EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING *Escherichia coli* IN READY–TO-EAT BUSH MEAT AND FRESH PRODUCE IN LAGOS AND OGUN STATE.

BY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED SCIENCES, MOUNTAIN TOP UNIVERSITY, MAKOGI, IBAFO, OGUN STATE, NIGERIA.

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SEPTEMBER, 2022.

DECLARATION

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

__________________________ __________________________

AKINYEMI, O. Oluwaseyi Date

CERTIFICATION

This is to certify that this project titled: **'EXTENDED SPECTRUM BETALACTAMASE *Escherichia coli* IN READY-TO- EAT BUSH MEAT AND FRESH PRODUCE IN LAGOS AND OGUN STATE.** " was carried out by, **AKINYEMI, Oluwaseyi Oluwayemisi** with matriculation number **180101010027.** This project meets the requirements governing the award of Bachelor of Science (B.Sc.) Degree in Microbiology department of biological sciences of Mountain Top University, Ogun State, Nigeria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this project to God Almighty and my wonderful family for their support and love towards me

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I Appreciate the Almighty God for strengthening me, for directing my path, for his continuous guidance, and for giving me the grace to complete this project.

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// ABSTRACT

Extended spectrum β-lactamases (ESBL) provides resistance to a broad-spectrum of cephalosporins; it is usually found in transmissible plasmids which encode for ESBL in the host organism. The β-lactamases are usually susceptible to inhibitors such as clavulanic acid and sulbactam, which may assist with identification of these strains in the laboratory. Studies on pathogens in ready-to-eat (RTE) bush meat and fresh fruit have already been conducted with the potential of antibiotic resistance factor. The aim of this study was to detect ESBL(s) produced by *Escherichia coli* isolated from bushmeat and fresh produce in Nigeria. A total of sixty one (61) samples comprising of 55 bush meat and 6 fresh produce samples were collected from different South-western states (Osun, Ogun, and Ondo and Lagos states) in Nigeria. Isolation of pathogenic *Escherichia coli* was carried out on SMAC (Sorbitol MacConkey agar) and MAC (MacConkey agar). The double disc synergy test was performed to detect ESBL production in the *E. coli* isolates. Phenotypically 6 out of 11 isolates were confirmed as ESBL-producing *E.coli*. The strains MS1U1, MD1U2, MK1P2 tested positive for *bla*SHV, *bla*CTX-M, *bla*TEM genes using PCR. This study shows the presence of ESBL *E. coli* in RTE bushmeat and fresh fruit produce and possible risk of transfer antimicrobial resistant *E. coli* to game meat handler and consumers which would difficult to treat.

Keywords: Extended spectrum beta-lactamase, Bush meat, Fresh fruit, and *Escherichia co*

CHAPTER ONE
INTRODUCTION

1.1 STATEMENT OF PROBLEM

Extended-spectrum β-lactamases (ESBLs) are a rapidly evolving group of β-lactamases which share the ability to hydrolyse second and third-generation cephalosporins and aztreonam but are inhibited by clavulanic acid(Schulman,2017). The ESBL hydrolyse the β-lactam ring in of the antibiotics, as a result these antibiotics become resistant and ineffective. They serve as the first illustration in which fundamental alterations in the enzymes' substrate spectra led to Beta-lactamase-mediated resistance to beta-lactam antibiotics (Schulman, 2017). Amongst the most commonly identified bacteria producing extended-spectrum beta-lactamases is *E. coli*, whose reservoirs include the environment (soil and water), wild animals, domesticated animals, food, and pets (Carattoli, 2009). Infections caused by ESBL producers are associated with increased mortality, length of hospital stay and increased cost of treatment.

Bushmeat is a major source of income for many families living in the forest (Gulland *et al.*, 2003). Many consumers consider bushmeat to be a safe, wholesome alternative to the factory-farmed meat sold in supermarkets. Bush meats are preferred because of their superior flavour and because it is thought that industrially produced meats contain chemicals and other additives (Vliet *et al.*, 2011). Zootherapy, commonly known as the use of materials derived from animals to cure human illnesses, is practiced in some societies (Alves *et al.*, 2013). These medicinal cures derived from animals have been employed by numerous cultures throughout history and are still in use today in many parts of the world as either primary or supplementary therapies (Alves *et al.*, 2013). Despite the fact that it commonly recognized that foods derived from wild animals, such as bush meat, have the potential to cause serious diseases by consuming or using of body parts from these animals which makes it easier for widespread of zoonotic diseases (Alves *et al.*, 2013). Bushmeat are majorly sold as dried or smoked animal products and the usually ready-to-eat with no further processing or cooking required.

Fresh fruit and vegetable consumption has increased globally as consumers' interest in their nutritional benefits and associations with a healthy diet grows; as a result, the World Health Organization (WHO) recommends consuming at least 400 grams of fruit and vegetables every day (WHO, 2003). Fresh fruit and vegetable are minimally processed without cooking before consumption, hence, a major substrate for pathogenic organisms and foodborne illness

connected to fresh produce due to microbial load of fruits and vegetables is a significant source of concern for human health (Callejón *et al*., 2015). Fresh fruit and vegetable is a vehicle for the transmission of antibiotic-resistant bacteria or antimicrobial resistance genes to humans, which may happen through the eating of infected fresh produce, in addition to aiding in the spread of foodborne pathogens (Verraes *et al*., 2013). Therefore, this study assessed the significance of fresh produce and RTE bush meat for direct human consumption as carriers of antibiotic-resistant *Escherichia coli* strains that produce extended-spectrum beta-lactamases.

1.2 JUSTIFICATION OF STUDY

Firsthand consumption of fresh produce and bushmeat has led to major health issues worldwide, such as foodborne infections. The presence of pathogenic *E. coli* on fresh fruit and bushmeat has been linked to these health issues. This study has been designed to investigate the extended spectrum beta lactamase producing *E. coli* linked to ready-to-eat fresh fruit and bushmeat from Ondo, Oyo, Osun, and Lagos state in light of the fact that pathogenic *E. coli* has posed a threat to human health.

1.3 AIMS AND OBJECTIVES OF STUDY

This study's objective is to identify and characterize Extended spectrum beta lactamase-producing Escherichia coli in fresh fruit and vegetables and bush meat using phenotypic and molecular techniques (PCR).

The specific objectives are:

(1) Isolate and identify *E. coli* strains that produce fresh fruit and meat that have an extended spectrum of beta lactamases.

(2) Simplex PCR characterization of *E. coli* generating extended spectrum beta lactamases

CHAPTER TWO
LITERATURE REVIEW

2.1 GAME MEAT (BUSH MEAT)

The term "game meat" (sometimes known as "bush meat") refers to meat from animals that are hunted for human consumption. For those who live in humid tropical forest regions of Africa, Latin America, and Asia, bush meat serves as both a major supply of animal protein and a source of income. Bush meat is a crucial source of sustenance for the impoverished, especially in rural areas. (Brown *et al*., 2007). Game meat is not raised on a farm. People who come into touch with game meat run the danger of contracting a zoonotic disease, a disease that spreads from animals to humans because game meat serves as a reservoir for viruses. Hunters, people who prepare bush meat, and those who eat uncooked bush meat are those who are most at danger (cooking meat all the way through kills the pathogens). (Jani, 2019). For instance, researchers claim that chimpanzees, bonobos, and other primates spread HIV to those who hunted or butchered them. The Ebola pandemic in western Africa, which claimed the lives of over 11,000 people between 2014 and 2016, was also likely started by fruit bats. (Jani , 2019).

2.2 TYPES OF GAME MEAT

Game meat comes in a variety of forms and classifications, although it may be broadly divided into three (3) primary categories: avian game, venison, small game, big game, species birds, and exotic game. The deer meat which is the most popular type of game meat is venison, which refers to all deer meat, whether it was obtained by hunting or farm-raised deer. Steaks, sausages, tenderloins, and ground meats can all be made from venison. Game bird: These are birds with established licensed hunting seasons. Pheasants, grouse, doves, quail, turkeys, partridges, and specific varieties of ducks can fall under this category. Big game consists of creatures such as bison, elk, buffalo, bear, etc. Big games usually have a strong and powerful flavor.

Small game: Rabbits and hare are the most popular types of small game, though squirrel and porcupine are also considered small game.

General examples of game meat (bush meat)

Hedgehog, antelope, porcupine, civet cat, rabbit, monkey, snake, deer, buffalo, bush fowl, Guinea fowl, bush dog, Waterbuck, bush buck, pangolin, alligator, wild goat, Palm bird, Raven, bat, wild pig, duck, elk etc.

2.3 PREPARATION OF BUSH MEAT: SOURCE OF CONTAMINATION

Bush meat can be prepared in many ways like boiling, smoking, roasting but the most common way to prepare bush meat is either by smoking or roasting. Preparation method includes the following; Killing or slaughtering, skinning, evisceration, trimming, washing and perseveration. Killing or slaughtering: majority of contaminations acquired by bush meat are from slaughtering process, cross contamination from the equipment used for killing animal can lead to contamination.

