OCCURRENCE OF *LISTERIA* SPECIES AND DETECTION OF LEAD AND CADMIUM IN FROZEN, SMOKED AND CANNED FISH IN KADUNA NORTH LOCAL GOVERNMENT, NIGERIA

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF AMASTER OF SCIENCE DEGREE IN VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE,

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MAY, 2017

DECLARATION

I declare that the work in this thesis, entitled "**OCCURRENCE OF *LISTERIA* SPECIES AND DETECTION OF LEAD AND CADMIUM IN FROZEN, SMOKED AND CANNED FISH IN KADUNA NORTH LOCAL GOVERNMENT, NIGERIA"** was performed by me in the Department of Veterinary Public Health and Preventive Medicine, under the supervision of Prof. M. Bello and Prof. J.K.P. Kwaga.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any Institution.

Musa Alhaji **MUSA**	……..	……
Name of Student	Signature	Date

CERTIFICATION

This project thesis entitled "**OCCURRENCE OF *LISTERIA* SPECIES AND DETECTION OF LEAD AND CADMIUM IN FROZEN, SMOKED AND CANNED FISH IN KADUNA NORTH LOCAL GOVERNMENT, NIGERIA"** carried out by MUSA ALHAJI MUSA meets the regulations governing the award of the degree of Master of Science of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to the Almighty God, to my parents, Family and Lawan Babale Gashu'a.

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Abstract

TheOccurrence of *Listeria* species and detection of Lead and Cadmium metals in frozen, smoked and canned fish was investigated in Kaduna North Local Government Area of Nigeria. A total number of 180 fish samples comprising of 60 frozen, 60 smoked and 60 canned, were purchased from the fish vendors at the three major markets in Kaduna North L.G.A (Kawo Market, Abubakar Gumi market and Unguwar Rimi market). Sampling was done once weekly. *Listeria* species were isolated using pre-enrichment, selective medium and were identified by conventional biochemical tests and confirmed with Microbact ID-System. Heavy metal analysis was done using wet digestion method. Levels of lead and cadmium in digested fish samples were determined using Atomic Absorption Spectrophotometer (AAS). Results show that from the 180 samples, 24 (13.3%) were found to be contaminated with *Listeria* spp. Out of the 24 isolates, Microbact 12L –ID system showed that 9(37.5%) were *L.grayi* and 15(62.5%) *L. ivanovii* isolates respectively. Distribution of the occurrence of *Listeria* spp by sample type showed that smoked fish had the highest occurrence of 19 (31.7%) when compared with frozen fish which had 5 (8.3%) whereas canned fish had 0 (0%). Distribution of *Listeria* isolates by location showed that Unguwar Rimi market had the highest 15(25.0%) followed by Kawo market 5(8.3%) and the least contaminated was Abubakar Gumi market 4(6.6%). Antibiotic susceptibility testing showed that*Listeria* sppisolates were susceptible to gentamicin (the first choice antibiotic) used in the treatment of listeriosis. The sensitivity regimens were 24(100%) for Gentamicin as the most effective, followed by 22(92%) for Chloramphenicol, 21(88.0%) for Amoxicillin and 19(79%) for Oxacillin. Antimicrobial resistance pattern Erythromycin (ER) occurred in 5 of the 24*Listeria* species isolates. The distribution of maximum residue limit of the metals were cadmium 35(58.3%) in smoked fish, 23(38.3%) in frozen and 20(33.3%) in canned fish. Lead (Pb) had 47(78.3%) in smoked fish, 36(60%) in frozen fish and 42 (70%) in canned

fish were above safe limit. There wasan association between occurrence of *Listeria* species and Lead and Cadmium levels in the fish sampled at $p \leq 0.05$. The findings of this work showed a potential threat to health and safety of the public. It is therefore recommended that good hygienic practices be implemented during fish processing and handling. Additionally, use of antibiotics in both human and veterinary medicine should be appropriately monitored.

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ABBREVIATIONS

%	Percentage
µg	Microgram
^{0}C	Degree Celsius
AAS	Atomic Absorption Spectrometer
ABU	Ahmadu Bello University
AFLP	Amplified fragment length polymorphism
AIDS	Acquired Immune Deficiency Syndrome
Ar	Arsenic
ATSDR	Agency for Toxic Substances and Disease Registry
Aw	Water activity
Ca	Calcium
CAC	Codex Alimentarius Commission
Cd	Cadmium
CDC	Centers for Disease Control and Prevention
-COOH	Carboxyl group
Cr	Chromium
Cu	Copper
DNA	Deoxyribonucleic acid
EU	European Union
FAO/WHO	Food and Agriculture Organization / World Health Organization of United Nations
FDA	Food and Drug Administration

FSIS	Food Safety and Inspection Service
HACCP	Hazard Analysis and Critical Control Point
Hg	Mercury
HIV	Human Immunodeficiency Virus
HNO_3	Nitric acid
$HCLO_4$	Perchloric acid
H_2SO_4	Sulphuric acid
ISO	International Organization for Standardization
LEB	*Listeria* Enrichment Broth
LGAs	Local Government Areas
LLO	Listeriolysin
MAFF	Ministry of Agriculture, Fisheries and Food
MHA	Mueller-Hinton Agar
ML	Milliliters
Nacl	Sodium Chloride
$-NH_2$	Amino group
PAHO	Pan American Health Organization
Pb	Lead
PCR	Polymerase chain reaction
PFGE	Pulsed-Field Gel Electrophoresis
RTE	Ready -to- Eat
SPP	Species
SPSS	Statistical Package for Social Science

TSB	Tryptose Soy Broth
USDA-FSIS	United States Department of Agriculture – Food Safety and Inspection Service
Zn	Zinc

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Food safety is a major public health concern worldwide. During the last decades, the increasing demand for fish consumption has been recommended because it is a good source of high quality protein, minerals, vitamins and omega-3 poly unsaturated fatty acids (FAO, 2014). The later composite protects consumers against coronary heart disease, reducing arrhythmias and thrombosis and risk of fatal heart attack (FAO, 2014). The intake of fish is beneficial to children's growth and development and acts against some diseases such as rheumatoid arthritis, psychiatric disorders and lung diseases (WHO, 2016).

Beside good health benefits of fish, there are many reports about fish contamination by microbes and chemicals in the environment (Habib *et al.*, 2009). Fish is soft and easily damaged; therefore rough handling and bruising results in contamination of fish flesh. Fish will become unfit for human consumption within one day of capture, unless it is subjected to some form of processing or preservation. Even after the fish has been processed, particularly if traditional methods have been used, the fish is still subject to many forms of loss and spoilage (Shewan, 2000). Fish, because of their soft tissues and aquatic environment are extremely susceptible to microbial contamination(USDA, 2008). Millions of bacteria, many of them potential spoilers, are present in the surface slime, on the gills and in the intestines of live fish, although the flesh itself is normally sterile(Center for Food Safety, 2014).Bacterial growth and invasion of the fish are prevented by the body's natural defense system during life but after death the defense system breaks down and the bacteria multiply and invade the flesh (Center for Food Safety, 2014).

Immediately a fish dies, it remains in first class quality only for two hours (Clucas and Ward, 1996). However, spoilage immediately sets in which is occasioned by an increase in the ambient temperature that triggers favourable conditions (pH) for microorganisms to thrive(kris-Etherton *et.al*, 2003).

The Gram-positive bacterium *Listeria monocytogenes* was probably first recognized in two rabbits in Sweden 1910(Murray *et al.*, 1926). Over a decade later, Murray *et al.* (1926) in the United Kingdom and Pirie (1927) in South Africa recognized a disease in laboratory rabbits, guinea- pigs and gerbils caused by a Gram-positive bacillus. Recognition of *L. monocytogenes* as a significant food borne pathogen emerged only in the early 1980s, with demonstration of food borne listeriosis outbreak (Schlecht *et al*., 1983). *L. monocytogenes* is widely distributed in the environment and occurs in almost all food raw materials from time to time (karunasagar, 2000).

The disease listeriosis usually occurs in high-risk groups, including pregnant women, neonates and immunocompromised adults, but may occasionally occur in persons who have no predisposing underlying condition(Rocourt *et al.*, 2001). Listeriosis is one of the most severe food borne infections, with low morbidity but high mortality of up to 30% (Rocourt *et al.*, 2001). *L. monocytogenes* is able to multiply in high salt concentrations even at refrigeration temperatures with or without oxygen. It is resistant to diverse environmental conditions and it can survive in industrial environments for years regardless of cleaning procedures (Rocourt *et al.*, 2001; Hoffman *et al.*, 2003). *L. monocytogenes* is ubiquitous in nature and therefore also aquatic creatures are potential bacterial sources. Apart from seafood products which undergo various processing steps that inactivate the bacterium, if present in the raw product, *L. monocytogenes* cross-contamination of products, presents a major problem especially for ready-to-eat products (Thimothe *et al.*, 2004). Furthermore, there are seafood products that are eaten

raw, without any listericidal step, such as cold-smoked and cold-salted fish. A lot of work have been done to study the sources of *L. monocytogenes* as well as means to control its growth and contamination in different food sectors (Chasseignaux *et al.,* 2002; Pak *et al*. 2002; Gudbjörnsdóttir *et al.,* 2004; Thimothe *et al.,* 2004). In addition to contamination by *Listeria*,there are certain heavy metals that contaminate water such as lead, cadmium, mercury and copper, which in turn may contaminate marine species (Olalade *et al*., 2011).

The contamination of water bodies with a wide range of pollutants has become a matter of great public health concern. In many countries including Nigeria, industrial wastes, agricultural run offs and burning of fossil fuels create a potential source of heavy metal pollution in the aquatic environment (Oluwa *et al*., 2010).

Discharge of heavy metals into rivers or any aquatic environment can change both aquatic species diversity and ecosystems due to their toxic and accumulative behavior (Heath, 1987; Allen, 1995). Aquatic organisms such as fish and shell fish accumulate metals to concentrations many times higher than present in water or sediment (Olaifa, 2004; Gungum, 1994). They can take up different metals and then concentrate them in their different body organs and tissues (Khare, 2002).

Humans exposed to heavy metals through the consumption of contaminated fish have developed various biochemical disorders (Wagboje *et al.,* 2001; Omoniyi, 2004; Waqar, 2006). Heavy metals may enter fish bodies through three possible ways, the body surface, the gill and the digestive tract (Javed, 2005). The heavy metals of the most widespread concern to human health are Lead, Copper, Mercury and Cadmium (Kris Etherton *et al.,* 2003; Chen *et al.,* 2007; Din *et al*., 2008; Bhouri *et al.,* 2010).

Heavy metals are recognized as toxic substances due to their low rate of elimination from the consumer's body; either man or animals (WHO, 1992; Harris, 2007; Wafaa *et al.,* 2003). When ingested in excess amounts, heavy metals combine with the body's bio-molecules like proteins and enzymes to form stable bio-toxic compounds, thereby damaging their structures and hindering them from bio-reactions of their functions (Duribe *et al.,* 2007).

The World Health Organization (WHO)/Food and Agriculture Organization (FAO) have determined a maximum tolerable weekly intake of 7µg Cd/kg of body weight (USFDA, 1993).The World Health Organization (WHO) have determined a maximum tolerable daily intake of 1.00µg/g for Cd and 2.00µg/g for Pb (WHO, 1989; 1993). The European Union's (EU) maximum residue limit (MRL) permitted in fish is 0.20µg/g for Lead (Pb), and 0.05µg/g for Cadmuim (Cd) (E.C, 2005; Ozturk *et al.,* 2009). Data from European Food Safety Authority (EFSA) have related exposure to Cd and Pb to effects like neurotoxicity, nephrotoxicity, carcinogenicity, endocrine and reproductive failures in adults (Herreros *et al.,* 2008).

