OCCURENCE OF PATHOGENIC MICROORGANISMS IN STREET VENDED SPICED GRILLED MEAT PRODUCT (SUYA) SOLD IN MAGBORO, OGUN STATE

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

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CERTIFICATION

This is to certify that this research project titled **"OCCURENCE OF PATHOGENIC MICROORGANISMS IN STREET VENDED SPICED GRILLED MEAT PRODUCT (SUYA) SOLD IN MAGBORO, OGUN STATE"** was carried out by DAVIES, Priscilla Anu with matriculation number 16010101004. This project meets the requirement governing the award of Bachelor of Science (B.Sc.) Degree in Microbiology department of biological sciences of the Mountain Top University, Ogun State, Nigeria and is approved for its contribution to knowledge and literary presentation.

DR. O.E FAYEMI

(Project Supervisor) Date

DR. O.E FAYEMI

(Head of Department) Date

DECLARATION

I hereby declare that this project report written under the supervision of Dr. Fayemi O.E 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 research project report has not been previously presented anywhere for the award of any degree or certificate.

_____ _____

DAVIES A. PRISCILLA Date

DEDICATION

I dedicate this project to God Almighty for giving me life, good health and all I needed to make this work a success and secondly to my alluring parents, Pastor and Pastor (Mrs.) Davies and my dear brother Emmanuel Davies, for their guidance, understanding and sacrifice. I also dedicate this work to my course-mate and friends for their support in the course of my four years study of Microbiology in Mountain Top University. May the Almighty God bless you all! Amen.

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ABSTRACT

Street vended foods sold are sources of food readily available to people all over the world, and the microbial quality and safety of these foods is always uncertain. Street food is the main source of foodborne diseases in developing countries because it provides a source of cheap nutrients for most low-income groups. This study was aimed at determining the prevalence of *Salmonella, Escherichia coli* and Yeasts and moulds in street vended suya sold at Magboro area of Ofada/Mokoloki LCDA, Ogun state. The samples were cultured on Eosin methylene blue agar, Potato dextrose agar, Nutrient agar, Sorbitol-MacConkey Agar, MacConkey agar and Xylose lysine deoxycholoate agar. The highest count of pathogenic *E. coli* was 3.6 Log10 CFU/g while for Total viable count was 6.2 Log10 CFU/g. *Salmonella* was not detected in 25g of the food sample. This study has demonstrated that suya sample sold in Magboro market constitute a potential health hazard to consumers as a result of the pathogenic microorganisms isolated from the food that rendered them microbiologically unsafe and unacceptable. Hence, the need for monitoring of this nutrition products by educating processors and consumers on good sanitary practices during processing displaying and sale of the products and the possible danger of contaminated products.

Keyword: Street vended suya, *Salmonella, Escherichia coli,* Yeasts and Moulds, microbial quality, good sanitary practices.

CHAPTER ONE

1.0 Introduction

Ready-to-eat foods (RTEs) are street foods that are eaten without any further processing or preparation. Traditionally or industrially processed packaged meals can typically be considered to contain, immediately or later consumed publicly sold foods (Cerna-Cortes *et al.,* 2015; FAO and WHO 2004; Von Holy and Makhoane, 2006). Consumers, mainly urban workers, enjoy these foods because of their taste, low cost, nutritional value, varieties, and availability for immediate consumption (Abdalla *et al.,* 2009).

Similar to other areas, due to ease of processing, availability, affordability, and palatability, RTEs are more consumed in low-and middle-income countries (LMICs) (Al Mamun *et al.,* 2013a; Al Mamun *et al.,* 2013b; Mensah *et al.,* 2002). The RTEs can be processed into foods that may be liquid, semi-solid, or solid in consistency from single or mixed raw ingredients, such as cereals, fish, meat, nuts, and spices (Adebayo-Oyetoro *et al.,* 2017; Ceyhun Sezgin and Sanher, 2016; Feglo and Sakyi, 2012). Many RTE vendors in LMICs often lack knowledge of good hygiene practices that may predispose foods to microbial contamination (Al Mamun *et al.,* 2013a, 2013b; WHO, 2010).

Bacteria and fungi in the soil and around us are widespread and abundant which infects food readily. (Bukar *et al.,* 2010). Meat is an essential food that contains a large amount of protein and promotes the growth of large amounts of microorganisms because of its cut surfaces. Bacterial pathogens are associated with the most severe safety issues that result in immediate health problems for customers (Sousa, 2008). Suya meat is a form of RTE food that has become widely popular and is consumed in Nigeria by the majority. Concerns about the hygienic standards of Suya roadside processing and safety were posed (Obadina *et al.,* 2014).

The protection and hygienic implications of street food sold in Kogi state, Nigeria, were examined by Metiboba and Kakwagh (2014). They discovered that most food sold was unfit for human consumption. The poor construction and vending sites, as well as the lack of facilities such as refuse collection facilities, have been described as the key factor responsible for the lack of food safety and quality. Street vendors play an important role in meeting the food demands of urban dwellers, according to Chukuezi (2010), but their activities are associated with a range of health hazards.

As in other developing countries, Nigeria's street food faces challenges. Food safety officers are inadequately supervised (Oyeneho and Hedberg, 2013); there is also a lack of food safety

training and good hygiene practices among food handlers. Therefore, street food, sometimes at all levels of handling, is at risk of contamination. Street foods are often processed and sold at unsafe temperatures from sales locations that include kiosks, make-shift housing, push carts and other temporary structures. They are prepared with waste water and garbage disposed of nearby in much polluted areas, providing nutrients and breeding ground for rodents and vermin (Barro *et al.,* 2006). Washing of hands and crockery is performed in bowls or buckets and often without soap (Abdalla *et al.,* 2008). In most instances, running water is not accessible at vending sites. In addition, the conditions under which street food is cooked and sold are compounded by the inadequate enforcement of applicable legislation on the environment and public health.

The target of this study is to determine the presence of pathogenic microorganisms that pose a threat to the safety of consumers and investigate the microbiological quality of street vended suya samples from Magboro, Ogun state in order to evaluate its hygiene standard.

CHAPTER TWO

2.0 Literature Review

It is because of its rich and nutritious nature, meat and meat products have gradually become part of the daily human diet. Beef and chevon were reported to contain high-quality proteins, minerals, vitamins and fat (Iroha *et al.*, 2011; Francis *et al.*, 2015). Due to the rise in demand for meat and its ingredients, livestock slaughtering continues to increase (Warris, 2010). The highly nutritious essence of meat provides an ideal habitat for the growth of species that are pathogenic, nonpathogenic and cause spoilage (Aleruchi *et al.*, 2016).

To many Nigerians, street-sold meat products such as chicken and beef suya are popular delicacies. In almost every neighborhood with a dense population, chicken and beef suya vendors are found for different formal or informal daily economic activities. Suya is a meat product that is processed on the lane, roasted and sold (Ologhobo *et al.*, 2010). Usually, Suya is made with skewered beef, ram, or chicken. Innards are also used, such as the intestine, liver and tripe (Eke *et al.*, 2012). In different spices, which include peanut cake, salt, vegetable oil, and other flavorings, the thinly sliced meat is marinated and then barbecued (Egbebi and Seidu, 2011; Eke *et al.*, 2012). Helpings of dried pepper combined with spices and sliced onions are eaten with Suya.

A few steps are involved in the process of processing Suya meat, first the grounding of peanuts. Upon grinding into a fine powder using mortar and pestle, the shell and the skin are separated from the peanut or crushed with a rolling pin. If the powder is oily, it is rolled and squeezed for a minute or two with the absorbent material. Then the peanut powder is combined with ground pepper, garlic and ginger which is mixed properly. In a bowl containing the peanut-spice blend, the meat is then cut into small sizes or thinly slices, dipped and rolled then allowed to coat fully. After the meat slices are threaded into skewers, brushed with vegetable oil and roasted for fifteen to twenty minutes on the glowing charcoal fire, the marinated meat is then held for thirty minutes or more for the peanut cake to adhere to it (Olaoye *et al.*, 2016).

Suya meat has become widely popular and is consumed in Nigeria by the majority. Most of suya's processors were located in strategic locations and were individuals with minimal experience in proper handling and hygiene of food. Traditional methods of handling, processing and packaging the goods are still used in the process of suya production, which are considered unhygienic, unhealthy and can result in rapid degradation of processed meat if not consumed within a short period of time (Rokade *et al.*, 2012).

Concerns about the hygienic standards of Suya roadside processing and safety have been raised (Obadina *et al.,* 2014). Suya is typically sold wrapped in old newspaper, which has been criticized as a potential source of pollution for serving (Apata *et al.,* 2013). The use of old newspapers in Suya packaging is a concern as pigments, colorants, binders, additives and photo initiators can cling to the printed inks on the sheets, and become hazardous to the consumer's health (Rokade *et al.,* 2012).

