ISOLATION AND ANTIBIOTICS SUPSCEPTIBILITY OF *E. COLI 0157H7* FROM MEAT SAMPLES SOLD AT MARKETS IN IKORODU.

BY

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CERTIFICATION

We certify that this research work was carried out by **JIMOH FATIMA ATINUKE** in the Department of Microbiology and biotechnology, College of Pure and Applied Sciences, Caleb University, Lagos. The research work is considered adequate in partial fulfillment of the requirements for the award of B.Sc. in Microbiology.

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I, **JIMOH FATIMA ATINUKE,** do hereby declare that this project is entirely my work and composition. The work embodied in this project has not been submitted in candidature for any degree and is not concurrently being submitted for any other degree. All references made to works of other persons have been duly acknowledged.

Signature ……. **Date ……....**

DEDICATION

This project is dedicated to the Almighty God for his faithfulness and divine enablement that has seen me through these years. I also want to dedicate it to my wonderful parents, siblings and my loved ones for their moral, spiritual and financial backing all through the period of this study.

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ABSTRACT

E.coli 0157:H7 is a major enteric pathogen known to cause food borne disease in human and animals. This study was aimed at determining the frequency of occurrence of *E.coli 0157:H7* in meat samples sold in three selected markets in Lagos State. A total of 20 samples was examined for the presence of *E.coli 0157:H7* using Sorbitol MacConkey (SMAC) agar based after initial pre-enrichment of the meat samples for 2hours, agar enhanced with MUG was used. Using a sterile wire loop, a loop of the meat samples was taken and inoculated into Sorbitol Macconkey Agar plates by streaking. The plates were incubated at 37 degree Celsius for 24 hours after which they were observed for colourless, nonsorbitol fermenting colourless characteristics of *E. coli* 0157:H7.

The isolates were subjected to morphological examination and biological test and their identity were confirmed using the characteristics. The test carried out include Gram's staining, Catalase Test, Potassium hydroxide test (KOH), Haemolytic Test, Sugar Fermentation Test, Indole Test, Starch Hydrolysis Test, Mannitol broth Test, Urease Test, Hydrogen sulphide Test, Motility Test, and Citrate Test. *E. coli* O157:H7 isolates were found from 20 samples. Antibiotic susceptibility testing revealed that *E. coli* O157:H7 isolates were antibiotic resistant. The resistance patterns were as follows; Ceftazidime (64.3%), Cefuroxime (85.7%), Gentamicin (71.4%), Cefixime (71.4%), Ofloxacin (64.3%), Augmentin (92.9%), Nitrofurantoin (71.4%0, and Ciprofloxacin (50%). This result shows that isolate had highest resistance to Augmentin and lowest resistance to Ciprofloxacin. The majority of *E. coli* O157:H7 isolates in this investigation displayed multidrug resistance to antibiotics in varying degrees.

The presence of *E.coli 0157:H7* may be as a result of the observation of poor proper hygiene, prevent and the mixture of the gastrointestinal tract contents with the meat and also poor meat handling.

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CHAPTER ONE

INTRODUCTION

In underdeveloped countries, food-borne infections are the major cause of disease and mortality, costing billions of naira in medical care and social costs. One of the most common causes of food poisoning is contaminated raw meat. In the production and processing environment, a diverse diversity of bacteria can colonize meat surfaces. Microbial contamination of raw meat occurs as a result of processing and begins at slaughter, when the carcass is infected with bacteria found on the animal's external surfaces, gastrointestinal tract, and lymph nodes, as well as in the plant environment (Nazir *et al.,* 2014). The Enterobacteriaceae bacterium family is the most common bacterial contamination found in raw and processed beef products around the world. *Salmonella, Escherichia coli (E. coli), Proteus*, and *Klebsiella* are the most common bacteria found in all cases of food poisoning linked to certain meat items. *E. coli* is the most well-known member of the Enterobacteriaceae family, and it is also the well-studied. It has been implicated as the cause of food poisoning in a variety of foods, including raw milk, cream, creamed fish, dates, and raw or badly cooked meat and poultry. Beef appears to be the main source of contamination for this bacterium. Several *E. coli* strains have emerged as powerful food-borne pathogens. One strain in particular (O157:H7) has been recognized as one of the deadliest for humans, causing a number of deaths each year. Because *E. coli* is a commensal bacterium in the intestines of food-producing poultry, cattle, and pigs, resistant strains from the gut easily contaminate beef carcasses (Khan *et al.,* 2017).

Cross-contamination of beef with other ready-to-eat meals has also occurred in kitchens and food service companies. Because different sampling/reporting procedures are used in different countries, comparing data from multiple countries is challenging. However, according to a WHO

summary *E. coli 0157:H7* is a global concern, with prevalence in meat ranging from 0.1 percent to 5% and prevalence in cattle ranging from 1.5 percent to 28 percent (WHO, 1997). Infections and deaths linked to tainted ground beef and other food products have been documented in nations all over the world.

The feces on the hide and digestive tract content in the intestines of the slaughtered cattle are the source of *E. coli O157:H7* on beef carcasses. The prevalence on the external surface of recently slaughtered animals' hides is similar to, or somewhat higher than, the frequency on cattle being kept for slaughter (Hancock, 1998).

Initially, the percentage of cattle with *E. coli O157:H7* in their feces was estimated to be less than 5%. (Hancock, 1998). During the months of highest prevalence, July and August, a later study using a more sensitive analytical method discovered that 28 percent of cattle entering US slaughtering plants were actively shedding *E. coli O157:H7* or non-motile *E. coli O157* in their feces. Eleven percent of the hide surfaces were also favorable (Elder *et al*., 2000).

The primary source of infections that contaminate carcasses, according to more recent studies employing superior methodology, is cattle hides (Koohmaraie *et al.,* 2005) Cattle colonization is short-lived (1-2 months), according to studies, and long-term carriers have yet to be discovered.

