OCCURRENCE AND ANTIBIOGRAM OF *ESCHERICHIA COLI* O157: H7 IN READY TO EAT BEEF *'SUYA'* SOLD IN ZARIA METROPOLIS, NIGERIA.

BY

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DECLARATION

I declare that the work in this thesis entitled **"Occurrence and antibiogram of *Escherichia coli* O157: H7 in ready to eat beef *'Suya'* sold In Zaria metropolis, Nigeria"** has been carried out by me in the Department of Veterinary Public Health and Preventive Medicine. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this project report was previously presented for another degree or diploma at this or any institution

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CERTIFICATION

This dissertation entitled **"OCCURRENCE AND ANTIBIOGRAM OF *ESCHERICHIA COLI* O157: H7 IN READY TO EAT BEEF *'SUYA'* SOLD IN ZARIA METROPOLIS, NIGERIA"** by Ayuba Sini IBRAHIM 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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DEDICATON

This project work is dedicated to the Lord Almighty, my parents, siblings and all my friends. May God continue to bless you all.

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ABSTRACT

Meat and meat products especially ready to eat beef *'suya'* are hazardous group of foods that can potentially transmit pathogens to humans. In Nigeria, the incidence of toxigenic and multidrug resistant *E. coli* O157: H7 in human cases is said to be on increase. Previous studies in kano and Benin cities indicate the presence of *E. coli* O157: H7 in ready to eat beef *'suya'* sold. Hence, it's considered a major public health risk. The specific objective of the study was to determine the occurrence and antibacterial resistance patterns of *E. coli* O157: H7 in ready to eat beef *'suya'* sold in Zaria metropolis, Nigeria. A total of three hundred samples (fifty each) from six purposively selected sample areas using three target locations, namely; Motor parks, Main Markets and Educational institutions as selection criteria. ISO 2001 isolation and identification method was used. Isolates were screened using the conventional biochemical characterization and further confirmed using Rapid latex agglutination test for *E. coli* O157: H7. Agar disc diffusion method was used to evaluate the antibacterial resistant pattern; data obtained were interpreted using clinical laboratory standard institutes 2011. A total of 4 (1.3%) *E. coli* O157: H7 and 6 (2.0%) *E. coli* O157 were isolated. The isolates exhibited one hundred percent resistance to Gentamicin, Streptomycin, Ciprofloxacin and Trimethoprim while the isolates one hundred percent demonstrated susceptibility to Beta - lactam group (Penicillin and Ampicillin). The isolates were moderately resistant to Kanamycin, Chloramphenicol and Tetracycline. However, none of the isolate was multidrug resistant. The high resistance to four commonly used antibiotics has raised a serious public health concern. Therefore, this finding of the occurrence of *E. coli* O157: H7 in ready to eat beef *'suya'* should prompt the public to keep in mind that food-borne illnesses due to mishandling of foods should be avoided.

Thus, Education and public awareness programs are important measures in sensitizing the general public and people at risk on dangers of self-medication so as to minimize the increased rate of antibacterial drug resistance. There is need to legislate and enforce laws to limit the prescription and indiscriminate dispensing of antibiotics to only qualified professionals.

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ABBREVIATIONS AND SYMBOLS

F	Fimbrial antigen
H	Flagellar antigen
O	Somatic antigen
K	Capsular antigen
CT-SMAC	Sorbitol MacConkey agar supplement with Cefixime and Tellurite
DAEC	Diffuse-adherent *E. coli*
EAEC	Enteroaggregative *E. coli*
EHEC	Enterohaemorrhagic *E. coli*
EIA	Enzyme Immunoassay
EIEC	Enteroinvasive *E. coli*
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. coli*
HC	Heamorrhagic Collitis
HUS	Heamorrhagic Ureamic Syndrome
LT	Heat Labile toxin
MAR	Multiple Antibiotic Resistance
MDR	Multi-Drug Resistance
MR	Methyl Red
mTSB	Modified Tryptone Soya Broth
PCR	Polymerase Chain Reaction
SIM	Sulphide, Indole, Motility medium
ST	Heat Stable toxin
TTP	Thrombotic Thrombocytopenic Purpura
VP	Voges Proskaue

VTEC — Verocytotoxic *E. coli*

et al — And others

mm — Milimeters

ml — Mililiters

g — Grams

µl — Microliters

µg — Microgramm

S/N — Serial Number

^{0}C — Degree Celsius

+ — Positive reaction

- — Negative reaction

> — Greater than

% — Percentage

< — Less than

CHAPTER ONE

INTRODUCTION

1.1 Background of the Research Study

Meat is one of the most popular and nutritious components of human diet and also an important source of food-borne pathogens (Abdulllahi *et al*., 2006). In terms of meat consumption, beef is the most commonly consumed in urban areas (Obayelu *et al*., 2009). The Centre for Disease Control and Prevention, United States, listed the following Meat products as vehicles of *E. coli* O157: H7 infection: beef roast, hamburger, ground beef and beef steak (CDC, 2002).

'Suya' is a popular, traditionally processed, roasted, spiced meat product, which may be served or sold along streets, in club houses, at picnics, parties, restaurants, motor parks and within institutions (Igene and Mohammed, 1983). Its preparation and sales along streets are usually not done under strict hygienic conditions. *'tsire'* is a type of *'Suya'* , that is roasted, boneless meat of mutton or beef that is cooked around glowing fire in which the meat pieces are stacked on wooden sticks and spiced with groundnut cake powder, spices, vegetable oil, maggi cube and salt (Alonge and Hikko, 1981). The prepared *'tsire'* when being sold are usually packaged in old newspapers and sometimes in cellophane. Most of the stages of *'tsire'* preparation, materials used in its preparation and packaging, the handlers and the surrounding environment can serve as sources of contaminants to the meat product (Umoh, 2001).

Even though meat from freshly slaughtered, healthy animals is supposed to have low microbial populations, laboratory evidence suggests that they could be contaminated to an unsafe level at the point of consumption (Umoh, 2001). The fact that there are sporadic cases of gastroenteritis and symptoms of food borne infection after consumption of *'Suya'* indicate that the products indeed constitute a food safety risk (Odusote and Akinyanju, 2003; Inyang *et al*., 2005).

Etok (1998) identified insufficient roasting / heating duration, uneven distribution of temperature and exposure to unhygienic environment as crucial factors of infection and contamination. *'Suya'* is usually prepared in a rush when the buyers are in a haste, or they are roasted and kept exposed to environmental contaminants, since the aim of the vendors is to maximize profit and at the same time have ready to eat (prepared) *'Suya'* at all times to meet the demand.

1.2 Statement of Research Problem

Most food produced and consumed in Africa escape adequate inspection (Delia *et al.,* 2008). The World Health Organization (WHO) (2000) reports indicate that illness due to contaminated food is the most widespread health problem in the world and an important cause of reduced socio-economic productivity. Food safety is a significant and growing public health problem in Nigeria and food-borne disease is an important contributor to the huge burden of illness and death caused by diarrhoea. The 1997 Local Government Health System profile for Nigeria reported on the leading causes of deaths in different zones, showed that diarrhoeal cases accounted for 25.0% of mortality followed by malaria (21.0%) and accidents (9.8%) (FAO/WHO, 2002) The Federal Ministry of Health reported 90,000 cases of food poisoning in 2007, which is

certainly a gross underestimate. World Health Organization estimates 200,000 deaths from diarrhoea due to bacterial infection each year (WHO, 2008). In Nigeria, there is lack of accurate information on the prevalence and impact of food-borne diseases. However, it is known that diarrhoea, the most common manifestation of food-borne disease is a major cause of illness and death. Studies agree that the most important causes of diarrhoea are toxigenic *E. coli*, *Salmonella* spp., Rotavirus and *Shigella* spp. Bacterial causes of diarrhoea (most of which are zoonotic) appear to be of relatively high importance in Nigeria and diseases associated with toxigenic *E. coli* and non-typhoid salmonellosis are high and increasing, confirming the identification of these as key hazards by stakeholders. Studies on hazards in food show high levels of contamination, with meat being particularly problematic (Umoh, 2001). Abattoirs are extremely unhygienic and appear to be critical points for contamination. Although cooking temperature kills most pathogens, a few may withstand the cooking temperature. Cooked meat may be re-contaminated by food handlers during processing or from the environment (Wagner, 2010).

Several studies have also indicated the presence of *E. coli* O157: H7 in meat and meat products (Enabulele and Uraih, 2009; Tafida *et al.,* 2012). Similar trend has also been observed by Okeke *et al*. (2000) among children in primary health care centers in southern-western Nigeria. Smith *et al*. (2003) and Olorunshola *et al*. (2000) reported 7% and 6% prevalence *E. coli* O157: H7 in healthy animals and hospitalized patients in Lagos State, respectively.

A number of researchers have reported the incidence of *E. coli* O157: H7 in food outbreaks (Hancock *et al.,* 1994; Karch *et al;* 1996; Agbogu *et al.,* 2006; Uzeh *et al.*, 2006). Mead *et al* (1999) have estimated the incidence of *E. coli* O157: H7 to be 50.0% among Entero-

haemorrhagic *E. colli* serotype in relation to public health problems. Eating meat, particularly ground beef that has not been cooked sufficiently to kill *E. coli* O157: H7 is thought to be the primary cause of infection. Among other known sources of infection are consumption of contaminated sprouts, lettuce, salami, unpasteurized milk and fruit juice, swimming in or drinking contaminated water and contact with the stools of infected animals or people (FAO/WHO, 2003). Additionally, unchlorinated water, cold sandwiches and vegetables have been implicated as sources of some outbreaks of *Escherichia coli* O157: H7. Other implicated foods include unpasteurized apple cider and juice, salad containing mayonnaise, home-made yoghurt, frozen meats, turkey roll and clams (FAO/WHO, 2003).

The pathogen (*E. coli* O157: H7) produces large quantities of one or more related potent toxins, called Shiga toxins that cause severe damage to the lining of the intestine and to other target organs, such as the kidneys (FDA, 2002). Some victims, particularly the very young, may develop Haemolytic Uremic Syndrome (HUS) characterized by renal failure and haemolytic anaemia and can lead to permanent loss of kidney function. In the elderly, combination of HUS with fever and neurologic dysfunction is characteristic of (TTP) Thrombotic Thrombocytopenic Purpura. When Left untreated, TTP has a mortality of about 95%; however, early diagnosis and treatment yield a survival rate of 80–90% (Abumuhor and Kearns, 2002).

Increasing antibacterial resistance has become one of the most common concerns of foodborne bacterial zoonosis. Research has shown that the frequency of antibiotic-resistant bacteria is closely related with the volume of antibiotics used (Salyer, 2002). 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 (Castaine-cort *et al.,* 1999). Use of antibiotics in animals selects the growth and spread of resistant strains, which will accumulate in the farm environment and may become the dominant strain under selective pressure. This will limit the therapeutic options available to veterinarians and physicians in treatment of bacterial infections (FAO/WHO, 2003).

1.3 Justification

Enterohaemorrhagic *Escherichi coli* O157: H7 is an important food borne pathogen first recognized as a cause of haemorrhagic colitis in 1982 during an outbreak of severe bloody diarrhoea in Oregon and Michigan, (USA) which was later traced to contaminated hamburgers (Benjamin and Datta, 1995). It has since been implicated in a number of outbreaks of intestinal distress; the most severe outcome in the general population is typically hemorrhagic colitis, a prominent symptom which is bloody diarrhoea, and other life threatening complications (CDC, 1982).

In developing countries, 50-60% of diarrhoea aetiological agents are bacterial in origin. In Nigeria, the proportion may be higher at 65-80% (Ifaenyi *et al*., 2010). Most of the bacterial causes of diarrhoea are zoonotic, transmissible between animals and humans. The most important bacterial zoonoses are: *E. coli* infection, campylobacteriosis, S*taphylococcus aureus* infection, salmonellosis, listeriosis, and *Bacillus cereus* food borne infection.

Most processed meat *'Suya'* is sold in outdoor markets. Meat-sellers-ready to eat beef *'Suya'*-are often inadequately and poorly dressed (Okoli *et al*., 2006). The humid tropical environment

encourages the breeding of flies which swarm and perch on meat displayed for sale, form a major nuisance at these markets and aid in the spread of disease agents of significant public health importance (Okoli *et al.,* 2006).

Cattle are important reservoirs of entero-toxigenic *E. coli* (ETEC) and contaminated meat is a major vehicle for its transmission from animals to humans (Paton and Paton, 1998). In Nigeria, The incidence of TEC organisms in human cases of diarrhoea is said to be on the increase (Smith *et al*., 2003). Studies in Kano city by Dahiru *et al.,* (2008) revealed the prevalence rate of 53.0% and 25.3% in fresh and roasted beef respectively. Stephen and Nduka, (2009) in Benin City reported a prevalence of 2.78%, 6.94%, and 4.17% in fresh abattoir meat, fresh meat in open market and ready-to-eat beef *'Suya'* respectively.

