INCIDENCE OF *SALMONELLA* SPECIES AND DIARROGENIC *ESCHERICHIA COLI* IN '*KILISHI*' SOLD IN MAGBORO, OGUN STATE

BY

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CERTIFICATION

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ABSTRACT

The reoccurrence of foodborne disease outbreaks linked to pathogenic *Escherichia coli*; the causative microorganisms of diarrhea and *Salmonella* spp. has led to worldwide sensitization on the routes of contamination of these pathogens and their pathogenicity in humans. The mode of transmission is mainly through the faecal-oral routes in humans, specifically through ingesting contaminated foods. The main focus of this investigation was to ascertain the prevalence of these pathogens in street vended *kilishi*. Microbial counts, biochemical characteristics and molecular typing of pathogens found in *kilishi* were elucidated. Three commercially available brands of packaged *kilishi* acquired from Makogi, Ogun state, were analysed; namely, Sample A (K1), Sample B (K2) and Sample C (K3). Total viable count was 14×10^{-3} Log_{10} cfu/g and 15×10^{-3} Log_{10} cfu/g for both Samples B and C respectively. A growth of 2×10^{-3} Log_{10} cfu/g was observed on Sorbitol MacConkey agar for Sample A only. Yeast and mould count of 2×10^{-3} Log_{10} cfu/g for Sample A only on Potato Dextrose Agar. Food safety cultural practises should be ensured in the packaged *kilishi* processing chain during manufacturing, handling, and packaging. Surveillance of the route of entry of pathogenic *Escherichia coli* and *Salmonella* should be monitored to reduce the risk of microbial contamination.

Keywords; Salmonella spp, Escherichia Coli, yeast and moulds.

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CHAPTER 1

1.0 INTRODUCTION

Kilishi is a Nigerian-Hausa version of the beef-jerky. It is a peppered form of dried beef, made from deboned meat of a goat, cow or sheep. Selected muscles from the animal are processed and sliced into thin sheets for ease of drying, the meat is then spiced and packaged (Omojola *et al.,* 2008). Pre-packaged and fresh meat have over the years been shown to be a suitable vehicle for the transmission and growth of enteropathogenic bacteria such as *E. coli*, *Salmonella* spp worldwide (Erickson, 2007). Fresh and processed meat are the most available sources of protein worldwide (Smil, 2002). The prominent role of proteins in human diet for the growth, development, replenishment and renewal of muscle mass and tissue has been a key selling point in their consumption and continuity of their availability in the food industry. In general, cattle, pig, sheep (and to a lesser extent) horse and goats are meat animals of overwhelming importance. The most important poultry birds are chickens, turkeys, ducks and geese. All of these animals and poultry are domesticated and reared specifically for meat production (Varnam *et al.,* 2000). The process of slaughtering, product packaging and handling often contributes to the entry point of various enteropathogenic microorganisms such as *E. coli* and *Salmonella* species. It is therefore extremely detrimental to the health of the average individual and the society as a whole who depend on this readily available source of protein to be privy to pathogenic contaminants and disease outbreak via the consumption of meat products. Enteric pathogens such as *E. coli* and *Salmonella* spp. have been recorded to have high levels in food products, for example, studies on the retail meats sold at the markets of Ouagadougou, Burkina-faso, had 44% of the beef and 29% of the chicken samples which were contaminated with Diarrheagenic *Escherichia coli* (DEC) with typical virulence genes (Kagambèga et al. 2012). An outbreak of gastroenteritis occurred among at least 47 persons attending a school potluck. Illness was associated with

consumption of ground beef (estimated odds ratio, 16.3; 95% confidence interval, 2.2 to 338.3). Salmonella Typhimurium was isolated

from infected individuals of the implicated ground beef (Mclaughlin et al. 2006). In New Mexico, between 1966 and 1995, eight stomach flu outbreaks thanks to consumption of contaminated meat jerky were reported, with 250 illnesses. Primarily concerned was a regionally made jerky, carne seca, made by soaking beef strips in a very spicy marinade so dehydrating them (Lingle, 2000). the method uses no different preservation methods, love salt natural action or the addition of chemical preservatives. Organisms isolated from samples enclosed many varieties of Salmonella spp. (Lingle, 2000). The presence of these pathogens has resulted in dire health related predicaments such as food poisoning, food intoxication, diarrhoea, all which could in some cases result in death. Therefore, this study will endeavour to determine the incidence of *Salmonella* spp. and diarrheagenic *Escherichia coli* in processed *kilishi* (beef jerky) meat products sold in Makogi and environs, Ogun state.