The usual shedding pattern in a herd throughout time includes epidemics of shedding interspersed with prolonged periods of rare or non-shedding animals. These outbreaks are more common in the summer, suggesting that environmental proliferation may play a role in *E. coli O157:H7* epidemiology (Hancock, 1998). It's worth noting that *E. coli O157:H7* has no negative consequences in cattle. Only microbiological tests can reveal its existence in a herd or an individual animal. Because colonization and shedding are intermittent, and within-herd frequency appears to

be low, regular and repeated testing is required to identify the status of herds and animals within them. Contamination routes for *E. coli O157:H7* on farms and population dynamics are currently unknown, so interventions and control options are limited to those recommended in general guidelines for hygienic practices, quality assurance programs, and/or application of HACCP principles to the extent possible (CCFH, 2005).

Types of feed, feeding regimens, and the delivery of probiotics or vaccines to animals are examples of potential therapies that have yet to be proven successful and/or require further research, but which could help eliminate the pathogen in the long run. Stress and extended periods of transportation, whether to farms, feedlots, or slaughterhouses, promote EHEC feces shedding. Efforts to lessen cattle stress prior to travel should be employed to reduce EHEC shedding after they arrive at their destination.

Meat is a good source of protein, vital amino acids, B vitamins, and minerals, among other things. As a result, it provides an ideal habitat for harmful germs to thrive. In addition, poultry meat is a good growth medium for a wide range of microorganisms, including pathogens and spoiling bacteria. Chicken and turkey, on the other hand, are the most common poultry meats. Chicken meat accounts for almost two-thirds of total global production.

Pathogens regularly contaminate meat and poultry carcasses and parts, which enter the carcasses via the digestive tract or fecal debris on feet and feathers. The bacteria *Escherichia coli* is frequently employed as a surrogate indication; its presence in food generally indicates fecal contamination, both direct and indirect (Khan *et al*., 2017). Traditional approaches for detecting bacterial pathogens in foods rely on morphological, biochemical, and immunological features of bacteria to be identified using selective growth medium. Methods based on polymerase chain

reaction (PCR) have been established as a powerful diagnostic tool for pathogenic microorganism detection. (Saad *et al*.2011).

1.1 STUDY OBJECTIVES

The major goal of this research is to determine the prevalence of E. coli (O157:H7) in the beef samples.

The specific objectives are:

• To isolate *E. coli* O157:H7 from meat samples sold in stated markets in ikorodu Lagos.

• To identify the *E. coli* O157:H7 strains found in the samples using biochemical test.

• To determine the antibiotics susceptibility of *E. coli O157:H7* isolated.

CHAPTER TWO

LITERATURE REVIEW

2.1 MEAT

Meat is animal flesh that is eaten as food. Humans have hunted and killed animals for meat since prehistoric times. The advent of civilization allowed the domestication of animals such as chickens, sheep, rabbits, pigs and cattle. This eventually led to their use in meat production on an industrial scale with the aid of slaughterhouses. Meat is mainly composed of water, protein, and fat. It is edible raw, but is normally eaten after it has been cooked and seasoned or processed in a variety of ways. Unprocessed meat will spoil or rot within hours or days as a result of infection with and decomposition by bacteria and fungi. (Merriam Webster, 2021).

Meat is important in economy and culture, even though its mass production and consumption has been determined to pose risks for human health and the environment. Many religions have rules about which meat may or may not be eaten. Vegetarians and vegans may not eat meat because of concerns about the ethics of eating meat, environmental effects of meat production or health effects of consumption. Meat is widely consumed as a source of protein and it is either been eaten in a processed form or cooked to avoid contamination and spoilage. This meat is also very rich in micro minerals such as iron, selenium, zinc, copper and manganese. All of them are essential, because of their very important role in key metabolism pathways and in the anti-oxidative enzymatic system.

When it comes to meat's lipid composition, fat supplies necessary nutritional energy as well as critical nutrients including essential fatty acids and fat-soluble vitamins. Meat's lipid

content influences its cooking qualities, palatability, and overall organo-leptic aspects. However, approval of meat by consumers is determined by cholesterol levels and saturated fatty acid makeup and conditions its nutritional value in accordance with the usual dietary recommendations. Additional nutritional benefits of beef or lamb meat include a high amount of B vitamins, including B12, B2, PP, and B6. The vitamins included in red meat are the most important source of vitamins for people of all ages (Cabrera and Saadoun, 2014)

2.2 BIOCHEMICAL COMPOSITION

Numerous aspects of the biochemical composition of meat vary in complex ways depending on the species, breed, sex, age, plane of nutrition, training and exercise of the animal, as well as on the anatomical location of the musculature involved. Even between animals of the same litter and sex there are considerable differences in such parameters as the percentage of intramuscular fat.Adult mammalian muscle flesh consists of roughly 75 percent water, 19 percent protein, 2.5 percent intramuscular fat, 1.2 percent carbohydrates and 2.3 percent other soluble non-protein substances. These include nitrogenous compounds, such as amino acids, and inorganic substances such as minerals.Muscle proteins are either soluble in water (sarcoplasmic proteins, about 11.5 percent of total muscle mass) or in concentrated salt solutions (myofibrillar proteins, about 5.5 percent of mass).

There are several hundred sarcoplasmic proteins. Most of them – the glycolytic enzymes – are involved in the glycolytic pathway, i.e., the conversion of stored energy into muscle power. The two most abundant myofibrillar proteins, myosin and actin, are responsible for the muscle's overall structure. The remaining protein mass consists of connective tissue (collagen and elastin) as well as organelle tissue. Fat in meat can be either adipose tissue, used by the animal to store energy and consisting of "true fats" (esters of glycerol with fatty acids), or intramuscular fat, which contains considerable quantities of phospholipids and of unsaponifiable constituents such as cholesterol.

2.3 MEAT CONTAMINATION

On raw meat the muscle tissues of healthy living animal are free of microorganisms and the under skin of animal carcass too becomes sterile immediately after slaughter. Hence, contamination of raw meat may be due to the slaughtering procedures of the stressed animals, as well as contact with external surfaces such as hair, gastrointestinal and respiratory tracts and/or other ambient environmental hazards. In abattoirs, the contamination of these raw meat occurs when the microorganisms are been introduced directly to meat samples and come in contact with surfaces in operations performed during offloading, weighing, processing, cutting and storage, as well as at the points of sale and distribution. (Shilenge *et al,* 2016) further stated that microbial pathogens are transmitted passively from a contaminated source such as raw poultry to cooked food that is prepared for later consumption as cold foods. Microorganisms that are typically and usually prevalent in raw meat include *Listeria monocytogenes, Salmonella, Staphylococcus aureus,* (on poultry), *Escherichia coli* amongst others.

