BACTERIOLOGICAL QUALITY AND OCCURRENCE OF *SALMONELLA* SPECIES AND *ESCHERICHIA COLI* O157:H7 IN ROASTED RAT (*Arvicanthis niloticus*) MEAT SOLD IN ZARIA, NIGERIA

BY

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BY

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DECLARATION

I hereby declare that this project titled "**Bacteriological quality and occurrence of *Salmonella* species and *Escherichia coli* O157:H7 in roasted rat (*Arvicanthis niloticus*) meat sold in Zaria, Nigeria"** was done by me in the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, under the supervision of Prof. E.C Okolocha and Dr. B.V. Maikai. The information derived from the literature have been duly acknowledged in the list of references provided. No part of this work has been presented for another degree or diploma in any institution.

Terseer Iyene ADDAI _____ _____

(Signature) (Date)

CERTIFICATION

This dissertation entitled "**BACTERIOLOGICAL QUALITY AND OCCURRENCE OF *SALMONELLA* SPECIES AND *ESCHERICHIA COLI* O157:H7 IN ROASTED RAT (*ARVICANTHUS NILOTICUS*) MEAT SOLD IN ZARIA, NIGERIA"** by Terseer Iyene ADDAI meets the regulation governing the award of Master of Science in the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This project work is dedicated to the Lord Almighty, my parents, siblings, all my friends, and in loving memory of my father, Late Mr. J.H. Addai.

ACKNOWLEDGEMENT

My immeasurable gratitude goes to the Almighty God for the grace given to me to accomplish a part of my dream. My unquantifiable gratitude goes to my parents, Mr. J.H. Addai (Late) and Mrs. E. Addai and my siblings, Terna, Aondongu, Arita and Aondofa, for their relentless support in all ramifications throughout the course of this work. Words are not enough to express my appreciation to my sedulous supervisors and role models; Professor E.C. Okolocha and Dr. Mrs. B.V. Maikai for their guidance through every fragment of this work. My heart filled appreciation also goes to the wonderful lecturers in the Faculty of Veterinary Medicine especially in the Department of Veterinary Public Health and Preventive Medicine for tutoring and mentoring me through this program. Special thanks also to Dr. M. Chia (Department of Botany, Faculty of Life Science) and Chief M.B. Odoba (Department of Veterinary Public Health and Preventive Medicine) who gave fatherly advice and support to the work. My heart filled gratitude also goes to the staff in the Bacterial, Parasitic and Viral Zoonoses laboratories especially Mallam Mahmoud and Mallam Yahuza Maitala. I also want to thank the staff of Central laboratory, City campus, Usman Danfodio Univerisity for their technical support during the molecular analyses. I also extend my profound gratitude to the Department of Zoological, Department of Microbiology, Faculty of Life Science and to Prof. Inabo and Dr. Gadzama for their support in this project.

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work a success; space and time is not enough to acknowledge you all by your names. May the Almighty God bless you.

ABSTRACT

Bacterial contamination of roasted ready-to-eat rat meat and occurrence of foodborne pathogens may be injurious to human health. In Nigeria, the incidence and multidrug resistance of *Salmonella* spp. and *E. coli* O157:H7 in human cases is on the increase and these organisms have been found associated with meat and meat products in several studies. A cross-sectional study was carried out to determine the bacteriological quality and occurrence of *Salmonella* spp. and *Escherichia coli* O157:H7 in roasted ready-to-eat rat (*Arvicanthis niloticus*) meat sold in Zaria, Nigeria. A total of 384 samples were examined from four purposively selected districts in the study area; Samaru, Basawa, Jushi and Sabon Gari. Total aerobic plate count was determined using spread plate technique and bacteria load determined. Isolates were screened for *Escherichia coli* O157:H7 and *Salmonella* using the conventional biochemical characterization, standardized micro-substrate (Microgen GN-ID A+B) detection kit for Gram negative bacteria and further confirmed using Rapid latex agglutination test for *E. coli* O157:H7 and PCR for *Salmonella*. Agar disc diffusion method was used to evaluate the antibacterial resistance pattern; data obtained were interpreted using clinical laboratory standards institutes (CLSI). Mean log and standard deviation of total aerobic plate counts (TAPC) were 10.01±0.27, 10.03±0.26, 10.10±0.29 and 9.9±0.29, from four sampling areas, Samaru, Sabon Gari, Jushi and Basawa. The average TAPC ranged from 12 x 10^9 $cfu.g^{-1}$ in Samaru to 15 x 10^9 $cfu.g^{-1}$ in Jushi sampling districts. *Salmonella* was isolated from 2(0.5%) and *E. coli* O157:H7 isolated from 5 (1.3%) of the total 384 samples. Multidrug resistance of the *Salmonella* and *E. coli* O157:H7 was observed. Of the total *Salmonella* isolates, 100% were found to be resistant to tetracycline, doxycycline, amoxicillin/clavulanic acid, trimethoprim/sulphamethoxazole and ampicillin, 83.3% resistant to chloramphenicol and cefixime, 67% resistant to gentamicin, 33.3% resistant to azithromycin, 17% resistant to kanamycin and cefuroxime sodium, with no isolate (0%) resistant to ciprofloxacin. Of the total *E.*

coli O157:H7 isolates, 100% were found to be resistant to ampicillin and penicillin, 80% resistant to streptomycin, 60% resistant to trimethoprim/sulphamethoxazole and doxycycline, 40% resistant to chloramphenicol and gentamicin, with no isolate (0%) resistant to imipenem. Ninety-six structured questionnaires were used to assess the knowledge and practices of retailers and consumers of roasted ready-to-eat rat meat in the study area. Fifty-one percent of the consumers and 67% of the retailers of roasted ready-to-eat rat meat in Zaria, use bare hands as a means of selection in bargaining. Air drying is the major means of preservation, as documented by 52% of the retailers and 42% of the consumers. The roasted ready-to-eat rat meat is consumed either in soup or with spices with no further reheating. The high bacterial load beyond the permissible level, the occurrence of *Salmonella* spp. and *E. coli* O157:H7 and the presence of multidrug resistant strains of *Salmonella* and *E. coli* O157:H7 in roasted ready-to-eat rat meat sold in Zaria, are of serious public health significance. Therefore, there is for public health enlightenment of the consumers and retailers of roasted ready-to-eat rat meat sold in Zaria, so as to avoid bacterial contamination of the roasted rat meat.

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ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of Variance
CDC	Center for disease Control and Prevention
CT-SMAC	Sorbitol MacConkey agar supplemented with Cefixime and Tellurite
CFSK	Center for food safety Korea
DAEC	Diffuse-adherent *E.coli*
DNA	Deoxyribonucleic Acid
EFSA	European Food Safety Authority
EAEC	Enteroaggregative *E. coli*
EHEC	Enterohaemorrhagic *E. coli*
EIA	Enzyme Immunoassay
EIEC	Enteroinvasive *E. coli*
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. coli*
F	Fimbrial antigen
FAO	Food and Agriculture organization
HUS	Haemorrhagic Uremic Syndrome

HC	Haemorrhagic Collitis
H_2S	Hydrogen Sulphide
H	Flagellar antigen
Log	Logarithm
ICMSF	International Commission on Microbiological Specification for Foods
ISO	International Standardization Organization
In vitro	In an artificial environment outside a living organism
Inv A	Invasion gene A
MDR	Multidrug resistant
MLVA	Multi Locus Variable Number Tandem Repeat Analyses
MAR	Multiple Antibiotic Resistance
MR	Methyl Red
O	Somatic antigen
NaCl	Sodium Chloride
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
Ph	Measure of the Acidity or Alkalinity of Water

SIM	Sulphide, Indole, Motility medium
S/N	Serial Number
TTP	Thrombotic thrombocytopenic Purpura
TAPC	Total Aerobic Plate Count
VP	Voges Proskaue
VTEC	Verocytotoxic *E. coli*
USA	United States of America
^{o}C	Degree Celsius
A	Alpha
B	Beta
µg	Microgram
%	Percentage
<	Less than
>	Greater than
+	Positive reaction

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Meat refers to mostly skeletal muscles and associated fat but it may also refer to organs, including lungs, livers, skin, brains, bone marrow, kidneys, and a variety of other internal organs as well as blood. It is animal tissue used as food (Hammer, 1987). The term "bush meat" stands for meat that has been sourced from wild animals for human consumption and could be consumed fresh, smoked, salted, or sun-dried (Gideon and Joseph, 2018). Bush meat is central to the livelihood of many poor rural dwellers that consume and trade in it and the high demand in the urban regions means that the bush meat trade has become a lucrative business (ACET 2014; Gideon and Joseph, 2018). Nearly 5 million tons of bush meats are being traded in Central and West Africa and in Nigeria about 25 percent of the population relies solely on resources of animal protein from bush meat (Wilkie and Carpenter, 1999; Fa *et al.,* 2002; Gideon and Joseph, 2018).

Rodents are accepted as a popular source of protein and more than 71 genera and 89 species of hammerrodents, (mostly Hystricomorphs) have been consumed by man in the tropical world (Oyarekua and Ketiku, 2010). Rats are a regular staple in different parts of the world; Cambodia, Laos, Myanmar, parts of the Philippines and Indonesia, Thailand, Ghana, China and Vietnam (Doyle, 2014). They are tinned in the Philippines, sold as "STAR" meat (rats spelled backwards) in supermarkets, often eaten at weddings in Vietnam, and usually considered a delicacy by most South East Asians" (Doyle, 2014).

Among rodent species widely consumed and traded in Africa are the African grass rat (*Arvicanthis niloticus*), the cane rat (Thryonomys), the African giant rat (*Cricetomys gambianus*). (Ajayi and Tewe, 1978; Fiedler, 1990; Tee *et al.,* 2012). The African grass rat is traded in many parts of Nigeria and it is the most traded bush meat species in Makurdi, Nigeria (Tee *et al.,* 2012).

Food-borne diseases represent an important public health problem, significantly affecting the health of the population with major economic consequences (FAO, 2002). Regarding the issues of meat safety to consumers, bacterial pathogens are especially of most serious concern (Sofos, 2008). Many pathogenic bacteria are known to be associated with processed meat products, including *Escherichia coli* O157 and *Salmonella* spp. These microorganisms can cause serious foodborne illnesses with symptoms, such as severe vomiting and chronic diarrhea, which can lead to dehydration and, in some instances, death (March and Ratnam, 1986; Eppinger *et al*, 2011).

Salmonella species belong to the family *Enterobacteriaceae*. They are Gram negative, aerobic or facultatively anaerobic, motile or non-motile and non-spore forming rods, non-motile exceptions are *S*. Gallinarum and *S*. Pullorum (Popoff, 2001; Chao *et al.,* 2007; Bastiaan and Aize, 2007). They are catalase-positive, oxidase-negative, and chemo-organotrophic with the ability to metabolize nutrients by both respiratory and fermentative pathways (Doyle and Beuchat, 2007). These pathogens grow on citrate, forming acetate, formate and carbon dioxide as by-products, and catabolizing D-glucose with the production of acid and gas (Doyle and beuchat, 2007; Bott, 1997). The genus *Salmonella* is a facultative intracellular pathogen, causing localized or systemic infections as well as a chronic asymptomatic carrier state in poultry (Shivaprashad, 1997). According to Le Minor (1984) based on the lipopolysaccharides (O), flagella protein (H) and sometimes the capsular (Vi) antigens, the *Salmonella* species are divided into serovars (serotypes).

Escherichia coli are facultative anaerobic non-spore forming gram negative bacteria. They part of the common microbial floras of gastrointestinal tract of poultry and human beings but may become pathogenic to both (Jawetz *et al*., 1984; Levine, 1987). *E. coli* are capable of reducing nitrates to nitrites, when growing fermentatively on glucose or other carbohydrates; it produces acid and gas (Rodney, 2006). *Escherichia coli* O157:H7 is an enterohaemorrhagic strain of the bacterium *E. coli* and a cause of food borne diseases (Tafida *et al.,* 2014). These strains are responsible for disease in animals and man, and have emerged to be important zoonotic agents (Karch *et al.*, 2005; Nataro and Caper, 1998) and are also referred to by their toxin producing capabilities, verocytotoxin producing *E*. *coli* (VTEC) or Shiga-like toxin producing *E*. *coli* (STEC) according to Paton and Paton (1998).

Microbial resistance to antibiotics is a worldwide problem (Schroeder *et al.,* 2002). Antimicrobial resistance in *Enterobacteriaceae* poses a critical public health threat, especially in the developing countries (Karlowsky *et al.*, 2003; WHO, 2008). The development of resistance to antimicrobials is known to occur through stable genetic change heritable from generation to generation through specific mechanisms including mutation, transduction, transformation and or conjugation (Goodman *et al.*, 1990).

1.2 Statement of Research Problem

Meat has been particularly problematic, in studies on hazards in food, showing high levels of contamination (Umoh, 2001). In major cities in Nigeria, bush meat is sold in the open market exposed to the sun and dust without packaging and as such are exposed to microbial contamination, some of which may be pathogenic (Ebabhamiegbeho *et al.*, 2011). This informal and unregulated

bushmeat supply chain offers a realm of opportunities for human exposure to wildlife that potentially harbor a diversity of zoonotic pathogens (Peeters *et al*., 2002; Apetrei *et al*., 2005; Pourrut *et al*., 2011).

Escherichia coli O157:H7 is an emerging public health concern in most countries of the world and are recognized as an important human pathogen of public health concern (Schlundt, 2001; Bettelheim and Beutin 2003). World Health Organization estimates 200,000 deaths from diarrhoea due to bacterial infection each year (WHO, 2008) and studies show that the most important causes of diarrhea are toxigenic *E. coli, Salmonella* spp. and *Shigella* spp.

Total cost associated with food-borne diarrhoea in Nigeria is put at US$3.6 billion and the cost associated with beef-borne diarrhoea amounts to US$156 million (Iheanacho *et al.,* 2011). Each year, 17.62 million pounds worth of antibiotics are used to prevent and treat animal diseases and 2.8 million pounds are used for animal growth promotion (Castanie-Cornet *et al*., 1999). Research has shown that the frequency of antibiotic-resistant bacteria is closely related with the volume of antibiotics used (Salyers, 2002). The family *Enterobacteriaceae* is known to habour series of antibiotic resistant genes which can be transferred horizontally to other bacteria species and are persistently resistant to various classes of antibiotics (Iroha *et al.*, 2011)

1.3 Justification

Food borne diseases occur commonly in developing countries particularly in Africa because of the prevailing poor food safety laws, weak regulatory systems, lack of financial resources to invest in safe equipment and lack of education for food handlers (WHO, 2004).

Several studies have indicated the presence of *E. coli* O157:H7 in meat and meat products (Enabulele and Uraih, 2009; Tafida *et al.,* 2014) and studies agree that the most important causes of diarrhoea are toxigenic *E. coli* and *Salmonella* spp. (Smith *et al.,* 2003).

Antibiotic therapy in food animal production is increasingly coming under close scrutiny, largely because of the fear of increased levels of resistance in food-borne human pathogens, such as *Salmonella*, *Campylobacter* and *Escherichia coli* (Threfall *et al*., 1998). Smith *et al.,* (2003) reported multidrug resistance in isolates of *E. coli* O157:H7 and resistance of clinical isolates of *Salmonella* species to first line drugs has also been reported by Davies *et al.* (2005). The implication of this is that life expectancy could fall due to people dying from diseases that are readily treatable today (Sandle, 2014).

Much has been reported on *E. coli* O157:H7 and *Salmonella* spp. from different food items, but to the best of our knowledge no work has been done on the bacteriological quality and occurrence of *Salmonella* spp. and *E. coli* O157:H7 in roasted ready-to-eat rat meat sold in Zaria. In this light, this study was aimed at assessing the bacteriological quality and occurrence of *E. coli* O157:H7 and *Salmonella* spp. in roasted ready-to-eat rat (*Arvicanthis niloticus*) meat sold in Zaria, Nigeria.

1.4 Aim

The aim of the study was to determine the bacteriological quality and occurrence of *Salmonella* spp. and *Escherichia coli* O157:H7 in roasted ready-to-eat rat (*Arvicanthis niloticus*) meat sold in Zaria, Nigeria.

1.5 Objectives

The objectives were to:

1. determine the total aerobic plate counts of roasted ready-to-eat rat meat sold in Zaria, Nigeria using spread plate technique.
2. isolate and identify *Salmonella* species and *E. coli* O157:H7 in roasted ready-to-eat rat meat sold in Zaria, Nigeria using conventional biochemical, serological and Molecular techniques.
3. determine the antibiotic susceptibility patterns of the *Salmonella* and *E. coli* O157:H7 isolates using Kirby-Bauer disc diffusion technique.
4. assess knowledge and practices of the consumers and retailers of roasted ready-to-eat rat meat sold in Zaria, Nigeria.

1.6 Research questions

1. What is the bacteriological quality of roasted ready-to-eat rats sold in Zaria, Nigeria.
2. Are *E. coli* O157:H7 and *Salmonella* spp. present in roasted ready-to-eat rat meat sold in Zaria, Nigeria?
3. What is the antibiogram of *E. coli* O157:H7 and *Salmonella* species with respect to commonly used antibiotics?
4. What are the knowledge and practices of retailers and consumers of roasted ready-to-eat rat meat sold in Zaria, as regards the consumption of roasted ready-to-eat rat meat in Zaria, Nigeria?

CHAPTER TWO

LITERATURE REVIEW

2.1 Microbiology of meat

Meat spoilage is usually associated with gram-negative proteolitic bacteria which literally decompose the protein with production of offensive odour (Haman, 1997). Furthermore, microbial growth on meat to unacceptable levels may contribute significantly to change in meat structure, colour and flavor and cause meat spoilage (De Filippis, 2003). During slaughter, there is contamination of the sterile tissue with intestinal flora such as gram-negative organisms which includes *Escherichia coli* as well as contaminants associated with humans, animals and their environment (Haman, 1997).

The development of resistance to antibacterial is known to occur through stable genetic change heritable from generation to generation through specific mechanism including mutation, transduction, transformation and or conjugation (Goodman *et al.,* 1990). Extensive use of antibiotics in both human and veterinary medicine, particularly in disease prevention and growth promotion in animal production is a considerable cause of the selection and prevalence of antibiotic resistant *E. coli* O157:H7 (Schroder *et al.,* 2002; Callaway *et al.,* 2003).

2.2 Bacterial contamination of meat

Meat and meat products derived from animals contaminated with meat borne bacteria can cause human infections and intoxications leading to morbidity and/or mortality to both food handlers and consumers (Muma, 1998). The processing of meat from live animals presents many opportunities for contamination with a range of pathogens (Sheridan, 1998).

The most frequently identified bacterial pathogens associated with consumption of meat are *Salmonella* spp., *Campylobacter* spp., *Staphylococcus aureus., Escherichia coli* O157:H7, *Listeria monocytogenes, Clostridium perfringens, Yersinia enterocolitica, Bacillius cereus* and *Vibrio parachaemolyticus* (Biswas *et al.,* 2011).

Studies on hazards in foods in Nigeria show high levels of contamination, with meat being particularly problemtic (Iheanacho *et al.,* 2011). In a study, Iroha *et al.,* (2011) investigated 300 raw meat samples comprising beef, chicken and chevon in Abakaliki, Nigeria of which 79 (29.3%) were contaminated with bacteria species such as *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella* Typhi, *Shigella dysenteriae* and *Staphylococcus aureus.* In another study, Adetunji and Isola (2011) investigated swab samples from meat tables for *Listeria* sp count, *Listeria monocytogenes* presence of these bacteria above acceptable levels. Bello *et al.* (2011) assessed the level of beef carcass contamination with *Escherichia coli* including O157 strains before and after washing with water at kano, Sokoto, and Zango abattoirs in Notherwestern Nigeria and reported isolation rates of 58.3%, 70.8% and 76.7% respectively for *E. coli* in each of 120 swab samples collected at the abattoirs.