Skinning: Bacterial such as *E.coli* contamination which are the normal skin flora as well as organism from soil and feces, which also on the skin.

Evisceration: this is the process of removing the intestinal part of the animal. During this process the carcass can the contaminated with *E.coli.*

Trimming and washing: Trimming and washing are done to improve the appearance of the carcass and to remove blood, bone-dust, hair and soil. Washing removes some bacteria and redistributes some organisms from one site to another.

Preservation: Spoilage organisms grow rapidly on meat, which is a highly perishable commodity. Thus, trade in meat, even at the local level, depends on some degree of preservation that controls the spoilage flora. Even after production, displaying bushmeat in the open market can lead to environmental contamination

2.4 CONSUMPTION OF GAME MEAT AND FRESH PRODUCES IN NIGERIA

According to the World Health Organization, animals have been the primary source of more than 75% of all newly developing infectious diseases during the past ten years (WHO,2020).The trade and consumption of bush meat have all been related to the outbreaks of HIV, Ebola, and even the most recent Covid 19. Through these activities, this disease has potentially spread, having a difficult impact on both health and the economy.

(Keresh *et al.*, 2005). Comparing subsistence use in rural areas to commercial trade feeding major urban centers, one can see that the risk and rate of outbreak are substantially higher.

For instance, the West African Ebola outbreak in 2014–2015 claimed the lives of over 11,000 people. (Cell Press, 2015).Several countries launched extensive media campaigns to persuade people not to eat bush meat (Luiselli, 2016).The preference of customers soon changed away from bushmeat, particularly fruit bats and monkeys (Akani *et al.*, 2015), and toward substitutes like fish (Bonwitt *et al.*, 2018).Bushmeat vendors vehemently lamented their dismal sales during the pandemic (Ajayi, 2014).However, by 2018, Nigerian bushmeat sales had recovered. (Mohamed, 2018). Nigeria has become the main African transit country for the sale of pangolin scales and ivory to Asian nations due to the country's growing bushmeat consumption. Between 2016 and 2019, the nation was connected to roughly half of all pangolin scale seizures worldwide (Wildlife, 2020).Although the penalties and rules governing wildlife were strengthened in 2016, they are commonly misunderstood by the general public and law enforcement personnel and are frequently not enforced by the relevant authorities. While many rural areas have little options for food, urban consumers choose to eat bushmeat for a variety of reasons, including health, taste, culture, and worries about chemicals in imported frozen chicken and turkey. Bushmeat is one of the many protein options available in cities, but it is sometimes only bought for special occasions because it is frequently more expensive than conventional meat or seafood. (Petrozzi *et al.*,2016). In Nigeria, consumption of bushmeat is common; 71 percent of respondents report having done it at some point in their life, with 45 percent having done so recently. One in sixteen people who have eaten bush meat in the past year do so on a weekly basis. Nearly half of consumers (47%) claim that their parents had an influence on their decision to consume, while 40% claim that they were the ones who made the choice. In Nigeria, 44 percent of consumers of bushmeat eat glasscutters, whereas 25 percent eat antelope or deer, 21 percent eat snake, and 10 percent eat wild pig (15 percent). Other animals eaten include monkeys (11%), porcupines (10%), tortoises (9%), crocodiles (8%) and

monitor lizards (7%) as well as bats (6%) and sea turtles (4%) and chimpanzees, pangolins, hedgehogs, and civets (approximately 2 percent each).The consumption of other bushmeat species besides those mentioned above was reported by 32% of consumers of bushmeat. These may also include animals such the genet, squirrel, giant rat, rock hyrax, guinea fowl, mongoose, and buffalo, which are frequently seen in bushmeat markets. Consumers who had consumed bushmeat during the previous year believed it to be 51 percent more delicious than farmed meat and 30 percent a part of their culture. Up to 28% think it is healthier and fresher than commonly found domestically farmed meat and fish because it has less chemicals.

The consumption of fresh fruits and vegetables ("fresh produce") has increased substantially since the 1980s due to consumer demand for a healthier lifestyle and more nutritious foods, especially in high-income countries. The World Health Organization recommends a daily intake of 400 grams of fresh produce (WHO, 2003). A diet rich in fresh produce has been shown to prevent certain chronic diseases such as diabetes, cancer, cardiovascular disease, hypertension, and obesity (WHO, 2003). Due to demand, globalization of trade has aided the distribution of fresh produce worldwide, surmounting the seasonality and location of certain commodities. Government and non-government agencies continuously promote nutrition education and encourage consumption of fresh produce to promote public health.

2.5 HEALTH BENEFIT OF GAME MEAT AND FRESH PRODUCE CONSUMPTION

One of the good thing about bush meat is the healthy benefit, it contains a lot of nutrient that nourish the body (Adebiyi *et al*., 2008). Bush meat are proteinuos in nature, the following are the health benefit of bush meat; Bush meat contain less fat in it than our common beef and it also contain more iron and protein than beef e.g. deer, a deer contains plenty of B12, B6, Riboflavin, and Zinc; thus, reduced risk of heart or nutrition related illness.(Adebiyi *et al*., 2008) Bush meat like elk which is similar to deer contains plenty of Niacin, Riboflavin, and Vitamin B. Additionally, elk contains plenty of Zinc, which is an excellent mineral that helps with your immune system. A small serving of elk has nearly half your daily recommendation of Zinc. (WHO, 2019)

Fresh fruits and vegetables are an important part of a healthy diet. They contain essential vitamins, minerals, fiber and other nutrients that are essential for good health. In fact, research has shown that a healthy diet rich in fruits and vegetables may reduce the risk of cancer and other chronic diseases. (Rediers *et al*., 2009)

Table 2.1 Key nutrient in fruit and vegetables (Rediers *et al*., 2009)

Nutrient	Health Benefit	Found In
Vitamin A	Healthy eyes and skin; protects from infection	Apricots, Cabbage, Cantaloupe, Carrots, Grapefruit, Greens, Leaf and Romaine Lettuce, Mangos, Spinach, Sweet Potatoes, Tomatoes, Watermelon
Vitamin C	Healthy teeth and gums; helps heal cuts and wounds	Bell Peppers, Broccoli, Brussels Sprouts, Cabbage, Cantaloupe, Cauliflower, Grapefruit, Oranges, Pineapple, Strawberries, Tomatoes
Calcium	Healthy teeth and bones	Greens, Kale, Okra, Rhubarb, Spinach
Fiber	Healthy digestive system; Reduced risk of heart disease	Apples, Bananas, Beans, Broccoli, Brussels Sprouts, Lentils, Peaches, Pears, Raspberries, Spinach
Folate	Wound healing; normal cell division	Asparagus, Broccoli, Peas, Beans, Greens, Spinach, Strawberries
Iron	Healthy blood; learning ability	Beans, Lentils, Spinach
Magnesium	Healthy bones	Beans, Spinach
Potassium	Healthy blood pressure	Bananas, Beans, Broccoli, Potatoes, Sweet Potatoes, Tomatoes

2.6 DISEASES ASSOCIATED WITH GAME MEAT AND FRESH PRODUCE

In Nigeria's largest towns, vibrant bushmeat markets are selling both legal and illegal bush meat. The majority of this commerce is still unregulated. The hazards of novel disease introduction and transmission are considerably increased by the process of trapping and transporting wild animals in demanding and unhygienic settings where they come into contact with people and domesticated animals. (Baragona, 2017). The types of infections that might be encountered while engaging in bushmeat-related activities are outlined here, along with a few significant and well-researched examples. Each antimicrobial pathogen strain that has recently arisen was identified separately in their study of worldwide trends in emerging infectious diseases (EIDs). (Jones *et al*., 2008) report that bacterial or rickettsial pathogens account for the vast majority of EID-causing pathogens, followed by viral or prion pathogens, protozoa, fungi, and helminthes. Viruses have been ranked as being more common in other research (Taylor *et al*., 2001) Only four emerging infectious diseases (EIDs) analyzed among 335 incidents between 1940 and 2004 list bushmeat as a driver; other important drivers included socioeconomic variables like human population density. All four of these bushmeat-related emerging infectious diseases (EID) incidents—Ebola, human immunodeficiency virus (HIV) 1, monkey pox virus, and SARS—were substantial occurrences, indicating that viruses are the most relevant pathogens in terms of spillover from bushmeat-related activities. (Kilonzo *et al*., 2014). By pathogen category (viruses, bacteria, helminthes, protozoa, fungi, and prions), a research from sub-Saharan Africa in relation to bushmeat species, was examine highlighting the tremendous potential for pathogens not yet connected to bushmeat-related activities to also be involved. Only a small number of studies have taken into account every possible zoonotic in a territory or taxonomic group (Magwedere *et al*., 2011)