Meat cutting is important in meat processing as carcasses are deboned and cut into smaller and more desirable cuts, using hand tools and machines, the risk of meat contamination is mostly

determined by the food handlers' health, personal cleanliness, and knowledge and practice of food hygiene.

2.4 MICROBIAL PATHOGENS ASSOCIATED WITH MEAT

2.5 Escherichia coli

Escherichia coli also known as *E. coli* refers to as one of the largest groups of bacteria that are mostly found in the flora of the intestines of humans and animals. *Escherichia coli* are gram negative, aerobic rod with certain strains that are pathogenic and produce an enterotoxin, but many of these enterotoxin strains are not harmful. *E. coli* are a large and diverse group of bacteria, The Genus *Escherichia*, Phylum proteobacteria, Class Gammaproteobacteria, Order Enterobacterales, and Family Enterobacteriaceae.

Although most strains of *E. coli* are harmless, others can make you sick. Some kinds of *E. coli* can cause diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses. (Brunder*et al.,* 1996).

These pathogenic bacteria can become pathogenic only when they reach tissues outside of their normal intestinal or other less common normal flora parts. Raw beef can be an important transport in the transmission of *E. coli* during slaughtering, processing or from cross-contamination as a result of food handling unsafety. Its presence in meat is usually a result of fecal contamination or when the intestinal tract is punctured (Ernest, 2015). Symptoms of *E. coli* infection usually start within three to four days after exposure, but the period incubation can be as short as a day or as long than ten days or even more. The disease which is most commonly associated with travelers show a number of varied symptoms that may vary from person to person. However, they often include severe stomach cramps, diarrhea, vomiting and fever. Safe food handlers and Proper hygiene procedures such as good slaughtering techniques, hygiene during slaughtering and

dressing together with prompt adequate cooling are keys to preventing the spread of all food-borne illnesses including *E. coli* (PHAC, 2014).

2.6 CLASSIFICATION AND TYPES OF *E. COLI*

Escherichia coli is the most well-known bacterial species It is also the most diverse.There are many beneficial *E. coli* strains, such as those that aid in food digestion in the intestines of humans. However, *E. coli* strains that cause urinary tract infections, meningitis, and intestinal infections are also present.

Enterotoxigenic (ETEC), Enteropathogenic (EPEC), Enteroinvasive (EIEC), Enterohemorrhagic (EHEC), and Enteroaggregative (EAEC) are the five types of *E. coli* infections found in the intestine.

ENTEROAGGREGATIVE STRAINS (EAEC)

People in poor nations are frequently infected with enteroaggregative (EAEC) strains. Although the exact mechanisms of these strains are unknown, it is believed that *E. coli* cells are able to adhere to intestinal cells and form a biofilm. In most cases, there are no lesions or inflammation. Watery and mucoid diarrhea is common symptoms, and they can persist for weeks.

ENTEROPATHOGENIC STRAINS (EPEC)

EPEC strains are uncommon in developed countries, but they are a common cause of newborndiarrhea in poor countries. It is believed to be transmitted through faeces-oral transfer or contaminated water. It's proven challenging to determine immunity and infectious dose. These strains are less likely to produce traveler's diarrhea, and they've been discovered on things near affected little children, indicating a low

infectious dose for babies. EPEC strains do not produce LT or ST toxins; instead, they adhere to the intestinal cell wall and establish a micro colony, which causes intestinal cells to change. The attachment and effacing (AE) lesions are another name for this process. The actual cause of watery-diarrhea is not known, but likely due to bacterial invasion of host cells and disruption to intestinal absorption.

ENTEROTOXIGENIC STRAINS (ETEC)

When visiting developing countries, ETEC *E. coli* strains are the most common cause of traveler'sdiarrhea. In many nations, it is also a leading cause of neonatal infection and death. Adults who live in endemic areas are frequently immune to these strains. These strains have a high infectious dosage and are spread by contaminated food and drink. These strains are only present in humans and are not found in animals.

Colonization factor antigens (CFA) are found in ETEC *E. coli* strains to help them adhere to intestinal cells and deliver toxins. These strains produce heat-labile toxins (LT) and/or heat-stable toxins (ST), which aid in the release of water and electrolytes from intestinal cells, resulting in watery diarrhea.

ENTEROINVASION STRAINS (EIEC)

In terms of disease-causing processes and dysentery symptoms, Enteroinvasive (EIEC) strains are remarkably similar to *Shigella*. EIEC strains invade intestinal cells and grow, causing cell death, inflammation, and ulcers in the process. Infections are largely limited to children in developing countries, with outbreaks in developed countries typically due to contaminated food or water. Only humans have been detected with EIEC species, and a high infectious dosage is usually necessary to produce sickness.

ENTEROHEMORRHAGIC STRAINS (EHEC)

Enterohemorrhagic (EHEC) strains, which include *E. coli*, are the most well-known. *E. coli* O157:H7 and Shiga toxin-producing *E. coli* O157:H7 These strains have a low infectious dosage and are spread by contaminated food, particularly raw meat and unpasteurized beverages. EHEC strains are distinct from other *E. coli* strains. In that they primarily target the colon and produce Shiga-toxins as well as AE lesions, they are similar to *E. coli* strains. AE lesions play a direct role in symptoms including non-bloody diarrhea.

2.7 ESCHERICHIA COLI 0157:H7

Among the enteric E. coli, shiga toxin-producing E. coli O157: H7 is the most significant foodborne pathogens that have gained increased attention in recent years. It has been the most commonly isolated serotype in association with abdominal cramps, bloody diarrhea, thrombotic thrombocytopenic purpura, hemorrhagic colitis, and hemolytic uremic syndrome in both outbreaks and sporadic cases.

E. coli 0157:H7 is a gram-negative, oxidase-negative strain that converted into hemorrhagic strains by the presence of lysogenic conversion after bacteriophage infection of the non-hemorrhagic cells.

