OCCURRENCE AND ANTIBIOGRAM OF *Escherichia coli* O157:H7 FROM NIGERIAN CURRENCY NOTES (NAIRA), COLLECTED FROM BUTCHERS AT THE MEAT MARKET IN KARU ABATTOIR, ABUJA. NIGERIA

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DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE,
AHMADU BELLO UNIVERSITY, ZARIA,
NIGERIA.

SEPTEMBER, 2015

DECLARATION

I declare that the work in this Dissertation entitled "**OCCURRENCE AND ANTIBIOGRAM OF *Escherichia coli* O157:H7 FROM NIGERIAN CURRENCY NOTES (NAIRA), COLLECTED FROM BUTCHERS AT THE MEAT MARKET IN KARU ABATTOIR, ABUJA. 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 reference provided. No part of this project report was previously presented for another degree or diploma at this or any institution.

Pindar Yawulda MBAYA _____ _____

Name of Student Signature Date

CERTIFICATION

This Dissertation entitled "**OCCURRENCE AND ANTIBIOGRAM OF *Escherichia coli* O157:H7 FROM NIGERIAN CURRENCY NOTES (NAIRA), COLLECTED FROM BUTCHERS AT THE MEAT MARKET IN KARU ABATTOIR, ABUJA. NIGERIA**" by Pindar Yawulda MBAYA meets the regulation governing the award of Masters of Science in the Department of 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 Dissertation is dedicated to the Lord Almighty, My Wife, Mrs Ruth Pindar , Parents, Dr and Mrs Yawulda Mbaya, Siblings Mrs Kunara, Dr Godiya, Mr Elisha and all my friends Dr Maryam Jelbis, Dr Isaac Igbaver, Dr Temala, Dr Amina, Dr Shehu Shamsudeen. May God continue to bless you all.

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ABSTRACT

Currency note is a medium of exchange for goods and services; buying and selling of meat, at meat markets. This present study was designed to evaluate the occurrence, Total viable count (TVC), serology and determination of antibiotic sensitivity pattern of *E. coli* O157:H7 from Nigerian currency notes (Naira) collected from butchers at the meat market in Karu Abattoir, Abuja. The 189 currency notes sampled from the eight currency denominations were subjected to cultivation and isolation using Eosine methylene blue and Sorbitol-MacConkay agar respectively, in which isolates were obtained. All the 189 (100%) naira notes sampled were contaminated with bacteria of the 189 samples, 12 (19.7%) were *E. coli*, of which 5 (41.7%) were *E. coli* O157:H7. Antibiotic sensitivity pattern of the 12 *E. coli* isolates from currency notes was done using antibiotic disc diffusion method and results were recorded according to CLSI standards. Antibiotic sensitivity pattern revealed that the *E. coli* isolates were observed to be 100.0% susceptible to vancomycin, oxacillin and penicillin G, while they were observed to be 33.3% resistant to ciprofloxacin, gentamicin and amikacin. Currency notes can potentially serve as fomite in transmitting microorganisms such as *Escherichia coli* O157:H7 which causes enteric diseases in humans. Also poor handling practices of currency notes poses a critical public health threat.

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Currency notes are widely exchanged for goods and services worldwide (Uneke and Ogbu, 2007). Naira note is the legal tender in Nigeria (Ogba, 2007), it is of two types Polymer and Paper. The paper naira note is a mixture of 75% cotton and 25% linen (Brady and Kelly, 2000) while the polymer is made from a polymer which is biaxially oriented polypropylene (BOPP)(https://en.wikipedia.org/wiki/Polymer_banknote). Currencies contaminated with pathogenic bacteria such as *Escherichia coli(E.coli)*can potentially be a vehicle for the spread ofthe microorganisms when it is exchanged within individuals (Pinner *et al.*, 1996). *E. coli* O157:H7 is commonly found in the intestines of cattle and cross contamination of any parts of the animal is possible when meat processing is not properly done. The bacterium is also found naturally in the intestines of other animals like pigs, sheep, goats and deer (Govindarajan, 1990; WHO, 2004; Yousuf *et al*., 2008).

According to Mir-Hassan *et al*., (2013) study, *E. coli* was frequently isolated from meat due to the transfer of faecal material during the slaughter process or via transfer from contact surfaces, their study further suggested that it was not unexpected to encounter *E. coli* on currency notes derived from meat outlets where transfer to currency notes was via improper handling of meat in exchange for currency notes. Uneke and Ogbu, (2007)observed that this route accounted for the high prevalence of enteric bacteria recovered from banknotes sampled from poultry meat shops. The findings also showed that contamination could be introduced outside the retail environment. Butchers with the bloody and dirty fingers receive and give currency notes, leading to the

contamination of the notes with microorganisms as reported by Mensah *et al*.(2002). Contaminated currency notes became a vehicle for the transmission of pathogenic organisms which were in circulation and contaminated the hands of other people in the process (Mensah *et al*., 2002; Uneke and Ogbu, 2007).

The survival of various microorganisms such as *E. coli* on money and other fomites, with their transmission via the hands of market men and women is often overlooked as enteric disease reservoir (Michaels, 2002). Pathogenic microorganisms that may survive on currency notes may serve as a potential source of Enteropathogens (Michaels, 2002; Cardoen *et al*., 2009; Lamichhane *et al.*, 2009). Contamination of objects by pathogenic microorganisms is of much public health concern as contaminated meat,butcher's hands, utensils, tables and the exchange of currency can be sources of transmitting pathogens such as *E. coli* (Michaels, 2002; Pope *et al.*, 2002).

Studies from different parts of the world have reported high rates of microbial contamination of currency notes in circulation (Michaels, 2002; Pope *et al.*, 2002).The microorganisms implicated were *Escherichia coli, Salmonella spp, Mycobacterium tuberculosis, Mycobacterium bovis, Staphylococcus spp* (Abrams and Waterman., 1972; Gadsby, 1998).

This study was carried out on isolation, serology,total viable count (TVC) and antibiotic sensitivity pattern of *E. coli* from Nigerian currency notes (naira) collected from butchers at the meat market in Karu Abattoir, Abuja. Nigeria.

1.2 Statement of Research Problem

Escherichia coli infection is one of the most significant (emerging) food borne pathogens that has gained increased attentionworldwide. Illnesses resulting from *E. coli* infection includeurinary tract infections, recurrent meningitis with clinical symptoms shown by susceptible individuals ranging from mild diarrhoea and haemorrhagic colitis to complicated infections such as haemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP).The pathogen causes infection with low infective dose of <100 cells.Diarrhoea caused by bacterial pathogens especially *E. coli* remains one of the major causes of morbidity and mortality in infants and young children in Nigeria (Akinyemi *et al.*, 1998; Okeke *et al.*, 2000a).

Contaminated currency notes serving as fomites, was suggested and has become a vehicle for the transmission of pathogenic organisms such as *E. coli* (Abrams and Waterman, 1972; Gadsby, 1988). This is often overlooked as a reservoir forenteric diseases. Studies from different parts of the world have reported high rates of microbial contamination of currency notes in circulation (Michaels, 2002; Pope *et al.*, 2002).

The major reservoir of *E. coli* among other animals is the cattle, its poor processing in Nigerian abattoirs and poor hygiene practice in transportation, sales and storage of the processed meat by butchers in meat outlets and also by meat buyers in the exchange of money for meat leads to cross contamination of the money and meat exchanged.

1.3Justification

Currency used as a means of exchange for goods and services is a daily activity and it involves people of different categories (buyers and sellers). Studies in different parts of the world have reported high rates of microbial contamination of currency notes in circulations,high levels of enteric pathogens were isolated from paper money samples obtained from butchers and fish mongers at local markets in Rangoon, Myanmar; Goktas and Oktay (1992) from butchers in turkey; Pope *et al.,* (2002) from fomites in Ohio, USA; Siddique*et al.,* (2003) in Manila, Philippine; Basavarajappa *et al*., (2005) in India; El–Dars and Hassan, (2005) in Egypt; Xu *et al*., (2005) in China; Blackenship, (2007) in Virginia, USA.

This research is particular about contamination with *E. coli* O157:H7on Nigerian currencynotes (naira) collected from butchers at the meat market in Karu Abattoir, Abuja.*E. coli* is frequently isolated from meat due to the transfer of faecal material during the slaughter process or via transfer from contact surfaces, meat produced in an unhygienic condition could pose threat to the health of the consumers as well as impair the keeping quality of such meatthrough the contamination of meat could result from contaminated working surfaces, equipment and the worker's hands used in the processing. The quality of water used in meat processing at the butcher shops also play a major role in reducing or increasing meat contamination, as only water was used in washing the tables, carcasses, blood from meat, equipment and worker's hands (Endale and Hailay, 2013). Bacterial contamination of meat products can also be unavoidablya consequence of poor meat processing (Jones *et al*., 2008). Without proper hygienic control, the environment in butcher's area can act as important sources of bacterial contamination (Gill *et al.,* 1998).

Hussein (2005b) observed that the animalswere slaughtered on the abattoir floor which was not properlydisinfected after processing meat, butchers and retailerswalk between carcasses as they transacted their business, while processed meat in the market were displayed on tables,exposed to contaminants. Hussein (2007)from his review of 3 decades publications recorded on *E. coli* O157:H7, had prevalence rates in therange of 0.2% to 27.8% for beef cattle at points of slaughter.

Improperly processed meat from the abattoir can contaminate the butcher's hands,utensils and tables (fomites), or unhygienic practices by the butchers or buyers of the meat themselves; such as not properly washing hands, utensils and tables with detergents, could all in turn contaminate currencies during buying and selling of the meat.Hand washing with soap and water by individuals, after handling currency, is not a common practice, these contaminated currencies can serve as potential source of spread of *E. coli* organisms which in turn cause enteric diseases in humans (Michaels, 2002).