Studies in other parts of Africa and developing country have also shown to be problematic in food hazards, showing high levels of contamination. Obeng *et al.* (2013) isolated *Staphylococcus, Escherichia coli* and *Salmonella* species from raw meat sold at retail outlets in the Tolon and Kumbungu districts of the Northern region of Ghana, ascribing the presence of these pathogens to low standards of animals and meat handling practices from pre-slaughter to post-slaughter, sales of meat, abattoir facilities and equipment.

2.3 Preparation of roasted rat meat

Usually in Nigeria, rats are hunted in bushy areas just like other bush meats, and consumed at home, sold within the areas of hunting or to other states where the rats are rarely available for hunting but with availability of consumers (data form, 2017). The rats are caught mostly by traps setting, hit on sight and bush burning to set them loose from hiding, mostly in large numbers, and either prepared at the site of catch or brought home for processing (personal observation and discussion, 2017).

The process of preparation involves a few steps. Firstly, the rats are killed by throat slitting or hitting them repeatedly on the ground. Secondly, based on preferences, they may be disemboweled or staked as a whole. The third step is the drying/smoking of the rat meat. This stage involves staking the rats by the fire to get rid of the furs till they are dried (personal observation and discussion, 2017).

They are then consumed with spices or in soup. For commercial purposes, they are displayed for consumers in market areas or exported in cartons to other locations with demand. They are usually staked four to five rat pieces per stick (personal observation and discussion, 2017).

2.4 Ready-to-eat (RTE) foods

Ready-to-eat products are food products prepared in advance, which can be eaten, when purchased without the need for cooking or other processing (Anonymous, 2013). “Ready-to-eat” is defined as the status of the food being ready for immediate consumption at the point of sale. It could be raw or cooked, hot or chilled, and can be consumed without further heat-treatment including re-heating.

Cooked, sliced meat and fish, pates, products intended to be cooked before use (like frankfurters, semi smoked sausages etc.) can be found in the everyday diet of almost every family (Monteiro, 2010). As RTE-meats are consumed mainly without re-heating, the processing, transport and handling by retailers and consumers can all potentially compromise the safety of the food (Anonymous, 2013; Stahl *et al*., 2015).

It is important that the food business operators use coordinated microbiological and safety criteria that make it possible to assess acceptability of foodstuffs in the production, processing and distribution stages (Melngaile, 2008; Marèenkova, 2010; Melngaile *et al*. 2014).

Microbiological contamination is the most frequent cause for spread of most foodborne diseases. European legislation stipulate that food shall not contain microorganisms, toxins or metabolites thereof in amounts that cause risk for human health. Food may not be distributed if not safe, including food that is microbiologically contaminated (Anonymous, 2005; Marèenkova, 2010).

Human salmonellosis is normally seen in the form of small family outbreaks and one of the final stages in the contamination chain is usually the cross-contamination of cooked food by raw food or by dirty working surfaces, the cooked food being left at room temperature for a number of hours (Forsythe and Hayes, 1998).

2.5 The genus *Salmonella*

2.5.1 Historical perspective

Karl Eberth (1835-1926) observed rod-shaped organisms in the smears of fluids from lymph nodes and spleens of typhoid patients in 1880 (Ellermeier and Slauch, 2006). Later in 1885, isolation and morphological description of *Salmonella* spp; *Salmonella choleraesius,* from a swine intestine by

Theobald Smith (1859-1934) under the direction of Daniel E. Salmon (1850-1914) (Ellermeier and Slauch, 2006) was made. The name *Salmonella* was subsequently adopted in honour of Dr. Salmon (Ferede, 2014). Later research, however, revealed that the bacterium isolated by Salmon and Smith rarely cause enteric symptoms in pigs and was therefore not the agent they were seeking (which was eventually shown to be a virus) (Schultz, 2010).

Over the decades following the work of Salmon and Smith, many other *Salmonella* species were isolated from both animals and humans (Wildal, 1896; Getenet, 2008). *Salmonella* Enteritidis was reported by Gaetner in 1888 and in 1889.

2.5.2 Nomenclature

The genus *Salmonella* consists of only two major species: *S. enterica* and *S. bongori* according to the latest nomenclature, which reflects recent advances in taxonomy (Grimont and Weill, 2007). Six subspecies are differentiated within *S. enterica* based on their biochemical and genomic characteristics: *S. enterica* subsp. Enterica (I), *S. enterica* subsp. Salamae (II), *S. enteric* subsp. Arizonae (IIIa), *S. enterica* subsp, d\Diarizonae (IIIb), *S. enteric* subsp. Houtenae (IV) and *S. enterica* subsp. Indica (VI) (Brenner *et al.*, 2000). *S. bongori* (V) was initially considered to be another subspecies but it has now been classified separately from the rest of the *S. enterica* lineages as a distinct species (Fabrega and Villa, 2013).

2.5.3 Serovars

Based on three major antigenic determinants, flagellar H antigen, somatic O antigen and capsular antigen (Vi), *Salmonella* spp. are classified into serovars, in line with the Kauffmann-White classification scheme (Pui *et al.*, 2011).

Somatic (O) and flagellar (H) antigens determine different serovars in each subspecies, in a total of 2,610 serovars today, as recognized by Kauffman-White scheme (Grimont and weil, 2007). Distribution according to species and subspecies is as follows: *Salmonella enterica* subsp. Enterica (1,547 serovars); *Salmonella enteria* subsp Salamae (513); *Salmonella enterica* subsp Arizonae (100): *Salmonella enterica* subsp Diarizonae (341); *Salmonella enteric* subsp Houtenae (73); *Salmonella enterica* subsp. Indica (13); *Salmonella bongori* (23) (Rodrigues, 2011).

Within *S. enterica* subsp. I, the most common O-antigen serogroups are A, B, C1, C2, D and E. Strains in this serogroup cause approximately 99% of *Salmonella* infections in humans and warm-blooded animals (Popoff and Minor, 1997). Serotypes in *S. enterica* subspecies II (*S. enterica* subsp. *salamae*), IIIa (*S.enterica* subsp. *arizonae*), IIIb (*S. enteric* subsp. *diarizonae*), IV (*S. eneteric* subsp. *houtenae*), IV (*S. enteric* subsp. *indica*). And *S. bongori* are usually isolated from cold-blooded animals and the environment, but rarely from humans (Farmer *et al.*, 1984). By newer convention, names are retained only for subspecies *enterica* serovars, and these names are no longer italicized. The first letter is a capital letter "S" followed by the serovar names of subspecies *enterica* (e.g. Typhimurium or Montevideo). At the first citation of the serotype the genus name is given followed by the word "serotype" or the abbreviation "ser" followed by the serotype name (Gonzalez, 2010).

2.5.4 Morphology of *Salmonella*

Salmonella belongs to the family *Enterobacteriaceae* (Grimont *et al.*, 2000) and tribe *Salmonellaea* (Ewing, 1986). They are gram negative, non-spore forming anaerobes and they are rod-shaped with size of about 2-3 x 0.4-0.6 µm (Yousef and Carlstrom, 2003; Montville and Matthews, 2008).

Salmonella are mesophilic with optimum growth temperature in the range of 32 – 37ºC but capable of growth within a wide range of 6 – 46º C. They are heat labile and can therefore be inactivated by ordinary cooking temperatures above 70ºC, although the cooling time and values for temperature and time could change depending on the serotype and the food matrix (Gonzalez, 2010).

Most strains are motile with peritrichous flagellar and can reduce nitrate to nitrite (Grimont *et al.,* 2000), and usually produce gas from glucose and hydrogen sulfide on triple-sugar iron agar. They are indole-negative and urease-negative and usually utilize citrate as a sole carbon source (Lindqvist, 2008). *Salmonella* are non-capsulated except *S.* Typhi, *S.* Paratyphi C and some strains of *S.* Dublin (Getenet, 2008).

2.5.5 Distribution and host range

The distribution of *Salmonella* can vary greatly depending on the serovar. Species such as *Salmonella* Enteritidis and *Salmonella* Typhimurium have established global niches (Ellermeier and Slauch, 2006).

They are regularly isolated from infected food-producing animals, animal feeds, animal foodstuffs especially of milk, meat or egg origin and even within the farm environment as a reservoir (Kinney, 2009). Salmonellosis has been recognized in all countries, but appears to be most prevalent in areas of intensive animal husbandry, especially of poultry or pigs (OIE, 2010).

The most significant human host-adapted organism is *S.* Typhi, the cause of typhoid fever (Imen *et al.,* 2012). Man remains the only known reservoir for this serotype. Other organism, such *S.* Typhimurium, have a broad host range and these serotypes are responsible for the majority of human infections (Imen *et al.,* 2012).

2.5.6 *Salmonella* infection in humans

These organisms are important causes of febrile illness among crowded and impoverished populations with inadequate sanitation who are exposed to unsafe water and food and also pose a risk to travelers visiting endemic countries (Whitaker *et al.,* 2009). Clinical manifestations include fever, headache, abdominal pain, and transient diarrhoea or constipation and infection can cause fatal respiratory, hepatic, spleen, and/or neurological damage. Without treatment, the mortality is 10 to 20%, decreasing to 1% among patients treated with the appropriate antibiotics (Ohl and Miller, 2001; Parry *et al.,* 2002).

Two major clinical syndromes caused by *Salmonella* infection in humans are enteric or typhoid fever and colitis/diarrhoeal disease (Fabrega and Villa, 2013). Enteric fever is a systematic infection caused by the human adapted pathogens *S.* Typhi and *S.* Paratyphi A, B, and C. In contrast, there are many non-typhoidal *Salmonella* (NTS) strains that cause diarrheal disease in humans and can in addition, infect a wide range of animal host (Ohl and Miller, 2001; Gordon, 2008). Most cases of non-typhoidal salmonellosis in humans are associated with the consumption of contaminated food products such as beef, pork, poultry meat, eggs, vegetables, juices and other kind of foods. It may also be associated with the contact between human and infected pet animals (Freitas *et al.,* 2010).

2.5.7 Virulence factors of *Salmonella*

Virulence factors such as toxins, fimbriae, and flagella allow the bacteria to colonize and survive the antimicrobial environment. The pathogenesis of *Salmonella* is contingent on the ability to evade and invade host cells in order to survive and replicate within the environment (Xiong, 2011). Others such as *Salmonella* pathogenicity islands (SPIs). Which contain secretion systems that

allow the bacterium to penetrate phagocytic and non-phagocytic cells, also contribute to pathogenesis and bacterial persistence (Xiong, 2011).

2.5.7.1 Salmonella invasion (inv) genes

The molecular characterization of a *S.* Typhimurium entry-defective mutant led to the identification of a genetic locus called *inv* (Galan and Curtiss, 1989). The *inv*A gene of *salmonella* contains sequences unique to the genus and has been proved as a suitable PCR target with potential diagnostic applications (Rahn *et al.,* 1992). This gene is recognized as an international standard for detection of *Salmonella* genus (Malorny *et al.,* 2003). Serotypes of *Salmonella* that do not possess the *inv*A gene are not capable of expressing *inv*ABC genes, making them unable to invade mammalian cells (Galan *et al.,* 1992).

2.5.7.2 Salmonella in meat and meat products in Nigeria

Kwaga *et al* (1985) in Zaria, reported thirteen different serotypes of *Salmonella* from the lymph nodes of slaughtered cattle and ten from raw beef. Tafida *et al.* (2013) in Zaria, revealed that meat and meat products are a hazardous group of foods that can transmit pathogens to humans. Studies carried out by Olayemi *et al* in 1979 showed an overall prevalence of 3.99% from gall bladder, small intestine and rectal swab. An overall incidence of 25% was found by Addo and Diallo. (1982) in a study that assessed the incidence of *Salmonella* within the premises of abattoirs and on processed carcasses. *Salmonella* Enteritidis was isolated by Uche and Agbo. (1985) from swabs colleted from butchers' hands, knives, tables and meat displayed for sale at Nsukka meat market. Another study conducted by Esona *et al.* (2004) on raw and processed meat products and bovine rectal swabs in Zaria for *Salmonella* reporting an overall prevalence of 6%. Despite several studies, there is still paucity of the prevalence of *Salmonella* in bush meat and bush meat products in several

parts of Nigeria. Therefore, the need for continued studies especially in processed ready-to-eat meat.

2.5.8 Antibiotic resistance among *Salmonella.*

Resistance of *Salmonella* to a single antibiotic was first reported in the early 1960s (Montville and Mathews, 2008). Since then, the isolation frequency of *Salmonella* strains resistant to one or more or more antibiotics has increased in several countries of the world (Pui *et al.*, 2011).

Recently, a multi-drug resistant strain of *Salmonella enteric* seriovar Newport has become established in the U.S and caused several outbreaks associated with retail meat and milk (Gupta *et al.*, 2004).

In 2003, 22.5% of non-typhoid *Salmonella* isolates from humans were resistant to at least one antimicrobial agent and the most common multi-drug resistance phenotype reported was to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracyclines, which was detected in 9.3% of isolates tested (USFDA, 2006).

Non susceptibility to ciprofloxacin has increased in human non-typhoidal *Salmonella* since 1996 and multidrug resistance of a common *Salmonella* serotype [I 4, (5), I 2: i] more than doubled from 18% in 2011 to 46% in 2013 (NARMS, 2015). While the use of antibiotics has been proven to be an effective means for the prevention and control of bacterial infection, their indiscriminate use can have adverse consequences by promoting the selection and prevalence of drug resistant microbial populations (Braude, 1978; Threfall *et al.*, 1997). Antimicrobial resistance can increase the morbidity, mortality and costs associated with disease. Moreover, it has social and economic consequences and requires strong scientific and public health efforts to improve the situation (Freitas *et al.*, 2010)

2.5.8.1 Antibiotic resistant Salmonella in Nigeria

Davies *et al.* (2005) noted multi-drug resistance among 19.4% of *Salmonella* Paratyphi serotypes detected in 60 clinical *Salmonella* spp isolates from humans in various health establishments in Abuja, Nigeria; 85.7% of the *S.* Paratyphi isolates possessed plasmid sizes of 4.7-5.7 kilobase, suggesting that transmissible R-plasmids might have been responsible for the drug resistance observed.

Salmonella organisms isolated in Nigeria were resistant to ampicilin, chloramphenicol, streptomycin, sulfonamides, tetracycline, nalidixic acid and cotrimoxazole, categorized as 'first line' drugs in the treatment of typhoid fever (Raji *et al.,* 2011). Tafida *et al.* (2013) reported an overall *Salmonella* spp. prevalence of 2.3% from 435 retailed beef and related meat products consisting of muscle meat, offal and processed meat products. All isolates exhibited multiple drug resistance. Simultaneous resistance to up to 8 antibiotics was found amongst the isolates. The isolates exhibited commonly, resistance to members of b-lactam family and other antibiotics classes including lincosamides, macrolides, aminoglycosides and nitrofurans.

2.5.9 Detection of *Salmonella*

With significant progress made in areas of sample preparation techniques for improved isolation and detection of *salmonella* in foods and food ingredients, several methods for the detection of *Salmonella* have been developed in the last decade (Odumeru and Leon-Velarde, 2012). Due to their sensitivity and selectivity, culture based methods remain the gold standard for the detection of *Salmonella* (Aloilja and Radke, 2003). Within the next decade, novel technologies such as the

application of biosensors, microarrays and nanotechnology are likely to become available for routine testing of food and food ingredients (Odumeru and León-Velarde, 2012).

2.5.9.1. Culture based method

Culture based methods are still the most widely used detection techniques and remain the gold standard for the detection of *Salmonella* due to their selectivity and sensitivity (Alocilja and Radke, 2003). The first step typically involves primary enrichment of the sample (Odumeru and Leon-Velarde, 2012) in media such as buffered peptone water or universal pre-enrichment broth (OIE, 2010) to recover sub-lethally injured cells due to heat, cold, acid or osmotic shock (Sandel *et al.,* 2003; Gracias and McKillip 2004).

Before being struck onto selective agars such as Xylose Lysine Deoxycholate agar (XLD agar), Bismuth Sulphite agar (BIS), modified semisolid Rappaport Vasiliadis (MSRV) or *Salmonella Shigella* agar, primary enrichment cultures are inoculated into secondary enrichment broths, such as Selenite Cystine broth (SC), Rappaport vasiliadis Soy broth (RVS), Tetrathionate Broth (TT), or Muller-kauffmann Tetrathionate-Novobiocin broth (MKTTn) and incubated at elevated temperatures (37^{o}C or 42^{o}C for 18-24 hours).

There are classical biochemical and serological tests which are well established confirmation and identification procedures for *Salmonella.* Key biochemical tests include the fermentation of glucose, negative urease reaction, lysine decarboxylase, negative indole test, H_2S production and fermentation of dulcitol. Serological confirmation tests typically utilize polyvalent antisera for flagellar (H) and somatic (O) antigens. Isolates with a typical biochemical profile, which agglutinate with both H and O antisera are identified as *Salmonella* species.

2.5.9.2 Control and prevention of Salmonella

The following preventive measures are recommended by the Centers for Disease Control and Prevention (CDC, 2016).

1. Poultry, ground beef and eggs should be cooked thoroughly. Foods containing raw eggs or raw (unpasteurized) milk should be avoided.
2. Hands, kitchen work surfaces and utensils should be washed with water and soap immediately after they have been in contact with raw meat and poultry.
3. Direct or indirect contact between reptiles and infants or immunocompromised persons should be avoided.
4. Cross-contamination of foods should be avoided. Uncooked meats should be kept separate from produce, cooked foods and ready-to-eat foods.

2.6. **History of *Escherichia coli***

In 1885 Theodor Escherich (German Scientist) first cultured '*Bacterium coli'* from the feces of a healthy individual and it was named *Escherichia coli* in 1919 in a revision of bacteriological nomenclature (Escherich, 1885; Law 2000). They are gram-negative, short rod-shaped bacteria which are usually motile with petrichous flagellae, possessing fimbriae and facultative anaerobes that are capable of fermentative and respiratory metabolism (Wilshaw *et al.*, 2000)

E. coli is commonly found in digestive tracts of human and animals and is considered as part of the normal bacteria of the intestine (Todar, 2005). Although most isolates are non-pathogenic they are considered as indicator of fecal contamination in food and about 10 to 15% of intestinal

coliforms are opportunistic and pathogenic serotypes (Barnes and Gross 1997) and cause a variety of lesions in immunocompromised hosts as well as in poultry.

Among the diseases caused by *E. coli*, some are often severe and sometimes lethal infections such as meningitis, endocarditis, urinary tract infection, septicemia, epidemic diarrhea of adults and children (Daini *et al.*, 2005) and yolk sac infection, omphalitis, cellulitis, swollen head syndrome, coligranuloma, and colibacillosis (Gross, 1994).

Escherichia coli is divided into sero-groups and sero-types by serological means. The initial sero-typing scheme was developed by Kaufman (1947) and is based on *E. coli* somatic (O), Flagellum (H) and capsular (K) antigens. The 'O' antigen is based on the antigenicity of the 'O' specific polysaccharide of the cell outer membrane Lipopolysaccharide. (Table 2.1 and Table 2.2). The 'O' antigens are the basis of classifying *E. coli* into subgroups (Krieg and Holt, 1984).