Packaging not only ensures that the quantity of the appropriate ingredient is contained and preserved by the food, but also increases sensory consistency as well as color stability. Food packaging has been shown to be capable of retarding product degradation, preserving the beneficial effects of manufacturing, extending shelf life and sustaining or increasing food quality and protection (Marsh and Bugusu, 2007). It is therefore necessary for food packaging materials to have adequate mechanical, thermal and optical properties for food products. In addition, in food packaging materials, anti-microbial and barrier functions against gases, vapour and scent are also important (Chin *et al.,* 2015). To prevent the growth of microbes, Suya meats should be stored between 50 to 60^{o}C (Uzeh and Akinyemi, 2012).

Suya can be made from beef or chicken in Nigeria, with the latter just gaining popularity. Without further processing or cooking, these foods are RTE, bought and consumed. In countries with high unemployment, low incomes and a weak social security programme, street food sales are a common occurrence (Ologhobo *et al.,* 2010). Customers of street-sold meat, however are little aware of the high health risks they face. At any point of processing and storage, street foods are subjected to different sources of contamination. Raw meat and meat products have been listed as important foodborne disease vehicles and have been implicated in outbreaks of foodborne diseases (Aleruchi *et al.,* 2016). Ingestion of infected products allows diseases to spread to humans by meat and meat products.

Diseases caused by foodborne pathogens pose a public health concern worldwide (Maha *et al.,* 2014). *Escherichia coli, Clostrudium perfringens,* fecal *Streptococci, Klebsiella pneumoniae*, as well as *Salmonella, Shigella, Bacillus, Staphylococcus* and *Listeria* species include meat pathogens (Iroha *et al.,* 2011). The health status of animals prior to slaughter and the hygiene status of the slaughterhouses contribute to the quality of those animals' meat (Nnachi *et al.,* 2014). Reports have suggested that animal slaughter is typically conducted under unhygienic conditions in rural communities within Nigeria. Potable water is not available in most situations, leaving butchers with unhealthy sources of water to use. In addition to storage at

high ambient temperature, humidity and improper handling methods, these aforementioned factors dispose raw meat to degradation and contamination (Nnachi *et al.,* 2014; Raji *et al.*, 2006).

2.1 Consumption and Contamination of Street Vended Foods

Many street food handlers in LMICs often lack knowledge of good hygiene practices that can predispose foods to microbial contamination (Al Mamun *et al.*, 2013a, 2013b; WHO, 2010). The method of selling RTEs in outdoor areas further complicates the situation. Consequently, aerosols, insects, and rodents that serve as sources of food pollutants are exposed to the food (Fowoyo and Igbokwe, 2014; Mensah *et al.,* 2002). Microbial contaminants of RTEs include bacteria such as species of *Bacillus*, coagulase negative *Staphylococci*, *Escherichia coli*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Pseudomonas spp*., and *Staphylococcus aureus* (Annan-Prah *et al.,* 2011; Fadahunsi and Makinde, 2018; Felgo and Sakyi, 2012; Gdoura-Ben Amor *et al.,* 2018; Kharel *et al.,* 2016; Tambekar *et al.,* 2009), fungi (such as diverse toxigenic species of *Aspergillus, Alternaria, Fusarium,* and *Penicillium* (Adjou *et al.*, 2012; Oranusi and Nubi, 2016), parasites (*Ascaris* lumbricoides and *Toxoplasma gondii* (Abd El-Razik *et al.,* 2014; Manyi *et al.,* 2014), and viruses (hepatitis A virus) (Yongsi, 2018).

Contaminating bacteria and fungi can also pose an increased risk to public health through the secretion of toxic compounds such as cereulide by *B. Cereus* (Ceuppens *et al.,* 2011) and mycotoxins (IARC, 2015) during food processing at various stages. For example, heavy metals, lead (Jalbani and Soylak, 2015) and pesticide residues such as tetradifon (Skretteberg *et al.*, 2015), mostly found in plant-based RTEs, as well as polyaromatic hydrocarbons from car fumes and other industrial sources, are non-microbial-related RTE pollutants (Proietti *et al.*, 2014).

Acute or chronic foodborne diseases (FBD) can result, depending on the type and concentration of the contaminant, the amount of food ingested by the consumer and the consumer's health status. On the other hand, continuous regular exposure, through the ingestion of polluted RTEs, to single or mixed food pollutants may produce a plethora of adverse health effects. Extreme complications such as tumors, neural tube defects, and even human deaths can vary from moderate to chronic nausea, vomiting, and diarrhea (Ceuppens *et al.,* 2011). Thus, poorly prepared RTEs in LMICs, where enforcement regulations and monitoring are grossly insufficient, can pose a huge public health risk (Gibb *et al.,* 2015; IARC, 2015; JECFA, 2017, 2018; Kamala *et al.,* 2018; Wild and Gong, 2010).

Food Borne Disease is thus a significant impediment to growth and development in the affected LMICs, since it is possible to redistribute resources used in the treatment of these preventable diseases to create other sectors of the economy (Alimi, 2016).

Hazard Analysis of Street Vended Foods

From the initial contamination of raw foods with pathogenic bacteria to subsequent contamination by vendors during preparation, the factors that should be considered for analyzing the hazards due to street foods are summarized in Table 2.1.

Table 2.1: Sources and type of hazard and the microbial risk involved. Source: Rane, 2011

S/N	Source	Hazard	Risk involved
1	Vendor location	Improper food handling	Transfer of pathogens like *Salmonella, E. coli, S. aureus* from human body and environment into foods
		Improper waste disposal	Transmission of enteric pathogens like *Salmonella, Shigella and E. coli* via vectors
2	Raw materials	Water	Passage of pathogens like *E. coli,* faecal streptococci, *Salmonella* and *Vibrio cholera*
		Vegetables and spices	Introduction spore formers like *Bacilli* and *Clostridium* and pathogens like *L. monocytogens, Shigella, Salmonella,* etc.
3	Utensils and equipment	Chemical contaminants	Leaching of chemical leading to poisoning
		Microbial contaminants	Cross contamination of food with *Staphylococcus aureus, E. coli* and *Shigella* due to contaminated water, dish cloth, handler.
4	Storage and reheating	Improper storage temperature and reheating of food	Likelihood of heat stable toxins produced by pathogens like *C. perfringens* and *B. cereus*.
5	Personal hygiene of vendors	Biological hazards	Introduction of *Staphylococcus*, *Salmonella* and *Shigella* via carriers.

2.2 *Salmonella*

Salmonella is an intracellular pathogen that causes a number of diseases, ranging from gastroenteritis to enteric fever in humans and animals, called salmonellosis (Lan *et al.,* 2007).

The genus consists of two different species, *Salmonella bongori* and *Salmonella enterica*, the former being a microbe of cold-blooded animal, while the latter consists of six subspecies: *enterica*, *salamae*, *arizonae*, *diarizonae*, *indica*, and *houtenae* (Levantesi *et al.*, 2011).

Salmonella, in some serotypes, are disease-causing intracellular pathogens. Non-typhoidal serotypes can be passed from animal to human and from human to human (Su and Chiu, 2007). They usually penetrate the gastrointestinal tract only and cause salmonellosis that can be treated without antibiotics. It is only possible to transfer typhoidal serotypes from human to human, causing food infection, typhoid fever and paratyphoid fever (Bethesda *et al.*, 2019). Gastroenteritis, accompanied by bacteremia and enteric fever, is the world's most common manifestation of *Salmonella* infection (Majowicz *et al.*, 2010).

2.2.1 Transmission of *Salmonella*

In nature, *Salmonella* is popular and often persists in a variety of foods. Poultry, eggs, and dairy products are the most common carriers of salmonellosis. In recent years, transmission vehicles have become increasingly concerned with fresh goods such as fruit and vegetables, where contamination can occur at multiple stages along the food chain (Bouchrif *et al.*, 2009).

First, since *Salmonella* can survive in the environment for a long time, the environment contaminated with *Salmonella* serves as the source of infection. After that, *Salmonella* is spread to vectors such as rodents, flies and birds where *Salmonella* is shed in their feaces for weeks and even months. The key risk factor for infection following direct transmission is moving animals such as pigs, cows and chickens (Newell *et al.*, 2010). As *Salmonella* is usually from the toxic environment and also from tainted feed, animal sources of this type are orally infected. Human beings get ill by eating food or drinking water infected with *Salmonella* from animal ponds. There is, however, no animal reservoir for *Salmonella Typhi* and *Salmonella* Paratyphi A, so infection will occur by eating infected foods handled inappropriately by individuals (Newell *et al.*, 2010). In addition, the transmission of *Salmonella* is also of great importance to food processing plants and food preparation equipment. The risk of salmonellosis can result from human consumption if carried by vectors and transferred to food. The *Salmonella* cells can bind to food contact surfaces such as plastic cutting boards that can turn into biofilms once attached and thus cause cross-contamination (Pui *et al.*, 2011).