However there is paucity of information on the incidence and prevalence of *E. coli* and risk factors associated with exchange of currency notes by butchers and meat buyers at meat markets in Nigeria.Knowledge of the microbial isolation, serology, TVC and antibiotic sensitivity pattern of *E. coli* on currency notes collected from butchers at the meat market in Karu Abattoir, can provide the basis for awareness of good hygienic practices, in people during and after currency handling and effective control of contaminationand infection.

1.4Aim of the Study

To determine the occurrence, serology, TVCand antibiotic sensitivity pattern of *E. coli* on Naira notes collected from butchers at the meat market in Karu Abattoir, Abuja.

1.5Objectives of the Study

The objectives of the study were to:

i. Determine if there is*E. coli* contaminationofNaira notes in circulation among butchers at Karu abattoirmeat market.
ii. determine the prevalence of *E. coli* O157:H7on naira notes from butchers at the Karu abattoir meat market,
iii. serotype *E. coli* O157:H7isolates obtained from naira notes collected from butchers at Karu abattoirmeat market,
iv. determine the relationship between the occurrences of *E. coli* on the different types (paper and polymer) and denominations of the naira notes in circulation among butchers in Karu abattoirmeat market and
v. assess the antibiogram of *E. coli* on naira notes collected from butchers at the Karu abattoir meat market.

1.6 Research Question

- Are naira notes collected from butchers at the meat market in Karu Abattoir, contaminated with *E. coli* O157:H7?
- What is the level of microbial contamination of*E. coli* on naira notes in circulation among butchers in Karu abattoir meat market?
- Are *E. coli* O157:H7 Serotypes found on isolates,obtained from naira notes collected from butchers at the Karu Abattoirmeat market?

- What is the relationship between the occurrences of *E. coli* on the differenttypes (paper and polymer) and denominations of the naira notes obtained from butchers at the Karu abattoirmeat market?
- What is the antibiogram of *E. coli* on naira notes from butchers at the Karu abattoirmeat market?

CHAPTER TWO

LITERATURE REVIEW

2.1Historical background 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*(*E.coli*) in 1919 in a revision of bacteriological nomenclature (Law, 2000). *E. coli* belongs tothe family Enterobacteriaceae, the organisms 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 (Willshaw, 2001).

E. coli has a natural habitat which is in the lower part of the intestine of most warm blooded animals (Heidelberg, 1981), asa part of the normal flora of the human intestinal tract, itplays 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).

E. coli are the major cause of neonatal septicemia, neonatal meningitis 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).

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

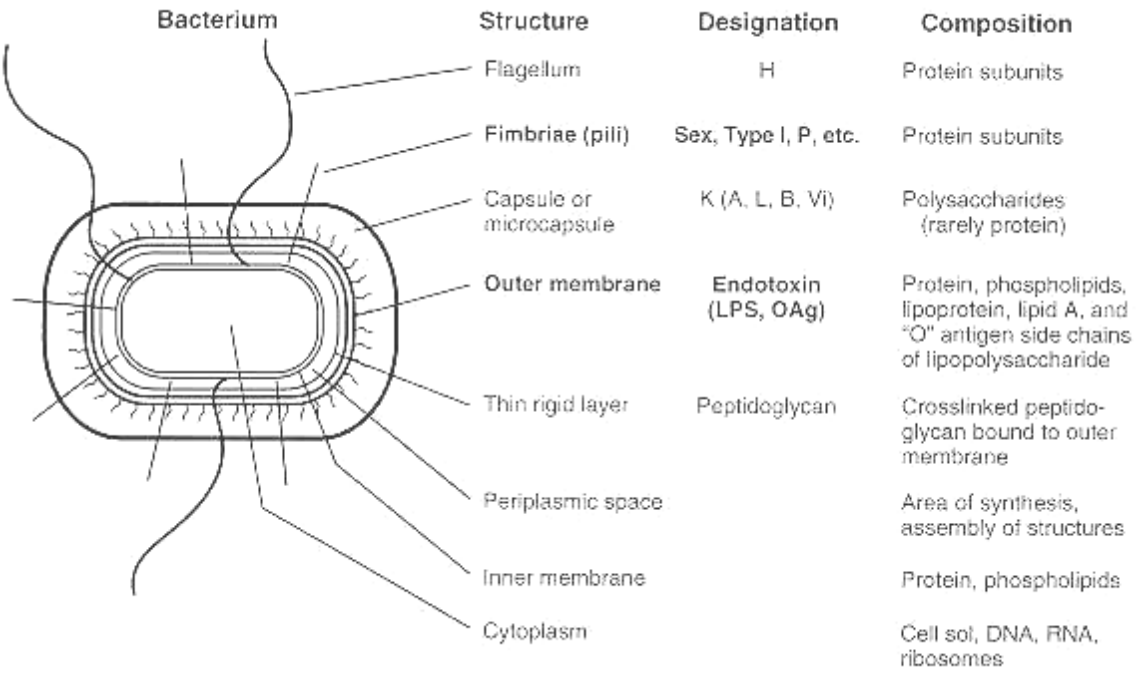


Figure 2.1 Structure and composition of *Escherichia coli* (Padhye *et al*., 1986)

2.2Pathotypes of *Escherichia coli*

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

I. Enteropathogenic *E. coli* (EPEC): Is the major cause of 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 heat-labiletoxins (Karper *et al*., 1997). They produce a non-fimbria 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 diarrhea with nausea, vomiting, abdominal cramps and fever. Onset of symptoms can range from 17-72 hours (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 found 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).

III. Enterotoxigenic *E. coli* (ETEC): These strain are 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 (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. coli* (EHEC):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 Vero 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). 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, thrombotic thrombocytopenic purpura (TTP) and death. (FDA, 2006).

In terms of zoonotic importance, Enterohaemorrhagic *E.coli,* causes severe disease. EHEC belongs to a large number of serogroup; the most important of these are *E. coli* O157, O111, O26, O103, O145, O91 and O22; most virulent of typical EHEC serogroup are O157, O111, O26 and O103. More than two hundred different serovars have been described for EHEC (Baljer and Weiler, 1999).

Entero-haemorrgic *E. coli* O157:H7 is one of the emerging food-borne infections. The pathogen causes exceptionally severe consequences of infection with low infective dose of 100 cells and high acidic tolerance (Buchanan and Doyle, 1997). The acidic tolerance of *E. coli* O157:H7is believed to play a key role in the pathogenesis and food borne illness (Buchanan and Edelson, 1996).

V. Entero-aggregative *E coli* (EaggEC): These pathotypes are self-adherent, tending to auto-agglutinate, giving the appearance under the microscope of a stack brick (Harrigan, 1998). The distinguishing feature of EaggEC strain is their ability to attach to tissue culture cells in an aggregative manner, they 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 (**Nataro*, et al.*,** 1998**)**. 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 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). Mayer and Wanke (1995) reported that EaggEC was recovered from diarrhoeal stool from AIDS patients. 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 harbors Afa/Dr adhesion which is associated with urinary tract infection (Archamband *et al*., 1988). 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 pyelonephritis in pregnant women (Archamband *et al*., 1988 and Servin, 2005).

2.3Epidemiology of *E. coli* O157:H7

2.3.1 Global situationof *E. coli* infection and contamination

Diarrhoea caused by bacterial pathogens especially *E. coli* remains one of the major causes of morbidity and mortality in infants and young children in many developing countries (John and Mathewson, 1992). *E. coli* O157:H7 has been isolated fromhuman specimens globally; these include USA, South Africa, Australia, Argentina, Canada, Chile, China, Czechoslovakia, France, Germany, India, Ireland, Italy, Japan, and the United Kingdom (Griffin*et al.*,1995). The U.K. experienced its largest outbreak of *E. coli* O157:H7 infection in Scotland, with 408 persons reported to have symptoms, 256 confirmed cases and 16 deaths. The vehicle was traced to

cooked beef and gravy from a specific butcher shop (WHO, 1997). Doyle *et al.,* (2006), also reviewed that most outbreaks occurred in developed countries; in particular, the United States, the United Kingdom and Canada. In the United States, *E. coli* O157:H7 causes≈73,000 infections and 60 deaths annually.

The largest *E. coli* O157:H7 outbreak took place in Sakai City of Japan in 1996 in which the radish sprouts in school lunches were contaminated, causing a total of 9451 cases with 12 deaths (Michino, 1996).

2.3.2 Nigeriansituationof *E. coli* infection and contamination

Diarrhoea caused by bacterial pathogens especially *E. coli* remains one of the major causes of morbidity and mortality in infants and young children in Nigeria(Akinyemi *et al.,* 1998; Okeke *et al.,* 2000). According to Nweze(2009) in his study done in Enugu and Onitsha, Southeast Nigeria, Out of the 920 stool samples investigated, 228 (25.10%) yielded bacterial isolates of which *E. coli* had the highest incidence of 44.74%. Ifeanyi *et al*., (2010) stated that, in FCT, Nigeria, on enteric bacteria pathogens associated with diarrhoea of children was as high as 65-80%.A study by Ademokoya*et al.,* (2014)has showed that 5.9% of diarrhoeic cases in health centers of Ondo State, were caused by *E. coli* O157:H7.