Table 2.1 Biochemical Characterization of *E. coli*

S/N	Biochemical tests	Reaction
i.	Urease	-
ii	Methyl Red	+
iii	Indole production	+
iv	Catalase	+
v	Lactose fermentation	+
vi	Citrate utilization	-
vii	Nitrate reduction	+
viii	Voges-Proskauer	-
	Acid from sugar	
	a) Salicin	+
	b) Mannitol	+
	c) Glucose	+
	d) Sucrose	+

Keys; + means positive while – means negative

Table 2.2 Virulence factors of *Escherichia coli*

S/N	Virulence factors	Proposed role(s) in pathogenesis
I	Col V Plasmid	Coded for a siderophore (aerobactin) for Fe chelation. Increases resistance to serum
ii	Haemolysin	Damages host cells and releases fe for red blood cells
iii	Enterochelin	Chelates Fe for bacterial uptake
iv	K1 antigen	Impedes phagocytosis and Blocks binding of c3b opsonin
v	p-pili	Allow bacteria to bind to P blood groups antigen on urinary tracts cells (especially in kidneys)
vi	Type 1 Pill	Allow bacteria to bind to (1) bladder opithelium, (2) Tamm-Horsfall glycoprotein, and (3) D-Mannose residues on a variety pf cells

2.6.1 Patho-types of *Escherichia coli*

Escherichia coli plays an important role in maintaining intestinal physiology. However, there are pathogenic strains that cause distinct syndromes of diarrhoeal disease (Adriana *et al.,* 2004). Six *E. coli* pathotypes associated with diarrhoea are currently recognized: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohaemorrhagic *E. coli* (EHEC) or Shiga toxin-producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC) (liliana *et al.,* 2008).

2.6.1.1 Enteroinvasive Escherichia coli

Both EIEC and *Shigella* spp. strains cause bacillary dysentery in humans by invading and multiplying within epithelial cells of the colonic mucosa, (Adriana *et al.,* 2004). This remarkable tropism results in an intense inflammatory response characterized by abscesses and ulcerations that damage the integrity of the epithelial cell lining of the colon, producing the pathognomonic symptoms of dysentery (Hale *et al* 1998). They cause high morbidity and mortality in young children in developing countries where sanitation and hygiene level are of a poor standard. The organism penetrates and multiplies within the epithelial cells of the colon causing widespread cell destruction (Todar, 2002), resulting in an inflammatory response accompanied by necrosis and ulceration of the bowel leading to release of blood and mucous in the stool (Hart *et al.,* 1993).

*E*scherichia *coli* (EIEC) belongs to the following O serotypes: O28ac:NM; O29:NM; O112ac:NM; O121:NM; O124:NM; O124:H30; O136:NM; O143:NM; O144:NM; O152:NM; O164:NM; O167:NM (Nataro and kaper 1998).

The entry of *Shigella* and EIEC bacteria into susceptible host target cells requires the coordinated expression of numerous genes that are activated in response to signals of the microenvironment. Symptoms include chill, fever, headache, muscular pain, abdominal cramps and diarrhea. These usually appear 8 to 24 hours after ingestion of contaminated food with infective dose of more than 10^6 *E. coli* per gram of food (FDA, 2006).

2.6.1.2 Entero-haemorrhagic Escherichia coli

Enterohemorrhagic *E*. coli (EHEC) strains, which include *E. coli* O157:H7, produce Shiga toxin that can cause diarrhoea, which may range from mild and non-bloody to stools that are virtually all blood but contain no fecal leukocytes. This pathotype produces a powerful cytotoxin, active against cultured vero cells hence they are often termed Vero cytotoxin *E. coli* (VTEC) but may also be known as shiga-like toxin producing *E. coli* (STEC). VTEC produces toxins designated as VT-1 and VT-2 which are toxic to the cero and Hela cells, as first reported by Konowalchuk *et al.* (1997). VT-1 is relatively heat stable high molecular weight Shiga-like toxin, whereas VT-2 is immunologically distinct (Doyle, 1991).

Symptoms include diarrhoea, severe abdominal cramps and vomiting. Others include bloody diarrhoea, haemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP) and death. The infective dose is very small. In some outbreaks 2 cells per 25 grams of food of EHEC were detected (FDA, 2006). Outbreaks have occurred in nursing homes, child care centers, schools, and the community. Major sources of infection have been ground beef, unpasteurized milk and juice, sprouts, lettuce, and salami. Waterborne transmission occurs through swimming in contaminated lakes, pools, or drinking contaminated water. Since low numbers of organisms

can cause infection, EHEC is easily transmitted from person to person and has been difficult to control in child care centers.

EHEC also possesses a number of other mechanisms such as adhesions, which are involved in virulence. EHEC cells adhere to the lower intestine, grow and produce toxins. The toxins play a role in the inflammatory response produced by EHEC strains and may explain the ability of EHEC strains to cause haemolytic uremic syndrome (HUS) (Todar, 2002).

2.6.1.3 Enteropathogenic E. coli (EPEC)

Enteropathogenic *E. coli* (EPEC), an important cause of infantile diarrhoea in the developing world (Nataro and Kaper, 1998), colonizes the small bowel and produces 'attaching and effacing' (A/E) lesions on the brush border surface of small intestinal enterocytes characterized by effacement of brush border microvilli, intimate attachment of bacteria and formation of an actin-rich pedestal beneath intimately attached bacteria (Moon *et al*., 1983).

They possess a number of virulence although they do not possess the colonization factors and do not produce any ST (heat-stable) or LT (heat labile) toxins (Karper, 1997). They produce a non fimbrial adhesin designated intimin, an outer membrane protein that mediates the final stages of adherence (Todar, 2002)

In children, symptoms include watery diarrhoea, fever, vomiting, abdominal cramps and fever. Onset of symptoms can range from 17-72 hours. Outbreaks have occurred due to contaminated water and cold meat pie (FDA, 2006).

2.6.1.4 Enterotoxigenic E. coli (ETEC)

ETEC are an important cause of diarrhoea in infants and travelers in underdeveloped countries or regions of poor sanitation. The disease requires colonization and elaboration of one or more enterotoxins (Todar, 2002). The strain is characterized by their production of well-defined heat-labile and or heat cholera toxin (CT) while heat stable enterotoxin (ST) is non-antigenic and of low molecular weight (Rhea and Fleming, 1994). Symptoms of ETEC include watery diarrhoea, fever, abdominal cramps, malaise and vomiting. Onset of the disease occurs 8-44 hours after ingestion of contaminated food. Infective dose is thought to be between 10^6 and 10^{10} *E. coli* cells (FDA, 2006).

2.6.1.5 Entero-aggregative E. coli

They are self-adherent, tending to auto-agglutinate, giving the appearance under the microscope of a stack brick (Harrigan, 1998). They also produce a toxin known as the enteroaggregative heat stable enterotoxin (EAST1) (Elliot and Nataro, 1995) and their aggregative adherence phenotype is mediated by at least two fimbriae called fimbriae 1 and 2 (Czeczulins *et al.*, 1997).

The patho-type are associated with persistent diarrhoea in children. It is however unknown how EaggEC causes diarrhoea except that it colonizes the intestinal mucosa, mainly that of the colon, followed by secretion of cytotoxin and enterotoxins. EaggEC differs from other diarrhoeagenic *E. coli* through its ability to adhere to epithelial cells such as HEp-2 in a stacked brick pattern (Nataro and Kaper, 1998)

2.6.1.6 Diffusely adhering E. coli

Its name comes from its ability to adhere to Hela cells in a diffuse pattern (Neidhardt, 1986). They also express adhesion of the Afa/dr family in 25% of cases of cystitis in children and 90% of cases of pyelonephiritis in pregnant women (Archamband *et al.,* 1998 and Servin, 2005). Two classes of DAEC strains have been identified (Servin, 2005). The first class habours Afa/Dr adhesion which is associated with urinary tract infection (Archamband *et al.,* 1998). The second class produces adhesion involved in diffuse adherence which is a potential cause of infantile diarrhoea (Benz and Schmidt, 1992).

2.6.2 Transmission of *E. coli* O157:H7

EHEC and EAHEC are transmitted by the faecal–oral route. Human disease caused by *E. coli* O157:H7 is often associated with contaminated and undercooked beef (CDC, 1997). Most human infections are associated with consumption of foods, which might get contaminated during slaughter or milking (Minihan *et al*., 2003) or, because of failures in food preparation, particularly preparation of undercooked ground beef (Meng *et al*., 1998). Both drinking and recreational waters have been the sources of *E. coli* O157:H7 that caused human illness outbreaks (Bruce *et al.*, 2003). Numerous *E. coli* O157:H7 outbreaks resulting from contact with cattle or their manure on farms, at fairs, and at petting zoos have also been reported (CDC, 2005). Transmission of the pathogen to humans via consumption of unpasteurized goat's milk (Mclntyre *et al.,* 2002), environmental contact with sheep manure (Ogden *et al.,* 2002) and sheep or goat contact at petting zoos (Payne *et al.,* 2003) has been reported.

2.6.3 Reservoir of *E. coli* O157:H7

Escherichia coli O157:H7 have been reported in dairy and beef cattle, in calves and adult animals, and in cattle in both feedlot and pasture-based production systems (Ogden *et al.,* 2004). *E. coli* O157:H7 has been isolated from numerous non-bovine animals and other sources as potential reservoirs or vehicles of *E. coli* O157:H7. It has been isolated from sheep (Chapman *et al.,* 1997), goats (Fox *et al.,* 2007), pigs and chickens (Doane *et al.,* 2007), horses (Hancock *et al.,* 1998), dogs (Hancock *et al.,* 1998) and turkeys (Doane *et al.,* 2007). Other wild animals demonstrated to carry *E. coli* O157:H7 include; raccoons (Shere *et al.,* 1998), wild deer (Heuvelink *et al.,* 2002), opossums (Renter *et al.,* 2003), rats (Cizek *et al.,* 2006), and rabbits (Scaife *et al.,* 2006). Among the numerous bird species that have been found positive for *E. coli* O157 are pigeons (Shere *et al.,* 1998), gulls (Wallace *et al.,* 1997), rook (Ejidokun *et al.,* 2006) and starlings (Nielsen *et al.,* 2004).

2.6.4 Geographical distribution of *E. coli* O157:H7

In Nigeria, the incidence in human cases of diarrhea is said to be on the increase (Smith *et al.,* 2009). Ogunsanya *et al.* (1994) reported more prevalence in diarrhoeic children (5.1%) than in the control non-diarrhoeic patients (3%). Olorunshola *et al.* (2000) investigated the prevalence of *E. coli* O157:H7 in 100 patients with diarrhoea by stool culture in Lagos. In Akwa Ibom State of Nigeria, Bloody diarrhoea accounted for 31% of all cases of diarrhoea in humans (Akinjogunla *et al.,* 2009). Smith *et al.* (2009) reported the prevalence of *E. coli* O157 in human stool samples to be 31.6%.

In Southern Nigeria, seven isolates belonging to serotypes O26, O111, O138 and O157 were isolated from 520 diarrhoeic faecal samples from patients with acute diarrhoeal disease in Enugu and Onitsha (Nweze, 2009). A significantly higher prevalence of EHEC O157 was observed in

Lagos (35.0%) with greater rate of meat consumption and more eateries than in Zaria (23.7%) which had a lower rate of meat consumption and fewer eateries (Smith *et al.*, 2009).

2.6.5 Clinical manifestation of EHEC O157: H7

There are three different principal manifestations of illness that have been attributed to *E. coli* O157:H7 according to Doyle and Padhye, (1989) which include haemolytic uremic syndrome (HUS), hemorrhagic colitis (HC), and thrombotic thrombocytopenic purpura (TTP).

Haemolytic uremic syndrome (HUS) is a tirade of an acquired haemolytic anaemia, thrombocytopenia and renal failure that occurs acutely in otherwise healthy individuals (Padhye and Doyle 1992). It is the most common cause of acute renal failure in children less than 4 years of age and a proportion of affected children die of this illness (Tzipori *et al.*, 1987).

Hemorrhagic colitis is a disease that is distinguished from dysentery described in shigellosis or invasive *E. coli* gastro-enteritis by the lack of fever and the bloody discharge resembling lower gastrointestinal bleeding (Padhye and Doyle, 1992). It is a distinct clinical syndrome that is characterized by sudden onset of abdominal cramps, followed within 24 hours by watery diarrhea which later becomes grossly blood and described as “all blood stool” (Padhye *et al.*, 1986).

Thrombotic thrombocytopenic purpura is a syndrome usually occurring in adults that consists of microangiophathic haemolytic anaemia, profound thrombocytopenia, fluctuating neuralagic signs, fever and mild azotemia (Kwaan, 1987). It is similar to HUS except that the central nervous system is involved (Padhye and Doyle, 1992)

2.6.6 Pathogenicity of *Escherichia coli* EHEC

The pathogenicity of enterohaemorrhagic *E. coli* (EHEC) strains is associated with the ability to produce either one or both of two phage-encoded Shiga toxins (Stx1 and Stx2), the production of EHEC haemolysin (EHEC-Hly) and the presence of an adherence factor intimin encoded by the eae gene, which is involved in the attaching-and-effacing lesions in the intestine (Paton and Paton, 1998). Antibiotic treatment of children with *E. coli* O157:H7 infection has been found to increase the risk of HUS (Wong *et al.,* 2000). Mortality rate is low (3-5%) in children but high in extreme age, particularly elderly (Dundas *et al.,* 1999). The most important sequelae are chronic renal insufficiency in a significant percentage of affected children (Elliot and Nichols, 2001). In both HUS and TTP, other infrequent consequences are encephalopathy, cardiomyopathy and diabetes mellitus resulting from involvement of the brain, myocardium and pancreas respectively (Coia, 1998).

2.6.7 Diagnosis of EHEC O157: H7 infection

Isolation of the organism from food or stool specimens involves first enrichment in a selective broth and then plating onto sorbitol MaConkey agar with additives (Griffin, 1995). Biochemical and serological tests are then done. Immunomagnetic beads coated with specific O157 antibody can be used to enhance isolation (Bennett *et al.,* 1995). Also, enzyme linked immune-sorbent assay (ELISA) methods are used to isolate and identify suspect colonies (Johnson, 1995).

Immunoassay methods are available to identify the presence of verotoxins (Su, 1995). Testing for verotoxin can also be done using toxin specific antibodies and genes using DNA probes (Willshaw *et al.,* 1993). Testing for verotoxin can identify verotoxin producing serotypes other than O157: H7.

Another method to detect *E. coli* O157:H7 rapidly, specifically and sensitively is by using DNA based polymerase chain reaction (PCR) methods. One multiplex PCR method amplifies simultaneously three different DNA sequences of verotoxins I and II, and a fragment of the 60-MDa plasmid (Deng *et al.*, 1996).

Molecular methods which are useful in distinguishing between outbreak related and unrelated isolates have been developed. The most commonly used DNA fingerprinting tests are based on restriction fragment length polymorphism (RFLP). A pattern or "fingerprint" is resolved for particular bacterial strains. Several RFLP methods have been developed, one uses pulsed-filled gel electrophoresis (PFGE) (Johnson, 1995), others are conventional gel electrophoresis (Samadpour, 1995).

2.6.8 Prevention and control of EHEC infection

The following preventive measures are recommended by the Centers for Disease Control and Prevention (CDC, 2010).

I. Wash all fruits and vegetables before eating.
II. Drink only pasteurized cider and milk.
III. Do not drink water from open streams and lakes
IV. Cook all ground beef or hamburger thoroughly until the meat is gray or brown throughout and juices run clear.
V. Make sure drinking water has been treated with adequate levels of chlorine or other effective disinfectants.

2.6.9. Treatment of EHEC O157: H7

There is evidence that bacterial isolates are resistant to some antibiotics (Aibinu *et al.,* 2007). In cases of HUS, treatment may involve: dialysis, medications such as corticosteroids, Transfusions of packed red blood cells and platelets and some people may have the liquid portion of their blood (plasma) removed and replaced with fresh (donated) plasma, or plasma is filtered to remove antibodies from the blood.

2.6.10 Prognosis of EHEC O157: H7

For complications such as blood clotting problems, Haemolytic anaemia, Kidney failure, Nervous system problems, thrombocytopenia, Uremia e.t.c. With proper treatment, more than half of patients will recover.

2.6.11 Antibiotic resistance of E. coli O157:H7

The development of resistance to antibacterial is known to occur through stable genetic change heritable from generation to generation through specific mechanisms including mutation, transduction, transformation and or conjugation (Goodman *et al.,* 1990). Extensive use of antibiotics in both human medicine and animal agriculture is suspected to have led to a widespread dissemination of antibiotic resistant genes (Callaway *et al.,* 2003).

Multidrug resistance in isolates of *E. coli* O157 strains obtained from farm animals and human infections in Lagos and Ogun States, Nigeria, has been reported by Smith *et al.* (2003, 2007). Carl *et al.* (2002) reported that 361 *E. coli* O157 isolates kept at the *E. coli* preference center at the Pennylvania State University were resistant to Sulphamethoxazole (26%), tetracycline (27%), cephalothin (17%) and ampicillin (13%).

5. Undercooked meat, poultry or egg should be avoided.
6. Vaccination of poultry against *salmonella* infections helps to achieve *Salmonella* free poultry and eggs that is safe for human consumption.

2.7 *Arvicanthis niloticus* (Nile grass rat)

The genus *Arvicanthis* contains seven currently recognized species (Musser and Carleton, 2005), the Nile grass rat *Arvicanthis niloticus* exhibits the widest distribution within the genus (Granjon and Duplantier 2009). It is rather generalist and inhabits steppes, savannahs as well as humid zones and human modified biotopes (e.g. villages, gardens, rice fields, sometimes within cities) of the Sahelian and Sudanian bioclimatic zones where it is considered a major agricultural pest (Granjon and Duplantier, 2009). These semi-diurnal, gregarious and herbivorous rodents (Rosevear, 1969) are common in the savannas and grasslands of sub-Saharan Africa, from the Atlantic coast in Senegal to Ethiopia, and down south to Zambia, with the exceptions of populations along the Nile Valley in Egypt, south west of the Arabian Peninsula, and a mention from the Hoggar mountains, south east Algeria (Musser and Carleton, 2005; Granjon and Duplantier, 2009).

Arvicanthis niloticus are a major reservoir of various tropical infections (Trape *et al.*, 1991). They have also been shown to carry a number of pathogens, such as *Leishmania* major (El Githure *et al.*, 1986), *Borrelia* spp. (Trape *et al.*, 1996), *Leptospira* spp. (Sebek *et al.*, 1989), *Rickettsia* spp. (Julvez *et al..*, 1997), *Schistosoma* spp. (Duplantier and Sene 2006) or *Toxoplasma gondii* (Mercier *et al.*, 2013).

The Nile rats (Arvicanthis niloticus) are among species of rat widely consumed and traded in Africa (Fiedler, 1990). They are consumed and traded in different parts of Nigeria and they constitute the most traded species of bush meat in Markudi, Nigeria (Tee *et al.*, 2012).

2.8 Polymerase chain reaction

The polymerase chain reaction (PCR) provides a new strategy for the detection of *Salmonella* (Rahn *et al.,* 1992) and the specificity of the reaction is determined by the uniqueness of a DNA target sequence. Briefly, extracted DNA is first subjected to denaturation into single stranded DNA. Next, specific short DNA fragments (primers) are annealed to the single stranded DNA strands, followed by extension of the primers complementary to the single stranded DNA with the aid of a thermostable DNA polymerase such Taq polymerase, an enzyme originally isolated from the bacterium *Thermus aquatic* (Chien *et al.,* 1976). Each new double-stranded DNA is then targeted during a new thermal cycle and thus the exponential amplification of the specific DNA sequence is achieved. The amplified project is then separated by gel electrophoresis and visualized by staining with fluorescent Ethidium bromide. The type of conventional or endpoint PCR, although sensitive and specific under optimized conditions, is time consuming and labour intensive due to post amplification steps, not sensitive enough to measure the accumulated DNA copies accurately and can only provide a qualitative result. Nevertheless, PCR techniques have expedited the process of pathogen detection and in some cases, replaced traditional methods for bacterial identification, characterization and enumeration in foods (McKillip and Drake, 2004).