2.7 *Escherichia coli*

The most extensively studied bacteria is *Escherichia coli.* It is one of the most significant pathogens in humans as well as a typical commensal resident of the gastrointestinal system. Therefore, among Gram-negative bacteria (GNB), *E. coli* is the most prevalent cause of bloodstream infection and urinary tract infections (UTIs). These isolates have specialized virulence factors that are absent from commensal and intestinal pathogenic strains, such as adhesins, toxins, iron-acquisition systems, polysaccharide coatings, and invasins. (Sannes

et al., 2004). Additionally, *E. coli* are the enteric Gram-negative bacteria that are most frequently found in the female genital tract. They can colonize the vagina and/or endocervical cavity and cause a variety of infections in pregnant women, including intra-amniotic and puerperal infections, as well as neonatal infections like early and late neonatal sepsis. (Guiral *et al*., 2011). *Escherichia coli* is a facultative anaerobe that ferments glucose to produce acid and gas. They are motile, peritrichous flagella that do not form spores, and they develop at a temperature of 37 °C. (Martinez et al., 1981). They are naturally colonizers (microflora) of the intestinal tracts of warm-blooded animals and members of the enterobacteriaceae family. Its breakouts have frequently been linked to fruits, vegetables, dairy products, and meat products. It falls to the bacterial domain. Proteobacteria, Gammaproteobacteria, Enterobacteriales, Phylum the Enterobacteriaceae family *Escherichia coli* is a species in the *Escherichia* genus.

2.8 CLASSIFICATION OF PATHOGENIC *Escherichia coli*

Escherichia coli (*E. coli*) is the predominant nonpathogenic facultative flora of the human intestine (Nataro *et al*., 1998). However, several strains of E. coli have developed the ability to cause disease in humans. Strains of *E.coli* that cause gastroenteritis in human can be grouped into six categories: Enteroaggragative(EAEC),enterohemorrhagic(EHEC),enteroinvasive(EIEC),Enteropathogenic(EPEC),Enterotoxigenic(ETEC),and Diffuse adherent (DAEC) (Benenso,1995).

Enterotoxigenic *E.coli* (ETEC): This is a type of pathogenic *E.coli* that causes watery diarrhea, which can range from mild, self-limiting disease to severe purging disease. (Sweeney *et al*.,1996)) The organism is an important cause of childhood diarrhea in the developing world and is the main cause of diarrhoea in travellers to developing countries Enterotoxigenic *E.coli* elaborate two (2) strain which are: heat-labile toxin (LT), heat-stable toxin (ST), or both toxins (Kaper *et al.,*1998).

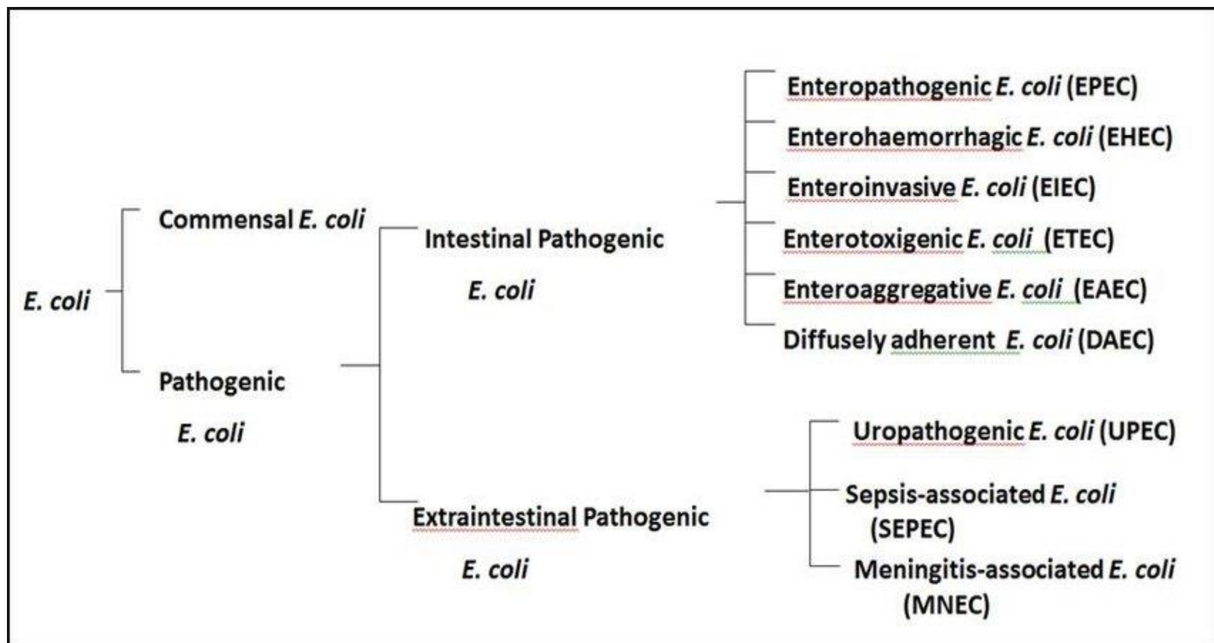

Figure 2.1: Classification of pathogenic *Escherichia coli* into group and sub group.

Source: (wakeham,2013)

2.9 PATHOTYPES AND THEIR INFECTION

Human gastroenteritis-causing *E. coli* strains can be divided into six categories: diffuse adherent (DAEC), enteroaggregative (EAEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC), and Enterotoxigenic (ETEC) (DAEC).

On the basis of their O (somatic), H (flagellar), and K (capsular) surface antigen profiles, pathogenic E. coli are serotyped (kaper et al., 1998). Each of the six groups above has its own unique pathophysiology and collection of O: H serotypes (Benenson, 1995).While *E. coli* O157:H7 is the predominant enterohemorrhagic E. coli serotype, other serotypes, including O111:H8 and O104:H21, can cause human diarrhoea (Benenson, 1995). Due to their strong resemblance to the Shiga toxin produced by Shigella dysenteriae, verotoxins, also known as Shiga toxins, are powerful toxins that EHEC excrete. Shiga toxin-producing *E. coli* is the name given to this particular group of organisms (STEC). STEC organisms can be located using a variety of techniques, such as DNA probes that locate genes that produce toxins (Benenson, 1995). Bloody stools without leukocytes or mild, non-bloody diarrhea are both possible symptoms (Benenson, 1995). Hemolytic-uremic syndrome can be brought on by STEC strains.

It appears non-O157 EHEC strains had existed for a while, but were only recently discovered. (kaper *et al.,* 1998). According to certain research, non-O157 serotypes represent the underlying cause of 20 to 50 percent of all EHEC illnesses. (kaper *et al*., 1998). It reportedly exhibits lower virulence than the O157 serotype. They are less likely to result in hemolytic uremic syndrome and bloody diarrhea, which are potentially fatal side effects of all STEC infections.(Tauxe *et al*., 1991). However, screening for Shiga toxin rather than the O157 antigen has been recommended for this population since younger patients have a higher risk for serious infections from non-O157 strains. The non-O157:H7 serotypes O26:H11, O103:H2, O111: NM, and O113:H21 are the most often identified ones to be linked to sickness in humans.

To become ill, only 100 to 200 organisms must spread an infection at a very low dose. (Kaper et al., 1998). There is a three- to eight-day incubation (Benenson, 1995). The majority of EHEC sickness treatment consists of supportive care. (kaper *et al*., 1998).animals are used in vaccine testing.

EHEC is primarily found in cattle, however it can also spread from person to person through humans. Consuming tainted food or water can potentially spread the disease. Ground beef, chicken, cheese, and ground pork have all been confirmed to have non-O157 serotypes.

(Tauxe *et al*., 1991).

2.10 VIRULENCE GENES IDENTIFICATION

E. coli virulence genes function in a variety of ways. The first mechanism is connected to blood stream invasion and is governed by genes that encode for amyloid curli and siderophores that support life outside of the gastrointestinal tract. (Hung *et al*., 2015). The second mechanism involves hemolysin and cytotoxin necrotizing factor damaging the cells and aiding *E. coli* in adhering to them via fimbria factors P and S. (Johnson *et al*., 1991). The presence of capsular K1, which prevents phagocytosis and complement-mediated death, is linked to the third mechanism. (Gaschignard *et al*., 2011). The fourth method involves specific strains of *E. coli* producing bacteriocins. (Micenkova *et al*., 2016)

2.11 ANTIBIOTICS FOR TREATMENT OF *E.COLI* INFECTIONS

Antibiotics are medications of natural or synthetic origin that have the ability to destroy or prevent growth of microorganisms (Pelae, 2006). They are frequently used to treat, prevent,

and control infections and illnesses (ventola, 2015). Shigella poisoning can cause hemolytic uremic syndrome, which may necessitate hospitalization, intravenous fluid replacement, blood transfusions, or dialysis. *Shigella* poisoning is treated with supportive care; medications are rarely utilized. The maintenance of electrolytes and hydration is the major objective of supportive care. The main therapies are ingesting electrolytes and fluids. For the most severe instances, IV fluids can be required. *E. coli* gut infections are rarely treated with medications. Antibiotics are rarely used in rare circumstances since they may increase the amount of toxins and the risk of hemolytic uremic syndrome. The use of anti-diarrheal drugs is not advised. They might impede colonic transit and lengthen the infection's persistence. (Pelae,2006)

A patient with severe hemolytic uremic syndrome and obvious kidney damage may receive an injection of the antibody Soliris (eculizumab) to stop future kidney damage. Soliris, however, has not been authorized for the management of HUS brought on by Shiga toxin. Only in severe cases of fever or persistent diarrhea are antibiotics used to treat some types of traveler's diarrhea and newborn diarrhea. As first-line treatments, fluoroquinolones like levofloxacin and ciprofloxacin are frequently used. The medication azithromycin is frequently used to treat invasive *E. coli* infections. The FDA has approved the use of the closely related antibiotics rifaximin and rifamycin SV to treat traveler's diarrhea brought on by noninvasive strains of *E. coli*.