A study at the metropolitan abattoirs and slaughter slabs of selected poultry farms in Lagos and Ibadan, Nigeria, investigated the contamination of meat from cattle and chicken slaughtered for human consumption with*E. coli* O157:H7. Out of the 800 meat samples collected, *E. coli* had the prevalence of 17.1%. (Olatoye *et al*., 2012). According to a study done by Enabulele and Uraih (2009) at market in Benin city, Nigeria overall prevalence rates of *E. coli* isolated from fresh meat samples from the abattoir and traditional open market, showed *E. coli* O157:H7 were

85.65% and 4.63% respectively, with the fresh meat samples from abattoir and traditional open market each, recording 100% *E. coli* prevalence. Agbeyegbe and Uraih (1982) observed high prevalence rate of *E. coli* in raw meat samples, the resultis also in agreement with the work of Hussein and Bollinger(2005a), who reviewed worldwide reports recorded *E. coli* O157:H7prevalence rates in therange of 0.2 to 27.8% for beef cattleat point of slaughter.

2.4 Meat processing and storage

2.4.1 Reservoirs of *Escherichia* spp.

Cattle have been identified by various studies to be a major reservoir of *E. coli* O157:H7 (Faith *et al.*, 1996; Chapman *et al.*, 1997; Hancock *et al*., 1997; Rice *et al*., 1997). The bacterium is found naturally in the intestines of animals like Cattle, pigs, sheep, goats and deer (Govindarajan, 1990; WHO, 2004; Yousuf *et al*., 2008).

Contaminated meat was found to be a major vehicle for *E. coli* transmission from animals to humans (Paton and Paton, 1998). 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*., 2007a), pigs,chickens and turkeys (Doane *et al*., 2007), horses and dogs (Hancock *et al.*, 1998). Other wild animals demonstrated to be reservoirs*E. coli* O157:H7 include; wild deer (Heuvelink *et al*., 2002), rabbits (Scaife *et al*., 2006), raccoons and Pigeons (Shere *et al.*, 1998), opossums (Renter *et al*., 2003), rats (Cizek *et al*., 1999), gulls (Wallace *et*

al., 1997), rooks (Ejidokun *et al*., 2006), and starlings are among the numerous bird species that have been found positive for *E. coli* O157 Nielsen *et al*., (2004).

2.4.2 Meat processing

E. coli is frequently isolated from meat due to the transfer of faecal material during the slaughter process or via transfer from contact surfaces, consequently *E. coli* wasencountered on currency notes derived from meat outlets where transfer to currency notes was due to improper meat handling(Mir-Hassan*et al*., 2013), this route also accounted for the high prevalence of enteric bacteria recovered from banknotes sampled from poultry meat shops, the findings further suggests that meat contamination could also be introduced outside the retail environment, currency notes from confectionary, meat shops, supermarkets or food service outlets had high carriage of *E. coli* (Mir-Hassan*et al*., 2013).

Animal products such as meat, fish and their products are generally regarded as high risk commodity in respect of pathogen contents, natural toxins and other possible contaminants and adulterants (Yousuf *et al*., 2008). Meat produced in an unhygienic condition could pose threat to the health of the consumers as well as impair the keeping quality of such meat. Contamination of meat can result from contaminated working surfaces, equipment and the worker's hands used in the processing of the meat (Lues et *al*., 2007). According to them, the quality of water used in meat processing at the butcher shops also play a major role in reducing or increasing meat contamination, as water is used in washing the working surfaces, carcasses, blood of meat, equipment and worker's hands. Bacterial contamination of meat products is unavoidable one of the consequence of poor meat processing (Jones *et al*., 2008). Without proper hygienic control,

the butcher's environment can act as important sources of bacterial contamination (Gill *et al.*, 1998).

According to the study carried out by Endale and Hailay (2013), 91.7% of the butchers handled money while processing meat, this finding agrees with (Muinde and Kuria, 2005) who observed that meat handlers are probable sources of contamination and all possible measures should be taken to reduce or eliminate such contamination.

Microbial load of 32% for *E. coli* were obtained from the butchers table atMekelle city, Ethiopia, in a study conducted by Endale and Hailay (2013), it showed the indication of the ineffectiveness of the method used in cleaning the tables, which were usually washed with water only and the presence of bacterial pathogens on meat contact surfaces contributed to the contamination of meat.

Enabulele and Uraih, (2009) from their study in Nigeriaalso observed, bacterial contaminants from workers, in foods, water and environmental samples,*E. coli* was the predominant isolate (32%) followed by *Staphylococcus species (28%)* and this is in close agreement to previous studies by (Okonko *et al*., 2009; Ukut *et al*., 2010) where they isolated almost similar organisms from meat, sea-foods and other ready to eat food stuffs. Higher rates of contamination of meat with these organisms was an indication of deplorable state of poor hygienic and sanitary practices employed right from the slaughtering, processing and transportation to butcher shops(Okonko *et al*., 2009; Ukut *et al*., 2010). Lawan*et al*., (2013) reported in their study that the use of non-portable water by the butchers in Kano, Sokoto, Kaduna Abattoirs might have contributed to carcass contamination as previously reported by Bello *et al*., (2011) who observed increase in *E. coli* counts and isolation of *E. coli* O157:H7 from beef carcasses in some Nigerian

abattoirs due to the use of non-portable water. Meat contaminated with microorganisms is the cause of many food-borne diseases (WHO, 1997). The source of these pathogenic microorganism may be the animals themselves or the surroundings where these animals are kept as well as the method used in processing after slaughtering, can also result in contamination with microorganisms (Adeyemo, 2002).

The isolation of bacterial species from meat may be due to improper/unhygienic handling and processing of the meat which was observed in a study by Obeng *et al.*, (2013)in Tolon and Kumbungu Districts of Ghana, that meat was normally transported to the markets in unhygienic meat vans, taxis, motor cycles, motor kings, and sometimes on bicycles. They, further observed that it was a common practice to see people carrying carcasses on their bare shoulders, this agreeswith the findings of Warris(2010), that *E. coli* presence in some outlets was an indication of faecal contamination of the meat, which wasalso due to unhygienic handling of meat right from slaughtering,processing, handling and transportation.

2.4.3Meat storage

According to Frazier (1976) meat preservation involves the application of measures to delay or prevent certain changes which makes beef unwholesome as food. These changes could arise from microbial decomposition, enzymatic/nonenzymatic reactions and damage from physical abrasions. Meat, an excellent source of protein in human diet is highly susceptible to microbial contaminations, which can cause its spoilage and food borne infections in human, resulting in economic and health losses (Komba *et al.*, 2012). In another study by Gill *et al*., (2000) they observed that meat, available at retail outlets came through a long chain of slaughtering and transportation, where each step posed the risk of microbial contamination, sanitary conditions of

abattoirs and its surrounding environment were major factors contributing in bacterial contamination of meat, contaminations was compounded during transportation, storage and handling of meat at butcher shops(Ahmad *et al*., 2013).

In a study by Lawan*et al*., (2013) on the evaluation of physical facilities of abattoirs, The cold rooms in Sokoto, Zango and Kaduna abattoirs were nonfunctional except Kano abattoir where the cold room was functional, they further observed that there was no importance attached to cold room in these abattoirs for the reason that animals were slaughtered, processed into carcasses and marketed to consumers the same day and therefore, no meat is stored in the abattoirs except in Kano abattoir where most of the meat in the cold room were those not sold in the market and thus returned to be preserved for sale the next day, which seriously affected the quality and shelf life of meat products.

2.5 Occurrence of *Escherichia coli* on currency notes

Money on which pathogenic microorganisms might survive is often overlooked as reservoir for enteric disease (Michael, 2002). The possibility that currency notes might act as environmental vehicles for the transmission of potential pathogenic microorganisms was suggested in 1972by Abrams and Waterman.

The environment plays important role in transmission of microbial agents to humans, with many environmental materials serving as vehicles (Anderson, 1991). Contamination of different objects by potential pathogenic microorganisms is of public health importance as contaminated materials can be possible sources of transmission of such pathogens as *E. coli*. Bacteria are

particularly very ubiquitous and their ability to contaminate objects such as the naira notes is very prevalent when compared to parasites (Jalaoso, 1991). Ordinarily, the exposure of naira notes to the atmosphere could even bring about contamination depending on the environment in question (Ameh and Balogun, 1997).

Paper currency is commonly handled by various categories of people during transaction (Oyero and Emikpe, 2007). Currency notes, can be contaminated by droplets during coughing, sneezing, touching with previously contaminated hands or other materials and placement on dirty surface. Paper currency is widely exchanged for goods and services in countries worldwide. It is used for every type of commerce, from buying foodstuffs at the market to paying for taxi fare; these currency notes provide large surface area as breeding ground for pathogens (Podhajny, 2004). Paper money is passed from hand to hand, this act makes it likely to be contaminated with disease-causing microorganisms, especially if handled with unclean hands, or kept in dirty surroundings, therefore this presents a particular risk to public health, since communicable diseases can spread through contact with fomites (Michaels, 2002; Pope *et al*., 2002; Michaels *et al*., 2003; Charnock, 2005; Xu *et al*., 2005).

Studies in different parts of the world have reported high rates of microbial contamination of currency notes in circulations; Khin *et al.*, (1989) revealed that high levels of enteric pathogens were isolated from paper money samples obtained from butchers and fish mongers at local markets in Rangoon, Myanmar; Goktas and Oktay (1992) from butchers in turkey; Pope *et al.*, (2002) from fomites in Ohio, USA;Siddique (2003) in Manila, Philippine; Basavarajappa *et al*., (2005) in India; El – Dars and Hassan, (2005) in Egypt; Xu *et al*., (2005) in China; Blackenship, (2007) in Virginia, USA.