CHAPTER THREE

3.0MATERIALS AND METHODS

3.1 Study area

The study was carried out in Zaria (Figure 3.1) which is positioned between latitude 11^{o} 07''N and 12^{o} 00'' N. and longitude 07^{o}44''E and 8^{o} 00''E. Zaria is located at the center of northern Nigeria and on a plateau at a height of 2200ft above sea level (Mortimore, 1970). The area is characterized by wet and dry seasons with fluctuation in temperature, with a monthly mean rising from January (21^{o}C) and attaining a maximum in April (29^{o}C) (Yakubu, 2009). Zaria metropolis is divided administratively into Zaria and Sabon Gari Local Government Areas (L.G.A), each comprising of six (6) and five (5) district areas respectively. Zaria LGA comprises Zaria city, Dutse Abba, Tudun wada, Gyelesu, Tukur Tukur and Jushi; while Sabon Gari comprises Muciya, Bassawa, Samaru, Bomo and Hanwa (Ministry of Economic Development, 1996).

The vegetation and atmospheric conditions as well as the presence of human settlement in the study area, Zaria, favours the survival of the Nile grass rat. Granjon and Duplantier (2009) reported that, the Nile grass rat thrives in the Savannas as well as human modified biotopes (e.g cities). The availability of rat meat is highly seasonal due to the difficulty in hunting after the onset of the raining seasons restricting sales to between January through May. The high demand for roasted rat meat in the study area as well as other states with low availability of the rats, favours the trade.

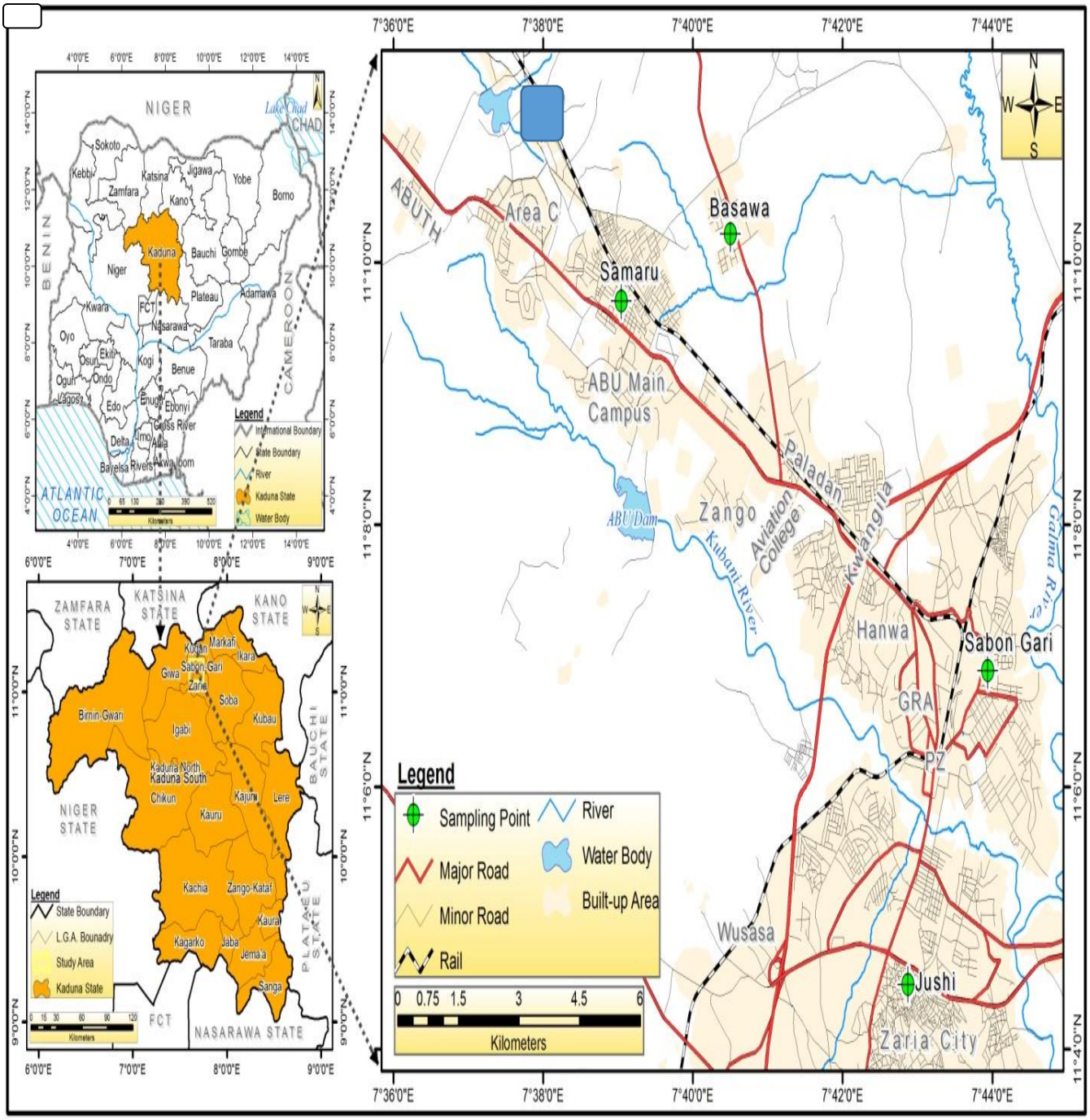


Figure 3.1 Map of Zaria showing Sampling areas.

Source: Modified from the Administrative Map of Zaria, Nigeria 2017.

3.2 Sample size

Sample size was determined using the formula described by Thrusfield (1997);

$N=Z^2pq \div d^2$

Where

N= Sample size

Z= Standard normal deviation for 95% confidence interval (1.96)

P= Prevalence 50%

d= desired precision (0.05)

q=1-p

$$N= \frac{1.96^2 \times 0.5 \times 0.5}{0.05^2}$$

N= 384.16

A total of 384 samples were collected.

3.3 Study Design

A cross-sectional study was carried out. Sampling was carried out from January to May, 2017. The rats are readily available between these months (January to May). The sampling was a Non-probability sampling and samples were collected from different locations in each sampling unit based on convenience.

The sampling frame constituted the two Local Government Areas (Sabon Gari and Zaria) that made up the study area. Four district areas were selected using purposive sampling method, these were; Samaru, Sabon Gari, Jushi and Basawa. Ninety-six (96) Samples each were collected from the four different sample areas selected.

3.4 Questionnaire administration

Closed ended structured questionnaires were administered to the retailers and consumers of roasted ready-to-eat rat sold in the study area. Ninety-six structured questionnaires were administered to retailers and consumers of roasted ready-to-eat rat meat in the study areas based on convenience. Markets/sales points in each district area were identified and documented as retailers while any customer who came to patronize the roasted rat meat was identified as a consumer. Consumers were asked orally if they have already participated in the research to avoid repetition.

3.5 Sampling

Samples were purchased and kept in sterile polythene bags just the way they are sold to the consumers. All samples were placed in separate sterile polythene bags to prevent cross contamination. They were immediately transported to the Bacterial Zoonoses Laboratory, Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria for bacteriological analyses.

3.6 Zoological Nomenclature

Unprocessed dead rat samples were randomly obtained from different retailers in the sampling areas and taken to the Department of Zoology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria for identification phenotypically based on keys (Desmarest 1822).

3.7 Laboratory Procedures

Conventional laboratory procedures (Fig. 3.2 and Fig. 3.3) were employed in assessing the microbial quality, occurrence and antibiogram of *Salmonella* spp. and *Escherichia coli* O157:H7 in roasted ready-to-eat rats sold in Zaria Metropolis, Nigeria. Buffered peptone water served as the non-selective pre-enrichment medium which constituted the stock. Total aerobic plate count was carried out from the stock to assess the bacteriological quality. The stock was further incubated for 24 h at 37^{o}C. One ml was further enriched in 9 mls of Rappaport Vassiliadis, a selective enrichment broth for *Salmonella* spp. and another 1 ml was aseptically taken and dispensed in 9 mls of EC broth, a selective enrichment broth for *E. coli.* Xylose Lysine Deoxycholate and Eosine Methylene Blue were the selective media used for the culturing for *Salmonella* spp. and *E. coli* respectively. Nutrient agar was used to store suspect colonies for further biochemical and serological analyses (ISO, 2002). Sorbitol MacConkey agar supplemented with Cefixime-Tellurite was used for the selective isolation of *E. coli* O157:H7 (March and Ratnam, 1986). Microgen GN-ID A+B kit for gram negative bacteria was further used to confirm biochemically confirmed isolates. *In vitro* susceptibility of isolates to commonly used antimicrobial agents was investigated according to the Kirby-Bauer disk diffusion susceptibility test protocol (Bauer *et al.,* 1996). Molecular technique, by the use of Polymerase Chain Reaction was further used to confirm isolates that conformed to conventional biochemical and serological tests (Figure 3.2 and Figure 3.3).

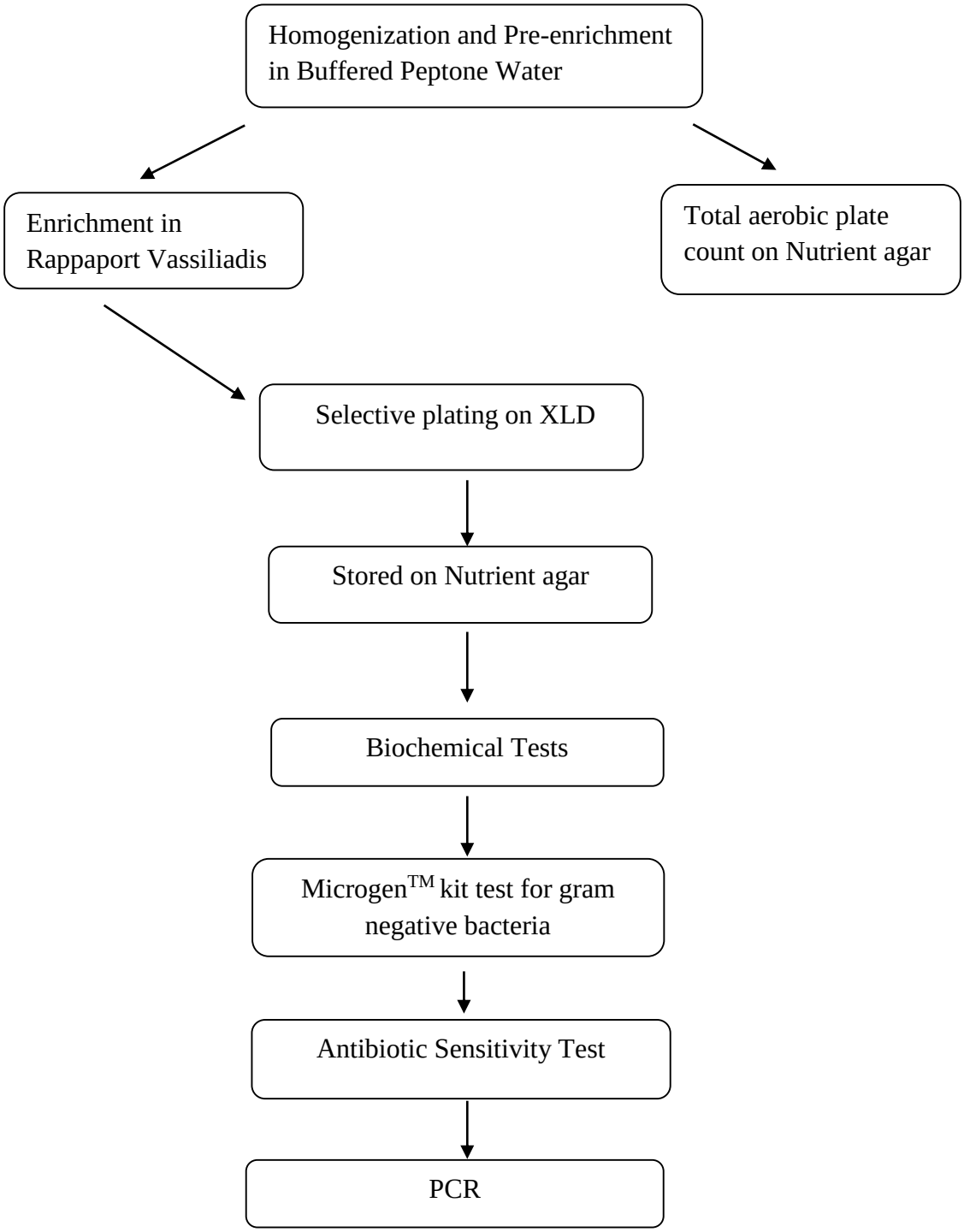


Figure 3.2 Flow Chart for Isolation and Identification of *Salmonella*

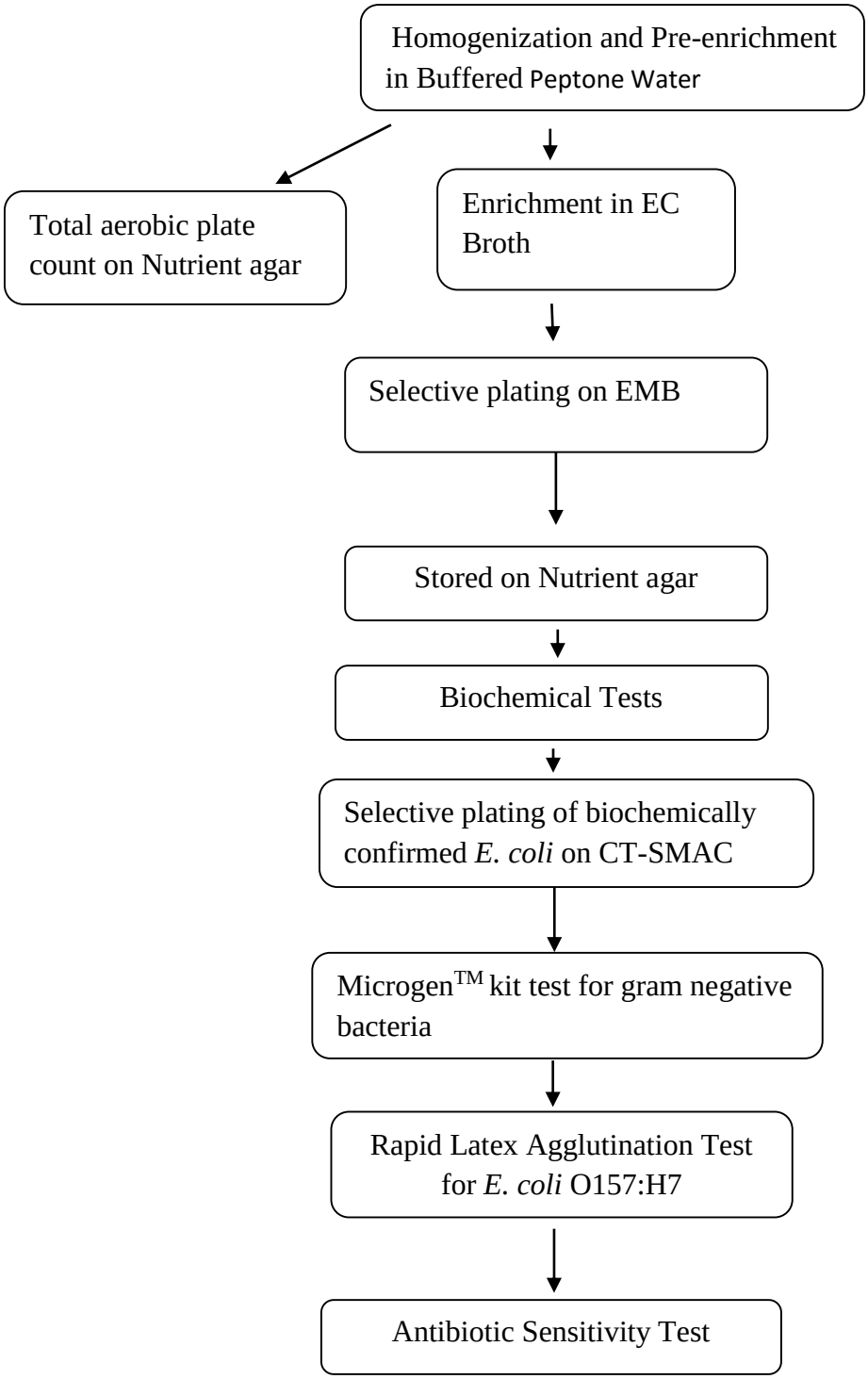


Figure 3.3 Flow Chart for Isolation and Identification of *E. coli* O157:H7

3.7.1 Non-Selective pre-enrichment

Different parts of the samples were cut aseptically with sterile scissors and ten (10) grams of each sample weighed aseptically, put in 90ml 1.0% buffered peptone water, and homogenized using a laboratory stomacher.

3.7.2 Total aerobic plate count

Standard aerobic plate count using spread plate methods as recommended by (Sanders, 2012) were used for the determination of total aerobic plate count. The homogenate, 10 g of the sample homogenized in 90 ml Peptone water, constituted the stock (10^{-1}) which was immediately used for serial dilutions. Serial dilutions of each sample was performed in hundred-fold to a dilution factor of 10^{-7} and plated out on Nutrient agar plates using spread plate technique (Cheesbrough, 2002).

Briefly, three sterile tubes containing 9.9ml of sterile saline were serially arranged in a test tube rack. A volume 0.1 ml of the 10^{-1} dilution was then transferred using a sterile pipette into 9.9ml of the diluents to give a 10^{-3} dilution. After shaking to mix, a fresh sterile pipette was used to transfer 0.1 ml of the 10^{-3} dilution to another 9.9 ml of diluents to give a 10^{-5} dilution. The test tube was then shaken to mix, after which a sterile pipette was further used to transfer 0.1 ml of the 10^{-5} dilutions to another 9.9ml of diluents to give a 10^{-7} dilution. Finally, 0.1 ml of the 10^{-7} dilution was then spread on nutrient agar plates, using a sterile hockey stick and the plates were incubated at 37ºC for 24 hours. The average bacterial loads of the roasted ready-to-eat rat meat samples collected were counted and expressed as colony forming unit per gram ($CFU.g^{-1}$) as described by Feng et al. (1998).

3.7.2.1 Expression of total aerobic plate counts

Colonies in the range of 30-300 were counted after incubation and the average counts expressed as colony forming unit per gram. Counts exceeding 300 colonies per plate were recorded as too numerous to count (TNTC) and counts below 30 were recorded as NIL.

3.7.3. Selective isolation of *Salmonella*

The homogenate was incubated at 37ºC for 24 h. From the homogenate, 1 ml of the pre-enriched culture was transferred using sterile pipette to 9ml of Rappaport Vassiliadis (RV) broth, which was then incubated at 37ºC for 24 hours for selective enrichment. A loopful of the enriched Rappaport Vassiliadis (RV) broth was streaked onto Xylose Lysine Deoxycholate plate and incubated at 37ºC for 24 hours.

3.7.3.1 Sub-culturing on nutrient agar plate

Two or three characteristic *Salmonella* colonies appearing pink, with or without black center on XLD agar were inoculated on nutrient agar slants and incubated at 37ºC for 24 hours and stored for biochemical confirmation.

3.7.4 Selective isolation of *E. coli*

From the homogenate incubated at 37ºC for 24 h, 1 ml of the homogenate was transferred using sterile pipette to 9ml of EC broth, which was then incubated at 37ºC for 24 hours for selective enrichment. A loopful of the enriched EC broth was streaked onto Eosin Methylene Blue (EMB) agar using sterile wire loop and the agar was incubated at 37ºC for 24 hours. Colonies with greenish

metallic sheen and dark centers suggestive of *E. coli* were picked, stored on nutrient agar slants, and refrigerated for further biochemical and serological analyses.