1.2 Statement of Research Problem

Listeria species has been isolated from river water and river sediment (Svanevik *et.al*. 2015), channels, ponds and lakes (Dijkstra, 1982;Svanevik *et.al*., 2015), as well as from sea water and water shorelines (Colburn *et al*., 1990). Because of that, it can be expected that *Listeria* speciescan be present in water organisms (Karunasagar and Karunasagar, 2000). Data accumulated during the last decade showed that there are foods with different and various risk levels as listeriosis vehicles (Rocourt *et al.,* 2000). Among fish, fish products and sea products, there is some high risk foods such as: molluscs (fresh and frozen shellfish, crustaceans – in the shell or de-shelled), fresh fish (that is intended to be consumed without thermal treatment), fish

products in brine (salted, marinated, fermented, cold smoked fish and fish in self sauce), mid-heat treated fish and crustacean products (cooking, hot smoking including pre-cooking and panning) (Rocourt *et al.*, 2000). Currently, *L. monocytogenes* infection is an important public health concern because of the high mortality rate (20% to 30%)worldwide in immuno-compromised individuals and the severity of the disease, which is often associated with septicaemia, meningitis, gastroenteritis, pneumonia, and spontaneous abortion (Jemmi and Stephan, 2006).

Frozen fish, smoked and canned fish have been regarded as major sources of protein in most developed and developing countries (FAO, 2014). It is therefore, imperative to investigate the quality and possible health implications, associated with the consumption of this class of protein in our society.

Heavy metals acquired through the food chain as a result of pollution are potential chemical hazards, threatening consumers. At trace levels, some heavy metals such as copper, cobalt, zinc, iron and manganese are essential for enzymatic activity and many biological processes. Other metals, such as cadmium, mercury, and lead have no known essential role in living organisms, and are toxic at high concentrations (Bryan, 1976; Bhouri *et al*., 2010).

Waqar (2006) suggested that, over one billion people are currently exposed to elevated concentrations of toxic metals and metalloids in the environment and several million people may be suffering from sub clinical metal poisoning.

Canned fish is widely consumed in many parts of the world because of its low saturated fat and omega fatty acids that support good health (Ikem and Egiebor, 2005). Canned fish such as sardines and geisha are packed in air-tight tin or steel can containers for distribution, storage or preservation. High levels of metals may be found in canned foods due to corrosion and leaching

of the metals from cans, or from tin foils used in packaging. These canned containers have a high potential of releasing metals into the foods. Chen *et al.* (2001) reported that heavy metals are widely recognized for their detrimental effects on human health. They may, also act as allergens, mutagens or carcinogens (Goyer, 1992).

In a study in Nigeria, Christopher *et al.* (2009) reported the overall concentration found in *Oreochromis niloticus*from Henshaw town beach market in Calabar is within acceptable limit for human health.

Because of its relatively low prices, a large sector of people in Kaduna North depend on fish as a source of protein which are imported from different geographical regions around the world. Since diet is the main route of exposure to heavy metals, and fish represent a crucial part of human diet, it is not surprising that polluted fish could be dangerous dietary sources of certain toxic heavy metals (Iwegbue, 2015).

1.3 Justification

Nigeria has been ranked as the highest importer of frozen fish in Africa, importing 740,000 tons of frozen fish annually (Jatto *etal*., 2013). Despite of being the highest importer of frozen fish in Africa, little or no attention is focused on its public health implications. Increased in environmental pollution problem where waste are indiscriminately discharged in aquatic environment can contain both infectious and non-infectious agents. Finding heavy metals or infectious agents can disqualify fish for human consumption. People trade in fish and the consumption of fish in the study area is habitual and thus, the need to assess the levels of some heavy metals and *Listeria* in the fish handled and consumed within the study area as it relates to public health. Currently, ongoing research seemed to have focused mainly on local fish, poultry

and dairy industry, with little attention being paid to imported fish. As such, the result of this study may provide baseline information that will guide public health policy development for the Nigerian fish industry.

Generally, there is limited information to the best of our knowledge about the extent of *Listeria* species contamination and the levels of heavy metals in frozen, smoked and canned fish supplied in the study area.

1.4. Aim of the Study

To determine the microbial contamination of frozen, smokedand canned fish by *Listeria* species and levels of lead and cadmiumin Kaduna North Local Government Area, Nigeria.

1.5 Objectives of the Study

i. To determine the microbial contamination with *Listeria* species of some frozen, smoked and canned fish sold in Kaduna North Local Government.
ii. To determine the levels of heavy metals (Lead and Cadmium) contaminants in the muscles of some frozen, smoked andcanned fish available in Kaduna North Local Government.
iii. To determine the association between the levels of lead, cadmium and occurrence of *Listeria* speciesin frozen, smoked and canned fish.

1.6 Research Questions

i. Is *Listeria* species present in some frozen, smoked and canned fish sold in Kaduna North?

ii. Are there Lead and Cadmium in the muscles of some frozen, canned and smoked fish in Kaduna North Local Government Area?

iii. Is there any association between the occurrence of *Listeria* speciesand the levels of Lead and cadmium in frozen, smoked and canned fish?

CHAPTER TWO

LITERATURE REVIEW

2.1Description of *Listeria*Specie

L. monocytogenes is a small (0.5 µm in diameter and 1-2 µm in length) organism, with cells that are found either singly, in short chains, arranged in V and Y forms or in palisades(Zhu *et al.*, 2005). Sometimes cells are coccoid and about 0.5 µm in diameter, colonies on blood agar are non-pigmented and may resemble those of β-haemolytic *Streptococcus*and occasionally appearing filamentous (Jacquet *et al.,* 2002). *Listeria* species are short, gram positive rods, catalase positive, oxidase negative and ferment carbohydrates (Beaufort *et al.,* 2007; Calo-Mata *et al.,* 2008). Among food isolates, serotype 1/2 is the most frequently found (Farber and Peterkin, 1991; Jacquet *et al.,* 2002).

Members of the genus are facultative anaerobes, non-sporing, non-acid fast bacteria and do not possess a capsule (Zhang*et al.,* 2007). *Listeria* species are motile by peritrichous flagella when grown at 20- 25°C and display a motility. The optimum growth temperature (but not for motility) is 30-37°C (Rocourt, 1999). *L. monocytogenes* is a ubiquitous gram-positive, non-spore forming, aerobic to facultative anaerobic psychotropic bacteria with low guanine ~plus~ cytosine (G+C) content of its DNA (Monk *et al.,* 2008). *L. monocytogenes* occurs naturally in fresh and salt

water, as well as in terrestrial environment such as livestock manure, decaying plant materials and in many raw foods associated with these environments (Gram, 2001; Zhang*et al.,* 2009; Ieren *et al.*, 2013).L. *monocytogenes*, the causative agent of listeriosis, was discovered more than 70 years ago by E.G.D. Murray and J. Pirie, who were working independently of each other (Rocourt, 1999). The first documented human listeriosis case involved a soldier who suffered from meningitis at the end of World War I (McLauchlin, 1997). Typical physiological characteristics of *L. monocytogenes* include a high tolerance to moderate and high levels of NaCl (up to 28% w/v) for short periods and resistance to freezing temperatures. Therefore, this organism can grow and multiply during refrigeration (4^{o}C), whereas, other competing organisms cannot, and it can also able to survive at relatively low water activity (Zhang*et al.,* 2005; Calo-Mata *et al.*, 2008)

2.2 Classification of *Listeria*Species

The genus *Listeria* belongs to the *Clostridium* sub branch, together with *Staphylococcus*, *Streptococcus*, *Lactobacillus*, and *Bronchothrix*. This phylogenetic position of *Listeria* is consistent with its low G+C DNA content (36^ 42%). The bacterial genus *Listeria* currently comprises 10 species; *Listeria monocytogenes*, *Listeria ivanovii*, *Listeria seeligeri*, *Listeria innocua*, *Listeria welshimeri*, *Listeria grayi*, *Listeria marthii*, *Listeria rocourtiae*, *Listeria fleischannii* and *Listeria welhenstephanensis* (Zhang *et al.*, 2007; Halter *et al.*, 2012). Among these species, only *L. monocytogenes* and *L. ivanovii* are pathogenic, while the other *Listeria* species are non-pathogenic (Liu, 2006). Human cases of listeriosis were caused almost exclusively by the species *L. monocytogenes* (European Food Safety Authority, 2013).

2.3 *Listeriosis* in Man and Animals

The term *listeriosis* encompasses a wide variety of disease symptoms that are similar in animals and humans. *Listeria monocytogenes* causes listeriosis in animals and humans. *L. ivanovii* has long been considered a ruminant pathogen with few reported cases of human infection (Cummings *et al*., 1994). However, it has recently been proposed that *L. ivanovii* is an opportunistic enteric human pathogen (Guillet *et al.,* 2010). Non involvement of central nervous system may be possibly a general characteristic of *L. ivanovii* infection regardless of host species(Guillet *et al.,* 2010).Encephalitis is the most common form of the disease in ruminant animals. In young animals, visceral or septicemic infections often occur(Guillet *et al.,* 2010). Intra-uterine infection of the foetus via the placenta frequently results in abortion in sheep and cattle (Doyle and Schoeni, 1986).

The incidence of listeriosis in humans is not known, because in the average healthy adult, infections are usually asymptomatic, or at most produce a mild influenza-like disease. Clinical features range from mild influenza-like symptoms to meningitis and/or meningo-encephalitis. Illness is most likely to occur in pregnant women, neonates, the elderly and immuno-compromised patients; however, apparently healthy individuals may also be affected. In pregnant women, the most usual symptom is a mild influenza-like illness without meningitis, while infection of the foetus is extremely common and can lead to abortion, stillbirth, or delivery of an acutely ill infant (David *et al.,* 1994).

In humans, overt listeriosis following infection with *L. monocytogenes* is usually sporadic, but outbreaks of epidemic proportions have occurred. In 1981, there was an outbreak that involved over 100 people in Canada. Thirty-four of the infections occurred in pregnant women, among whom there were 9 stillbirths, 23 infants born infected, and 2 live healthy births. Among 77 non-

pregnant adults who developed overt disease, there was nearly 30% mortality. The source of the outbreak was coleslaw produced by a local manufacturer (David *et al.,* 1994). In 1985, 142 people developed listeriosis in California. Of these, 93 cases were prenatal, and among the 49 cases that were in non-pregnant individuals, 48 were immuno-compromised. Thirty newborn infants and 18 adults were recorded death. The source of the infection was a certain brand of "pasteurized" soft cheese that apparently got contaminated with unpasteurized (raw) milk during the manufacturing process (Liu, 2006). However, a multistate outbreak of *Listeria monocytogenes* infections with 46 culture-confirmed cases, 7 deaths and 3 stillbirths or miscarriages in 8 states was linked to eating sliced turkey deli meat, suggesting that the plant might be the source of the outbreak (Liu, 2006).

Unlike most of the other foodborne pathogens, *Listeriamonocytogenes* can grow at refrigeration temperatures. In addition, *Listeria* is widely distributed in nature, and the organism has been recovered from farm fields, vegetables, animals and other environments such as food processing facilities, retail stores and home, kitchens, vegetables, raw milk and ready to-eat foods (Ieren *et al.,* 2013; Ndahi *et al.,* 2013).

2.4 Epidemiological Importance of *L. monocytogenes*

In the early 1980s, food was recognized as a primary source of *L. monocytogenes* infection in humans (Farber and Peterkin, 1991; Latorre *et al.,* 2007). In Nigeria and other developing countries, *L.monocytogenes* is one of the most important causes of death from food borne infections (Jemmi and Stephan, 2006). Several food borne listeriosis outbreaks have occurred in countries such as the United States of America, Japan, New Zealand, Germany, England and France over the past 2 decades, and although *L. monocytogenes* has been observed in a large

variety of foods, the greatest threat of this pathogen is associated with refrigerated products that have a long shelf life and products that are generally eaten with little or no prior heating (Bremer *et al.,* 2003). Seafood is the first among these high risk, ready-to-eat (RTE) products that were contaminated with *Listeria* species (Roberts*et al.,* 2003).