2.2.2 Food Materials Involved in *Salmonella* Outbreak

Salmonellosis is caused by eating raw and undercooked eggs, undercooked poultry and meat, raw fruits and vegetables that are infected (such as sprouts and melons), as well as raw milk

and other unpasteurized milk products (Raufu *et al.*, 2013). Various Gram-negative bacteria are known to contaminate food sold on the street. Amongst such, there have been widespread reports of *E. coli, Klebsiella species, Salmonella,* and *Pseudomonas*. In street-sold juice in Sudan (North Africa) and in zobo from Nigeria (West Africa), respectively, Abdallah and Mustafa (2010) and Bukar *et al.,* (2009) detected *Salmonella.*

2.2.3 Pathogenesis of *Salmonella*

Salmonella is capable of attacking epithelial cells, M cells, macrophages, and dendritic cells of different types of cells (LaRock *et al.,* 2015). They pass through the stomach of a person and colonize the small and large intestines when *Salmonella* bacteria are ingested (Grassl *et al.*, 2014). There, bacteria enter the mucosa of the intestine and proliferate. By inhibiting the fusion of digestive enzymes using a type three secretion system, bacteria can invade the lymphoid tissues of the gastrointestinal tract and spread to the bloodstream (Conner *et al.*, 2012). Bloodstream transmission relies on host factors and *Salmonella* strain virulence and occurs in less than 5% of infections. Any organ (such as the liver, gallbladder, bones, or meninges) can become infected if the infection spreads to the bloodstream.

It is possible to classify serotypes of *Salmonella* into two main groups: typhoid and non-typhoid. Non-typhoidal serotypes are more prevalent, usually causing self-limiting gastrointestinal disease. A variety of animals can be infected and are zoonotic, meaning that they can be spread between humans and other species (Conner *et al.,* 2012). Typhoid serotypes include human-adapted *Salmonella Typhi* and *Salmonella Paratyphi A* that are not present in other animals.

NONTYPHOIDAL *SALMONELLA*

NON-INVASIVE

Non-typhoidal *Salmonella* infection typically occurs when food containing a high bacterial concentration is consumed by a person (Grassl *et al.*, 2014). Infants and young children are much more vulnerable to infection, and a small amount of bacteria can easily be swallowed.

In healthy adults, the species enter through the digestive tract and must be consumed in significant quantities to induce disease. It is only after the living *salmonella* enter the gastrointestinal tract that an infection can begin. While the stomach kills some of the pathogens, the surviving ones enter the small intestine and multiply throughout the tissues. The death of the majority of ingested pathogenic bacteria is as a result of gastric acidity, but *Salmonella* has

established a degree of tolerance to acidic conditions that enables a subset of ingested bacteria to survive (Tischler and McKinney, 2010).

INVASIVE

Nontyphoidal serotypes are often present in developing nations as gastrointestinal diseases (*Salmonella enteritidis*). In the United States, about 45,000 cases a year are registered, but there could actually be as many as 2 to 3 million cases annually (WHO, 2012). The incubation period is just about 8 to 48 hours when the bacteria are in the body. As the bacteria multiply and invade the intestinal mucosa, they develop an enterotoxin and a cytotoxin that destroys the epithelial cells, the disease results from a true food-borne infection. The most common symptoms are stomach pain, cramps, diarrhea, nausea, vomiting, and fever, which typically lasts for 2 to 5 days but can last for several weeks (Conner *et al.,* 2012). As many as 1 billion *Salmonella* per gram of feces can be found during the acute phase of the disease. Most adult patients recover, but for children and elderly people, the lack of fluids may cause complications.

TYPHOIDAL *SALMONELLA*

Infection with *Salmonella typhi* is acquired by ingesting food or water that has been contaminated with faces of infected humans or by interaction between people (Tischler and McKinney, 2010). The bacteria spread to the lymphoid tissue, blood, liver, and bile, colonizing the small intestine, entering the epithelium (Tischler and McKinney, 2010; Bhan *et al.,* 2005). Unlike *S. typhimurium*, by preventing the initiation of early inflammatory response in the human intestine, *S. typhi* allows the colonization process in deeper body tissues. *S. typhi* performs this function with the help of a secretion system (Bhan *et al.,* 2005).

This behavior is accomplished by generating factors that inhibit inflammatory response and thus allow the gallbladder to be systemically invaded and colonized (Tischler and McKinney, 2010). There are two type III secretion systems (T3SS-1 and T3SS-2) encoded by SPI-1 that deliver bacterial proteins to non-phagocytic epithelial cells (Bhan *et al.,* 2005; Tischler and McKinney, 2010; Kaur and Jain, 2011) which triggered the invasion of intestinal mucosa. These variables interfere with the role of host cells while facilitating bacterial penetration into the intestinal epithelial cells and enabling the host macrophage to survive and multiply (Tischler and McKinney, 2010).

When infection reaches a threshold level determined by the number of bacteria, virulence, and host immune response, the bacteria are systemically disseminated, which initiates the process of bacteremia to colonize macrophages in other organs. The most common secondary infection sites are terminal ileum liver, spleen, bone marrow, gallbladder, and Peyer's patches (Kaur and Jain, 2011). It is an infection in the gallbladder that can turn a person into the pathogen's asymptomatic carrier.

2.2.4 Prevention of *Salmonella*

Prevention includes monitoring measures at all levels of the food chain, ranging from agricultural production to food processing, production and preparation in both commercial and domestic establishments. National and regional foodborne disease surveillance systems are effective means of understanding and tracking the situation of these diseases, as well as detecting and responding to early-stage salmonellosis and other enteric infections thus, preventing those from spreading further (WHO, 2018).

2.3 *Shigella*

One of the leading causes of diarrheal disease is *Shigella,* a highly virulent pathogen that causes bacterial dysentery, and contributes significantly to the worldwide burden. Shigellosis or Bacillary dysentery, a gastrointestinal disease caused by a species of *Shigella*, is recognized worldwide as a serious health problem. Owing to insufficient waste management, poor hygiene conditions and unsafe drinking water, it is often found in developing countries. It is primarily due to travel to unindustrialized countries in developed nations and use of polluted food materials (Izumiya *et al.,* 2009). Globally, in children under five years of age, mortality and morbidity due to shigellosis were found to be highest (Wen *et al.,* 2014).

Shigella is responsible for 80-165 million disease cases and 600,000 deaths worldwide each year, of which 1.5 million are registered in developed countries and 163 million in developing countries (Heiman and Bowen, 2013). Each year, around 500,000 cases of shigellosis are registered in the United States (Painter *et al.,* 2015).

2.3.1 Serovars and tranmsission of *Shigella*

The genus *Shigella* is composed of four species: *Shigella dysenteriae* (serogroup A), *Shigella flexneri* (serogroup B), *Shigella boydii* (serogroup C) and *Shigella sonnei* (serogroup D). These species are further distributed into multiple serotypes, according to biochemical characterization and serological properties, as *Shigella dysenteriae* has 15 serotypes, *Shigella flexneri* has 14 serotypes and subserotypes, *Shigella boydii* has 20 serotypes, and *Shigella*

sonnei has a single serotype (Livio *et al.,* 2014). Such species are also known as bacillary dysentery, the etiological agent of Shigellosis. Symptoms can range from mild watery diarrhea to extreme inflammatory dysentery when mucoid and bloody stools are passed through.

Abdominal cramping, fever, nausea, malaise, vomiting and convulsions are the other clinical manifestations. Septicemia, dehydration, joint pains, hypoglycemia, hemolytic uremia and neurological complications are other complications of shigellosis (Marteyn *et al.,* 2012).

The mode of transmission is through the fecal-oral route and through direct contact with the person infected. As only 10-100 organisms are necessary to cause disease and the bacteria are more immune to stomach acid and can easily pass through the gastric acid barrier, the *Shigella* species are highly infectious (Patil and lava, 2012). There is currently no protective vaccine targeting *Shigella*, but many *Shigella* vaccine candidates, including killed, live attenuated, ribosomal and conjugate vaccines, and are under development (WHO, 2006). Good hygiene, safe handling and processing of food, the use of clean vegetable cutting boards, adequate cooking of food, proper washing of raw vegetables prior to serving, the use of boiling water and the safety of food from flies are recommended to control shigellosis.