Smith *et al.,* (2003) reported multidrug resistance in isolates of *E. coli* O157: H7 strains obtained from farm animals and human infection in Lagos and Ogun states in Nigeria. *Escherichia coli* O157: H7 is an important pathogen with worldwide distribution (Schlundt, 2002).

It is in view of the above findings that this study was conducted in Zaria metropolis, providing data on the prevalence and antibiogram of *E*. *coli* O157: H7 in ready to eat beef *'Suya'* sold in Zaria metropolis, Nigeria.

1.4 Aim and Objectives

1.4.1 Aim

The aim of the study was to determine the occurrence and antibiograms of *E. coli* O157: H7 isolated from ready to eat beef *'Suya'* sold in Zaria metropolis, Nigeria.

1.4.2 Objectives

The specific objectives of the study were to:

1. Determine the prevalence of *E. coli* O157: H7 from ready to eat beef *'Suya'* sold within Zaria metropolis, Nigeria.

2. Determine the antibiotic resistance patterns of *E coli* O157: H7 isolated from ready to eat beef *'Suya'* sold in Zaria metropolis, Nigeria.

1.5 Research Questions

1. Do ready to eat beef *'Suya'* sold in Zaria metropolis, Nigeria. Contain *E. coli* O157: H7?

2. Are *Escherichia coli* O157: H7 in ready to eat beef *'Suya'* sold in Zaria metropolis, Nigeria. Are resistant to antibiotics?

CHAPTER TWO

LITERATURE REVIEW

2.1 History of *Escherichia coli*

Theodor Escherich (German Scientist) first cultured '*Bacterium coli*' in 1885 from the feaces of a healthy individual (Escherich, 1885). It was renamed *Escherichia coli* in 1919 in a revision of bacteriological nomenclature (Law, 2000). *Escherichia coli* are member of the family Enterobacteriaceae. They are gram-negative, short rod-shaped bacteria which are usually motile with peritrichous flagellae, possessing fimbriae, and are facultative anaerobes that are capable of fermentative and respiratory metabolism (Wilshaw, *et al.,* 2000). *E. coli* a natural habitat is in the lower part of the intestine of most warm blooded animals (Heidelberg, 1981).

As part of the normal flora of the human intestinal tract, *E. coli* plays a crucial role in food digestion by producing vitamin K from undigested material in the large intestine. Most strains

of *E. coli* are harmless commensal members of the intestinal flora of mammals in which some strains adhere to the intestinal mucosa while others are only temporary transient in the lumen of the colon. While some strains of *E. coli* live as commensals, many are opportunistic pathogens of humans and other animals (Levine, 1984).

Escherichia coli are the major cause of neonatal septicemia, neonatal meningititis and urinary tract infections in humans and of a variety of invasive diseases in mammals. It is also a leading cause of diarrhoeal diseases in humans and other mammals (Neidhardt, 1986).

Escherichia coli can respond to environmental factors such as Chemicals, pH, temperature and osmolarity in a number of ways, since it is a single celled organism (Todar, 2002). For example, in response to change in temperature and osomolarity, it can vary the pore diameter of its outer membrane pores to accommodate lager molecules (nutrient) or to exclude inhibitory substances (Todar, 2002).

Escherichia coli on E.M.B, it grew as large, smooth colonies surrounded with and covered in a dark, shiny metallic green characteristic of the species. The gram negative rods in pairs and doublets were determined to be motile facultative anaerobes. Biochemically, oxidase test is negative, while catalase is positive. The methyl red tests positive, glucose test positive along with gas production. Hydrogen sulfide production is negative. Lactose is positive, as were arabinose and sorbitol. Adonitol, dulcitol, and phenylalanine were negative. Lysine decarboxylase is positive, while ornithine decarboxylase is negative. (Table 2.1) Urease is negative, as is citrate. Indole is positive. *E. coli* is the most frequently and widely isolated organism (Todar, 2002).

Table 2.1: Biochemical Characterization of *E. coli*

S/N	Biochemical test	Reaction
i.	Lactose fermentation	+
ii.	Catalase	+
iii.	Citrate Utiliazation	-
iv.	Indole Production	+
v.	Nitrate Reduction	+

vi.	Methyl Red	+
vii.	Voges-Proskauer	-
viii.	Urease	-
	Acid from sugar:	
	a) Glucose	+
	b) Mannitol	+
	c) Salicin	+
	d) Sucrose	+

Keys: + means positive while - means negative

By serological means, *E. coli* is divided into sero-groups and sero-types. The initial sero-typing scheme was developed by Kaufman (1947) and it 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 Lipolysaccharide. As showed in figure 2.1. And

Table 2.2 The ‘O’ antigens are the basis of classifying *E. coli* into subgroups (King and Holt, 1984). Flagella antigens are heat-labile flagellar antigen compose of protein, ‘K’ antigens identified on the basis of their chemical composition, are acidic polysaccharide capsular antigens. Today, more than 180 O sero-groups and 60 H serotypes are recognized.

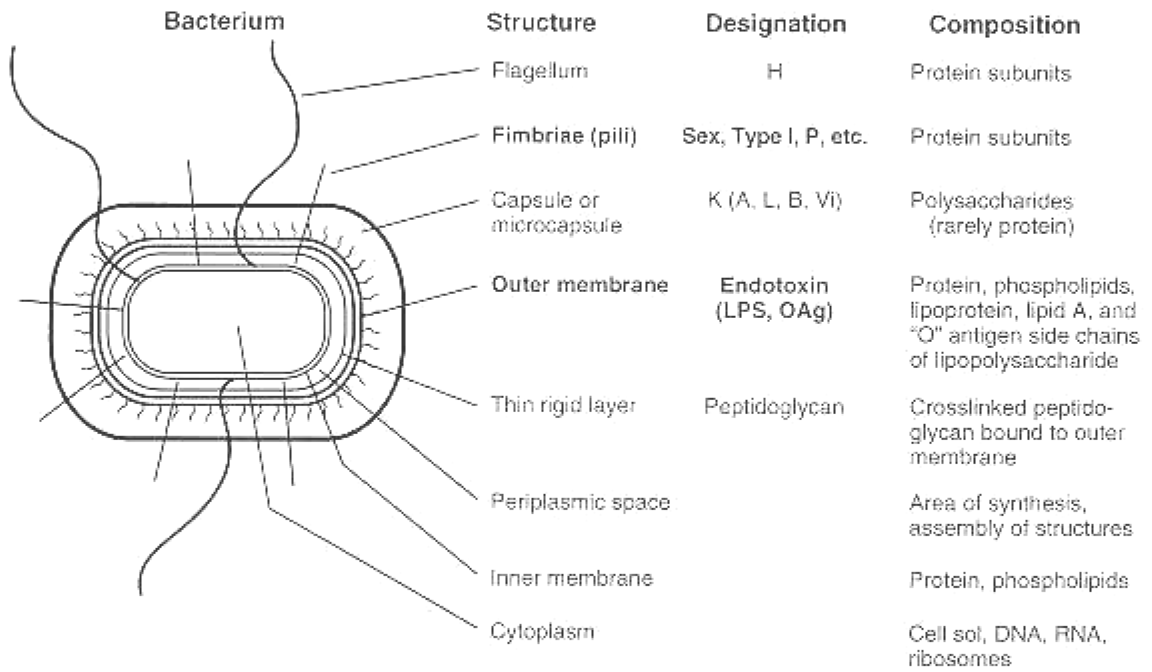


Figure 2.1: Structure and Composition of *Escherichia coli* (Padhye *et al*., 1986)

Table 2.2 Virulence Factors of *Escherichia coli*

S/N	Virulence Factor	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 opitehlium, (2) Tamm- Horsfall glycoprotein, and (3) D-mannose residues on a variety pf cells.

2.2 Patho -Types of *Escherichia coli*

E. coli that cause enteric diseases are divided into six patho-types:

I. Enteropathogenic *E. coli* (EPEC) is the major cause infantile diarrhoea worldwide, mostly in developing world. They possess a number of virulence factors although they do not possess the colonization factors and do not produce any ST (heat-labile) 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). The cells adhere to lining of the small intestine causing changes in the electrolyte secretion and structural damage of the small intestine. In children, symptoms include watery diarrhoea, fever, vomiting and abdominal pain. In adults, the symptoms include severe diarrhoea with nausea, 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)

II. Enteroinvasive *E. coli* (EIEC) These groups are responsible for a bacillary- like illness and share some virulence properties with *Shigella dysenteriae* (Hart *et al*., 1993). 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 cell 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) Symptoms include chill, fever, headache, muscular pain, abdominal cramps and diarrhoea. 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). Outbreaks have occurred due to the ingestion of contaminated water and soft cheese.

III. Enterotoxigenic *E. coli* (ETEC). These strain are characterized by their production of well define heat-labile and / or heat cholera toxin (CT) while heat stable enterotoxin (ST) is non-antigeic and of low molecular weight (Rea and fleming*,* 1994). 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) Symptoms of ETEC include watery diarrhoea, fever, abdominal cramps, malaise and vomiting. Onset of 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).

IV. Entero-haemorrhagic *E. colli* (EHEC) These 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 Vero and HeLa cells, as first reported by Konowalchuk, Spiers and Satvric (1997). VT-1 is relatively heat stable high molecular weight Shiga-like toxin, whereas VT-2 is

immunologically distinct (Doyle, 1991). EHEC also possess 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) Symptoms include diarrhoea, severe abdominal cramps and vomiting. Others include bloody diarrhoea, haemolytic ureamic syndrome (HUS), thrombatic thrombocytopenic purpura (TTP) and death. The infective dose is very small. In some outbreak 2 cells per 25 grams of food of EHEC were detected (FDA, 2006).

Entero-haemorrgic *E. coli* O157: H7 is one of the emerging food-borne infections. The pathogen causes exceptionally severe consequences of infection. Low infective dose and acids tolerance (Buchanan and Doyle, 1997). The acid tolerance of *E. coli* O157: H7 is believed to play a key role in the pathogenesis and food borne illness (Buchanan and Edelson, 1996).

V. Entero-aggregative *E coli* (EaggEC) they are self-adherent, tending to auto-agglutinate, giving the appearance under the microscope of a stack brick (Harrigan, 1998). The distinguishing feature EaggEC strain is their ability to attach to tissue culture cells in an aggregative manner. These patho - type are associated with persistent diarrhoea in children. It is however, unknown how EaggEC causes diarrhoea except that it colonize 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).They also produces 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 amd 2 (Czeczulin *et al*., 1997). This is the cause of great concern because diarrhoea is an important cause of morbidity in immune-compromised people.

VI. Diffusely adhering *E. coli* (DAEC) its name comes from its ability to adhere to HeLa cells in a diffuse patterns (Neidhardt, 1986). 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). They also express adhesion of the Afa/Dr family in 25% of cases of cystitis in children and 30% of cases of pyelonephiritis in pregnant women (Archamband *et al*., 1998 and Servin, 2005).

2.3 Preparation of Beef *'Suya'*

Usually in Nigeria, skinless, boneless flesh of cattle or chicken is used for commercial preparation of *'Suya'* meat. Finely grinded roasted peanut cake, red pepper, salt, grinded ginger, grinded garlic, chunked fresh tomatoes and minced fresh onions are required on suitable conditions depending on the quality of *'Suya'* meat required to be prepared (Jay, 2002 and Judge *et al.,* 2002).

The process of preparation involves a few steps, first is the grounding of peanut. The shell and the skin are removed from the peanut before grinding into fine powder using mortar and pestle or crushed with a rolling pin. If the powder is oily; it is wrapped with an absorbent paper and squeezed for a minute or two. Next, the grinded pepper, garlic, ginger are stirred into the peanut powder and mixed properly.

The meat is then cut into small sizes or thin sliced, dipped and rolled in a bowl containing the mixed peanut - spice and allowed to coat completely. The minced meat are then kept for thirty minutes or more for the peanut cake to stick to it after which the meat slices are threaded

unto skewer and brushed with vegetable oil and roasted on the glowing charcoal fire for fifteen to twenty minutes. It is finally removed from the skewer and served hot in a newspaper with sliced onions, tomatoes and cabbage (Judge *et al.,* 2002).

2.3.1 Microbiology of meat

During slaughtering process, there is contamination of the sterile tissue with intestinal flora such as gram-negative organisms which includes *Escherichia coli* as well as contaminants such as *Pseudomonas species* and gram-positive *lactic acid bacteria* and *Staphylococci* species associated with humans, animals and their environment. Meat spoilage is usually associated with gram-negative proteolitic bacteria which literally decompose the protein with production of offensive odour (Haman, 1997).

The addition of salt and drying of fresh meat have been an effective means to control the meat micro-flora and thus preserve the tissue for later consumption. The curing salt (sodium chloride) and subsequent proper handling method, favours the growth of gram-positive bacteria, primarily *Staphylococcus aureus* while inhibiting the proliferation of gram-negative bacteria (Boles *et al.,* 2000). The smoke from the fire has a preservative effect on the *'Suya'* meat.