The epidemiological patterns of human listeriosis include a background level of sporadic cases with occasional outbreaks (Gillespie *et al.,* 2010). Notably, reports from European Food Safety Authorityindicate that fish and shellfish products are frequently contaminated with L. *monocytogenes*. However, compared to listeriosis outbreaks associated with other foods, a low number of cases have been linked to seafood (European Food Safety Authority, 2013), potentially due to the following factors:

1) The low numbers of *L. monocytogenes* generally present in seafood,

2) The relatively low volumes of products and the nature of the distribution chain,

3) Increased consumer awareness that seafood must be chilled compared to other products,

4) reduced consumption of raw fish,

5) Decreased epidemiological tracking efficiency due to long incubation periods after the ingestion of contaminated food,

6) Decreased consumption of high-risk products, such as RTE seafood, by immune-compromised individuals.

2.4.1 Distribution of *listeria* in Nigeria

The first case of human listeriosis in Nigeria was recorded in the northern part of the country (Onyemelukwe *et al.,* 1983). Few sporadic cases of listeriosis have also been reported on smoked

fish in Jos, Nigeria (Chukwu *et al.,* 2006). Various food surveys conducted in Nigeria have reported on the detection of *L. monocytogenes* in different food products, including raw milk, smoked fish (Salihu *et al.,* 2008), beef, pork, goat meat, poultry, fish and vegetables (Ikeh *et al.,* 2010; Ieren *et al.,* 2013; Ndahi *et al.,* 2013; Shinkafi and Ukwaja, 2010).

2.4.2 *Listeria*in water, aquatic food products and processing Environments.

Although *L. monocytogenes* is not considered a marine organism, it can be isolated from marine water (most likely due to waterrun-off from the land), vegetation, sewage, animal feeds, farm environments, and food-processing locations (Embarek, 1994; Gram, 2001). Therefore, it is very likely that fish, fish-processing environments, and final seafood products are contaminated *Listeria*species (Gram, 2001).

Water run-off, particularly water that has passed through agricultural areas, is proposed to serve as the main route for the influx of *L. monocytogenes* into surface waters such as lakes, streams, and rivers (Gram, 2001; Lyautey *et al.,* 2007). Other sources of contamination, such as animal faeces, may also increase the abundance of the pathogen in aquatic systems because animals such as ruminants shed *L. monocytogenes* in their faeces (Hutchison *et al.,* 2004; Lyautey *et al.,* 2007). Lyautey *et al.* (2007) observed a significant direct link between the number of dairy farms in a catchment and increased *Listeria* prevalence in surface waters. Upstream proximity to a dairy plant was observed as another risk factor, and the majority of *L.monocytogenes*-positive water samples were located within a few kilometers downstream of a dairy plant. The researchers concluded that agricultural activities, such as the use of livestock manure followed by farming activities, affect (increase) the rateof *Listeria* prevalence in surface waters (Lyautey *et al.,* 2007).

Climate conditions have a potential effect on the prevalence of *Listeriaspp*., particularly *L.monocytogenes*. Among different climate conditions, rainfall is associated with the largest increase in bacterial contamination of surface waters (Mallin *et al.*, 2009; Stump *et al.*, 2010). Therefore, seafood farms have potentially greater risk of *Listeria* contamination because brooks, rivers, and other surface waters enter such farms after heavy rainfall (Miettinen and Wirtanen, 2006). Miettinen and Wirtanen, (2006) speculated that both soil borne *Listeria* and *L. monocytogenes* commonly present in livestock manure are simply washed into rivers and surface waters upstream of the fish farms, which is consistent with the link between surface-water contamination and farming activity.

Although studies have observed *L. monocytogenes* in freshwater ecosystems, El-Shenawy and El-Shenawy (2006) examined the *L. monocytogenes* contamination in fish farmed in freshwater and those with a partial or complete life cycle in seawater. The authors observed a correlation between the rates of contamination of L. *monocytogenes* in the Agba and Suez Gulf and in the Red Sea and a trend toward higher contamination levels in urban areas or areas with industrial/tourism activities. These observations suggested that the discharge of untreated sewage into seawater was involved in contamination of fish with L.*monocytogenes* (El-Shenawy and El-Shenawy, 2006). *Listeria* is component of the indigenous micro flora in surface water and other water bodies connected to river. Therefore, these microorganisms are most likely present on the external surface of fish that swim in contaminated water. *L. monocytogenes* has been observed on fish surfaces and in the stomach lining, gills, and intestines, but the flesh is usually free of the organism unless it has been contaminated from different sources (Miettinen and Wirtanen, 2005). There are 2 possible routes for contamination of fish with *Listeria*:

(1) The spread of *Listeria* from the intestinal contents to other fish tissues (including muscles), especially if the period between death and viscera removal takes a few more hours than the expected time;

(2) Cross-contamination (due to manipulation of fish using contaminated equipment and inappropriate transport means) (Gudmundsdottir *et al.,* 2006; Souza *et al.,* 2008). Therefore, contaminated raw materials can also affect the final products, especially RTE-seafood products (Miettinen and Wirtanen, 2005).

Jemmi and Keusch, (1994) studied the water resource and management practices of 2 rainbow trout farms; in their study, *L. monocytogenes* was not observed in 30 fish skin samples or 30 faecal content samples from the farm using concrete-walled ponds and hygienic management practices. In contrast, in the farm that used river water and naturally (earth) walled ponds, 33.33% and 40% of skin and faecal samples, respectively, contained *L. monocytogenes* (Jemmi and Keusch, 1994).

The location of *Listeria* in fish carcasses and the place of sampling are considered to affect *Listeria* screening results. Several studies (Al-Sheikh *et al.* (2012) reported 13.6% in Egypt; 12.34% by Kuzmanovic Jelena *et al.* (2011)in Belgrade, Serbia; 16.5% in Taiwan by Wang *et al.* (2012) and Salihu *et al.* (2008) reported 25% on smoked fish in Sokoto, Nigeria)have used samples from the fish surface to generate prevalence data; however, this approach may not always yield accurate results. When fish is contaminated with low number of *L.monocytogenes*, or when contamination is sporadic, selective sampling of the gills yields more accurate results than sampling of the skin (Miettinen and Wirtanen, 2005).

2.5 Lightly Preserved Seafood Products (LPSPs)

Lightly preserved seafood constitute a broad group of chilled, stored RTE foods with a pH > 5.0 and <6% NaCl in the water phase of the product (Lyhs *et al.,* 2002). Over the last 2 decades, *L. monocytogenes* has been frequently isolated from RTE foods and LPSPs, including cold and hot-smoked salmon, gravid salmon, fermented fish, and fish salads. This observation highlights the high risk of transmission of *Listeria* specie. Smoked seafood products are among the RTE foods reported as potential carriers of *L. monocytogenes* (Basti *et al.,* 2006).

Several factors result in contamination of smoked seafood products these include:

(a) the relatively high prevalence of *Listeria* specie immediately after final packaging;

(b) the ability of the pathogen to grow on smoked fish;

(c) Unhygienic production process providing multiple opportunities for contamination or recontamination.

Because *L. monocytogenes* is a significant food safety concern, the prevalence of *L. monocytogenes* in smoked fish products in Europe has been extensively studied(European Food Safety Authority, 2013). The presence of *L. monocytogenes* in cold-smoked salmon has been examined by legislators, researchers, and food-control agencies(Miettinen and Wirtanen, 2005; European Food Safety Authority, 2013). The overall reported prevalence rate for *L. monocytogenes* in cold-smoked salmon is approximately 10% (Rorvik and Yndestad 1991; Vogel *et al.,* 2001; Gram 2001; Gonz´alez-Rodríguez *et al.,* 2002; Azevedo *et al.,* 2005; Gudmundsdottir *et al.,* 2005; Miettinen and Wirtanen, 2006); however,researchesin Navarra (Spain) by Vitas *et al.* (2004) andin Sokoto (Nigeria) by Salihu *et al.* (2008). Observed a much higher prevalence (28% and 25% respectively). The overall rate is consistent with studies (with reported incidence levels between 5% and 35%) in specific European Union

member states (European Food Safety Authority, 2013). Different fish species and seafood products have different intrinsic characteristics (pH, water activity and salt concentration) that affect the presence and resistance of *L. monocytogenes* (Uyttendaele *et al.,* 2009) and these characteristics might underlie the differences in prevalence. The level of contamination in smoked fish is low (<10 CFU/g), but retail samples might occasionally contain viable bacterial counts ranging from 104 to 106 CFU/g (European Food Safety Authority, 2013).

2.6 Ready to Eat (RTE) Food.

RTE refrigerated food products are ready for consumption without additional treatment or cooking. Examples of RTE refrigerated foods include seafood salads, deli salads, soft cheeses, and pre packed fresh vegetables and fruits. The lack of any form of heating step before consumption necessitates hygienic preparation and appropriate storage conditions to ensure the safety of these foods throughout their shelf life. *L. monocytogenes* contamination is a major concern in refrigerated seafood salads. The presence of *L. monocytogenes* in these products may result from contaminated raw materials or from cross-contamination during processing, packaging or retail presentation (Little *et al.,* 2007). The safety of seafood salads can be ensured by a combination of refrigerated storage, preservative use, and addition of organic acids to decrease the pH of the final product. However, when the contamination involves a resistant/adapted pathogenic strain, this food-preservation strategy might be ineffective and thus compromise food safety (Foley *et al.,* 2005).

In processing environment, raw materials are the primary source of potential *L. monocytogenes* contamination in seafood products (Eklund *et al.,* 1995). The processing plant environment might also play an important role (Rorvik *et al.,* 1995, 2000; Reij and Aantrekker, 2004). Most seafood products undergo some form of heat treatment, which can eliminate *Listeria* before

consumption; therefore, if recommended hygiene regimes and good hygiene practices are implemented, cross-contamination can be avoided, and contaminated raw materials would not contaminate the finished products. Different areas within processing plants may have significantly different levels of *L. monocytogenes* contamination. There is a link between contamination and cross-contamination of seafood, especially RTE seafood products, during processing (Gudbjornsdottir *et al.,* 2004; Thimothe *et al.,* 2004; Gudmundsdottir *et al.,* 2005; Soultos *et al.,* 2007). It is extremely difficult to determine the specific source of environmental contamination in processing plants. Based on data from different types of RTE food-processing plants, knives, conveyor belts, and mainly drains and floors contained the highest levels of *Listeria* and *L. monocytogenes* (Rorvik *et al.,* 1997). Floors and drains are particularly difficult to clean and maintain *Listeria*-free.

Rorvik *et al.* (1997) observed that 33% of salmon smoke house plants were contaminated with *L.monocytogenes*, mainly because drains represent a niche of contamination. However, the authors did not observe a correlation between the contamination of the processing plants and the occurrence of *Listeria* in the final product. *Listeria* were present in 15.4% of the drains and in 5.1% of the employee contact surfaces (gloves and aprons) (2/39) but in none of the samples from food contact surfaces. Gudbjornsdottir *et al.* (2004) also observed that raw material and specific locations in a cold-smoked salmon processing plant, specifically the floors and drains, were potential sources of *L.monocytogenes* in final products.

Gudbjornsdottir *et al.* (2004) proposed that floor drains might contaminate equipment through aerosols or air currents. However, other studies did not observe *L. monocytogenes* in the drains of processing plants.