2.4 *Escherichia Coli*

The Gram-negative, facultative anaerobic and non-sporulating bacterium *Escherichia coli* (*E. coli*) is often known to be of fecal origin. In humans and warm-blooded animals, it is commonly found in the gut. Many *E. coli* strains are harmless. However, some strains, such as Shiga toxin-producing *E. coli* (STEC), can cause serious foodborne illnesses (WHO, 2018). It is capable of living in water and soil, under frozen and refrigerated temperatures, and under dry conditions for prolonged periods of time and can only be killed by extensive cooking or pasteurization (Davis and Kendall, 2012). Particularly in young children and adults in developing countries and in poor sanitation conditions, *E. coli* is a major cause of diarrhea, and *Escherichia coli* O157: H7 is a highly pathogenic strain of *Escherichia coli* (Davis and Kendall, 2012).

2.4.1 Strains of *E.coli*

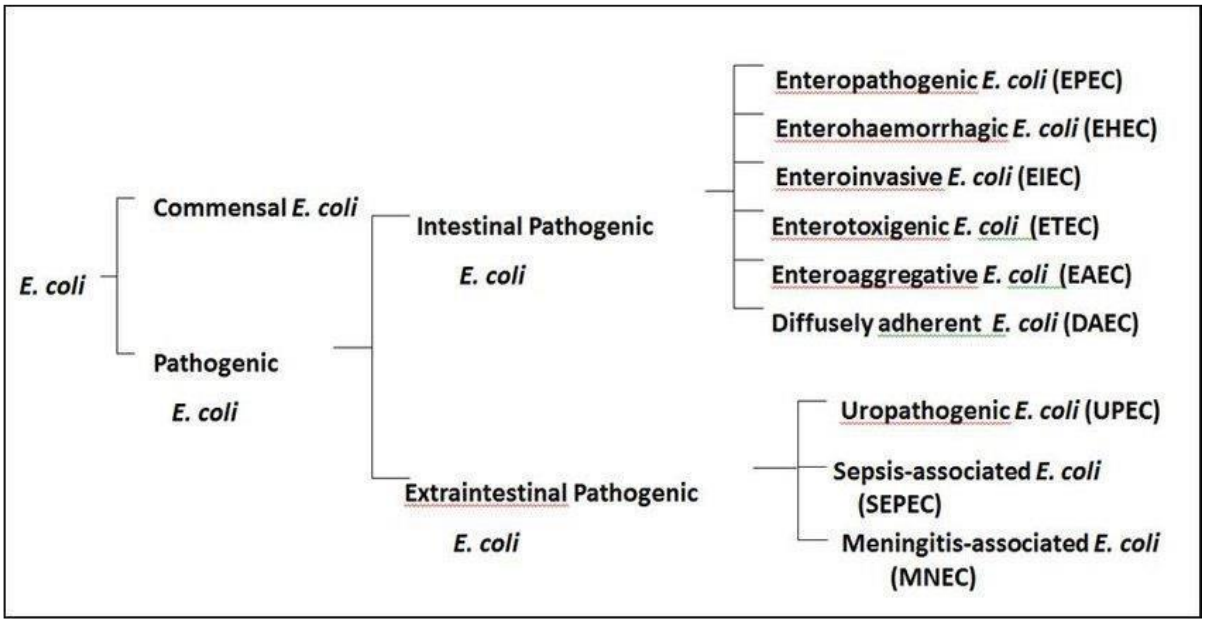


Figure 2.4.1: *Escherichia coli* species and its sub species classification. Source: Wakeham, (2013)

2.4.2 Shiga Toxin *E. coli* (STEC)

Vero toxigenic or Shiga-like toxigenic *E. coli* O157: H7 (VTEC or STEC) is known to be a major foodborne disease threat. *E. coli* O157: H7 was the first of several strains that can produce one or more Shiga toxins (also known as verocytotoxins and previously known as Shiga like toxins) classified as Entero-hemorrhagic *E. coli* or EHEC (Croxen, 2013). It is mainly transmitted to humans through the ingestion of infected foods, such as raw or undercooked ground meat products, raw milk, and raw vegetables and sprouts that are contaminated.

Because of its similarity to the toxin produced by *Shigella dysenteric*, STEC produces toxins, referred to as Shiga-toxins, STEC can grow at temperatures ranging from 7^{o}C to 50^{o}C, with an optimum temperature of 37^{o}C (2018: WHO). In acidic foods, some STECs may develop down to $_{P}$H 4.4 and in foods with a minimum water activity (a_w) of 0.95.

Launders *et al.* (2016) stated that the presence of STEC in potatoes is a concern as it may cause cross contamination with other foods that are consumed raw. In addition, the presence of *E. coli* in street vended meat from Bangladesh (Asia) and Cameroon (Central Africa), respectively was stated by Biswas *et al.*, (2010) and Yannick *et al.*, (2013), whereas diarrheagenic *E. coli* strains were recovered from grilled chicken in Burkina Faso (West Africa) (Somda *et al.*,

2018). Similarly, in street vended juice, *Pseudomonas* was detected in Pakistan, (Batool *et al.*, 2013).

Domestic animals and wildlife are also a potential source of pathogenic bacteria, particularly in the pre-harvest phase of lettuce and leafy greens along the California coast (Stuart *et al.*, 2006). Berger *et al.*, (2010) demonstrated that wildlife feces are involved in vegetable contamination and can cause *E. coli* O157: H7. Jay-Russell *et al.* (2014) studied a potential pathogenic reservoir of *E. coli* in coyote and dog feces. Insects can be a cause of plant contamination, too. Infected flies have been shown to pass *E. coli* to fruits or leaves.

Via thorough cooking of foods, STEC is killed until all parts hit a temperature of 70^{o}C or higher (WHO, 2018).

2.4.3 Transmission, symptoms and treatment of *Escherichia coli* O157:H7

E. coli is spread to humans through fecal food and water contamination, by cross-contamination, or through direct human interaction during food preparation. The key route of contamination tends to be through the ingestion of contaminated foods, such as ground meat products, raw milk and fresh produce, raw or undercooked (FAO, 2011).

According to the Center of Disease Control and Prevention (CDC), the signs of *E. coli* O157:H7 infection are bloody diarrhea, severe stomach cramps, vomiting and fever, which was first identified on 1982. The incubation time is usually about three to nine days (Marley, 2007). Symptoms ranging from mild to severe and bloody diarrhea are caused by this. The infection can lead to a life-threatening disease, such as hemolytic uremic syndrome (HUS) (FAO, 2011), in up to 10% of patients (especially young children and the elderly). There are, however, many ways to prevent this infection, such as by thoroughly cooking the ground meat and vegetables, avoiding the use of raw milk and unpasteurized milk products, and eventually practicing hand washing to avoid cross-contamination in food preparation (Marley, 2007).

Infection therapy is largely dependent on rehydration, whereas antibiotic therapy is frequently contraindicated because it can induce the release of Shiga toxin and thereby cause clinical deterioration with a possible evolution to HUS (CDC, 2011).

2.4.4 Prevention of *E. coli 0157: H7*

Prevention requires a multidisciplinary approach to the processing of animals and plants and risk-based approaches across the food supply chain. These include the implementation from farm to consumer of Good Agricultural Practices (GAP), Good Manufacturing Practices

(GMP), Good Hygiene Practices (GHP) and Hazard Analysis Critical Control Point (HACCP) (FAO, 2011).

Food workers have also been involved in unsafe food handling, facilitating microbial contamination of ready-to-eat products as various studies suggest. Typically, this happens because food handlers have poor personal hygiene or are asymptomatic carriers of pathogenic micro-organisms. Proper hand-washing and personal hygiene are measures to reduce the risk of contamination by food workers (Faour-Klingbeil *et al.*, 2016).

Five (5) simple measures are proposed by The World Health Organization (2019) to avoid food contamination with *E. coli* and other enteropathogens: (1) separating raw and cooked food, (2) keeping the working area clean, (3) cooking (food thoroughly), (4) keeping food at safe temperature and (5) using safe water and raw materials.

2.5 Yeasts and Moulds

Yeasts are eukaryotic singled celled microorganisms classified as members of the kingdom of the fungus. Hundreds of millions of years ago, the first yeast originated and 1,500 species are currently identified (Hoffman *et al.*, 2015). It is estimated that these constitute 1 percent of all fungal species described. Moulds are fungi that grow in the form of multi-cellular filaments called hyphae (Moore *et al.*, 2011). Yeasts are capable of growing in the presence of sugars, organic acids and other easily metabolized carbon sources in foods with a neutral or slightly acidic pH environment. On their surfaces, as in cheeses or meats, the growth of yeasts within food products is often seen, or by the fermentation of sugars in drinks, such as juices, and semi-liquid products, such as syrups and jams (Karabagias, 2018).