3.7.5 Conventional biochemical characterization of presumptive isolates

Presumptive *Salmonella* and *E. coli* isolates subcultured (18-24hrs) on nutrient agar plates were inoculated onto Triple Sugar Iron (TSI) agar (Oxiod, UK), Sulfide Indole Motility (SIM) agar (Oxoid, UK), Methyl Red-Voges Proskauer (MRVP) broth, Simmons Citrate agar and Urea agar (Oxoid, UK) (Cowan and Steel, 1993; Harley and Prescott, 2002). Isolates were further tested for fermentation of maltose, manitol, lactose, glucose, sucrose and manose. To inoculate TSI, a colony was picked using a sterile inoculating needle and stabbed into the butt before streaking across the entire slant. For SIM, a colony was picked using a sterile inoculating needle and stabbed approximately two-thirds of the way into the butt and then withdrawn along the same path. MR-VP broth was inoculated by selecting a light inoculum and suspending in the broth. For oxidase test, a sterile filter paper was moistened with a suspension of the oxidase reagent after which a colony was picked using a sterile toothpick and smeared on the moistened filter paper. This was observed for colour reactions. All other sugars and amino acids were inoculated in same manner as the MR-VP broth. The inoculated media were incubated for 24 hours at 37^{o}C. Biochemically confirmed isolates were stored on Nutrient agar slants for further tests.

3.7.6 Interpretation of conventional biochemical tests*:*

*3.7.6.1 Salmonella**:*** isolates that gave reactions suggestive of *Salmonella* species were set aside for further confirmation. Typical biochemical reactions observed were alkaline (purple) slant/ acid (yellow) butt on TSI, Yellow colouration (negative) of SIM agar upon addition of 1ml of Kovac's reagent, reddish colouration (positive) upon addition of 3-5 drops of methyl red reagent on MR,

tallow colouration (negative) after addition of 0.6ml and 0.2ml barrits reagents A and B respectively on VP, Bluish colouration (Positive) on Citrate agar, no change in colour (negative) on urea agar and oxidase reagent moistend filter paper.

*3.7.6.2 E. coli**:*** Isolates that gave reactions suggestive of *E. coli* species were set aside for further confirmation. Typical biochemical reactions observed were acid (yellow) slant/ acid (yellow) butt with gas on TSI, reddish ring colouration (negative) of the SIM agar upon addition of 1ml of Kovac's reagent, reddish colouration (positive) upon addition of 3-5 drops of methyl red reagent on MR, tallow colouration (filter paper.negative) after addition of 0.6ml and 0.2ml barrits reagents A and B respectively on VP, no change in colour (Negative) on Citrate agar, no change in colour (negative) on urea agar and oxidase negative.

3.7.8 Isolation of *E. coli* O157:H7

Biochemically confirmed *E. coli* were streaked on Cefixime-Tellurite Sorbitol MacConkey agar (CT-SMAC agar) supplemented with Cefixime 50µg/L and Potassium tellurite 2.5mg/L at 37^{o}C (March and Ratnam, 1986). The plates were then incubated at 37^{o}C for 24 hours. Suspected colonies of *E. coli* O157 appeared as non-sorbitol fermenters, which is characterized as having a slightly transparent, almost colourless with a weak pale brownish appearance. Three to four colonies from the CT-SMAC agar plates were selected and stored on nutrient agar slants plates, and incubated at 37^{o}C for 24 hours. These were stored in a refrigerator for further biochemical and serological analyses.

3.8 Bacterial characterization of presumptive isolates using the standardized micro-substrate detection kit for gram negative bacteria

The Microgen^{TM} (Oxoid, UK) Gram-negative system is a standardized micro-substrate system designed to simulate conventional biochemical substrates used for the identification of *Enterobacteriaceae* and common miscellaneous Gram-negative bacilli. Organism identification is based on PH change and substrate utilizations. The substrates are contained in 12 wells; 1: Lysine; 2: Ornithine; 3: Hydrogen Sulfide; 4: Glucose; 5: Mannitol; 6: Xylose; 7: O-Nitrophenyl-β-D-galacotopyranoside (ONPG); 8: Indole; 9; Urease; 10: Voges Proskauer (VP);11: Citrate and 12: Tryptophan Deaminase (TDA). A 18-24 hour culture of a typical *Salmonella* and *E. coli* O157:H7 isolate following conventional biochemical confirmation was obtained on selective medium. One to three (1-3) colonies were emulsified in 3 ml sterile saline. The Microgen wells were labelled and filled to $\frac{3}{4}$ levels with the corresponding isolate suspension and resealed. The sealed plates were then incubated for 24 hours at 37^{o}C for 24 hours. After incubation, two (2) drops of Kovac's reagent was added to well 8 and observed for 2 minutes. One (1) drop each of VP1 and VP2 reagents were added to well 10 and observed for 15-30 minutes, while a drop of TDA reagent was added to well 12 and interpreted immediately.

Interpretation of the Microgen^{TM} test: The utilization of substrate in each well was recorded as positive or negative based on colour changes. Interpretation of colours was aided by a color chat provided by the Manufacturer. Microgen^{TM} uses an octal coding system in which each group of three reactions produces a single digit of the code. Using the results obtained, the indices of the positive reactions were circled and the sum in each group of three reactions formed the code number. This code was entered into the computer package which immediately gave the probable identity of the organism tested in percentage. The percentage figure shown against the organism

was the percentage share of the probability for that organism as a part of the total probabilities for all choices.

3.9 Rapid Latex Agglutination Test for *Escherichia coli* O157:H7

Commercially available latex agglutinations kit having *E. coli* O157:H7 antisera was used to further confirm *E. coli* O157:H7 (Nataro and Kaper, 1998). The kit contains two test reagents (O157 test reagent and H7 test reagent). The O157 test reagent consists of a red latex particle coated with antibodies specific for *E. coli* O157 and H7 test reagent consists of blue latex particles coated with antibodies specific for *E. coli* H7 antigen. A drop of the reagent was mixed on a card with the suspension of *E. coli* isolate. Rapid agglutination occurs through the interaction of specific lgG and O157 lipopolysaccharide antigen.

3.9.1 Test procedure for O157 (somatic antigen)

Confirmed *E. coli* isolates after conventional biochemical tests were sub-cultured on CT-SMAC and incubated at 37^{o}C for 24 hours. Using a mixing stick, sufficient growth (Non-sorbitol fermenting cultures) was removed to just cover the blunt end of the stick. About 40µl of saline (0.85% NaCl w/v) was placed in two circles on reaction card to emulsify the sample of the culture in the saline by rubbing with the flat end of the stick. It was mixed thoroughly, but not vigorously. Using a separate stick, a similar amount of the sample was emulsified in the saline in the other circle. For each test, one drop of O157 test latex was added to each circle and one drop of the O157 control latex, then the content, mixed/rocked carefully spreading the latex over the entire area of the circle.

3.9.2 Test procedure for H7 (flagella antigen)

Cultures that were positive with O157 test latex antigen were grown overnight at 37^{o}C in EC broth at 37^{o}c. Using an applicator stick, sufficient growth was removed just to cover the blunt end of the stick. Approximately 40µl of saline (0.85% NaCl w/v) was placed in two circles on a reaction card to emulsify the sample of the culture in the saline by rubbing with the flat end of the stick. It was then mixed thoroughly but not vigorously, using a separate stick, then a similar amount of the sample was emulsified. For each test sample, one drop of H7 test latex in one circle and one drop of the H7 control latex in the other circle and the content was rocked carefully spreading the latex over the entire area of the circle. Positive results were indicated by agglutination with clear clumping of the latex particles. The rate of appearance of the agglutination was dependent on the quality and quantity of the cultured antigen. In a case of negative result, the latex will not agglutinate and the appearance of the suspension will remain substantially unchanged throughout the period of the reaction.

3.10 *In vitro* antibiotic susceptibility testing of isolates

In vitro susceptibility of the *Salmonella* isolates to 13 antimicrobial agents, and the *E. coli* O157:H7 isolates to 8 antimicrobial agents, was investigated according to the Kirby-Bauer disk diffusion susceptibility test protocol (Bauer *et al.,* 1996). The antibiotics tested were penicillin (10 µg), tetracycline (30µg), doxycycline (30µg), chloramphenicol (30µg), amoxicillin/clavulanic acid (30µg), ciprofloxacin (5µg), trimethoprim-sulphamethoxazole (25µg), cefixime (5µg), kanamycin (30µg), gentamicin (30µg), Azithromycin (15µg), imipenem (30µg), cefuroxime sodium (30µg), Streptomycin (10 µg) and ampicillin (10µg).

Four to five discrete colonies from nutrient agar plates were suspended in tubes containing 2ml normal saline adjusted to match with a 0.5 McFarland turbidity standard. A sterile cotton swab was dipped into the suspension, rotated several times, pressing firmly on the inside of the wall of the inside of the tube above the fluid level to remove excess inocula and swabbed uniformly over the surface of duplicate Mueller Hinton agar (Oxoid, England) plates. Using a multiple disc dispenser (Oxoid, UK), 6 antibiotics were dispensed per plate. The plates were incubated in inverted positions at 37^{o}C for 24 hours after which the diameter of the zones of inhibition were measured and classified according to interpretative standards of the Clinical Laboratory Standards Institute (CLSI, 2015).

3.11. Detection of *inv*A gene by PCR (Polymerase Chain Reaction) method

3.11.1 DNA extraction of isolated Salmonella species

An overnight suspension of the bacterial isolates was prepared by inoculating a loop full of the *Salmonella* isolates (including a PCR confirmed *Salmonella* positive isolate) into 10 mls of peptone water, incubated at 37^{o}C for 24 hrs.

3.11.2 DNA extraction

Extraction of DNA was performed by boiling. Two hundred microliters of molecular grade water was transferred into each tube, boiled in a water bath at 95^{o} for 30 minutes and centrifuged at 6000

rpm for 5 min. The supernatants were aseptically transferred into fresh sterile 1.5 micro liters ependorf tubes and used for amplification by PCR with *Salmonella* specific primers. Extracted DNA were quantified using a Nanodrop spectrophotometer (Nanodrop ®; Pretoria, South Africa).

3.11.3 Primer set and PCR amplification

Salmonella specific primers, S139 and S141 (Rahn *et al*, 1992) with the following nucleotide sequence based on the targeted *inv*A gene of *Salmonella*; 5´ - GTG AAA TTA TCG CCA CGTTCG GGC AA - 3´and 5´ - TCA TCG CAC CGT CAA AGG AAC C -3' for forward and reverse primers respectively were used. The primer set amplifies a 284 bp fragment of the *invA* gene. The components of the reaction mixture constituted:100 ng of template DNA, 1x PCR assay buffer with $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, (MBI Fermentas, USA), each dNTPs at a concentration of 200µM, 1 U of *Taq* DNA polymerase (Banglore Genei, India) and 15 picomol solution of each primer (SBS Genetech, India), in 25 µl PCR reaction mix. One µl of deionized water served as the negative control.

Amplification was carried out in an Eppendorf Mastercycler Gradient (Hamburg, Germany) using the following cycling condition; initial denaturation at 94ºC for 1minute, 35 cycles of denaturation at 94ºC for 1 minute, annealing at 64ºC for 30 seconds and elongation at 72ºC for 30 seconds, followed by 7 minutes final extension period at 72ºC.

3.11.4. Electrophoresis of PCR product

PCR amplicons were separated by 1.5% agarose gel in cooperated with 5 microliters of ethidium bromide and electrophoresed at 100 volts for 45 minutes. 100bp DNA Ladder was used. The gel was visualized by a Biorad gel documentations device.

3.12. Data Analyses

The result obtained were analyzed using Statistical Package for Social Sciences (SPSS version 21.0). Result obtained from total aerobic plate counts were converted to $\log_{10}$ (CFU/g) and expressed as logarithmic mean and standard deviation. One way Analyses of variance was used to test for association of microbial contamination between different sampling points. P value ≤ 0.05 was considered significant.

The frequency of isolation of *Salmonella* and *E. coli* O157:H7 from each sample point was calculated as: $\frac{\text{No. of positive samples in category}}{\text{Total no.of samples in category}} \text{ X } 100$

CHAPTER FOUR

4.0 RESULTS

4.1 Zoological nomenclature

The samples were identified as *Arvicanthis niloticus.*

4.2 Total aerobic plate count

Of the total 384 samples examined from the four sample areas, 297 (77%) yielded colonies within the range of 30-300, while 54 (14%) were too numerous to count and 33 (9%) yielded colonies below 30. Mean total aerobic plate counts were lowest (12×10^9) at Basawa and highest (15×10^9) at Jushi. Mean log ± SD TAPC from Samaru, Sabon Gari, Jushi and Basawa sampling points were 10.01±0.27, 10.03±0.26, 10.10±0.29 and 9.9±0.29 respectively. There was significant difference between the Mean log ± SD TAPC of the four areas sampled. A P-value of 0.04 was recorded, where a P-value ≤ 0.05 is considered statistically significant (Table 4.1).

4.3 Frequency of isolation of *Salmonella* and *E. coli* O157:H7 isolates

4.3.1 Frequency of isolation of *Salmonella*

Out of the 384 samples of roasted ready-to-eat rats screened for *Sallmonella* spp. from the four sample areas examined, 166 (43%) were positive with typical growth on XLD. The number and percentages of suspect *Salmonella* isolates with typical growth on XLD observed from Samaru, Sabon Gari, Jushi and Basawa sampling areas were 50 (50%), 33 (34%), 47 (49%) and 36 (38%) respectively (Table 4.2).

The number of biochemically confirmed *Salmonella* isolates were highest, (7) at Jushi sampling unit and lowest (2) at Sabon Gari sampling areas. (Table 4.3).

Table 4.1 Log_{10}Total Aerobic Plate Count (mean±SD) of Samples from the areas sampled.

S/no	Sample areas	N	n	Mean count CFU.g-1 (10^9)	Log_{10}(mean±SD)	p-value = 0.04
I	Samaru	90	70	12	10.01±0.27	
II	Sabon Gari	90	69	14	10.03±0.26	
III	Jushi	90	84	15	10.10±0.29	
IV	Basawa	90	74	12	9.9±0.29	
	Total	384	297			

Key: N = Number of samples collected**; n** - number of samples with colonies within countable range (30-300); **TAPC** = Total aerobic plate count; **CFU.g^{-1} =** Colony forming units per gram

Table 4.2 Frequency of distribution of suspect *Salmonella* isolates from four areas sampled.

S/N	Sample areas	Samples collected		Suspect *Salmonella* isolates *on* XLD		Biochemically confirmed *Salmonella* isolates	
		No	%	No	%	No	%
I	Samaru	96	25	50	52	5	10
II	Sabon Gari	96	25	33	34	2	6
III	Jushi	96	25	47	49	7	15
IV	Basawa	96	25	36	38	4	11
	Total	384	(100)	166	43	18	5

Key: XLD- Xylose Lysine Deoxycholate

Table 4.3 Isolates of *Salmonella* spp. from different sampled areas and their reactions to conventional biochemical tests.

S/NO	isolate I.D	TSI	S	I	M	UR	MR	VP	OX	CT	MN	LC	SC	GL	ME
1	S175	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
2	B65	K/A, H_2S	+	-	+	-	+	-	-	+	-	-	-	+	+
3	SR	K/A, H_2S	+	-	+	-	+	-	-	+	-	-	-	+	+
4	Sa5	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
5	Sf11	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
6	B125	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
7	Sa1	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
8	Srt7	K/A, H_2S	+	-	+	-	+	-	-	+	-	-	-	+	-
9	Sf11	K/A, H_2S	+	-	+	-	+	-	-	+	-	-	-	+	+
10	K8ST	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
11	Sa2	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
12	Sa3	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
13	B126	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
14	5Tkm	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
15	R23	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
16	R53	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
17	Rsb	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+
18	Rs5	K/A, H_2S	+	-	+	-	+	-	-	+	+	-	-	+	+

KEY; TSI- TRIPLE SUGAR IRON; S- SULPHIDE; I- IND-INDOLE; M- MOTILITY; UR-UREASE; MR- METHYL RED; VP- VOGES PROSKAUER; OX- OXIDASE; CT- CITRATE; MN- MANITOL; LC- LACTOSE; SC- SUCROSE; GL- GLUCOSE; ME- MANOSE; K/A- ALKALINE OVER ACID AND H_2S- HYDROGEN SULPHIDE

4.3.2 Frequency of isolation of *E. coli* O157:H7

Out of the 384 samples of roasted ready-to-eat rats screened for *Escherichia coli* O157:H7 from the four sample areas examined, 145 (38%) were positive with typical growth on EMB agar. The number and percentages of suspect *E. coli* isolates with typical growth on EMB observed from Samaru, Sabon Gari, Jushi and Basawa sampling areas were 37 (39%), 34 (35%), 43 (45%) and 31 (32%) respectively (Table 4.4).

Further screening of the isolates with conventional biochemical tests (Table 4.5) showed a decrease in the total number and percentages of suspect *E. coli* isolate*s* on EMB agar from 145 (38%) to 22 (6%). The number of biochemically confirmed *E. coli* isolates were highest, (8) at Jushi sampling unit and lowest (3) at Basawa sampling areas while Samaru and Sabon Gari had 4 and 7 biochemical confirmed *E. coli* isolates respectively (Table 4.4).

Biochemically confirmed *E. coli* isolates were further screened on Sorbitol MacConkey agar supplemented with cefixime and Tellurite (CT-SMAC) for the selective isolation of *E. coli* O157:H7. Of 37 biochemically confirmed *E. coli* isolates, 15 (4%) gave reactions typical of *E. coli* O157:H7 on CT-SMAC.

The distribution of *E. coli* O157:H7 on CT-SMAC for the four sampling points; Samaru, Sabon Gari. Jushi and Basawa are 4, 3, 5 and 5 respectively (Table 4.5).

Table 4.4. Frequency (%) distribution of suspect *E. coli* isolates from four (4) areas sampled.

S/N	Sampling area	Samples collected		Suspected *E. coli* isolates on E.M.B		Biochemically confirmed *E. coli*		Confirmed *E. coli* on CT-SMAC	
		No	%	No	%	No	%	No	%
I	Samaru	96	25	37	39	4	11	4	100
II	Sabon Gari	96	25	34	35	7	21	3	43
III	Jushi	96	25	43	45	8	19	5	63
IV	Basawa	96	25	31	32	3	10	3	100
	Total	384	100	145	38	22	15	15	68

Key: E.M.B - Eosin Methylene Blue, **CT-SMAC** – Cefixime Tellurite Sorbitol Maconkey agar

Table 4.5. Isolates of *E. coli*, from different sampled areas and their reactions to conventional biochemical tests.