Molecular sub typing methods such as pulsed-field gel electrophoresis (PFGE) or PCR-based finger printing (for example, Rep-PCR, RAPD, and MLST) provide in-depth information on the routes of contamination of *L. monocytogenes* (Chen *et al.,* 2010; Jadhav *et al.,* 2012; Zunabovic *et al.,* 2012; Schoder *et al.,* 2014). The transmission of the bacterium through the primary production areas is not well understood; however, this pathway is important to identify the etiology of contamination and outbreaks (Miettinen and Wirtanen, 2006). It is unclear whether the same subtypes are present in the raw materials, indoor environment during processing, and the final product. Few studies have observed the same subtype in the raw material and final product, indicating that raw material contamination is sometimes transmitted to the final product (Norton *et al.,* 2001; Nakamura *et al.,* 2006). By examining the steps between primary productions and processing to the final product, Miettinen and Wirtanen, (2006) detected 30 *L. monocytogenes* PFGE-pulsotypes in 15 Finland rainbow trout farms and fish processing plants. Most of the isolated strains were the same pulso-types as those from the processing area and the raw fish. Notably, only very few of the subtypes isolated in the indoor processing environment were detected in the final product, but a persisting subtype was observed in the final product (Norton *et al.,* 2001; Nakamura *et al.,* 2006). Almost every plant has an "in-house" subtype of *L. monocytogenes* (Rørvik *et al.,* 1995; Miettinen *et al.,* 1999; Norton *et al.,* 2001; Thimothe *et al.,* 2004), which may persist inside the facility for prolonged periods. The reason for persistence of those *L. monocytogenes* subtypes is unclear. The ability to form bio-films has been proposed as a crucial factor affecting the establishment of *L. monocytogenes* in seafood processing environments; furthermore, bio-film formation poses a challenge for bacterial elimination (Thimothe *et al.,* 2004). For example, *L. monocytogenes* can produce strong bio-films on plastic

surfaces (such as the conveyor belts used in fish factories), which is also observed with weak bio-film producing strains (Cerf *et al.,* 2010).

The large amount of water used during processing (as in hatcheries) is a crucial factor contributing to bio-film formation because the water quality is impaired by the detachment of bio-film-forming bacteria, irrespective of the type of water treatment (Cerf *et al.,* 2010). Hoffman *et al.* (2003) examined contamination by *L. monocytogenes* in a smoked fish-processing environment using Ribotyping analysis to distinguish between raw material and environment-associated contamination, and the authors concluded that environmental contamination independently introduced *L. monocytogenes* into seafood products. Furthermore, the automated ribotyping analysis revealed that raw fish contained a greater diversity of strains (16 of 46 isolates) compared to the processing environment (11 of 115 isolates), indicating separate bacterial populations (Hoffman *et al.,* 2003).

In principle, cleaning and sanitizing procedures can eliminate the pathogen from the processing line and equipment, but recontamination may occur after processing (Eklund *et al.,* 1995). The sporadically occurring subtypes are often sensitive to cleaning and disinfection procedures, but the strains belonging to persistent subtypes are not eliminated by such procedures (Cerf *et al.,* 2010). Eklund *et al.* (1995) observed that the external surfaces of fresh and frozen fish might act as inoculating agents that introduce *L. monocytogenes* into processing plants; this contamination is extended by further operations such as filleting, rinsing, and brining. Therefore, equipment, personnel, and surfaces might serve as secondary contamination sources of *L.monocytogenes*.

A survey of smoked salmon preparation practices during 16 site visits observed that only the largest companies (44%) evaluated their washing and cleaning practices to control *L. monocytogenes* contamination, and only 1 company periodically changed the sanitizers.

Furthermore, smaller companies even used household dishwashing detergents (Cerf *et al.*, 2010). Carpentier and Cerf (2011) reached important conclusions regarding the control of *Listeriaspp*. in manufacturing environments by avoiding water in sites of product exposure; this restriction is not easy during the processing of raw materials, but it may be possible for RTE food products after processing. Another important rule is to clean the floors surrounding the equipment to avoid contamination by bacteria dislodged from the floors(Hoffman *et al.,* 2003).

Furthermore, bacterial growth must be inhibited by temperature reduction, limitation of soiling, and drying. Secondary contamination during preparation in a processing line or at a retail store plays an important role in the contamination and/or recontamination of products. Furthermore, moist areas must be monitored carefully during processing because these areas frequently harbour *L.monocytogenes*, which may adapt and spread through the processing units(Hoffman *et al.,* 2003). It is important to consider factors such as appropriate design of food-processing equipment, detailed working instructions for employees, reasonable planned job rotations, and monitoring the effectiveness of cleaning and disinfecting procedures in the production facilities.

2.7 Specific Control Measures for Listeriosis

I. Industrial: Heat treatment with measures to ensure that processing contamination risks are reduced. For ready-to-eat, high-risk processed foods, reduction of all cross-contamination risks after processing; thermal processing, good hygiene practices during production and processing.

II. Food service establishment/household: Refrigeration of perishable foods and consumption within a short space of time. Pre-cooked refrigerated foods should be thoroughly reheated before consumption. Avoidance of certain high-risk foods e.g. sea foods, ready-to-eat meat such as pâté and raw products during pregnancy. Consumers, particularly pregnant women and other

vulnerable individuals should avoid eating raw foods of animal origin, such as cold, smoked or raw seafood, pâté.

2.7.1 Prevention/control of listeriosis.

The food industry and public health agencies play a pivotal role in the prevention of food-borne listeriosis by developing and implementing effective Hazard Analysis and Critical Control Point (HACCP) programmes to reduce the presence of *L. monocytogenes* at all critical points in the food production and distribution chain (from the farm to the market), (OIE Terrestrial Manual, 2014).

The following steps will help to reduce the risk of getting listeriosis and other food borne illnesses:

•Follow all food package labels and instructions about preparation and storage

•Set the refrigerator temperature to 4ºC (40ºF) or colder

•Refrigerate or freeze all perishable foods within two hours of purchase or preparation

•Separate raw meats, poultry and seafood, from produce and ready-to-eat (RTE) foods in your shopping cart and fridge

•Defrost food in the refrigerator, in cold water or in the microwave, never at room temperature

•Cook foods and reheat leftovers to an internal temperature of 74ºC (165ºF) or higher, Serve food right away

• Clean all knives, cutting boards, thermometers and utensils after contact with raw meats and poultry

• Thoroughly clean and sanitize all surfaces used for preparing food

• Wash produce (fruits and vegetables) well before eating

2.7.2 *Listeria* control plan

1. Sanitation and Good Manufacturing Practices (GMPs) e.g. (HACCP)

2. Training of Plant Personnel on personal hygiene

3. Plant Environmental Monitoring/Testing

4. Raw Material Controls

5. Minimize Growth in Finished Product, (National Food Safety Initiative Project, 2003).

However, Listeriosis can be treated with antibiotics. Early diagnosis is important, especially for pregnant women, older adults and people with weakened immune systems. Antibiotics given to pregnant women with listeriosis can often reduce the risk of infection in the newborn or the unborn child. There is no vaccine to prevent listeriosis. As with any disease causing diarrhea or vomiting, people infected should drink plenty of fluids to prevent dehydration. Likewise, the lack of well designed and tested vaccines for animal use, means that control of listeriosis in animals is most feasible by preventing the environmental conditions that favour its presentation (OIE Terrestrial Manual, 2014).

2.7.3 Bacterial resistance and tolerance

The word “resistance” has been widely used also for disinfectants. However, “resistance” should be used as a relative term, since disinfectants' efficacy related strongly to the testing method used. Strains that survive a concentration of disinfectant that kills the majority of strains of the same species could be defined as resistant (Langsrud *et al.,* 2003). As underlined in a previous paper (Cerf *et al.,* 2010; Carpentier and Cerf, 2011), the term “resistance” is a frequent cause of misunderstanding. So, it has been proposed that “resistance” should be preferred when the phenomenon being studied is killing and “tolerance” when it is the adaptation to inhibitory concentrations (Cerf *et al.,* 2010).

2.7.4 Adaptation of *Listeria* species to the environment.

Some of the longest known environmental adaptations of *L. monocytogenes* include those associated with tolerance to cadmium, lead, and arsenic. Such tolerance occurs with sufficient frequency to allow its use in subtyping applications (Lee *et al.,* 2013; Ratani *et al.,* 2012).

L. monocytogenes is a common dweller of soil, water and vegetables where it is normally exposed to heavy metals. Unlike antibiotics, metals are not subjected to degradation and can exert a long-term selective pressure (Ji *et al.,* 2012; Kolpin *et al.,* 2002; Stepanauskas *et al.,* 2006). Cadmium tolerance was more common among strains repeatedly isolated from sea foods than among those recovered sporadically (Harvey and Gilmour, 2001; Ratani *et al.,* 2012).

2.7.5 Survival of *Listeria* species in heavy metal contaminated environments.

Bacteria that demonstrate the capacity of surviving in toxic heavy metals concentrations have been isolated from different sources (Basu *et al*., 1997; Castro-Silva *et al*., 2003; Choudhury, 1998; Duxbury, 1986; Haefeli *et al*., 1984; Lima *et al*., 2007; Otth *et al*., 2005; Gilg, 2004; Mindlin *et al*., 2001). Many bacteria have specific genetic mechanisms of resistance to toxic metals (Silver, 1988; Foster, 1983).The presence of heavy metals lead to resistance in the environment (Gaulet *et al*., 1992). In the environment metals may select these resistant variants in a manner similar to the selection of antibiotic resistant strains. Indeed, it is relatively common the association of metal and antimicrobial resistance, since both resistance genes are frequently located on the same mobile genetic elements (Foster, 1983; McIntosh *et al*., 2008).

Microorganisms have developed mechanisms of resistance that lead to the selection of resistant varieties with the ability to tolerate metals (Verma *et al*., 2001). Malekzadeh *et al*. (1996) and Verma *et al*. (2001) reported on*L. monocytogenes* strainswhich did not show growth inhibition in

the presence of 1mM/L of cadmium (Cd), lead (PB), zinc (Zn), and copper (Cu). Kazem*i et al.* (2015) reported that unlike different concentrations of cadmium or mercury, which could be lethal for bacteria, different levels of lead did not generally have an effect on the growth of *L. monocytogenes.*

Consequently, it can be assumed that the selective pressure exerted by heavy metals contribute to the indirect co-selection of antibiotic resistance, particularly in environments contaminated with the two elements(Lima *et al*., 2012).

Lima *et al.* (2012) reported that, depending on the pollutant metal, structural characteristics of the cell wall may be one of the aspects determining the survival of microorganisms in contaminated environments.

2.8 Canning

Canning is a way of processing food to extend its shelflife. The idea is to make food available and edible long after the processing time. The "father of canning" is the Frenchman Nicolas Appert. In 1795, he began experimenting ways to preserve foodstuffs, placing food in sealed glass jars and then placing the jars in boiling water(Geoghegan, 2013). Appert devised a cheap and effective method of preserving large amounts of food. However, glass containers presented challenges of breakage. Shortly after, the British inventor and merchant Peter Durand patented his own method, this time in a tin can, creating the modern-day process of canning foods (Bryan, 1953).

A 1997 study found that canned fruits and vegetables are as rich with dietary fiber and vitamins as the same corresponding fresh or frozen foods, and in some cases the canned products were richer than their fresh or frozen counterparts (Petrick, 2008). The heating process

during canning appears to make dietary fiber more soluble, and therefore more readily fermented in the colon into gases and physiologically active by-products (Petrick, 2008).

2.8.1 Canned fish

Canned fish are fish which have been processed, sealed in an airtight container such as a sealed tin can. Canning is a method of preserving food, and provides a typical shelf life at room temperature ranging from one to five years (Huss, 1988). Fish have a low acidity at levels where microbes can flourish. From a public safety point of view, foods with low acidity (PH> 4.6) need sterilization under high temperature (116-130 °C). To achieve temperatures above the boiling point requires a method of pressurized cooking which is provided by the containment within the can (Huss, 1988). After sterilization, the container can prevent microorganisms from entering and proliferating inside. Other than sterilization, no method is perfectly dependable as a preservative. For example, the microorganism *Clostridium botulinum* (which causes botulism), can only be eliminated at temperatures above the boiling point. Such preservation techniques are needed to prevent fish spoilage and lengthen shelf life. They are designed to inhibit the activity of spoilage bacteria and the metabolic changes that result in the loss of fish quality. Spoilage bacteria are the specific bacteria that produce the unpleasant odours and flavours associated with spoiled fish (FAO, 2005).