CHAPTER 2

2.0 LITERATURE REVIEW

Processed meat has proven to be a fore runner in terms of consumption by humans as a food source throughout the world. Meat, refers to animal flesh consumed as food (Duffy *et al.,* 2006). Meat is highly available and it is a nutritious source of protein . It consists of muscle and tissue of animals, which could be cooked and garnished to suit the human palate. However, the cooking, garnishing and overall processing of meat has been observed to be subject to a plethora of unhygienic conditions, leaving it privy to numerous pathogenic microbes during slaughtering, cooking, garnishing and packaging.

2.1 *SALMONELLA* SPECIES

Salmonella species are non-spore-forming, preponderantly motile eubacteria with cell diameters between concerning 0.7 and 1.5 μm, lengths from a pair of to five μm, and covered flagella (all round the cell body). Enterobacteria species are facultative animate thing pathogens. S. enterica taxon are found worldwide all told warm-blooded animals and within the environment. S. bongori is restricted to cold-blooded animals, significantly reptiles (Ohad et al., 2014) Salmonella species are intracellular pathogens (Kumar and Valdivia, 2009); sure serotypes inflicting illness. Non-typhoidal serotypes is transferred from animal-to-human and from human-to-human. they sometimes invade solely the duct tract; causing salmonellosis, the symptoms of which might be resolved while not antibiotics. However, in sub-Saharan Africa, nontyphoidal Salmonella is invasive and cause paratyphoid fever fever, which needs immediate treatment with antibiotics (Ryan and Ray, 2004). Typhoidal serotypes can solely be transferred from human-to-human, and may cause food-borne infection, typhoid fever, and paratyphoid fever. typhoid is caused by Salmonella invasive the blood (the typhoidal form), or additionally spreads throughout the body, invades organs, and secretes endotoxins (the septic form). this may result in severe shock and septic shock, and requires medical care together with antibiotics (Seymour *et al.,* 2017).

2.2 SOURCES OF TRANSMISSION

Salmonella microorganism board the intestines of people, animals and birds (Pruthi, 2019) the majority are infected with Salmonella by consumption foods that are contaminated by faeces. normally infected foods include; aw meat, poultry and seafood. Faeces may get onto raw meat or poultry during slaughtering process, due to the unhygienic practises of food handlers, such as inadequate washing of hands, cross contamination due to improper evisceration processes, contamination due to bad personal hygiene; keeping of long nails, long unkept hairs, dirty garments and absence of proper professional butchering apparel; apron, Hair covers and overalls. Seafood may be contaminated if harvested from contaminated water. Raw egg is a major source of Salmonella contamination, and while an egg's shell seems as a perfect barrier to prevent contamination, (Keerthirathne *et al.*,2016) some infected chickens produce do infected eggs that contain *Salmonella* before shells are even formed. Some fresh produce, may be washed in the field with water contaminated with Salmonella. The kitchen is also a place where contamination occurs, when juices from raw meat and poultry come into contact with each other or uncooked foods, such as salads (Pruthi, 2019).

The points of contamination during the meat processing chain are numerous and can vary and range from unhygienic slaughtering processes, cross contamination from food handlers and line workers into product, inadequate heating or storage temperatures to improper packaging and transport of food product (Bacon *et al.,* 2000)

2.3 THE GENUS *SALMONELLA*

Salmonella enterica (formerly *Salmonella choleraesuis*) is a rod-shaped, flagellate, facultative aerobic, Gram-negative bacterium and a species of the genus *Salmonella*. A number of its serovars (a distinct variation within a species of bacteria or virus or among immune cells of different individuals) are serious human pathogens (Kurtz *et al.,* 2017). Most cases of salmonellosis are caused by food infected with *S. enterica*, which often infects cattle and poultry, though

other animals such as domestic cats (Forshey *et al.*, 2000). *S. enterica* has six subspecies each with subspecies and serovars with different specificity my antigenicity. S. enterica has more than 2500 serovars (Achtman *et al.*, 2012). *Salmonella bongori* was previously thought to be an *S. enterica* subspecies, but it is now considered to be the other the other species in the main genus *Salmonella*. Most of the human pathogen *Salmonella* serovars belong to subspecies enterica. These serogroups of S. enterica embrace S. Typhi, *S. Enteritidis, S. Paratyphi, S. Typhimurium, and S. Choleraesuis* (Chiu et al., 2004). *Salmonella bongori*: it's a Gram-negative, rod-shaped microorganism (bacillus), that causes a epithelial duct malady known as salmonellosis, characterised by cramping and diarrhoea. it's usually thought-about a germ of cold-blooded animals, in contrast to different members of the genus, and is most often related to reptiles. it absolutely was earlier called *Salmonella* taxon V or S. enterica subsp. *bongori* or *S. choleraesuis* subsp. *bongori* (Chiu et al., 2004)

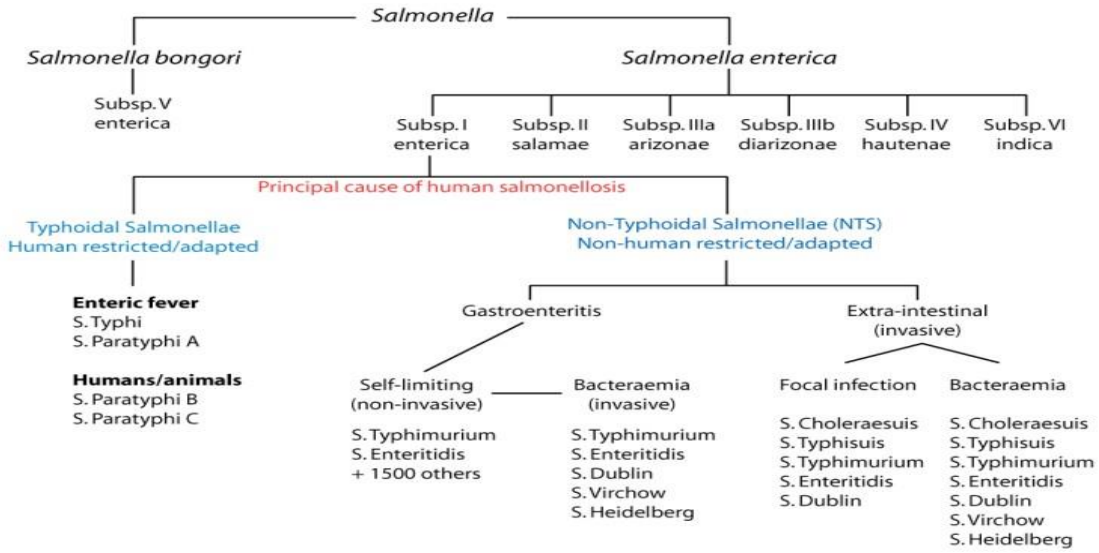

Figure 1.1: Classification of Salmonella. Adapted from Hurley *et al*., 2014

The most dangerous strain of *Salmonella* is *Salmonella enterica typhi* (referred to as *Salmonella Typhi* from now on), is an obligate parasite that has no known natural reservoir outside of humans, *Salmonella Paratyphi* as well which only resides in higher primates. There

are also prevalent serovars in various types of meat such as; Chicken which *Salmonella Infantis, Salmonella Typhimurium* and *Salmonella Enteritidis*, Beef with *Salmonella Anatum, Salmonella Montevideo* and *Salmonella Typhimurium.*

2.4 DIARROGENIC *ESCHERICHIA COLI*:

Escherichia coli is the sort species of the genus *Escherichia*, that contains largely motile gram- negative bacilli among the family Enterobacteriaceae and the genus *Escherichia*. Most *Escherichia coli* strains are commensal; however, there are many extremely tailored clones that have the capability to cause human illness. Strains that cause enteric infections are selected diarrheagenic *E. coli*, a gaggle that features emerging pathogens with public health connexion worldwide (Vidal, 2005). Diarrheagenic *Escherichia coli* is recognised because the predominant non-pathogenic facultative flora of the human intestine. On the 20th of September, the u. s. Department of Agriculture's Food Safety and examination Service (USDA-FSIS) investigated a multistate happening of Shiga toxin-producing *E. coli* O26 infections linked to beef from Cargill Meat Solutions, within which eighteen were hospitalized and one died of complications resulting in excretory organ failure (Kennedy *et al.*, 2006). On

15th of December 2006, seventy-one folks were recorded to be hospitalized with morbific *Escherichia coli* strain *E. coli* O157 in New Jersey in association with an area Taco Bell restaurant. Eight (8) of those persons developed lysis pathology syndrome (kidney failure) (Behravesh *et al.*, 2011)

2.5 CATEGORIES OF DIARROGENIC *ESCHERICHIA COLI*

The enteric pathogens are referred to as diarrheagenic *Escherichia coli* (DEC) of that six classes are characterized: Enteropathogenic *E. coli* (EPEC), Enterohemorrhagic *E.*

coli (EHEC)/ Shiga poison manufacturing *E. coli* (STEC), Enteroaggregative *E. coli* (EAEC),

Enteroinvasive *E. coli* (EIEC), Enterotoxigenic *E. coli* (ETEC), Diffusely adhering *E. coli* (DAEC) (Elias *et al.,* 2016).

Escherichia coli may be a microorganism that is ordinarily found within the gut of humans and homeothermic animals. Most strains of *E. coli* are harmless. Some strains however, adore Shiga toxin-producing *E. coli* (STEC), will cause severe foodborne disease. it's transmitted to humans primarily through consumption of contaminated foods, adore raw or undercooked ground meat products, raw milk, and contaminated raw vegetables and sprouts (Lebaron *et al.*, 2015).

2.6 MODE OF ACTION OF STEC

STEC produces toxins, referred to as Shiga-toxins as a result of their similarity to the toxins made by *Shigella dysenteriae*. STEC will grow in temperatures starting from 7 °C to 50 °C, with an optimum temperature of 37 °C (Paton *et al.,* 1998). Some STEC can grow in acidic foods, right down to a hydrogen ion concentration of 4.4, and in foods with a minimum water activity (a_w) of 0.95. STEC is destroyed by thorough preparation of foods till all components reach a temperature of 70 °C or higher. *E. coli O157:H7* is that the most vital STEC serotype in relevance public health; however, alternative serotypes have oft been concerned in irregular cases and outbreaks (Mathusa *et al.,* 2010). Symptoms of the diseases caused by STEC embrace abdominal cramps and looseness of the bowels which will in some cases get to bloody diarrhoea (haemorrhagic colitis). Fever and vomit can also occur (Loftus, 2011). The period will vary from 3 to eight days, with a median of three to four days. Most patients recover inside ten days, however in a very tiny proportion of patients (particularly young kids and also the elderly), the infection may result in a grievous disease, resembling hemolysis uraemic syndrome (H.U.S). It is characterised by acute nephritic failure, anemia and blood disorder (low blood platelets) (Lucas, 2015).

2.7 SOURCES AND TRANSMISSION

Most accessible info on STEC relates to serotype *E. coli* O157:H7, since it's simply differentiated biochemically from alternative Escherichia coli strains. The reservoir of this infective agent seems to be mainly; ruminants resembling sheep, goats, ruminant are thought- about important reservoirs, mammals (such as pigs, horses, rabbits, dogs, and cats), birds (such as chickens and turkeys) are found infected. Raw or undercooked ground meat product that is prepacked meat (chicken and turkey sausages), animal products such as milk and cheese and dirty contamination of vegetables (Wang *et al.*, 2015)

2.8 *E. COLI O157:H7* TRANSMISSION

Consumption of contaminated foods, akin to raw or undercooked ground meat product and milk. soiled contamination of water and alternative foods, also as cross-contamination throughout food preparation (with beef and other meat products, contaminated surfaces and room utensils), also will result in infection. samples of foods involved in outbreaks of *Escherichia coli O157:H7* resulting from undercooked hamburgers, dried cured salami, yogurt, and cheese made up of raw milk (Kubori and Galan, 2000). An increasing variety of outbreaks are related to the consumption of fruits and vegetables (including sprouts, spinach, lettuce, coleslaw, and salad) whereby contamination could also be due to Contact with excretory product from domestic or wild animals at some stage throughout cultivation or handling (Tilley, 2011). STEC has conjointly been isolated from bodies of water (such as ponds and streams), wells and water troughs, and has been found to survive for months in manure and water-trough sediments. Waterborne transmission has been reported, each from contaminated drinking-water and from recreational waters (Aljohani *et al.*, 2017). Person-to-person contact is a very important mode of transmission through the oral-faecal route. associate degree symptomless carrier state has been reported, wherever people show no clinical signs of illness however are capable of infecting others. The period of excretion of

STEC is regarding one week or less in adults, however will be longer in children. Visiting farms and alternative venues

wherever the final public would possibly get direct contact with stock has conjointly been known as a very important risk issue for STEC infection (Widiasih *et al.,* 2004).

2.9 PREVENTION

The keys to safer food are to keep it clean so to avoid contamination. It would also be more sanitary and safer to separate the raw and cooked meat products. The raw meat products when to be prepared for consumption must be cooked thoroughly and also be kept at safe temperatures to prevent growth of contaminating microbes. Use safe water and hygienic meat raw materials when cooking.

2.10 YEAST AND MOULD

Yeasts are eukaryotic, acellular microorganisms classified as members of the fungus life kingdom. the primary yeast originated 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 plant life species. On the opposite hand, moulds are fungus that grow within the sort of multi-cellular filaments known as hyphae (Denning *et al.,* 2011). Yeasts are able to grow in foods with a neutral or slightly acidic hydrogen ion concentration surroundings and in the presence of sugars, organic acids, and other simply metabolized carbon sources. the expansion of yeasts among food product is often seen on their surfaces, as in cheeses or meats, or by the fermentation of sugars in beverages, cherish juices, and semi-liquid products, such as syrups and jams (Karabagias, 2018). Moulds tend to grow on the surface of objects within the form of a noticeable 'mycelium' created of several cells. Moulds have each positive and negative effects on the food trade. Specific styles of moulds are useful for the food industry whereas different types of moulds is quite toxic and should turn out sensitivity and metabolic process problems, or produce poisonous substances known as mycotoxins (Karabagias, 2018). fungus genus mold, for instance, that is most frequently found on meat and poultry (as well as in environment), will cause an infection known as

Aspergillosis, which is really a gaggle of sicknesses starting from delicate to severe respiratory organ infections, or maybe whole-body

infections. one amongst the best issues concerning mould in food is that the mycotoxins that some varieties produce. one amongst the foremost researched mycotoxins is aflatoxin, a cancer-causing poison (Kurtzman, 2006).

2.11 FUNGAL CONTAMINATION IN STREET VENDED FOODS

Fungal contamination of street vended foods is common because of trafficker observe of displaying the foods brazenly in markets, such they're exposed to fungus spores. various fungal genera contaminate food materials, however the oftentimes occurring ones in street vended foods embrace genus Aspergillus, Fusarium, Mucor, Penicillium, and Rhizopus. Aspergillus and Fusarium were according to contaminate retailed *kulikuli* (peanut cake) and salads from Benin Republic, Togo, and Nigeria (Adjou *et al.,* 2012; Singh *et al.,* 2011). Similarly, Aspergillus and Penicillium were reported in street-vended doughnut, egg roll, and pie from Nigeria (Oranusi & Braide, 2012)

CHAPTER 3

3.0 MATERIALS AND METHODS

STUDY AREA: Sampling of processed meat products was done in Ofada / Mokoloki Local Council Development Area (LCDA), located in the state of Ogun. This LCDA is made up of Magboro, Ibafo, Makogi and Mowe for which the main area of concentration was the Magboro settlement in particular. The Magboro shopping malls where the point of acquisition of the *kilishi* samples.

SAMPLE COLLECTION: Processed and packaged *kilishi*: Sample A, Sample B and Sample C were acquired from choice locations of the Magboro shopping mall in Ogun state, Nigeria.

WORKING ASEPTICALLY: Proper sterilization was performed at any required step of the benches efforts to ensure aseptic conditions of work setting and materials. Work bench area were sterilized with 70% ethanol solution and working close to a flame from the bunsen burner to keep air clean and aseptic all over the work zone.

STERILIZATION

Eppendorf tubes test tubes and micro pipette tips were sterilized in the autoclave at 121 ° C for 15 min. While beakers, petri-dishes, jars, scotch jars and McCartney jars were sterilized in the oven at 160 ° C for 1 hour.

3.1 PREPARATIONS OF NECESSARY MATERIALS

BUFFERED PEPTONE WATER (BPW): The dehydrated media is measured out and dissolved in distilled water in accordance with the manufacturer's instructions to make a solution of 0.1 % of peptone water in a suitable beaker. The mixture is then HEATED to ensure proper dissolution of the solute. The boiled mixture is then autoclaved to achieve sterilization at 15lbs (121^{o}c) for 15-20 minutes. 9ml of the solution is then dispensed equally into individual test tubes as diluent.

SALMONELLA SHIGELLA (SS) AGAR 60g of the dehydrated medium was measured out and suspended in a litre of distilled water in a beaker. It was mixed well with a stirring rod and then set to boil with continuous agitation for 60 seconds. The media is not to be autoclaved. It is then left to cool to 45^{o}c and then poured into plates to solidify.

SORBITOL MAC-CONKEY AGAR (SMAC) 51g of Sorbitol MacConkey Agar is measured out and dissolved in one litre of deionized water in a suitable beaker. The mixture is mixed thoroughly with a glass stirring rod and then put to boil for 60 seconds. It is then sterilized via the autoclave at 121^{o}C for 15 minutes. The mixture is then left to cool till 45^{o}C and then poured out into plates.

NUTRIENT AGAR 28g of dehydrated Nutrient agar is dissolved in 1 litre of deionized water in a suitable beaker. The solute is mixed into the water thoroughly with the aid of a glass stirring rod. The mixture is then subjected to boil for 60 seconds to ensure complete dissolution of the solute. The medium is then sterilized by autoclaving at 15lbs (121 ^{o}C) for 15 minutes The resultant medium is then left to cool to 45 ^{o}C and then poured out into sterile petri dishes. The medium is then left to solidify.

SELENITE F BROTH 19g of dehydrated selenite F medium is dissolved in 750 ml deionized

water in a suitable beaker. 4g of sodium biselenite is dissolved in 250ml deionized water in a separate beaker. The 19g of selenite F and the 4g of sodium biselenite are mixed together in a suitable beaker. Resultant mixture is then dispensed into sterile test tubes. Mixture is warmed in boiling water to ensure complete dissolution and sterilization for 10-15 minutes.

EOSIN METHYLENE BLUE AGAR (EMB)

The dehydrated medium was dissolved in the appropriate volume of distilled water i.e. 36g in 1000ml distilled water based on manufacturer's instructions in a conical flask and mixed thoroughly. The mixture was then heated to completely dissolve the crystalline powder and was then sterilized in the autoclave at 121ºC for 15minutes.

POTATO DEXTROSE AGAR

The dehydrated medium was dissolved in the appropriate volume of distilled water i.e. 39g in 1000ml distilled water in a conical flask. The mixture was then heated for a while to completely dissolve the powder and was then sterilized in the autoclave at 121ºC for 15minutes . The potato dextrose agar was modified with 25mg of chloramphenicol to inhibit bacterial growth thus promoting growth of fungus and mould.

3.2 SAMPLE PREPARATION

Twenty-five (25) g of *kilishi*: Sample A(K1), Sample B (K2) and Sample C (K3) sample were each weighed and 225ml of 1% peptone water (enrichment broth) in a conical flask. The samples were homogenized for 4 minutes at 180rpm in a stomacher. The samples were serially diluted from the initial concentration to the 10^{-4} concentration respectively.

Primary enrichment: 25g from each of *kilishi* meat samples (k1, k2, k3) respectively were

transferred into 225 ml of 1% of sterilized buffered peptone water aseptically. The samples were homogenized for 4 minutes at 180rpm in a stomacher. Then incubated at 37°C for 24hrs.

Secondary enrichment: Further enrichment to increase the concentration of *Salmonella* in the sample.1 ml of the initial primary enrichment was transferred into 90mls of selenite F broth medium. The resultant solution was incubated at 37ºC for 24hours.

STREAKING ON SALMONELLA SELECTIVE MEDIA

After incubation, the test tubes were vortexed and a loopful of the incubated selenite F broth was streaked unto Xylose lysine deoxycholate (XLD) agar and incubated at 37 ºcfor 24hours.

SAMPLE PLATING

For the Nutrient agar, potato dextrose agar and Eosin methylene blue agar plates, spread plate technique was used for plating of inoculum (samples). 0.1ml of the inoculum directly from dilutions 10^{-2}, 10^{-3} and 10^{-4} were plated and spread throughout the surface of the agar.

However, for the detection of Salmonella in the K1, K2 and K3 samples, 0.1ml of inoculum from the secondary enrichment media were pipetted onto Salmonella-Shigella agar plates or streaked on XLD plates and then incubated.

CRYOPRESERVATION OF ISOLATES

A loopful of pure culture of each isolate was inoculated into a sterile Eppendorf tube containing

1ml of brain heart infusion, incubate at 37ºC for 24 h and 500ul of 20 % sterile glycerol as cryoprotectant and it was stored in a -20ºC freezer.

3.3 DNA EXTRACTION

Each selected isolate was subcultured via the streaking method nutrient agar and incubated overnight at 37 ºC. The resultant isolates were emulsified in 500ml double distilled water it was centrifuged at 14,000 RPM for 5 minutes.

The supernatant was decanted and 1ml of sterile water was added to the Eppendorf tube, centrifuged again at 10,000RPM for 2 minutes the process was repeated twice. Then 200ul of sterile water was added into the Eppendorf tube and centrifuged at 14,000RPM for 5 minutes.

It is then placed in a sterile to boil for 10-20 minutes and subsequently placed in a sterile ice box to cool and decanted. The supernatant contains the extracted DNA which is transferred into fresh Eppendorf tubes and stored for future use.

3.4 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 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.

3.4.1 AGAROSE GEL ELECTROPHORESIS

The agarose was prepared using 1g of agarose powder and 50ml of TAE buffer which is boiled and mixed until a clear solution is attained and 3ul of ethidium bromide is added. Contents are the transferred into the electrophoresis tank containing the gel combs and cast. The DNA samples are then pipetted into their respective wells. The tank is connected to the power source to begin electrophoresis.

Table 3.1 PCR REACTION COMPONENTS

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 MULTILEX 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	∞

CHAPTER 4

4.0. RESULTS AND DISCUSSIONS

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 microorganisms 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 contamination of the three different *Kilishi* samples (K1 (Sample A), K2 (Sample B) and K3(Sample C)) gotten from Magboro super markets, Ogun state were as follows:

4.1. MORPHOLOGICAL CHARACTERISTICS OBSERVED ON AGAR PLATES 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 concentration. Evidence of non-growth of glistening green colonies or any colonies for that matter on the surface of the eosin methylene blue agar goes to show the absence of a culturable level of *Escherichia coli* in the sample's diluent Eosin methylene blue agar offers a speedy and correct technique of distinguishing *E. coli* from different gram-negative mastitis pathogens (Leininger, 2001). Furthermore, complete absence of growth further points to the absence of a culturable level of coliforms in the sample cultured in question or inherent inablility of the agar used to culture visible levels of colonies due to nutritional deficiency.

4.2. MORPHOLOGICAL CHARACTERISTICS OBSERVED ON AGAR PLATES OF ISOLATES ON SORBITOL MAC CONKEY AGAR

Table 4.1

Sample	Isolate ID	Color	Shape	Elevation	Appearance	Surface	Number Log_{10} CFU/g	Opacity
K1	K1 10^{-2}	Pink	Circular	Raised	Glistening	smooth	2	opaque

In this particular culturing exercise evidence of growth was found on the spread plate culture of the 10^{-2} diluent Sample A *kilishi*. The colonies observed were raised and white in colour to attest to the inherent fermentative capabilities of the microbe to act and ferment lactose. The resultant colony count: 2 x 10^{-2} $\log_{10}$ cfu/g while on the low side were very distinct and easy to observe, they were also quite small in size.

This attests to the presence of *E. coli* O157:H7 STEC which is high pathogenic. Although the prevalence of this coliform in Sample A's diluent was proven by the microbial count to be quite low, the presence of a culturable amount would deem the food product unsafe for community consumption. There were no other visible microbial growths recorded on any of the other samples except Sorbitol MacConkey agar.

4.3. MORPHOLOGICAL CHARACTERISTICS OBSERVED ON AGAR PLATES OF ISOLATES ON MACCONKEY AGAR

There was no visible microorganism growth on the aesthetic MacConkey agar plates for any of the samples cultured in any concentration.

Nutritional deficiency of the agar powder used was taken into consideration on account of this result as the absence of growth on MacConkey agar should under normal circumstances equate to absence of substantial growth on Sorbitol MacConkey agar.

4.4. MORPHOLOGICAL CHARACTERISTICS OBSERVED ON AGAR PLATES OF ISOLATES ON POTATO DEXTROSE AGAR

Table 4.2

Sample	Isolate ID	Color	Shape	Elevation	Appearance	Surface	Number	opacity
K1	K1 10^{-1} (2)	Creamy	circular	raised	Solid	glistening	1 x 10^{-3} log^{10}	opaque
K1	K1 10-1	White with creamy center	circular	Raised	Filamentous	Filamentous	1 x 10^{-3} log^{10}	transcluscent

Evidence of various growth colonies of cultured media of Potato Dextrose agar which had been modified with antibiotic chloramphenicol to inhibit bacterial growth point to the presence of fungal microbes within the cultured serial diluent samples. While *Kilishi* has been tested and observed to have a ph range of 5-6 which is the ideal growth ph range for 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 suitable environment to multiply. The presence of filamentous colonies were observed for the 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 x 10^{-3} log^{10} cfu however points to the prevalence of a low amount of fungal contamination of the sample.

4.5. MORPHOLOGICAL CHARACTERISTICS OBSERVED ON AGAR PLATES OF ISOLATES ON NUTRIENT AGAR

Table 4.3

Sample	Isolate ID	Color	Shape	Elevation	Appearance	Surface	Number Log_{10} cfu/g	Opacity
K1	10-2	Creamy	Circular	Raised	Solid	glistening	TNTC	opaque
K2	10-3 (2)	Creamy	Circular	Raised	Solid	glistening	14 x 10^{-3} log_{10} cfu/g	opaque
K3	10-4	Creamy	Circular	Raised	Solid	glistening	15 x 10^{-3} log_{10} cfu/g	opaque

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 in order 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 x 10^{-3} log_{10} cfu/g (SANS, 2011). However, Sample A (K1) far surpassed the acceptable standard of microbial load for a processed and dried meat product with a TNTC amount of colonies, Sample B (K2) and Sample C (K3) also were slightly higher than the acceptable amounts with 14 x 10^{-3} log_{10} cfu/g distinct and 15 x 10^{-3} log_{10} cfu/g distinct colonies identified respectively.

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

4.6. MORPHOLOGICAL CHARACTERISTICS OF ISOLATES ON XYLOSE LYSINE DEOXYCHOLATE AGAR

There was no visible microorganism growth on the cultured and incubated xylose lysine deoxycholate agar plates for any cultured samples on any concentration. 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.

CHAPTER 5

5.0 CONCLUSIONS AND RECOMMENDATIONS

The analysis of the microbiological quality of processed and packaged *kilishi* (beef jerky) sold at Makogi, Ogun state showed presence of pathogenic microorganisms in all three brands. All three brands of *kilishi* have very low microbial load as demonstrated from the microbial counts identified from each sample. There was however an incidence of the isolation of *E. coli* O157:H7 from Sample A on Sorbitol Macckonkey agar which is a known selective media for pathogenic *E. coli*. Although, microbial counts recorded were quite low; the infective dose of *E. coli* O157:H7 is very low because it is highly pathogenic.

RECOMMENDATIONS

Stringent food safety measures should be applied to the processing and production of *kilishi*, close attention should be paid to the appropriate temperatures for processing and the resultant packaging choice should be reviewed by the producers. A thorough Hazard Analysis and Critical Control Point survey should be taken on the production line of *kilishi* production chain and resultant critical control points should be treated with outmost caution and hygiene.