In the shape of a visible 'mycelium' made up of many cells, moulds tend to grow on the surface of objects. For the food industry, moulds have both positive and negative effects. For the food industry, specific types of moulds are beneficial, while other types of moulds can be quite toxic and can generate allergic reactions and breathing problems or produce poisonous substances called mycotoxins (Karabagias, 2018). For example, Aspergillus mold, which is most frequently found in meat and poultry (as well as in the environment can cause an infection called Aspergillosis, which is actually a group of diseases ranging from mild to severe lung infections or even infections of the whole body. The mycotoxins that some varieties produce are one of the biggest concerns regarding mould in food. Aflatoxin, a cancer-causing poison, is one of the most investigated mycotoxins (Kurtzman, 2006).

2.5.1 Fungal Contamination in Street Vended Foods

Due to the vendor's practice of displaying the foods freely in markets, fungal contamination of street foods sold is prevalent, such that they are exposed to fungal spores. Various fungal genera contaminate food products, but *Aspergillus, Fusarium, Mucor*, *Penicillium*, and *Rhizopus* are commonly found in street foods. Retailed kulikuli (peanut cake) and salads from the Republic of Benin, Togo, and Nigeria have been confirmed to be contaminated with *Aspergillus* and *Fusarium* (Adjou *et al.*, 2012). Similarly, *Aspergillus* and *Penicillium* were reported in street-vended doughnut, egg roll, and meat pie from Nigeria (Oranusi and Braide, 2012).

2.6 Prevalence of Foodborne Illness

The United Nations World Health Organization (WHO) has reported that LMICs, particularly those in the sub-regions of Africa and South-East Asia, suffer significantly from the burden of FBD (Havelaar *et al.*, 2015), sometimes resulting in enormous economic losses. According to a recent report by the World Health Organization (WHO), over 91 million people are affected in Africa. It has also been reported that in developing countries, 2.2 million children die of diarrhea each year, while more than 600,000 children are reported to have died on an annual basis due to the consumption of unhealthy food in Southeast Asia (WHO 2015).

2.6.1 Prevention of Foodborne Illness

The quest for measures aimed at ensuring food safety, inclusive of RTEs, worldwide has been marked by both successes and difficult efforts (FAO/WHO, 2018). In order to minimize contamination from street foods sold, good personal hygiene is required during actual food preparation as well as during food packaging. Since food is biological in nature, the growth of microorganisms and foodborne diseases resulting from the ingestion of infected foods and food products can be supported (Nyenje *et al.*, 2012).

The leading cause of serious and fatal foodborne illnesses is foodborne bacterial agents. Of the many thousands of different bacterial species, *Staphylococcus, Salmonella, Clostridium, Campylobacter, Listeria, Vibrio, Bacillus*, and Enteropathogenic *Escherichia coli* species cause more than 90% of food-poisoning diseases (Tambekar *et al.*, 2008). The control of the key risk factors associated with street foods should be improved in order to ensure street food safety for the protection of consumers against unhealthy foods, and successful preventive measures need to be taken at an appropriate stage (Sezgin and Şanlıer, 2016).

The viability of interventions in low-income countries with large populations, sanitation or environmental conditions and awareness of food safety issues among producers, suppliers and

consumers of street foods are key factors for mitigating foodborne hazards (Oluwadamilola *et al.*, 2020) .Many foodborne diseases can be avoided by proper food handling and storage. Street food trade stakeholders, particularly street food suppliers, consumers and governments, need to be involved in ensuring the safety of food sold (Alimi, 2016). Strengthening food safety policies and strict implementation of food safety regulations will dramatically deter, minimize, or reduce risks to safe levels that are appropriate. This is critical because street food safety policies either do not exist or are poorly implemented in most developed countries (Liu *et al.*, 2014). Engagement of food and health-related experts to develop recommendations for the management of street food activities and implementation of the farm-to-fork definition of hazard analysis and vital control points (HACCP) (Sezgin and Şanlıer, 2016).

It is important to officially recognize street foods and street food vendors in developed countries to help eradicate patterns in running this extremely risky food sector in hiding and thereby exposing the public to health problems. This calls for well-structured standards and/or legislation to be established specifically for the food service industry. In order to ensure smooth enforcement of food safety regulations aimed at the provision of healthy and nutritious foods, proper and timely coordination of different departments within the governments involved in food safety issues is also of utmost importance (Imathiu, 2017).

2.6.2 Public Health Implication of Foodborne Diseases in Meat

Due to the lack of basic infrastructure and facilities and also the challenge of placing the large number of street food vendors under effective control measures, food from street vendors is considered to be a serious public health danger (Rane, 2011). In developing countries the main cause of food-borne illness is food sold by street vendors. While food products from these outlets are often prized for their distinctive taste and convenience, their microbiological protection is not always certain (Islam *et al.*, 2015). *Bacillus cereus* causes vomiting and diarrhea, *Clostridium perfringens* causes stomach cramps and diarrhea. Food-borne bacterial pathogens usually contained in street-sold foods. Vomiting, diarrhea, loss of appetite, extreme abdominal cramps and mild fever are triggered by *Salmonella* species that cause gastrointestinal tract typhoid, food poisoning and irritation and inflammation (Hasan *et al.*, 2018; Sharma *et al.*, 2015).

Many foodborne disease outbreaks are underreported or underestimated in developed countries. Nigeria, for instance, is a country with over 170 million inhabitants. However it has been estimated that there are just 90,000 cases of foodborne diseases each year. Australia is a

developing nation with only 24 million residents, compared to Nigeria, equal to 1:7. And, despite the high standard of living, healthy water supply, proactive government policies, and food safety measures, over 5.2 million people are estimated to have foodborne diseases annually in Australia. In developing countries, food borne diseases caused by microorganisms are a significant national and international health issue and an important cause of death (Garode and Waghode, 2012).

The economic and public health implications of foodborne diseases in developing countries should not be overestimated. Collaborative efforts should be made between developed countries' governments, politicians, researchers, and the general public to reduce the incidence of foodborne diseases (Odeyemi, 2016).

CHAPTER THREE

3.0 Materials and Methods

3.1 Description of Study Area

The study area was Magboro market located in Obafemi-Owode Local government area in Ogun State and is one of the many towns around the state that share a close proximity with the ever-bustling Lagos. Magboro is a semi-urban settlement with a population estimated to be over a million inhabitants.

3.2 Collection of Samples

Suya samples were bought on the street; at random from different street vendors at popular spots in Magboro market. Two types of suya were purchased; 'Meat suya' from animals like goat, sheep and cow while 'offal suya' from chicken gizzard. The samples were transported to the laboratory aseptically for microbial identification and analysis.

3.3 Materials and Equipment Used

Materials used: Petri-dishes, beakers, conical flasks, hockey stick, measuring cylinder, Eppendorf tubes, micro pipette (with their tips), test tubes (with their racks), spatula, filter paper, inoculating loop, wash bottles.

Equipment used: Autoclave, incubator, weighing balance, thermal cycler, centrifuge, stomacher blender, distiller, Lamina air flow cabinet, Magnetic stirrer water bath (set at 50°C and 100°C), Bunsen burner.

3.4 Media Used

PEPTONE WATER

Peptone water is a microbial growth medium composed of peptic digest of animal tissue and sodium chloride. The pH of the medium is 7.2±0.2 at 25 °C and is rich in tryptophan. Peptone water is also a nonselective broth medium which can be used as a primary enrichment medium for the growth of bacteria.

Preparation

1. The dehydrated medium was dissolved in the appropriate volume of distilled water to make up 0.1% and 1% peptone water based on manufacturer's instruction's instructions in a conical flask and mixed thoroughly.

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

3. 9ml of the 0.1% was then dispensed into various test tubes for serial dilution.

4. 225ml of the 1% was then dispensed into conical flask.

SORBITOL-MACCONKEY AGAR (SMAC)

Sorbitol MacConkey agar is a selective and differential media used for detecting sorbitol non-fermenting *Escherichia coli* O157: H7.

Preparation

1. The medium (50g) was suspended in 1000ml distilled water and mixed thoroughly.
2. The mixture was heated with frequent agitation to completely dissolve the powder and autoclaved at 121^{O}C for 15minutes.
3. The agar was allowed to cool to 45^{O}C and poured aseptically into sterile petri dishes and left to solidify.

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.