2.8.2 Sardines

Sardines are nutrient-rich fish commonly consumed by humans. They are commonly served in cans, but fresh sardines are often grilled, pickled, or smoked. Sardines, or pilchards, are several types of small, oily fish related to herrings, family *Clupeidae* (EtymologyDictionary, 2012). The term *sardine* was first used in English during the early 15th century, and may come from the

Mediterranean island of Sardinia, around which sardines were once abundant(BBC Worldwide, 2009). The terms sardine and pilchard are not precise, and what is meant depends on the region. The United Kingdom's Sea Fish Industry Authority, for example, classifies sardines as young pilchards (Stummer, 2003). One criterion suggests fish shorter in length than 6 inches (15 cm) are sardines, and larger ones pilchards(Codex *Alimentarius*, 1995).The FAO/WHO Codex standard for canned sardines cites 21 species that may be classed as sardines (Codex *Alimentarius*, 1995). Good quality sardines should have the head and gills removed before packing (Food Standards Agency, 2004). They may also be eviscerated before packing (typically the larger varieties). If not, they should be purged of undigested or partially digested food or faeces by holding the live fish in a tank long enough for them to empty their digestive systems (Food Standards Agency, 2004).

2.9 Heavy Metals

"Heavy metals" are terms given to the group of metals or metalloids with high relative density of $5g/cm^3$ or higher, often toxic to organisms (Dufus, 2002). Heavy metals are present in industrial, agricultural, municipal, and urban runoff, and they continuously accumulate in various environmental media (Qu *et al.* 2012; 2015). They have received attention as environmental contaminants, potential toxicological hazards, economic, social and political issues (Ezemonye and Enuneku, 2005).

2.9.1 Mechanisms of action

(a) Inhibition of Enzymatic Activities: This is so because some metals such as Pb, Hg and Cd have affinity for sulphur and therefore attack sulphur bonds in enzyme, thus immobilizing them.

Other sites of attack include the free amino (-NH2) and carboxyl (-COOH) groups in protein (Tucker, 2007).

(b) Attacks on Cell Membrane and Receptor: The heavy metals bind to cell membrane and receptor, thereby altering their structures. This affect transport and other inter or intra cellular processes in the body. Cadmium inhibits oxidative phosphorylation in the body (Tucker, 2007).

(c) Interference with Metabolic Cations: Heavy metals interfere with the metabolism of essential cations such as absorption, transportation, decomposition and storage. Cd follows the pathway of Zn and Cu metabolisms. Pb replaces Ca in bones (Tucker, 2007).

(d) Action on the Artery: Heavy metals can increase the acidity of the blood. The body draws Ca from the bones to help restore blood pH. Toxic metals set up conditions that lead to inflammation in arteries and tissues, causing more Ca to be drawn to the area as a buffer. The Ca coats the inflamed area in the blood vessel and creating another by the hardening of the artery walls and its progressive blockage of the arteries. This leads to osteoporosis (Tucker, 2007).

2.9.2 Cadmium (Cd)

Cadmium is a metal with no known beneficial properties that support life. There is no evidence that it is either biologically necessary or beneficial (Eisler, 1985; Eisler, 2000; Nordberg *et al.*, 2007). At low concentrations, it is toxic to all life, including plants, fish, birds, mammals (including humans), and microorganisms (Eisler, 1985; Eisler, 2000; Nordberg *et al.*, 2007; ATSDR, 2008). It causes cancer, birth defects and genetic mutations (Eisler, 2000, Nordberg *et al.*, 2007). In a study that used comparative acute toxicity testing of 63 heavy metals, cadmium was the most toxic metal (Borgmann *et al.*, 2005). In its elemental form, cadmium does not break

down but it can change form and compounds and can bind strongly to soil or sediment particles, depending primarily on the acidity of the surrounding water (ATSDR, 2008).

2.9.3 Cadmium behavior and toxicity

Cadmium and other metals released from mining sites can contaminate drinking and other water sources (Peplow and Edmonds, 2004). Metal contamination in aquatic system is of particular concern because metals are both persistent and toxic (Clark, 1992). Animals that accumulate cadmium in their bodies can be eaten by others, and so on; such that cadmium would both accumulate and bio magnify in the food chain (EPA, 2000). Fish can accumulate cadmium from the water and eating foods contaminated with cadmium (food). It is important to note that bioaccumulation and magnification occurred when a substance cannot be easily metabolized or excreted (ATSDR, 2008).

Bio accumulation occurs when an animal accumulates cadmium from the environment into its body. Bio magnification of cadmium occurs when the concentration of cadmium increases from one link in the food chain to another link in the food chain. A bigger fish eats multiple small fish and accumulates the cadmium from those fish in its body tissue. As that fish ages, it keeps accumulating the cadmium from all of the contaminated smaller fish it eats. The cadmium further accumulates or magnifies as a bigger fish eats that fish, and so on.

Cadmium would be acutely toxic when an animal dies from exposure to a high concentration over a short period of time. Cadmium would be chronically toxic when the animal is exposed to cadmium over a long period of time at a lower concentration (ATSDR, 2008). Chronic exposure can lead to mortality; sub lethal effects such as reduced growth and reproductive success are more common. Cadmium may also accumulate in aquatic biota chronically exposed to sub lethal concentrations (Schaider *et al.*, 2007).

Cadmium bio magnifies in the food chain and can accumulate in humans. For example, cadmium can enter freshwaters due to mining activities (Edmonds and Peplow, 2000) where it is taken up by algae, insects, and passed up the food chain to fish, which can accumulate it in their gills, liver and kidneys (Eisler, 1985; Nordberg *et al.*, 2007; ATSDR, 2008; Edmonds and Peplow, 2000). Humans accumulate cadmium primarily in the kidneys and liver, and its impacts are greatest to the lungs (from inhalation) and kidneys (from ingestion) (ATSDR, 2008).

The most likely source of cadmium toxicity in humans is contaminated foods (ATSDR, 2008). People who regularly consume shellfish, fish organ and meats (liver) may have increased cadmium exposure (ATSDR, 2008).

2.9.4 Sources of exposure to cadmium

Cadmium can be released to the environment in a number of ways, including: Natural activities, such as volcanic activity, weathering and erosion, and river transport; Human activities, such as tobacco smoking, mining, smelting and refining of nonferrous metals, fossil fuel combustion, incineration of municipal waste (especially cadmium-containing batteries and plastics), manufacture of phosphate fertilizers, and recycling of cadmium-plated steel scrap and electric and electronic waste (UNEP, 2008); Remobilization of historic sources, such as the contamination of water sources by drainage water from metal mines. Cadmium released can be carried to and deposited on areas remote from the sources of emission by means of long-range atmospheric transport (WHO, 2000).

Food and drinking-water: Cadmium contained in soil and water can be taken up by certain crops and aquatic organisms and accumulate in the food-chain (WHO, 2007). Food constitutes the main environmental source of cadmium for non-smokers (WHO, 2007). Highest cadmium levels are found in the kidney and liver of mammals fed with cadmium-rich diets and in certain species

of oysters, scallops, mussels and crustaceans. Lower cadmium concentrations are found in vegetables, cereals and starchy roots. Owing to the larger consumption of such food items, they represent the greater part of daily cadmium intake in most populations (IPCS, 1992; WHO, 2010). Some crops, such as rice, can accumulate high concentrations of cadmium if grown on cadmium-polluted soil. Acidification of cadmium containing soils may increase the cadmium concentrations in crops.

2.9.5 Health effects of cadmium

The kidney is the critical target organ. Cadmium accumulates primarily in the kidneys, and its biological half-life in humans is 10–35 years (WHO, 2008). This accumulation may lead to renal tubular dysfunction, which results in increased excretion of low molecular weight proteins in the urine. This is generally irreversible (WHO, 2008).

High intake of cadmium can lead to disturbances in calcium metabolism and the formation of kidney stonesWHO, 2010). Softening of the bones and osteoporosis may occur in those exposed through living or working in cadmium-contaminated areas(IARC, 2009). In a study in Japan where soil has been contaminated with cadmium from zinc/lead mines, Itai-itai disease used to be widespread and is still seen in women over 50 years of age. It is characterized by osteomalacia, osteoporosis, painful bone fractures and kidney dysfunction (WHO, 2008).

High inhalation exposure to cadmium oxide fume results in acute pneumonitis with pulmonary oedema, which may be lethal. Long-term, high-level occupational exposure is associated with lung changes, primarily characterized by chronic obstructive airway disease. There is sufficient evidence that long-term occupational exposure to cadmium (e.g. through cadmium fume) contributes to the development of lung cancer(IARC, 2009). There is limited evidence that

cadmium may also cause cancers of the kidney and prostate. The International Agency for Research on Cancer (IARC) has classified cadmium and cadmium compounds as carcinogenic to humans (Group 1), meaning that there is sufficient evidence for their carcinogenicity in humans (IARC, 1993; IARC, 2009).

2.9.6 Lead (Pb)

Lead is a metallic element belonging to group IV A of the Periodic Table (atomic number: 82 and relative atomic mass: 207.2), (U.S.EPA, 1998). Pure lead is a silvery-white metal that oxidizes and turns blue-grey when exposed to air. It is soft enough to be scratched with a fingernail. It is dense (11.3 g/cm3), malleable, and readily fusible. Its properties include: a low melting point; malleable; ductile; easy to cast; high density; low strength; easy to work; acid resistance; electrochemical reaction with sulphuric acid; chemical stability in air, water and earth; and the ability to attenuate sound waves, ionising radiation and mechanical vibration (U.S.EPA, 1998). Lead is hardened by alloying it with small amounts of arsenic, copper, antimony, or other metals. These alloys are frequently used in manufacturing various lead-containing products (Saeed, 2015).

2.9.7 Lead in aquatic environments.

In the aquatic environment, lead can occur in ionic form (highly mobile and bio-available), organic complexes with dissolved humus materials, attached to colloidal particles such as iron oxide or to solid particles of clay or dead remains of organisms (OECD, 1993). The speciation of lead in the aquatic environment is controlled by many factors, such as: pH, salinity, sorption and biotransformation processes. Lead is typically present in acidic aquatic environments as $PbSO_4$, $PbCl_4$, ionic lead, cationic forms of lead hydroxide and ordinary hydroxide $Pb(OH)_2$

(U.S. EPA, 2005). The speciation of lead in water is also dependent on the presence of other molecules in water. Lead is known to form strong complexes with humic acid and other organic matter (Denaix *et al*., 2001; Gao *et al*., 1999; Guibaud *et al*., 2003; U.S. ATSDR, 2005). Lead-organic matter complexes are stable to a pH of 3, with the affinity increasing with increasing pH but decreasing with increased water hardness (U.S. ATSDR, 2005).

In sea water, there is the presence of lead complexes to Fe-Mn oxides, which is due to the content of these oxides in sea water (U.S. ATSDR, 2005). Sorption of lead to polar particulate matter in fresh water and estuarine environments is an important process for the removal of lead from these surface waters. Changes in water chemistry (e.g. reduced pH or ionic composition) can cause sediment lead to become re-mobilized and potentially bio available to aquatic organisms (U.S. EPA, 2005). Methylation may result in re-mobilization and reintroduction of lead into the aqueous environment compartment and its subsequent release into the atmosphere (SRC, 1999; U.S. EPA, 2005). However, methylation is not a significant environmental pathway controlling the fate of lead in the aquatic environment(WHO/UNECE, 2007).

2.9.8 Human exposure to Lead

Exposure to lead occurs as a result of ingestion of foodstuffs, water and other beverages, and from air. Ingestion of contaminated soil, dust and old lead-based paint due to hand to-mouth activities may also be important regarding lead intake in infants and young children. When tap-water systems with leaded pipes are used, lead intake via drinking-water can be an important source, especially in children. Inhalation exposure may be significant when lead levels in the air are high (WHO/UNECE, 2007).