The *E.coli* serotype iO157:H7 can be found naturally in the intestinal contents of cattle, goats, and even sheeps, the digestive tract of cattles lack the Shiga toxin receptor globotriaosylceramide thus, making it asymptomatic carriers of the bacterium (Pruimboom-Brees*et al.,* 2000).

Infection with *E. coli* iO157:H7 can result from ingesting food or drink that has been tainted or oral contact with tainted surfaces as an example, ground beef that is undercooked, raw milk and leafy vegetables in conversion after bacteriophage infection of the non-hemorrhagic cells.

Escherichia coli O157:H7 was first recognized as a pathogen in 1982 as a result of an outbreak involving hamburgers in two US states. The organism was found to produce a toxin that is capable of damaging the kidneys (hemolytic-uremic syndrome (HUS)) and affecting the central nervous system (CNS) (thrombotic thrombocytopenic purpura (TTP)), often preceded by bloody diarrhea (hemorrhagic colitis (C)). The toxin has been called both a verocytotoxin (affecting the vero kidney cells of the green monkey) and a Shiga-like toxin (similar to a toxin produced by Shigella). In fact, there are a number of similar toxins that make up the verotoxin/Shiga toxin category. This division of nomenclature remains today with both terms verotoxin-producing E. coli (VTEC) and Shiga toxin-producing E. coli (STEC) being used to describe the same group of organisms. Other virulence factors that may be necessary for severe infections are an attaching and effacing protein (adhesin/intimin) and a hemolysin. An earlier outbreak in Canada with one death in 1980 was likely caused by the same organism after apple cider (unfermented squeezed apple juice) was consumed at a farmer's market. Verotoxin was found in the intestines of the dead youth but VTEC were not successfully isolated. Outbreaks from apple cider have since been occasionally reported in Canada and the USA. In fact, outbreaks of E. coli O157:H7 and similar organisms producing verotoxin/Shiga toxin have occurred in many countries outside North America including Australia, Japan, the UK, and many other European countries. It is interesting that the more northerly countries seem to have the higher case rates, for example, Canada, Scotland, and Scandinavian countries. This may relate to farm practices for cattle and sheep that are the main reservoirs for the pathogen.

Supershedders (animals that excrete large numbers of the pathogen in their feces) may be a major transmission factor in the field or feedlot and during and after transportation to the slaughterhouse.

In 1993, a major outbreak of E. coli O157:H7 infection affected approximately 500 people in four northwestern US states. Many children developed HUS, and four died as a result. This episode forced the US government to declare that E. coli O157:H7 was an adulterant in ground beef (and later in nonintact beef). Another large outbreak caused by this pathogen occurred in Africa in 1992, affecting probably thousands of people, with an undocumented number of cases of HUS; drinking water and cooked maize were the identified vehicles of transmission. In 1996, in an outbreak of E. coli O157:H7 in Japan, 6309 schoolchildren and 92 school staff members were affected, with two deaths; the epidemiological investigation identified fresh radish sprouts (kaiware-daikon) as the probable cause.

This was the largest outbreak ever recorded from this pathogen. However, subsequently two very large and well-publicized outbreaks occurred in Scotland in 1996–97 (400 ill with 20 deaths of elderly persons) and Wales in 2005 (>170 schoolchildren and others, and 1 child died). Both were caused by butchers who managed catering businesses and also worked with slaughtered animals. The outbreaks were traced to contaminated meat dishes that were delivered to residences and schools. Another well-published outbreak originated from Californian bagged fresh spinach in 2006, with 205 confirmed cases of illness and 3 deaths. The ultimate source was not determined but upstream cattle, feral pigs, and irrigation water may have been involved in field contamination, and the washing and disinfection processes for the spinach were insufficient to prevent E. coli O157:H7 from entering the bags.

This was one of the three leafy green outbreaks caused by E. coli and other pathogens in 2006 involving leafy greens, mainly lettuce, over the previous decade. The leafy greens industry is assessing how to minimize risks to avoid similar problems. Other outbreaks have been linked to contaminated unpasteurized milk, alfalfa sprouts, salami, recreational water, and contact with infected live animals.

2.8 PROTEINS INVOLVED IN PATHOGENESIS OF *E.COLI* O157:H7

There are hirty-five proteins that are presumably involved in the pathogenesis of *Escherichia coli O157:H7* infections, Only 19 genes have been identified so far, including a hemolysin, a catalase-peroxidase (katP), a serine protease (espP), a type II secretion system apparatus (etp), a zinc metalloprotease (stcE), and a putatidyltransferas (ecf) (Brunder*et al.,* 1996; Schmidt *et al.,* 1994).

2.9 EFFECT OF *E. COLI* 0157:H7 INFECTION

Food safety is becoming more and more of a public health concern. Unsafe food causes a variety of acute and chronic illnesses, ranging from diarrhea to various cancers. Approximately 2.2 million people die each year from foodborne and waterborne diarrheal illnesses, with 1.9 million of them being children. As a result, foodborne infections in both affluent and developing countries place a considerable burden on society. Meat has long been thought to be the source of a major amount of human foodborne illness. Meat can be contaminated with a wide range of pathogens and spoilage germs, and it would be difficult to keep track of all of them. *E. coli* can contaminate food in a number of ways, including bowel rupture during evisceration, indirect contamination with sewage and polluted water, and finished product handling and packaging. Meats are a particularly common source of *E. coli*

contamination, which can be acquired through fecal contact during slaughter. Pathogen germs can contaminate food at any point in the supply chain, including production, processing, distribution, retail marketing, and handling or preparation. Furthermore, some *E. coli* strains are human and animal pathogens. An individual can become infected by swallowing or taking even a small amount of *E. coli* bacteria. Infection can occur as a result of any of the following processes: Eating ground meat contaminated with *E.coli* that has not been cooked thoroughly enough to destroy the bacterium. Bacteria from the animal's intestines can sometimes make their way into the meat during preparation. Unpasteurized milk: Using milk that has not been cooked to kill microorganisms. *E. coli* can enter milk via a cow or milking equipment, which is especially dangerous for youngsters who drink from a bottle.