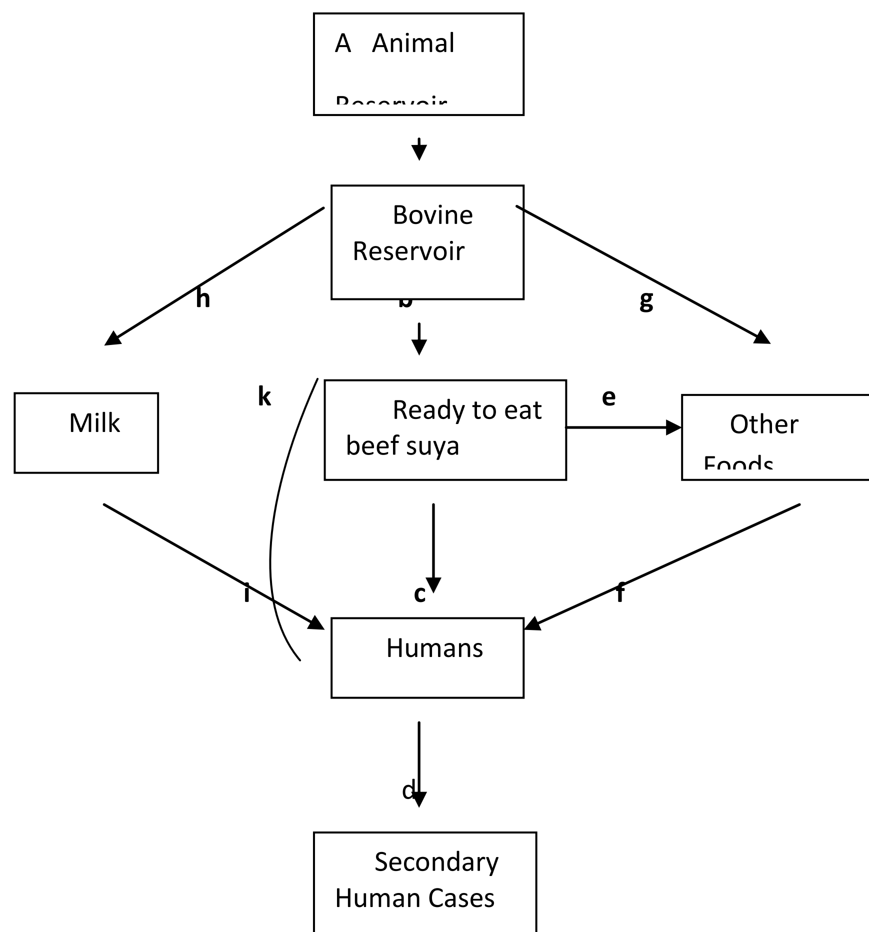
Extensive use of antibiotics in both human and veterinary medicine, particularly in disease prevention and growth promotion in animal production is a considerable cause of the selection and prevalence of antibiotic resistant *E coli* O157: H7 (Schroeder *et al.*, 2002; Callaway *et al.,* 2003). The development 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). Because some antibiotics may cause bacterial lysis and liberate the free shigatoxin in the intestinal tract (Karch *et al.*, 1996, Wong *et al.,* 2000), the antibacterial treatment is contraindicated for human *E. coli* O157: H7 infections. However, such treatment may be recommended for cystitis and pyelonephritis other than hemorrhagic colitis all caused by *E. coli* O157: H7. Despite, these limitations of using antibacterial agents in *E. coli* O157: H7, it is believed that the organism may still be susceptible to most antibacterial (Griffin and Tauxe, 1995).

2.4 Transmission of *E. coli* O157: H7

Human disease caused by *E. coli* O157: H7 is often associated with contaminated and undercooked beef (CDC, 1997). However, improved outbreak surveillance for this organism has revealed many other modes of transmission of *E. coli* O157: H7 to humans, as shown in figure 2. 2 many of which can be linked directly or indirectly to cattle. Other bovine-derived food vehicles include unpasteurized milk (Bhat *et al*., 2007), raw milk cheese and butter (Rangel *et al*., 2005), and dry-cured salami (CDC, 1995). Produce has become a common source of food borne *E. coli* O157: H7 since the first U.S. outbreaks were reported in 1991 (Rangel *et al.,* 2005); produce items associated with outbreaks include spinach, lettuces, apple cider and juice, coleslaw, and sprouts (CDC, 2006). Both drinking water 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 (McIntyre *et al*., 2002), environmental contact with sheep manure (Ogden *et al*., 2002), and sheep or goat contact at petting zoos (Payne *et al.*, 2003) was been reported.



Key: **a**-Direct transmission, **b**- Faecal contamination, **c**- Food-borne transmission, **d**- Person to person transmission, **e**- Cross contamination, **f**- Food-borne transmission, **g**- Faecal contamination, **h**- Faecal contamination, **i**- Food-borne transmission and **k**- Direct transmission

Figure 2.2: Animal Reservoir and Mode of Transmission of *Escherichia coli* O157: H7

2.5 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). In addition, *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, including other livestock and domestic animals, many kinds of wild animals and birds, flies, water, feed, and manure. *E. coli* O157: H7 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; wild deer (Heuvelink *et al*., 2002), rabbits (Scaife *et al*., 2006), raccoons (Shere *et*

al., 1998), opossums (Renter *et al*., 2003), and rats (Cizek *et al*., 1999). Pigeons (Shere *et al*., 1998), gulls (Wallace *et al*., 1997), rooks (Ejidokun *et al*., 2006), and starlings (Nielsen *et al*., 2004) are among the numerous bird species that have been found positive for *E. coli* O157.

2.6 Geographical Distribution of EHEC O157: H7

Escherichia coli O157: H7 was first described in 1975 in California after it was isolated from a woman with bloody diarrhoea, but its identification as an enteropathogen was notdocumented until two, nearly simultaneous, U.S. outbreaks in 1982 (Ingham *et al.*, 2006).

In Nigeria, The incidence in human cases of diarrhoea 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, Nigeria. Six (6%) of the 100 samples examined were positive for EHEC O157: H7. Five of the six patients were children below five years of age and one was a teenager. Local fast-food restaurants commonly referred to as "*bukkas*" were regarded as likely sources of infection.

In Southeastern 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). In Akwa Ibom State of Nigeria, bloody diarrhoea accounted for 31% of all cases of diarrhoea in humans (Akinjogunla, *et al*., 2009). EHEC strains were more prevalent in females (68.4%) than in males (30.6%). Incidence of EHEC was high in people within age group 1 – 15 years old (95%), 16 -30 years old (80%) and 46 – 60 years old

(70%) but comparatively low in people within age group 31 – 45 (55%) and 61 years old and above (45%) (Akinjogunla, *et al.,* 2009) Smith, *et al*. (2009) reported the prevalence of *E. coli* O157 in human stool samples to be 31.6%.

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).

2.7 Clinical Manifestation of EHEC O157: H7

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

Hemorrhagic colitis is a distinct clinical syndrome that is characterized by sudden onset of abdominal cramps, followed within 24 hours by watery diarrhoea which later becomes grossly blood described as "all blood and no stool" (Padhye *et al*., 1986). The disease is distinguished from dysentery described in Shegellosis or invasive *E. coli* gastro-enteritis by the lack of fever and the bloody discharge resembling lower gastrointestinal bleeding (Padhye and Doyle, 1992).

Haemolytic uremic syndrome (HUS) 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). Padhye and Doyle (1992) Stated that the disease is a tirade of an acquired haemolytic anaemia, thrombocytopenia and renal failure that occurs acutely in otherwise healthy individuals. In the 1980s, this proved to be correct when a novel category of diarrhoeagenic *E*. *coli*, so called enterohmorrhagic *E*. *coli* (EHEC), that was causally associated with the syndrome of hemorrhagic colitis (Bitzan *et al*., 1988) and subsequently with HUS was discovered (Karmali, 1989). Padhye and Doyle, (1992), reported that the pathogenesis of HUS appears to be associated with toxin which damages endothelial cells, thus triggering the clotting mechanism.

Thrombotic thrombocytopenic purpura is similar to HUS except the central nervous system (CNS) is involved (Padhye and Doyle, 1992). It is a syndrome usually occurring in adults that consists of microangiopathic haemolytic anaemia, profound thrombocytopenia, f luctuating neuralgic signs, fever, and mild azotemia (Kwaan, 1987). Patients often develop blood clots in the brain and death frequently results. Other unusual clinical complications associated with *E*. *coli* O157: H7 infection includes hemorrhagic cystitis and banalities (Grandsen, *et al.*, 1985), convulsions, sepsis with another organism, and anaemia (Rowe, *et al.*, 1991).

The complete form of HUS, which includes the three characteristics haemolytic anaemia, thrombocytopenia and acute renal dysfunction, was noted in eight patients, while an

incomplete form of HUS, which did not include all three characteristics, was noted in seven patients.

2.8 Pathogenesis and Consequences of EHEC O157: H7

As Shiga-like toxins elaborated by the *E. coli* are absorbed into the systemic circulation, HUS and TTP-like syndrome are produced. These toxins bind preferentially to specific glycol-lipid receptors on vascular endothelial cells, inhibit protein synthesis causing cell death, leading to endothelial injury and localized intravascular thrombosis. The vascular endothelium of kidney is specifically susceptible, so renal failure results in the infected individuals.

Injury to the renal endothelium is probably the primary etiological event in HUS. The lumen of glomerular capillaries is narrowed as a result of swelling of the injured endothelial cells, hypertrophy of mesangial cells and detachment from the underlying basement membrane. Further occlusion of the narrowed glomerular capillaries and afferent arterioles occurs with the formation of platelet thrombi and fibrin generation and ischemic glomerular and tubular necrosis leads to renal injury. Similar, although pathologically different, events occur in TTP, the other thrombotic microangiopathy which may occur following VTEC O157 infection. The onset of HUS/TTP occurs very fast, usually 2 to 14 days after the onset of diarrhoea, and by the time the syndrome manifests, diarrhoea may have resolved completely (Elliott and Nichols, 2001).

In children, these microangiopathic syndromes are often self-limited and resolve with supportive care, which may include temporary haemo-dialysis. However, 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 extremes of age, particularly elderly (Dundas, *et al.,* 1999). The most important sequelae are chronic renal insufficiency in a significant percentage of affected children (Elliott and Nichols, 2001). Besides, other vascular territories may also be affected, causing ischemic symptoms and neurologic manifestations. 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).

2.9 Diagnosis of EHEC O157: H7

It is difficult to detect *E. coli* O157: H7 in raw meats and food due to much higher levels of other sorbitol non-fermenting bacteria. Isolation of the organism from food or stool specimens involves first enrichment in a selective broth and then plating onto sorbitol MacConkey agar with additives (Griffin, 1995). Biochemical and serological confirmation 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).

Another method to detect *E. coli* O157: H7 is to look for verotoxin or verotoxin genes. Immunoassay methods are available to identify the presence of verotoxins (Su, 1995). Testing forverotoxin 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.

Identification of *E. coli* O157: H7 can also be done rapidly, specifically and sensitively using DNA based polymerase chain reaction (PCR) methods. One multiplex PCR method amplifies simultaneously three different DNA sequences of *E. coli* O157: H7: a specific fragment of the *eae* gene, conserved sequences of verotoxins I and II, and a fragment of the 60-MDa plasmid (Deng *et al* 1996). Since this test detects other virulence markers besides verotoxin, it is more specific than tests which only identify verotoxin genes. PCR methods however are affected by many laboratory variables and are less reproducible between laboratories than other methods, and are often less sensitive than direct culture.

Molecular methods for inter-strain differentiation of *E. coli* O157: H7 have been developed. These methods are useful in distinguishing between outbreak related and unrelated isolates. The most commonly used DNA fingerprinting tests are based on restriction fragment length polymorphism (RFLP) methodology where restriction enzymes are used to cut genomic DNA into fragments that are separated by agarose gel electrophoresis. A pattern or “fingerprint” is resolved for particular bacterial strains. Several RFLP methods have been developed, one uses pulsed-field gel electrophoresis (PFGE) (Johnson, 1995) others use conventional gel electrophoresis (Samadpour, 1995).