Some surveys done in Nigeria on the pathogens, carried via currency have also illustrated that *E.coli*, *Salmonella* and *S. aureus*, amongst others, are highly prevalent on notes and coins handled at food retail outlets (Igumbor *et al*., 2007; Uneke and Ogbu, 2007).In another study conducted by Umeh *et al.*, (2007), in Nigeria reported that 89% of the paper currency notes (naira) tested were contaminated with a variety of microorganisms, some of which were potentially pathogenic, this was also in accordance with studies done by Okeke *et al*., (2000) in Osunwhich showed aprevalence of 56.8%, Olorunshola *et al.,* (2000) in Lagoswith a prevalence of6%,Yakubu *et al.,* (2014), in Benin, Nigeria had 28.5% prevalence.

2.5.1 Handling of currency notes at meat markets

Currency note has a contact surface area whereby pathogens can be transferred within a population (Igumbor *et al*., 2007; Bhat *et al.,* 2010). Given that currency is frequently handled and transferred over large geographical areas there is the potential to readily disseminate contamination across the globe (Pop *et al*., 2002; Igumbor *et al*., 2007; Bhat *et al.,* 2010).

With respect to risk factors, it can be considered that contamination carried on currency would be most significant in food retail outlets whereby pathogens can be transferred to high risk products such as meat that can support growth of introduced microbes to hazardous levels (Jiang and Doyle, 1999; Lamichhane, 2009; Feglo and Nkansah, 2010; Matur *et al*., 2010)

Several studies from United States reported contamination of coin and paper bills and identification revealed presence of pathogenic or fecal indicator microbes that included*E.coli,S. aureus*, *Klebsiella* and *Enterobacter* (Oyero and Emikpe, 2007; Ahmed *et al*., 2013). According to the results of the study done byEndale and Hailay, (2013), most of the butcher shop workers

handle money while processing the meat. Since the money is being contaminated withmicrobes, it can also contaminate the food. Handling of foods with bare hands may also result in cross contamination, hence introduction of microbes on safe food. Because meat handlers are probable sources of contamination for microorganisms, it is important that all possible measures be taken to reduce or eliminate such contamination (Muinde and Kuria, 2005).

2.5.2 Prevalence of *E. coli* on currency notes

A study by Mir-Hassan *et al.,* (2013) showed a prevalence of *E. coli* on Iranian currency notes to be 48.14%. Goktas and Oktay in Turkey(1992) 17.%, Feglo and Nkansah in Ghana (2010) 1.79%, Md. Shakir *et al*., (2010) in Dhaka city of Bangladesh 58%, Umeh *et al*., 2007 in Nigeria 80%, Neel (2012) in Tanga, Tanzania 51.71%.In Osun, Nigeria, which showed aprevalence of 56.8%, Olorunshola *et al*., (2000) in Lagos with a prevalence of6%,Yakubu*et al.,* (2014), in BeninCity, Nigeria had 28.5% prevalence.

2.6Pathogenesis and consequences of *E. coli* O157:H7 infection

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 is 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 mellitusresulting from involvement of the brain, myocardium and pancreas, respectively (Coia, 1998).

2.6.1 Transmission of *E. coli* O157:H7infection

The forms of transmission are animal-to person, waterborne and person to person (Armstrong *et al.*, 1996; Coia, 1998).Human disease caused by *E. coli* O157:H7 can be linked directly or indirectly to cattle (Bhat *et al*., 2007). Numerous *E. coli* O157:H7 outbreaks resulted from contact with cattle or their manure have also been reported (CDC, 2005). *E. coli* O157:H7 survives in cattle feces for up to 18 weeks at15ºC (Fukushima *et al.,* 1999). Fecal

materialcontaminated with*E. coli* O157:H7may in turncontaminate meat during slaughter, animals could transport these bacteriafrom a fecal source to drinking water or foods. (Pennington, 2000).Person-to-person spread of *E. coli* O157:H7 has been the primary mode of infection in many outbreaks in day cares, schools and hospitals, particularly wherethere have been lapses in hygiene (Belongia *et al.*, 1993; Reida *et al*., 1994; Pennington, 2000). Foods can also be contaminated with *E. coli* O157:H7 by cross contamination during food preparation and by infected workers with poor hygiene (Jackson *et al.*, 1998).

2.6.2Clinical manifestation of*E. coli* O157:H7infection

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; haemorrhagic colitis (HC), HUS and TTP.

Haemorrhagic 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).

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 triad 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*, called EHEC, that was causally associated with the syndrome of haemorrhagic 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, fluctuating neuralgic signs, fever, and mild azotemia (Kwaan, 1987). Patients often develop blood clots in the brain and death. Other unusual clinical complications associated with *E. coli* O157:H7 infection includes haemorrhagic cystitis and banalities (Grandsen*et al.*, 1985), convulsions, sepsis with another organism, and anaemia (Rowe*et al.*, 1991).

2.6.3 Diagnosis of*E. coli* O157:H7infection

Isolation of the organism from meat and currency notes specimens involves first enrichment in a selective broth and then plating onto Sorbitol MacConkey agar (Griffin*et al.*,1995). Biochemical test using Microbact® 12E,rapid latex agglutination technique (Wellcolex), also enzyme linked immune-sorbent assay (ELISA) methods are used to isolate and identify suspect colonies (Johnson*et al.*, 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 and Brandt, 1995). Testing for verotoxin producing serotypes other than O157:H7 can also be done using toxin specific antibodies and genes using DNA probes (Willshaw*et al.*, 1993).

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).

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 *et al.* 1995) others use conventional gel electrophoresis (Samadpour, 1995).

2.7Clinical signs of *E. coli* O157:H7 infection

The disease caused by *E. coli* O157:H7 is hemorrhagic colitis and is characterized by abdominal pain and diarrhea, other symptoms may include vomiting and mild fever. The illness lasts an average of 8 days (USDA, 1997), Complications are HUS and TTP. HUS primarily affects infants and young children and is characterized by renal failure and hemolytic anemia. TTP primarily affects the elderly, and is characterized by HUS plus two other symptoms, fever and neurologic symptoms (Su and Brandt, 1995). TTP has a mortality rate as high as 50%. (USFDA, 1992). In TTP, there is typically less kidney damage than in children, but neurologic signs including stroke, seizures and central nervous system (CNS) deterioration are more common.

Death occurs most often in cases with serious extrarenal disease such as severe CNS signs. Approximately 65–85% of children recover from HUS without permanent damage; however, long-term renal complications including hypertension, renal insufficiency and end-stage renal failure also occur. (http://www.cfsph.iastate.edu/Factsheets/pdfs 2009).

2.8 Treatment of *E. coli* O157:H7 infection

Treatment for *E. coli* O157:H7 infection is primarily supportive, including management of dehydration and complications such as anemia and renal failure (Su and Brandt, 1995). Antimotility agents should not be given and antibiotic treatment does not appear to diminish the severity of illness or prevent the development of HUS (Thom, 1994;Griffin, 1995;Su and Brandt, 1995). Antibiotic treatment will result in elimination of competing bowel flora by the antibioticgiving a competitive advantage to *E. coli* O157:H7, and also lysis of *E. coli* O157 leading toincreased release of verotoxin (Su and Brandt, 1995).

2.9Prevention and control of *E. coli* infection

To reduce the risk of an *E. coli* infection, the following preventive measures are recommended by the Centers for Disease Control and Prevention(CDC, 2004).

i. Education and public awareness programs to improve meat hygiene, are important measures in sensitizing the general public and people at risk.
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 color of the meat is not a reliable indicator.
iii. Wash hands thoroughly after using the toilet, handling currency, meat or livestock.

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

v. If ill with diarrhoea, do not prepare or handle food that others will be eating.

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

2.9.1Resistance of*E.coli*to antibiotics

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), this is as a result of extensive use of antibiotics in both human medicine and animal agriculture which is suspected to have led to a widespread dissemination of antibiotic resistant genes (Callaway *et al.,* 2003).

Smith *et al*. (2003; 2009) reported multidrug resistance in isolates of *E. coli* O157 strains obtained from fresh faecal droppings of farm-animals (cattle, pig, chicken and sheep) and human stool comprising of diarrhoeic (150) and non diarrhoeic (50), in Lagos and Ogun State in Nigeria. Carl *etal*., (2002) reported that 361 *E. coli* O157 isolates recovered from samples collected from humans, cattle and swine, kept at the *E. coli* Reference Centre at the Pennsylvania State University were resistant to sulphamethoxazole (26%), tetracycline (27%), cephalothin (17%) and ampicilin (13%). A study done by Walsh *et al*.(2006),on antibiotic resistance in Irish isolates of Verotoxigenic *E. coli* showed that all the *E. coli* O157 isolated were resistant to at least one antibiotic especially ampicillin but were all susceptible to gentamycin, ciprofloxacin and Trimethoprim.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Study area

3.1.1 Location

Karu is one of the satellite towns in Abuja Municipal Area Council (AMAC) of the Federal Capital Territory, Nigeria. It is located about 7km north east of the Federal Capital City (FCC), off the Abuja–Keffi express way. It liesbetween latitudes 8° 59’ 38.6”N and 9° 01’39.6”N and longitudes 7° 33’ 17.19”E and 7°34’49.61”E. Karu has an area of about 275 squarekilometers. It

is bordered to the north byNyanya, to the south by Jikwoyi, to the west by Kugbo and to the east by Mararaba (Karu LGA, in Nasarawa State). Karu abattoir, which is the study area, is located close to a residential area (Balogun, 2001).

3.1.2 Climate

It experiences three weather conditions annually. These include a warm, humid rainy season and a blistering dry season. In between the two, there is a brief interlude of harmattan occasioned by the northeast trade wind, with the main feature of dust haze and dryness.