2.9.10 susceptible population groups

Susceptibility in the general population depends on many factors, including age, genetic characteristics, nutritional status, tobacco smoking, alcohol consumption and health status. Young children (below 6 years old) are most susceptible to lead exposure, even at low levels. Lead may also be transferred to the feotus in *utero*, both from exogenous sources, and as a result of its mobilization from the bones of the mother. Such mobilization can occur in women whose exposure ceased many years earlier, and even at low blood lead levels (Bellinger, 2005). In some children the habit of pica predisposes them to exposure if lead is present in the substances ingested. Susceptibility to lead exposure and, more specifically, to retained lead may also be linked to a genetic factor, as the gene which codes for amino levulinic acid dehydrogenase, a critical enzyme in the production of haeme may be altered (Bergdahl, 1997; Kelada, 2001). People working with lead under unsafe conditions are especially exposed and may be considered as another susceptible population group, especially if they are children or young adults (Ide and Parker, 2005).

2.9.11 Lead absorption

Absorbed lead is rapidly taken up by blood and soft tissue, and then slowly redistributed to bone. Bone accumulates lead during much of the lifespan and may then serve as an endogenous source, releasing lead slowly back into the blood after the exposure stops. Lead is readily transferred to the human foetus throughout gestation (IPCS, 1995).

The human skeleton begins to accumulate lead during foetal development and continues up to about 60 years of age (Pounds *et al*., 1991). Interest in bone lead, and its measurement invivo, stems from concern that skeletal lead is not metabolically inert, but can be mobilized by physiological and pathological states, for example, during pregnancy and lactation (Flora *et al.*, 2011; Bampidis, *et al.*, 2013) and osteoporosis, with possible adverse effects. Recent research by

Bampidis, *et al.*(2013),indicates that re-release of stored lead from bones into the blood and soft tissues may occur as bones become thinner with age(Bampidis, *et al.,* 2013). A number of potential health effects associated with this re-release have been identified in the elderly. In this way, lead exposure that occurred decades earlier may be a source of ill-health in the elderly (Flora *et al.,* 2011; Bampidis, *et al.*, 2013).

2.9.12 Lead from food containers

The influence of lead-soldered tin cans used for storage of food was noticeable (Tukker *et al.*, 2001). Glazes on ceramics, earthen ware, bone china, and porcelain often contain lead and are a potential source of exposure (MMWR, 2004). Reports from Brazil, Mexico, Morocco, Tunisia, and other countries refer to some traditionally manufactured pottery, in which the glazing materials contain lead (Belgaied, 2003; Morocco's submission, 2005). If the manufacturing process is carried out at low temperatures, the lead in the glaze is not fixed and may subsequently leak into food and beverages (Mexico comments, 2006).

2.9.13 Mechanisms of action of lead

There are a number of toxic mechanisms of lead, and more is becoming known about its molecular effects. Lead binds to the sulfhydryl (SH) groups of proteins, altering their structure and function. Among other effects, lead substitute's calcium and zinc, affecting various biological processes, such as metal transport, energy metabolism, apoptosis, conduction of ions, cell adhesion and signaling, enzymatic processes, protein maturation, and genetic regulation (Saeed, 2015). Lead has an affinity for the cell membrane, interferes with mitochondrial oxidative phosphorylation, and impairs the activity of calcium-dependent intracellular messengers and protein kinase (Saeed, 2015; Flora *et al.*, 2011; Bampidis, *et al.,* 2013). Lead

may inhibit DNA repair, have genotoxic effects, and affect sodium, potassium and calcium ATPase (Lidsky and Schneider, 2003; Toscano and Guilarte, 2005). The toxic effects of lead may, therefore, involve several organ systems and functions (Sallman, 2001; Saleh *et al.*, 2009; Moshen, *et al.*, 2011).

2.9.14 Signs and symptoms

Lead toxicity may involve the gastrointestinal system, the blood, or the neurological system. Chronically exposed individuals may have a blue line on the gum margins and anaemia. Children often appear asymptomatic even with elevated blood lead concentrations (U.S. CDC, 2002).

2.9.15 Health effects in humans

Exposure to lead can result in a wide range of biological effects, depending on its level, duration and timing. Lead is toxic to multiple organ systems, and effects may range from enzyme inhibition and anaemia to disorders of the nervous, immune and reproductive systems, impaired kidney and cardiovascular functions, and even death. Effects occur over a broad range of doses (IPCS, 1995). Because of their rapid growth and maturation, biological characteristics and behaviour, children are more vulnerable than adults to the effects of lead exposure. There is substantial evidence to show that high Pb level in the environment could affect blood Pb level, intelligence and behavior (Bellinger*et al*., 1990; Lanphear *et al*., 1998).

Due to their toxicity and accumulation in biota, determining the levels of heavy metals in commercial fish species has received considerable attention in different countries all around the world. Such interests were aimed at ensuring the safety of the food supply and minimizing the potential hazardous effects on human health (U.S. CDC, 2002).

2.9.16 Review of the heavy metals situation in different part of the world and Africa

Heavy metal pollution occurs when humans are exposed to heavy metals beyond certain limits. This has become an international medical issue since the Minamata metal poisoning in the 1960s which claimed the life of thousands of people in Japan (Wilcox *et al*., 2008).In an American study, Carrigan and co-workers found a strong association between pancreatic cancer and chromium, selenium and molybdenum (Carrigan *et al*., 2007). The authors quantified the heavy metal composition of pancreatic juice collected from patients with pancreatic cancer with no occupational exposures. In a similar study in an Egyptian population, serum cadmium levels were assessed in 31 newly diagnosed cancer patients. Compared to controls, the study found high cadmium levels in pancreatic patients who were exposed to the cadmium-polluted environment (Kriegel *et al.,* 2006).Similarly, an observational study in Taiwan sought to investigate possible involvement of specific heavy metals as risk factors of oral cancer in addition to cigarette smoking and betel quid chewing. Using spatial analysis and Geographic Information System, the authors analysed 22,083 patients diagnosed with oral cancer over a period of 20 years alongside nation-wide levels of heavy metals in the soil. The study found geographical relationship between the incidence rate of oral cancer and the corresponding soil levels of arsenic and nickel in the patients' residential areas (Su *et al.,* 2010).In a Chinese study to investigate probable association between long-term environmental exposure to lead and cadmium and cancer incidence, the authors found higher incidences of gastrointestinal and lung cancers among populations exposed to contaminated river over 30 years. In that ecologic study, the levels of heavy metals were determined in the drinking water and agricultural products consumed by the residents. Blood levels were then determined and compared to controls. Cancer mortality among the residents of the contaminated areas was found to correlate with cadmium and lead levels (Wang *et al.,* 2011).

Human exposure to heavy metals in the Sub-Saharan Africa has become a major health risk. This has received the attention of national and international environmentalists and concerned health bodies. Rapid population growth, increasing urbanization and the increasing appearance of slums and townships as a consequence of poor planning coupled with increasing industrial activities have resulted in overwhelming production of waste without adequate disposal systems. It has been reported by the United Nations Center for Human Settlements that only one-third of the solid waste generated in urban areas across the continent is collected and less than 2% recycled (Scheinberg *et al.,* 2011). Increasing mining activities, use of lead gasoline, indiscriminate dumping and burning of toxic waste, concentration of production factories within residential areas and poor pollution legislations have contributed to unprecedented heavy metal pollution in the last decades. According to a WHO study on the environmental burden of disease, 25% of the total burden of disease and 2.97 million human deaths are yearly attributed to environmental risk factors (Oberg *et al.,* 2010).

2.9.17 Review of the Heavy metals situation in Nigeria

Chukwujindu, (2015) determined metal concentrations in selected brands of canned fish in South East and South Western Region, Nigeria. He estimated the dietary intakes and target hazard quotients and reported the mean concentrations of Cd and Pb in the different brands of canned fish varied between 0.04 and 0.83µg/g for Cd, and 0.05 to 2.98µg/g for Pb was found to have the highest concentration in the tuna samples than that of mackerel and sardine samples. The concentrations were above their respective EU specified limits 0.05µg/g as limits for Cd and 0.2µg/g for Pb in sardine and mackerel respectively (European Union, 2005). Abubakar *et al.*(2014) reported the levels of heavy metals were above their corresponding permissible limits recommended by FAO/WHO and concluded that the risk of human exposure to heavy metal

contamination through fish was significant. Babalola *et al.,* (2014) reported Cd concentrations of less than the limit of detection to 0.028µg/g in canned mackerel, sardine, and tuna sold in Nigeria. Results presented in a Comparative study of accumulation of heavy metals in the different organs of the fishes consumed in Benue state, showed that iron concentration was high in both gills (0.025mg/L) and (0.0028mg/L) flesh, while lead was the lowest in the two species of fish studied (Beetseh and Abrahams, 2013).In another study levels of Pb, Cd, and nickel (Ni) was investigated in three highly popular sea foods in Nigeria; fish (*Tilapia zilli*), crab (*Callinectes sapidus*), and periwinkle (*Littorina littorea*), in Ondo State, an oil-polluted coastal Region in Nigeria. The study revealed that anthropogenic enrichment of Pb, Cd, and Ni could pose potential threats to the ecology and health of the area.

Saiful Islam *et al.,* (2015) studied the Apportionment of heavy metals in soil and vegetables and associated health risks assessment reported the concentration of Cr, Ni, As, Cd, and Pb about 47 % of vegetable samples, respectively, exceeded the maximum level of the tolerance limit of contaminants in vegetables. In a second study, Ihedioha and Okoye, (2012) assessed the concentrations of Cd and Pb in the muscle, liver, kidney, intestine, and tripe of cows in Nigeria, and concluded that Cd was accumulated mainly in the kidney, while Pb accumulated mostly in the liver, and both toxic metals were above the maximum, international permissible levels in most samples.

Ibrahim and Aliyu, (2010) reported that 355 people across 6 villages in Zamfara State, northwest Nigeria sustained lead (Pb) poisoning, majority of the victims were children,Dooyema *et al.,* (2011) reported that 97% of the children had elevated blood lead levels above 45µg/dL, the CDC-recommended threshold for commencing chelation therapy. Eighty five percent of blood samples exceeded the maximum detection limit of Lead care II blood lead analyzer (> 65 µg/dL).

The study also showed that children are exposed to lead primarily through hand-to-mouth or the inhalation of lead dust; the main sources of lead exposure in the state being the artisanal gold ore mining and processing activities that are prevalent in many villages in the state. The failures to understand long-term impacts on health and avoid the hazards of artisanal mining were seen in 2010 in Zamfara State in northwest Nigeria with the death of at least 400 children as a result of lead poisoning. Human Rights Watch (HRW) described this tragedy as the "worst lead poisoning epidemic in modern history" (Dooyema *et al.,* 2011).

In a study conducted in Otukpo, south-western Nigeria; the Blood Lead Level (BLL) among children <6 years old ranged from 2.1 to 31.8 μg/dl (mean = 9.4 μg/dL; SD = 4.2), with 35% of the children exhibiting a BLL > 10 μg/dL (Ogunseitan *et al.,* 2007). A study on blood lead and tap water lead levels in Nigeria found mean BLL of 11.4 and 12.3 μg/dL for females and males respectively (Omokhodion, 1994).In a United Nation Environment Programme (UNEP) report on environmental assessment in Ogoniland, southeastern Nigeria, released in 2011, drinking water, air and agricultural soil in 10 communities contain over 900 times permissible levels of hydrocarbon and heavy metals. The report acknowledges that recovery after extensive compliance with recommendations may take 30 years.In a more recent study by Ibeto and Okoye (2010) in Eastern Nigeria, blood samples from 240 residents of an urban area including children and pregnant women were analysed for heavy metals. The results showed values significantly higher that the WHO recommended limits for nickel, manganese and chromium. The sampled population who are non-miners demonstrated the prevalence heavy metal pollutions in Nigeria among ordinary people.