2.9.1 Prevention and control of EHEC O157: H7

To reduce the risk of an *E. coli* infection, the following preventive measures are recommended by the Centers for Disease Control and Prevention

i. Education and public awareness programs are important measures in sensitizing the general public and people at risk and the greatest improvements of meat hygiene have been demanded.

ii. Cook ground beef thoroughly to an internal temperature of 71°C (160°F) and use a meat thermometer to ensure this temperature is reached. The colour of the meat is not a reliable indicator.

iii. Drink only pasteurized apple cider and milk.

iv. Wash all fruits and vegetables before eating.

v. Wash hands thoroughly after using the washroom, handling diapers, pets, or livestock and before preparing or eating food.

vi. Clean and sanitize counter tops, cutting boards and utensils after contact with raw meats and poultry. Use 5mL (1tsp) unscented chlorine bleach in 750mL (3 cups) water to sanitize.

vii. Do not drink water from open streams and lakes.

viii. If ill with diarrhoea, do not prepare or handle food that others will eat. (CDC, 2004)

x. Cook all ground beef or hamburger thoroughly until the meat is gray or brown throughout and juices run clear.

xi When dining in restaurants, send undercooked hamburger back for further cooking.

xii. Avoid raw, unpasteurized milk and milk products.

xiv. To reduce person-to-person transmission, make sure that infected persons, especially children, wash their hands frequently with soap.

xv. Make sure your drinking water has been treated with adequate levels of chlorine or other effective disinfectants.

2.9.2 Treatment of EHEC O157: H7

Although antibiotics are not recommended for treatment of *E. coli* O157: H7 infections in humans, However, the study of antibacterial susceptibility of *E. coli* O157: H7 may have more therapeutic significance as recent studies have indicated possible role of early administration of antibacterial in preventing the progression of haemolytic uremic syndrome and hemorrhagic colitis cause by *E. coli* 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 the plasma is filtered to remove antibodies from the blood.

2.9.3 Prognosis of EHEC O157: H7

Complications such as Blood clotting problems, Haemolytic anemia, Kidney failure, Nervous system problems, thrombocytopenia, Uremia etc. Generally, HUS is a serious illness in both children and adults, and it can cause death. With proper treatment, more than half of patients will recover. The outcome is better in children than adults.

2.9.4 Antibiotic resistance of *E. coli* O157: H7

Extensive use of antibiotics in both human medicine and animal agriculture is suspected to have lead to a widespread dissemination of antibiotic resistant genes (Callaway *et al.,* 2003). The development of resistance to antibacterials 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).

Smith *et al*. (2003, 2007) reported multidrug resistance in isolates of *E. coli* O157 strains obtained from farm-animals and human infections in Lagos and Ogun state in Nigeria. They had collected a total of 350 fresh faecal droppings of animals (cattle, pig,chicken and sheep) and human stool comprising of diarrhoeic (150) and non diarrhoeic (50). Carl *et al*. (2002) reported that 361 *E. coli* O157 isolates kept at the *E. coli* reference centre at the Pennsylvania State University were resistant to sulphamethoxazole (26%), tetracycline (27%), cephalothin (17%) and ampicilin (13%). These isolates were recovered from samples collected from humans, cattle and swine.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

The following bacteriological media were used; Modified tryptone soya broth (OXOID), Cefexime-Tellurite Sorbitol MacConkey agar (OXOID), Nutrient agar (OXOID), Triple sugar iron agar (OXOID), Urea agar (OXOID), Mueller-Hinton agar (OXOID), Methyl Red Voges Proskauer (OXOID), Buffered peptone water (OXOID), Eosin methylene blue (OXOID), Sulphide Indole Motility agar (OXOID) and Simmons citrate agar (OXOID),

Antibiotic discs (OXOID) were used at various concentrations: Ampicillin (10µg), Augmentin (30µg), Penicillin (10iu), Gentamicin (10µg), Ciprofloxacin (5µg), Chloramphenicol acid (30µg), Streptomycin (30µg), Tetracycline (30µg) and Trimethoprim (5µg).

The following consumables were used sterile swab sticks, cotton wool, disposable Petri dishes, Pasteur pipettes, Polythene bags, Aluminum Foil, Test tubes,

The following equipments were used: Stomacher laboratory blender (SEWARD, London), water bath, Electric Autoclave, Hot air oven, Refrigerator, Antibiotic disc dispenser (OXOID) and weigh balance. Others include; conical flasks, Measuring cylinder, Bunsen burner, wire loop and pin, bijou bottles, incubator, Needle and Syringes,

3.2 Study Area

The study was carried out in Zaria metropolis of Kaduna State, Nigeria. Located between latitudes 11^0 $7^{'}$, 11^0 $12^{'}$ N longitude 07^0 $41^{'}$ E. As shown in figure 3.1. It is a medium sized city with an estimated population of 547,000 and a growth rate 3.5% per annum (Mortimore, 1970).

Zaria metropolis is divided administratively into Zaria and Sabon gari Local Government Areas (L.G.As), each comprising of six district areas. Zaria LGA comprises Zaria city, Tudun wada, Gyelesu, Tukur Turkur, Wuciciri and Dutse Abba; while Sabon Gari comprises Hanwa, Muciya, Samaru, Bassawa and Bomo (Ministry of Economic Development, 1996)

Fifty to seventy five percent of the working populations of Zaria metropolis derive their livelihood from farming. Agriculture in Zaria can be divided into two types: Rainfall (from May to October) and irrigation farming in the dry season (from November to April). It is characterized by a tropical climate, a monthly mean temperature ranging from 13.8 to 36.7^0C and annual rainfall of 1092.8mm. Dry season farming is the second most prevalent agricultural activity in Zaria with vegetables being the common produce, but in some cases fruits are sandwiched among cereal crops (Agbogu *et al*., 2006).

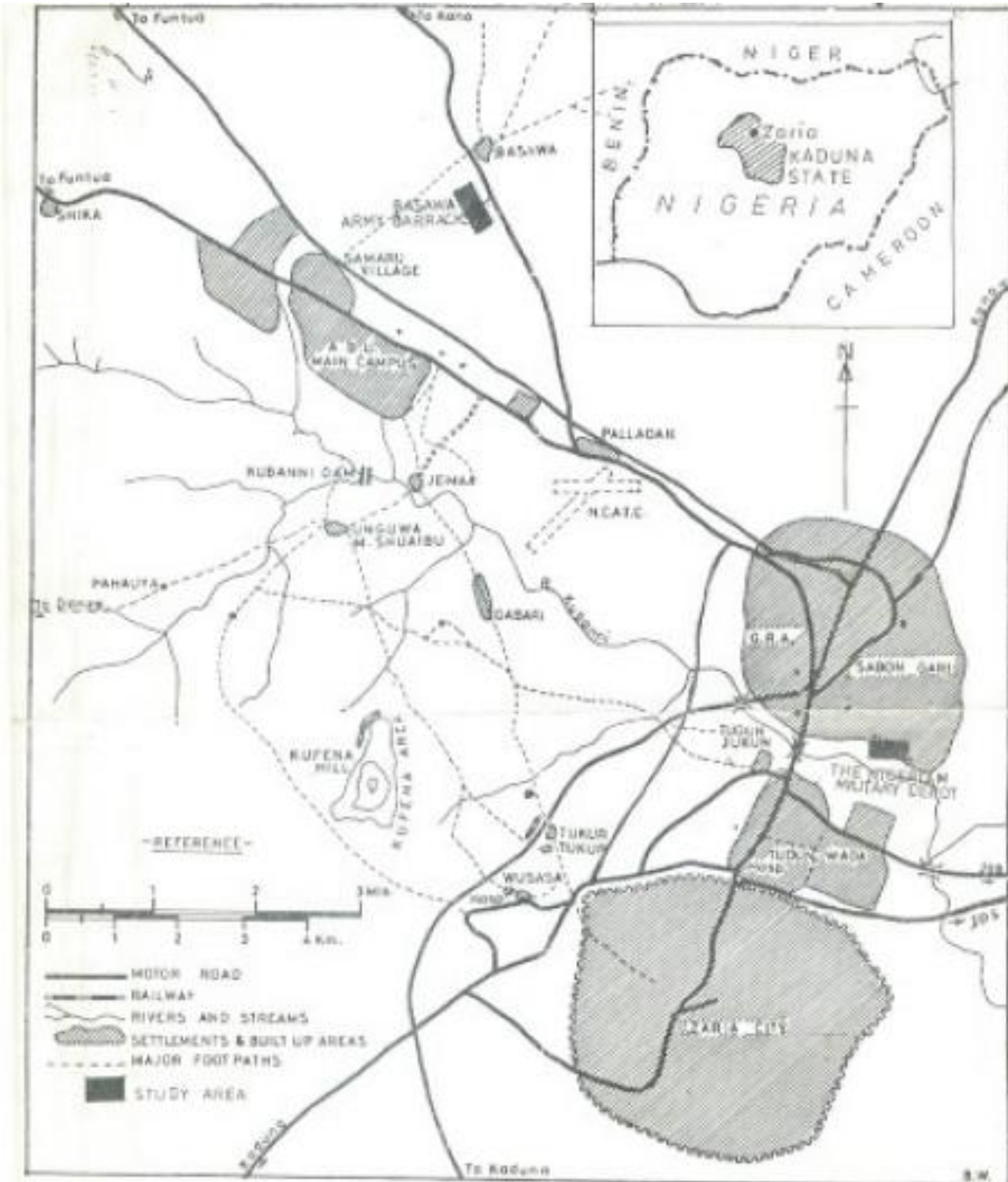


Figure 3.1: Map of Zaria showing the sampling area (Mortimore, 1970).

3.3 Sample Size

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

$N=Z^2Pq/d^2$

Where

N= Sample size

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

P= prevalence 25.3% (Dahiru *et al.,* 2009)

d= desired precision (0.05)

q= 1-p

$$n= \frac{1.96^2 \times 0.253 \times (1-0.253)}{(0.05)^2}$$

N= 290.40

A total of 300 samples were collected.

3.4 Study Design:

The two local government areas (Sabon gari and Zaria) that made up the study area constituted the sampling frame. Six district areas were selected using purposive sampling method, these were: Danmagaji, Zaria city, Tudun wada, Samaru, Kwangilla and Sabon gari. (Figure 3.1)

Three target locations, namely; Motor parks, Main markets, and Educational institutions were used as selection criteria, with each of the six districts having at least two target locations because these locations are characterized with high commercial activities that attract *'Suya'* vendors. The markets that were considered include Samaru, Sabongari, Tudun wada and Zaria city; the motor parks include Funtua garage located in Samaru and those at Sabo, Tudun wada, Danmagagi and Zaria city, while Educational institutions include Ahmadu Bello University, Samaru main Campus, Ahmadu Bello University Kongo Campus, Federal College of Education, Zaria and Nuhu Bamali Polytechnic, Zaria.

Fifty (50) samples each were collected from ready to eat beef *'Suya'* vendors in the six different sample areas.

