MOLECULAR CHARACTERIZATION OF PATHOGENIC *ESCHERICHIA COLI* IN READY-TO-EAT GAME MEAT AND FRESH PRODUCE FROM SOUTHWESTERN REGION OF NIGERIA

BY

ADEGBOYE, SIMISOLA PRECIOUS

18010104002

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DECLARATION

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

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ADEGBOYE, SIMISOLA PRECIOUS

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Date

CERTIFICATION

This is to certify that this report titled "**MOLECULAR CHARACTERISATION OF PATHOGENIC *ESCHERICHIA COLI* IN READY-TO-EAT GAME MEAT AND FRESH PRODUCE FROM SOUTHWESTERN REGION OF NIGERIA**" was carried out by **ADEGBOYE, Simisola Precious** with matriculation number **18010104002**. This project meets the requirements governing the award of Bachelor of Science **(B.Sc.)** degree in **Biotechnology** from the Department of Biological sciences, Mountain Top University, Ogun State, Nigeria and is approved for its contribution to knowledge and literary presentation.

_____ (Signature and Date)

Dr. G.B Akanni

Supervisor

_____ (Signature and Date)

Dr. C.I. Ayolabi

Ag. Head of Department

DEDICATION

This project is dedicated to God Almighty for providing me with good health, and the grace to complete this project; to my beloved parents, Mr. and Mrs. Adegboye, for their prayers, counsel, and sacrifice.

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ABSTRACT

The consumption of ready-to-eat (RTE) game meat and fresh produce has increased and it serves as a very important part of human diet. Foodborne diseases linked to contaminated RTE game meat and fresh produce is a public health concern. This study investigated the pathogenic *E. coli* in these RTE foods sold in various cities in south western, Nigeria. The identification of *E. coli* were performed using sorbitol MacConkey agar (SMAC) and molecular characterization of virulence genes. The Kirk-Bauer disk diffusion test was used to determine antibiotics susceptibility tests of the *E. coli* strains. A total of 55 samples RTE game meat and 11 samples of fresh produce were analyzed for pathogenic *E.coli*. All thirty (30) isolates from fresh produce were identified as potential pathogenic E. coli. Further identification with multiplex PCR revealed that four of the isolates were positive based on the band size (as compared with the predicted band size) which directly linked them to potential pathotypes. Overall, 4 out of 30 isolates coded for 3 genes which are; *Vtx* 1 coded for verotoxigenic *E. coli* while *est*A porcine and human *est*A coded for enterotoxigenic *E.coli*. It was observed that the total viable count for *E.coli* from game meat and fresh produce were respectively high. The isolates were confirmed using multiplex PCR for the game meat and using simplex PCR to determine the ESBL of the fresh produce. The presence of *E.coli* in RTE game meat and fresh produce in south western part of Nigeria poses publc health concerns which could lead to food borne illness.

Keywords: Ready-to-eat game-meat, Fresh produce, pathogenic *E.coli*, Food borne illness

CHAPTER ONE

1 INTRODUCTION

Background of Study

Outbreaks and foodborne pathogens pose a significant threat to human public health, leading to a substantial economic burden both in developed and less developed countries(Akhtar *et al*., 2014). More than 250 known foodborne diseases could be caused by food contaminated with bacteria, viruses, parasites, and toxins, which continue to be a public health problem in the world. Bacteria cause a large proportion (approximately 90%) of all foodborne illnesses(Bari and Yeasmin, 2018).The bacterial pathogens are commonly found in slaughtered livestock (cattle, sheep, and swine) and poultry (chicken and turkey), as well as ready-to-eat (RTE) foods including smoked/dried game meats and fresh produce (fruits and vegetables). Meat and poultry carcasses and their offal are frequently contaminated with pathogens which contaminate the carcasses from fecal material (Smith and Fratamico, 2018).

Shiga-toxin producing *Escherichia coli* O157:H7 (STEC), is a strain of the *Enterohemorrhagic E. coli* group, is recognized as an organism whose presence in any food material can lead to serious disease outbreak (Abreham *et al*., 2019). In the human gastrointestinal tract (GIT), *E. coli*O157:H7 is known to produce large quantity of Shiga-toxins, which can cause severe damage to the lining of the intestine and other organs of the body (Ingber, 2022). The organism is particularly associated with the development of hemolytic uremic syndrome, known to result in a mortality rate of 2 - 10% (Kim *et al*., 2020). The potentially high mortality associated with *E. coli* O157:H7 infection, therefore make its presence in any food material worrisome and of serious public health concern. Most outbreaks recorded has been traced to consumption of beef and vegetables (lettuce) contaminated with the *E. coli* O157:H7 strain (Bedasa *et al*., 2018). Although, undercooked ground beef meat has been identified as a leading food vehicle of *E .coli*O157:H7, fresh raw vegetables are also becoming increasingly important vehicle of transmission (Ngene *et al*., 2020). Many outbreaks of *E .coli*O157:H7 infections were associated with contaminated leafy lettuce, radish sprout, alfalfa sprout, potatoes. Contamination of vegetables with *E. coli* O157:H7 may occur at different stages from cultivation to transportation

(Mostafidi *et al*., 2020). Vegetables grown in soil fertilized by animal manure have a great chance to be contaminated with *E. coli* O157:H7 (Iwu *et al*., 2021). *E. coli* O157:H7 can enter the lettuce tissue when lettuce seeds are grown in manure fertilized soil or by irrigation with water mixed with sewage or by contaminated surface water irrigation (Bintsis, 2018).

The present study aims to investigate the presence of pathogenic *E. coli* in ready-to-eat fresh produce and RTE game meats sold at open markets from various locations in Southwest, Nigeria. The prevalence of STEC and other pathogenic *E. coli* in RTE game meat and fresh produce will be determined with the selection of pathogenic *E. coli* based on the virulence genes and antimicrobial resistance patterns (Patterson *et al*., 2022;Hozzari*et al*., 2020).

Statement of the Problem

Pathogenic *E.coli* particularly Shiga toxin-producing *E. coli* (STEC) are capable of causing severe foodborne illness (Park *et al*., 2020). Raw or undercooked ground meat products, raw milk, and fecal contamination of vegetables are the primary causes of STEC outbreaks (Gourama, 2020).Ready-to-eat foods including game meat and fresh produce have been known to be a source of *E.coli* infections as several strains are known to produce toxins that can cause diarrhea (Abebe, 2020). Therefore, the need to know the prevalence of pathogenic *E.coli* strains in RTE foods sold in open markets in various locations in Nigeria exists. Antimicrobial resistant bacteria is a source of concern, it will be essential determine the antimicrobial resistance of the*E.coli* strains food in RTE foods in these areas in order to determine the public health risks for consumers (Duze *et al*., 2021).

Significance of the Study

The *E. coli* O157:H7 serovar is frequently used as the target organism in studies describing the survival of *E. coli* in foods (Duc *et al*., 2020). Ready-to-eat street food is a potential source of spreading pathogenic *E. coli* which are resistant to antimicrobial agents (Zurita *et al*., 2020). The presence of this organism in RTE game meat and fresh produce would indicate the food safety levels of these food in terms of hygiene and fecal contamination.

Aims and Objectives

- To isolate pathogenic *E. coli* found in RTE game meat and fresh produce food samples sold in open markets in Southwest, Nigeria.
- To characterize the various *E. coli* pathotypes isolated from these food samples using culture-based and molecular techniques.
- Determine the antimicrobial resistance patterns of the *E. coli* strains.

CHAPTER TWO

LITERATURE REVIEW

2 BACKGROUND

Foodborne illnesses affect an estimated one-third of the population annually in developed nations (Fouladkhah *et al*., 2019). According to the World Health Organization, food can cause or spread more than 200 different diseases or illnesses (Yang *et al*., 2020). But among the most prevalent foodborne pathogens are *Campylobacter*, *Bacillus cereus*, *Clostridium botulinum*, *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Staphylococcus aureus*, and *Clostridium botulinum* (Gourama, 2020). The incidence of foodborne illness, which includes a wide range of illnesses brought on by pathogenic microorganisms, is currently increasing globally and raising public health concerns. Each year, these microorganisms cause an estimated 48 million illnesses and 3000 deaths in the United States (Sinkel *et al*., 2018). Ready-to-eat foods do not require any additional preparation, and these foods are frequently eaten as it is; whether raw, cold or pre-cooked (Ema *et al*., 2022).

Ready-to-eat foods do not require any additional preparation, and these foods are frequently eaten as it is; whether raw, cold or pre-cooked (Ehuwa *et al*., 2021). All over the world, people regularly eat various ready-to-eat foods in public settings (Adeosun *et al*.,2022). Due to the critical role that food plays in human life, it is crucial to maintain food safety in order to protect people from foodborne illnesses and other related health risks (Kamboj *et al*., 2020).In addition to being valued by consumers for their accessibility, affordability, variety, and distinctive organoleptic properties, ready-to-eat foods play a significant role in meeting the nutritional needs of many consumers (Mengistu *et al*., 2020). Ready-to-eat foods, however, can act as an ideal environment for a number of pathogenic microorganisms of public health concern to grow and multiply if they are not handled safely and in a hygienic manner (Mahros *et al*., 2021). The prevalent poor hygienic and sanitation conditions or practices, lax food safety and regulatory systems, lack of resources, and lack of education, however, make foodborne diseases common and one of the main causes of illness in developing nations (Aluh *et al*., 2021). Health

organizations and other concerned groups are working harder to improve food quality and safety and prevent foodborne illness as a result of these issues. (Todd, 2020).

In many countries, bacterial food-borne zoonotic infections are the leading cause of human intestinal disease (Heredia and Garcia, 2018). As a result, increased research and surveillance efforts from government agencies are required, as well as special attention and awareness from the food industry (Kerr *et al*., 2018). Shiga toxin-producing Escherichia coli (STEC) are currently thought to be an important group of food-borne zoonotic pathogens that cause diarrhoea, haemorrhagic colitis (HC), and the potentially fatal haemolyticuraemic syndrome (HUS) in humans. Domestic ruminants, particularly cattle, are thought to be a major reservoir of STEC (Chen *et al*., 2020). Large game animals such as red deer (Cervuselaphus) and wild boar (Susscrofa) are also known to be healthy carriers of O157:H7 and non-O157 STEC (Dias *et al*., 2022).Fresh meat and ready-to-eat meat products derived from deer have been identified as a significant source of food-borne E. coli O157:H7 and non-STEC O157 to humans (Meng and Doyle, 2020). Despite this, the microbiological contamination levels allowed for large game meat and meat products are not subject to any official regulation. Furthermore, data on the microbiological quality of game meat for some pathogens is limited (Soare *et al*., 2022). A complicating factor is that efforts to monitor the health of wild game rely on disease detection through visual inspection and recommended hygienic practices to limit the spread and multiplication of biological hazards (Jamwal and Phulia, 2021).