The rainy season begins from April and ends in October, daytime temperatures reach 28 °C (82.4 °F) to 30 °C (86.0 °F) and night time is between 22 °C (71.6 °F) to 23 °C (73.4 °F). In the dry season, daytime temperatures can soar as high as 40 °C (104.0 °F) and night time temperatures can fall to 12 °C (53.6 °F). Even the chilliest nights can be followed by daytime temperatures well above 30 °C (86.0 °F). The high altitudes and undulating terrain of Karu act as a moderating influence on the weather of the territory("Abuja Nigeria Tourist Information". Touristlink.com. 2013).



Figure 3.1 Map of Abuja showing location of Karu http://mapsof.net/map/fct-map

3.2 Study Design

3.2.1 Sample Size Determination

Sample size for *E.coli* was determined using the formula by Thrushfield (1997) at 95% confidence level.

$$N = \frac{Z^2pq}{d^2}$$

Where

N=sample size

q=1-p

p=prevalence (9% for *E.coli*, Matur*et al*.**,** 2010)

d=level of significance (0.05)

.

. . $N=\frac{1.96^2 \times 0.09 \times (1-0.09)}{(0.05)^2}$

$=3.8416 \times 0.09 \times 0.91$

$= \frac{0.314627}{0.0025}$

$=125.85 \approx 126$ samples

However, a total of 189 currency samples were collected from the butchers at the meat market in Karu abattoir,Abuja, Nigeria.

3.2.2Materials required for the study

3.2.2.1Bacteriological Culture Media

The following bacteriological media were used; Modified Tryptone Soya Broth (OXOID), Cefexime-Tellurite(OXOID), Sorbitol MacConkey agar (OXOID), Nutrient agar (OXOID), Mueller-Hinton agar (OXOID), Buffered Peptone water (OXOID), Eosine Methylene Blue (OXOID), Plate Count agar(OXOID), *E. coli* Biochemical Test Kit (Microbact® 12E)and *E. coli* O157:H7 Rapid Latex Agglutination Test kit (Wellcolex).

3.2.2.2 Antibiotic impregnated discs

A total of twelve (12) antibiotics were used for this study in various concentrations. They include Trimethoprim (5µg), Amikacin (30µg), Nalidixic acid (30µg), Penicillin G (10 IU), Gentamicin (10µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Streptomycin (10µg), Oxacillin (1µg), Vancomycin (30µg), Amoxicillin (10µg) and Cefoxitin (30µg) (Oxoid, UK).

3.2.2.3 Equipment, apparatus and disposable items

Other materials used include Cotton wool, Swab sticks, Durham tubes, Test tubes, Test tube racks, Conical flasks of varying calibrations, Masking tape, Aluminum foil, Permanent marker, Refrigerator (Haier Thermocool), Incubator, Electronic weighing balance, Measuring cylinders, Autoclave, Hot air oven, Disposable gloves, Pipettes, Petri dishes, Sterile Sample bottles, Polythene bags, Bunsen burner, Pasteur loopand Distilled water, multiple disc dispenser.

3.2.2.4 Paper and polymer naira notes

189 Nigerian currency notes (all the 8 denominations) were collected from butchers at the meat market in Karu Abattoir, FCT. The currency notes studied for Paper naira noteswere;28 One hundred Naira Notes (N100), 20 Two hundred Naira Notes (N200), 21 Five hundred Naira Notes (N500) and 19 One thousand Naira Notes (N1, 000) and polymer naira noteswere;25 Five Naira

Notes (N5), 24 Ten Naira Notes (N10), 24 Twenty Naira Notes (N20), 28 Fifty Naira Notes (N50).

3.2.3Sampling techniqueof naira notes

Sampling technique adopted for this study was convenience sampling technique where a total of 189 naira notes were collected from butchers at the meat market in Karu abattoir, FCT. Abuja. On each day of sampling, based on informed consent, availability of currency and acceptance of the butchers to participate in the studyand were given an equivalent of the currency collected in exchange.

3.2.3.1 Sampling procedure and data collection

This study was conducted from September to December 2014 (three months) in which 189 Nigerian currency notes (all the 8 denominations) were collected from butchers at the meat market in Karu Abattoir, FCT. The currency notes studied were25 Five Naira Notes (N5), 24 Ten Naira Notes (N10), 24 Twenty Naira Notes (N20), 28 Fifty Naira Notes (N50), 28 One hundred Naira Notes (N100), 20 Two hundred Naira Notes (N200), 21 Five hundred Naira Notes (N500) and 19 One thousand Naira Notes (N1,000).

A total of 189 samples of the currency notes, were collected for a period of four weeks. With the aid of a pair of sterile forceps, each currency note were collected and transferred aseptically unto a sterile surface, swab stick dipped in peptone water was used to swab both surfaces of the currency notes. The swab stick was then placed in the sample bottle containing 20 ml of peptone water and the butcher was given a currency of equal value as a replacement. The sample bottle was capped, placed in a cooler with ice packs and then transported after collection to the Bacterial Zoonosis Laboratory in the Department of Public Health and Preventive Medicine,

A.B.U., Zaria for processing, (isolation, TVC, serotyping, and determinationof the antibiotic sensitivity pattern of *E.coli).*

3.2.3.2Isolation and identification of E.coli

The isolation of *E. coli* was conducted according to the procedure ofISO, (2001). The sample from the 1% buffered peptone water was incubated at 37^0C for 24 hours, a loop full was inoculated directly on Eosine Methylene Blue (EMB) agar, streaked using a sterile loop and incubated at 37^0C for 24 hours. Greenish metallic sheen colony growth suggestive of *E. coli* which was stored on nutrient slant and refrigerated for further biochemical and serological analysis.

3.2.3.3Total viable count

Serial dilutions were made from 1ml of the sample and 9ml of the normal saline solution, 2 drops were surface plated on plate count agar (PCA)for total viable count (TVC). Plates were incubated at 37^0C for 24 hours. The number of distinct colonies on each plate were enumerated using a colony counter, colony forming units (CFU) per ml or cm^2 of sample were calculated, using the dilution factor of each and converted to log10, CFU/cm or ml values. Mean values of (TVC)were determined and reported.

3.2.3.4Biochemical tests using MICROBACT® 12E identification system.

A24 hour pure culture of the organismgrowth suspected to be*E. coli* was obtained from nutrient agar slant.1 to 3 isolated colonies was selectedfrom the pure culture of the organism and emulsified in saline. A Test Strip or Microplate was placed in a holding tray and the seal was peeled back. 4 drops of the bacterial suspension was added to each well.2 drops of Mineral Oil

(MB1093A) was added to the black wells. The seal was replaced and incubated at 35°C ± 2ºC for 24 hours. It was then removed from the incubator and appropriate reagents were added. The results were recorded on the report forms and were interpreted using the Microbact®Identification Package.

3.2.3.5 Rapid latex agglutination test for E. coli O157:H7.

Latex agglutinations kitfor *E. coli* O157:H7 (Wellcolex)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 consist of a Red latex particles coated with antibodies specific for *E. coli* O157 and H7 test reagent consist of Blue latex particles coatedwith antibodies specific for *E. coli* H7 antigen. A drop of the reagent was mixed on a card with the suspension of *E. coli* isolate.

(a). Test procedure for O157 (somatic antigen)

E. coli isolates obtained from samples on nutrient agar slants were sub-cultured on CT-Smacand 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. 40µl of saline (0.85% NaCl w/v) was placed in two circles on the 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 into 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 other and then 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 indicating rapid agglutination occurred through the interaction of specific IgG and O157 lipopolysaccharide antigen.

(b). 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). Using a mixing stick, sufficient growth was removed to just cover the blunt end of the stick. 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, and then, a similar amount of the sample was emulsified. For each test sample, one drop of H7 test latex was placed in one circle and one drop of the H7 control latex in other, and then 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 indicating rapid agglutination occurred through the interaction of specific IgG and O157 lipopolysaccharide antigen.

3.2.3.6 Antimicrobial resistance test

A total of twelve (12) antibiotics were used for this study in various concentrations. They include trimethoprim (5µg), amikacin (30µg), nalidixic acid (30µg), penicillin G (10 IU), gentamicin (10µg), chloramphenicol (30µg), ciprofloxacin (5µg), streptomycin (10µg), oxacillin (1µg), vancomycin (30µg), amoxicillin (10µg) and cefoxitin (30µg) (Oxoid, UK). These antibiotic impregnated discs were all obtained from Oxoid, UK.

Mueller-Hinton agar plates were prepared by adding 38gof the powdered medium to 1 liter of distilled water, it was brought to the boil to dissolve the solute completely. It was then sterilised

by autoclaving at 121°C for 15 minutes, sterile swabs were dipped into the nutrient agar slants and used to streak the Mueller-Hinton agar in three directions at 180^0C until the entire surface was streaked. The plates were allowed to dry at room temperature and the antimicrobial discs were dispensed into the plates using the multiple disc dispensers (OXOID). Sterile forceps were further used to ensure sufficient contact with the medium. The petri dishes were then inverted and incubated at 37^0C for 24 hours. Clear zones of inhibition were measured to the nearest millimeter using a transparent ruler and interpreted based on Clinical Laboratory Standard Institute (CLSI, 2012) cut-off point recommendations. Isolates were classified as susceptible, intermediate and resistant.

3.2.4Data analysis

Data was analyzed using the statistical package for social sciences (SPSS) version 20. Frequency of occurrence for*E. coli* on currency notes was determined and is presented in tables, charts and graphs. Chi square and independent T-test were used, where necessary to test for relationship between the occurrence of *E. coli* and the currency denominations. Percentage of polymer to paper currency notes.