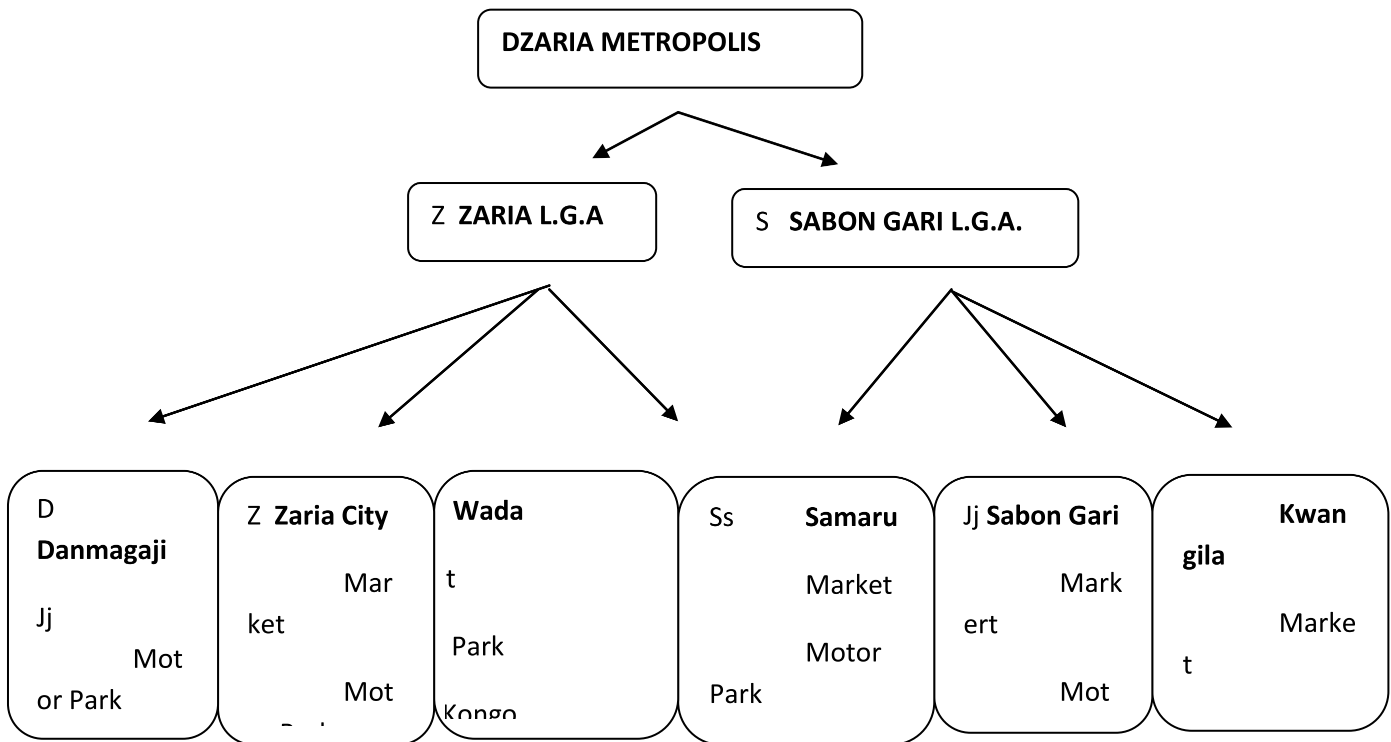

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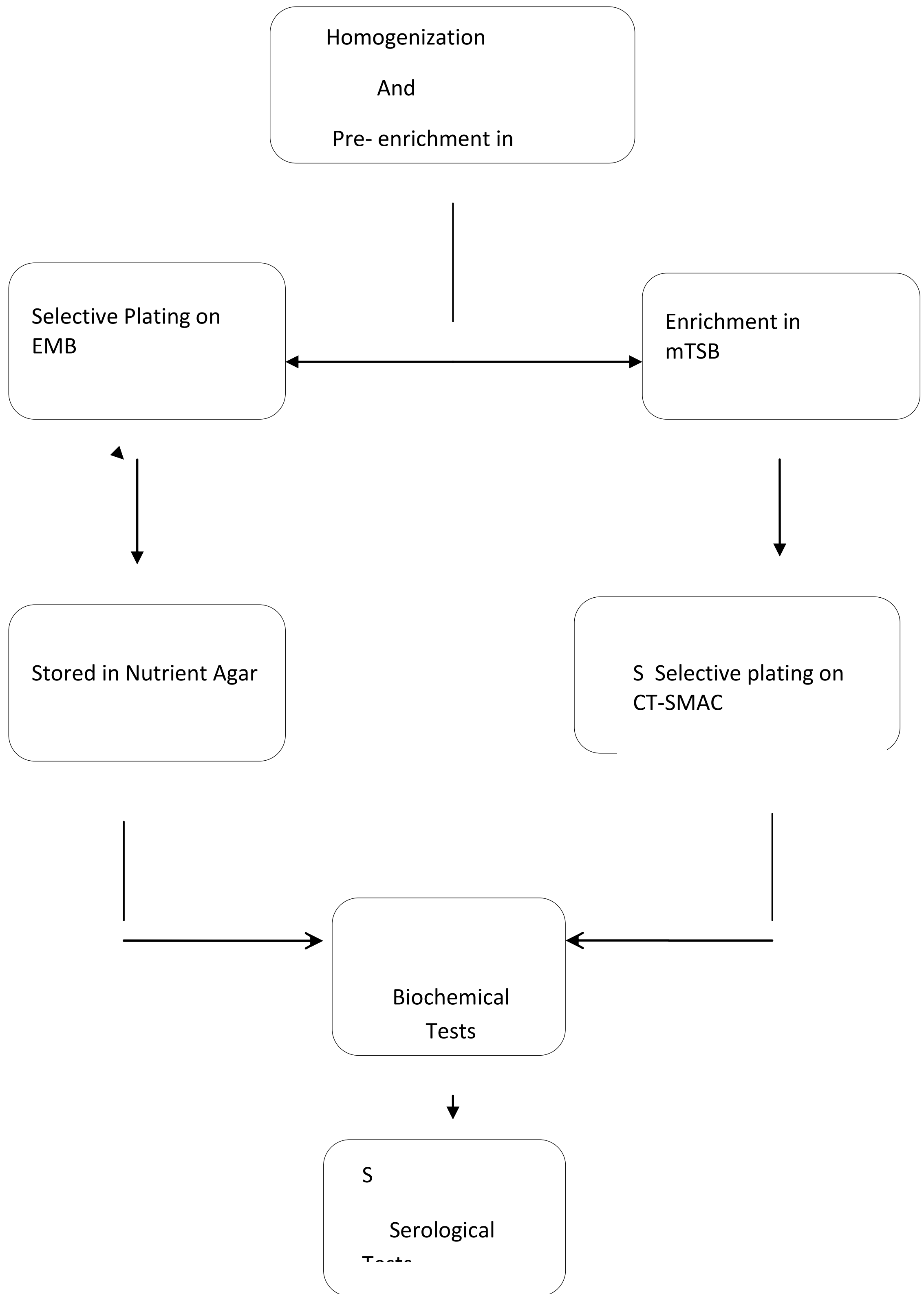
Figure 3.2: Schematic representation of the sampling areas

3.5 Sample Collection

Ready to eat beef *'Suya'* samples were purchased from meat vending outlets, wrapped the way they are sold. All samples were placed in separate sterile polythene bags to prevent cross contamination. These samples were appropriately labeled and transported to the Bacterial

Zoonosis Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria.


Homogenization
And
Pre- enrichment in
Selective Plating on EMB
Enrichment in mTSB
Stored in Nutrient Agar
S Selective plating on CT-SMAC
Biochemical Tests
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Serological

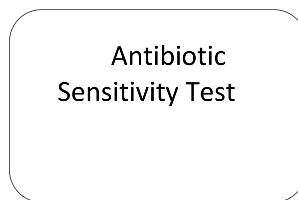

Figure 3.3 Flow Chart for Isolation and Identification of *E. coli* O157: H7 Used in the Study

3.6 Laboratory Procedures

3.6.1 Isolation and identification of *Escherichia coli*

The isolation of *E. coli* O157: H7 was conducted according to the procedure of ISO, (2001). Ten grams of each sample (read to eat beef *'Suya'*) was homogenized and pre enriched in 90ml of 1.0% buffered peptone water, in a sterile polythene bag and incubated at 37^0C for 18-24 hours. Enrichment broth was inoculated into two different media as follows: a loopfull was inoculated directly by streaking on Eosin Methylene Blue (EMB) agar. The inoculated plates were streaked using a sterile loop and incubated at 37^0C for 24 hours. Greenish metallic sheen colonies growth suggestive of *E. coli* were picked stored on nutrient agar slant and refrigerated for further biochemical and serological analyses. Another one ml of the broth was further enriched in 9ml of Modified Tryptone Soya Broth (mTSB), incubated at 37^0C overnight and streaked on Cefixime-Telluritte Sorbitol MacConkey agar (CT-SMAC agar) supplemented with

Cefexime 50µg/L and Potassium tellurite 2.5mg/L at 37°C (March and Ratnam, 1986). This was then incubated at 37⁰C for 24 hours. Suspected colonies of *E. coli* appeared as non-sorbitol fermenters, which is characterized as having a slightly transparent, almost colourless with a weak pale brownish appearance. About 4-5 colonies from the CT-SMAC agar plates were selected separately and sub-cultured on nutrient agar slants plates, and incubated at 37⁰C for 24 hours. These were stored in a refrigerator for further biochemical and serological analyses.

3.6.2 Biochemical tests

Biochemical characterization was by conventional biochemical tests (Barrow and Feltham, 1993). Five different biochemical media were used. A characteristic colony of *E. coli* was identified on the basis of sugar utilization, IMViC patterns and production of hydrogen sulphide (H_2S). All biochemical media were prepared according to manufacturer's instructions.

3.6.2.1 Urease test

This test was aimed at identifying isolates that produce urease, which hydrolyzes urea to give alkaline ammonia (NH_4) and carbon dioxide.

The presence of urease is tested by preparing urea in a broth base containing a pH indicator which is yellow at the acidic pH and red at the basic. Urease liquid media, stored in refrigerator, was poured in three to five milliliters portion in to sterile screw-top glass tubes. The tubes were inoculated with the isolate using a sterile wire loop and incubated at 37^0C for 18-24 hours and examined, for a pink colour indicating positive test while a yellow colouration is considered negative.

3.6.2.2 Citrate utilization test

This test is based on the ability of an organism to use citrate as its source of carbon. Simon's citrate agar medium was prepared as slant in test tubes. A sterile wire loop was used to inoculate the isolate onto the agar slant medium and incubated at 37^0C for 24 hours after which it was examined for colour change. A bright blue colour in the medium gave a positive citrate utilization test while green colouration is considered negative.

3.6.2.3 Methyl red voges - proskauer test (MRVP)

This test was used to identify organisms that produce acetymythylcarbinol (acetone) a natural product formed from pyruvic acid in the course of glucose fermentation. The organism was inoculated into a MRVP media and incubated at 37^0C for 24 hours. The broth was divided into two halves, 2-3 drops of methyl red was added to one broth and observed for formation of pink colour which indicates positive result. To the remaining half, 5 drops of 40.0% potassium hydroxide (KOH) was added followed by the addition of 5 drops of 5.0% of alcoholic alpha-

Naphthol and shaken gently. The cap of the tube placed in a sloping position. The development of a red colour starting from the liquid air interface within 15-30 minutes was indicative of a positive test.

3.6.2.4 Indole test, Hydrogen Sulphide and Motility tests,

This test was carried out for indole production by test organism which is important in identifying enterobacteria. A sterile wire loop was used to inoculate a colony of test organism into a prepared SIM (Sulphide, Indole and Motility) media. The tube was stoppered and incubated at 37^0C for 24hours. Four drops of Kovac's reagent was added to the medium using a sterile pasteur pipette to determine the production of indole by the organism. Observation of red coloration on the surface layer within ten minutes showed a positive result while appearance of a colourless layer is indicative of a negative result.

The suspected isolates when inoculated into the medium using a sterile straight wire followed by incubation by 37^0C for 24 hours. The upper layer of the inoculated medium and line of stabbing was observed for the presence of dark colouration which is indicative of a positive H_2S test.

The motility of bacteria can be determined using a single test medium. A semi-solid media containing beef extract, peptone, and 0.5% agar. The lower agar content allows bacteria with flagella for movement to demonstrate their motility by moving away from the stab line in an agar deep. Motility can be determined by examining the agar deep. Obligate aerobes require an atmosphere containing concentrations of oxygen similar to room air. Micro aerophiles

require oxygen, but less than that of room air. Anaerobes do not require oxygen for life, and can be either obligate anaerobes, which grow only in anaerobic environments, or facultative anaerobes, which grow in any oxygen environment. In terms of test results, obligate aerobes appear only at the top of the deep, micro aerophiles grow below the top of the stab line, obligate anaerobes grow only in the bottom portion of the stab line, and facultative aerobes grow evenly throughout the stab and on surface of the culture. Motility test medium was poured into test tubes and allowed to solidify. The tubes were inoculated with at sterile wire pin and allowed to incubate at 37°C for twenty-four hours before tests were read.

3.6.2.5 Carbohydrates fermentation test in Triple sugar Iron Agar

This is used to test for ability to ferment glucose, lactose and sucrose, Triple Sugar Iron Agar (TSI) is a variation of the broth sugar tests. TSI Agar slants contain 2% polypeptone, 1% lactose, 1% sucrose, 0.1% glucose, phenol red pH indictor, and ferric ammonium citrate. Utilization of sugars proceeds in much the same way as in sugar broth tests, with acid production changing a pH indicator.

Specifically, fermentation of lactose/sucrose and glucose causes the entire tube to be yellow. Fermentation of glucose alone causes the butt of the culture to be yellow, but the shallow slant portion turns red as glucose is oxidatively exhausted and peptone is metabolized, producing NH_3 resulting in an alkaline pH Gas production during the utilization of sugar is indicated by fissures or pockets in the slant. Some bacteria also produce hydrogen sulfide (H_2S) by reducing thiosulfate in the medium or breaking down cysteine in the peptone. Ferric

ammonium citrate reacts with hydrogen sulfide to produce a black precipitate in the butt of the agar. The slants were made from commercially prepared TSI mix and poured to allow the butt of the agar to be four centimeters deep, and the slants were inoculated using a sterile wire loop to inoculate a colony of test organism into a prepared TSI (triple Sugar iron) slant. Observable colour changes as red and pink were interpreted as alkaline and acids with or without gas formation.

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

Commercially available latex agglutinations kit having *E. coli* O157 and H7 antisera was used to further confirm *E. coli* O157: H7. The kit contains two test reagents (O157 test reagent and H7 test reagent). The O157 test reagent consists of a red latex particles 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 IgG and O157 lipopolysaccharide antigen.

3.7.1 Test procedure for O157 (somatic antigen)

Confirmed *E. coli* isolates after conventional biochemical tests were sub-cultured on CT-Smac and incubated at 37^0C for 24 hour. 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 in the, then we mix/rock the content carefully spreading the latex over the entire area of the circle.

3.7.2 Test procedure for H7 (flagella antigen)

Cultures that were positive with O157 test latex antigen were grown overnight at 37^0C in tryptone soya broth (TSB) at 37^0C. Using a applicator stick, sufficient growth was removed to just cover the blunt end of the stick. Approximately 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. Then mixed thoroughly, but not vigorously, using a separate stick, I then, emulsified a similar amount of the sample. For each test sample, one drop of H7 test latex in one circle and one drop of the H7 control latex in other circle, and rocked the content carefully spreading the latex over the entire area of the circle. Positive results showed agglutination with clear clumping of the latex particles. The rate of appearance of the agglutination is dependent on the quality and quantity of the cultured antigen. In case of negative result, the latex does not agglutinate and the appearance of the suspension remains substantially unchanged throughout the period of the reaction.