Fruit and vegetables: An individual can become infected by eating fresh vegetables or fruit that have been polluted by bacteria-infested water. This is particularly common when surrounding animals' excrement mixes with the water supply or source. Beverages and foods: *E. coli* can be obtained from unpasteurized fruit juices and yogurt and cheese made from raw milk. Food in the kitchen can also get contaminated if a knife or cutting board that has touched uncooked meat (like chicken) come into contact with food that will be eaten raw (like a salad).

Water: Water contaminated with *E. coli* and swallowed perhaps while swimming in a pool, lake, or pond.

People: An individual might get *E. coli* from another person that is already a carrier, such as an infant. The bacteria can be contacted during the process of cleaning up an infected child and then forget to wash your hands properly before touching the mouth.

Animals: In petting zoos or animal exhibits at fairs *Esherichia coli* can be present. Most *E.coli* bacteria cases are initiated with non-bloody diarrhea and self-resolve without further

complication however, some patients progress to bloody diarrhea or Hemorrhagic Colitis (HC) in 1-3 days after getting infected. 5-10% of Hemorrhagic Colitis disease in patient can progress to a life-threatening sequelae Hemolytic Uremic Syndrome (HUS) or thrombocytopenic purpura (TTP).

Water and other foods (e.g., lettuce, sprouts, fruit juices, vegetables, raw milk) have also been implicated as transmission vehicles. Person-to-person transmission is a common route of infection in day care settings. Direct contact with infected animals is also a well-known cause of infection (WHO, 1997). Infection with *E. coli O157:H7* can cause mild to severe illness, with the majority of deaths occurring in children under the age of five and the elderly (AGA, 1994; Tarr, 1994; Duffy et al., 2005). According to studies conducted in the United States, 13 to 27 cases of infection occur in the community for every verified case recorded (Mead et al., 1999). In the four years from 1996 to 1999, the annual laboratory confirmed case rate per 100,000 populations in the US FoodNet sites ranged from 2.1 to 2.8. Between 2000 and 2004, the annual incidence rate dropped from 2.9 to 0.9 incidences per 100,000 people. Between 1998 and 2003, the annual incidence rate for cases in Ireland ranged from 0.9 to 2.1 per 100,000 people (Duffy *et al.*, 2005).

2.9.1 SIGNS AND SYPTOMS OF *E. COLI* 0157:H7 INFECTION

i. Stomach pains and cramps.

ii. Diarrhea that may range from watery to bloody.

iii. Fatigue.

iv. Loss of appetite or nausea.

v. Vomiting.

vi. Low fever < 101 °F/ 38.5 °C (not all people have this symptom).

vii. The disease usually lasts 5 to 10 days and causes little or no temperature.

viii. The infection can induce haemolytic uremic syndrome (HUS), which is a condition in which red blood cells are damaged and the kidneys begin to fail, in some people, particularly children under the age of five, immune compromised patients, and the elderly. (Benjamin and Datta, 1995; Arnold and Kaspar, 1995; Leyer et al., 1995; Cheville *et al.,* 1996).

2.9.2 *ESCHERICHIA COLI* AS AN INDICATOR OF OTHER ORGANISMS

Indicator organisms, such as *Escherichia coli*, are bacterial groupings that signal the presence of potentially harmful organisms and may point to the source of microbial contamination. Its presence in raw foods is thought to indicate fecal contamination, either direct or indirect. As a result, it's employed as a marker for the presence of enteric pathogens in food and drink. Enteric and diarrheal diseases, urinary tract infections, sepsis, and meningitis are all caused by pathogenic *E. coli* strains. They are capable of producing disease in some circumstances, such as when the immune system is weakened or when disease is caused by an environmental exposure. Foodborne *E. coli* may be a significant cause of urinary tract infections, albeit indirectly through the host fecal flora. They're

becoming more widely recognized as the leading source of foodborne illness and outbreaks around the world. The most probable number (MPN) technique using Lauryl Sulphate Tryptose is a standardized method for the detection and enumeration of *E. coli.* The majority of EHEC isolates are acid tolerant, meaning they can survive in acidic environments and during stomach transit (Benjamin and Datta, 1995; Arnold and Kaspar, 1995; Leyer et al., 1995; Cheville et al., 1996). *E. coli O157:H7* is one of the more recently identified foodborne pathogens, having been discovered during an outbreak involving ground beef in 1982. Since then, it has become a rapidly rising cause of foodborne illness around the world. There are a variety of enterohaemorrhagic E. coli serotypes, some of which may be more important in certain parts of the world than *E. coli O157:H7* (WHO, 1997; Acheson, 2000). The main reservoir of *E. coli O157:H7* appears to be cattle. Contamination of carcasses after slaughter is the most common way for ground beef to become contaminated.

2.9.3 ISOLATION AND IDENTIFICATION OF *ESCHERICHIA COLI* 0157:H7

E. coli O157:H7 has the somatic (O) antigen 157 and flagella (H) antigen 7, as well as the ability to ferment D-sorbitol for longer than 24 hours and the inability to produce ß-glucuronidase, which can separate a synthetic molecule, 4-methyl-umbelliferyl-D-glucuronide (MUG) (JS Thompson *et al.,* 1990). For the detection of *E. coli* O157:H7, Sorbitol MacConkey (SMAC) agar enhanced with MUG has been used and is still used. Cefixime, potassium tellurite, and vancomycin added to SMAC agar plates to inhibit the growth of other Gram-negative flora increase the selectivity for *E. coli* O157:H7. A commercially accessible latex agglutination assay can further validate the O157 and H7 characteristics.

2.9.4 SUSCEPTIBILITY OF *E. COLI* 0157:H7 TO ANTIBIOTICS

The main virulence factors responsible for hemorrhagic colitis and haemolytic uremic syndrome in humans are Shiga toxins generated by *E. coli* O157:H7 (Karmali *et al*).

Antibiotic treatment is not recommended for human *E. coli* O157:H7 infections because some drugs, such as fluoroquinolones, produce Shiga toxin-encoding bacteriophages in vivo and boost Shiga toxin gene expression (Zhang *et al*). Despite the fact that antibiotics should not be used to treat *E. coli* O157:H7 infections, some data suggests that antibiotic resistance is rising in isolates (Kim *et al.,* 1984). Because many *E. coli* O157:H7 infections in humans are caused by eating undercooked contaminated beef, it's crucial to figure out if the bacteria develop antibiotic resistance during food animal production. It's also crucial to figure out if resistant *E. coli* O157:H7 is a possible reservoir for resistance factors to spread to other microorganisms. Antibiotic use in agriculture, according to the Food and Drug Administration, the Centres for Disease Control and Prevention, and others, is responsible for the majority of increases in antibiotic-resistant human isolates (Tollefson *et al.,*)

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Description of study area

Ikorodu is a city and Local Government Area in Lagos State, Nigeria. It shares boundary with Ogun State. Its geographical latitude is 6°36'3"N and geographical longitude 3°29'17" E.

3.2 Apparatus and equipment used

The apparatus and equipment includes the autoclave, incubator, microscope, Petri dishes, burnsen burner, test tubes, test tube rack, spatula, measuring cylinder, conical flask, distilled water, ethanol, cotton wool, permanent marker, paper tape, gloves, and glass slides. petri-dishes, foil paper, syringes, masking tape, autoclave, refrigerator, water bath, incubator, wire loop, spirit lamp, beaker, and cryovial tubes.

3.3 Media used

The media used include Sorbitol MacConkey agar, Nutrient Agar, Tryptone soya Broth media, water, distilled water, Hydrogen Peroxide, decolorizer, fructose, lactose, maltose, glucose, sucrose, Blood Agar, Glycerol broth, crystal violet, ethanol, iodine, acetone, safranin, oil immersion, and peptone water.

Reagents used

Kovac reagent, Safranin, crystal violet, iodine, The gram staining materials include crystal violet, iodine, alcohol/acetone, safranin, oil immersion.

3.4 Collection of samples

A total of 20 samples were obtained from different shops and sellers at 5markets at Ikorodu local Government area, Lagos State. The samples were transported to the microbiology laboratory of the Department of Biological science and Biotechnology Caleb University, they were kept in the refrigerator at 4°C until microbiological analysis.

3.5 Sterilization Techniques

Work bench: The work bench was thoroughly cleaned with cotton wool soaked with ethanol.

Glass wares: Conical flask, test tube, beaker were washed thoroughly washed with soap and water and sterilized at high temperature of 160°C for 1 hour.

Growth media: The growth media used were Nutrient agar (NA), Sorbitol MacConkey agar, Triple sugar iron(TSI), fructose, lactose, maltose, glucose, sucrose, Tryptone soy Broth media.

3.6 Sample enrichment and isolation of E. coli

The samples were initially prepared by inoculating the samples into a sterile broth containing (tryptone soy broth) in McCartney bottles for enrichment for about 1-2hours after which the samples were plated on Sorbitol MacConkey agar by streaking and the incubated for48 hours at 37°C. After this the plate were observed for colonies with transparent, and flat doughnut like appearance characteristics of E.coli

3.7 Characterization and identification of the isolates

Characterization and identification of the bacteria groups were carried out using colonial, Morphological and biochemical characteristics. All isolates were sub-cultured to obtain pure culture, characterized and identified using the tests described below:

3.7.1 Gram Staining

The Gram stain divides bacteria into groups i.e. Gram positive (purple color) and Gram negative (pink or reddish color), it was conducted as follows; A smear of the culture was prepared on a clean, grease free slide by emulsifying a little quantity of the growth on a drop of distilled water. The smear was heat fixed and replaced on the slide which was then stained with crystal violet for

30-60 seconds and rinsed off. The slide was further stained with iodine solution and allowed for 60 seconds then rinsed with water but not blotted, then smear was briefly decolorized for about 5 seconds with acetone-alcohol, the smear was rinsed quickly to avoid excess discoloration but not blot dried. The smear was counter stained with safranin for 30-60 seconds; this was rinsed off and allowed to dry. A drop of oil immersion was placed on the stained smear and was viewed under a microscope using objective lens x100. Then the cell shape and Gram's reaction was determined and recorded.

3.7.2 Catalase test

Catalase is an enzyme found in bacteria. It catalysis the breakdown of hydrogen peroxide with

the release of free oxygen gas, it is used to differentiate those bacteria that produce enzyme

catalase such as Staphylococci (positive) and non-catalase producing such as Streptococci (negative). A loopful of 3% hydrogen peroxide was put on a grease free, clean slide and then the bacteria culture to be tested is emulsified with it. Production of gas in form of gas bubbles indicates a positive result, while the absence of gas production showed that the organism is catalase negative.

3.7.3 Potassium hydroxide test (KOH)

0.3g of KOH was mixed with 10ml of distilled ater and kept in a syringe for use. Colonies of the isolates were picked with sterile loop and dropped on a clean grease free microscopic slides, a drop of potassium hydroxide was added to the colony and stir. A mucoid solution indicates Negative result while non mucoid is positive.

3.7.4 Indole Test

The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole. The organism was incubated into peptone broth and was later incubated for 48hrs. KOVAC'S reagent was added to the media to detect if indole has been produced. After a quick shake, the presence of cherry red coloured ring on the top of the media indicate a positive result. The absence of this color indicates negatives.

3.7.5 Sugar Fermentation Test

This is the ability for each of the isolates to utilize sugar. One Gram of each sugar (fructose, lactose, maltose, glucose, sucrose) was weighed appropriately into each beaker containing 100ml peptone (served as a nutrient base). 1% Phenol red indicator was added to the beakers and dispensed into the test tubes and sterilized after which they were inoculated with the isolates for 48hrs to 72hrs. A positive result was indicated by colour change from red to yellow, while red indicates a negative result. When many e. coli 0157:H7 ferment glucose they produce gases, mainly carbon dioxide and hydrogen. These gases bubble up through the medium and escape into the atmosphere. Tubes of broth media can be made with inverted tubes called Durham tubes which are filled with medium after sterilization.

3.7.6 Citrate Utilization Test

This test is based on the ability of an organism to use citrate as source of carbon. It was used to identify the Enterobacteria. The utilization of citrate depends upon the presence of enzyme citrate pemease produced by organisms that helps it transport into the cell Simon's citrate agar medium was prepared in an agar slant, then using a sterile wire loop, organism was inoculated onto the slant medium and incubated at 37°C for 48hours after which it was examined for color formation. A bright blue colour in the medium gave a positive citrate test. While no colour change indicates negative.