Table 2.1 Medication for the treatment of *E.coli* infections

Drug Name	**Drug Class**	**Administration Route**	**Standard Dosage**	**Common Side Effects**
Cipro (ciprofloxacin)	Antibiotics	Oral	750 mg as a single dose or 500 mg twice daily for three days	Nausea, diarrhea, vomiting
Levaquin (levofloxacin)	Antibiotics	Oral	500 mg once daily for one to three days	Nausea, headache, diarrhea
Zithromax (azithromycin)	Antibiotics	Oral	1000 mg as a single dose or 500 mg once daily for three days	Diarrhea, nausea, abdominal pain
Xifaxan (rifaximin)	Antibiotics	Oral	200 mg three times daily for three days	Nausea, dizziness, headache
Aemcolo (rifamycin SV)	Antibiotics	Oral	388 mg twice daily for three days	Constipation, headache

2.11 ANTIBIOTICS RESISTANCE IN *E.coli*

Pathogenic and spoilage microbe prevalence can be significantly increased in ready-to-eat (RTE) meats by post-cooking handling activities, exposure time at points of sale, and meat storage conditions (Henriquez et al., 2015).*Salmonella* enterica, *Staphylococcus aureus*, *E. coli*, and *Clostridium perfringens* are the most prevalent harmful bacteria detected in RTE meats (Yuste et al., 2010).The most prevalent pathogen in the Enterobacteriaceae family and the most frequent facultative anaerobic species in both animal and human gastrointestinal tracts is *E. coli*. (Pitout, 2012) Shiga toxins are produced by specific strains of E. coli, which are associated to a number of illnesses and several fatalities each year (Painter *et al*., 2013).

The majority of ready-to-eat bush meat is sold on the street; meats and other commodities sold there are frequently exposed to germs that cause food poisoning, including *E. coli.* Food animal abuse has significant effects on public health because it encourages the emergence of germs resistant to antibiotics and the transmission of resistance genes to people (Smith, 2002). Farmers that purposefully or inadvertently use antibiotics without the necessary understanding are engaging in abuse. Additionally, farm animals' gastrointestinal tracts serve as a reservoir for germs. When antibiotics are used on certain microorganisms, they may become resistant to them. (Huijbers et al., 2016) Consuming undercooked meat from such farm animals could therefore result in transmission to humans. The environment, pets, and wild animals can all contribute to antibiotic resistance in people. Multiple antibiotic-resistant microorganisms can occasionally be found in large numbers in the environment, in pets, and in wild animals. Resistant bacteria that enter humans through the oral-fecal route or through handling are spread by the environment, pets, and wild animals, which act as reservoirs and reintroduce them into the food chain (Muloi, 2018).

2.12 EXTENDED SPECTRUM BETA LACTAMASE PRODUCIND *E. coli*

Extended-spectrum beta-lactamase (ESBL) producers are Gram-negative bacteria that produce enzymes that bestow resistance to most beta-lactam antibiotics like penicillins, cephalosporins, and the monobactam aztreonam (Silvia *et al*., 2014). These ESBL producers have been noticed mainly in the Enterobacteriaceae family of bacteria which may harbor several antibiotic resistance determinants making treatment of infections caused by these pathogens more difficult (Rotteir *et al*., 2012). Extended-spectrum beta-lactamase producers have a complex epidemiology; the most prominent bacteria involved include *E. coli* whose reservoirs comprise the environment (soil and water), wild animals, farm animals, food, and pets (Carattoli,2008) .

In some communities, bush meat may disseminate antimicrobial-resistant ESBL producers in the environment as previously observed. Different mechanisms, such as drug inaccessibility to the target, target modification, and/or drug inactivation by specialized enzymes known as beta-lactamases, can lead to the development of beta-lactam resistance. The genes encoding beta-lactamases frequently coexist with other determinants of antimicrobial resistance and can be linked to transposons or integrons, increasing the probability that the resistance genes will spread among bacterial species and lead to the emergence of multidrug resistant (MDR) microorganisms. (Li *et al*., 2007). The emergence of extended spectrum beta-lactamase (ESBL) and carbapenem resistance in Enterobacteriaceae, which has spread globally in the previous decade, is one of the most urgent areas of drug resistance. (WHO, 2014). One of the most crucial therapeutic choices for severe infections caused by extraintestinal *Escherichia coli* in humans is cephalosporin antimicrobial therapy, which includes cefotaxime, ceftazidime, ceftriaxone, and cefepime. (Pitout, 2012). As they are frequently the last line of defense against multi-drug resistant invasive Enterobacteriaceae microorganisms, the development of resistance against carbapenems (ertapenem, imipenem, meropenem, and doripenem) is of particular concern. (Pitout, 2012). The most significant mechanism of beta-lactam resistance among *E. coli* is still the synthesis of beta-lactamases. It is difficult to classify beta-lactamases, and there are two main approaches: molecular classification (Ambler classification) and functional classification (Bush Jacoby classification) (Bush, 2010). The Bush Jacoby categorization divides beta-lactamases into three groups: cephalosporinases, serine beta-lactamases, and metallo beta-lactamases. It does this by using substrate or inhibitor profiles.

In the 2000s, there was a steady rise in the number of ESBL-producing *E. coli*, and there have been numerous reports of nosocomial and community isolates that are resistant to these antimicrobial classes. (Pitout, 2012). The ESBL pandemic in *E.coli* is primarily associated with CTX-M beta-lactamases, particularly CTX-M-15 (Pitout, 2012), but additional enzymes may be in charge of inactivating beta-lactams. For instance, the SHV or TEM types accounted for the majority of ESBLs during the 1980s and 1990s. (Paterson, 2005).

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 STUDY AREA

The fresh produce samples were collected from Magboro market which is located in Obafemi-Owode Local government area in Ogun State due to close proximity the commercial hub Lagos and for its large human population. The study Site for the bush meat was Olomore market Abeokuta, Sango Garage Ogun State which is the main market for bush meat.

3.2 SAMPLES COLLECTION

Ready-to- eat game meat (bush meat) such as antelope, Guinea Fowl, Alligator, Hedgehog, Wild Rabbit, Grasscutter, Pangolin, Sparrow, Bush rat were collected from different bush meat market in Ondo, Osun, Ogun and Lagos State, while the fresh produce were Cabbage (Brassica oleracea var. capitate), carrots (Daucus carota subsp. Sativus), Pineapple, Watermelon, Cucumber, Lettuce were bought from Magboro market. After buying from the vendor the samples were collected in a zip-lock bag and then in kept in the fridge to prevent the samples (fresh produce and bush meat) from spoilage. The bags containing the samples were taken to the laboratory for further analysis.

3.3 MATERIALS, REAGENTS AND EQUIPTMENTS USED

MATERIALS

Petri dish, Glass spreader, Inoculating loop, cotton wool,70% Ethanol, latex, Bunsen burner, Beaker ,Wash brush, Makers, Measuring cylinder, Conical flask, Test tubes, Racks, Centrifuge, Cork borer, Eppendorf tube, Sterile tips, Micropipette, Incubator, Distilled water, Autoclave, Paper tape, Foil paper, Inoculating loop, Bunsen burner, Wash bottles, Spatula, Hockey stick

REAGENTS

20% Glycerol, Brain Heart Infusion Broth (BHI), 0.1% Buffer Peptone Water, Nutrient Agar (N.A), Sorbitol MacConkey Agar (SMAC)

EQUIPMENTS

Autoclave, Distillers, Water bath, Oven, Incubator, weighing balance, Vortex meter, PCR, Gel documentation and electrophoresis.

3.4 PREPARATION OF CULTURE MEDIA

BUFFER PEPTONE WATER

Preparation

1. The dehydrated medium was dissolve in 225ml volume of distilled water to make up 0.1% peptone water based on manufacturer's instruction's instructions in a conical flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminum foil.