Preparation

1. The medium (48.5g) was suspended in 1000ml distilled water and mixed thoroughly.
2. The mixture was heated with frequent agitation to completely dissolve the powder and autoclaved at 121^{O}C for 15minutes.
3. The agar was allowed to cool to 45^{O}C and poured aseptically into sterile petri dishes and left to solidify.

NUTRIENT AGAR

Nutrient agar is a general-purpose nutrient medium used for cultivation of microbes supporting growth of a ride range of non-fastidious organisms.

Preparation

1. The medium (28g) was suspended in 1000ml distilled water and mixed thoroughly.
2. The mixture was heated with frequent agitation to completely dissolve the powder and autoclaved at 121^{O}C for 15minutes.
3. The agar was allowed to cool to 45^{O}C and poured aseptically into sterile petri dishes and left to solidify.

EOSIN METHYLENE BLUE (EMB AGAR)

Eosin methylene blue agar is a differential medium used to isolate coliforms. It provides a color indicator distinguishing between organisms that ferment lactose (e.g., E. *coli*) and those that do not (e.g., *Salmonella, Shigella*).

Preparation

1. The medium (36g) was suspended in 1000ml distilled water and mixed thoroughly.
2. The mixture was heated with frequent agitation to completely dissolve the powder and autoclaved at 121^{O}C for 15minutes.
3. The agar was allowed to cool to 45^{O}C and poured aseptically into sterile petri dishes and left to solidify.

POTATO DEXTROSE AGAR (PDA)

Potato dextrose agar is a general-purpose medium for yeasts and moulds that can be supplemented with acid or antibiotics like Chloramphenicol, Tartaric Acid and Chlortetracycline can be added as selective agents and to inhibit bacterial growth.

Preparation

1. The medium (39g) was suspended in 1000ml distilled water and mixed thoroughly.
2. The mixture was heated with frequent agitation to completely dissolve the powder and autoclaved at 121^{O}C for 15minutes.
3. The agar was allowed to cool to 45^{O}C and poured aseptically into sterile petri dishes and left to solidify.

XYLOSE LYSINE DEOXYCHOLATE AGAR (XLD AGAR)

Xylose Lysine Deoxycholate Agar is a selective growth medium used for the isolation of *Salmonella* and *Shigella spp.* From clinical and food samples.

Preparation

1. The medium (57g) was suspended in 1000ml distilled water and mixed thoroughly.
2. The mixture was heated with frequent agitation to completely dissolve the powder.
3. It was transferred to the water bath at 50^{O}C. it is not to be autoclaved as instructed by the manufacturer.

4. The agar was allowed to cool to 45^{O}C and poured ascetically into sterile petri dishes and left to solidify.

SELENITE F BROTH

Selenite F Broth is the medium used for the selective enrichment of *Salmonella spp* from both clinical and food samples. It is a buffered Lactose Peptone Broth to which Sodium Biselenite is added as the selective agent.

Preparation

1. The dehydrated media (19g) of selenite F was dissolved in 750 ml distilled water in a sterile conical flask. (Part A)

2. Sodium biselenite (4g) was dissolved in 250ml distilled water in another conical flask. (Part B).

3. PART A and PART B was mixed together and heated to dissolve the medium completely. Distribute in sterile test tubes

4. It was then sterilized in a water bath or free-flowing steam for 10mins. It is not to be autoclaved as instructed by the manufacturer.

3.5 Sample Preparation

Twenty-five (25 g) of each suya samples was weighed into a sterile stomacher bag containing 225 ml of sterile 1% buffered peptone water and homogenized in a stomacher machine set at 180 rpm for 4 minutes and then incubated at 37^{O}C for 24hours. The resultant homogenate was diluted serially up to 10^{-4}.

ISOLATION OF *E. COLI*, COLIFORMS AND TOTAL VIABLE COUNT

The resultant homogenate was serially diluted to 10^{-4}. From the appropriate dilutions, 0.1 ml was plated in duplicate onto SMAC Agar, EMB Agar, MAC Agar an Nutrient Agar for the isolation of enteropathogenic *E. coli*, coliforms and for the Total viable count the using the spread plate technique. The plates were incubated at 37 ºC ± 2 for 18- 24 hours.

ISOLATION OF YEAST AND MOULD

Aliquots (0.1 ml) of each dilution was plated on mPDA and spread out using a glass spreader. The plates were inverted and kept at 25^{O}C for 2 days. Yeast appear as creamy and white colonies while Mould appear as filamentous colonies.

NOTE: PDA was modified with 25mg of chloramphenicol to inhibit the growth of bacteria.

ISOLATION OF *SALMONELLA SPP.*

Primary enrichment

Twenty five gram (25g) of the samples was aseptically added to 225ml peptone water which was incubated at 37 ºC for 24 h.

Secondary Enrichment

One ml (1ml) from the pre-enrichment broth was inoculated into 9ml of Selenite F Broth contained in test tubes and was incubated at 37^{O}C for 24hours to allow selective enrichment for *Salmonella spp*. This enrichment was peculiar to *Salmonella* alone.

After incubation, the test tubes were vortexed and a loopful of the incubated selenite F broth was streaked unto Xylose lysine deoxycholate agar. The plates were inverted and incubated at 37^{O}C for 24hours. The plates were examined for typical *Salmonella* colonies.

Sub Culturing

The plates were checked after the required duration for the growth a sub-culturing needs to be done. Sub culturing was done to purify the isolated bacterial colonies from a mixed culture to a new and single culture, the bacterial isolates transferred or sub-cultured were those were differentiated on the basis of their colony morphology, shape, color, elevation and other physical characteristics.

Presumptive colonies obtained after incubation were sub- cultured unto fresh nutrient agar plates using the streaking method procedure by taking a loopful of preferred isolate using the inoculating loop (the inoculating loop is heated using the Bunsen burner and allowed to cool for about 5 seconds before taking the loop from the original mixed culture and streaked onto the new petri-dish). The plates were inverted and incubated at 37^{O}C for 18- 24 hours.

3.6 Cryopreservation of Isolates

A loopful of each isolate was inoculated into a sterile Eppendorf tube containing 1ml of brain heart infusion incubated at 37 ºC for 24 h and 500ul of 20 % sterile glycerol as cryoprotectant and it was stored in a -20 ºC freezer.

Molecular identification of isolates

DNA Extraction

Boiling method

Each isolate was streaked out on nutrient agar and incubated overnight at 37^0C. The loopful actively dividing cells were emulsified in 500ml double distilled water until it was turbid, it was centrifuged at 14,000 rpm for 5 minutes and the supernatant was decanted, 1ml of sterile water was added to the Eppendorf tube, vortexed and centrifuged again at 10,000RPM for 2 minutes the process was repeated twice, 200ul of sterile water was pipetted into the Eppendorf tube, vortexed and centrifuged at 14,000 rpm for 5 minutes and then it was placed in the heating block to boil for 10-20 minutes, it was then placed in the fridge for a while, the content of the Eppendorf tube was then vortexed and centrifuged finally at 14,000 rpm for 5 minutes, a new set of Eppendorf tubes were labelled and the supernatant containing the extracted DNA was then transferred into the new Eppendorf tubes and they were placed in racks and stored in the freezer for further use.

PCR Protocol

16S rRNA amplification

Partial 16S rRNA gene amplification using forward primer fD1 (5´-AGA GTT TGATCC TGG CTC AG-3´) and reverse primer rD1 (5´-AAG GAG GTG ATC CAG CCG CA-3´) (Weisburg *et al*., 1991). The components of the PCR and constituent mixes were summarized in Table 3.6 below. 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 16s rRNA Amplification

No.	Component	1 rxn
1	Mastermix	5ul
2	fD1	0.4ul
3	rD1	0.4ul
4	DNA	2ul
5	dH_2O	2.2ul
6	Total	10ul

Table 3.2: Multiplex PCR Protocol

No	Component	1 rxn
1	Master mix	7.5ul
2	STX1F	0.186ul
3	STX1R	0.186ul
4	STX2F	0.186ul
5	STX2R	0.186ul
6	EAEF	0.186ul
7	EAER	0.186ul
8	DNA	2ul
9	dH_2O	4.36ul

Table 3.3: Procedure for Thermalcycler

Analysis	Step	Temperature	Time
1x	Initial denaturation	95^0c	5 min
35x	Denaturation	95^0c	2 min
	Annealing	42^0c	30 sec
	Polymerization	72^0c	4 min
1x	Final polymerization	72^0c	10 min
1x	Hold	4^0c	∞
Cycler			

AGAROSE GEL ELECTROPHORESIS

The agarose was prepared using dry agarose powder, 1g of the agarose powder was then dissolved in 50ml of TAE buffer the mixture was then boiled until a clear solution was gotten 3ul of ethidium bromide was added to the mixture using a micropipette it is swirled and left to cool but not solidify, the content of the flask is then transferred into the gel cast with the combs in place, after, it is left to solidify and the gel is gently removed and put in an electrophoresis tank containing TAE buffer. 4ul of the PCR products are then pipetted into each well that was formed after removing the comb. The tank is connected to the power pack and left to run till it

gets to one-third of the gel and then it is turned off and the gel is viewed under the UV transilluminator.