Escherichia coli can cause health problems primarily during the preparation and storage of contaminated RTE meat and fruits and vegetables (Giri *et al*., 2021). Ready to eat (RTE) meats, on the other hand, have been identified as transmission routes for foodborne bacteria such as E. coli, posing a significant microbiological risk (Ema *et al*., 2022). As a result, food-traceability systems are urgently needed, particularly for meat and meat-derived products, to improve the quality of food-processing events and ensure safe food for final consumers (Ema*et al*., 2022). E. coli 0157:H7 has been connected to life-threatening illnesses like hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (Koolebogile, 2020). Majority of *E.coli* strains are non-pathogenic, a few are very pathogenic and cause watery and bloody diarrhea (Schuetz, 2019). The contamination of RTE foods with E. coli has been discovered (Wilson et al., 2018). It is simple to transfer this species from surfaces, like hands, to

foods. The likelihood of contracting diseases of animal origin, like pathogenic E. coli, has increased as more people consume beef, milk, and poultry. E. coli, an Enterobacteriaceae family member, is the most common organism found in the digestive tracts of both humans and animals (Ekici and Dumen, 2019). The enteric and faecal pathogen E. coli has been identified as an indicator species (Devane *et al*., 2020).HC and HUS are two serious human gastrointestinal disorders that have been linked to some E. coli strains, despite the fact that the majority of these strains are not pathogenic (Liu *et al*., 2022). Enterohemolysin (hlyA), intimin (eaeA), and shiga toxins (stx1 and stx2) are virulence factors that are important in the emergence of these disorders (Elsyaed and Mounir., 2020). Additional research has been done on E. coli and other pathogens in RTE foods.

2.1 Fresh Produce

Fruits are the edible parts formed from leaves of plants. They are rich in vitamins, fibres, anti-oxidants, minerals and carbohydrates (Muronga *et at*., 2021). Fruits and vegetables have been reported to be vectors of pathogens and other contaminants and this can be traced to fact that they are always consumed without further processing (or even minor heat processing) and most of the time they contain pathogens from harvesting practices, transportation channel and human handling(Ehuwa *et al*., 2021).

2.2 Potential Sources of Produce Contamination

Due to the fact that fresh produce cultivation is an open system, it is vulnerable to contamination from a variety of sources (Alegbeleye *et al*., 2018). This is due to the fact that each farm has its own unique set of environmental risk factors, including topography, land-use interactions, and climate. The prevalence and transmission of foodborne pathogens, as well as the danger of produce contamination, are influenced by a combination of these unique environmental risk variables (Rasool *et al*., 2021).Pathogens can contaminate produce ‘on-field' through different mechanisms, including air deposition, uptake from contaminated soils, and groundwater contamination (Gong *et al*., 2018), use of raw (or poorly treated) manure and compost, exposure to contaminated water (irrigation or flooding), insect transfer, or fecal contamination caused by cattle or wild animals (Pradhan *et al*., 2019). Several researchers have looked at the source of contamination, with soil, water, biological amendments, and wild animal activities all being mentioned as possible routesfor human infections (Angelici and Karanis., 2019); (Olaimat&*Holley*, 2012). In perfect conditions, pathogens would be absent from the soil, water,

and biological modifications, preventing contamination (Zhang *et al*., 2021). Pathogens, on the other hand, can survive in the environment for long periods of time and become extensively spread (Suleyman *et al*., 2018).

2.2.1 Pre-harvest contamination of Fresh Produce

The main sources of pre-harvest contamination are soil and inadequately composted animal dung used as organic manure (Ramos *et al*., 2019). Due to use of animal feces as manure, the soil is prone to be a natural reservoir for a diversity of human pathogens, including *E. coli* pathogens (Jonas *et al*., 2019). *E. coli O157: H7* can live in the soil for 7 to 25 weeks, depending on the soil type, humidity level, and temperature (Luna-Guevara *et al*., 2019). This bacterium can also survive during the storage and distribution of crops. According to [Launders et al., 2016], the presence of STEC O157 in potatoes poses a risk because it may cause cross contamination *E. coli O157: H7* with other raw foods (Ngene et al., 2020). Furthermore, animal manure is widely used in the production of organic foods (Khalil et al., 2019).

2.2.2 Post-harvest contamination of Fresh Produce

In some circumstances, the presence of *E. coli* in vegetables such as alfalfa sprouts, fresh spinach, and raw clover sprouts is much higher at the end of the postharvest process than at the beginning (Iwu and Okoh, 2019). This could be due to later direct contamination or pathogen proliferation during raw vegetable postharvest operations (Lenzi*et al*., 2021). The presence of E. coli in postharvest packing stages could imply fecal contamination and the presence of enteric pathogens from feces (Allende *et al*., 2018). When *E. coli* O157: H7 was isolated from specific types of fresh vegetables, the prevalence was rather low, these bacteria can cause disease in consumers (Zada *et al*., 2022).

Table 2.1: Contamination Sources during the Pre and Post-Harvest Stages in produce

Stage	Contamination sources
Pre-harvest	Insecticides, fungicides, irrigation water, manure that has not been properly composted, human handling, and seasons are all factors to consider (fall, winter, and spring)
Post-harvest	Poor hygiene in harvesting and transporting equipment, contaminated water for washing and distributing equipment, grimy cutlery, and dirty processing equipment

2.2.3 Outbreaks Linked to Fresh Produce

Foodborne disease outbreaks linked to fruits and vegetables have increased dramatically (Fig. 2.1). *Salmonella* and *E. coli* are the most dangerous pathogens. *E.coli O157:H7,* despite the fact that a wide spectrum of dangerous bacteria exists in theory (Lin *et al*., 2022). At any step in the supply chain, microorganisms can taint fresh produce. Sprouting seeds, tomatoes, and leafy greens have all been linked to high-profile foodborne disease outbreaks, with sprouted seeds, tomatoes, and leafy greens being the most prevalent. (Riggio *et al*., 2019). The underlying causes for certain product kinds being linked to the bulk of outbreaks can be explained in part by market volume (Bugos and Ivanov, 2021).The *E. coli* O157:H7 outbreak related to baby spinach in America in 2006 was unique in that the pathogen's strain was isolated from affected patients, spinach in unopened bags, and the farm where the outbreak occurred (Mulaosmanovic *et al*., 2021). The source of the spinach contamination was thought to be *E. coli* O157:H7 transmission from a nearby cow ranch by infected wild pigs that gained access to the crop through a damaged fence (Lama and Bachoon, 2018). However, a survey of the Salinas valley in the summer of 2006 discovered a significant incidence of *E. coli* O157:H7, indicating that the true route might have been via polluted irrigation water (Coulombe *et, al.*, 2020).

Table 2.2: Outbreaks Linked to Fresh Produce

Date	Pathogen	Produce	Comments
December 2005	*Salmonella*	Mung bean sprouts	Canada,618 confirmed cases
February 2006	*Salmonella*	Alfalfa sprouts	Canada, sprout recall due to suspected contamination.
February 2006	*Salmonella*	Alfalfa sprouts	Australia,100 confirmed cases
June 2006	*E. coli*O121:H9	Lettuce	United states ,4 confirmed cases
July 2006	*Salmonella*	Fruit salad	U.S.A and Canada ,41 confirmed cases
September 2006	*E.coli*O157:H7	Spinach	U.S.A ,205 confirmed cases;3 deaths
September 2006	*Clostridium botulinum*	Carrot juice	U.S.A and Canada; 6 cases
October 2006	*E.coli* 0157:H7	Lettuce	U.S.A:81 confirmed
October 2006	*E.coli* 0157:H7	Lettuce	Canada: recall for suspected contamination
October 2006	*Salmonella*	Tomatoes	U.S.A:183 cases
August 2007	Shigella sonnei	Carrots	Canada, 4 cases
June 2008	*Salmonella*	Tomatoes	Unitedstate and Canada 1442 confirmed cases.
September 2008	*E.COLI* 0157:H7	Lettuce	United state and Canada,134 confirmed cases.
September 2008	*Salmonella*		United states,14 confirmed cases
November 2008	*Salmonella*	Alfalfa sprouts	UK, 32 confirmed cases
December 2008	*Salmonella*	Basil Alfalfa sprouts	United states, recall for the suspected contamination.

2.3 Game Meat

The majority of ready-to-eat game meats, particularly in West Africa, are typically those that are produced locally by smoking and drying (Ikoafe *et al*., 2021). Therefore, the method does not completely protect the meat from microbial attack by bacteria, fungi, or the toxic substances these bacteria produce (Gokoglu, 2019). When this type of meat is used as a source of food by an individual or group of people, especially when it is not properly cooked before consumption, it poses a serious threat to their health (Zupo *et al*., 2020).The vast variety of species that make up game meat includes donkeys, leopards, monkeys, grass cutters (Thryonomys and Swindenanns), African elephants, and antelope (Alcalaphinae) (MIkeh *et al*., 2021).

Similar to ready-to-eat meat, bush meat is typically used as a source of income because it can be sold for money or capital, is a cheaper source of protein than other sources, and can be exported or sold domestically (Tang *et al.*,2019). When bush meat is properly dried, which is a labor-intensive process involving many important steps beginning with the slaughter of the animal, carcass trimming selection of the raw material, proper cutting and pre-treatment of the pieces to be dried, the meat can be consumed (Hassan, 2020).

Additionally, to prevent severe rancidity, ready-to-eat bush-meat with a high fat content should be consumed as soon as possible after cooking (Otoo, 2019). These game meats must also be regularly checked for spoilage-related off odor, which results from improper handling and/or drying of the meat (Charmpi *et al*., 2020). It is imperative to thoroughly sort out any deteriorating bush meat and not cook it (Chaves *et al*., 2019).The availability of water, its activity, pH value, redox potentials, moisture content, temperature, relative humidity, and nutrient content are all important factors that contribute to the microbial contamination of ready-to-eat bush meat (Nowshad *et al*., 2021). Because of the meat's nutritive value or other characteristics, bush-meats are frequently consumed by a variety of people, especially in Nigeria, irrespective of their age and race (Onyekuru *et al*., 2018). But they are also vulnerable to microbial attack, bacterial growth, and fungal proliferation when not handled properly, which could cause food-borne illnesses or diseases in consumers because these microbes are pervasive and cause bush-meat to deteriorate, lowering its acceptability and economic benefits to people (Chi mang, 2021).When these microorganisms infiltrate game meat, they have the potential to ruin its appealing appearance, turn its pleasant smell foul, and perhaps even change its flavor to one that is soured and unappealing to the consumer (Niman, 2021).

2.4 Zoonotic Diseases and Risks

Animal species consumed as bushmeat can be natural reservoirs for diseases that can be passed on to humans (zoonoses) (Hilderink and Winter, 2021). Indeed, many pathogens (viruses, bacteria, protozoa, and parasites) found in a range of bushmeat species are transmissible to humans (Rahman *et al*., 2020). In Africa, there are twenty-five different parasites (including *Trichuris* sp., *Ancylostoma* sp., roundworms, *Toxoplasma gondii*, and *Strongyloidesfuelleborni*), nine major virus types (including SIV, HTLV, Marburg virus, Lassa virus, Ebola virus, Nipah virus, and herpes), and eight types of bacteria (including *Escherichia coli*, *Salmonella* spp., and *Campylobacter* spp.) that have been detected in bushmeat and can be spread to humans (Fa *et al*., 2019).Not all transmissions happen via ingestion. In actuality, the majority of zoonoses are transmitted to people by coming into contact with an animal's bodily fluids and excrement during the preparation and butchering of raw meat before cooking (Schweon and Vitale, 2020). In cases of zoonosis transmission between animals and humans, rodents, rats, monkeys, and small antelope (duikers and chevrotains) are the most frequently mentioned species (Fa *et al*., 2020). While viral zoonotic disease outbreaks like HIV and Ebola typically receive the most media attention, bacterial and parasitic infections acquired through bushmeat consumption are a significant cause of serious illness among populations residing in tropical and subtropical forest regions (Milbank and Vira, 2022).