2. The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15mins.

3. It was then dispensed by pipetting into various test tubes for serial dilution. Three types of media were used for the isolation of Escherichia coli; MacConkey agar (MAC), Nutrient agar (NA), Sorbitol-MacConkey Agar (SMAC)

3.5 SORBITOL–MACCONKEY AGAR (SMAC)

Preparation

1. The dehydrated medium was dissolved in the appropriate volume of distilled water i.e. 51.5g of SMAC in 1000 ml distilled water based on manufacturers' instruction's instructions in a conical flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminum foil.

2. The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15minutes.

3. The medium was then allowed to cool to a range of 45-50°C and poured aseptically into sterile petri dishes and left to solidify. This medium is reddish-purple in color.

MACCONKEY AGAR

MacConkey Agar is used for gram-negative enteric bacteria isolation and lactose fermentation differentiation from non-lactose fermenting bacteria and lactose fermenting bacteria but provides pink colonies on MacConkey Agar as Escherichia coli.

Preparation

1. The dehydrated medium was dissolved in the appropriate volume of distilled water i.e. 48.5g of MacConkey in 1000 ml distilled water based on manufacturers' instruction's instructions in a conical flask and mixed thoroughly. The conical flask is then closed in cotton wool that is wrapped in aluminium foil.

2. The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15minutes. Avoid overheating.

3. The medium was then allowed to cool to a range of 45-50°C and poured aseptically into sterile petri dishes and left to solidify. The medium is neutral red in colour

NUTRIENT AGAR

Nutrient Agar is a general purpose, nutrient medium used for the cultivation of microbes supporting growth of a wide range of non-fastidious organisms. Nutrient agar is popular because it can grow a variety of types of bacteria and fungi, and contains many nutrients needed for the bacterial growth.

Preparation

1. Nutrient agar was prepared according to the manufacturer's instruction

1. 28 g of nutrient agar powder was suspended in 1 liter of distilled water in a conical flask and mixed thoroughly.th conical flask is then closed with a cork (cotton wool that is wrapped with aluminum foil).

2. The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15minutes.

3. The medium was then allowed to cool to a range of 45-50°C and poured aseptically into sterile petri dishes and left to solidify. The medium appears opalescent and is light amber in color

3.6 SAMPLE PREPARATION

25g of each fresh produce and bush meat was put in a sterilized conical flask containing 225ml of 1%BPW after which serial dilutions were performed and appropriate dilution were plated on Sorbitol Mac Conkey (SMAC) and MacConkey (MAC) plate.

3.7 SERIAL DILUTION

0.1 mililitre of the samples were pipetted using the micropipettes (set at 100ul) into test tubes containing 9ml of 0.1% BPW to obtain 10^{-2}, followed by transfer of 0. 1ml from 10^{-2} into a new test tube (containing 9ml of 0.1% of BPW) to create 10^{-3} dilution, the test tubes are then put in the vortex mixer for even mixing. The dilution factor was repeated factor for 10^{-3}, 10^{-4} and 10^{-5}. The test tube were labelled for easy identification.

3.8 PLATING (SPREAD PLATE TECHNIQUE)

Spread plates technique was used for plating of inoculum (samples) for the SMAC and MacConkey agar plates. About 15-20ml of agar were poured into sterilized petri dishes (observing aseptic methods and conditions), then allowed to cool, set and solidify. 0.1 ml of the inoculum directly from dilutions 10^{-2}, $10^{-3}$and 10^{-5} were plated (using pipettes) onto appropriately labelled agar-containing petri-dishes for SMAC and MacConkey agar, this will be used for the identification and isolation of Escherichia coli strains. After dispensing, the

hockey stick is used to spread the inoculum around the agar (the hockey stick was dipped into alcohol and then flamed in the Bunsen burner before spreading so as to maintain aseptic conditions).

3.9 SUBCULTURING

The plate were checked after the required duration for the growth (24hrs) a sub-culturing needs to done. Sub-culturing was done to purify the isolated bacterial colonies from a mixed cultures to a new and single culture, the bacteria isolate sub-cultured were those differentiated on basis of their colour (pink and white) and the differentiated characteristics are transferred onto fresh petri dishes containing nutrient agar. A loop of the isolate will be taken by inoculating loop which is heated using the Bunsen burner and is allowed to cool for 5 seconds and then the isolate will be taken and streaked onto the new petri-dish.

3.10 CULTURE PRESERVATION

The preservation of isolates (*E.coli*) was done using 20% glycerol and BHI (Brain Heart Infusion).70µl of BHI was dispend into Eppendorf tube and then cultured E.coli was taken from nutrient agar with an inoculating loop, the loop containing the culture was dipped inside the Eppendorf tube containing the BHI and incubated for 37˚c for 24hours, 750µl of 20% glycerol is added to the Eppendorf tube and then stored in the freezer for cryopreservation.

3.11 ANTIMICROBIAL SUCEPTIBILITY TESTING

The antimicrobial was performed using Gram negative disc, Muller Helton agar and the activated isolate.

Procedure: *E. coli* isolates were activated by culturing 50µl in 9ml BHI and incubate at 37˚C for 24hours. The Muller Helton plate is inoculated with the test organism (50µl of the activated isolate into each plate) and spreading method was done using a sterile spreader, the culture was allowed to stand for 10-15mins to allow penetration into the agar. After 15mins the gram negative antibiotics disc containing different antibiotics like ceftizoxime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cephalexin (30 µg), amoxicillin (30 µg), imipenem (10 µg), cefepime (30 µg), cefoxitin (30 µg), gentamycin (30 µg), tetracycline (30 µg), trimethoprim/sulfamethoxazole (30 µg), nalidixic acid and ciprofloxacin (30 µg) was place in

at the center of the plate and it was incubated at 37˚C for 24hours,after incubation result were taken.

3.12 DNA EXTRACTION

1000µl (1ml) of pure BHI was prepared and dispersed in 2ml of Eppendorf tubes and autoclaved i.e. wrapped with foil paper and then 50ul of each isolates of E.coli was added to the Eppendorf tubes and then incubated at 370c , each of the isolates in the Eppendorf tubes was then centrifuged at 5000g for 3minutes then the BHI supernatant was dispersed into waste leaving the pellet , 500µl of distilled water was added into the Eppendorf (washing the residual cell from media)and then vortex to mix well and it is centrifuged at 5000g for 3minutes,the supernatant was discarded leaving the pellet, 200ul of distilled was added and vortexed , samples were ready for DNA extraction using heating block, the Eppendorf tubes containing the samples were placed in the heating block at 1000c for 15minutes and then it is covered to prevent the cap from opening ,after 15minutes the tubes are immediately placed inside ice to cool for 5minutes(it allows cell membrane to break). It is centrifuged at 7000g for 6 minutes, the DNA is then extracted into sterile tube.

3.13 MOLECULAR IDENTIFICATION OF *E.coli* PATHOTYPE

The components of the PCR and constituent mixes were summarized in Table below. After the PCR cocktail has been prepared it was place into the Thermocycler. The PCR was carried with initial denaturation at 95 °C for 5 min; 35 cycles of 95 °C for 2 min; 42°C for 30 s and 72 °C for 4 min; and a final elongation step at 72 °C for 10 min. The PCR products were confirmed by electrophoresis and visualized under UV light with a Gel Doc system (Cleaver Scientific Ltd, Warwickshire, United Kingdom)

AGAROSE GEL ELECTROPHORESIS

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

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

PRECAUTIONS

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

Table 3.1: Gene targets, primer sequences, primer concentrations and amplicon sizes for the multiplex PCR (Persson *et al.*, 2007)

Gene Target	Virulence factor/gene	Sequence (5´-)	Final concentration(μm)
Human estA	STIh	TTTCGCTCAGGATGCTAAACCAG CAGGATTACAACACAATTCACAGCAGTA	0.4
Porcine estA	STIp	CTTTCCCCTCTTTTAGTCAGTCAACTG CAGGATTACAACAAAGTTCACAGCAG	0.4
vtx1	VT1	GTTTGCAGTTGATGTCAGAGGGA CAACGAATGGCGATTTATCTGC	0.25
Eae	Intimin	GGYCAGCGTTTTTTCCTTCCTG TCGTCACCARAGGAATCGGAG	0.15
vtx2	VT2	GCCTGTCGCCAGTTATCTGACA GGAATGCAAATCAGTCGTCACTC	0.5
EltA	LTI	AAACCGGCTTTGTCAGATATGATGA TGTGCTCAGATTCTGGGTCTCCT	0.45
IpaH	IPaH	TTGACCGCCTTTCCGATACC ATCCGCATCACCGCTCAGAC	0.1
16SrDNA	16SrDNA	GGAGGCAGCAGTGGGGAATA TGACGGGCGGTGTGTACAAG	0.25

