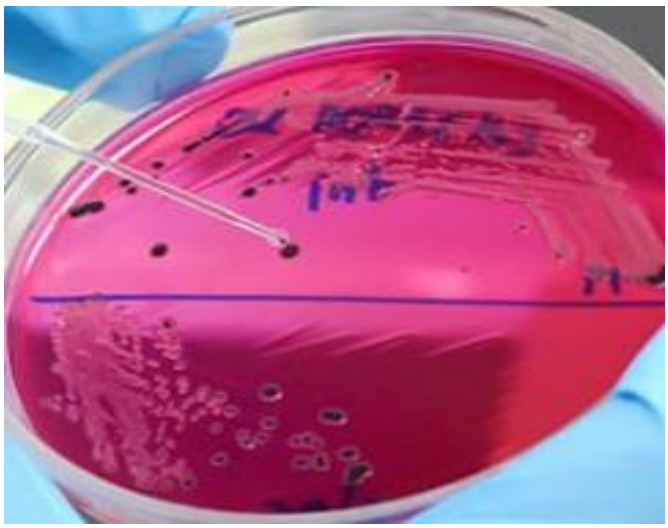

Plate 4.3: Example of *Salmonella* spp colonies on XLD agar

The existence of presumptive *Salmonella* colonies was checked on the XLD plates. On XLD plates, the development of with black colonies was observed.