S/NO	isolate I.D	TSI	S	I	M	UR	MR	VP	MT	CT	MN	LC	SC	GL	ME
1	C17	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
2	R15	A/A, G	-	+	+	-	+	-	-	-	-	+	+	+	-
3	F10	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
4	K9	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
5	P.Z5	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
6	C24	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
7	P.Z1	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
8	F1	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
9	C6	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
10	P.Z7	A/A, G	-	+	+	-	+	-	+	-	+	+	+	+	-
11	T13	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
12	C3	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
13	K12	A/A, G	-	+	+	-	+	-	-	-	+	+	-	+	-
14	Z11	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
15	C16	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
16	P.Z9	A/A, G	-	+	+	-	+	-	-	-	+	+	-	+	-
17	R10	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
18	Z9	A/A, G	-	+	+	-	+	-	+	-	+	+	+	+	-
19	Z15	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-
20	C30	A/A, G	-	-	+	-	+	-	-	-	+	+	+	+	-
21	R62	A/A, G	-	+	+	-	+	-	-	-	+	+	-	+	-
22	C21	A/A, G	-	+	+	-	+	-	-	-	+	+	+	+	-

KEY; TSI- TRIPLE SUGAR IRON; S- SULPHIDE; I- IND-INDOLE; M- MOTILITY; UR-UREASE; MR- METHYL RED; VP- VOGES PROSKAUER; MT- MALTOSE; CT- CITRATE; MN- MANITOL; LC- LACTOSE; SC- SUCROSE; GL-GLUCOSE; ME- MANOSE; A/A- ACID OVER ACID AND G- GAS (CO_2)

4.4 Biochemical Identification of isolates using the Standardized micro-substrate (Microgen GN-ID A+B) detection kit for gram negative bacteria

Eighteen isolates that largely conformed to typical *Salmonella* reactions, following conventional biochemical test and 15 isolates with typical *E. coli* O157:H7 reactions on CT-SMAC were tested with the Microgen^{TM} system for gram negative bacteria. Of these 6 yielded typical *Salmonella* reactions and 5 yielded typical *E. coli* reactions as indicated by the standardized micro-substrate detection kit for gram negative bacteria.

All sample areas had at least one *Salmonella* isolate confirmed by the Microgen^{TM} system with Samaru and Jushi the only sampling areas with two standardized micro-substrate detection kit confirmed isolates. Basawa sampling unit was the only unit without a standardized micro-substrate detection kit confirmed *E. coli* isolate, with Samaru having one and both Sabon Gari and Jushi having two standardized micro-substrate detection kit confirmed *E. coli* isolates each (Table 4.9).

Of the organisms identified by the standardized micro-substrate detection kit system, *Escherichia coli* had the highest frequency with five isolates. *Salmonella arizonae* with four isolates had the second highest frequency, while *Salmonella* spp. and *Salmonella* Typhi had one isolate each. Twelve other members of the family *Enterobacteriaceae* were also identified (Table 4.10).

Table 4.6 Distribution of *Salmonella* and *E. coli* screened using the Standardized micro-substrate (Microgen GN-ID A+B) system for gram negative bacteria, obtained from four (4) sampling areas.

S/no	Sampling area	No of *Salmonella* isolates confirmed	No of *E. coli* Confirmed
I	Samaru	2	1
II	Sabon Gari	1	2
III	Jushi	2	2
IV	Basawa	1	0
	Total	6	5

Table 4.7 Identified organisms using the Standardized micro-substrate (Microgen GN-ID A+B) system for gram negative bacteria

S/N	Identified organisms	Frequency
I	*Klebsiella ozanae*	6
II	*Escherichia coli*	5
III	*Citrobacter freundi*	5
IV	*Salmonella arizonae*	4
V	*Salmonella enterica* serovar Typhi	1
Vi	*Salmonella* spp.	1
Vii	*Pseudomonas agglomerance*	1

4.5 Results after further confirmatory tests using commercial Latex Agglutination Kits

Five of the isolates confirmed as *E. coli* by the standardized micro-substrate detection kit for gram negative bacteria tested positive for *E. coli* O157:H7 after a further confirmatory test using commercial Latex Agglutination kits. All but Basawa sampling unit had at least one isolate that gave typical *E. coli* O157:H7 reactions. Sabon Gari and Jushi sampling areas had two isolates each while Samaru had a single isolate that gave typical *E. coli* O157:H7 reactions (Table 4.11).

Table 4.8 Frequency distribution of confirmed *E. coli* O157:H7 isolates confirmed using the Latex agglutination kit.

S/N	Sampling Unit	No. of *E. coli* O157:H7
I	Samaru	1
II	Sabon Gari	2
III	Jushi	2
IV	Basawa	0
	Total	5

4.6 Polymerase Chain Reaction (PCR)

4.6.1 Virulence Marker of *Salmonella* Isolates of Polymerase Chain Reaction (PCR)

Following PCR amplification and electrophoresis of the targeted *inv*A virulence gene, the expected 284bp DNA fragment unique to *Salmonella* species was detected in 2 of the 6 *Salmonella* isolates (Plate 1).



Plate I: Amplicons of *inv*A gene in 6 *Salmonella* isolates on 1% agarose gel. Lane 1: 100 base pair markers; Lanes 3,4,5,6,7,8 are *Salmonella* isolates; Lane 9: negative control; Lane 2: positive control.

4.7 Antibacterial resistance

4.7.1 *In vitro* Susceptibilty of *Salmonella* Isolates to 13 Antimicrobial Agents

The percentage susceptibilities of *Salmonella* Isolates to the 13 antimicrobial agents tested are provided on Table 4.12.

All isolates showed hundred percent (100%) resistance to Ampicillin (Beta lactam), doxycycline (tetracycline), sulphamethoxazole/trimethoprim (sulfonamide/tetrahydrofolic acid inhibitor), amoxicillin/clavulanic acid (beta lactam/beta lactamase inhibitor) and tetracycline (tetracycline) with eighty three percent (83.3%) resistance to chloramphenicol (phenicol), eighty three percent (83.3%) resistance to cefixime (cephems) and sixty seven percent (66.7%) resistance to gentamicin (aminoglycoside) (Table 4.12).

The isolates were sixty-seven percent (66.7%) susceptible to imipenem (monobactam), sixty-seven percent (66.7%) susceptible to azithromycin (macrolide), and fifty percent (50%) susceptible to kanamycin (aminoglycoside) (Table 4.12).

Of all antimicrobial classes tested, all isolates exhibited multidrug resistance with each isolate showing resistance to at least 6 and the highest to 11 antimicrobials within the class of beta lactams, tetracyclines, phenicols, aminoglycosides, sulphonamide/tetrahydrofolic acid inhibitor, monobactam, cephems and polymyxins (Table 4.12).

Five resistant patterns were observed, with one isolate resistant to a panel of 10 antibiotics (highest) and six isolates (lowest) resistant to a panel of five antibiotics (Table 4.13).

Table 4.9 *In vitro* susceptibility of 6 *Salmonella* isolates to 13 antimicrobial agents.

Drug class	**Antibiotic**	**Resistance pattern (n=6)**		
		S (%)	**I (%)**	**R (%)**
Carbapenem	Imipenem (30µg)	66.7	33.3	0
Tetracyclines	Tetracycline (30µg)	0	0	100
	Doxycycline (30µg)	0	0	100
Beta-lactam	Amoxicillin/Clavulanic acid (30µg)	0	0	100
Aminoglycosides	Gentamicin (30µg)	0	33.3	66.7
	Kanamycin (30µg)	50	33.3	16.7
Cephems	Cefixime (5µg)	16.7	0	83.3
	Cefuroxime Sodium (30µg)	33.3	50	16.7
Macrolides	Azithromycin (15µg)	66.7	0	33.3
Phenicols	Chloramphenicol (30µg)	16.7	0	83.3
Penicillin	Ampicillin (10 µg)	0	0	100
Folate Pathway inhibitors	Trimethoprim/Sulphamethoxazole (25µg)	0	0	100
Fluoroquinolones	Ciprofloxacin (5 µg)	100	0	0

Key: µg = Microgramm**; n =** Number of isolates**; S =** Susceptible**; I =** Intermediate**; R =** Resistant

Table 4.10 Resistance Patterns of 6 *Salmonella* isolates against 13 antimicrobial agents

Resistance pattern	Number of isolates resistant to panel of antibiotics
TE, AMC, AMP, DO, RL, C, CN, AZM, CFM, CXM	1
TE, AMC, AMP, DO, RL, C, CN, AZM	2
TE, AMC, AMP, DO, RL, C, CN,	3
TE, AMC, AMP, DO, RL, C	5
TE, AMC, AMP, DO, RL	6

Key: TE= Tetracycline; AMC= Amoxicillin/Clavulanic acid; AMP= Ampicillin; DO= Doxycycline; CXM= Cefuroxime Sodium; RL= Sulphamethoxazole; C= Chloramphenicol CN= Kanamycin, AZM= Azithromycin

4.7.2 *In vitro* Susceptibilty of *E. coli* O157:H7 Isolates to Antimicrobial Agents

The percentage susceptibilities of *E. coli* O157:H7 Isolates to the 8 antimicrobials tested are provided on Table 4.14

All isolates showed hundred percent (100%) resistance to ampicillin (beta lactam) and penicillin (penicillin). with 80% resistance to streptomycin (aminoglycoside), 60% resistance to doxycycline (tetracycline) and 60% resistance to sulphamethoxazole/trimethoprim (sulfonamide/tetrahydrofolic acid).

The isolates were hundred percent (100%) susceptible to imipenem (monobactam), 60% susceptible to chloramphenicol (phenicol) and 60 susceptible to gentamicin (aminoglycoside).

Of all antimicrobial classes tested, all isolates exhibited multidrug resistance with each isolate showing resistance to at least 2 and the highest to 7 antimicrobials within the class of beta lactams, tetracyclines, phenicols, aminoglycosides, sulphonamide/tetrahydrofolic acid inhibitor, and monobactam, as shown on Table 4.14

Three resistant patterns were observed, with two isolates showing resistance to a panel of seven antibiotics (highest) and five isolates showing resistance to a panel of two antibiotics (lowest). Three isolates showed resistance to a panel of four antibiotics (Table 4.15).

Table 4.11 *In vitro* susceptibility of 5 *E. coli* O157:H7 isolates to 8 antimicrobial agents.

Drug class	**Antibiotic**	**Resistance pattern (n=6)**		
		S (%)	**I (%)**	**R (%)**
Carbapenem	Imipenem (30 µg)	100	0	0
Tetracyclines	Doxycycline (30 µg)	40	0	60
Penicillins	Penicillin (10 µg)	0	0	100
	Ampicillin (10 µg)	0	0	100
Aminoglycosides	Gentamicin (30 µg)	60	0	40
	Streptomycin (10 µg)	0	20	80
Phenicols	Chloramphenicol (30 µg)	60	0	40
Folate Pathway inhibitors	Trimethoprim/ Sulphamethoxazole (25 µg)	40	0	60

Key: µg – Microgramm; n = Number of Isolates; S = Susceptible, I = Intermediate; R = Resistance

Table 4.12 Resistance Patterns of 5 *E. coli* O157:H7 isolates against 8 antimicrobial agents

Resistance pattern	Number of isolates resistant to panel of antibiotics
AMP, P	5
AMP, P, S, DO	3
AMP, P, S, DO, CN, C, RL	2

Key: AMP = Ampicillin; DO = Doxycycline; RL= Trimethoprim; C = Chloramphenicol; CN= Kanamycin; S = Streptomycin; P = Penicillin

4.8 Demographic features of retailers and consumers of roasted ready-to-eat rat meat in Zaria

Table 4.13 gives the demography of retailers and consumers of roasted ready-to-eat rat meat sold in Zaria. Majority of the consumers were males (64%). The highest age range was 19-36 years, which was recorded by 40% of the consumers. Seventy-six percent of the consumers had tertiary education. Ninety percent of the consumers claimed to be aware of meat safety. Majority of the retailers were males (83%). The highest age range recorded by the retailers was 19-36 years (58%). Seventy-five percent of the retailers had no formal education. Ninety-two percent of the retailers were without meat safety training/certificate.

4.9 Practices of consumers of roasted ready-to-eat rat meat sold in Zaria, Nigeria.

Eighty-eight percent of the consumers buy the roasted ready-to-eat rat meat from markets, while 12% source the rats within households. Fifty-one percent use bare hands in collecting the roasted ready-to-eat rat meat from the sellers while 49% collect the roasted ready-to-eat rat meat from the sellers in polythene bags. Fifty-six percent consume the roasted ready-to-eat rat meat after cooking in soup while 44% spice with salt and pepper then consume, without reheating. Air drying is the major mode of preservation of roasted ready-to-eat rat meat as reported by 52% of the consumers while 48% employ roasting in preserving roasted ready-to-eat rat meat not consumed immediately after purchase. Seventy-three percent of the consumers attested to not been satisfied with the surrounding where the roasted ready-to-eat rat meat is placed by the sellers (Table 4.14).

4.10 Practices of retailers of roasted ready-to-eat rat meat sold in Zaria, Nigeria.

All the retailers (100%) get the rats from outdoor bushy areas. The roasted ready-to-eat rat meat that aren’t sold off immediately are preserved by air drying by 42% of the retailers while 58%

smoke/reheat. Sixty-seven percent of the retailers present the roasted ready-to-eat rat meat to the buyers using bare hands while 33% use polythene bags. Highest sales are recorded from January-April as documented by 100% of the retailers. Sixty-seven percent of the retailers also make sales to other areas outside the study area with demand. All retailers (100%) record 100% regularity in patronage (Table 4.15).

Table 4.13 Demography of retailers and consumers of roasted ready-to-eat rat meat sold in Zaria.

	Demography	No of Respondents	Frequency (%)
Consumers of smoked ready-to-eat rat meat	**Age (years)**		
	<18	12	14
	19-36	34	40
	37-54	24	29
	55-72	10	12
	>72	4	5
	Sex		
	Male	54	64
	Female	30	36
	Level of education		
	Primary	4	5
	Secondary	10	12
	Tertiary	64	76
	No formal Education	6	7
	Meat safety knowledge/ Awareness		
	Yes	76	90
	No	8	10
Retailers of roasted rat meat	**Age (Years)**		
	<18	0	0
	19-36	7	58
	37-54	5	42
	55-72	0	0
	>72	0	0
	Sex		
	Male	10	83
	Female	2	17
	Level of education		
	Primary	0	0
	Secondary	1	8
	Tertiary	2	17
	No formal education	9	75
	Meat safety training/		

Certificate		
Yes	1	8
No	11	92

Table 4.14 Practices of consumers of roasted ready-to-eat rat meat sold in Zaria, Nigeria.

knowledge/practices	No of respondents	Percentage (%)
Source of rat meat		
Purchase from market	74	88
Caught within households	10	12
Method of consuming smoked rat meat		
After cooking with soup	47	56
After spicing without further reheating	37	44
Method of collecting smoked rat meat from sellers		
By using bare hands	43	51
In polythene bags	41	49
How do you preserve smoked rat meat not consumed? immediately after purchase		
Air drying	44	52
Smoking/ Reheating	40	48
Are you satisfied with the surrounding where the rat meat is displayed by the seller?		
Yes	23	27
No	61	73

Table 4.15 Practices of retailers of roasted ready-to-eat rat meat sold in Zaria, Nigeria.

Practices	No of respondents	Percentage (%)
Source of rats		
Caught within households	0	0
Caught from outdoor bushy areas	12	100
Mode of preserving smoked rat meat not sold Immediately		
Air drying	5	42
Smoking/ Reheating	7	58
Mode of presenting the smoked rat meat to the buyers		
By bare hands	8	67
By use of gloves/polythene bags	4	33
Do you wash your hands before and after handling the Smoked rat meat		
Yes	12	100
No	0	0
Period of highest sales		
January-April	12	100
May-August	0	0
September-December	0	0
Sales of smoked rat meat in other states		
Yes	8	67
No	4	33
Regularity in patronage by customers		
Yes	12	100
No	0	0

CHAPTER FIVE

5.0 DISCUSSION

Results of this study showed that there was bacterial contamination of roasted ready-to-eat rat meat sold in Zaria, Kaduna State, Nigeria. The contamination could be attributed to unhygienic practices observed in processing, packaging and preservation of the roasted ready-to-eat rat meat as Mead *et al.* (1999) reported that meat may be easily contaminated with different pathogens if not handled appropriately. Also, the means of transportation and packing methods used during bushmeat transportation are other factors that may cause the contamination of bushmeat by pathogens (Vliet *et al.*, 2017).

Regarding the frequencies of samples exceeding the acceptable Total aerobic plate count limits, there were significant difference among the sample locations. The high values of TAPC observed; $>10^9$ is unsatisfactory according to FAO/WHO (2002), Microbial guidelines for food, which states that for smoked ready-to-eat meat and fish, the total aerobic plate counts of $<10^6$ is satisfactory while $\geq 10^7$ is unsatisfactory. High values of bacterial contamination in locally processed meat have also been observed in other studies. Salihu *et al.* (2010) reported a TAPC of between 6.70×10^8 and 9.30×10^9 Cfu/g from traditionally prepared fried ground beef (Dambun nama) in Sokoto, Nigeria. Syne *et al.* (2015) reported a TAPC of 9.0×10^9 Cfu/g from locally processed meat sold in retail outlets in Trinadad and Tobago while Gideon and Joseph (2018) reported a TAPC of 2.3×10^8 from cane rats, in an assessment of microbial count loads of bush meats sold in different markets in Benin city, Nigeria.

The high TAPC observed in this study could be due to the poor hygiene practices observed in the handling, processing and packaging practices as Abolagba and Iyeru (1998) who reported that a

high microbial load of smoked products would be as a result of lack of proper hygienic handling and smoking.

Contamination of meat at unacceptable levels may contribute significantly to changes in meat structure, color and flavor and cause meat spoilage (De Filippis, 2013). Meat and meat products derived from animals contaminated with meat borne bacteria can cause infections and intoxications leading to morbidity and/or mortality in both food handlers and consumers (Muma. 1998).

The results of *Salmonella* isolation in the present study demonstrates the presence of the pathogen in roasted ready-to-eat rat sold in Zaria, Nigeria. The prevalence of *Salmonella* detected in the study, despite the poor/unhygienic processing, handling and preservation practices appears low. Notwithstanding, *Salmonella* is an important food borne pathogen and its presence in foods is of risk to human health and poses serious concerns to public health. The *inv*A gene which is essential for full virulence in *Salmonella* and is thought to trigger internalization required for invasion of deeper tissue (Khan *et al*., 2000), was detected in two (2) of the (6) screened *Salmonella* isolates, giving an overall prevalence of 0.5%. The absence of the gene in the screened *Salmonella* isolates may be as a result of lack of invasiveness by those isolates. This agrees with other reports of Bacci *et al.* (2006) who reported the *inv*A gene in 62 of 63 strains of *Salmonella* screened and Oludairo *et al.* (2013) who reported 5 out of 8 *Salmonella* isolate harboring the *inv*A gene. These findings however defer from that of Tafida *et al.* (2013) who reported the *inv*A gene in all *Salmonella* isolates tested. Culture based methods are still the most widely used and remain the gold standard in the detection of *Salmonella* due to their selectivity and sensitivity even though PCR techniques have received much attention recently in the detection of *Salmonella* spp. as they have the advantage of being fast, less laborious and reliable as using *inv*A gene primers specific for

Salmonella considerably decreases the number of false positive results, in PCR assay. (Alocilja and Radke, 2003; Amini *et al.,* 2010).

Other studies have shown the presence of *Salmonella* spp. in the study area. Kwaga *et al*. (1985) obtained a prevalence of 8.4% in raw beef and 1.5% in cattle rectal swabs respectively in Zaria while Esona *et al*. (2004) reported a 6% isolation rate of *Salmonella* from beef in Zaria. The low prevalence in the present study agree with the findings of Abd El-Atty and Meshref. (2007) who detected *Salmonella* with a prevalence of 4% in sausages and 2% in spiced minced meat and the findings of Tafida *et al.* (2013) who reported an overall *Salmonella* spp. prevalence of 2.3% from 453 retailed beef and related meat products. This further corroborates the low rate of isolation of *Salmonella* in the study, due to the fact that these foods are equally exposed to prolong high temperature as a result the low moisture content of the products leads to reduced water activity, which does not promote the growth and survival of microorganisms (Tafida *et al.,* 2013).