Greater focus is needed on these widespread illnesses, which are frequently correlated with unkempt conditions in areas where meat is butchered and prepared (Mensah *et al*., 2022). Two potential tactics for preventing this kind of transmission are increased access to clean water and the use of gloves and contemporary utensils (Murray and Saiman, 2022). Given the likelihood that bushmeat consumption will increase in the future, it is becoming more and more urgent to think about the best methods for growing, transporting, and processing bushmeat in accordance with culturally appropriate health and hygiene standards (Saylors *et al*., 2021).

2.5 *Escherichia coli*

Escherichia coli are typical inhabitant of the human large intestine (Cieplak*et al*., 2018). The majority of strains are non-pathogenic; however some strains can develop enterotoxins or invasion factors from bacteriophages or plasmid DNA and become pathogenic (Yim et al., 2021). These virulent strains are responsible for diarrheal diseases globally, including neonatal meningitis, septicemia and urinary tract infections (UTIs) (Riley, 2020).E coli are gram-negative bacilli in the Enterobacteriaceae family (Azimi *et al*., 2021). They are nonsporulating facultative anaerobes. Ecoli strains containing the K1 capsular polysaccharide antigen cause approximately 40% of septicemia and 80% of meningitis (Rahim, 2019). Different strains of E coli are linked to a variety of different diarrheal illnesses. Enterotoxigenic E coli (ETEC), enteroinvasive E coli (EIEC), and Shiga toxin-producing E coli (STEC) are among them. The prototypic strain of STEC is E coli O157:H7 (Santos *et al*., 2020). Each E coli class has unique somatic (O) and flagellar (H) antigens as well as virulence characteristics(Gebisa *et al*., 2019).

2.5.1 Epidemiology

Diarrheogenic E coli strains are found worldwide. The infection is spread through the fecal-oral route, primarily through contaminated water and food (Puvaca and Frutos, 2021). STEC, particularly E coli O157:H7, is shed in ruminant feces such as cattle, sheep, deer, and goats. Infection in humans is spread through contaminated food or water, or through direct contact with an infected person (Kim *et al*., 2020). Ground beef, animal exposure in public settings (petting zoos), contaminated apple cider, and water contamination in recreational areas have all been linked to outbreaks (Rani *et al*., 2021). Most *E. coli* strains require 10 hours to 6 days incubation. The incubation period for E coli O157:H7 is typically 3 to 4 days (Song and Kang, 2022). In neonatal infections, *E. coli* and other gram-negative bacterial pathogens are frequently transmitted through the maternal genital tract (Viet *et al*., 2021).Person-to-person transmission from nursery personnel or environmental sites can result in hospital acquisition of gram-negative organisms (Berhanu and Pal, 2020). The incubation period varies, with the onset of infection ranging from birth to several weeks after birth (Puopolo *et al*., 2018).

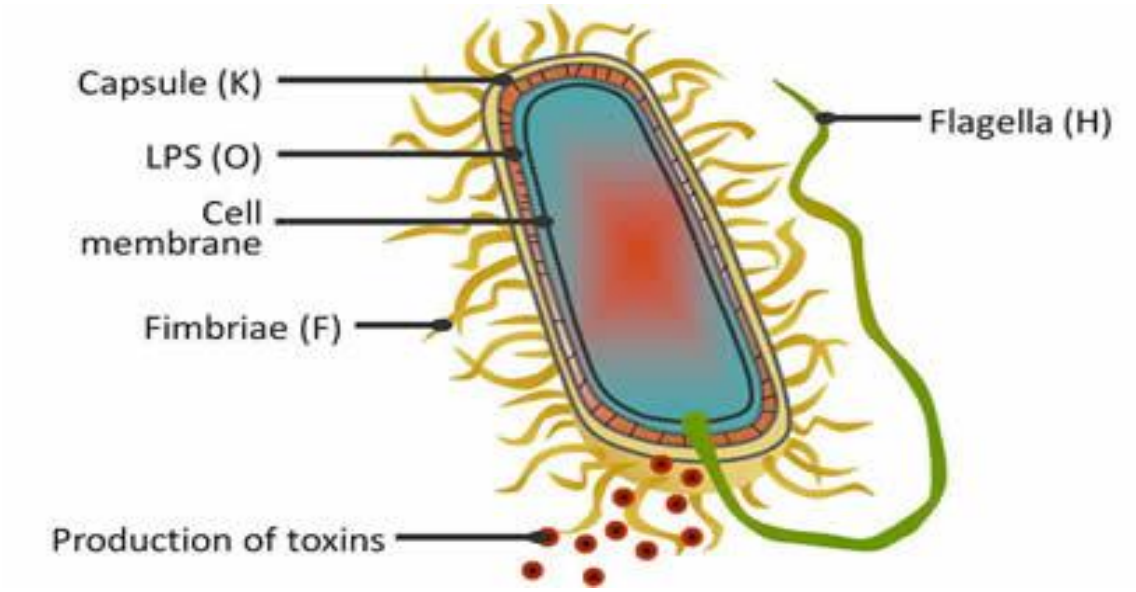

Figure 2.1: Typical Structure of Pathogenic *E.coli*

2.5.2 Classification of *E.coli*

Escherichia coli are Gram-negative facultative anaerobic rods that are part of the typical gut microbiota in humans and animals (Okposhi*et al*., 2022). Although the majority are nontoxic, pathogenic variations cause either enteric (diarrhoeagenic *E. coli* (DEC)) or extra-intestinal (extra-intestinal pathogenic *E. coli*) infections in humans (ExPEC). ExPEC cause urinary tract infections, as well as mastitis, septicemia, peritonitis, Gram-negative pneumonia, and meningitis to a lesser extent (Solorzano*et al*., 2019).

The diarrhoeagenic *E. coli* are divided into seven groups based on virulence traits and mechanism of pathogenicity and include;

- Shiga toxin-producing *E. coli* (STEC).
- Enteropathogenic *E. coli* (EPEC).
- Enterotoxigenic *E. coli* (ETEC).
- Enteroinvasive*E. coli* (EIEC).
- Enteroaggregative *E. coli* (EAEC).
- Diffusely Adherent *E. coli* (DAEC).
- Adherent Invasive *E. coli (*AIEC).
- Enteropathogenic *E. coli* (EPEC).

2.5.2.1 Enteropathogenic *E. coli* (EPEC).

Enteropathogenic *E. coli* (EPEC) carry *eae,* but are *stx* negative, and thus belong to the group of bacteria known as attaching and effacing (A/E) pathogens, forming A/E lesions in the small intestine (Moxley, 2022). EPEC are subdivided into typical (tEPEC) and atypical (aEPEC) strains depending on the presence (or absence) of the EPEC Adherence Factor (EAF) plasmid which includes the bundle forming pili (Bfp) operon encoding the pili required for localised adherence on epithelial cells (Munhoz*et al*., 2021). In general, EPEC are non-invasive and do not produce heat-labile (LT) or heat-stable (ST) enterotoxins. EPEC infection is characterised by watery or bloody diarrhoea with the occurrence caused by tEPEC decreasing with age due to the loss of specific EPEC receptors and/or the

development of immunity (Hassan *et al*.,2021). aEPEC infections, once considered to predominate in developed countries, are now known to exceed those caused by tEPEC throughout the world (Carlino, 2019).

2.5.2.2 Diffusely-adherent *E. coli*

The diffusely adherent *E. coli* (DAEC) are comprised of a heterogenous group of *E. coli* strains with variable virulence and that do not display the patterns of adherence observed with other *E. coli* pathotypes (Aijuka*et al*., 2018). They are identified by their adherence to HEp-2 as well as HeLa cells in a diffuse pattern and are divided into two classes (Javadi*et al*., 2020). The first class carry afimbrialadhesins (Afa) or Drori antigen (Dr) adhesins and have been found to be associated with urinary tract infections (UTIs) (pyelonephritis, cystitis and asymptomatic bacteriuria) and with various enteric infections (Mathebula, 2018). In Afa/Dr DAEC, the F1845 and DR adhesins bind to the brush border-associated decay-accelerating factor (DAF) molecule, common on the surface of polarised epithelial cells, destroying or rearranging the microvilli and forming brush border lesions (Turniak and Sobieszczanska, 2019). This manifests as watery diarrhoea that may be persistent and severe in young children (Sharma *et al*., 2022). Adults may be asymptomatic, but carriage may lead to chronic inflammatory intestinal diseases such as Crohn's disease (Rogler*et al*., 2021). The second class of DAEC strains includes *E. coli* strains that express an adhesin involved in diffuse adherence (AIDA-I) , which is a potential cause of infantile diarrhea (Waititu, 2020).

2.5.2.3 Enteroaggregative *E. coli*

The ability of enteroaggregative E. coli (EAEC) to adhere to tissue culture cells in a distinctive "stacked brick-like" manner is one of their distinguishing characteristics (Schiller *et al*., 2021). This ability is typically mediated by aggregative adherence fimbriae (AAF), which are encoded by the aggR genes (Boisen*et al*., 2020). A general classification of typical (aggR positive) and atypical (aggR negative) groups has been made as a result of the fact that not all EAEC strains are aggR positive (Petro et al., 2020). Additionally, the astA genes' encoded enteroaggregative heat stable toxin (EAST1) is produced by them. Acute or ongoing diarrhea, frequently accompanied by mucus, nausea, vomiting, a low-grade fever, and occasionally bloody stools are among the symptoms (Ellis, 2018). EAEC cause both endemic and epidemic diarrheal diseases worldwide, infecting both children and adults (Ghosh et al., 2022).

2.5.2.4 Enterotoxigenic *E. coli*

Enterotoxigenic *E. coli* (ETEC) are a major cause of traveller'sdiarrhoea and are endemic in most developing countries with significant mortality rates in children (Khalil*et al*., 2021).They are a diverse group of many different serotypes. ETEC cells adhere to the epithelium of the small intestine via one or more colonisation factor antigens (CFA) followed by the expression of heat labile (LT) or heat stable (ST) enterotoxins (Smith et al., 2022). Both are involved in the deregulation of ion channels in the epithelial cell membrane. The diarrhoea may be accompanied by cramps, nausea and headaches but fever is usually absent. In a study published in 2004, Wennerås and colleaguesestimated that there were approximately 840 million cases of ETEC annually in developing countries with 280 million of these being in children less than 4 years of age(Porter, 2021). ETEC are usually transmitted via contaminated water and food.ETEC can grow in a variety of environments, including rivers, drinking water, irrigation water, and fresh produce. (Chigor*et, al.*,2020).