Table 3:2 Components used for Multiplex PCR

NO	Reagents	Initial concentration(µm)	Final concentration(µm)	Volume(µl)
1	Master mix	5x	1x	2
2	*StFp*	20 µm	0.5µl	0.25 µl
3	*StRp*	20 µm	0.5 µl	0.25 µl
4	*eltaF*	20 µm	0.45 µl	0.225 µl
5	*eltaR*	20 µm	0.45 µl	0.225 µl
6	*eaeF*	20 µm	0.15 µl	0.075 µl
7	*eaeR*	20 µm	0.15 µl	0.075 µl
8	Mgcl2	25 mm	1.5 µl	0.6 µl
9	dH20			4.3 µl
10	DNA			2 µl

Table 3.3: PROTOCOL FOR THERMOCYCLER

Analysis	Step	Temperature	Time
1x	Initial denaturation	95˚C	15min
35x	Denaturation	94 ˚C	50sec
	Annealing	57 ˚C	40sec
	Polymerization	72 ˚C	50sec
1x	Final polymerization	72 ˚C	3min
1x	Hold	4 ˚C	∞

3.14 ESBL RESISTANT GENES

Double disc synergy method was used for ESBL genes. Muller Helton plate was inoculated with 50µl of the activated isolate and spread plate was performed, the plates were allow to stand for 15mins and disc containing cefotaxime and ceftazidime 30µg are applied either side of one with co- amoxiclavulanic acid 20+10µ; and are placed 20mm away (Centre to Centre) from it the plated were incubated at 37˚c for 24hours .ESBL production is recorded when the zone of either the cephalosporin is expanded by the clavulanate. Zone of inhibition like bean shape around the antibiotics can also be used to detect ESBL production.

Table 3.4 Sequences, annealing temperatures and expected product sizes of primer sequences targeting the specified ESBL genes (Oduwu-mensah *et al*., 2016)

Gene	Primer	Annealing temp(˚C)	Expected product size(bp)
blaTEM	f: 5′-AAA CGC TGG TGA AATA-3′ r: 5′-AGC GATCTG TCT AT-3′	45	720
blaSHV	f: 5′-ATG CGT TAT ATT CGC CTTG-3′ r: 5′-TGC TTT GTT ATT CGG GCAA-3′	60	726
blaCTX-M	f: 5′-GAC GAT GTC ACT GGC TGAGC-3′ r: 5′-AGC CGC CGA CGC TAA TAC A-3′	55	499

Table 3.5: Components used for Multiplex PCR for ESBL (Oduwu-mensah *et al*., 2016)

NO.	Reagents	Initial Concentration	Final Concentration	Volume/rxn
1	Master mix	5x	1x	2 µl
2	*BlaTemF*	20 µm	0.2 µl	0.1 µl
3	*BlaTemR*	20 µm	0.2 µl	0.1 µl
4	*BlasHvF*	20 µm	0.2 µl	0.1 µl
5	*BlasHvR*	20 µm	0.2 µl	0.1 µl
6	*BlactxmF*	20 µm	0.2 µl	0.1 µl
7	*BlacxmR*	20 µm	0.2 µl	0.1 µl
8	Mgcl2	25 mm	0.5 µl	0.2 µl
9	dH20			5.2 µl
10	DNA			2 µl

CHAPTER FOUR

RESULT AND DISCUSSION

4.1 RESULTS

The microbial analysis of bush meat and fresh produce samples gotten from Ogun, Lagos, Ibadan, Osun and Ondo state. The microbial analysis were carried out for total viable count, for general E.coli and pathogenic E.coli. All samples had pink and white, raised, circular and smooth colonies on selective media SMAC (Sorbitol MacConkey agar) and circular, translucent or opaque on Nutrient agar (NA).MacConkey, Sorbitol MacConkey agar and Nutrient agar and also the morphological characteristics of samples cultured on Sorbitol MacConkey agar using spread plate method with bacterial isolates collected from bushmeat sampling from 9 locations in Lagos, Osun, Ondo, Ogun and Oyo states. Table 4.4 shows the results of ESBL producing *E. coli* with bacterial isolates collected from bushmeat and fresh fruit sampling from different locations in Lagos, Osun, Ondo, Ogun and Oyo states.

In Lagos state, Monkey had the highest viable count which was 9.5 log_{10} CFU/g and Quail had the lowest viable count of 4.1 log_{10} CFU/g. In Ogun State, Antelope had the highest total viable count of 8.6 log_{10} CFU/g and Guinea fowl had the lowest of 4.8 log_{10} cfx/g. In Ondo State, Antelope has the highest total viable count of 8.8 and Guinea fowl has the lowest TVC of 4.4 log_{10} CFU/g (Table 4.1). In Osun State, Antelope has highest TVC of 8.6 log_{10} cfx/g then Hare has the lowest tvc of 4.8. In oyo state, pangolin has the highest TVC of 7.1 and Eta has the lowest of 4.3. The microbial analysis of RTE game meat samples in south western part of Nigeria were reported and it was discovered that all samples were positive with *E.coli*. The characteristics of E.coli was recorded when incubated on the SMAC and MAC plate and a white and pink colour was recorded and also they were also circular colonies.

Table 4.1: **Various game meat and their sampling location showing the total viable count**

	GAME-MEAT	NUMBER OF SAMPLES	TOTAL VIABLE COUNT (cfu/ml)
	Pangolin		8.6×10^6
	Quail		4.1×10^6
	Deer		8.1×10^6
Lagos State	Bush dog	25	6.4×10^6
	Grasscutter		8.5×10^6
	Etu		5.5×10^6
	Wild Cat		7.3×10^6
	Atika		6.3×10^6
	Agbonrin		4.5×10^6
	Antelope		8.7×10^6
	Monkey		9.5×10^6
	Rabbit		7.5×10^6
	Porcupine		8.3×10^6
	Antelope		8.6×10^6
	Grasscutter		8.4×10^6
Ogun State	Rabbit		7.8×10^6
	Bush rat	12	6.2×10^6
	Igala		6.7×10^6
	Hedgehog		5.2×10^6
	Guinea fowl		4.8×10^6
	Alligator		7.3×10^6
	Civet Cat		7.2×10^6
	Rabbit		7.4×10^6
Ondo State	Antelope	9	8.8×10^6
	Grasscutter		8.3×10^6
	Guinea Fowl		4.4×10^6
	Hare		4.8×10^6
Osun State	Sese	5	6.8×10^6
	Antelope		8.6×10^6
	Aparo		5.5×10^6
Oyo State	Eta	4	4.3×10^6
	Esii Tuku		7.1×10^6
	Guinea Fowl		5.0×10^6
Total		55	

Various game meat samples showing the total viable count of each sample from different locations (Table 4.1).

Table 4.2: Total viable count in various Fresh fruits and their sampling location.

LOCATION	FRESH FRUIT SAMPLE	NUMBER OF SAMPLES	TOTAL VIABLE COUNT (cfu/ml)
Ogun State	Pawpaw	11	3.2×10^6
	Cucumber		4.1×10^6
	Lettuce		8.6×10^6
	Cabbage		6.4×10^6
	Pineapple		6.0×10^6
	Watermelon		5.5×10^6
	Carrot		4.5×10^6

The various Fresh fruit showing the highest total viable count is lettuce (8.6×10^6) while Pawpaw had the lowest count of 3.2×10^6 (Table 4.2).