The study also revealed the presence of *E. coli* O157:H7 in roasted ready-to-eat rat meat sold in Zaria, Nigeria. The low isolation rate, 1.3 %, observed in this study, could be attributed to the fact that the rat meat are exposed to high temperatures, reducing the moisture content thereby reducing microbial activity despite the high rate of contamination. The presence of interfering bacteria during isolation (e.g *Pseudomonas* spp.) may be a possible reason for the low prevalence of *E. coli* O157:H7 (Szabo *et al.,* 1986; Vernozy-Rozand, 1997; Lejeune *et al.,* 2001). The low isolation rate of 1.3 % of *E. coli* O157:H7 observed in this study agree with the findings of Tafida *et al.* (2014) who reported 2.2 % prevalence from raw meat analyzed in Zaria. Also, (Enabulele and Uraih, 2009) reported a 2.3 % prevalence of *E. coli* O157 from raw meat and "suya" in Benin, Nigeria. The low isolation rate observed in this study however is at variance with the findings of Dahiru *et*

al. (2008) who recorded a 25% prevalence of *E. coli* O157:H7 from roasted beef (suya) in Kano, Nigeria.

The standardized micro-substrate (Microgen GN-ID A+B) kit for gram negative bacteria confirmed six (6) of eighteen (18) suspected *Salmonella* isolates identified by conventional biochemical tests and five (5) of the fifteen (15) suspected *E. coli* O157:H7 isolates identified by the CT-SMAC. The standardized micro-substrate (Microgen GN-ID A+B) kit for gram negative bacteria as a means of identification of *Enterobacteriaceae* proved to be a more sensitive and convenient method for the identification of *Salmonella* and *E. coli* over the conventional method of identification normally employed. Among the organisms identified by the standardized micro-substrate (Microgen GN-ID A+B) kit for gram negative bacteria were *Salmonella arizonae, Escherichia coli, Salmonella* Typhi, *Citrobacter freundi, Pseudomonas agglomerans,* and *Klebsiella ozaenae.* Other bacteria detected were also of the *Enterobacteriaceae* family. Some members of this family, in addition to their role in food borne illness, are associated with food spoilage and therefore contribute to substantial economic losses and food wastage (Baylis *et al.,* 2011). Five (5) of the *E. coli* isolates confirmed by the standardized micro-substrate (Microgen GN-ID A+B) kit for gram negative bacteria were further confirmed as *E. coli* O157:H7 by the Rapid latex agglutination test.

Resistance to antibiotic poses a great threat in both veterinary and human medicine, and bacteria resistance could be caused by a variety of factors. van den Bogaard *et al*. (2001) explained that usage of antibiotics was the most significant factor responsible for antimicrobial resistance in bacteria.

It was observed in this study that multiple antibiotic resistance was common among *E. coli* O157:H7 and *Salmonella* spp. These results are in agreement with previous reports in Nigeria (Raji *et al*., 2007; Olonitola *et al*., 2015). The resistance could be as a result of indiscriminate use of antibiotics by the populace within the study area.

Antibiogram of the *Salmonella* isolates from this study revealed multi-drug resistance to commonly used antibiotics, with each isolate resistant to at least five (5) of the antibiotics tested. There has been an increasing resistance to commonly used antimicrobials, in Nigeria, over the past 25 years (Okeke *et al.,* 2000; Iwalokun *et al.,* 2001) and other parts of the world such as Kenya (Kariuki *et al.,* 2006), Singapore (Ling *et al.,* 2002) and the Netherlands (Kivi *et al.,* 2005). In the present study, all *Salmonella* isolates showed 100% resistance to ampicillin, doxycycline, tetracycline, amoxicillin/clavulanic acid, and trimethoprim/sulphamethoxazole. A high resistance of 83.3% to chloramphenicol, 83.3% to cefixime and 66.7% to gentamicin was observed. Hundred percent susceptibility of the isolates to ciprofloxacin and 66.7% susceptibility to both imipenem and azithromycin was recorded in the study. This agree with the findings of Davies *et al.* (2005) who reported resistance of 7 clinical *Salmonella* isolates to at least 3 antimicrobials including ampicillin, tetracycline, chloramphenicol, nalidixic acid and cotrimoxazole, categorized as first line drugs in the treatment of typhoid fever.

The 100% susceptibility of *Salmonella* spp. observed against the fluoroquinolone (ciprofloxacin) is similar to a report by Campos *et al*. (1990), Sonstein and Burnham. (1993) and Mathew *et al.* (2015). However, in Lagos, Nigeria, Akinyemi *et al*. (2007) reported 18% reduced susceptibility of *Salmonella* spp. to ciprofloxacin. The high susceptibility of the *Salmonella* spp. to fluoroquinolones recorded in this study may be connected to relatively high cost of ciprofloxacin (Akinyemi *et al*., 2007), thereby discouraging its indiscriminate use.

The high (66.7%) susceptibility of the *Salmonella* isolates to imipenem observed in this study agrees with the findings of Mathew *et al.* (2015) who reported 100% susceptibiity of *Salmonella* enterica Serovar Typhi to imipenem, in Kaduna Metropolis, Nigeria.

The high resistance of the *Salmonella* isolates, 100% to Ampicillin and 83.3% to chloramphenicol could be due to the fact that these are the most commonly used drugs in human and poultry (Threlfall *et al*., 2001), therefore the development of resistance over time. The high percentage resistance of the *Salmonella* isolates to ampicillin and amoxicillin/clavulanic acid, which belongs to same class of antibiotics are similar with the findings of Mshelbwala *et al.* (2018), Suresh *et al.* (2006) and Mathew *et al.* (2015).

Resistances to sulphamethoxazole-trimethoprim among *Salmonella* isolates have been documented from other parts of the world, Senegal (Bada-Alambedji *et al.,* 2006), Mexico (Zaidi *et al.,* 2006) and USA (Zhao. *et al.,* 2006). A 100% resistance of the *Salmonella* was observed in this study which is as opposed to the low resistance (55.1%) reported by Agada *et al.* (2014).

The *Salmonella* isolates in this study showed 100% resistance to the tetracyclines (tetracycline and doxycycline), which is similar to the findings of Kalu *et al.* (2008) and Mathew *et al.* (2015). This finding was not surprising as both antibiotics belong to the same class.

Eighty-three percent resistance of the *Salmonella* isolates to cefixime, a cephalosporin was observed in this study. This trend is of particular concern because the extended spectrum cephalosporins are the antibiotics of choice for children (Weill *et al*., 2004).

Also the antibiogram of *E. coli* O157:H7 reported in this study shows multidrug resistance to various antibiotics tested at various percentages. This result is in agreement with the findings by other researchers like Rueben *et al.* (2013), Schroeder *et al.* (2002) and Kim *et al.* (1994) who

reported multidrug resistance among *E. coli* O157:H7 isolates. A 100% resistance to ampicillin and penicillin by the *E. coli* O157:H7 isolates was observed. The *E. coli* O157:H7 isolates showed resistance to 80% streptomycin and 60% resistance to both doxycycline and trimethoprim/sulphamethoxazole. All the isolates (100%) were sensitive to imipenem,

The high resistance (100%) observed of the *E. coli* O157:H7 isolates to ampicillin and penicillin (Beta-Lactams) are similar with the findings reported by Shintandi and Sternesjo (2001), Al Haj *et al* (2007), Olatoye (2010), Reuben *et al.* (2013) and Tafida *et al.* (2014). These are the most commonly available antibiotics used as growth promoters and routine chemoprophylaxis among livestock in Nigeria (Olatoye, 2010) which may be a probable reason for the high resistance to these antibiotics observed in this study. Another probable reason for the high resistance of the isolates to doxycycline (a tetracycline) and penicillin could be due to the fact that Tetracycline and penicillin (ampicillin) are first-line drugs which are routinely prescribed or readily purchased over the counter for self-medication (Ayukekbong *et al.,* 2017). The development of antimicrobial resistance might limit their use leading to treatment failure and onset of complications (Reuben and Owuna, 2013).

Above the average resistance (60%) of the *E. coli* isolates to sulphamethoxazole / trimethoprim is similar with findings of Rueben *et al.* (2013) who observed 84.2% resistance of *E. coli* O157:H7 isolates from fermented milk samples to this antibiotic in Nasarawa State, Nigeria. This antimicrobial is commonly used to treat respiratory infections, diarrhoea, mastitis, and other infections in beef and dairy cattle (Rueben *et al.,* 2013), which could be a reason for the high resistance observed in this study.

Eighty percent resistance of the *E. coli* O157:H7 was observed against streptomycin. This finding was rather surprising as Cheesbrough (2000) reported that the antibiotic is administered intravenously thereby restricting indiscriminate use. Rueben *et al.* (2013) however, found resistance of *E. coli* O157:H7 isolates to streptomycin to be relatively low which is dissimilar to the high resistance observed in this study.

Also the *E. coli* O157:H7 isolates were observed to have with 60% susceptibility to gentamicin and chloramphenicol. This finding is in agreement with reports by Walsh *et al.* (2006) and Okolocha (2006) who observed that all *E. coli* isolates tested were highly susceptible to gentamicin and trimethoprim. The quinolones ciprofloxacin and ofloxacin, the aminoglycoside gentamicin and the phenicol chloramphenicol all showed great activity, agreeing with the findings of Iwu *et al*. (2017) that they are the drug of choice for E. coli O157 infections.

The high susceptibility (60%) of the *E. coli* O157:H7 isolates to chloramphenicol in this study was not in agreement with an earlier study in Zaria by Raji *et al*. (2007) however agrees with the findings of Ejeh *et al.* (2017).

Susceptibility of the *E. coli* O157:H7 isolates to imipenem was observed to be the highest (100%). This makes this antibiotic a considerable drug in treatment of *E. coli* O157:H7 infections. This high susceptibility to imipenem was also observed by Goncuoglu *et al.* (2010) who documented a 100% susceptibility of *E. coli* O157:H7 isolates to the carbapenem. Among the several hundreds of known betalactams, carbapenems possess the broadest spectrum of activity and greatest potency against Gram-positive and Gram-negative bacteria. They are often referred to as “antibiotics of last resort” and are administered when patients with infections are suspected to be harboring resistant bacteria or become gravely ill (Torres *et al*., 2007).

According to Aarestrup (1995) and Levin *et al*. (1997), multiple resistances capable of regional dissemination can emerge as a result of antimicrobial selection pressure in either livestock or humans. Evidence has been found which indicates that resistant strains of pathogens can be transmitted to humans through food (Oosterom, 1991; Khachatourians, 1998) which predisposes the consumers of roasted ready-to-eat rat meat to resistant strains of these pathogens.

The multidrug resistance observed in the study is of serious public health concern and requires urgent attention. The finding that all *Salmonella* and *E. coli* O157:H7 isolates were susceptible to ciprofloxacin and imipenem, with high percentage susceptibility of the *Salmonella* isolates to cefixime and azithromycin and high percentage susceptibility of the *E. coli* O157:H7 isolates to chloramphenicol and gentamicin, gives assurance of the need to continually maintain a susceptible *Salmonella* and *E. coli* O157:H7 population to these antibiotics. Also the 100% resistance observed by the *Salmonella* isolates to tetracycline amoxillin/clavulanic, ampicillin, doxycycline and trimethoprim and 100% resistance of *E. coli* O157:H7 to ampicillin and penicillin, calls for urgent attention and public health concerns to find alternatives to these drugs and regulate their indiscriminate use by enacting stringent laws.

From the questionnaires administered, it was recorded that majority of the retailers were males between the ages of 19-36 and 37-54 years. This is not surprising as the profession demands energy and time, from hunting of the rats to processing them. This finding agrees with reports by Salifu and Teye (2006), on meat handling profession being energy demanding and requiring men who are strong and the youth who can cope with the business. The fact that majority (75%) of the retailers had no formal education and majority (92%) were without meat safety training/certificate is critical to the consumers' health as FAO (2003), documented that to handle food hygienically, food handlers should have the necessary knowledge and skills. Also, according to Adams and

Moss (2008), an integral part in ensuring safe products to the consumer is training of food handlers regarding the basic concepts and requirements of personal hygiene. Majority of the consumers had tertiary education and claimed to be aware of meat safety. However, the knowledge and practices of the consumers documented in this study generally shows poor meat safety practices employed.

Majority of the consumers (56%) consume the smoked rat meat in soup which ensures that the meat is further cooked, thereby obviating the action of microbes. A large percentage (44%) of the consumers consume the smoked rat meat with spices without further reheating. Although, spicing has been shown to have antimicrobial effect in roasted beef (Shelef, 1984), this practice of consuming the smoked rat meat without further reheating could be hazardous to the consumers as Sidorowicz (1974) and Vliet *et al.* (2017) reported that smoked meat constitutes a high risk if only superficially or inadequately smoked. Seventy-three percent of the consumers expressed their dissatisfaction with the surroundings in which the smoked rat meat is displaced. This gives an idea of a poor environmental hygiene by the retailers. Personal hygiene plays a critical role in meat safety. Majority of the consumers (51%) documented that they collect the smoked rat meat from the sellers by the use of bare hands and a larger percentage (67%) of the retailers reported that they use bare hands to present the smoked rat meat to the customers. This could be a potential source of meat contamination if the hands are not properly washed/disinfected (FAO, 2017). Air drying technique, as a means of preservation was employed by majority (52%) of the consumers and 42% of the retailers. This could present health hazards to immediate consumers because even though air drying is one of the oldest and inexpensive food preservation techniques used to prolong shelf-life of foods (Akhtar and Pandey, 2015), it is very prone to contaminants (Kumar *et al.,* 2015). All the retailers reported that they wash their hands before and after handling the smoked rat meat.

This is a good step in personal hygiene in reducing the risk of contamination in handling smoked meat (Abolagba and Iyeru 1998; FAO, 2017).

The regularity in customer patronage documented by all the retailers, sales in other states outside the study area as reported by a high percentage (67%) of the retailers suggests that the sales of smoked rat meat could be a lucrative business. However, the fact that the availability of the rats is highly seasonal and restricts sales to the first quarter of the year (January – April) may pose a disadvantage to this profession.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Based on the findings and deductions from the results of this study, it is evident that the bacteriological load of roasted ready-to-eat rat meat sold in Zaria metropolis is above the permissible value by FAO/WHO (2002). The study also established the presence of *Salmonella* spp. and *Escherichia coli* 0157:H7. *Salmonella* spp. had an overall frequency of 0.52%, with frequencies of 0.26% each in Samaru and Jushi sampling unit and this has critical implications for public Health. *Escherichia coli* O157:H7 had an overall frequency of 1.3%, with its presence in all but one of the areas sampled.

Multidrug resistance of the *Salmonella* and *E. coli* O157:H7 isolates was observed in the study. The *Salmonella* isolates showed highest sensitivity to ciprofloxacin and imipenem, and showed 100% resistance to tetracycline, amoxicillin/clavulanic acid, ampicillin, doxycycline and sulphamethoxazole. The *E. coli* O157:H7 isolates showed highest sensitivity (100%) to imipenem and showed 100% resistance to penicillin and ampicillin.

The unhygienic practices in processing, handling and preservation techniques implored, could be a probable reason for the high bacteria count observed in this study. The above findings reported in this study have serious public health concern on the burden of *E. coli* O157:H7 and *Salmonella* spp. in Zaria and Nigeria as a whole. The occurrence of these pathogens, suggest that the consumers of roasted ready-to-eat rat meat are exposed to food borne hazards. This study further confirms that food hygiene, handling practices and packaging remain a great challenge in a developing country such as Nigeria.

6.2 Recommendations

Based on the findings in this study, the following recommendations are made:

1. The government should set up strict microbial standards for the processing of roasted ready-to-eat rat meat to reduce the risk of food borne pathogens.
2. The unregulated supply chain of these roasted rat meat, can make outbreak of diseases difficult to trace. Regulatory bodies should be set up to monitor the processing and distribution of roasted rat meat, and if possible, register sales points.
3. Laws to limit the prescription and indiscriminate dispensing of antibiotics by only qualified medical practitioners should be enacted by the government.
4. Effective susceptibility test should be conducted by medical practitioners before prescribing and administering antibiotics to patients.

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APPENDICES

APPENDIX I: Preparation of Media and Reagents

The following media were used

Sorbitol MacConkey agar (SMA)

Composition in gram per litter; 20.0 g peptone; 10.0 g sorbitol; 5.0 g NaCl; 1.5 g bile neutral red; 300 mg neutral red; 1.0 g crystal violet; 15.0 g agar

Preparation; 51.5 g Sorbitol MacConkey agar powder per litter of distilled water in a sterile conical flask. Mixed by shaking and boil to dissolve completely, autoclaved at 121^{o}C for 15 minutes. Supplemented with (0.2 g/L) with Cefixime 50 µg/L and Potassium tellurite 2.5 mg/L at 45^{o}C to enumerate *E. coli* O157:H7. 20 ml were dispensed per petri dish

Nutrient agar (NA)

Composition in grams/litter; 1.0 g Lab. Lemco powder; 2.0 g Yeast extract; 15.0 g Sodium Chloride; 5.0 g Agar; 5.0g Peptone; 7.4 pH

Preparation: 23 g of NA per 1 L of distilled water. Mixed to by boiling to dissolve completely and aseptically dispensed into bijou bottle autoclaved at 121^{o}C for 15 mins. Medium allowed to dry in slant position and kept in the fridge for further use.

Eosin Methylene Blue (EMB)

Composition in grams per litter; 1.o g Lactose; 5.0 g Bile Salts; 5.0 g Sodium Chloride; 0.075 g Neutral red; 12.0 g Agar; 1000ml Distilled water; 7.6 PH

Preparation; 28 g of EMB agar powder per 1 L ofdistilled water. Mixed by shaking and boiling to dissolve then autoclaved at 121^{o}C for 15mins. Dispensed 20 ml per petri dish.

Buffered Peptone Water (PW)

Composition in grams per liter; 10.0 g Peptone; 5.0 g Sodium Chloride; 1000 ml Distilled water; 7.6 pH

Preparation; 20g per 1 L distilled water. Autoclaved at 121^{o}C for 15 minutes

Urea Agar (UA)

Composition in grams per litter; 1.0 g Peptone; 5.0 g Sodium Chloride; 2.0 g Potassium dihydrogen-Sulphate K H_2PO_4; 5.0 g Glucose; 20.0 g Agar powder; 1000ml Distilled water

Preparation; 2.4 g was suspended in 95ml of distilled water dissolved by boiling. Autoclaved at 115^{o}% for 20 minutes. Cooled to about 45^{o}C and 5 ml of 40% v/v sterile urea solution was added. Medium was dispensed into culture tubes.

Simmon Citrate Agar (SCA)

Composition in grams per liter; 2.0 g Magnesium sulphate; 0.8 g Sodium ammonium sulphate; 0,2 g Ammonium dihydrogen sulphate; 2.9 g Sodium citrate tribasic; 5.0 g Sodium Chloride; 0.08 g Bromoethyl molblue; 15.0 g Agar No.3; 1000 ml Distilled water; 6.9 pH

Preparation; autoclaved at 121^{o}C for 15 minutes. Dispensed into culture tubes and the medium was allowed to solidify.

Kovacs reagent for Indole (Cowan and steel, 1974)

It contains: 5.0 g g p-dimethylaminobenzaldehyde; 75 ml amyl alcohol; 25 ml conc. HCL

Preparation: Dissolve the aldehyde in the alcohol by gentle warming in a water bath about 50-55^{o}C. Cool and add the acid protect from light and store at 4^{o}C.

Triple sugar iron Agar (TSI) (Cowan and Steel, 1974)

Composition per liter; 3.0 g beef ectract; 3.0 g yeast extract; 20 g peptone; 1.0 g glucose; 10.0 g lactose; 10.0 g sucrose; 200 mg $FeSO_4.7H_2O$; 5.0 g NaCl; 300 mg $Na_2S_2O_3.5H_2O$; 20.0 g agar; 12.0 ml phenicol red (0.2 %)

Preparation: Dissolve the solids by heating, sterilize at 115^oC 20 minutes and cool to form slants with deep butts.