Prevalence was calculated using the formula

$$\text{Prevalence} = \frac{\text{Total no. of positive samples}}{\text{Total no. of samples}} \times 100$$

CHAPTER FOUR

RESULTS

4.1 Contamination of currency notes (naira) with *E. coli* collected from butchers at Karu abattoir, meat market. Abuja.

All the 189 (100%) naira notes sampled from butchers at the Karu abattoir, in Abuja, were contaminated; 168 (88.9%) had colonies that could be counted ≤ 300 while 21 (11.1%) of the samples collected produced colonies that were too numerous to count (TNTC) >300 (Figure 4.1).

The mean microbial load on the currency notes with countable colonies, obtained from the butchers at the Karu abattoir meat market, was 190.08±83.92 cfu/cm^2. Distribution of the microbial load fromthe currency notes showed that the currency denominations on which the TVC withthe highest load of microbial contamination, was the five Naira notes (219.67±67.01 cfu/cm^2), this was followed by microbial contamination on one hundred Naira notes

(212.64±77.66 cfu/cm^2). The notes that the least microbial contamination among all the currency denominations that were sampled was Fifty Naira notes (150.68±87.81 cfu/cm^2) (Table 4.1)

The result shows that paper currency had higher microbial load (190.97±83.58) than polymer currency notes (189.11±84.81). The relationship was however not statistically significant (t=-0.143, p=0.887) (Table 4.2).

A statistically significant difference was observed between the nature of microbial contamination and type of currency notes. All (100.0%) of the paper currency notes had bacterial growth that was countable and none of them had colonies TNTC while 79.2% of the polymer notes had bacterial colonies that were countable and 20.8% of the notes had colonies that were TNTC (Table 4.3).

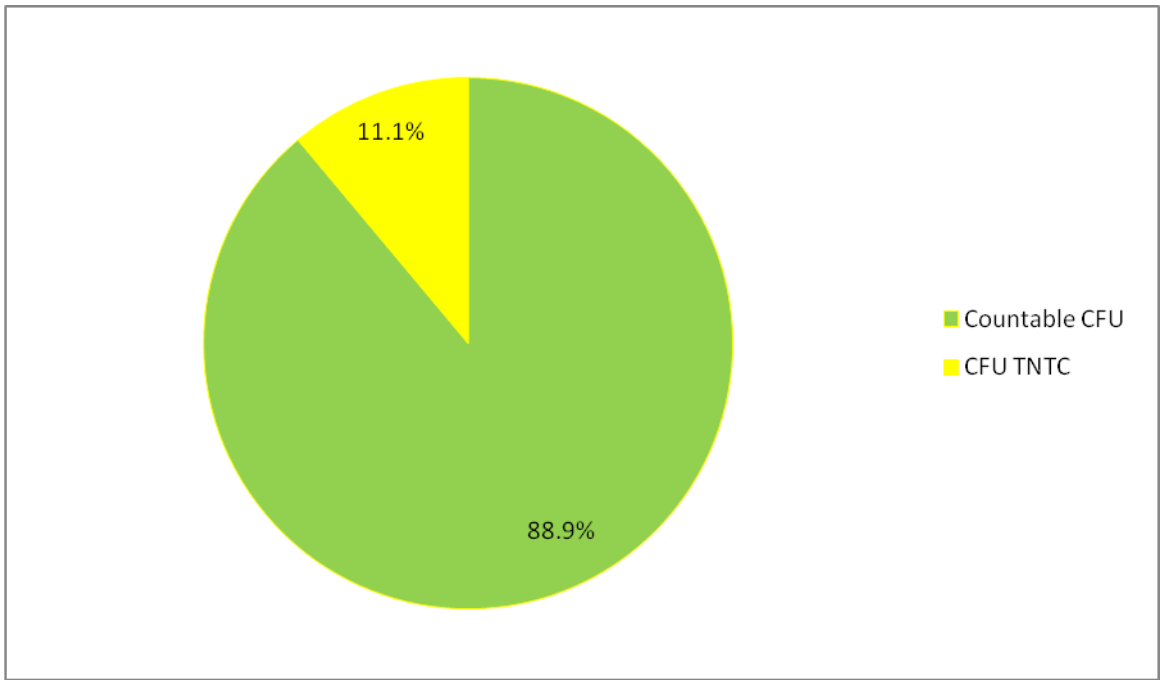


Figure 4.1 Level of microbial contamination among currencies sampled from butchers at the Karu abattoir,meat market Abuja.

4.1.1 Level of microbial contamination among currencies sampled from butchers at the Karu abattoir, meat market Abuja.

All the 189 (100%) naira notes sampled from butchers at the Karu abattoir, in Abuja, were contaminated; 168 (88.9%) had colonies that could be counted ≤ 300 while 21 (11.1%) of the samples collected produced colonies that were too numerous to count (TNTC) >300 (Figure 4.1)

Table 4.1 Mean microbial load from currency notes of the eight denominations sampled from butchers at Karu abattoir, meat market. Abuja.

Currency Denominations	No of samples collected	No. of samples with TVC	Mean (±SD)
N5	25	15	219.67±67.01
N10	25	25	189.28±79.44
N20	25	18	210.39±89.88
N50	26	22	150.68±87.81
N100	28	28	212.64±77.66

N200	21	21	187.48±85.73
N500	20	20	174.45±85.73
N1,000	19	19	180.26±91.19
Total	189	168	190.08±83.92

4.1.2 Mean microbial load from currency notes of the eight denominations sampled from butchers at Karu abattoir, meat market. Abuja.

The mean microbial load on the currency notes with countable colonies, obtained from the butchers at the Karu abattoir meat market, was 190.08±83.92 cfu/cm^2. Distribution of the microbial load from the currency notes showed that the currency denominations on which the TVC with the highest load of microbial contamination, was the five Naira notes (219.67±67.01

cfu/cm^2), this was followed by microbial contamination on one hundred Naira notes (212.64±77.66 cfu/cm^2). The notes that the least microbial contamination among all the currency denominations that were sampled was Fifty Naira notes (150.68±87.81 cfu/cm^2) (Table 4.1)

Table 4.2 Mean microbial load among currency types sampled from butchers at the Karu abattoir meat market. Abuja.

Currency type	No. of samples with TVC	Mean ±SD	Statistic	
Polymer	80	189.11±84.81	t=-0.143	p=0.887

Paper	88	190.97±83.58
Total	168	190.08±83.92

4.1.3 Mean microbial load among currency types sampled from butchers at the Karu abattoir meat market. Abuja.

The result from the table shows that paper currency had higher microbial load (190.97±83.58) than polymer currency notes (189.11±84.81). The relationship was however not statistically significant (t=-0.143, p=0.887) (Table 4.2).

Table 4.3 Relationship between level of microbial load and currency type obtained from butchers at Karu abattoir meat market. Abuja.

Currency type	Level of microbial load		Total
	TVC	TNTC	
Polymer currency	80 (79.2)	21 (20.8)	101 (53)
Paper currency	88 (100)	0 (0.0)	88 (47)
Total	168 (88.9)	21 (11.1)	189 (100)

χ^2=20.584 df=1 p=0.000

4.1.4 Relationship between level of microbial load and currency type obtained from butchers at Karu abattoir meat market. Abuja.

A statistically significant difference was observed from Table 4.3, between the nature of microbial contamination and type of currency notes. All (100.0%) of the paper currency notes had bacterial growth that was countable and none of them had colonies TNTC while 79.2% of the polymer notes had bacterial colonies that were countable and 20.8% of the notes had colonies that were TNTC (Table 4.3).

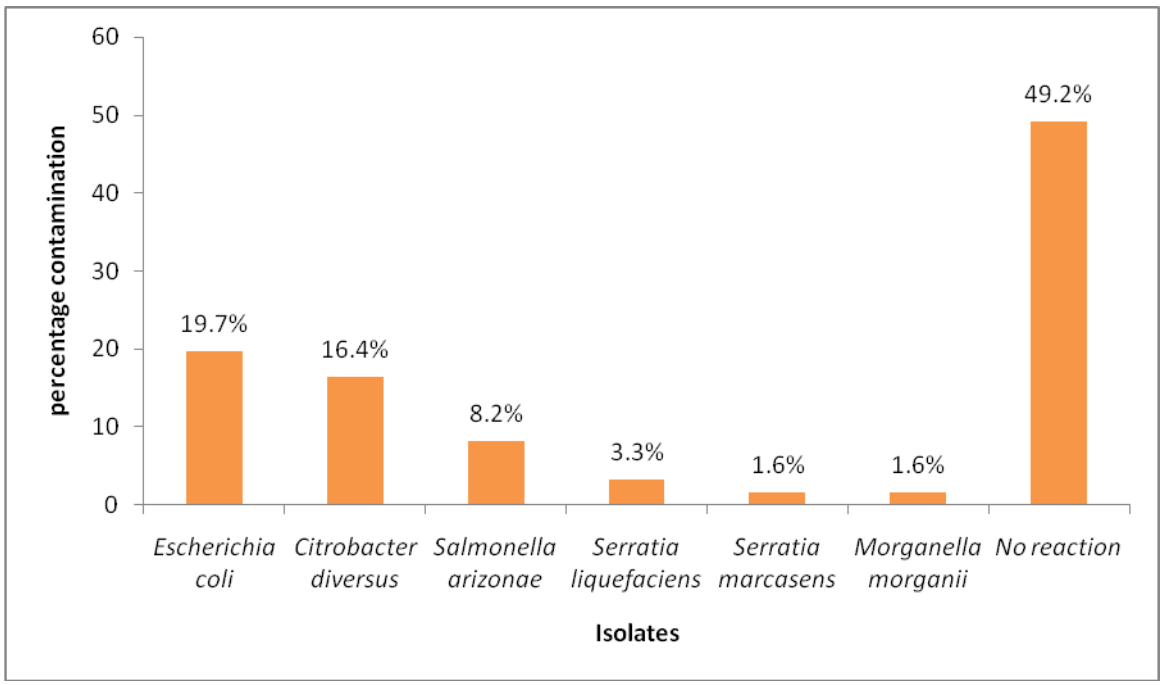


Figure 4.2 Percentage distribution of 61 isolates from 189 currency notes collected from Butchers at Karu abattoir meat market following confirmation with Microbact® 12E.