2.5.2.5 Enteroinvasive*E. coli* (EIEC)

Enteroinvasive *E. coli* (EIEC) and *Shigella* spp are facultative intracellular pathogens that cause a mild form of dysentery, characterised by the appearance of blood and mucus in the faeces (Hassan *et al*., 2021). The early stage of this infection is usually characterised by mild watery diarrhoea, fatigue, malaise, fever and anorexia but as the infection develops the patient may also suffer abdominal cramps, tenesmus and scanty stools often accompanied by blood and mucus(Settanni*et al*., 2021). In the absence of medical attention, the patient may also show signs of dehydration. Most cases are self-limiting although severe life-threatening complications may occur, especially in developing countries where the host may be malnourished, immune-compromised and without access to adequate treatment (Upadhyay, 2021). There are 21 major serotypes of EIEC, the majority of which are non-motile and lacking the H antigen. *Shigella* includes 49 sero- and subserotypes clustered into 4 species including *S. dysenteriae, S. flexneri, S. boydii* and *S. sonnei*. EIEC and *Shigella* spp. carry a 220 kb virulence associated invasion plasmid including the invasion plasmid antigen (Ipa) proteins encoded on the *ipa* operon, which confers an ability to enter and disseminate between intestinal epithelial cells (Raso, 2021). Thus, these bacteria are highly invasive. Transmission is usually mediated by contaminated food and/or water via the faecal-oral route, but direct person-to-person transmission has also been reported (Hansson *et al*., 2018).

2.5.2.6 Adherent Invasive *E. coli* (AIEC)

This pathotype is identified by its capacity to: 1) adhere to Caco-2 intestinal epithelial cells that have undergone differentiation and/or have not; 2) invade I-407 cells; 3) induce host cell action polymerization and microtubule recruitment in bacterial uptake; and 4) persist and replicate inside J774-A1 macrophages (Govindarajan *et al*., 2020. Consistently detecting invasive determinants in all AIEC has not yet been accomplished (Rossi *et al*., 2022). They are currently thought to be the most likely factor contributing to the onset of Crohn's disease in genetically susceptible individuals (Sharif *et al*.,, 2018). The stx genes may be acquired by any of the aforementioned pathotypes (Singh *et al*., 2019). For instance, it has been demonstrated that Shigella spp., EPEC, and EAEC can all acquire the stx gene and produce a condition resembling STEC (Moxley, 2022).

2.5.2.7 Enterohaemorrhagic *E. coli*

The entero-hemorrhagic *E. coli* (EHEC) strains cause bloody and non-bloody diarrhea (Meng and Doyle, 2020). The most infamous piece of this pathotype is strain O157:H7, which causes bloody diarrhea and no fever (Rani *et al*., 2021). EHEC can cause hemolytic uremic condition and unexpected renal failure (Detzner *et al*., 2020). It utilizes bacterial fimbriae for connection (*E. coli* basic pilus, ECP), and is tolerably intrusive and has a phage-coded Shiga poison that can cause extraordinary provocative responses (Sadiq, 2020).

2.5.2.8 Shiga toxin producing *Escherichia coli* (STEC)

The most regular facultative anaerobe identified in the gastrointestinal tracts of warm-blooded animals and humans is *Escherichia coli*(Martinez-Medina, 2021). Virulence genes are hardly found in *E. coli* strains (Desvaux*et al*., 2020). Pathogenic strains distinguished by their ability to generate verotoxins (also known as Shiga toxins) are referred to as verocytotoxigenic *E. coli* (VTEC) or Shiga toxin-producing *E. coli* (STEC) (Kim et al., 2020). Morbidity and mortality associated with recent major outbreaks of gastrointestinal illness caused by Shiga toxin-producing *Escherichia coli* (STEC) have emphasized the threat these organisms pose to public health (Ekici and Dumen, 2019). These types of epidemics have the potential to strain acute care services, even in nations with highly developed health-care systems (Kain and Fowler,2019). This pathogen group, the toxin, its structure and function, its interaction with host cell receptors,

and signs and symptoms of illness receives a lot of attention (Yumoto *et al*., 2019). The ability to manage STEC illness in people and lower epidemic rates is based on prompt diagnosis and identification of the source of infection (Valilis*et al*., 2018). Significant advances in awareness of the pathology of STEC infection have occurred in recent years, contributing to the development of improved diagnostic tools as well as treatment and preventive effortsrevealed the characteristic which differentiates STEC from other types of pathogenic *E. coli*, namely, the synthesis of a toxin with a severe and permanent cytopathic impact on Vero (African green monkey kidney (Joseph *et al*., 2020) cells. Verotoxigenic *E. coli* strains were related to instances of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) in the early 1980s (Thomas *et, al.*,2018). STEC strains belong to a wide spectrum of serotypes and can cause significant human disease (Nguyen *et al*., 2021). O157:H7 is a prominent STEC serotype in many regions of the world and has been the type most often connected with major outbreaks (Good, 2022).

Serotype data has been used as a determinant for identifying STEC strains that have the potential to cause major human infections since the discovery of STEC serotype O157:H7 as a prominent foodborne pathogen (Huang *et al*., 2021). When non-O157 STEC strains were linked in outbreaks and other serotypes were recognized as being of health concern, the focus on serotypes remained. However, serotype is not a virulence factor in and of itself, and not all STEC serotypes have been linked to human infections (Butt *et al*., 2021). As a result, some people have devised the term enterohemorrhagic *E. coli* to refer to a subgroup of STEC that contains pathogenic strains, the majority of which have eae.Serotype O157:H7 is the most common EHEC strain, but others from serogroups O26, O111, O103, and O145, to mention a few, have also caused serious human sickness. Alternate EHEC strains, such as those with the serotypes O113:H21, O91:H21, O104:H4, and others, do not contain eae but cause HUS , indicating that these viruses have other attachment mechanisms (Panel *et al*., 2020). Because many STEC virulence genes are migratory and can be lost or transferred to other bacteria, STEC strains of the same serotype may or may not have the same virulence genes or pose the same threat (Bai *et al*., 2022). The likelihood of a STEC strain causing severe disease or the severityofSTEC-relatedillness.

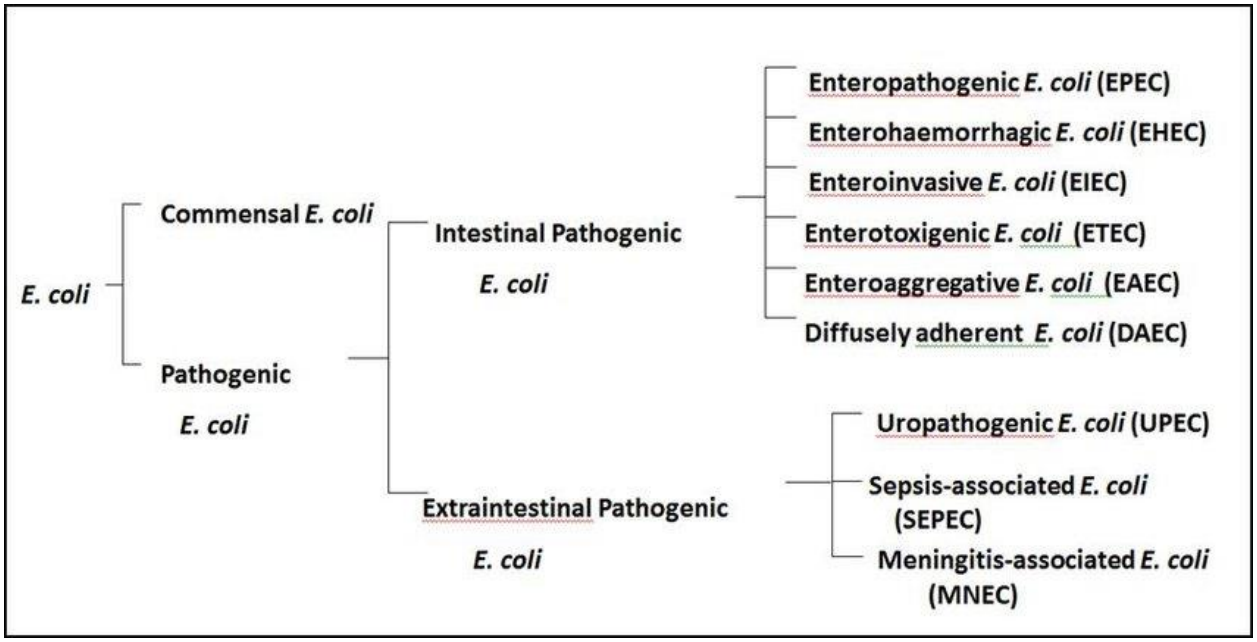

Figure 2.2: Classification of *E. coli* into three main groups: commensal, intestinal pathogenic and extraintestinal pathogenic adapted

2.5.3 STEC as a Zoonotic Pathogen

STEC are zoonotic pathogens that enter the human body via contaminated food and water. Individual cases and outbreaks have been linked to direct animal contact (for example, farm visits), environmental contamination, and fecal-oral transmission (Dallman *et al*., 2021). Shiga toxins (Stx), named after the toxin produced by *Shigella* dysenteriae serotype 1, characterize STEC infections (Lee and Tesh, 2019).

However, not all STEC are capable of infecting humans, and only a subset of these is virulent, belonging to the pathovar widely recognized as enterohemorrhagic *E. coli*(EHEC) (Santos *et al*., 2020). Most EHEC contain the locus of enterocyte effacement (LEE), a chromosomal pathogenicity island that encodes a type III secretion system, an adhesin called intimin, and its receptor Tir. The eae gene encodes intimin, which allows bacteria to adhere to the epithelia, causing attaching and effacing lesions (Gebisa *et al*., 2019). It is shared by enteropathogenic *E. coli* (EPEC) strains (Martins *et al*., 2020). Enterohemorrhagic *E. coli* carrying LEE are referred to as typical EHEC, while those that do not are referred to as atypical EHEC (Schwidder *et al*., 2019).

There are two types of Shiga toxins (Stx1 and Stx2), and the stx toxin genes are carried by lambdoid bacteriophages that have been integrated into the *E. coli* genome (Pinto *et al*., 2021). The *E. coli* chromosome; the stx1 gene has four subtypes (a, c, d, and e), whereas the stx2 gene has twelve (a to l). There have been no reports of strains with more than one stx1 subtype. A given strain, however, may have both a stx1 and a stx2 subtype gene, or more than one stx2 subtype gene. Many STEC are attaching and detaching (A/E) bacteria, they move the eae gene on the locus of enterocyte effacement (LEE) and form distinguishable lesions on the surfaces of intestinal epithelial cells (Gill et al., 2022) . The most common STEC serogroup related to human illness is O157, and its molecular pathogenesis has indeed been extensively researched (Joseph *et al*., 2020). it is divided into three genetic lineages, *E. coli* O157:H7 (I, II, and I/II) as a result of an ancestral clone's geographical spread and subsequent regional expansion (Lawal *et al*., 2022) .

Table 2.3: Classification of *E. coli* associated with Diarrhoea

PATHOTYPE	EPIDEMIOLOGY	TYPE OF DIARRHOEA	MECHANISM OF PATHOGENESIS
Shiga-toxin producing *E.coli*	Hemorrhagic colitis and hemolyticuremic syndrome in all ages	Bloody or nonbloody	Shiga toxin production, large-bowel attachment, coagulopathy
Enteropathogenic *E.coli*	Acute and chronic endemic andepidemic diarrhea in infants	Watery	Small-bowel adherence and effacement
Enterotoxigenic *E.coli*	Infant diarrhea in resource-limited countries and traveler's diarrhea in all ages	Watery	Small-bowel adherence, heat stable/heat-labile enterotoxin production
Enteroinvasive*E.coli*	Diarrhoea with fever in all ages	Bloody or nonbloody; dysentery	Adherence, mucosal invasion, and inflammation of large bowel
Enteroaggregative *E.coli*	Acute and chronic diarrhoeain all ages	Watery,occasionally bloody	Small- and large-bowel adherence, enterotoxin and cytotoxin production

2.5.4 Ecology of Pathogenic *E.coli*

E. coli bacteria are continuously released into the immediate environment of the animals through their feces, contaminating the pens, litter, and floor of animals kept indoors as well as the soil for animals kept outdoors (Delsart *et al*., 2020). They can survive for extended periods of time, possibly longer than 10 weeks and are spread through slurry and manure, which are applied to fertilized fields and crops as well as ground and surface water (Soares *et al*., 2021). E. coli is spread from one animal to another by contaminated feed, handlers, drinking water, and possibly farm to farm by means of machinery like transport trucks (Rasschaert *et al*., 2020). Infection occurs either orally or, in the case of birds, through inhalation of contaminated dust (Menanteau *et al*.,2018).Humans can also contract E. coli from animals through direct contact, ingesting tainted food or water after manure has been spread, or eating meat after carcasses have been contaminated at the butcher shop (Anyanwu *et al*., 2020). ETEC-related intestinal infections and oedema disease STEC in pigs is frequently communicable; the same strain has been identified in large populations, in numerous sick pigs, and from one batch to another (Ledwaba, 2020). After infection, these strains are typically only shed for a few days, most likely as a result of the emergence of immunity. ExPEC infections behave differently from contagious diseases (Day *et al*., 2020). Each animal has a unique strain makeup, and multiple strain infections can frequently be found in the same animal. For extraintestinal infections like mastitis and urogenital tract infections, the faecal microflora serves as a reservoir. Similar to this, EPEC are frequently found in the intestines and feces of healthy animals, but they can sicken animals who have compromised immune systems (Haley et al., 2022).

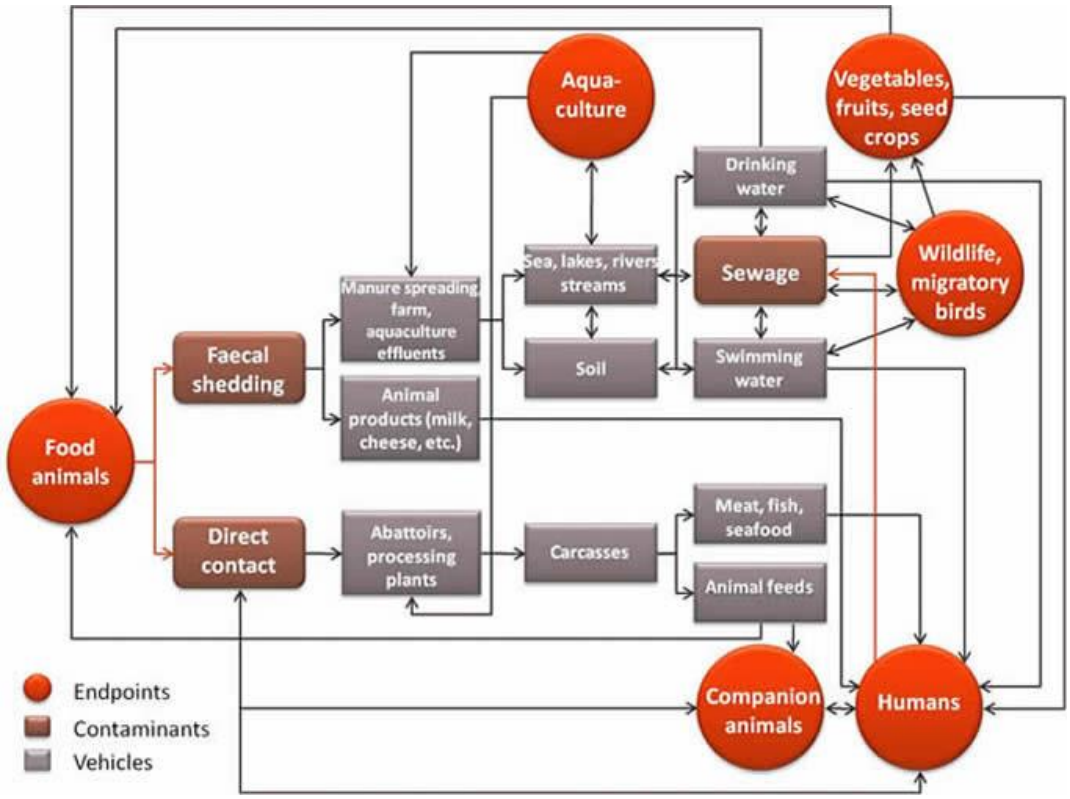

Figure 2.3: Ecology of *E.coli*

2.5.5 Virulence Factors of *E.coli*

The ability of *Escherichia coli* to produce toxins enhance its ability to infect a host with disease (Duan et al., 2019). It produces α-hemolysin toxin which is a pore-forming cytotoxin, it inserts into the plasma membrane of the host cells thereby causing leakage of the host's cytoplasmic contents and eventually leading to cell death (FitzGerald *et al*., 2020). Another toxin it produces is one which is similar to the shiga toxin and inhibits protein synthesis by ribosomal binding. Also, it produces labile toxin (LT) (Menge, 2020). The widespread species Escherichia coli includes a broad variety of different types, ranging from highly pathogenic strains causing worldwide outbreaks of severe disease to avirulent isolates which belong to the normal intestinal flora or which are well known and safe laboratory strains (Santos *et al*., 2020). The pathogenicity of a given strain is mainly determined by specific virulence factors which include adhesins, invasins, toxins and capsule (Jajere, 2019) (Table 1.2). They are often organized in large genetic blocks, called pathogenicity islands located either on the chromosome or on large plasmids and which are often transmitted by bacteriophage or other mobile elements (Novick, 2019)

Table 2.4: Virulence Factors in *E.coli*

Primary virulence factors	Secondary virulence factors
Shiga toxins (Stx1, Stx2)	Adherence factors/Fimbriae (P-, S-, F1C-fimbriae, Bfp, AAF)
Heat labile toxins (LTI, LTII)	Colonization factors (CFA/CS)
Heat stable toxins (STa, STb, EAST)	Invasion factors (Ipa)
Hemolysins (Hly, Ehx/Ely)	Iron transport systems (aerobactin/Iuc)
Cytotoxic necrotizing factors (CNF1, CNF2)	Capsule
Type III secretion system(s) (Sep)	
Intimin (Eae)	

2.5.6 Clinical Manifestations

Septicemia and meningitis can occur in newborns, both term and preterm. Early-onset infection, particularly in the first two days after birth, indicates vertical transmission, whereas late-onset infection suggests nosocomial or community acquisition (Odabasi and Bulbul, 2020). Early-onset meningitis is more likely to be caused by group B Streptococcus, E coli, and Listeria monocytogenes, whereas late-onset meningitis can be caused by other gram-negative organisms and staphylococcal species (Wong *et al*., 2021). The pathotype of E coli that causes meningitis and sepsis is known as neonatalmeningitis-associated E coli. The K1 capsule of neonatal meningitis-associated E coli contains sialic acid, which increases the bacteria's ability to cross the blood-brain barrier (Le Guennec *et al*., 2020).

Clinically, E coli-caused neonatal septicemia or meningitis cannot be distinguished from infection caused by other agents (Riley, 2020). Fever, temperature instability, abnormal heart rate, respiratory distress, apnea, cyanosis, lethargy, irritability, jaundice, vomiting, diarrhea, and abdominal distention are all clinical signs of septicemia (Naik *et al*., 2019). Maternal intrapartum infection, gestation of less than 37 weeks, low birth weight, and prolonged rupture of membranes are all risk factors for neonatal gram-negative bacterial infections (Puopolo *et al*., 2018). Neonates with defects in the integrity of their skin or mucosa, as well as gastrointestinal or genitourinary tract abnormalities, are also at increased risk (Ogunrinola *et al*., 2020).

ETEC strains have been linked to self-limited gastrointestinal illness with abdominal cramping and watery stools that lasts 1 to 5 days (Kotloff, 2022). ETEC is common in infants in resource-limited countries, but it is uncommon in the United States as a cause of diarrhea (Schuetz, 2019). These strains, however, are a major cause of traveler's diarrhea, with infection typically caused by consuming contaminated food or water (Baker-Autin *et al*., 2018).Toxins produced by STEC strains are similar to those produced by *Shigella dysenteriae* type 1 (Fogolari *et al*., 2018). These bacteria have been linked to diarrhea, hemorrhagic colitis, hemolytic uremic syndrome (HUS), and postdiarrheal thrombocytopenic purpura (usually in adults) (Joseph *et al*., 2020). STEC O157:H7 is the most pathogenic E coli prototype (Tolen *et al*., 2018). STEC can cause bloody diarrhea or occult positive diarrhea. A third of the cases have fever and severe abdominal pain (Sell and Dolan, 2018).

The EIEC strains are biochemically similar to Shigella and cause disease by invading intestinal epithelial cells (Pakbin*et al*., 2021). These strains, like Shigella, can cause watery diarrhea, fever, crampy abdominal pain, and tenesmus (Talaat*et al*., 2021). In children under the age of two, enteropathogenic E coli strains cause watery diarrhea and, in some cases, severe dehydration (Snehaa*et al*., 2021). These illnesses are most common in developing countries. Children who have chronic diarrhea may experience growth retardation (Chifunda and Kelly, 2019). In children and adults, diffusely adherent E coli causes watery, sometimes bloody diarrhea (Suleiman *et al*., 2022). Pathogenicity has not been determined definitively, but it involves the bacteria adhering to the epithelial cells of the large intestine in a diffusion manner (Dubruil, 2020).

HUS is a serious side effect of STEC enteric infections, defined by the triad of microangiopathic hemolytic anemia, thrombocytopenia, and renal failure (Exeni*et al*., 2018). In North America, the most common serotype is E. coli O157:H7, which usually appears 2 weeks after the onset of diarrheal symptoms (Laura *et al*., 2018). It affects up to 20% of children suffering from E coli O157:H7 diarrhea. 50% of the cases are severe enough to necessitate dialysis, and 3% to 5% of patients die as a result of the illness (Yinen*et al*., 2020).

UPEC strains cause approximately 80% of community-acquired UTIs and 30% of nosocomial UTIs. Infections in children are frequently caused by urinary tract blockages, which result in pools of stagnant urine (Momoh and Ayodele-Asowata, 2022). UPEC can live in the colon before being introduced into the urethra (Roussel *et al*., 2022). The colonization of the periurethral area by enteric pathogens is the first step in the development of a UTI (Mestrovic *et al*., 2020). Bacteria can enter the bladder and kidney thanks to a variety of virulence factors. E. coli has pili, which are hairlike appendages on the cell surface that improve the bacteria's ability to adhere to the uroepithelium (Zhu *et al*., 2018). Furthermore, UPEC strains contain type 1 and P fimbriae, which increase virulence and play a role in initial urethral colonization, and many UPEC strains produce hemolysin, which may be involved in the progression of kidney disease (Bessaiah *et al*., 2021).

2.5.7 Diagnosis of *E. coli*

E coli septicemia, UTIs, and meningitis are diagnosed by the growth of E coli in blood, urine, or cerebrospinal fluid (Oldendorff *et al*., 2022). Diagnosis of diarrhea-associated E coli infection is typically difficult because most clinical laboratories cannot distinguish diarrhea-associated E coli strains from stool flora E coli strains (Mare *et al*., 2021). E coli O157:H7 is an exception, as it can be identified using selective media (for example, MacConkey agar base with sorbitol) (Hinenova *et al*., 2020). 90% of human intestinal E coli strains ferment sorbitol quickly, whereas O157:H7 strains do not. In addition, serologic diagnosis using enzyme immunoassays to detect serum antibodies to the O157:H7 lipopolysaccharide is available for outbreak investigations at the Centers for Disease Control and Prevention (Al-Awwal *et al*., 2022).

2.5.8 Treatment of *E.coli*

Children, especially infants, who are suspected of having a systemic E coli infection should be given intravenous antibiotics until the organism is isolated from cultures (Walker *et al*., 2019). Due to the fact that approximately 50% of E. coli are resistant to amoxicillin or ampicillin, an aminoglycoside or a third-generation cephalosporin is recommended as empiric therapy, pending sensitivity data (Morris and Cerceo, 2020). Once susceptibility results are available, a more specific antibiotic can be chosen. The duration of therapy is determined by the patient's clinical response and the location of the infection (Khatri *et al*., 2019). The typical duration of therapy for uncomplicated bacteremia is 10 to 14 days, 7 to 14 days for UTIs, and a minimum of 21 days for meningitis (Kaufman *et al*., 2019).Infections with multidrug-resistant E coli are becoming more common, with resistance mediated by the production of extended-spectrum b-lactamase (Ali *et al*., 2020) (ESBL). These isolates are most commonly isolated from hospitalized patients, but they are also becoming a more common cause of community-acquired infections (Fallah *et al*., 2019). Prior antibiotic administration, the presence of urinary or vascular catheters, and longer hospital or intensive care unit stays are all risk factors for infection (Allaw *et al*., 2022). Most b-lactam antibiotics, including third-generationk cephalosporins, can be hydrolyzed by ESBLs. They may also be resistant to trimethoprim-sulfamethoxazole, fluoroquinolones, and aminoglycosides (Yekani *et al*., 2018). Carbapenems are widely regarded as the drug of choice for treating ESBL-E coli infections (Yekani *et al*., 2018). The treatment of E. coli-associated diarrhea is primarily supportive, with special attention paid to hydration and electrolyte balance

(Joseph *et al*., 2020). Antimotility drugs should not be given to children who have inflammatory or bloody diarrhea (Viegelmann *et al*., 2021). ETEC diarrhea is usually self-limiting, but if it persists, antibiotic therapy may help to shorten the illness (Khalil *et al*., 2021). Azithromycin or a fluoroquinolone, such as ciprofloxacin, are effective, but fluoroquinolones are not approved for routine pediatric use (Gibani *et al*., 2020). A meta-analysis failed to confirm that children with hemorrhagic colitis caused by STEC have a higher risk of developing HUS if treated with an antimicrobial agent; however, most experts agree that children with E coli O157: H7 enteritis should not be treated with an antimicrobial agent (Tarr and Freedman, 2022).

2.5.9 Prevention

Good hand cleanliness and contact isolation of persons who are ill, particularly those who have ESBL infections, are preventive interventions for E coli infections (Lemmen and Lewalter, 2018). All ground beef should be completely cooked and raw milk should not be consumed to avoid contracting E coli O157:H7 illnesses (Asime *et al*., 2020). People with diarrhea brought on by E. coli O157:H7 should refrain from using recreational facilities like swimming pools and water slides for 2 weeks after symptoms subside due to the potential for waterborne transmission of the illness (Gonzalez and Michaels, 2021). Public health authorities should be made aware of any outbreaks, especially in child care facilities, as O157:H7 infection is a reportable condition (Astill *et al*., 2020).Doctors should encourage families to check their refrigerators for recalled items and not cook them if found in the event that an E. coli outbreak is reported in the media (Detwiler, 2020). Additionally, people should follow food safety precautions, refrain from consuming raw or undercooked beef, wash their hands, kitchen surfaces, and utensils with soap and water right away after coming into contact with raw ground beef, and refrain from contaminating other goods in their refrigerators (Koch *et al*., 2022). If they believe they may have become unwell after consuming recalled food goods, they should also be recommended to see a doctor. Symptoms often appear 2–7 days after ingestion(Thomas and Feng, 2020).Travelers should be urged to only consume bottled or canned beverages, refrain from using ice, and avoid consuming raw products and peeled fruits in order to prevent traveler's diarrhea (Long-Marin and Smith, 2021). However, they can consume fruits that they themselves peeled. When brushing teeth, only bottled water should be used. Antimicrobial medications are not advised for the prevention of traveler's diarrhea, but they may be necessary if the condition is severe or

accompanied by fever, bloody stools, or both (Leung *et al*., 2019). The diverse pathogens that can cause diarrhea impede the use of vaccines to protect against traveler's diarrhea (Levine *et al*., 2020). A number of studies suggest that an oral treatment, killed whole-cell vaccine combined with the nontoxic B subunit of cholera toxin (Dukoral) protects travelers from ETEC infection (Barry *et al*., 2019). This vaccine was approved for use as a traveler's diarrhea vaccine in the United States in late 2006 (Riddle *et al*., 2018). However, a conservative estimate based on the global prevalence of ETEC infection and the vaccine's efficacy suggested that it could prevent 7% or less of traveler's diarrhea cases (Seo *et al*., 2020).

2.5.10 Antimicrobial Resistance of *E. coli*

Antibiotics have long been used in human and veterinary medicine to reduce morbidity and mortality as well as the economic impact of bacterial infections (Thapa *et al*., 2020). However, E. coli has developed antibiotic resistance to one or more antibiotics, raising public health concerns (Davis *et al*., 2018). The widespread and increasing use of antibiotics is linked to the prevalence of resistant bacteria (Lazar *et al*., 2018). In the food production process, antimicrobials are used to prevent and control illnesses, improve growth, and increase feed efficiency in food-producing animals (Ma and Suzuki, 2018). The use of these antibiotics at low doses for extended periods of time, for example, to feed animals, can result in the selection and spread of antibiotic resistance to other microbes in the food chain (Khan *et al*., 2020). However, plant-based foods especially salads and RTE street foods/meals play a significant role in the spread of antibiotic resistance and are a growing source of concern (Ema *et al*., 2022).

Significant public health concerns have been raised after multiple researchers isolated multidrug-resistant (MDR) and extended-spectrum beta-lactamase (ESBL) producing E. coli from raw meat, vegetable salad, egg surface, unpasteurized milk, raw fish, and water. Studies on pathogenic E. coli serotypes in RTE foods must be continued to ensure total food safety (Sivakumar *et al*., 2021). It is now known that a variety of foods, especially those with animal origins and those that have been contaminated by sewage, can transmit pathogens to people (Larsson and Flach, 2022). Because drug-resistant strains of E. coli are becoming more common, treating infections with them has become more challenging globally (Mousavi *et al*., 2021). The health of consumers is seriously threatened by the emerging resistance found in E. coli strains to most antibiotics (Dagher *et al*., 2021).

Resistance can be acquired via plasmids and drug efflux systems, also resistance of amoxicillin, cotrimoxazole (due to presence of TEM-1 and TEM-2 betalactamse) and trimethoprim has increased over the years (caused by the frequent carriage on plasmids and integrons of *dhfr* resistance genes) (Mutuku *et al.*, 2022).

CHAPTER THREE

METHODS AND METHODLOGY

3 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.

3.1 Sample 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.

3.2 MATERIALS, REAGENTS AND EQUIPMENTS 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.

3.3 Preparation of Culture media

3.3.1 Buffered Peptone water

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. The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15mins. It was then dispensed by pipetting into various test tubes for serialdilution .Three types of media were used for the isolation of Escherichia coli; MacConkey agar (MAC), Nutrient agar (NA), Sorbitol-MacConkey Agar (SMAC).

3.3.2 Sorbitol-MacConkey Agar

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

3.3.3 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.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.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.The medium was then allowed to cool to a range of45-50°C and poured aseptically into sterile petri dishes and left to solidify. The medium is neutral red in colour.

3.3.4 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. Nutrient agar was prepared according to the manufacturer's instruction 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). The mixture was heated for a while to dissolve the powder completely and was then sterilized by autoclaving at 121°C for 15minutes.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 for isolation, to obtain 0.1% BPW, 1 gram of peptone powder was dissolved in one liter distilled water and is then autoclaved at 121^0C for 15 minutes.

3.4 Primary Enrichment

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 SMAC and MAC plate.

3.5 Serial Dilution

0.1mililitre 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.

For the SMAC and MacConkey agar plates, spread plates technique was used for plating of inoculum (samples). 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).

3.6 Sub-Culturing

The plate were checked after the required duration for the growth 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.

3.7 Preservation of Cultures

The *E.coli* isolates is preserved with the use of 20% glycerol and BHI (brain heart infusion). 750ul of BHI was dispensed into eppendorf tube and then E.coli culture was taken from the nutrient agar with an inoculating loop and then the loop containing the culture is dipped inside the eppendorf containing BHI and it is incubated at 37^0c for 24hours and then after 24hours 750ul of 20% glycerol is added and is placed inside the freezer for preservation.

3.8 DNA Extraction

1ml of pure BHI will be prepared and dispersed in 2ml of eppendorf tubes and autoclaved and then 50ul of each isolates of E. coli was added to the eppendorf tubes and then incubated at 37^0c , 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 , then 750ul of distilled water was added into the eppendorf and then vortex to mix well and it is centrifuged at 5000g for 3minutes,the supernatant was discarded leaving the pellet and then 750ul of distilled water is added again and it is centrifuged at 5000g for 3mins and then the supernatant is discarded leaving the pellet. 200ul of distilled was added and vortexed and then the eppendorf tubes containing the samples were placed in the heating block at 100^0c 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 a tube

3.9 Antimicrobial Susceptibility 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.

3.10 Molecular Identification of *E.coli*

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).

Table 3.1: PCR Reaction Components used for pathogenic E.coli amplification

REAGENT	INITIAL CONC	FINAL CONC	VOLUME/REACTION(**µl)**
Master Mix	5x	1x	2
StxIf	20	0.4	0.2
StxIR	20	0.4	0.2
VtxIf	20	0.25	0.125
VtxIR	20	0.25	0.125
Vtx2R	20	0.5	0.125
Vtx2R	20	0.5	0.125
PaLf	20	0.1	0.05
IPaLR	20	0.1	0.05
Mgcl	20	1.5	0.16
DH_2O	20		4.16
DNA	25		2
		2 µl	10

Table 3.2:PCR reaction components used for pathogenic *E.coli a*mplification

REAGENT	**INITIAL**	**FINAL**	**VOLUME/REACTION(µl)**
Master Mix	5x	1x	2
Stx2f	20	0.5	0.25
Sex2R	20	0.5	0.25
EltaF	20	0.45	0.225
EltaR	20	0.45	0.225
EaeAf	20	0.15	0.075
EaeaAR	20	0.15	0.075
$MgCl_2$	25	1.5	4.3
dH_2O			
		2	10

Table 3.34: Gene targets, primer sequences, primer concentrations and amplicon sizes for the multiplex PCR of pathogenic *E.coli*

Gene Target	Virulence factor/gene	Sequence (5´-)	Final concentration(µm)
Human estA	STIh	TTTCGCTCAGGATGCTAAACCAG CAGGATTACAACACAATTCACAGCAG TA	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

Table 3.4: Protocol for Thermal cycler for amplification of Diarrheagenic *E.coli*

Analysis	Step	Temperature	Time
1x	Initial denaturation	95℃	15 min
35x	Denaturation	94℃	6 min
	Annealing	57℃	40 sec
	Polymerization	72℃	50 sec
1x	Final polymerization	72℃	3 min
1x	Hold	4℃	∞

Table 3.5:ESBL Primer sequence (Oduro-Mensah*et al., 2016)*

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

CHAPTER FOUR

4 Results

The microbial analysis of the Ready to eat game meat and fresh produce samples gotten from Oyo, Ondo, Osun, Lagos and Ogun state were reported. All samples had pink (non-O157) and white (O157) raised, circular and smooth colonies on SMAC and MAC which indicates the presence of *E. coli* in the samples.

The results of the findings were summarized in the table below in Table 4.1 showing the Total Viable Count of Pathogenic *E.coli* isolates on Sorbitol MacConkey agar, MacConkey agar and Nutrient agar. While Table 4.2 shows the Antimicrobial susceptibility patterns of Pathogenic *E.coli* isolates

4.1 Total Viable Counts

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 $\log_{10}$ cfu/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. In Osun State, Antelope has highest tvc of 8.6$\log_{10}$ cfu/g then Hare has the lowest tvc of 4.8. In oyo state, Esii Tuku has the highest tvc of 7.1 and Eta has the lowest of 4.3. It is shown in table 4.1 below.

Table 4.1: Total Viable Counts for *E. coli* in gamemeat

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

4.2 Microbial Analysis of Ready-to-eat Game Meat

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* as shown in Figure 4.1 below. 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.

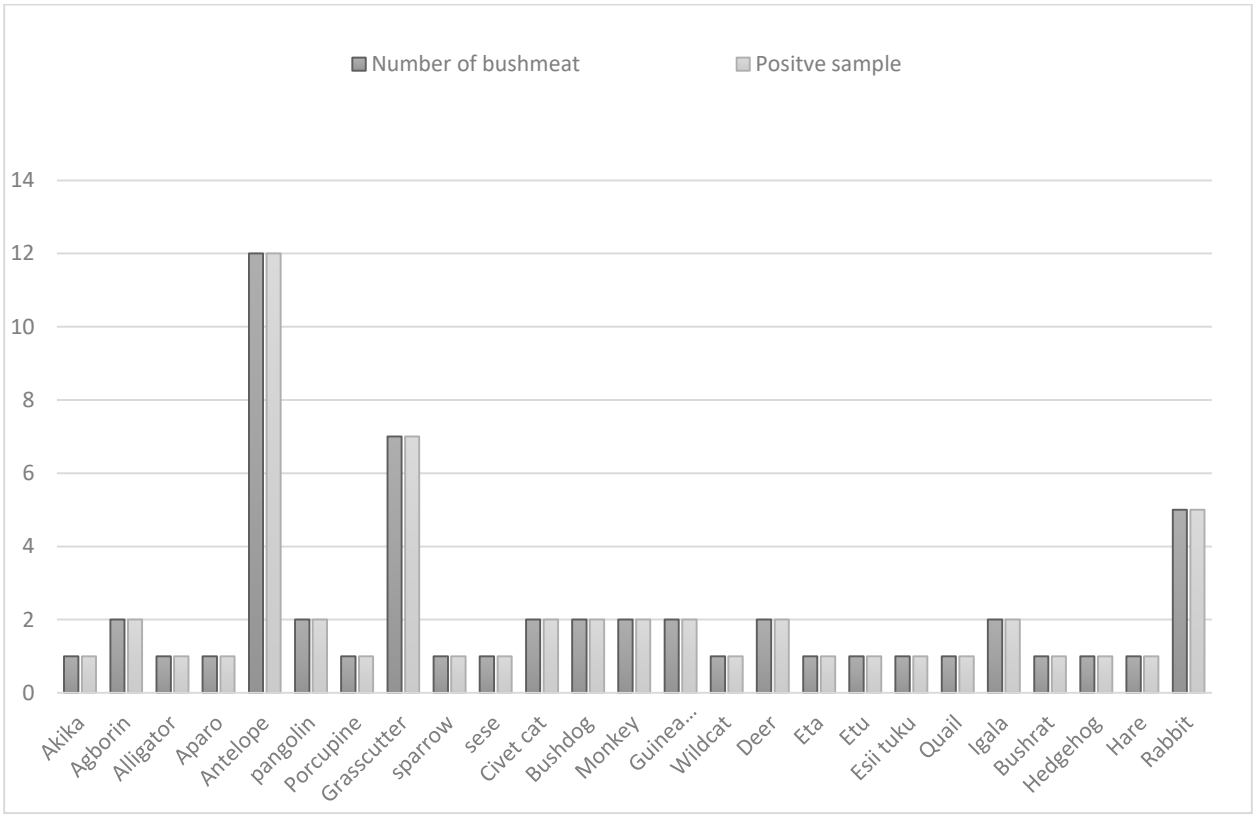

Figure 4.1: A Chart Representation of Microbial analysis of all Positive Game meat Samples

4.3 Microbial Analysis of Fresh Produce Samples

For fresh produce samples, they were gotten from ogun state with different locations, the fresh produce show all the characteristics of E.coli show white and pink on SMAC and MAC plate when it was incubated at 37^0C for 24hrs which shows circular colonies.

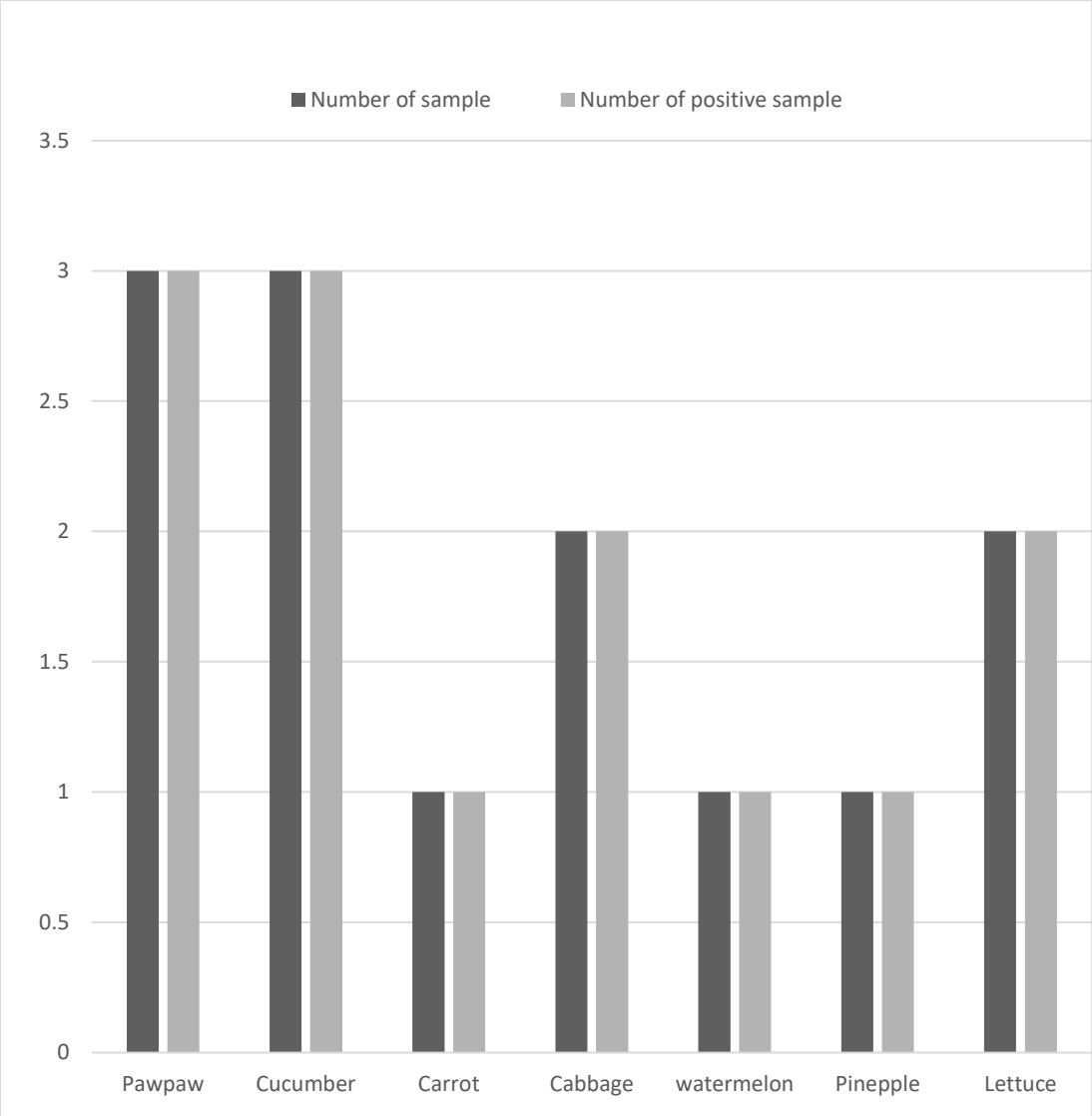

Figure 4.2: A Chart Representation of Microbial Analysis of all Positive Fresh Produce

4.4 Antimicrobial susceptibility pattern of Pathogenic *E.coli* isolates from ready-to-eat game meat and fresh produce from Southwestern region of Nigeria.

Majority of the Pathogenic *E.coli* isolates from Ready to eat game meat and fresh produce were resistant to the antibiotic class of Cephalosporin (Cefuroxime, Ceftriaxone, Cefotaxime, Ceftazidime**)** (Fig 4.3). It was noted that the Pathogenic *E.coli* isolates tested were highly susceptible to Aminoglycoside majorly Amikacin (Table 4.2).