Methyl Red Solution (Cowan and Steel, 1974)

Composition; 40 mg methyl red; 40.0 g ethanol

Peparation; Methyl red dissolved in ethanol and dilute to volume with distilled water.

Citrate Media (Cowan and Steel, 1974)

Composition per liter; 5.0 g NaCl; 200 mg $MgSO_4$; 1.0 g K_2HPO_4; 1.0 g NH_4HPO_4

Preparation: Dossiole the salt in distilled water, and 3g of Sodium citrate or 2.77 g of hydrated form to the salt solution and sterilize at 115^oC for 20 minutes

MRVP Medium (Oxiod, UK)

Composition in grams per liter; Peptone 7.0; Glucose 5.0; Phosphate buffer 5.0

Preparation: Dissolve 17 g in 1L of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121^oC for 15 mins.

Sulphide Indole Motility (Oxiod, UK)

Composition in grams per liter; Tryptone 20.0; Peptone 6.1; Ferrous Ammonium Sulphate 0.2; Sodium thiosulphate; Agar 3.5; pH 7.3±0.2 at 25^oC.

Preparation; Suspend 30 g in 1L of distilled water and boil to dissolve the medium completely; Dispense into final containers and sterilize by autoclaving at 121^{o}C for 15 mins.

Muelletr Hilton agar (Titan Biotech, India)

Composition in grams per liter; Beef infusion from casein 300.0; Acid hydrosylate 17.5; Agar 17.0; Starch 1.5 pH 7.3±0.2 at 25^{o}C

Kovacs reagent

Composition in grams per liter; P-dimethylaminobenzyldehyde – 10g, Isoamyl alchohol (amyl alcohol) – 150 ml, Concentrated hydrochloric acid (HCL) – 50 ml

Preparation; Add 50 ml of concentrated HCL to 150 ml of amyl alcohol, Dissolve 10 g of p-dimethylaminobenzaldehyde in the solution,

Normal saline 8.5g/l (BDH Chemical, England)

Concentration in grams per liter; Sodium Choride (NaCl) – 8.5g, Distilled water – 1L

Preparation: Weigh the NaCl and transfer into conical flask, Add distilled water to the 1 liter mark. Mix thoroughly until the salt is fully mixed,

Oxidase reagent (KEM light labs, India)

Composition: Tetramethyl-p phenylenediamine dichloride – 0.1g, Distilled water – 10 ml

Preparation: Dissolve 10g of the reagent in 11 of distilled water. Use fresh

APPENDIX II: Roasted ready-to-eat rat meat displayed for sale



APPENDIX III: Roasted rats sold in a compound selected by bare hands



APPENDIX VI; Roasted ready-to-eat rat meat



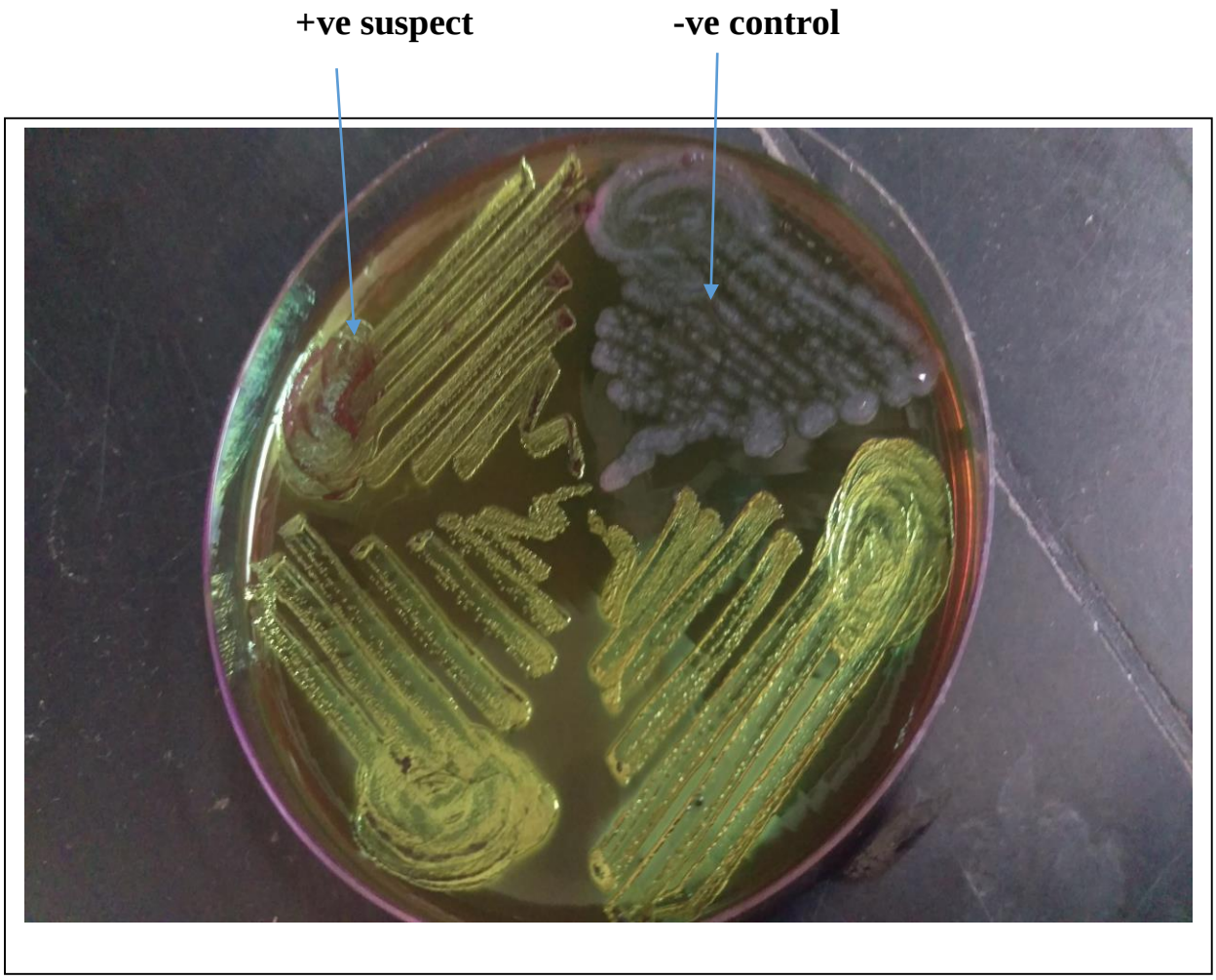
APPENDIX V: Poorly roasted rat, showing traces of fresh blood



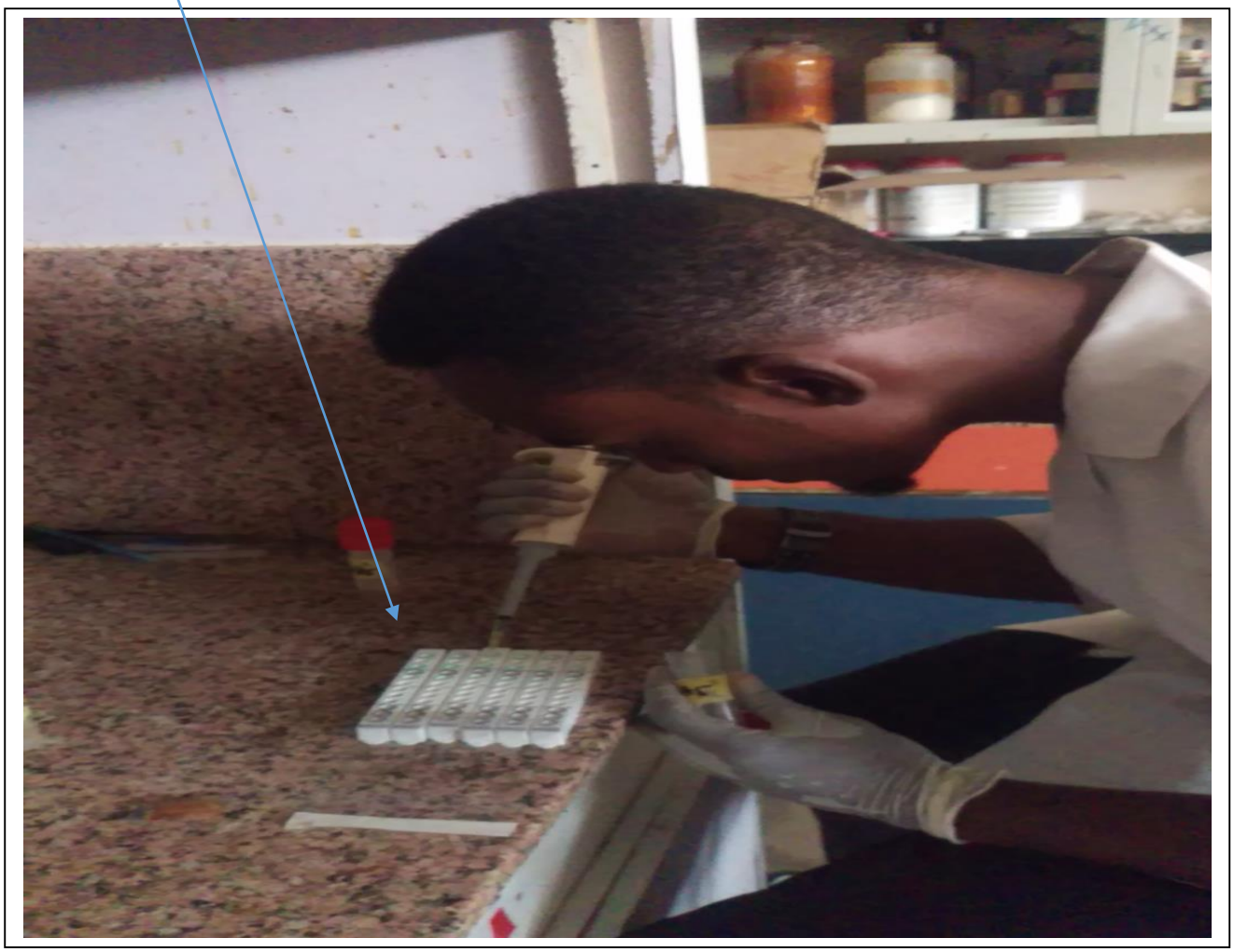
APPENDIX VI: Roasted ready-to-eat rat meat packaged in a carton to transported out of Zaria.



APPENDIX VII: Suspect *E.coli* colonies on E.M.B



APPENDIX VIII: Student carrying out the Microgen™ test



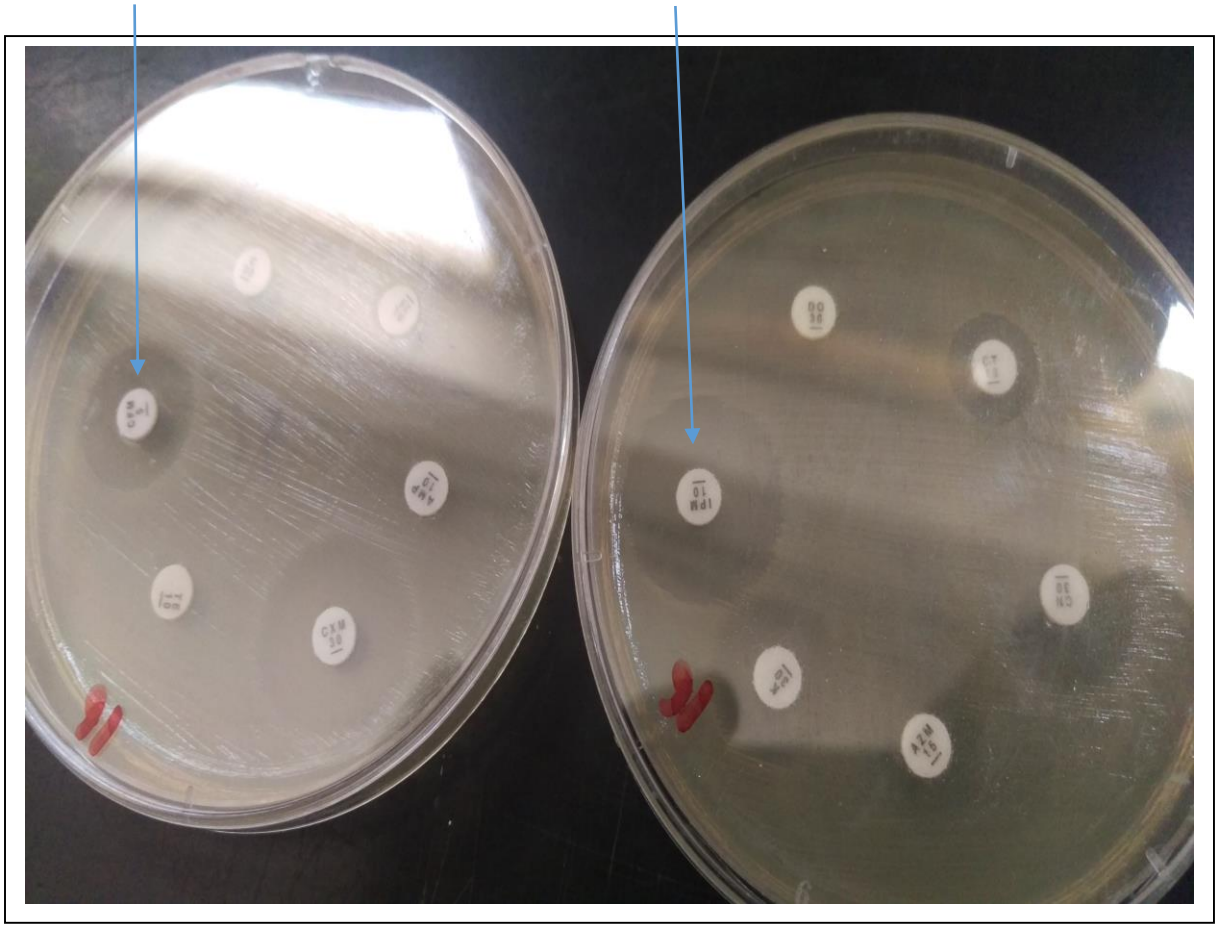
APPENDIX IX: MICROGEN^{TM} TEST SHOWING REACTIONS IN WELLS



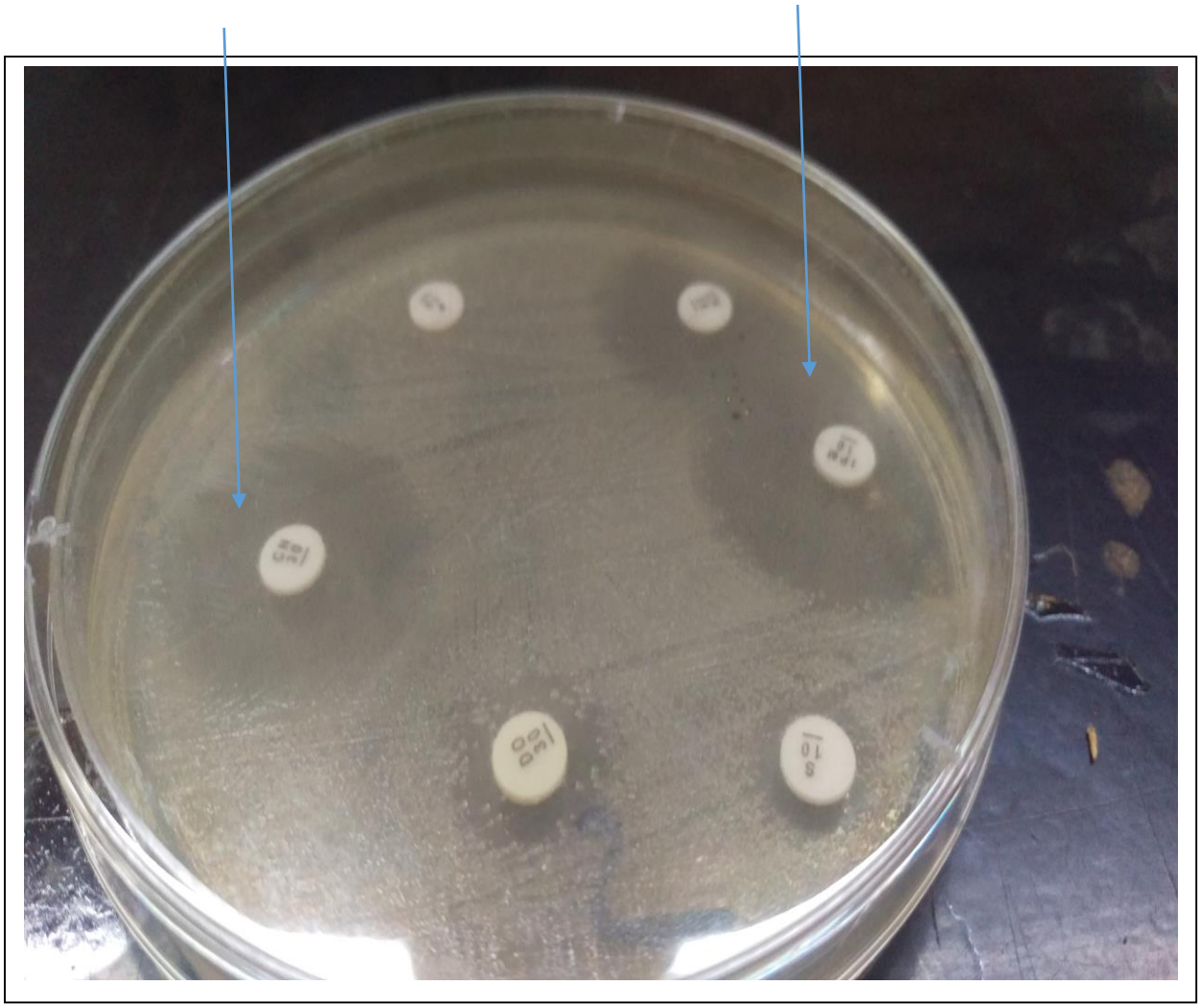
APPENDIX X: Antibacterial susceptibility pattern of Salmonella isolates to 2 antibiotics showing zone of inhibition.



APPENDIX XI: Antibacterial susceptibility pattern of *Salmonella* to 12 antibiotics showing zone of inhibition.



APPENDIX XII: Antibacterial susceptibility pattern of E. coli O157:H7 to 6 antibiotics showing zone of inhibition.



APPENDIX XIII: Clinical Laboratory Standard Institute cut-off points Chart Used in the study

Antimicrobial agent	Disc content	Zone diameter interpretive criteria		
		S	I	R
Ampicillin	10 µg	≥17	14-16	≤13
Amoxicillin-clavulanate	20/10 µg	≥18	14-17	≤13
Cefepime	30 µg	≥25	-	≤18
Cefuroxime	30 µg	≥18	15-17	≤14
Cefixime	5 µg	≥19	16-18	≤15
Imipenem	10 µg	≥23	20-22	≤19
Gentamicin	10 µg	≥15	13-14	≤12
Kanamycin	30 µg	≥18	14-17	≤13
Streptomycin	10 µg	≥15	12-14	≤11
Tetracycline	30 µg	≥14	12-14	≤11
Doxicycline	30 µg	≥16	11-13	≤10
Ciprofloxacin	5 µg	≥31	21-30	≤20
Trimethoprim-Sulphamethoxazole	25 µg	≥16	11-15	≤10
Chloramphenicol	30 µg	≥18	13-17	≤12
Penicillin	10 µg	≥17	14-16	≤13