2.9.18 PH significance of heavy metals

PH is an indication of the acidity or alkalinity of a substance and is measured in pH units. PH is defined as the negative logarithm of the hydrogen concentration. The pH scale goes from 0 to 14 with pH 7 as the neutral point. As the amount of hydrogen ions in the substance increase the pH decreases thus becoming acidic. From pH 7 to 0 the substance is increasingly more acidic and from PH 7 to 14 the substance is increasingly more alkaline or basic.

pH is one of the factors which influence the bioavailability and the transport of heavy metal in the substance (Smith and Giller, 1992), heavy metal mobility decreases with increasing pH (8 and above) due to precipitation of hydroxides, carbonates or formation of insoluble organic complexes (Smith and Giller, 1992). In another study Sauve *et al.,* (2000) observed that heavy metals increased significantly due to the acidic nature of the soil pH. The amount of heavy metals mobilized in soil environment is a function of pH, properties of metals, redox conditions, soil chemistry, organic matter contents, cation exchange capacity and the metals are generally more mobile at pH < 7 than at pH > 7 Sauve *et al.,* (2000). Chaun *et al*. (1996) showed low solubility of Pb, Zn, Cd, and Cu at pH 6 to 6.5 and an increase by several orders at pH 2. Similarly, Pb, Cd, and Zn exhibited weak solubilities at slightly alkaline condition (pH 8); while at pH 3.3 solubility were higher (Chaun *et al*., 1996). The Type 1 metals (silver, cadmium, chromium, lead and titanium) can be removed efficiently if the pH is raised significantly above the acid breakpoint i.e. a pH of 9 will successfully remove the metal (David *et al.,* 1994).

2.9.19 Prevention / control of heavy metals

•As a preventable phenomenon, concerned and interested parties must expend more resources and attention in the likelihood that heavy metal pollution in Sub Saharan Africa play significant role in cancer epidemiology.

• Legislation and regulatory activity as they pertain to mining and environmental degradation should be enhanced and enforced.

• Reducing consumption of raw materials and products that include cadmium as impurity or use of low-cadmium raw materials such as phosphate rock with low cadmium content as a raw material or; remove the cadmium in the manufacturing of phosphate fertilizers.

• Controlling cadmium and lead releases through low-emission process technologies and cleaning of off gases and wastewater; Cadmium can be emitted from processes by fugitive emissions or though flue gas systems by moving these activities to completely enclosed buildings, which may be equipped with ventilation systems with suitable controls.

• Reducing consumption of raw materials and products that include lead as an impurity; include the choice of an alternative raw material, such as using natural gas for power generation instead of coal.

•Regulatory authorities also need to be more stringent in enforcing existing rules in other to protect the citizens from avoidable heavy metal contamination e.g. planting of edible fruits and other agricultural products in close proximity with roadside mechanic shops, petrochemical and other mining industries since the consumption of such food products may contribute to health hazards of the populace.

Management of lead-containing waste

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Culture media

Bacterial culture media were obtained from Oxoid (Basingstoke, England) and they comprise *Listeria* Enrichment Broth, *Listeria* Selective agar, Nutrient agar, Tryptone Soya agar, Blood agar base, Mueller- Hinton agar, Andrane Peptone Water, Buffered peptone water and Sodium Chloride.

3.1.2 Chemical reagents

Nitric acid (HNO_3), perchloric acid ($HClO_4$), 70% ethanol, sulphuric acid (H_2SO_4), distilled water, Crystal violet, Lugol's iodine, Safranin dye, Hydrogen peroxide, acetone, Sanitizer, Detergents, Acetone- alcohol, deionized water.

3.1.3 Glass wares and laboratory equipment

Glass and disposable Petri dishes, Microscopic Glass slide, Measuring cylinders, conical flasks, Universal bottles, sample bottles, Test tubes, Pipettes, polythene bags, beakers, weighing balance, hot plates, funnels, filter papers, tin opener, cotton wool, hand gloves, aluminum foil, Sterile Swab stick, Ice-man cooler, Masking tape, Markers, Wire loop and pin, Needle and syringe, Antibiotic disc dispenser, Water bath, Incubator, Refrigerator, incubator (Astell Scientific- JBF044), autoclave, Refrigerator, Stomacher laboratory blender (SWARD, London), atomic absorption spectrophometer (AA240FS Varian- U.S

3.1.4 Antibiotic discs and their concentrations

Chloramphenicol (30µg), Gentamicin (30µg), Amoxicillin (25µg), Ciprofloxacin (5µg), Streptomycin (10µg), Tetracycline (30µg), Oxacillin (1µg), Erythromycin (15µg), Trimethoprim (5µg), (Oxoid, Basingstoke, England)

3.1.5 Rapid kits were obtained from Oxoid (Basingstoke, England).

The isolates were also tested using the Microbact^{TM} 12L *Listeria* identification system (Oxoid, MB1128A) according to the manufacturer's instructions.

3.1.6 Supplements

Listeria Enrichment Supplement, *Listeria* Selective Supplements (Oxoid, Basingstoke, England)

3.1.7 Sugars

Rhamnose, Xylose, Mannitol, Sucrose and Lactose (Oxoid, Basingstoke, England).

3.2 Methods

3.3 Study Area

The study was conducted in Kaduna North Local Government Area of Kaduna state. Kaduna state is located at Latitude $10^{o}20$'N and Longitude 7^{o} 45' East. The city is located in the North West Geo-political zone of Nigeria. It is a trade centre and a major transportation hub for the surrounding Agricultural area with rail and road junction. Kaduna North has an area of 72 km Sq. and a population of 364,575 according to 2006 census (KDSG, 2008).

Kaduna North falls within the Sudan savannah region; it's characterized by rainy and dry seasons with a little period of harmattan. Its headquarters is located at Magajin Gari in the heart of Kaduna town. It has (3) districts; Doka district, Kawo district and Gabasawa district respectively (KDSG, 2008). Most of the inhabitants are mostly traders in various businesses and civil servants. In addition, it has livestock, poultry and fish farms with fish marketing outlets which will enable field based research (FAO, 2007).

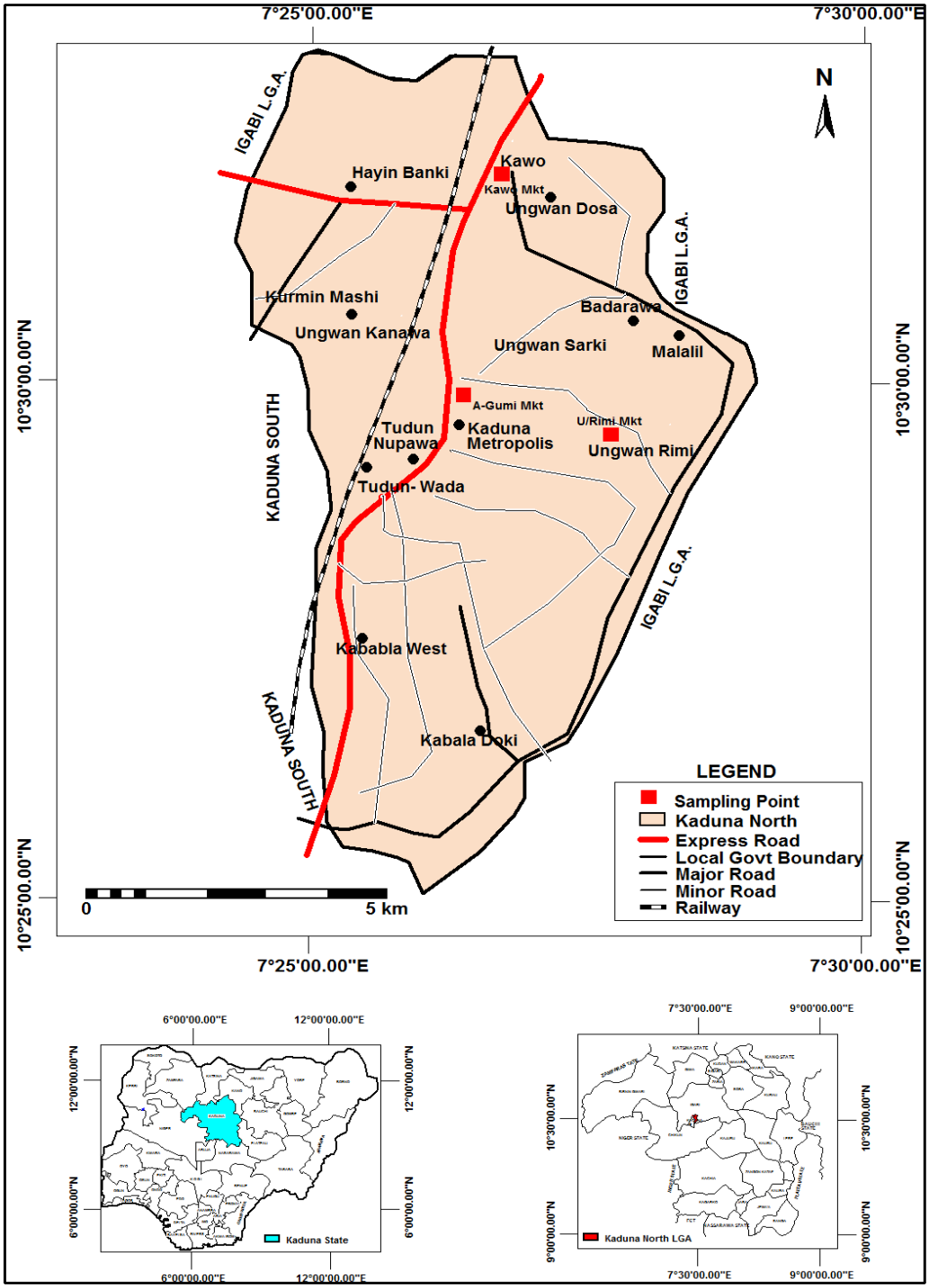


Figure 3.1: The Map of Kaduna North L.G.A, Nigeria showing the sampling areas.

Source: nigeriazipcodes.com Retrieved October 10 2015. 12:36 GMT

3.4Study Design

A cross sectional study was carried out. 60 Frozen, 60 smoked and 60 canned fish were purchased from three major markets of the three districts in the study area based on convenience for a period of 10 weeks (October, 2015- January, 2016). During each week of the sampling, eighteen (18) fish samples were collected consisting of 2 frozen, 2 smoked and 2 canned fish from each of the three markets in the study area. The samples were not collected from the same fish vendors.

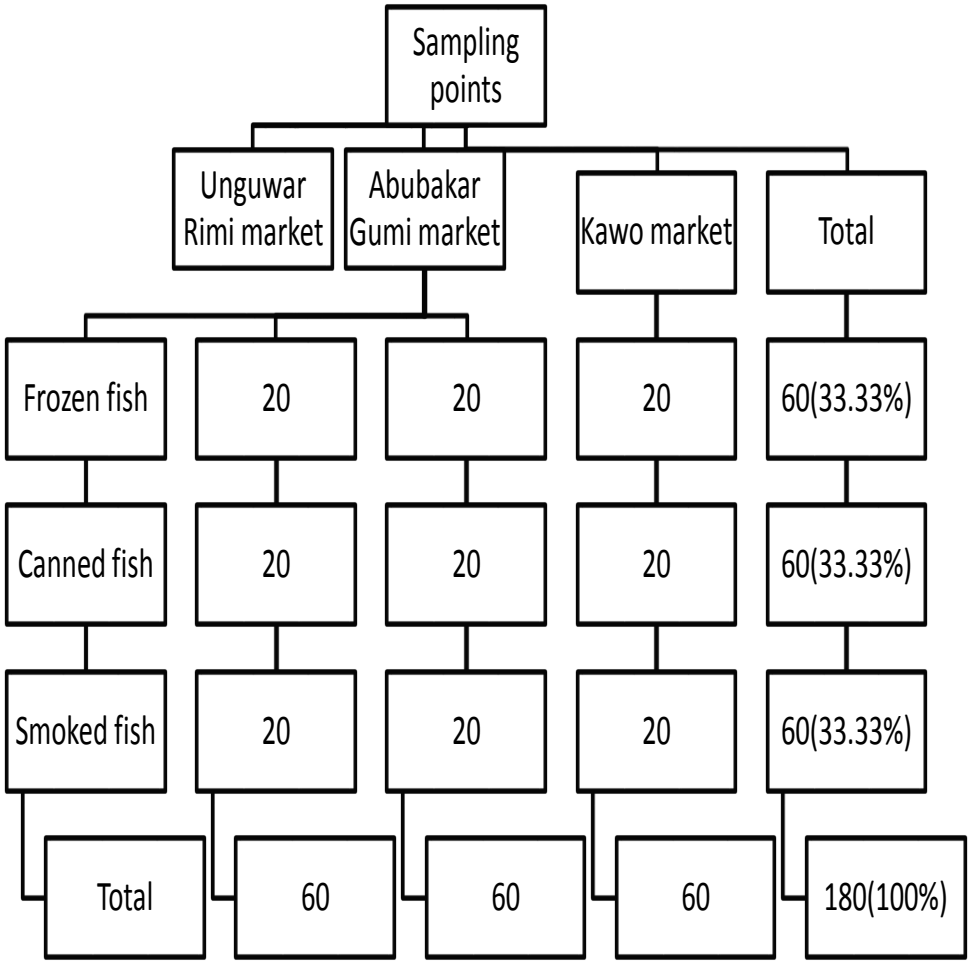


Figure 3.2: The distribution of frozen, smoked and canned fish purchased from three (3) markets in three districts of Kaduna North L.G.A.