3.7.3 *in vitro* antibacterial resistance test

Escherichia coli O157: H7 isolates were tested for susceptibility to the following antibiotics at concentrations indicated: Ampicillin (10µg), Augmentin (30µg), Penicillin (10 i.u), Gentamicin (10µg), Ciprofloxacin (5µg), Chloramphenicol acid (30µg) Oxacillin (5µg) Streptomycin (30µg), Tetracycline (30µg) and Trimethoprim (5µg).Confirmed *E. coli* O157: H7 isolates were first sub-cultured on CT-Smac agar and incubated at 37^0C for 24 hours. Two to three colonies of the appropriate cultures were inoculated into 5ml of0.1tryptone soy broth and incubated at 37^0C until the turbidity approximated 0.5 McFarland' s standard. Mueller-Hinton agar plates were prepared and used according to the manufacturers' instructions. Sterile swabs were dipped into the broth culture and used to streak the surface of Mueller-Hinton agar in three directions at 180^0 until the entire surface was covered. The plates were allowed to dry at room temperature and the antibacterial discs were dispensed into the plates using the multiple disc dispensers (OXOID). A pair of sterile forceps was further used to ensure sufficient contact with the medium. The petri dishes were then inverted and incubated at 37^0C for 18-24 hours. Clear zones of inhibition were measured to the nearest millimeter using a transparent ruler and interpreted based on Clinical Laboratory Standard Institute (2011) cut-off point recommendations. Isolates were classified as susceptible, intermediate and resistant.

3.8 Statistical Analyses

Microsoft excel 2010 was used. The prevalence of *E. coli* was calculated by dividing the total number of positive samples by the total number of the samples examined and expressed in percentages. The results obtained were presented using tables, graphs and charts.

CHAPTER FOUR

RESULTS

4.1 Frequency (%) Distribution of Suspected *E. coli* Isolates from Eosin Methylene Blue and Cefexime Tellurite Sorbitol Maconkey agar

Out of the 300 samples of beef *'Suya'* screened for *E. coli* 135 (45.0%) were positive with typical growth on EMB as compared to 76 (25.0%) on CT-SMAC. The results showed that Danmagaji had the lest number of *E. coli* positive isolates both on EMB 14 (28.0%) and CT-SMAC 9 (18.0%) out of fifty samples. In addition, there was more positive growth on EMB 135 (45.0%) as compared to CT-SMAC 76 (25.0%).

Further screening of the isolate with conventional biochemical tests showed a decrease in total number and percentages of confirmed *E. coli* isolate on both EMB agar and CT-Smac from 135 (45.0%) and 76 (25.0%) to 19 (6.3%) and 8 (27.0%), respectively.

Table 4.1 shows the frequency (%) distribution of suspected *E. coli* isolated from EMB and CT-SMAC.

Table 4.1 Frequency (%) distribution of suspected *E. coli* isolates obtained from six sampling areas of ready to eat beef *'Suya'* sold in Zaria metropolis, Nigeria.

S/N	Sampling Area	No of Sample Collected	No. (%) Suspected *E .coli* Isolates on E.M.B agar	No. (%) Suspected *E. coli* 0157Isolates on CT-Smac
i.	Kwangilla	50	28 (56.0)	10 (20.0)
ii.	Tudun Wada	50	17 (34.0)	14 (28.0)
iii.	Sabon gari	50	27 (54.0)	18 (36.0)
iv.	Zaria City	50	23 (46.0)	10 (20.0)
v.	Danmagaji	50	14 (28.0)	9 (18.0)
vi.	Samaru	50	26 (52.0)	15 (30.0)
	Total	300	135 (45.0)	76 (25.0)

Key: E.M.B- Eosin Methylene Blue, CT-SMAC- agar

Table 4.2 Frequency (%) distribution of confirmed *E. coli* isolates after conventional biochemical tests from six sampling unit of ready to eat beef *'Suya'* sold in Zaria metropolis, Nigeria.

S/N	Sampling Unit	No. (%) confirmed *E. coli* Isolates from E.M.B agar	No. (%) confirmed *E. coli* Isolates from CT-Smac
i.	Kwangilla	3 (11.0)	1 (10.0)
ii.	Tudun Wada	5 (29.0)	0 (0,0)
iii.	Sabon gari	2 (6.0)	3 (14,0)
iv.	Zaria City	4 (17.0)	1 (10.0)
v.	Danmagaji	2 (14.0)	1 (11.0)
vi.	Samaru	3 (12.0)	2 (13.0)
	Total	19 (6.3)	8 (2.7)

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

4.2 Results after Further Confirmatory Tests Using Commercial Latex Agglutination Kits

A total of six isolates tested positive for *E. coli* O157. The prevalence of *E. coli* O157 from various sample areas after serology showed one isolate from Kwangilla, two isolates from Sabon gari and two from Samaru. Only four isolates tested positive for *E. coli* O157: H7 as shown on table 4.4. This gave a prevalence of 1.3% for *E. coli* O157: H7 and 2.0% *E. coli* O157: H7.

Table 4.3 Frequency distribution of confirmed *E. coli* O157 isolate after serology for *E. coli* O157 of ready-to eat beef *'Suya'* sold in Zaria metropolis. Nigeria.

S/N	Sampling Unit	No. confirmed *E. coli* O157 Isolates on E.M.B agar	No. *E. coli* confirmed as O157 from CT-SMAC
i.	Kwangilla	0	1
ii.	Tudun Wada	0	0
iii.	Sabon gari	1	1
iv.	Zaria City	0	0
v.	Danmagaji	1	0

vi.	Samaru	0	2
	Total	2	4

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

4.3 Antibacterial Resistance of *E. coli* O157: H7

Using the Clinical Laboratory Standard Institute (CLSI) 2011 cut off points to interpret results. Nearly all the isolates of *E. coli* O157: H7 showed less than 50.0% resistance to aminoglycosides group (Streptomycin and Kanamicin) with the exception of Gentamicin with 100.0% susceptible. Unlike other groups, all isolates showed zero or no resistance to Beta-lactam (Penicillin and ampicillin). However, all isolates (100.0%) were resistance to flouroquinolones (ciproflaxacin) and sulfonamides (trimethoprim). Other antibiotic agents such as tetracycline had 14.0% resistantce. The result showed no multidrug resistance. Table 4.5 showed the antibiotic resistance of seven confirmed isolates of *E. coli* O157: H7 against five different classes of nine antibiotics.

The antibacterial resistance patterns are shown in Table 4.6 the most common patterns were Kanamycin - Ampicillin- Ciprofloxacin with a frequency of 3 (33.0%) followed by Ampicillin 2 (11.0%). Streptomycin - Ampicillin 1 (22.0%) and Streptomycin – Ampicillin - Tetracycline 1 (22.0%) Ampicillin showed the highest level of multidrug resistance observed among the various patterns.

Table 4.4 Antibacterial agents against confirmed *E. coli* O157: H7 isolates from six sampling units of ready to eat beef *'Suya'* sold in Zaria Metropolis, Nigeria.

S/N	Antibacterial Agents (Concentration in µg)	% Susceptible, Intermediate & Resistance of the isolates against antibacterial agents		
		S	I	R
1.	Gentamicin (10µg)	100	0	0
2.	Streptomycin (10µg)	29	43	29
3.	Kanamicin (30µg)	43	43	14
4.	Penicillin (10iu)	0	0	0
5.	Ampicillin (10µg)	0	0	100
6.	Chloramphenicol (30µg)	43	43	14
7.	Ciprofloxacin (5µg)	100	0	0
8.	Trimethoprim (25µg)	100	0	0
9.	Tetracycline (30µg)	86	0	14

Key: S - Susceptibility, I - Intermediate and R - Resistance

Four resistance patterns each were observed from table 4.6. All the isolates exhibited multidrug resistance, with the least (two isolates) showing one antibiotic and the highest showed resistance to only one. All the isolates showed resistance to Ampicillin (AMP).

Table 4.5 Resistance pattern of *E. coli* O157: H7 isolates against 9 antibacterial agents in Zaria

S/N	Antibiotics	Number (%) Resistance
I	S,AMP	1 (22)
Ii	AMP	2 (11)
Iii	K,AMP,C	3 (33)
Iv	S,AMP,TE	1 (22)

**S-Streptomycin, AMP - Ampicillin, K - Kanamycin, C - Ciprofloxacin and TE -Tetracycline

Table 4.6 Percentage susceptibility of *E. coli* O157: H7 isolate against nine antibacterial agents in Zaria metropolis

S/N	Antibacterial Agents / disc potency	Susceptibility (%)
i	Gentamicin (10µg)	100
ii	Trimethoprin (25µg)	100
iii	Ciprofloxacin (5µg)	100
iv	Tetracycline (30µg)	86
v	Kanamycin (30µg)	43
vi	Chloramphenicol (30µg)	43
vii	Streptomycin (10µg)	26
viii	Ampicillin (10µg)	0

ix	Penicillin (10iu)	0

CHAPTER FIVE

DISCUSSION

The data obtained revealed that culturable *Escherichia coli* O157: H7 were isolated from ready eat beef *'Suya'* sold in Zaria metropolis, Nigeria. Previous studies also indicated the presence of *E. coli* O157: H7 in meat and meat products (Enabulele and Uraih, 2009, Tafida *et al.,* 2012). Similar trend has also been observed by Okeke *et al*. (2000) among children in primary health care centers in southern-western Nigeria. Olorunshola *et al*. (2000) and Smith *et al*. (2003) in Lagos state, Nigeria. Reported 7.0% prevalence in healthy animals.

Globally, Magwira *et al.* (2005) in Botswana reported a prevalence of 2.3% and 3.8% from fresh beef sausage and raw ground beef respectively. Likewise, Abd El-Atty and Meshref (2007) in Egypt obtained a prevalence of 2.0% in sausage which he attributed to contamination from faeces of infected animals as well as unsatisfactory hygienic measures during handling.

The low prevalence of 1.3% obtained in this study, could be due to the fact that most ready to eat meat vendors warm before serving their customers. One possible reason may be the fact that most buyers are aware of the health implication of consuming over stayed ready to eat beef *'Suya'*. Hence, the sellers have to prepare it fresh always.

Another reason for low prevalence of *E. coli* O157: H7 may be due to the presence of interfering bacteria during isolation (e.g *Pseudomonas* species) (Szabo *et al*., 1986; Vernozy-Rozand, 1997; Lejeune *et al.,* 2001). Also, the isolation procedure may be contaminated by the presence of other negative facultative anaerobic rods which also colonize fresh beef meat and do not ferment sorbitol, appearing as colourless colonies on CT-SMAC agar such as *Aeromonas*,

Plesiomonas, and *Morganella* species (Fujisawa *et al*., 2002). Hence, there is a possibility that the actual prevalence may be higher than reported in this study.

Previous studies have shown that cattle are the principal reservoirs of *E. coli* O157: H7 (Renter *et al.,* 2003 and Lejeune *et al.,* 2004). Hence, they serve as possible source of contamination during and after processing. Other sources of contaminants include; materials used in its preparation and packaging, handlers and the environment (Umoh, 2001).

There was no significant difference (P<0.05) in the occurrence of *E. coli* O157: H7 isolated from ready-to-eat beef *'Suya'* samples with respect to the various six sample units, indicating that the beef *'Suya'* produced and marketed in these areas may have similar microbiological quality. This may be due to the fact that similar handling procedures are employed during processing at the various areas (Umoh, 2001).

The isolation of the pathogen (*E. coli* O157: H7) in Zaria metropolis suggests that contaminated ready eat beef *'Suya'* sold to consumers exposes them to food borne hazards. Furthermore, it shows that food hygiene and handling practices remain a great challenge. The socio-economic implication is that resources and time are wasted on medication. The effects of food-borne infections are also greatly felt by immune-compromised individuals such as people living with HIV/AIDS, pregnant women, children, diabetic patients (Shisana *et al*., 2005). Isolation of the pathogen may also be an indication of poor sanitary environment under which animals are slaughtered, meat processed and sold (Abdullahi *et al*., 2006).

Overuse or misuse of antibacterial agents in different fields, such as human, agriculture and veterinary medicine as prophylactic or growth promoting agents in the food animal, has created enormous pressure of antibacterial resistance among bacterial pathogen and endogenous microflora (WHO, 2000). Antibacterial resistance poses a critical public health threat especially in developing countries. This has a direct clinical implication which involved the development and transfer of resistant gene by these bacteria, thereby making it difficult to effectively control bacterial infections (WHO, 1997 and Karlowsky *et al*. 2003).