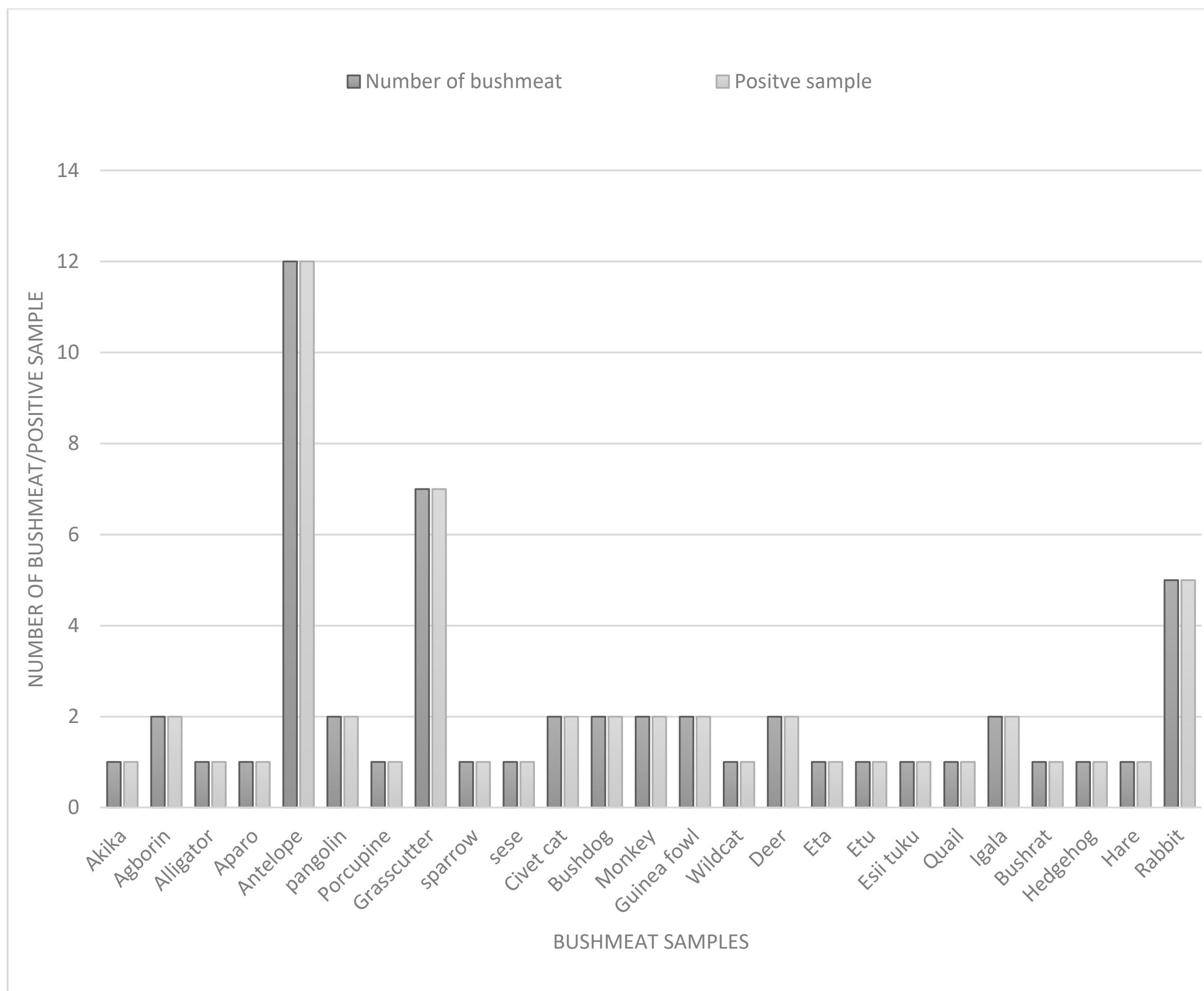

Figure 0.1: Chart of microbial analysis showing the prevalence of *Escherichia coli* in the bushmeat samples

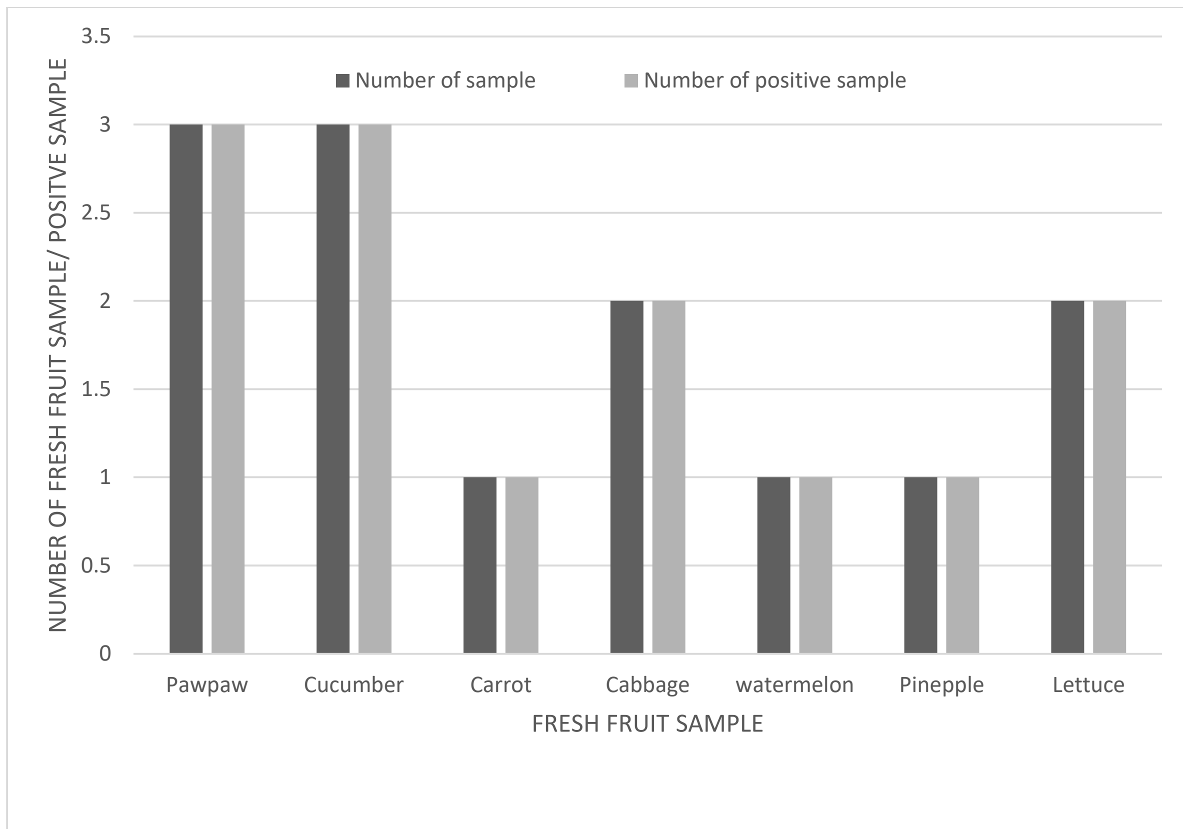

Figure 0.2: Chart of microbial analysis showing the prevalence of *Escherichia coli* in the Fresh fruit samples

Table 4.3: Antimicrobial Susceptibility Pattern of Selected *Escherichia coli* Isolates from bushmeat samples.

Classes of Antibiotics	Antibiotics	Disc Code	Antibiotic Disc Content (µg/disc)	Number of *E.coli* Isolates (n =11)		
				R	I	S
Sulfonamides	Cotrimoxazole	COT	25	7	-	4
Aminoglycosides	Gentamycin	GEN	10	5	-	6
	Amikacin	AMK	30	-	1	10
Cephalosporin	Cefotaxime	CTX	30	11	-	-
	Cefoperazoen	CPZ	30	11	-	-
	Ceftriaxone	CTR	30	11	-	-
Fluoroquinolones	Ciprofloxacin	CIP	5	8	-	3
Anti 50S Ribosomal	Chloramphenicol	CHL	10	6	2	3
Tetracycline	Tetracycline	TET	10	8	1	2
Carbapenem	Meropenem	MEM	10	8	-	3
Glycopeptides	Vancomycin	VAN	10	11	-	-

E.coli isolates were viewed on the disc and it was interpreted using the CLSI and it was classified into susceptible(S) , intermediate (I) and resistance(R).

Table 4.4: ESBL Test Performed on isolates

ISOLATE	Cefotaxime (CTX)	Amoxiclavulanic acid (AMC)	Ceftazidime (CAZ)
MK1P1	-	+	+
MS1U1	+	+	+
MA1U2	+	+(Not large)	+
MA1A2	+	+	+
MDIU3	+	+(Not large)	+
MKPs2	+	-	+
MS1C2	+	+	+
MD1U2	+	+	+

Note: Positive (+) means presence of ESBL and Negative (-) means absence of ESBL

PCR AMPLICATION IMAGE

The genetic characterization shows DNA band on agarose gel of the pathogenic *E. coli* and ESBL after PCR amplification. This confirms the results from the morphological test.