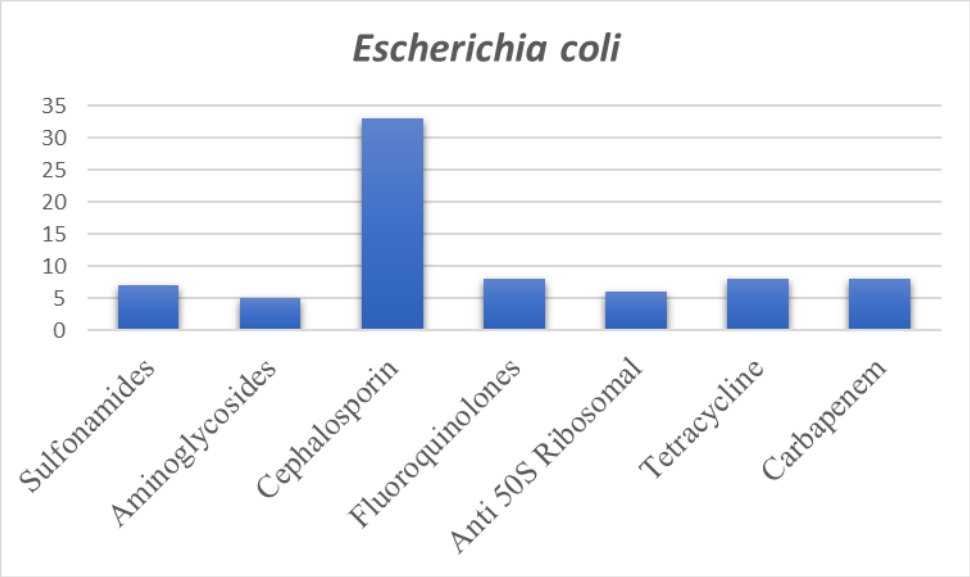

Figure 4.3:AMR profile among *E. coli* game meat and Fresh Produce isolates. This shows the resistance profile of all the *E. coli* isolates recovered in the study

Table 4.2: Antimicrobial susceptibility pattern of Pathogenic *E.coli* isolates from ready-to-eat game meat and fresh produce from Southwestern region of Nigeria

Classes of Antibiotics	Antibiotics	Disc Code	Antibiotic Disc Content (µg/disc)	*E.COLI* (n =11) Isolates		
				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	-	-
	Cefoperazone	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

4.5 ESBL Identification of E.coli using Double disc (Fresh Produce)

7 Pathogenic *E.coli* isolates that were ESBL producers; . The anitbiotics used is the CTX, AMC and CAZ

Table 4.3: ESBL Identification of *E. coli* using Double disc (Fresh Produce)

ISOLATE ID	CTX	AMC	CAZ
MK_1P_1	-	+	+
MS_1U_1	+	+	+
MD_1U_2	+	+	+
MK_1P_2	+	+	+
MP_1W_2	+	+	+
MD_1U_3	+	-	+
MS_1C_2	+	+	+
MA_1P_3	+	+	+
MMC_1	+	+	+

4.6 Molecular Identification of *E. coli using Multiplex PCR*

Multiplex pcr was used to determine the *E.coli* pathogen and its was done using two treatment . As shown below in figure 4.4 treatment 1 shows that 8 *E.coli isolates* were positive while in figure 4.5 treatment 2 shows that 2 *E.coli* pathtype was positive.

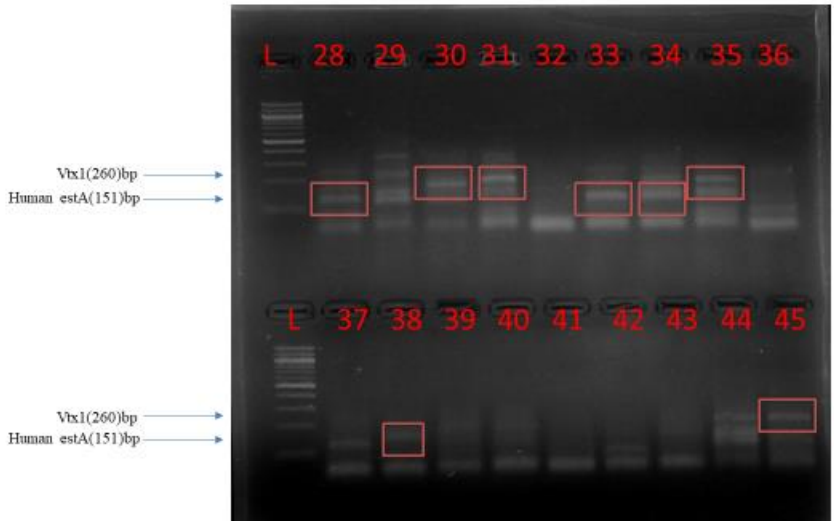

Plate 4.1: Illustrative agarose gel electrophoresis image of multiplex-PCR products (Human *estA, Porcine estA, vtx1, vtx2, ipaH, eae, eltA*). Lane L: marker (100-bp ladder), lane 28: *E.coli* isolate (Human *estA*), lane 30:(*vtx1*).

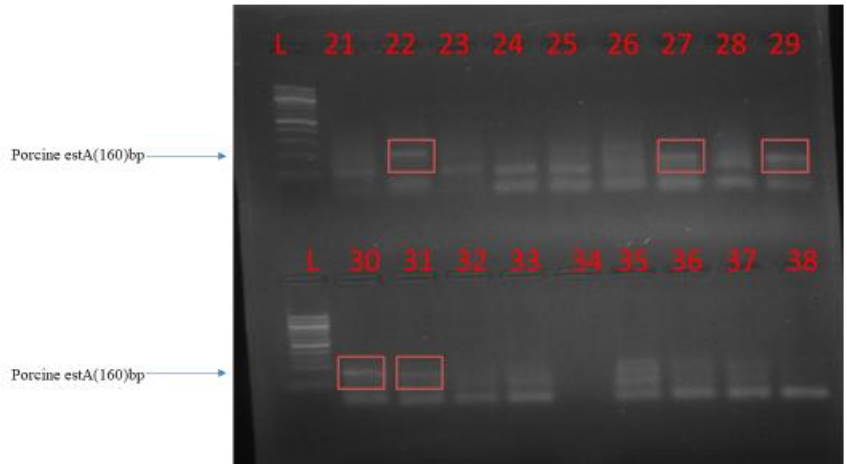

Plate 4.2: Illustrative agarose gel electrophoresis image of multiplex-PCR products (Human *estA, Porcine estA, vtx1, vtx2, ipaH, eae, eltA*). Lane L: marker (100-bp ladder), lane 22: *E.coli* isolate (Porcine *estA*), lane 30:(Porcine *estA*)).

4.7 ESBL Identification using Simplex PCR

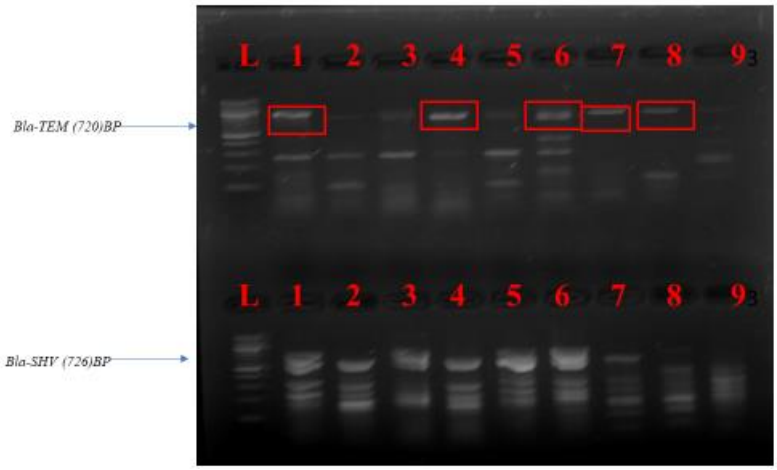

Plate 4.3: Illustrative agarose gel electrophoresis image of multiplex-PCR products (*Bla-TEM*, *Bla-SHV*); Lane L: marker (100-bp ladder),

4.8 Discussion

RTE game meat and Fresh meats are sometimes contaminated with bacteria, which can be harmful to the human body. The major bacterial pathogens include: *Salmonella, S. aureus, C. botulinum, Clostridium perfringens, B. cereus* and *E. coli*. The sources of these microbes in meat could be inherent micro-flora in normal tissues of animals, air, environment, or contamination due to unhygienic slaughtering, handling and processing conditions. The isolate is *E.coli* and is circular in shape showing pink and white on SMAC plate. Each isolate was subjected to various tests to study their characteristic features in order to identify them. Considering the marked importance of *E. coli* infection organisms as food-borne pathogens, we aimed in this study to evaluate the levels of contamination by those organisms in RTE game meat and fresh produce in southwestern part of Nigeria. The isolated strains were characterized at the molecular level using PCR and evaluated their antimicrobial resistance patterns to different antimicrobials. The total viable count was able to indicate the microbiological quality of the produce examined and it was able to show the level at different states in south west in Nigeria of which their microbial load ranges. After the total viable count examination, Antibiotic susceptibilty test was done on the isolates by using mueller hinton agar and antibiotic disc, then after molecular identification was done using the multiplex pcr for game meat and fresh produce. In multiplex pcr, 55 isolate from game meat were used and 7.3% of the isolate were tested positive for vtx1 gene which was used to detect VTEC (verotoxigenic *E.coli*/STEC) which causes diarrhoeal in humans , and 18% of the *E.coli* isolate were positive for estA which is used to dectect ETEC (enterotoxigenic *E.coli*). In simplex pcr, 11 samples were used from fresh produce for the detection of esbl and 27.3% were positive the bio tem, 18.2% were positive for bio shv.

All *E.coli* found in the fresh produce is due to contamination through post- harvest and pre - havest contamination of fresh produce. In the location the fresh produce were gotten from, the fresh produce is carelessly piled on top of surfaces; occasionally, fresh produce is cut in half for customer affordability. Most of the time, the split surfaces are left uncovered to prevent contamination from the environment. Fresh produce is occasionally sold in polyethylene bags, but most of the time it is not packaged and is left open in the air, making it vulnerable to contamination by airborne pathogens. Therefore, it is crucial that the government enact laws

governing proper food handling and general hygiene. According to all results, all samples had presumptive for *E.coli* in the game meat isolates in them. This shows the probability of an unhygienic handling, slaughtering and marketing of gamemeat. *E.coli* presence in these samples suggests faecal-oral route contamination from the game meat handler, as well as coming in contact with the infected animal which leads to spread of zoonotic diseases. However there are consequences, game meat presents numerous routes of opportunity for transmission of zoonotic pathogens, including airborne and blood-borne during hunting and the butchering of carcasses, as well as foodborne risks associated with preparation and consumption. Sixty-two percent of all newly emerging infectious diseases are zoonotic, and more than seventy percent of those zoonoses involve wildlife reservoirs, making human interaction with wildlife a significant channel for endemic and emerging infectious diseases. It is therefore important that the government should implement rules concerning general hygiene and proper game meat handling and also creates public awareness.

CHAPTER FIVE

5 Conclusions and Recommendations

5.1 Conclusion

This study showed the possible public health harzard related to RTE game me and fresh produce in south western part of Nigeria. Based on the findings of this study and the deductions derived from there, it could be concluded that RTE Game meat and fresh produce gotten from some of the states across Nigeria are contaminated with strains of pathogenic *E. coli*. In addition, the study has demonstrated that game meat and fresh produce may contribute to the prevalence of *E. coli* illnesses.

5.2 Recommendations

Consumers need to be informed about the potential risk of consuming unhygienic game meat and fresh produce. Regulatory and educational efforts from the government officials are needed to improve the safety of fresh meat that are intended for use as ready to eat products in Nigeria. Further precautions are needed during the processing and handling of game animals by the hunters, butchers and retailers as the hygienic environment and proper handling can have a greater influence on RTE Game meat and fresh produce

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