The results from this study showed that all the isolates were resistant to at least one antibiotic especially ampicillin but were all susceptible to gentamicin, ciprofloxacin and trimethoprim. The susceptibility results obtained is similar to reports by Walsh *et al*. (2006) and Okolocha *et al.* (2006) observed that all the *E. coli* isolates tested were highly susceptible to gentamicin, ciprofloxacin and trimethoprim. The susceptibility exhibited by all the isolates could be as a result of the mode of action of the antibiotic (Brown, 1988), high cost of the antibiotics. Hence, less frequent prescription of gentamicin, ciprofloxacin and trimethoprim by physicians.

In this study, the susceptibility of all the isolates to Gentamicin, Ciprofloxacin and Trimethoprim could be due to the fact that these antibiotics are very expensive compared to others and therefore, not indiscriminately used (Idika, 1999; Okeke *et al*. 2000). However, reports have shown that any resistance to the quinolones can spread rapidly (Hoge *et al*., 1998), hence, the need to monitor and enforce control. The policy of strict reservation of antibiotics for special cases might further reduce the rate of development of resistance in humans and animals.

Contrary to the above findings, a study in Zaria reporting the occurrence of plasmid-mediated multidrug resistant *E. coli* O157 where one aquatic isolate contained two plasmids and was resistant to seven drugs including, ampicillin, cefuroxime, ciprofloxacin, cotrimoxazole, nalidixic acid, nitrofurantoin and tetracycline (Chigor *et al*., 2010). In the present study, no multidrug resistant *E. coli* O157: H7 was seen. This could be attributed to difference in host habitat.

The antibacterial sensitivity tests results showed high resistance by the *E. coli* O157: H7 isolates to Ampicillin and penicillin (Beta- Lactams). This is in agreement with the findings reported by Shintandi *et al.* (2001), Al Haj *et al* (2007) and Olatayo (2010), The high resistance obtained in this study may be due to the fact that it is the most commonly available antibiotics used as growth promoter and routine chemoprophylaxis among livestock in Nigeria (Olatayo, 2010).

This, poses a major challenge in both human and animal medicine because these drugs are commonly used in the treatment of human patients. Uncontrolled usage of antibiotics in treatment of animals and their incorporation in animal feeds has accounted significantly for the increase in antibiotics resistance in bacterial isolates (WHO, 2000; Galland *et al*., 2001).

Findings in this study agree with a report of a study in Abeokuta, Southwest-Nigeria where human strains had a lower frequency of resistance to ampicillin and higher rate against chloramphenicol (Akinduti *et al.*, 2008). It seems the toxicity of chloramphenicol restricts its

being commonly used (Okeke *et al*., 1999).The isolates showed no resistance profile against the beta - lactam group (Penicillin and Ampicillin) of antibiotics which are used against gram positive bacteria.

Surprisingly, in this study, the rate of resistance to tetracycline among isolates was low (14%).

Studies in developing countries have shown that they are among the most frequently used antibacterial agents because they are inexpensive and are usually obtained over the counter (OTC) of patent medicine shops without recourse to prescription by physicians (Hart and Kariuki, 1998; Okeke *et al.*, 1999). Moreso, it is common to find these antibiotics used locally for prophylaxis and treatment of diarrhoea (Idika, 1999). Smith *et al*. (2003) in Lagos, Nigeria reported that high antibiotic resistance observed in animal isolates could be consequence upon widespread use of the drug in animal feeds.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The results based on the findings and deductions from this study showed that ready-to-eat beef *'Suya'* sold in Zaria metropolis were contaminated by a potentially pathogenic enteroheamorrhagic *Escherichia coli* O157: H7 that have public health implications. Although a relatively low prevalence of 1.3% was observed in this study, the presence of the *Escherichia coli* O157: H7 is still of public health concern because the organism has low infective dose of less hundred one cells. It is important to stress that management of meat safety risks should be based on an integrated effort and approach that applies to the general public. From the producers, distributors, packers, handler, food vendors authorities and consumers. Therefore, the report of the presence of *E. coli*O157: H7 should prompt the public to keep in mind that food-borne illnesses are due to mishandling of foods in a way we should avoid. Thus, consumer education and environmental sanitation should be the major targets in efforts to improve meat safety.

In addition, the isolates exhibited one hundred percent resistance to Gentamicin, Streptomycin, Ciprofloxacin and Trimethoprim while the Beta - lactam group (Penicillin and Ampicillin) one hundred percent susceptibility. The isolates were moderately resistant to Kanamycin, Chloramphenicol and Tetracycline. However, none of the isolate was multidrug resistant. The high resistance to four commonly used antibiotics raised a serious public health concern.

6.2 Recommendations

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

i. There is need for awareness campaign to sensitize meat handlers and vendors on the need for cooking through either by increasing the temperature, time of cooking or a combination of both, without compromising taste and adherence to hygienic principles during food processing, storage and marketing as part of the measures to promote good health and prevent the transmission of pathogens from ready to eat beef *'Suya'* to humans.

ii. In Nigeria, food-borne diseases occur predominantly as isolated sporadic cases rather than taking the form of outbreaks and many, if not most, cases of food-borne infections are unrecognized, un investigated and undocumented. Therefore, the need for epidemiological surveillance and periodic monitoring of food-borne illness.

iii. In order to have accurate judgment about the possibility and range of transmission of *E. coli* O157: H7. It is recommended that further studies be conducted to determine the prevalence of *E. coli* O157: H7 in other ready to eat meat products such as shredded fried meat (damdu), spiced sun dried meat (Kilishi) etc.

iv. Education and public awareness programs are important measures in sensitizing the general public and people at risk on dangers of self-medication so as to minimize the increased rate of antibacterial drug resistance.

v. There is need to legislate and enforce laws to limit the prescription and indiscriminate dispensing of antibiotics to only qualified professionals.

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APPENDICES

APPENDIX I: Preparation of Media and Reagents

The following media were used

1.1 Sorbitol MacConkey agar (SMA)

Composition in grame per litter

20.0 g peptone

10.0 g sorbitol

5.0 g NaCl

1.5 g bile salts

300 mg neutral red

1.0 g crystal violet

15.0 g agar

Preparation:

Fifty one point five grams of Sorbitol Mac conkey Agar powder was weighed and transferred into one liter of distilled water contained in a sterile conical flask. Cover neck of flask with aluminum foil. It was then mixed by shaking and boiled so as to dissolve completely, autoclaved at 121^0C for 15mins. The medium was supplemented with (0.2 g/liter) with Cefexime 50µg/L and Potassium tellurite 2.5mg/L at 45^oC to enumerate *E*. *coli* O157: H7. Reagent was added to heat-sterilised (121 °C, 15min) molten (47 to 50 °C) SMA to yield CT-SMA. It was allowed to cool to $45°C$ and mixed well before dispensing aseptically in 20ml volume into disposable Petri dishes. The medium was allowed to solidify on slant position and was preserved in the fridge for used thereafter. Prior to incubation, the surface of the agar was dried by partial exposure at 37^0C. The appearance of the plate was clear pink/red. The media was preserved in the fridge.

1.2 Nutrient Agar (NA)

Composition in grams/liter

1.0g Lab. Lemco powder

2.0g Yeast extract

15.0g Sodium chloride

5.0g Agar

5.0g Peptone

7.4 pH

Preparation:

Twenty three grams of nutrient agar powder was transferred into one liter of contained sterile conical flask. Cover neck of flask with aluminum foil. It was then mixed and to boil so as to dissolve completely and aseptically dispensed into bijou bottle autoclaved at 121^0C for 15mins. The medium was allowed to solidify on slant position was preserved in the fridge for used thereafter.

1.3 Eosin Methylene Blue (E.M.B)

Composition in grams per liter

1.0g Lactose

5.0g Bile salts

5.0g Sodium chloride

0.075g Neutral red

12.0g Agar

1000ml Distilled water

7.6 PH

Preparation:

Twenty eight grams of Eosin Methylene Blue agar powder was weighed and transferred

One liter of distilled water contained in a sterile conical flask. Cover neck of flask with

Aluminum foil paper. It was then mixed by shaking and brought to boil so as to dissolve

and then autoclaved at 121^0Cfor 15mins. It was allowed to cool to $45°C$ and mixed before dispensing aseptically in 20ml volume into disposable Petri dishes. Prior to incubation the surface of the agar was dried by partial exposure at 37C. The appearance of the plate clear pink/red. The media was preserved in the fridge.

1.4 Buffered Peptone Water (PW)

This medium was used to enrich and develop the inoculums that were used to inoculate

agar plates. It was also used to maintain the culture for some biochemical tests.

Composition in grams per liter

10.0g Peptone

5.0g Sodium Chloride

1000ml Distilled Water

7.6 pH

Preparation:

The powdered medium was used and it was prepared as directed by the manufacturer. Twenty grams of the powdered medium (Oxoid) was dissolved in 1000ml of distilled water medium was sterilized by autoclaving at 121°C for 15minutes.

1.5 Modified-Tryptic Soya Broth (MTSB) (Enrichment Media)

Composition in grams per liter:

17.0 g casein

5.0 g NaCl2

3.0 g soybean powder

2.5 g K2HPO4

2.5 g glucose

1.5 g casmino acid

10 mg Acriflavine

1.5 g bile salt

Preparation:

The powdered medium was used and it was prepared as directed by the manufacturer.

Point five grams of the powdered medium (Oxoid) was dissolved in 1000ml of distilled water medium was sterilized by autoclaving at 121°C for 15minutes.

1.6 Urea Agar (U.A)

This medium was primarily used for urease test.

Composition in grams per liter

1.0g Peptone

5.0g Sodium Chloride

2.0g Potassium dihydrogen- Sulphate K H_2PO_4

5.0g Glucose

20.0g Agar powder

1000ml Distilled water

Preparation:

The powdered urea agar (oxoid) was used and was prepared as directed by the manufacturer Urea agar was prepared by suspending 2.4g of the powdered medium in 95ml of distilled dissolved by boiling. The medium was sterilized by autoclaving at 115°C for 20 minutes. Medium was cooled to about 50°C and 5ml of 40% v/v sterile urea solution was added.

Medium was dispensed into culture tubes in 15ml aliquote and were allowed to solidify slant positions. They were thereafter used.

1.7 Simmon Citrate Agar (SCA)

This medium was used for the differentiation of Enterobacteriaceae. Based on the of citrate as the sole source of carbon.

Composition in grams per liter

2.0g Magnesium sulphate

0.8g Sodium ammonium sulphate

0.2g Ammonium dihydrogen sulphate

2.9g Sodium citrate tribasic

5.0g Sodium chloride

0.08g Bromoethyl molblue

15.0g Agar No.3

1000ml Distilled water

6.9 pH

Preparation:

The medium was constituted as above and sterilized by autoclaving at 121°C for 15 minutes. Two drops of concentrated hydrochloric acid (HCL) were added to the medium to adjust the

pH of the medium to the accepted level. About 15ml aliquote of the medium was dispensed in to culture tubes and the medium was allowed to solidify in these tubes in slant positions. They were thereafter used.

1.8 Kovacs reagent for indole (Cowan and Steel, 1974)

It contains:

5.0 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 °C.

Cool and add the acid protect from light and store at 4 °C.

1.9 Triple – Sugar Iron Agar (TSI) (Cowan and Steel, 1974)

Composition per liter:

3.0 g beef extract

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 phenol red (0.2 %)

Preparation:

Dissolve the solids by heating, add the indicator sol., mix and tube, sterilize at 115 °C

20 minute and cool to form slopes with deep butls.