4.1.5 Occurrence of *E. coli* from currency notes obtained from butchers at Karu abattoir meat market. Abuja.

Among the 61 isolates that were suspected from Sorbitol-MacConkay agar, 12 (19.7%) were confirmed as *E. coli* using the Microbact® 12E identification system, while 10 (16.4%) were identified as *Citrobacter diversus,* 5 (8.2%) as *Salmonella arizonae,* 5 (8.2%) as *Serratia liquefaciens,* 2 (3.3%) as *Serratia marcasens,*1 (1.6%) as *Morganella. morganii* and 30 (49.2%) of the suspected isolates showed no reaction on the Microbact® 12E identification system (Figure 4.2).

Table 4.4 Relationship between the occurrence of *E. coli* and currency types collected from butchers at Karu Abattoir meat market, Abuja.

Sample category	Bacterial Isolate Category		Total
	E.coli	Other bacterial organisms	
Polymer currency	8 (40.0)	12 (60.0)	20 (64.5)
Paper currency	4 (36.4)	7 (63.6)	11 (35.5)
Total	12 (38.7)	19 (61.3)	31 (100.0)

χ^2=0.040 df=1 p=0.842

4.1.6 Relationship between the occurrence of *E. coli* and currency types collected from butchers at Karu Abattoir meat market, Abuja.

Statistical test done to determine the relationship between the type of currency and occurrence of *E. coli* shows that, of the 12 *E. coli* isolates, more of the polymer currency notes 8 (40.0%) were found to be contaminated with *E. coli* than the paper currency notes 4 (36.4%). The findings were however, not statistically significant as shown in Table 4.4.

Table 4.5 Serology using Rapid Latex Agglutination Test for the 12 *E. coli* isolates

S/NO	Currency Denomination	Isolates of *E.coli*	
		O157:	**H7**
1	**N10**	-	-
2	**N10**	+	+
3	**N10**	-	-
	N20	+	-
4	**N50**	-	-
5	**N100**	+	-
	N100	-	-
6	**N200**	+	-
7	**N500**	+	+
	N500	+	+
8	**N500**	+	+
9	**N1,000**	+	+
10			
11			
12			
	Total	**8**	**5**

4.1.7 Rapid latex agglutination test for *E. coli* isolatesobtained from currency notes from butchers at the Karu Abattoir meat market.

Using the Serological test; from the 12 *E. coli* isolates that were confirmed using the Microbact® 12E identification system; Serological test using Rapid Latex Agglutination Test for *E. coli* **(**Wellcolex *E. coli* O157:H7 kit) indicated that 5 of the *E. coli* isolates were confirmed to be Positive for *E. coli* O157:H7 (ten naira, five hundred, five hundred, five hundred and one thousand naira notes), 3 were positive for *E. coli* O157 (twenty, two hundred and one hundred naira notes) while 4 were Negative for *E. coli* O157:H7 (ten, ten, fifty and one hundred naira notes). According to currency denomination (Table 4.5).

Table 4.6 Percentage for Serology using Rapid Latex Agglutination Test for the 12 *E. coli* isolates

S/NO	*E. coli* Isolates	Confirmation	Percentage (%)
1	*E. coli* O157:H7	Positive	41.7
2	*E. coli* O157	Negative	25
3	*E. coli* O157:H7	Negative	33.3

4.1.8Percentage for Serology using Rapid Latex Agglutination Test for the 12 *E. coli* isolates.

Using the Serological test; from the 12 *E. coli* isolates that were confirmed using the Microbact® 12E identification system; Percentage forSerological test using Rapid Latex Agglutination Test for *E. coli* **(**Wellcolex *E. coli* O157:H7 kit) indicated that 41.7% of the *E. coli* isolates were confirmed to be Positive for *E. coli* O157:H7, 25% were positive for *E. coli*,while 33.3% were Negative for *E. coli* O157:H7 (Table 4.6).

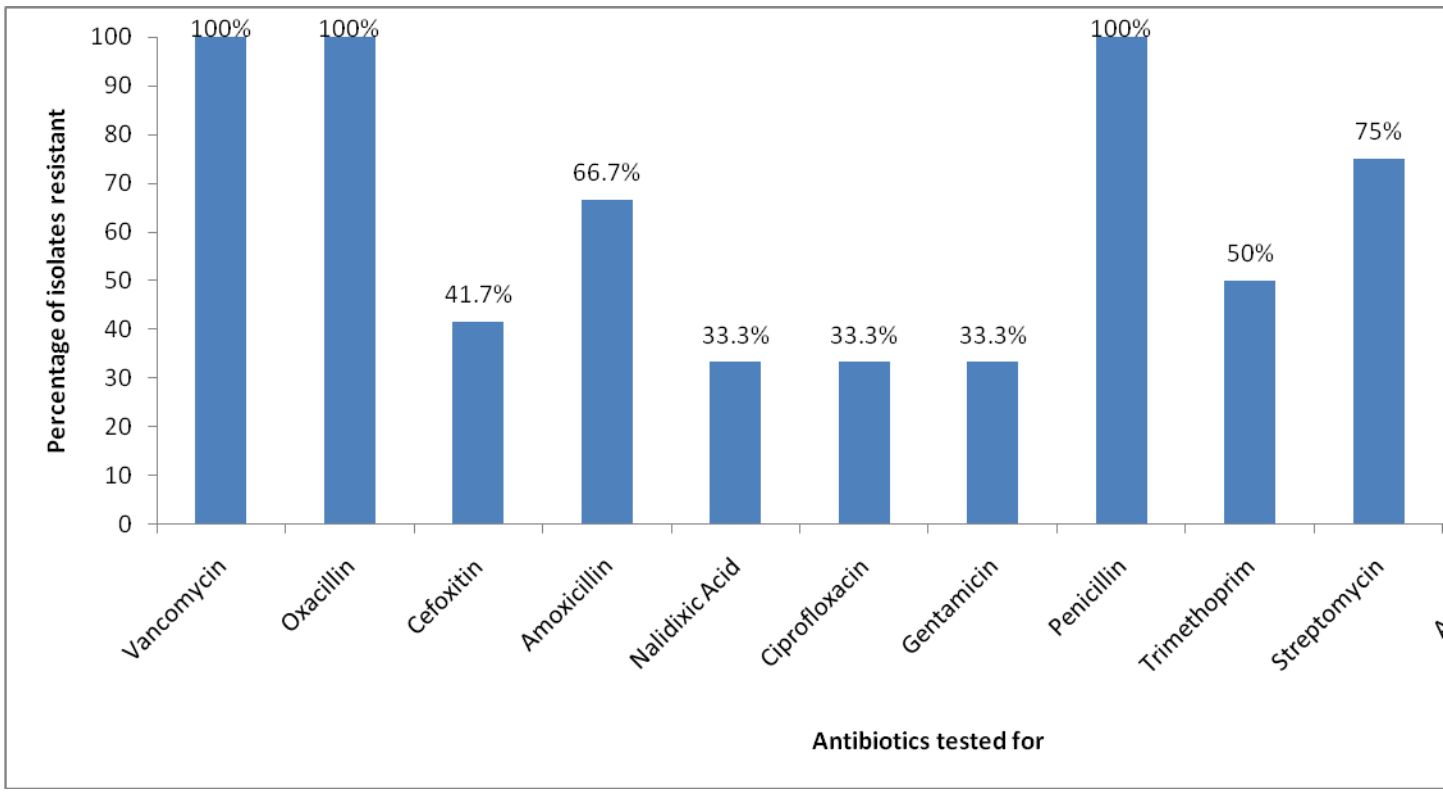


Figure 4.3. Percentage distribution of Antibiotic resistance of *E. coli* isolates on Nigerian currency notes at Karu Abattoir, Abuja.To tested antimicrobial agents.

4.1.9. Antimicrobial resistance of *E. coli* isolates from currency notes obtained from butchers at the Karu abattoir meat market, Abuja.

Among the 12 antimicrobial agents for which susceptibility and resistance was tested for by the disc diffusion technique, vancomycin, oxacillin and penicillin G were observed to be 100.0% ineffective against *E. coli* isolates obtained from this study. Streptomycin 75.0%, amoxicillin 66.7%, trimethoprim showed to be 50.0%, nalidixic acid, chloramphenicol and cefoxitin 41.7% respectively, ciprofloxacin, gentamicin and amikacin were observed to be the most effective against the *E. coli* isolates with only 33.3% of the isolate showing resistance to each of these antimicrobial agents (Figure 4.3).

CHAPTER FIVE

5.0 DISCUSSION

From this study, the data obtained showed that culturable *Escherichia coli* O157:H7 was isolated from currency notes obtained from butchers at the Karu Abattoir meat market, Abuja. All the 189 (100%) naira notes sampled from butchers at the Karu abattoir, in Abuja, were contaminated; 168 (88.9%) had colonies that could be counted ≤ 300 while 21 (11.1%) of the samples collected produced colonies that were too numerous to count (TNTC) >300. The reason could be due to the fact that these currency notes got into the hands of several people including butchers who may have contaminated the currency notes with blood that serves as a good medium for bacteria growth, other handlers of the Naira notes may have been traders, beggars and people who carry out other jobs that could contaminate hands and without cleaning or washing such hands, could result to cross contamination. The currency notes were also observed to be kept in clothes, pockets, wallets, purses and bags which are hardly washed, as a result, these pockets, wallets, purses and bags could also be the source for contamination of the currency notes.