3.7.7 Mannitol broth Test

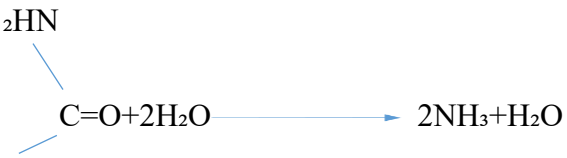
This test is used to test an organism's ability to ferment the sugar of glucose as well as the ability to convert the end product of glycosis, pyruvic acid into gaseous by products. Mannitol broth agar is prepared, phenol red is added for colouring and dispensed into the cryovial tubes, the isolates are then inoculated and incubated. Change in colour from red to yellow indicates positive, no change indicates negative.

3.7.8 Motility test

It is a differential medium used to determine whether an organism is equipped with flagella. Half strength of Nutrient agar is dispensed into tubes and stabbed with the bacterial colonies, Incubated at 37 degree Celsius for 24 hours. The results of motility agar are often difficult to interpret generally, if the entire tube is turbid, this is an indications that the bacteria moved away from the stab mark (motile), and if however, the stab mark is clearly visible and the rest of the tube is not turbid, the organism is likely non motile.

3.7.9Urea Hydrolysis Test

This test was aimed at identifying enterobacteria that produce urease enzyme, which hydrolyze urea to give ammonia and carbon dioxide. Some microorganisms have the ability to produce enzyme urease, Urease is a hydrolytic enzyme, which attacks the amide linkage liberating Ammonia.



Urea agar based (Christensen agar base) was prepared and sterilized, 5g of urea agar was dissolved in 5ml of sterilized water and added to the urea base agar prepared, the agar was then dispensed aseptically into McCartney bottles in slant position and allowed to solidify, a loop full of pure isolates was then inoculated into the agar and incubated at 35-37°C for 18-24hours. Color change was observed on all growth medium. If the colour changed from light orange to magenta (pinkish red), the organism is urease positive (produces the enzyme urease). If no colour change was detected, the samples organism is urease negative.

3.8.0 **Hydrogen sulphide Test**

Trypticase Soy Agar (TSA) is prepared, dispensed into cryovial bottles. After which it is inoculated with the isolates, Positive result was indicated by a black coloration while no coloration indicates a negative result

3.8.1 Starch Hydrolysis Test

This test is used to identify bacteria that can hydrolyze starch (amylose and amylopectin) using the enzymes a-amylase and oligo-1,6-glucosidase. The iodine reacts with the starch to form a dark brown color. Thus, hydrolysis of the starch will create a clear zone around the bacterial growth. This test is used for the identification of bacteria that hydrolyses starch. Starch agar is prepared, pure isolate is streaked, and incubated at 37 degrees Celsius for 24 hours. Iodine is added after growth which reacts with the starch, and forms a dark brown colour, starch hydrolysis will create a clear zone around the bacterial growth which indicates positive, No zone formation indicates negative.

3.8.2 Hemolysis Test

This is the breaking down of red blood cells. The ability of bacterial colonies to induce hemolysis when grown on blood agar is used to classify certain microorganisms. This is particularly useful in classifying some microorganism like streptococcal species. A substance that causes hemolysis is a hemolysin. Blood agar was prepared by preparing nutrient agar, allowed to cool and human blood sample is added, poured into a sterile petri-dish, and allow solidifying. After which the culture was streaked on the medium, incubated at 37°C for 24 hours. Clear zone around the colony indicates beta haemolysis, partial indicates alpha haemolysis and no clear zone indicates gamma haemolysis. Haemolytic is carried out to know if the organism is pathogenic or non-pathogenic

3.8.3 Isolates susceptibility testing

Every bacterial isolate will be tested for antimicrobial susceptibility using MuellerHinton Agar (MHA) (Oxoid, England) and the Abtek antibiotic disc using Mc

Farland standard procedures. MHA is prepared at 121 degrees Celsius for15 minutes allowed to cool and poured into the petri dish to solidify. Three to five pure bacteria colonies would be selected and moved to a tube containing 5ml of sterile normal saline, where they would be gently combined to create a homogeneous suspension. The suspension's turbidity is calibrated to meet the McFarland turbidity norm of 0.5. To uniformly spread the bacteria around the entire surface of MHA, a sterile swab will be used. The inoculated plates would be left at room temperature to dry for 3 to 5 minutes, and a set of antibiotic discs would be placed on the inoculated plates using sterile forceps and would be allowed to stand for 30 minutes. The plates would be incubated at 35°C for 16 to 18 hours, and the diameter of the zone of inhibition which would be determined by the break points of antimicrobial discs would be measured with a ruler and interpreted according to the standards of 2014 Clinical and Laboratory Standards Institute (CLSI, 2014).

CHAPTER FOUR

RESULTS

The occurrence of *E. coli0157:H7* in the meat samples collected and used in this study is shown, 11 out of 20 meat samples was found to contain *E. coli0157:H7*.

Table 4.1The occurrence of E. *coli0157:H7* in the meat samples.

Tables 4.2 Shows the morphological characteristics and gram reaction of *E. coli0157:h7* isolated from meat samples with the isolates mainly smooth in texture and transparent.

Tables 4.3 Shows the biochemical characteristics and probable identity of the isolates from meat samples, with total eleven *E. coli0157:H7* isolates and they are sugar fermenting.

Table 4.4 Antibiotic Resistant phenotypes in the E. coli 0157:H7 isolates

Tables 4.5 Shows the antibiotics susceptibility of *E. coli0157:H7* isolates from meat samples sold at markets in ikorodu. The isolates were susceptible to Ceftazidime, Gentamicin, Ofloxacin, Nitrofurantoin, Ciprofloxacin, and resistant to Augmentin, Cefuroxime, Cefixime.

Table 4.1: The occurrence of E. *coli0157:H7* in the meat samples.

Sample location	Number of meat samples	Number of *E. coli0157:H7*
Meat samples itamaga	5	3
Meat samples ikorodu garage	11	6
Meat samples at sabo	4	2

Tables 4.2: Morphological Characteristics of *E. coli0157:H7* Isolated from Meat Samples

Samples code	Form	Size	Elevation	Texture	Color	Gram
Ms2	Round	Large	Flat	Smooth	Transparent	-
Ms3	Irregular	Tiny	Convex	Smooth	Transparent	-
Ms5	Round	Large	Flat	Smooth	Transparent	-
Ms7	Round	Large	Flat	Smooth	Transparent	-
Ms9	Round	Large	Flat	Smooth	Transparent	-
Ms13	Irregular	Tiny	Convex	Smooth	Transparent	-
Ms14	Round	Large	Flat	Smooth	Transparent	-
Ms15	Round	Large	Flat	Smooth	Transparent	-
Ms16	Round	Large	Flat	Smooth	Transparent	-
Ms19	Round	Large	Flat	Smooth	Transparent	-
Ms20	Round	Large	Flat	Smooth	Transparent	-

Tables 4.3 The Biochemical Characteristics and Identification of *E. coli 0157:h7* isolates

SAMPLES	CATALASE	KOH	UREASE	CITRATE	INDOLE	MANNITOL	MALTROSE	SUCROSE	FRUCTOSE	LACTOSE	STARCH HYDROLYSIS	GLUCOSE
Ms2	+	-	+	-	-	+	+	+	+	+	-	+
Ms3	+	-	+	-	-	+	-	+	+	-	+	+
Ms5	+	-	+	-	+	+	+	+	+	+	+	+
Ms7	+	-	+	-	+	+	+	+	+	+	+	+
Ms9	+	-	+	-	+	+	+	+	+	+	+	+
Ms13	+	-	+	-	+	+	-	+	+	-	-	+
Ms14	+	-	+	-	+	+	-	+	+	-	-	+
Ms15	+	+	+	-	+	+	+	+	+	+	+	+
Ms16	+	-	+	-	+	+	+	+	+	+	-	+
Ms19	+	-	+	-	+	+	+	+	+	+	+	+
Ms20	+	-	+	-	+	+	+	+	+	+	-	+

KEYS- POSITIVE (+), NEGATIVE (-)

Table 4.4 Antibiotic Resistant phenotypes in the *E. coli 0157:H7* isolate

ISOLATES	AUG	NIT	CPR	CAZ	CRX	GEN	CXM	OFL
Ms2	R	S	S	S	R	S	R	S
Ms3	R	R	S	R	R	S	R	S
Ms5	R	S	R	S	S	S	R	R
Ms7	R	R	S	R	R	S	R	S
Ms9	R	S	S	S	R	S	S	S
Ms13	R	R	S	R	R	S	R	S
Ms14	R	S	S	R	R	S	R	S
Ms15	R	R	S	R	R	S	R	R
Ms16	R	S	R	R	R	R	R	S
Ms19	R	S	S	S	S	S	S	S
Ms20	R	S	S	S	R	S	R	S

Antibiotics: CAZ – Ceftazidime, CRX – Cefuroxime, GEN – Gentamicin, CXM – Cefixime, OFL – Ofloxacin, AUG – Augmentin, NIT – Nitrofurantoin, CPR – Ciprofloxacin

Tables 4.5 The Antibiotics Susceptibility of *E. coli0157:h7* isolates

Antibiotics	**S**(n%)	**R**(n%)
Ofloxacin	**9 (17%)**	**2 (6%)**
Augmentin	**0**	**11 (31%)**
Nitrofurantoin	**8 (15%)**	**3 (8%)**
Ciprofloxacin	**9 (17%)**	**2 (6%)**
Ceftazidime	**5 (10%)**	**6 (17%)**
Cefuroxime	**2 (9%)**	**9 (25%)**
Gentamicin	**10 (19%)**	**1 (3%)**
Cefixime	**9 (17%)**	**2 (6%)**

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.0 DISCUSSION

E. coli0157:H7 is found all over the world and is usually found in the normal flora of humans and animals' intestines. Spores of the organism can be found in soil, sediments, and locations contaminated by human or animal feces (Mafruza *et al.,* 2012)

E. coli0157:H7 was not detected or isolated in the meat samples in this study. This confirm that meat samples are vehicles of *E. coli0157:H7* as reported in a similar study conducted by Nashwa *et al.,* (2016). These occurrences recorded in this study may be as a result of poor hygiene practice by the meat vendors and also the contamination of the meat with the intestinal content of the animal. Poor storage conditions including environmental hygiene were the main reason why there was reproduction of spores which have led to the increase in bacterial count in the meat samples.

5.1 CONCLUSION

Selected meat samples from markets in ikorodu, Lagos State as used in this research has shown that inappropriate sanitation of meats can lead to infections by some pathogenic bacteria like *E. coli 0157:H7* capable of endangering lives, and can eradicated with the following antibiotics.

5.2 RECOMMENDATION

Working with meats samples requires concentration and caution. Cultured meats samples are to not exceed the required temperature and time in order to avoid over growth. All equipment used are to be cleansed with ethanol or by sterilizing to kill

pathogens to avoid infections to the scientist. Proper use of nose mask, gloves and lab coat, always wear a well-covered shoe in the laboratory.

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APPENDIX

ANTIBIOTICS SUSCEPTIBILITY

ISOLATES	AUG	NIT	CPR	CAZ	CRX	GEN	CXM	OFL
Ms2	0	9	24.5	11	0	15.3	0	25.5
Ms3	0	0	18	0	0	15	0	26
Ms5	0	17.5	0	12	22.5	11.5	0	0
Ms7	0	0	31	0	0	16	0	28
Ms9	0	15	26	23.5	0	15.5	30	30
Ms13	0	12	23.5	0	0	17	0	22
Ms14	0	15.5	18	0	0	14.5	0	23
Ms15	0	0	17	0	0	18	0	15
Ms16	0	14.5	0	0	0	0	0	0
Ms19	0	15	21	17	26	17.5	29	22
Ms20	0	13	25	12.5	0	15.5	0	26.5

ANTIBIOTIC SUSCEPTIBILITY

Antibiotics: CAZ – Ceftazidime, CRX – Cefuroxime, GEN – Gentamicin, CXM – Cefixime, OFL – Ofloxacin, AUG – Augmentin, NIT – Nitrofurantoin, CPR – Ciprofloxacin