PRECAUTIONS

• Aseptic techniques were observed at every stage of work.

• Personal protective technique was also observed, such as wearing of covered shoe, nose cover, gloves, lab coat, etc.

• Ensured that the inoculating loop cooled before picking the organism when sub-culturing in order not to kill organism of interest.

• Ensured that the petri-dish was incubated inverted.

• Ensured proper timing, most especially during autoclaving.

CHAPTER FOUR

RESULTS AND DISCUSSION

The study was empirically undertaken to establish the occurrence of common food-poisoning pathogens in selected street-vended meat. Pathogenic microorganisms are either not allowed at all to occur in the foodstuff or they are limited to a specified number of cells per gram food. If limits are exceeded, this might have tremendous consequences for consumer's health. Therefore, a zero tolerance per 25 g of food sample is predicted for most of all common pathogens (*Salmonella, Campylobacter, E. coli*, etc.) (SANS, 2011). Table 4.1, 4.2 and 4.3 showed the Morphological characteristics of presumptive general *E. coli,* Shiga toxigenic *E. coli, Salmonella and Yeasts and Moulds* on Eosin methylene blue, Xylose lysine deoxycholate, Sorbitol-MacConkey Agar, Nutrient Agar and Potato dextrose Agar. *Salmonella* was not detected in the food samples which was cultured on XLD agar. Table 4.4 showed the results of *Escherichia coli,* Yeasts and moulds and Total viable counts grown on Eosin methylene blue, Xylose lysine deoxycholate, Sorbitol-MacConkey Agar, Potato dextrose Agar and Nutrient Agar.

This study shows the presence of Yeasts and moulds and pathogenic *E. coli* found in street vended suya. The presence of the of these microorganisms can be as a result of unhygienic work place, lack of proper hygiene of the vendors, use of unhygienic equipment for food preparation etc. Their presence in ready-to-eat food renders it unfit and unsafe for consumption by humans.

Table 4.1: Morphological characteristics of bacterial isolates on Eosin methylene blue, Xylose lysine deoxycholate and Sorbitol-MacConkey Agar

Samples	Isolate ID	Colour	Shape	Size	Elevation	Appearance	Texture	Opacity	Margin
Meat Suya	S1 EMB	Green metallic sheen	Circular	Moderate Large	Raised	Shiny	Smooth	Opaque	Entire
	S1 SMAC	Pink White	Circular	Punctiform Moderate	Raised	Shiny	Smooth	Opaque	Entire
	S1 XLD	Yellow	Circular	Large	Raised	Shiny	Smooth	Opaque	Entire
Gizzard Suya	S2 EMB	Green metallic sheen	Circular	Small Moderate	Raised	Shiny	Smooth	Opaque	Entire
	S2 SMAC	Pink White	Circular	Small	Raised Flat	Shiny	Smooth	Opaque	Entire
	S1 XLD	Yellow	Circular	Large	Raised	Shiny	Smooth	Opaque	Entire

Table 4.2: Morphological characteristics of isolates on Potato dextrose agar

Sample	Isolate ID	Colour	Shape	Size	Elevation	Appearance	Texture	Opacity	Margin
Meat Suya	S1 PDA 1	White Creamy	Circular Irregular Filamentous	Small Moderate Large	Raised	Shiny	Smooth	Opaque	Entire
	S1 PDA 2	White Creamy	Circular Irregular Filamentous	Punctiform Moderate Large	Raised Flat	Shiny	Smooth	Opaque	Entire
Gizzard Suya	S2 PDA 1	White Creamy	Circular Filamentous	Small Moderate Large	Raised	Shiny	Smooth	Opaque	Entire
	S2 PDA 2	White Creamy	Circular Irregular Filamentous	Small Moderate	Raised	Shiny	Smooth	Opaque	Entire

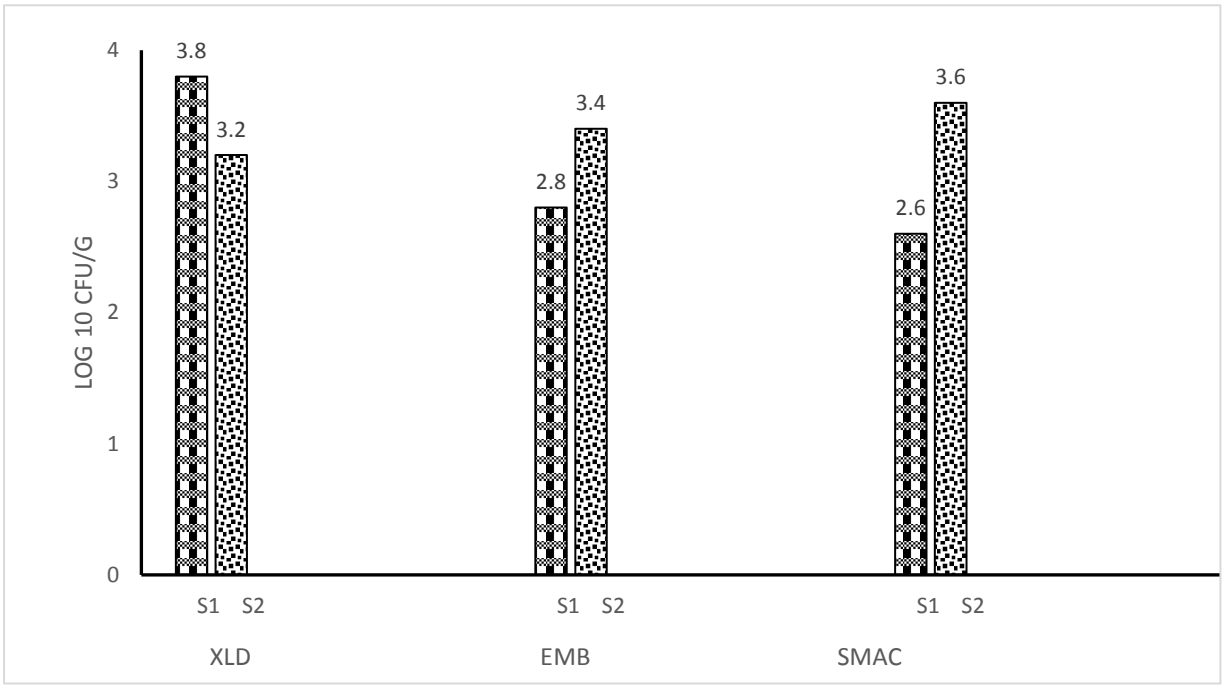
Table 4.3: Morphological characteristics of isolates on Nutrient Agar

Sample	Isolate ID	Colour	Shape	Size	Elevation	Appearance	Texture	Opacity	Margin
Meat Suya	S1 NA	White	Circular	Small Moderate Large	Raised	Shiny	Smooth	Opaque	Entire
Gizzard Suya	S2 NA	White	Circular	Small Moderate Large	Raised	Shiny	Smooth	Opaque	Entire

Table 4.4: The results of *Escherichia coli,* Yeasts and Moulds and Total viable count grown on Eosin methylene blue, Xylose lysine deoxycholate, Sorbitol-MacConkey Agar, Potato dextrose Agar and Nutrient Agar.

Samples	**Sample ID**	**Dilution**	**Number of Colonies**	**Log 10 Cfu/g**
1st Sampling Meat Suya	S1 EMB	10^1	6	2.8
	S1 NA	10^3	150	6.2
	S1 SMAC	10^2	4	2.6
	S1 XLD	10^1	60	3.8
	S1 PDA	10^1	78	3.9
	S1 PDA	10^2	3	3.5
2nd Sampling Gizzard Suya	S2 EMB	10^1	26	3.4
	S2 NA	10^3	72	5.9
	S2 SMAC	10^2	4	3.6
	S2 XLD	10^1	17	3.2
	S2 PDA	10^1	22	3.3
	S2 PDA	10^2	2	3.3

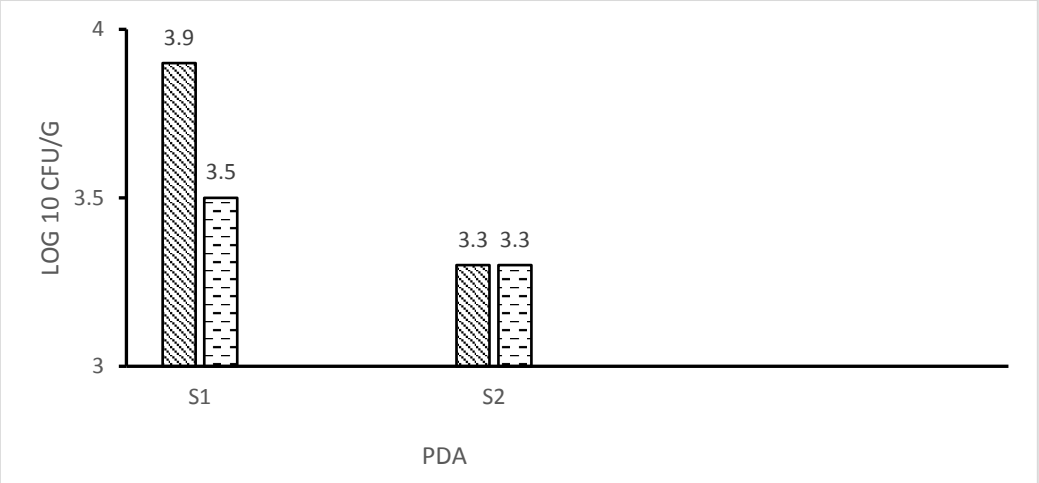
Log 10 Cfu/g = log (level of dilution plated x number of colonies counted / volume plated)



Keys: S1: Sample 1 (Meat Suya) S2: Sample 2 (Gizzard Suya).

Figure 4.1: Microbial counts of coliforms and pathogenic *E. coli* (STEC) in Suya sample collected from Magboro market, Ogun State.

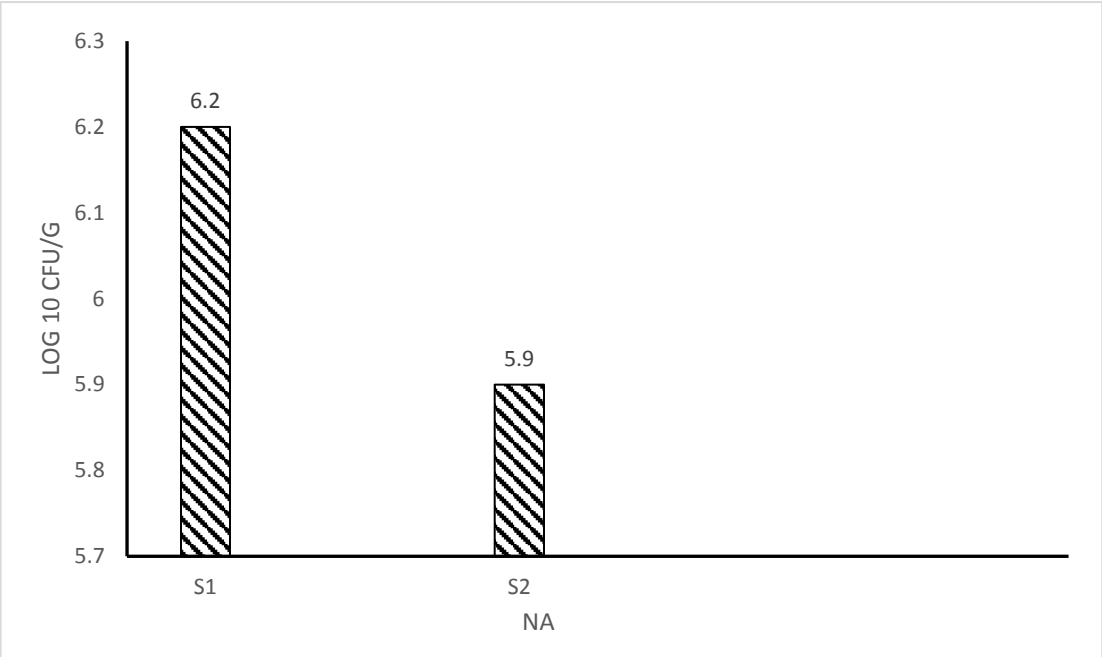
According to SANS 2011, the microbiological specification for coliforms, *E. coli* and *Salmonella* should be < 2 Log_{10} cfu/g, < 1 Log_{10} cfu/g and 0/25g respectively. *Salmonella* was absent in 25g of the suya sample examined. This implies the sample is satisfactory for *Salmonella* count but not within acceptable range as *E. coli* was identified in the sample. The incidence of foodborne pathogens in suya are of growing concern since it is widely consumed by among people.



Keys: S1: Sample 1 (Meat Suya) S2: Sample 2 (Gizzard Suya).

Figure 4.2: Incidence of Yeasts and Moulds in street vended suya from Magboro market, Ogun State.

The microbiological specification for Yeasts and moulds in dried meat products should be <3 log cfu/g according to SANS, 2011. Some species of Aspergillus are known to produce powerful mycotoxins which are harmful to man, thus their occurrence in suya is undesirable. The presence of molds could have come from contaminated spices used and wrapping with contaminated wrap before serving (Shamsudeen and Oyeyi, 2008).



Keys: S1: Sample 1 (Meat Suya) S2: Sample 2 (Gizzard Suya).

Figure 4.3: Total viable counts in Suya from Magboro market, Ogun State.

The Total viable counts (TVC) in dried meat products should be less than 6 log cfu/g (SANS, 2011). The TVC for S1 was moderately higher than the microbial specification with a count of 6.2 log10 cfu/g. Although S2 has a count of 5.9 log10 cfu/g, it should not be considered safe as the microbial load present is likely to cause illness to consumers.

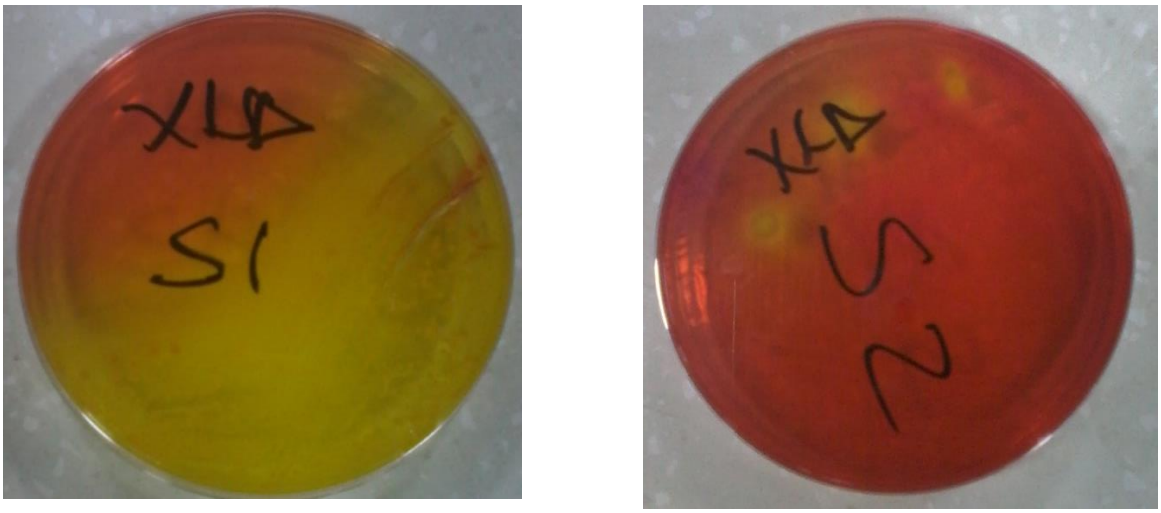


Figure 4.4: Suspected colonies of *Salmonella* on XLD agar

CHAPTER 5

5.0 Conclusions and recommendations

This study demonstrated that meat and suya sold in Magboro market constitute a potential health hazard to consumers as a result of the pathogenic microorganisms isolated from the food that rendered them microbiologically unsafe and unacceptable. There is a need for proper monitoring of the production and processing of suya from farm-to-fork. Processors and consumers need to be educated on good sanitary practices during processing, display and sale of suya and the possible danger of contaminated products. Safety measures should be employed in preparation/production of roasted meat (suya) by the vendors to prevent food poisoning as a result of presence of fungi and other microorganisms. The suya should be kept close to the fire/heat at all time to inhibit growth of microorganisms most especially fungi and bacteria. Raw meat and all utensils should be properly washed using clean water. The awareness of Hazard Analysis Critical Control Points (HACCPs) system in suya preparation by vendors could reduce or eliminate contaminations of the suya products when put into proper use.

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