The contamination of currency notes by coliforms shows that humans are the major source of currency contamination similar to a study by Ngwai *et al*., (2011) which showed that all newly printed currency notes obtained from commercial banks prior to their introduction into circulation and subsequent handling by the public revealed no bacterial growth when compared to currency notes collected from different groups of people. This is similar to a study by Abrams

and Waterman (1972), who stated that there is a possibility that currency notes might act as environmental vehicles (fomites) for the transmission of potential pathogenic microorganisms.

The isolation of the pathogen *E. coli* O157:H7 in Karu urban area, indicated that contaminated currency notes exchanged hands between butchers and buyers of meat which could also be due to improper processing and transporting from the abattoir which agrees with a similar study by Nkaga and Uriah, (1981) or cross contamination from the buyers with poor hygiene practice themselves as observed by Oyero(2007) or outside the retail environment (Mir-Hassan*et al*., 2013). This also agrees with various studies which identified cattle to be a major reservoir of *E. coli* O157:H7 (Faith *et al.*, 1996; Chapman *et al.*, 1997; Hancock *et al*., 1997; Rice *et al*., 1997). It could also be due to improper washing the utensils and tables as observed by Gill *et al*., 1998; Jones *et al*., 2008; Endale and Hailay, 2013. Human diseases caused by *E. coli* O157:H7 can be linked directly or indirectly to cattle (Bhat *et al*., 2007). This shows further that meat hygiene and currency notes handling practices remain a great challenge.

The contamination was found more in lower currency denomination (5 Naira-200 Naira notes) than in higher currency denomination (500-1000 Naira notes) as shown by the total viable counts. This can be attributed to the fact that the lower denomination currency notes are frequently more used in daily, petty transactions than the higher currency (500-1000 Naira notes). This agrees with the findings in in the studies conducted in Idah of Kogi State, Federal Capital Territory and Benin City all in Nigeria (Mailafia *et al*., 2011; Ukwuru and Gabriel, 2012; and Yakubu *et al*., 2014).

From the data analysis, T-test showed that there was no statistically significant association between the nature of the currency and microbial contamination as paper currency notes had

higher contamination rate when compared to the polymer currency notes as seen in the amount of total viable count which is 190.97±83.58 and 189.11±84.81 for paperand polymer currency notes respectively, however,this was contrary to a study conducted by Gedik *et al*., (2013) whoobserved that the structure of polymer notes allows growth and transmission of pathogenscompared with that of paper currency notes.

More microorganism were obtained on polymer note (53%) than their counterpart, paper notes (47%) currency. Polymer notes form the smaller denomination of Nigerian currency and are more in circulation than their paper counterpart which has higher denominations handled by business people in the higher class who are probably cleaner, this was in agreement to a study by Mailafia*et al*., (2011).

Vancomycin, oxacillin and penicillin G were observed to be 100.0% susceptible to the*E. coli*isolates obtained from this study, which is in agreement with a study by Jane-Francis *et al*., (2014).Ciprofloxacin, gentamicin and amikacin were observed to be the most resistant against the *E. coli* isolates with only 33.3% of the isolate showing resistance to each of these antimicrobial agents, this was however contrary to a study by Akshay *et al*., (2011), which showed resistance only to ampicilin with ciprofloxacin, gentamicin and amikacinobserved to be susceptible.

The antibiotic resistance indicates the misuse or overuse of antimicrobial 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 antimicrobial resistance among bacterial pathogen and endogenous micro flora as observed by WHO (1997), 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 of bacterial infections (Karlowsky *et al*., 2003; WHO, 1997).

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.0Conclusion

The results of this study showed that Nigerian currency notes (Naira) collected from butchers at the meat market, in Karu abattoir, were contaminated by a potentially pathogenic enteroheamorrhagic *Escherichia coli* O157:H7, with a prevalence of 2.6%, have public health implications, because *E. coli* O157:H7 has low infective dose of < 100 cells.

The occurrence of *E. coli* O157:H7 on naira notes collected from butchers at the meat market, should make the public to be mindful that microorganisms such as *E. coli* O157:H7 could be found on currency notes acting as fomites.

The result of the study also indicated that the *E. coli* O157:H7 were 100% susceptible to antimicrobials; vancomycin, oxacillin and penicillin G, while ciprofloxacin, gentamicin and amikacin were observed to be resistant against the *E. coli* isolates with only 33.3%.

6.1Recommendation

Based on the findings of this study, the following recommendations were made:

i. There is need to educate the public through awareness campaign to sensitize butchers, meat handlers and buyers on the need for proper hygienic practices (such as proper washing of hands) before and after handling currency notes.

ii. The butchers should also be educated on the need for proper washing of butcher's utensils and tables with detergents to prevent the transmission of pathogens from fomites to humans.

iii. In order to have accurate judgment about the possibility and range of transmission of *E. coli* 0157:H7. It is recommended that further studies be conducted to determine the prevalence of *E. coli* O157:H7 in other Meat markets of different locations.

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APPENDIX 1

1.0 Preparation of Media and Reagents

The following media were used

1.1 Sorbitol MacConkey agar (SMA)

Composition in gram per litre

20.0g Peptone

10.0g Sorbitol

5.0g NaCl

1.5g Bile salts

300mg Neutral red

1.0g Crystal violet

15.0g Agar

1,000ml Distilled water

Preparation:

51.5g of Sorbitol Mac conkey Agar powder was weighed and transferred into 1L of distilled water contained in a sterile conical flask. The neck ofthe conicalflask was covered with aluminum foil. It was then mixed by shaking and heated to boiling so as to dissolve completely, autoclaved at 121^0C for 15minutes. The medium was supplemented with (0.2 g/litre) 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, 15minutes) 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 and was preserved in the fridge for further use. Prior to incubation, the surface of the agar was dried by partial exposure at 37°C. The appearance of the plate was clear pink/red. The media was preserved in the fridge.

1.2 Nutrient Agar (NA)

Composition in grams/litre

1.0g Lab. Lemco powder

2.0g Yeast extract

15.0g Sodium chloride

5.0g Agar

5.0g Peptone

7.4 pH

1,000ml Distilled water

Preparation:

23g of nutrient agar powder was transferred into 1L of sterile peptone contained in the conical flask. Cover the neck of flask with aluminum foil. It was then mixed by shaking and brought to boil so as to dissolve completely and aseptically dispensed into bijou bottle autoclaved at 121°Cfor 15minutes. The medium was allowed to solidify on slant position was preserved in the fridge for use thereafter.

1.3Eosine Methylene Blue (E.M.B)

Composition in grams per litre

1.0g Lactose

5.0g Bile salts

5.0g Sodium chloride

0.075g Neutral red

12.0g Agar

1,000ml Distilled water

7.6 pH

Preparation:

28g of Eosine Methylene Blue agar powder was weighed and transferred into 1L of distilled water contained in a sterile conical flask. Cover neck of flask with Aluminum foil. It was then mixed by shaking and brought to boil so as to dissolve and then autoclaved at 121°Cfor 15minutes. 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 37°C. The appearance of the plate clear pink/red. The media was preserved in the fridge.

1.4Buffered Peptone Water (PW)

This medium was used to transport, 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 litre

10.0g Peptone

5.0g Sodium Chloride

1,000ml Distilled Water

7.6 pH

Preparation:

The powdered medium was used and it was prepared as directed by the manufacturer. 20g of the powdered medium (Oxoid) was dissolved in 1,000ml of distilled water medium was sterilized by autoclaving at 121°C for 15minutes.

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

Composition in grams per litre:

17.0g Casein

5.0g NaCl2

3.0g Soybean powder

2.5g K2HPO4

2.5g Glucose

1.5g Casmino acid

10mg Acriflavine

1.5g Bile salt

1000ml Distilled water

Preparation:

The powdered medium was used and it was prepared as directed by the manufacturer. 0.5g of the powdered medium (Oxoid) was dissolved in 1000ml of distilled water medium was sterilized by autoclaving at 121°C for 15minutes.

1.6 Plate Count Agar (PCA)

Composition in grams per litre:

5g Tryptone

2.5g Yeast extract

1.0 Glucose

9.0 Agar

7.0 pH

Preparation:

17.5g was addedto 1 litre of distilled water. The solution was dissolved by bringing to the boil with frequent stirring, mixing and pouring into final containers. Sterilize by autoclaving at 121°C for 15 minutes.

1.7 Sorbitol MacConkey Agar (CT-Smac)

Composition in grams per litre:

20g Peptone

10g Sorbitol

1.5g Bile salts No 3

5.0g Sodium chloride

0.03g Neutral red

0.001g Crystal violet

15g Agar

7.1 pH

1.8 Cefexime-Tellurite Supplement

Vial contents

2.5mg Potassium Tellurite

0.05mg Cefexime

Preparation:

51.5g Suspended in 1 litre of distilled water. Brought to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Allow to cool to 50°C. Pour into sterile Petri dishes.

If required, reconstitute one vial of C-T supplement per 500ml medium, according to instruction in the product leaflet. Aseptically add this to cooled medium before pouring into sterile Petri dishes

1.9Mueller-Hinton agar

Composition in grams per litre:

300g Beef, dehydrated infusion

17.5g Casein hydroysate

1.5g Starch

17g Agar

7.3 pH

Preparation:

38g was added to 1 litre of distilled water. Brought to the boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes.