INCIDENCE OF PATHOGENIC MICROORGANISMS IN STREET VENDED '*KILISHI*' (BEEF JERKY-LIKE PRODUCT) FROM MAGBORO, OGUN STATE, NIGERIA

BY

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CERTIFICATION

This is to certify that this project was carried out by OLUJIMI, OLAIDE OMOTADE with matriculation number 17010101020 of the Department of Microbiology, College of basic and applied sciences in Mountain Top University under the supervision of DR G.B. AKANNI

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ABSTRACT

Safety of meat and meat products is a key issue of public health concern. Street food is readily available to people around the world but the microbial safety and consistency of such food is always unknown. In developing countries, street food provides the majority of low-income groups with a source of inexpensive nutritious meal, however, it is the primary source of transmission of foodborne diseases. The goal of this study was to determine the incidence of *Salmonella* spp, *Escherichia coli* and yeast and moulds in street-vended *kilishi* (beef jerky) sold in the Ofada / Mokoloki LCDA area of Magboro, Ogun state. Identification of these pathogens in the samples were performed using culture based and molecular methods. Total viable count was around

14×10^{-3} Log_{10} CFU/g in all the samples while the highest count of pathogenic *E. coli* was 15×10^{-4} Log_{10} CFU/g. *Salmonella* was also detected in the samples. The growth of Yeast and mould was 1×10^{-1} Log_{10} CFU/g. The implication of the very high microbial count could result in food poisoning, spoilage of food, lack of safe and quality food. The high level of microbial load in *kilishi* is of public health concern.

Keywords; Salmonella spp, *Escherichia Coli*, yeast and moulds, Virulence Factors

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CHAPTER ONE

1.1 INTRODUCTION

Street foods are ready-to-eat (RTE) foods and beverages prepared and/or sold by vendors and hawkers especially in public places street stalls, markets, and other similar places. Due to the uniqueness in taste, flavor, convenience, and the role which they play in the cultural and social heritage of societies, street vended foods have also become crucial for the conservation of nutritional standards globally (Rane, 2011). However, street vended foods have been highlighted as the vehicle of pathogens transmission of foodborne diseases linked to the consumption of street foods (Todd, 2016). Street food marketing has become a vital public health issue and a serious concern to everyone due to widespread foodborne diseases (Rane, 2011). Although street vended food is enjoying a good vary of acceptability, microorganisms from the genus *Campylobacter, Clostridium, Salmonella, Escherichia, Listeria, Shigella, Yersinia, Vibrio, Staphylococcus,* and *Norovirus* are commonly isolated pathogens and recognized as the causative agents in foodborne outbreaks (Bintsis, 2017).

Street vended meats and meat products in Nigeria are mostly prepared on the street or at individuals' homes but consumed without further processing. Examples are 'Suya' and *Kilishi* (a beef jerky-like product) which are RTE foods (Ologhobo, 2010). *Kilishi* is hawked and sold around the streets, schools, fuel stations, transportation garages, highway traffic lanes, market places, and anywhere people gather (FAO/WHO, 2005). The presence of *Salmonella, Listeria monocytogenes*, and *Escherichia coli* 0157:H7 in ready-to-eat (RTE) meat merchandise is taken into account a serious concern for food management authorities worldwide (Tareq 2013; Shaker, 2014), therefore stringent measures must be ensured that these pathogens are not found in ready-to-eat (RTE) meat product such as *Kilishi* in Nigeria.

1.2 Statement of the Problem

Ready to eat meat products such as *kilishi* (Beef Jerky-like meat product) is believed to be easily contaminated by various pathogenic microbes, due to unhygienic methods employed during its processing and preservation.

The implication of pathogens in RTE foods is of great public health concern, mostly the health of children under 5 years old and the aged people. Therefore, food safety standards must be adhered to during the processing, packaging, and sale of these meat products.

1.3 Justification of this study

The investigation of RTE meat products especially Beef Jerky *(Kilishi*) is necessary to know the particular agent that may be present in the meat product that may cause ill-health or mortality in individuals. A survey was conducted by administering a structured questionnaire during the study to various categories of families and individuals to establish the frequency of consumption of each type of RTE meat product in their household. The possible pathogen that may be present in *kilishi* were examined which would in turn results in ill-health.

1.5 Objectives of this study

1. To identify the pathogenic microorganisms present in the RTE meat products *kilishi*.
2. To establish that there is a survival of pathogens in the meat product at the point of sale and consumption.
3. To establish the potential risk involved in the consumption of street vended RTE meat products in Magboro, Ogun State.
4. To create awareness on the level of food safety of street vended foods and its importance.

Chapter Two

2.1 Literature Review

The increase of human population and urbanization, the per capita income, globalization, the changes in client trends (more macromolecule within the diet) have redoubled the consumption of animal products (Dhama et al., 2014). Estimates advocate that the intake of those animal products will increase to 376 million lots by 2030 (Dhama et al., 2013). This high demand for ANimal merchandise provokes intensive animal production and process of products, with an accumulated movement of foods globally

Food-generating animals which includes cattle, chickens, pigs, and turkeys are essential reservoirs for lots foodborne pathogens which includes *Campylobacter species,* non-Typhi serotypes of *Salmonella enterica,* Shiga toxin-generating lines of *Escherichia coli,* and *Listeria monocytogenes* (Norma, 2018).

The zoonotic potential of some foodborne pathogens and their ability to cause diseases or even death is sufficient. Microbial pathogens can cause disease by the consumption of animal products contaminated with microorganisms or their toxins.

In many countries, foodborne pathogens account for millions of cases of sporadic illness and

chronic complications, as well as large and challenging outbreaks. For instance, a major proportion of the 1.5 billion annual diarrheic episodes in kids lower than three years getting on worldwide are caused by enteropathogenic microorganisms, which ends up in additional than 3 million deaths per annum (EFSA, 2016). Surveys estimate that within the united states alone, microorganism enteric pathogens cause 9.4 million episodes of foodborne health problem in humans, 55,961 hospitalizations, and 1,351 deaths each year (Scallan et al., 2011). The importance of food-producing animals as carriers of unhealthful microorganism is real; for example, beef has been reportable because the vector of transmission for 7% of the 1.7 million cases of the foodborne illness that was recorded from 1996 to 2000 in European nation and Wales (Anderson et al., 2009). Meat and meat product contamination could be a serious concern because it is difficult to control. Several factors can be concerned in contamination, as well as those from the surroundings (associated fauna, water from completely different sources, and

animal manure disposal, etc.), and human-related animal handling (slaughtering and process practices, and storage procedures, etc.) (Sofos, 2008)

The presence of microorganisms in RTE meat products such as *kilishi* can be the result of contamination of the raw materials used for its preparation or a low level of hygiene during meat preparation, packaging, and/consumption. In preparation of the meat, there could be microbial contamination on the equipment /utensils used due to poor hygiene or ineffective cleaning and disinfection of utensils used which eventually contaminates the production processing (Nørrung, 2008; Heredia, 2018).

Table 2.1 Potential sources of contamination during meat processing, type of hazard and the microbiological risk involved (Source: Rane, 2011)

S/N	Source	Hazard	Risk Involved
1	Vendor Location	Improper food handling	Transfer of pathogens like Salmonella and *E. coli* from human body and environment into foods.
			Transmission of enteric pathogens like Salmonella, Shigella, and *E. coli* via vectors
		Improper waste disposal	
2	Raw Materials	Water	Passage of pathogens like *E. Coli*, salmonella
		Vegetables and Spices	Introduction sporeformers like Shigella, Salmonella,e.t.c.
3	Utensils and pieces of equipment	Chemical Contaminants	Leaching of chemical leading to poisoning
		Microbial Contaminants	Cross-contamination of food with *E.coli* and Shigella due to contaminated water, dishcloth, handler.
4	Storage and Reheating	Improper storage temperature and reheating of food	Likelihood of heat-stable toxins produced by pathogens
5	Personal hygiene of Vendors	Biological Hazards	Introduction of Salmonella and Shigella via carriers

2.2 *Escherichia coli*

Escherichia coli are commensal organisms that colonize the intestinal microflora of humans and other mammalian intestines (Krogfelt, 2013). Ruminants, especially cattle, have been implicated as a principal reservoir of one of the enterovirulent *E. coli* pathotypes (Osman, 2012). *Escherichia coli* pathotypes associated with diarrhoeal diseases have gained a growing interest in all parts of the world; numerous reports of the *E. coli* strains are available isolated from diarrhea patients (Tamura, 1996). The classification of pathogenic *E. coli* pathotypes was largely based on historical and epidemiological observations because the mechanism by which *E. coli* caused diarrhoeal diseases was used. (Clements, 2012).

Diarrheagenic *E. coli* that cause human illness have been classified based on specific sets of virulence genes they carry and the characteristics of the disease they cause (Kaper et al., 2004). These pathotypes include the enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive E. coli (EIEC), enteroaggregative *E. coli* (EAEC), Shiga toxin-producing *E. coli* (STEC), diffusely adherent *E. coli* (DEAC), and adherent invasive *E. coli* (AIEC) that have been associated with Crohn's disease (Jafari, 2012).

There are presently ~186 completely different *E. coli* O-groups associate degreed 53 H-types, so serotyping is extremely complex. There also are several infective teams of *E. coli* that cause sickness in humans and animals, together with diarrheagenic *E. coli* and also the extra-intestinal pathogenic *E. coli* (ExPEC) that cause illness outside of the GI-tract (Fratamico, 2016). There are also hybrid pathotypes, including the enteroaggregative harm *E. coli* (EAHEC) that carry STEC- and EAEC-associated virulence sequences. As an example, EAHEC serotype O104:H4, an EAEC that nonheritable the bacteriophage that carried the Shiga poison gene of STEC, caused an oversized occurrence in 2011 related to illness in over 3800 people and 54 deaths (Weber et al., 2012).Certain *E. coli* serotypes are usually related to specific pathotypes, admire STEC O157:H7 and O103:H21 that are necessary STEC, often observed as enterohemorrhagic *E. coli* (EHEC). Therefore, pathogenic *E. coli* constitutes a genetically heterogeneous family of bacteria, and they continue to evolve (Kaper et al., 2004).

2.2.1 Shiga Toxin *E. coli* (STEC)

Vero toxigenic or Shiga toxigenic *E. coli* (VTEC or STEC) O157: H7 is considered to be a significant danger to foodborne diseases. *E. coli* O157: H7 was the first of several strains known as enterohemorrhagic *E. coli* or EHEC, which can contain one or more Shiga toxins (also known as verocytotoxins and previously known as Shiga like toxins). It is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products, raw milk, and contaminated raw vegetables and sprouts (Croxen, 2013). Biswas *et al.,* (2010) and Yannick *et al.,* (2013) reported the presence of *E. coli* (STEC) in street vended meat from Bangladesh (Asia) and Cameroon (Central Africa), respectively, whereas *diarrheagenic E. coli* strains were recovered from grilled chicken in Burkina Faso (West Africa) (Somda *et al.,* 2018).

2.2.2 Sources of STEC contamination

E. coli is transmitted to humans through fecal contamination of foods and water, additionally as cross-contamination, or by direct human contact throughout food preparation. The first exposure route seems to be through the consumption of contaminated foods, cherish raw or undercooked ground meat products, raw milk, and fresh manufacture (FAO, 2011). Cattle and life also represent a possible supply of pathogenic bacteria, notably for lettuce and foliaceous greens at pre-harvest stages on the coast of American state and in Yuma, AZ (Stuart et al., 2006). Wildlife feces are involved in the contamination of the vegetables and can cause *E. coli O157*:*H7* (Berger, 2010). Research on a possible pathogenic *E. coli* reservoir in feces from coyotes and dogs was also conducted (Jay-Russell, 2014). STEC can also colonize other animals and birds, although the incidence of STEC is lower than in ruminants (FSANZ 2013; Meng et al. 2013).

Human ailment related to RTE dried meats that have been contaminated with STEC and other pathogenic microbes can have severe consequences; the main animal reservoir for STEC are ruminants; other animals and birds can also harbor STEC, uncertainty around the level of reduction in microbial load risk due to the high-temperature treatment during drying as this process differs amongst manufacturers presents a risk (FDA 2012).

Major Foodborne infective STEC strains embody O26, O45, O103, O111, O121, O145, O157 (FDA 2012), and O104 (ECDC/EFSA 2011). The growth of E. coli will occur at temperatures between seven – 46°C, pH scale of 4.4 – 10.0, and a minimum water activity of 0.95 at optimum conditions. Some STEC strains can survive at pH 2.5 – 3.0 for over 4 hours. STEC can survive frozen storage at -20°C, however, it's freely inactivated by cooking (FSANZ 2013; Meng et al. 2013). However, STEC is destroyed by thorough cooking of foods until all components reach a temperature of 70^{o}C or higher (WHO, 2018).

2.2.3 Virulence Factors in *E. coli*

The ability of *Escherichia coli* to produce toxins enhance its ability to infect a host with the disease; it produces an α-hemolysin toxin which is a pore-forming cytotoxin, it inserts into the plasma membrane of the host cells thereby causing leakage of the host's cytoplasmic contents and eventually leading to cell death; another toxin it produces is one which is similar to the Shiga toxin 17 and inhibits protein synthesis by ribosomal binding. It produces a labile toxin (LT) (Ryan and Ray, 2004)

Shiga toxin-producing *Escherichia coli* (*E. coli*) (STEC), is also known as verocytotoxin-producing *E. coli* (VTEC), it is a pathogen that can cause food-borne infections, severe and illnesses that can cause death in humans for example; hemorrhagic colitis (HC), hemolytic uraemic syndrome (HUS) that is the main cause of acute renal failure in infants (Mohammad, 2015). One of the foremost potent microorganism toxins is understood as Shiga toxin stx and is found in *Shigella dysenteriae* one conjointly in some serogroups of *Escherichia coli* called stx1 in *E. coli*. Some *E. coli* strains turn out the second kind of stx called stx2 that has constant mode of action as stx/stx1 however antigenically distinct. Shiga toxin (stx) causes fluid accumulation in ileal loops of some animals and excretory organ harm in most animals, however, humans encounter *shigella* as a consequence of *shigella dysenteriae* kind 1 or certain serogroups of E. coli similar to the O157:H7. There are 2 immunologically distinct teams of Stx supported.

Most outbreaks of HC and HUS are attributed to strains of the enterohemorrhagic serotype O157:H7 (Mora, 2007). The power of E. coli O157:H7 to cause severe illness in humans is said to their capability to secrete shiga toxins (Stx1and Stx2) (Prendergast, 2011). Another virulence-

associated issue of most STEC isolates related to severe disease is intimal, a 94-kDa outer membrane protein, that is encoded by the eae sequence on a ca. 34 K chromosomal pathogenicity island termed the locus of enterocyte effacement; this locus is related to the shut adherence of E. coli to animal tissue cells, initiation of host signal transduction pathways, and therefore the formation of attaching-and-effacing intestinal lesions (Leotta, 2008).

2.2.4 Symptoms and Treatment of *Escherichia coli* O157:H7

According to the Centers for Disease Control and Prevention (CDC), the symptoms of *E. coli* O157:H7 infection, which were first identified in 1982, are bloody diarrhea, severe stomach cramps, vomiting, and fever. Normally the incubation period is around three to nine days (Marley, 2007). It causes symptoms ranging from mild to severe and bloody diarrhea. In up to 10% of patients (particularly young children and the elderly), the infection may lead to a life-threatening disease, such as hemolytic uremic syndrome (HUS) (FAO, 2011).

However, there are several ways to prevent this infection for example by cooking the ground meat and vegetables thoroughly, avoid consuming raw milk and unpasteurized dairy products, and finally practicing handwashing to prevent cross-contamination in food preparation (Marley, 2007). The treatment of the infection is mainly based on rehydration, while antibiotic treatment is often contraindicated as it may activate Shiga toxin release and therefore cause clinical deterioration with a potential evolution to HUS (CDC, 2011).

2.2.5 Prevention of *E. coli* 0157: H7

The hindrance needs a multidisciplinary approach in animal and plant production yet as risk-based approaches on the whole food supply chain. These embrace the appliance of Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP), Good Hygiene Practices (GHP) and Hazard Analysis Critical Control Point (HACCP) from the farm to the buyer (FAO, 2011).

Several studies had shown that food handlers are involved in unhygienic handling of foods, thereby encouraging microbial contamination of ready-to-eat products. This happens because food handlers have poor personal hygiene or they are asymptomatic carriers of pathogenic micro-organisms; measures to minimize the risk of contamination by food handlers require adequate hand-washing and personal hygiene (Faour-Klingbeil *et al.,* 2016).

The World Health Organization (2019) proposes 5 simple measures to avoid food contamination with *E. Coli* and other enteropathogens; namely, separating raw and cooked food, keeping the working area clean, thoroughly cooking, keeping food at a safe temperature, and using safe water and raw materials.

2.3 Salmonella

One of the major public health concerns contributing to the economic burden of both industrialized and underdeveloped countries through the financial implication associated with surveillance, prevention, and treatment of disease is *Salmonella* (Crump et al, 2004). The most common manifestation of Salmonella worldwide is Gastroenteritis, followed by bacteremia and enteric fever (Majowicz et al, 2010). There are a lot of serotypes of salmonella, some are found in one kind of animal in a single place and many others found in several animals globally; some cause severe illness when they infect while some cause mild illnesses; there are currently more than 2,500 serotypes (serovars) of *Salmonella*, Less than 100 serotypes are responsible for most human infections; the nomenclature for the genus *Salmonella* has evolved from the initial one serotype-one species based on the serologic identification of O (somatic) and H (flagellar) antigens (CDC, 2020). Each serotype was considered a separate species (for example, *S. paratyphi A, S. newport, and S. enteritidis*); this concept, if used today, would result in 2,463 species of Salmonella. The proposals for nomenclature changes in the genus had been revised by Brenner (2000).

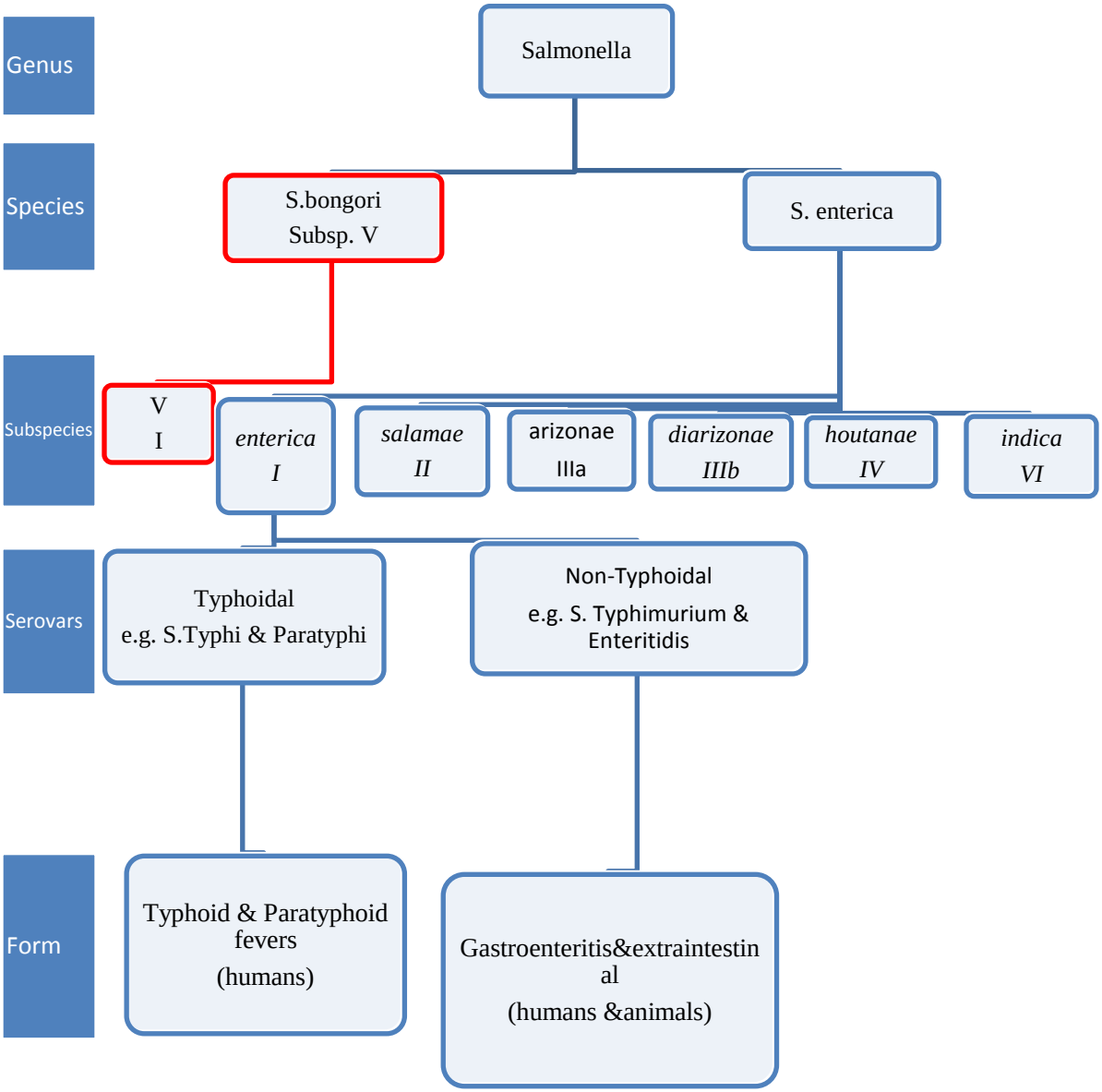

Fig 2.2 Classification of *Salmonella* species and Sub-species. Adapted from Hurley et al., (2014).

2.3.1 Pathogenesis of *Salmonella (Salmonella typhi)*

Whenever a contaminated food is ingested, *Salmonella* strains (*Salmonella typhi*) pass the intestinal epithelium and spreads to systemic sites like the spleen, bone marrow, gall bladder, and liver. Symptoms of infection (headache, muscle aches, fever, stomach pain, constipation, and diarrhea) develop within the first 10-14 days of ingestion (Parry, 2002). After treatment of

infection with appropriate antibiotic treatment, some individuals will continue to shed the microorganism for several months to years (Gunn, 2014). In carriers, *S. typhi* and S. paratyphi may persist in an asymptomatic form within the gall bladder (Dongol, 2012). Due to the restriction of typhoidal serovars to humans, carriers are a key reservoir that contributes to the transmission and spread of typhoid (Pitzer, 2014).

2.3.2. Epidemiology of *Salmonella*

Salmonella is a prominent cause of diseases and illnesses in both humans and animals which has been estimated to cause about 93.8 million cases of human gastroenteritis and 155, 000 deaths worldwide each year (Majowicz, 2010).

2.3.3. Prevention of *Salmonella*

Prevention needs management measures in the least stages of the food chain, from agricultural production to processing, manufacturing, and preparation of foods in each industrial institutions and at home. National and regional surveillance systems on foodborne diseases are necessary suggests that to understand and follow things of those diseases and additionally to notice and answer food poisoning and different enteric infections in early stages, and therefore to stop them from additional spreading (WHO, 2018)

2.3.4. *Shigella*

Shigella, a extremely virulent infectious agent that causes microorganism dysentery, is one in every of the leading causes of diarrhoeal sickness and contributes considerably to the burden worldwide. Dysentery or Bacillary dysentery, a channel disease caused by Shigella species, is recognized as a heavy pathological state throughout the world. It's principally found in developing countries because of improper waste management, poor sanitary condition, and unsafe drinking water. In industrial nations, it is mostly due to visit unindustrialized countries and consumption of contaminated food material (Izumiya, 2009). Globally, mortality and morbidity due to shigellosis were found to be highest in children under five years old (Gu,2017). Worldwide, *Shigella* is responsible for 80-165 million cases of disease and 600,000 deaths annually, of which 1.5 million in developed countries and 163 million are reported in developing countries (Heiman and Bowen, 2014). In the United States, about 500,000 cases of shigellosis are reported each year (Painter, 2015).

2.3.5 Serovars and transmission of *Shigella*

The *Shigella* genus is split into four species that are *Shigella dysenteriae* (serogroup A), *Shigella flexneri* (serogroup B), *Shigella boydii* (serogroup C), and *Shigella sonnei* (serogroup D). consistent with organic chemistry characterization and medical science properties, these species are any distributed into many serotypes, as *Shigella dysenteriae* have fifteen serotypes, *Shigella flexneri* has fourteen serotypes, and subserotypes, *Shigella boydii* have twenty serotypes, and *Shigella sonnei* with one serotype (Livio et al., 2014). These species are the etiological agent of bacillary dysentery conjointly referred to as bacillary dysentery. The symptoms will vary from gentle watery symptom to severe inflammatory dysentery with the passage of mucoid and bloody stools. The opposite clinical manifestation embraces abdominal cramping, fever, nausea, malaise, vomiting, and convulsions. Alternative complications of bacillary dysentery include septicemia, dehydration, joint pains, hypoglycemia, lysis uremia, and neurologic complications (Marteyn et al., 2012). The mode of transmission is via the fecal-oral route and by direct contact with an infected individual. The *Shigella* species are extremely infectious, as solely 10-100 organisms are enough to cause malady and also the microorganism are additional proof against abdomen acid and might simply undergo the stomachic acid barrier (Patil, 2012). Currently, there's no protecting immunizing agent targeting *Shigella*, however many vaccine candidates for enterobacteria are beneath development as well as killed, live attenuated, ribosomal and conjugate vaccines (WHO, 2006)

To control shigellosis good hygiene, safe handling, and processing of food, using clean cutting boards for vegetables, adequate cooking of food, properly washing of raw vegetables before serving, use of boiled water, and protection of food from flies are recommended.

2.4 *Bacillus SPP*

The *Bacillus cereus* group presently consists of seven *Bacillus* species, i.e., *B. anthracis, B. cereus, B. mycoides, B. pseudomycoides, B. thuringiensis, B. weihenstephanensis,* and the most recently recognized member of the group, *B. cytotoxicus,* which is thermotolerant; these species are so closely related that they could be within one species but differentiated from *B. anthracis,* which has specific large virulence plasmids (Ehling-Schulz, 2019).

The broad spectrum of *B. cereus* toxicity ranges from avirulent strains used as probiotics for humans to highly toxic strains responsible for food-related fatalities; *B. cereus* can cause two different types of foodborne illness: the diarrheal type, which was first recognized after a hospital outbreak associated with vanilla sauce in Oslo, Norway, in 1948, and the emetic type, which was described 20 years later after several outbreaks associated with fried rice in London, England (Lereclus, 2019).

The diarrhoetic variety of foodborne sickness} is caused by associate cytotoxin created throughout vegetative growth of *B. cereus* within the small intestine, whereas the emetic illness is caused by a toxin that's preformed by *B. cereus* while growing in the food (Koehler, 2019); once *B. cereus* has been exposed to sublethal acid treatment conditions it's custom-made acid resistance of the strain will cause a food safety problem as a result of it should end in increased protection for cells that are afterwards exposed to deadly heat or peroxide stress (Kim et al, 2013). Whereas numerous foodborne pathogens are famous to cause foodborne illnesses, B. cereus has been usually found in most of the cases to be liable for food-borne outbreaks (Velusamy et al. 2010).

2.4.1 CHARACTERISTICS OF THE ORGANISM

Traditionally, all aerobic endospore-forming bacteria have been placed in the genus Bacillus. Over the past three decades, this group has been spread to more than 100 species (6), and the Bacillaceae family has been divided into nine separate genera. The first genus is Bacillus, which includes members of the *B. cereus* group *B. anthracis*, *B. cereus*, *B. mycoides*, *B. thuringiensis*, and more recently, *B. pseudomycoides*, *B. weihenstephanensis*, and *B. cytotoxicus*. These bacteria have highly similar 16S and 23S rRNA sequences, It suggests that, relatively recently, they have diverged from the traditional evolutionary line. Although *B. anthracis is related to other species within the B. cereus* range based on rRNA sequences, it is the most distinctive member of this group, both in its highly virulent pathogenicity and taxonomically (Baron, 1996).

2.4.2 VIRULENCE FACTORS/MECHANISMS OF PATHOGENICITY

The 2 forms of B. Cereus foodborne sickness are caused by terribly differing kinds of toxins. The nauseant toxin, cereulide, is a 1.2-kDa dodecadepsipeptide synthesized by a nonribosomal amide

synthetase whereas diarrhetic unwellness is caused by enterotoxins of macromolecule such as protein (Granum, 2010).

2.5 Yeast and Mould

Yeasts are eukaryotic, one-celled microorganisms called a part of the kingdom of the fungus; the primary yeast was developed many lots of years ago, and 1,500 species are presently known. (Hoffman et al., 2015). These are calculable to represent 1% of all represented fungal species. On the opposite hand, molds are a fungus that grows within the sort of multi-cellular filaments referred to as hyphae (Moore et al., 2011).

Yeasts will grow in foods with a neutral or slightly acidic pH environment and therefore the presence of sugars, organic acids, and alternative simply metabolized carbon sources. the expansion of yeasts inside food products is usually seen on their surfaces, as in cheeses or meats, or by the fermentation of sugars in beverages, such as juices, and semi-liquid products, such as syrups and jams (Karabagias, 2018).

Molds, on the other hand, tend to grow on the surface of objects in the shape of a visible 'mycelium' made up of many cells. Molds have both positive and negative effects on the food industry. Specific types of molds are beneficial for the food industry while other types of molds can be quite toxic and may produce allergic reactions and respiratory problems, or produce poisonous substances called mycotoxins (Karabagias, 2018).

The mold; *Aspergillus* spp, for instance, which is most often found on meat and poultry (as well as in the environment), can cause an infection called Aspergillosis, which is a group of illnesses ranging from mild to severe lung infections, or even whole-body infections. One of the best considerations relating to mold in food is that the mycotoxins that some varieties produce; one in every of the foremost researched mycotoxins is aflatoxin, a cancer-causing poison (Kurtzman, 2006); food-borne diseases, the major cause of morbidity and mortality are reported to be a serious threat to public health all over the world. *Bacillus cereus* has emerged as a major food-borne pathogen during the last few decades and is often present in a variety of foods, such as starchy foods (rice), animal origin foods (meat, milk, and dairy products (Meena, 2000).

CHAPTER THREE

3.0 Materials and Methods

3.1 Study Area

The study site was Magboro market which is located in the Obafemi-Owode Local government area in Ogun State and is one of the many towns around the state that share proximity with the ever-bustling Lagos. A town once occupied by only the natives is now an urban settlement with a population estimated to be over a million inhabitants and still growing.

3.2 Collection of Samples

Samples of *kilishi* were bought at random from different street vendors at popular spots in Magboro market. The beef jerky samples were taken to the laboratory aseptically for identification and microbial analysis.

3.3 Materials and Equipment Used

Materials used: Petri-dishes, beakers, conical flasks, hockey stick, measuring cylinder, Eppendorf tubes, micropipette (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, Laminar airflow 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 that 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 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 a conical flask.

2. *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.

3. *MACCONKEY AGAR*

MacConkey Agar is used for gram-negative enteric bacteria isolation and lactose fermentation differentiation from non-lactose fermenting bacteria and lactose fermenting bacteria.

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.

4. *NUTRIENT AGAR*

Nutrient agar is a general-purpose nutrient medium used for the cultivation of microbes supporting the growth of a wide 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.

5. *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 the 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.

6. *POTATO DEXTROSE AGAR (PDA)*

Potato dextrose agar is a general-purpose medium for yeasts and molds 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, supplemented with 25 mg chloramphenicol, and poured aseptically into sterile Petri dishes, and left to solidify.

7. *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.

8. *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. Dehydrated media of selenite F (19g) 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 were mixed 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 food samples were weighed into a sterile stomacher bag containing 225 ml of 1% buffered sterile peptone water and blended in a stomacher machine set at 180 rpm (revolution per minute) for 4 minutes to form a homogenate of the food sample and was incubated at 37 ^{O}C for 24hours. The resultant homogenate was diluted serially up to 10^{-4}.

3.6 Serial Dilution

One milliliter (1ml) of the samples were pipetted using the micro-pipette (set at 1000ul) into test tubes containing 9ml of BPW (0.1%) to obtain 10^{-2}, followed by the transfer of 1ml from 10^{-2} into a new test tube (containing 9ml of BPW) to create 10^{-3} dilution, the test tubes are then put in the vortex mixer for even mixing. The dilution factor was repeated for 10^{-3}, 10^{-4}. The test tubes were labeled for easy identification.

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

From the appropriate dilutions, 0.1 ml was plated in duplicate onto SMAC Agar, EMB Agar, MAC Agar, and Nutrient Agar for the isolation of enteropathogenic *E. coli*, coliforms, and the Total viable count using the spread plate technique. The plates were incubated at 35^{O}C- 37^{O}C for 18- 24 hours.

3.8 ISOLATION OF YEAST AND MOULD

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

3.9. ISOLATION OF *SALMONELLA SPP.*

3.9.1 Primary enrichment

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

3.9.2 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.

3.9.3 Sub Culturing

Subculturing was carried out to purify the isolated bacterial colonies from a mixed culture. Bacterial isolates transferred or sub-cultured were differentiated based on their colony morphology, shape, color, elevation, and other physical characteristics.

Presumptive colonies obtained after incubation were sub-cultured onto fresh nutrient agar plates by the streaking method. The plates were inverted and incubated at 37^{O}C for 18- 24 hours.

3.9.4 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 24h and 500ul mixed with an equal volume of sterile 20 %glycerol as cryoprotectant and it was stored in a -4 ºC freezer.

3.9.5DNA Extraction

3.9.5.1 Boiling method

Each isolate was streaked out on nutrient agar and incubated overnight at 37^0 C. The loopful actively dividing cells were emulsified in 500µl 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 10,000 rpm 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 14,000RPM for 5 minutes, a new set of Eppendorf tubes were labeled 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.

3.9.6 PCR Protocol

3.9.6.1 *16S rRNA amplification*

Partial 16S rRNA gene amplification using forward primer fD1 (5´-AGA GTT TGA TCA 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 below. The PCR was carried with an 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(ul)
1	Mastermix	5
2	fD1	0.4
3	rD1	0.4
4	DNA	2.0
5	dH_2O	2.2
6	Total	10

Table 3.2 MULTIPLEX PCR PROTOCOL

No	Component	1 rxn (ul)
1	Master mix	7.5
2	STX1F	0.186
3	STX1R	0.186
4	STX2F	0.186
5	STX2R	0.186
6	EAEF	0.186
7	EAER	0.186
8	DNA	2.00
9	dH_2O	4.36
10	Total	

Table 3.3 PROCEDURE FOR THERMOCYCLER

Analysis	Step	Temperature(^{0}c)	Time(min.)
1x	Initial denaturation	95	5
35x	Denaturation	95	2
	Annealing	42	0.5
	Polymerization	72	4
1x	Final polymerization	72	10
1x	Hold	4	∞
Cycler			

3.9.7 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.

3.9.8 PRECAUTIONS

• Aseptic techniques were observed at every stage of work.

• Personal protective technique was also observed, such as wearing of a 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 the organism of interest.

• Ensured that the petri-dish was incubated inverted.

• Ensured proper timing, most especially during autoclaving.

CHAPTER FOUR

4.1 RESULTS AND DISCUSSION

This study reveals the presence of *salmonella* in food samples; *salmonella* suspect isolates on SMAC which is not in line with the food safety standard for salmonella; which explains pathogenic microorganism*s* should not be found within 25g of food samples; this study aims to create an awareness in the aspect of consuming RTE meat product such as *Kilishi* which individuals usually do not consider to have a certain level of the microbial count.

The microbial investigation of pathogens present in the three different *Kilishi* samples (K1, K2, and K3) from Magboro supermarkets, Ogun state was as follows:

4.2 MORPHOLOGICAL CHARACTERISTICS OF ISOLATES ON EOSIN METHYLENE BLUE AGAR

There was no visible microbial growth on the cultured eosin methylene blue agar plates for any of the samples cultured in any dilution factor. The absence of glistening green colonies on the surface of the EMB agar shows the absence of *E. coli* in the sample. EMB agar provides a rapid and accurate method of distinguishing *E. coli* from other gram-negative pathogens. However, since growth was found of SMAC, there is a possibility of an error with the EMB growth media.

4.3 MORPHOLOGICAL CHARACTERISTICS OF ISOLATES ON SORBITOL MAC CONKEY AGAR

Sample	Isolate ID	Color	Shape	Elevation	Appearance	Surface	Log CFU/g	Opacity
K1 (KZ *kilishi*)	K1 10^{-2}	pink	Circular	Raised	Glistening	Smooth	2 X 10^{-2} distinct	Opaque

Table 4.1

There was evidence of growth found on the spread plate culture of the 10^{-2} diluent Sample A *kilishi*. Colonies seen were raised and pink in color which shows the fermentative ability of the microbe to ferment lactose. Colony count: $2x10^{-2}$ CFU/log_{10} while on the low side were very distinct and easy to observe, they were also quite small in size.

E. coli 0157:H7 was suspected which is fecal coliform, although the presence of this coliform in Sample A was very low according to the microbial count when there is the presence of a visible amount or a certain level of high microbial count the food product is considered not safe for consumption; there were no other visible microbial growths recorded on any of the other samples for Sorbitol Macconkey agar.

4.4 MORPHOLOGICAL CHARACTERISTICS OF ISOLATES ON MACCONKEY AGAR

There was no visible microbial growth on the cultured macconkey agar plates for any of the samples cultured in any concentration.

4.5 MORPHOLOGICAL CHARACTERISTICS OF ISOLATES ON POTATO DEXTROSE AGAR

Sample	Isolate ID	Color	Shape	Elevation	Appearance	Surface	Number	opacity
K1 (KZ *kilishi*)	K1 10^{-1} (2)	Creamy	Circular	Raised	Solid	Glistening	$1x10^{-2}$cfu/log_{10}	opaque
K1 (KZ *kilishi*)	K1 10-1	White with a creamy center	Circular	Raised	Filamentous	Filamentous	$1x10^{-1}$cfu/log_{10}	translucent

Table 4.2

Potato Dextrose Agar which was prepared and the antibiotic named chloramphenicol was added to the PDA to inhibit bacterial growth on it were some growth suspected to be fungal growth which shows that this is from the samples used. The pH range of *kilishi* was tested and observed to be between the range 5-6 which supports the growth of most fungi, it would present a suitable substrate for fungal spores to be inoculated on. Further-more the evidence of low moisture content of 10.00±0.15c (Omojola *et al.,* 2008) which is the ideal range for *kilishi* is also an encouraging factor to provide fungal spores with a suitable environment to multiply. The presence of filamentous colonies was observed for Sample A (K1) 10^{-1} sample serial diluent and solid circular raised colonies were also observed for the same concentration and sample. The

evidence of a low amount of 1 CFU/log however points to the prevalence of a low amount of fungal contamination of the sample.

There were no other visible microbial growths observed for any other sample plated and their respective concentrations.

4.6 MORPHOLOGICAL CHARACTERISTICS OF ISOLATES ON NUTRIENT AGAR

Sample	Isolate ID	Color	Shape	Elevation	Appearance	Surface	Number	Opacity
K1	10-3	Creamy	Circular	Raised	Solid	Glistening	TNTC	Opaque
K2	10-3 (2)	Creamy	Circular	Raised	Solid	Glistening	$14x10^{-3}log_{10}$cfu/g distinct	Opaque
K3	10-4	Creamy	Circular	Raised	Solid	Glistening	$15x10^{-4}log_{10}$cfu/g distinct distinct	Opaque

Table 4.3

Morphological characteristics and culturing of each sample on Nutrient agar was taken into consideration for the total viable microbial count of the samples in question. The total viable microbial count is observed to estimate the total level of culturable contaminants present in the sample as a whole. As stated in the South African National Standards for processed meat products, The Total viable count (TVC) in dried meat products should be less than 6 log cfu/g (SANS, 2011). However, Sample A (K1) far surpassed the acceptable standard of microbial load

for a processed and dried meat product with >300 CFU/log, Sample B(K2) and Sample C(K3) also were slightly higher than the acceptable amounts with 14 distinct and 15 distinct colonies identified respectively.

There were no other visible microbial growths observed for any other sample plated and their respective concentrations.

4.7 MORPHOLOGICAL CHARACTERISTICS OF ISOLATES ON XYLOSE LYSINE DEOXYCHOLATE AGAR

While this is a known selective growth agar for Salmonella and Shigella, a total absence of growth from and of the primary enrichment broth will point to the absence of a culturable amount of both Salmonella and Shigella microbes.

There was no visible microbial growth on the cultured xylose lysine deoxycholate agar plates for any of the samples cultured in any concentration.

CHAPTER FIVE

5.1 Conclusions and recommendations

Commercial brands of *kilishi* sold in Magboro proved to have pathogenic microorganisms. The presence of STEC and Salmonella in the RTE meat product is of great concern as diseases could occur from consumption of this beef jerky (*Kilishi*). Street vended meat products sold in (Magboro axis) area of Ogun state contain presumptive pathogenic STEC and Salmonella which can create a public health hazard. Therefore, RTE meat products such as *kilishi* should be adequately passed through open flame and/or roasted just for a while before selling to ensure that the meat products are free of disease-causing microorganisms.

The general public should ensure the safety of commercially sold meat products by checking thoroughly the manufactured date and expiry date before consumption. Poor storage condition is also a means these RTE meat products get contaminated, proper storage facility should be present and prepared before the transportation and selling of *Kilishi*.

Owners of the livestock used in making the beef jerky should ensure that proper hygiene is adequately considered and the animal is completely healthy before, during, and after processing of the meat. As an addendum surveillance systems should be set in place by the government and its concerned agencies, to trace the occurrence and prevalence of foodborne diseases occurring as a result of consumption of meat products with a high level of microbial load capable of causing diseases and increase mortality rate.