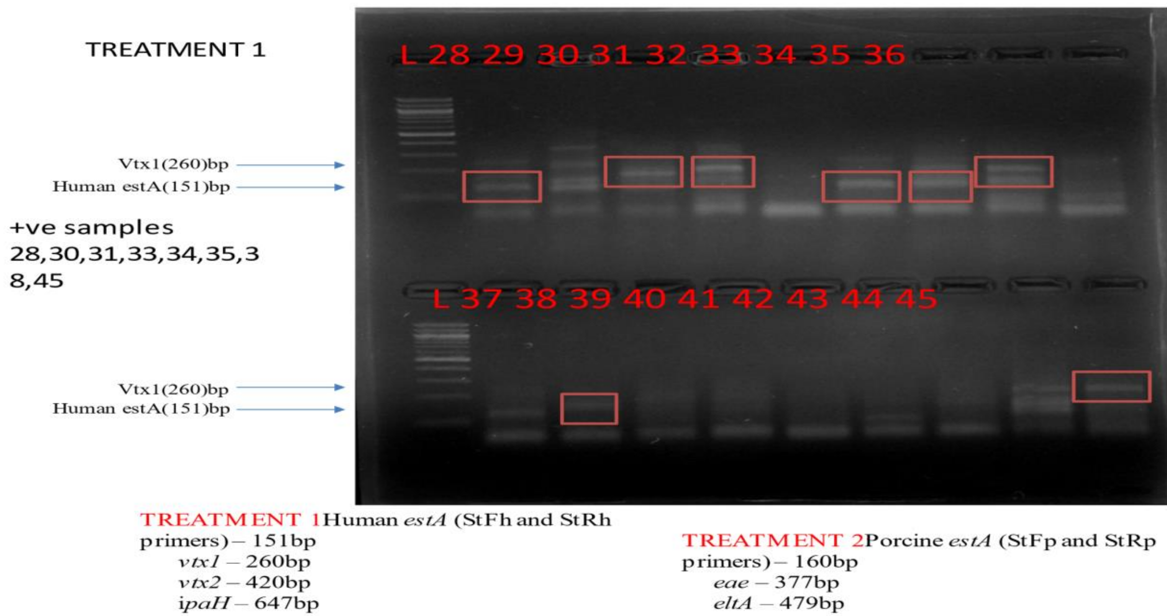

Plate 4.1: Pathogenic *E. coli* Multiplex PCR Result

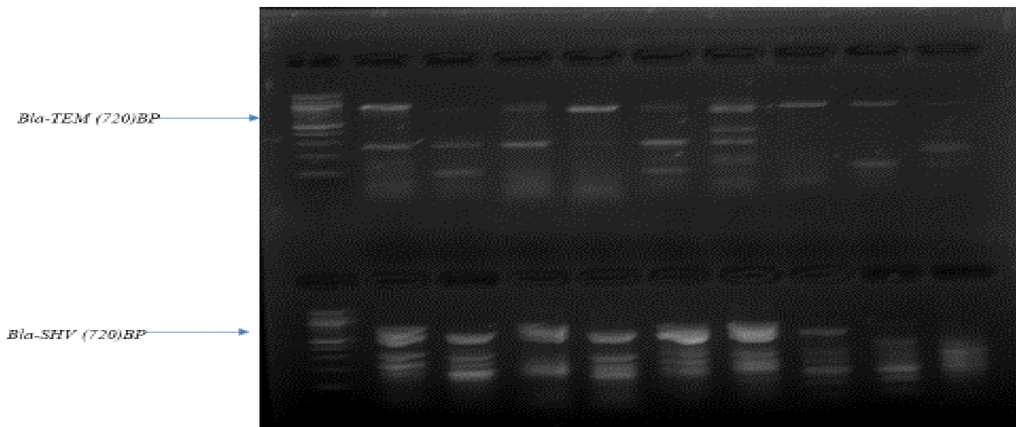

Plate 4.2: ESBL Simplex PCR Result

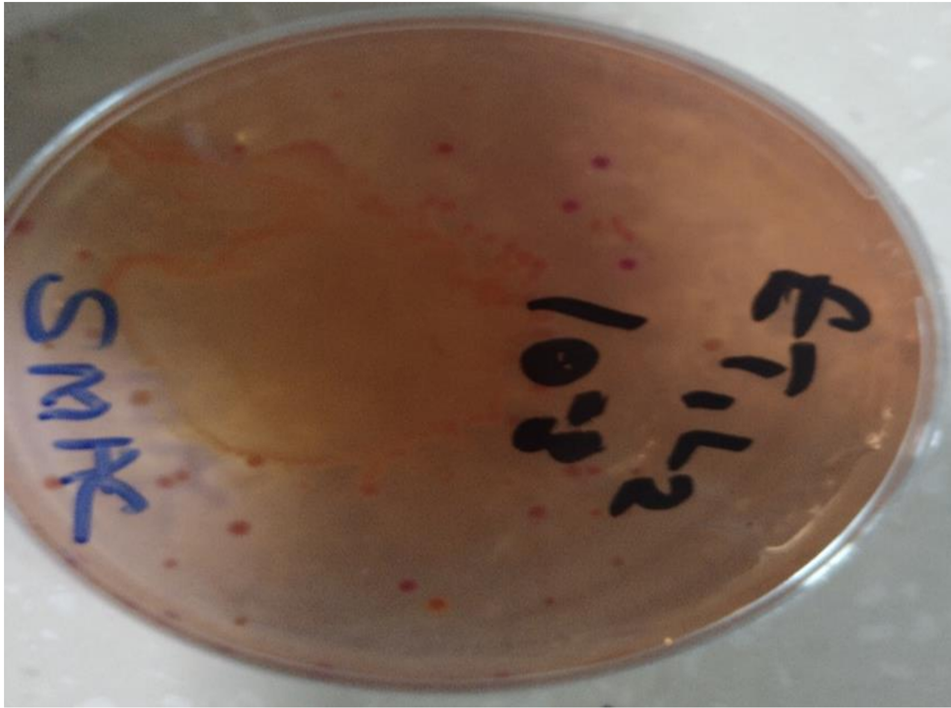

Figure 4.3 Sorbitol MacConkey agar plates showing growth of pathogenic *E. coli*

4.2 DISCUSSION

Nigerians have an advanced food supply chain and consume a lot of bushmeat. Animals that are trafficked the most are mammals, particularly ungulates and rodents (Petrozzi et al., 2016).Frequently, microorganisms that can be harmful to humans are found in bushmeat (Burgess *et al*., 2017). Salmonella. The main bacterial pathogens include: *C. aureus Clostridium perfringens, botulinum, and E. and Cereus coli* (Hedman et al., 2020).The natural microflora in an animal's normal tissues, the air, the environment, or contamination as a result of unclean handling, processing, and slaughtering circumstances are possible sources of these bacteria in meat (Pal et al., 2019).The morphological characteristics of individual microorganisms serve as preliminary criteria for identification. Of the several morphological properties, and colonial characteristics were employed in the identification of isolates. This study was carried out to identify the prevalence and number of foodborne pathogens specifically *Escherichia coli* in 55 bushmeat samples and 6 fresh fruit produces (15 Antelopes, 7 Grasscutters, 6 Rabbits, 4 Civet cats, 4 Guinea Fowls, 3 Deers, 2 Alligators, 2 Pangolins, 2 Hedgehogs, 2 Sparrow, 3 Bush dogs, 2 Monkeys, 1 Hare, 1 Porcupine and 1 Quail, Cucumber, Pawpaw, Watermelon, pineapple, lettuce and cabbage) obtained from different market in Ogun, Lagos, Osun, Oyo and Ondo state. Noticeably, more than 40% food samples from different parts of India were found to be contaminated with *E. coli* wherein >10% isolates were ESBL producers (Bhoomika *et al*., 2016). However, <10%ESBL producing isolates of *E. coli* has also been reported (Abayneh *et al*., 2018). A higher prevalence of 73.58% ESBL producers were observed in human clinical samples in North East India (Bora *et al*., 2014).In this study, high presence of E. coli was observed in game meat samples (54.54%)of the isolate were tested positive for *Vtx*1 gene which was used to detect VTEC (verotoxigenic *E.coli*/STEC) which causes diarrhoeal in humans and for *estA* which is used to detect ETEC (Enterotoxigenic *E.coli*) followed by fresh produce samples of which 3(27.25%), 2(18.17%) and 2(18.17%) out of 11 samples harbour ESBL producing *E.coli* in fresh produces and bushmeat respectively. Presence of ESBL producing *E. coli* in ready-to-eat foods and cooked foods is of public health significance. (Lavilla *et al*., 2008) Previous research have demonstrated that foods are a vector for the spread of ESBL-producing bacteria, most likely from reservoirs, food animals, and food handlers, and that once contaminated, they can result in an outbreak (O'Connor et al., 2017).

The samples from meat vendors had the highest E. coli contamination (54.36%), followed by milk vendors (48.88%) and egg vendors (45.20%). ESBL-producing *E. coli* also showed a similar trend, with 16.1%, 11.11%, and 2.05%, respectively.

When samples from the two cities were compared, it was found that samples from Delhi (17.54%) had more ESBL-producing E. coli than samples from Bareilly (6.96%). A final food product is frequently contaminated by raw foods and the environment, and this was seen in the current investigation as well. While (Sarma et al., 2013) observed 75% prevalence in chevon, pork, and poultry meat from Mathura city of India, (Bhoomika et al., 2016) similarly noted the highest incidence of E. coli in raw milk samples (81.11%), followed by chicken meat (66.32%) and chevon meat (46.34%).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

This study indicated ESBL *E.coli* is present in RTE bush meat and fresh fruits and vegetables, and possible risk of transfer to game meat handler and consumer. This suggests that the bush meat product was improperly prepared and handled in an unhygienic environment. The high counts of *Escherichia coli* contamination suggests that fresh fruit and bush meat have gone through a variety of unhygienic preparation and distribution processes.

5.2 RECOMMENDATIONS

The following recommendations were offered in view of the findings of this study that sales of some fresh vegetables and bushmeat in public markets and open stalls should be conform to strict food safety standards to reduce the chance of contamination. Additionally, the process for producing bushmeat needs to be updated to a more automated form to guarantee hygienic preparation. Finally, to prevent cross-contamination, fresh produce and vegetables should be stored separately from raw meats and other animal products; also the development of innovative handling, conservation and cooking practices, adapted to each socio-cultural context, should help reduce the negative impacts of bushmeat and fresh fruit consumption on human health.

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