1.10 Methyl Red Solution (Cowan and Steel, 1974)

It consists of`

40 mg methyl red

40.0 g ethanol

PREPARATION

Dissolve the methyl red in the ethanol and dilute to volume with the distilled water

1.11 Citrate Media (Cowan and Steel, 1974)

Composition per liter

5.0 g NaCl

200 mg $MgSO_4$

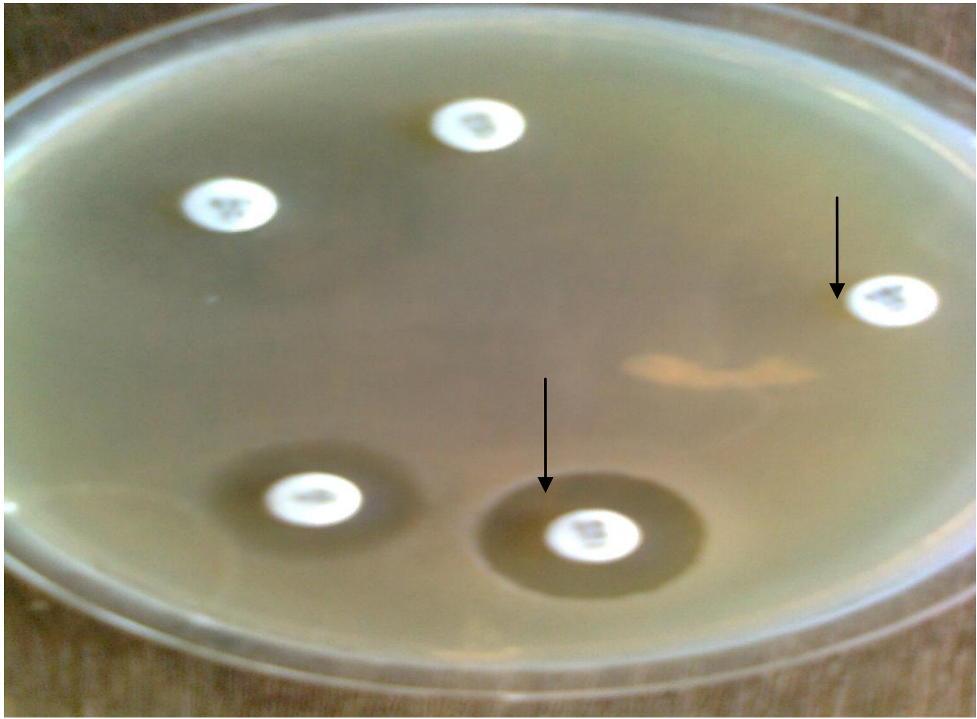
1.0 g K_2HPO_4

1.0 g NH_4HPO_4

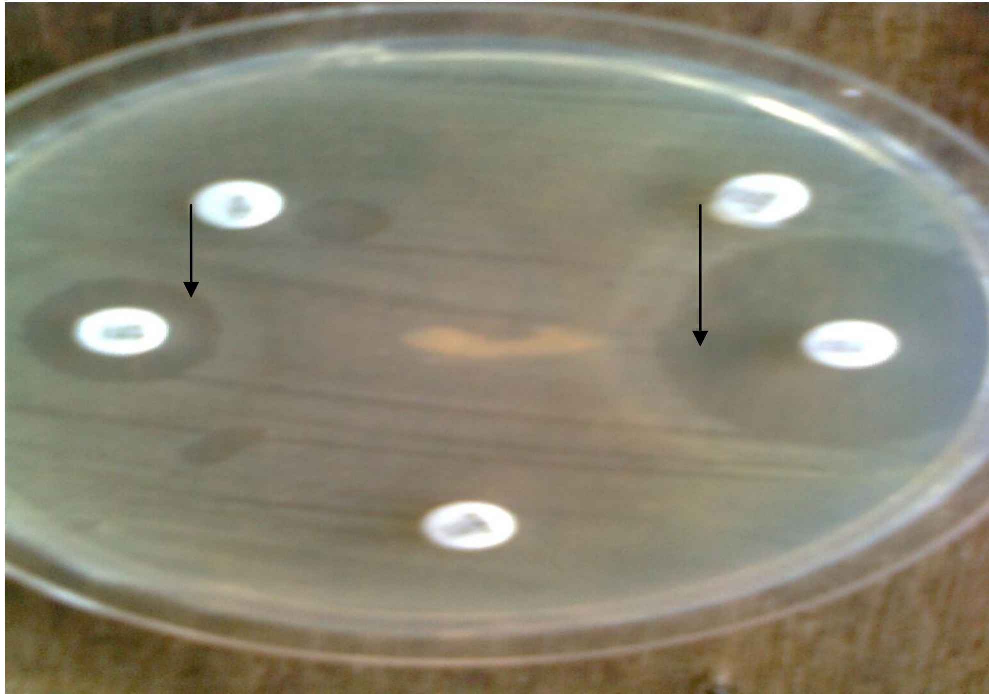
Preparation

Dissolve the salt in distilled water, and 3 g of sodium citrate or 2.77 g of hydrated form to the salt solution and sterilize at 115 °C for 20 minute.

APPENDIX II: Antibacterial susceptibility pattern of *E. coli* O157: H7 showing zone of inhibition

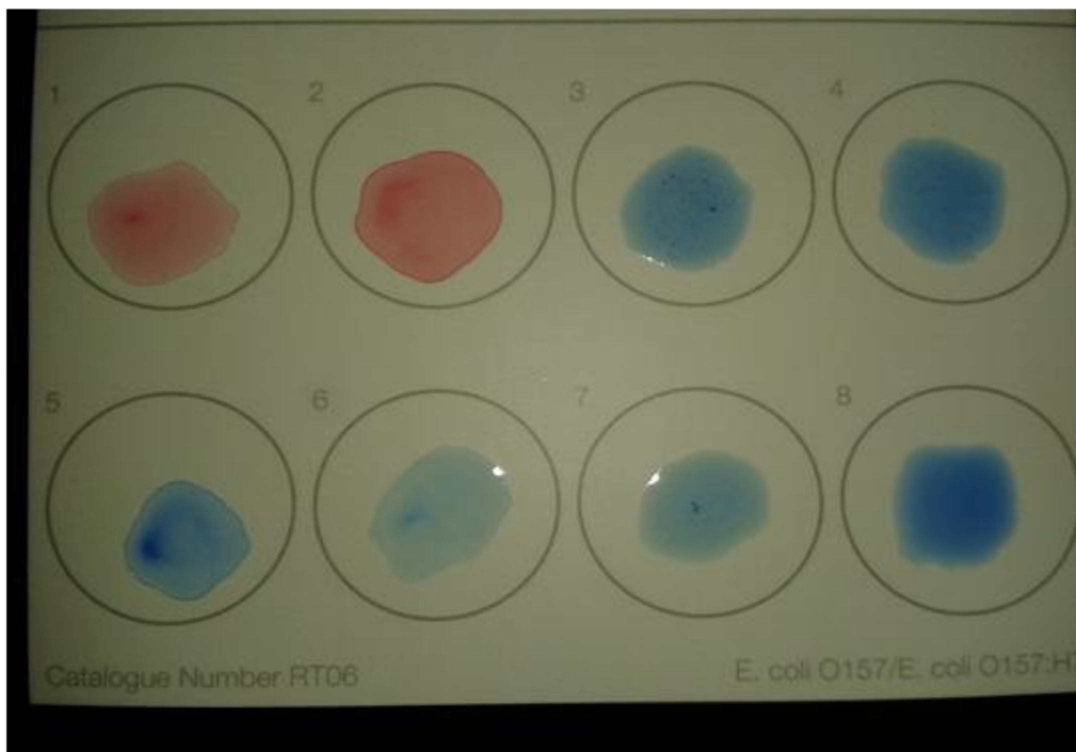


APPENDIX III: Antibacterial susceptibility pattern of *E. coli* O157: H7 showing zone of inhibition

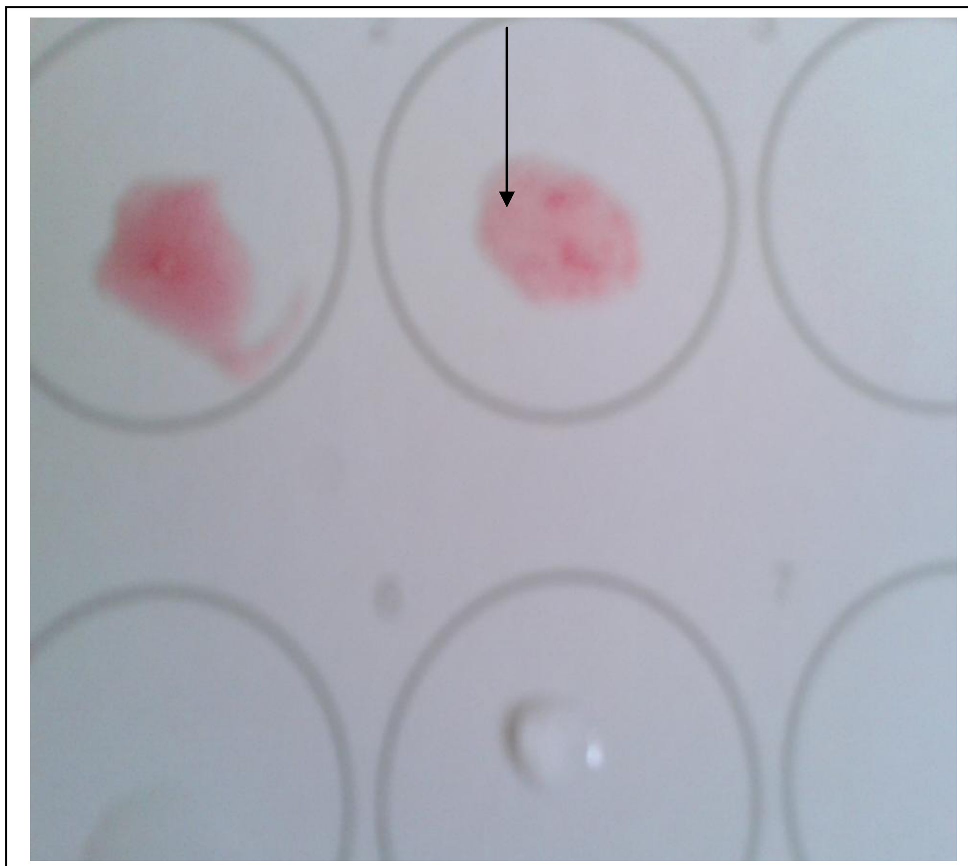


APPENDIX IV: Latex agglutination test reagent with antisera for *E. coli* O157: H7

APPENDIX V: Negative agglutination of *E. coli* O157 with test reagents



APPENDIX VI: Latex agglutination of *E. coli* O157 with antisera



APPENDIX VII: Clinical Laboratory Standard Institute Cut-Off points Chart Used in \
the study

S/N	Antibacterial Agents	CLSI Recommendation Cut- Off Points		
	(Concentration in µg)	S	I	R
1	Gentamicin	≥15	13-14	≤12
2	Streptomycin	≥15	12-14	≤11
3	Streptomycin	≥18	14-17	≤13

4	Penicillin	≥17	14-16	≤13
5	Ampicillin	≥17	14-16	≤13
6	Chloramphenicol	≥18	13-17	≤12
7	Ciprofloxacin	≥21	16-20	≤15
8	Trimethorim	≥16	11-15	≤10
9	Tetracyline	≥15	12-14	≤11

Adopted from Clinical Laboratory Standard Institute 2001 using Muller-Hinton Agar

Keys: S= susceptible, I=intermediate and R=resistance.

APPENDIX VIII: Measured Zone of Inhibition of Nine Antibacterial Agents on Muller-Hinton

S/N	Antibacterial Agents	*E. coli* O157: H7 Isolates						
	(Concentration in µg)	Measures Zone of Inhibition (mm)						
		Kw^{17}	Sg^{14}	Vt^{02}	Dm^{03}	Dm^{04}	Sm^{01}	Sg^{26}
1.	Gentamicin (10 µg)	21	20	21	16	17	16	23
2.	Streptomycin (10 µg)	0	15	18	13	0	13	12
3.	Kanamicin (30 µg)	23	17	13	10	18	10	24
4.	Penicillin (10 iu)	0	0	0	0	0	0	0
5.	Ampicillin (10 µg)	0	0	0	0	0	0	0
6.	Chloramphenicol(30µ)	21	30	12	0	16	0	33
7.	Ciprofloxacin (5 µg)	30	32	35	40	23	35	33
8.	Trimethoprim (25 µg)	30	29	32	24	27	20	20
9.	Tetracycline (30 µg)	30	23	33	26	0	20	26

APPENDIX IX: Antibiotic result of *E. coli* 0157:H7 Intepreted as S=Susceptible, I=Intermediate and R=Resistant

	Antibacterial Agents (Concentration in µg)	*E. coli* O157: H7 Isolates						
		Kw^{17}	Sg^{14}	Vt^{02}	Dm^{03}	Dm^{04}	Sm^{01}	Sg^{26}
1.	Gentamicin (10 µg)	S	S	S	S	S	S	S
2.	Streptomycin (10 µg)	R	S	S	I	R	I	I
3.	Kanamicin (30 µg)	S	I	R	R	S	R	S
4.	Penicillin (10 iu)	R	R	R	R	R	R	R
5.	Ampicillin (10 µg)	R	R	R	R	R	R	R
6.	Chloramphenicol(30µ)	S	S	R	R	I	R	S
7.	Ciprofloxacin (5 µg)	S	S	S	S	S	S	S
8.	Trimethoprim (25 µg)	S	S	S	S	S	S	S
9.	Tetracycline (30 µg)	S	S	S	S	R	S	S

APPENDIX X: The following table shows the result obtained with the *E. coli* O157 Latex Reagents and the *E. coli* O157 Positive Control should be interpreted

O157 Latex Reagents	Negative Control Latex Reagents	Remarks
+	-	Presumptive for *E. coli* O157
+	+	Auto-agglutinating or cross reacting strain present. Perform further test to rule out *E. coli* O157
+	-	Auto-agglutination
-	Not done	Indicates absence of *E. coli* O157
Stringy or mucoid Appearance	Not done	Uninterpretable. Make fresh suspension of colonies in saline and allow clumps to settle out. Retest supernatant.