3.5 Sample Size

Convenience sampling was used for this study.A total of one hundred and eighty (180) samples of fish (60 frozen fish; 60 smoked fish and 60 canned fish) were used for this study.

3.6 Sample Collection and Transportation

A total of one hundred and eighty (180) samples which comprised of 60 frozen (20 samples from each market), 60 smoked (20 samples from each market) and 60 canned fish (20 samples from eachmarket) were purchased from fish vendors and retail outlets of the major markets in three (3) districts of Kaduna North L.G.A. namely; Kawo Market, Abubakar Gumi market and Unguwar Rimi market, based on convenience. Sampling was done once weekly for a period of 10 weeks (October, 2015- January, 2016), so as to ensure collection of new batches of fish samples.

Each sample was wrapped in sterile aluminum foil, packed and labeled appropriately in sterile polythene bags. Frozen fish were transported in a Coleman box containing ice packs to the Bacterial Zoonoses Laboratory in the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria for microbiological analysis and Multi-user Post Graduate Research Laboratory in the Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria for heavy metal analysis. Information on the container/labels was recorded to include National Agency for Food and Drug Administration and Control (NAFDAC) number, manufacture and expiry dates, batch number and Manufacturer's address where available. Canned containers were examined for evidence of defects before purchase; those with defects were excluded from sampling.

3.6 Enrichment and Isolation of *Listeria* Species

3.6.1 Isolation and identification of *Listeria* species

The International Standard Organization (ISO) 11290-1 (1996) method for qualitative isolation and identification of *L. monocytogenes* was used as described by Indrawattana *et al.* (2011). About 10 grams from each fish sample was weighed and suspended into 90 ml of 0.1 % peptone water. Homogenization was done using a Stomacher (Stomacher Lab Blender 400) for one minute, the sample was allowed to settle for 2 to 3 minutes. One ml of the homogenized sample was suspended into the 9ml of prepared *Listeria* enrichment broth incorporated with *Listeria* selective supplement (0.1 ml) and incubated at 37°C for 18 to 24 hours.

A loop-full of the overnight culture was streaked on Oxford *Listeria* agar (Oxoid, Basingstoke, UK), and incubated at 37°C for 24-48 h. The colonies were identified by colonial morphology for *Listeria* colonies viz grayish colonies surrounded by black halos with sunken center (Hitchins, 1995; Aygun and Pehlivanlar, 2006). Colonies showing black to brown colonies surrounded by black halos with sunken center on *Listeria* selective agar were sub-cultured onto Tryptone Soya Agar (Oxoid, M290) supplemented by 0.6% of Yeast Extract Powder (Oxoid, LP0021) (TSYEA) and incubated at 37ºC for 24 hours. The colonies were subjected to gram staining and catalase test. The suspected colonies of L*isteria* specieswere inoculated into nutrient agar slant and incubated at 37°C for 24 hours and then stored at 4°C for further identification as described by Janzten *et al.* (2006), and Jemmi and Stephan (2006).

3.6.1.2 Biochemical characterization of isolates

The suspected *Listeria* isolates were subjected to conventional biochemical tests (Gram staining, catalase, β-haemolysis on 7% sheep blood agar), and carbohydrate fermentation test using mannitol, rhamnose, maltose, sucrose and xylose as described by Janzten *et al.*, (2006), and

Jemmi and Stephan (2006). Other tests carried out are motility test, aesculin hydrolysis and oxidase test.

3.6.1.3 Catalase test

A drop of 3% hydrogen peroxide (H_2O_2)) was placed on a glass slide; a portion of growth was removed from *Listeria* Selective Agar with a wire loop. The evolution of gas (oxygen) was indicated by formation of bubbles on the glass slides and also indicated positive result.

3.6.1.4 Gram's stain

Gram staining was done using suspected *Listeria* species colonies on *Listeria* selective agar plates. A pure colony of *Listeria* was emulsified in a drop of sterile distilled water on a slide were smeared and air dried. The slides were fixed by rapidly passing through a Bunsen burner flame in two quick successions and allowed to cool. The fixed smear was covered with crystal violet stain for 1minute. The stain was rapidly washed off with clean slow running tap water. The smear was covered with Lugol's iodine for another 1minute and washed off with clean tap water and decolourized with alcohol for 30 seconds then washed immediately with clean tap water. The smear was then counter stained with Safranin dye for 30 seconds and washed off with clean tap water. The stained slides were air dried and were examined microscopically using oil immersion objective lens (×100).

Organisms that appeared purple to blue in the Gram-stained slides were tentatively to be *Listeria* species.

3.6.1.5 Motility test

The motility medium was prepared according to manufacturer's instruction (Oxoid). The suspected colonies of *Listeria* were inoculated by stabbing at the centre of the motility medium

with straight wire loop and incubated at 37^0C for 24 hours. The diffusion of the growth within the stabbing layer was observed, like an umbrella signifying that the presumptive isolates were motile.

3.6.1.6 Sugar fermentation testing

The sugars were prepared according to manufacturer's instruction (Oxoid).
1g of each sugar and 1.5g of andrates peptone waterwere suspended in 100ml of sterile peptone water. Three (3) ml of each of the sugar was dispensed into bijou bottles and autoclaved at 110°C for 15mins. The *Listeria* isolates were inoculated into sterile sugar and incubated at 37°C for 24 – 48hrs. Pink to red colour development indicated positive for the sugar fermentation while yellow colour was considered negative.

3.6.1.7 Haemolysis

Seven percent sheep blood agar base (Oxoid, CM0854) was used to determine the presence of listerio-lysin (LLO) exo-toxin in *L.monocytogenes* and *L. ivanovii* strains. The presumptive *Listeria* isolates were inoculated onto blood agar and incubated at 37^oC for 24hours. After 24hours, blood agar plates were observed for α and β haemolysis. β haemolysis presented with a clear around reddish colouration while α haemolysis presented with a greenish colouration around the presumptive colonies (Morobe *et.al.,* 2009).

3.6.1.8 Aesculin hydrolysis

The isolates were grown on bile aesculin agar for 24hours.A positive result was indicated by black colouration of the whole medium, after 24hours of incubation, while a negative reaction was indicated by a lack of colour change throughout the medium.

3.6.1.9 Oxidase test

Whattman filter papers were wetted with a one drop of a dilute solution (1%) of the oxidase reagent, tetra methyl-p-phenylene diamine dihydrochloride. A loopful of the presumptive colony from Bile Aesculin Agar with a wire loop and was smeared on the wet paper. The developments of an intense purple colouration by bacterial cells in the smear within 10 – 15seconds were recorded as positive test.

3.7 Identification of Presumptive Isolates by Microbact^{tm} 12l - Id System

Prior to inoculation into the Microbact^{TM} 12L, isolates were checked to ensure they are *Listeria* species(Short Gram positive bacillus, motile at 25^0C, catalase positive and oxidase negative) by subjecting them to conventional biochemical tests namely catalase test, aesculin hydrolysis test, haemolysis, oxidase test and sugar fermentation test. The suspending broth was brought to room temperature after storage at -4^0C before inoculation of micro well test strips. A single well – isolated colony from an 18-24hours culture was selected and emulsified in a vial of *Listeria* suspending medium (2.5ml). The medium was mixed thoroughly until a homogenous medium was obtained. Micro well test strips were removed from the foil pouch, placed on the holding frame and the lid removed. Using a sterile Pasteur pipette, 4 drops (approximately 100µL) of the bacterial suspension was transferred to each well of the micro well test strips.

As a purity check, 1 drop of the organism suspension was transferred onto nutrient agar plate. The plate was incubated aerobically at $35\text{-}37^0$C for 18-24 hours. One drop of the haemolysin reagent was added to well 12 and the lid was replaced onto the micro well test strip and incubated at 37^o C for 24hours. After incubation, lids were removed from the micro well test

strip and the result was recorded on the report form provided. The various octal codes were ran on Microbact 12L software version (Oxoid, MB1244A) to identify various species of *Listeria*

3.8 Antibiotic Susceptibility Testing

The antibiotic susceptibility of the isolates was determined by Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (Oxoid, Basingstoke, UK). The antibiotics that were tested included: Amoxicillin (25µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Erythromycin (15µg), Gentamicin (30µg), Streptomycin (10µg), Tetracycline (30µg), Trimethoprim (5µg) and Oxacillin (1µg).Three to five well isolated colonies of *Listeria* were transferred into 5ml on Mueller-Hinton broth (Oxoid) and incubated at 37°C for 16 to 18 hrs. The overnight culture broth was diluted using sterile distilled water to a turbidity equivalent to 0.5 McFarland standard (Harakeh *et al.,* 2005), and swabbed onto the entire surface of the dried Mueller-Hinton Agar (MHA, Oxoid) using sterile swab stick. The inoculated MHA plates were allowed to dry at room temperature before placing the antibiotic discs followed by incubation at 37°C for 24hrs. The zone of inhibition were measured in millimeter and interpreted in accordance with the Clinical and Laboratory Standards Institute Standards guidelines (CLSI, 2011). The multiple antibiotic resistances (MAR) index was determined for each of the isolates. MAR index was calculated by dividing the number of antibiotics to which each isolate was resistant (a) by the total number of antibiotics to which the isolate was tested (b) (Singh *et al.*, 2010)

MAR index = a/b

MAR index = Multiple- antibiotic resistance indices above the bench score of 0.20

a = number of antibiotics to which each isolate was resistant to

b = total number of antibiotics to which the isolate was tested.

3.9 Digestion of Samples (Wet Digestion Technique):

The samples were prepared and digested according to the technique described by Shibamoto and Bjeldanes (1993).

Washing of equipment is an important process to avoid contamination especially when trace elements or heavy metals are to be analyzed. Briefly; after washing, one gram from each sample of dorsal muscle was digested by 10 ml of digestion mixture (60 ml Nitric acid 65% and 40 ml Perchloric acid 70-72 %) in screw capped tube after maceration by sharp scalpel. The tubes were tightly closed and the contents were vigorously shaken, allowed to stand overnight at room temperature and heated for 1 hour at 100^{o}c to ensure complete digestion of samples. Cooled samples were diluted with 10 ml deionized water, thoroughly mixed, filtered with Whattman filter paper and making it up to 20ml with deionised water, kept at room temperature until analyzed for heavy metal contents. Blank solutions were prepared in the same manner. Digested sample solutions were analyzed for lead and cadmium contents by Atomic Absorption Spectrophotometer (AAS) (AA240FS VARIAN Spectrophotometer).

3.9.1 Basic principle of atomic absorption spectrophotometer (AAS) for heavy metals analysis.

The concentration of lead and cadmium (µg/g) were estimated according to the following equation: C=R x D/W

C=concentration of Lead and Cadmium (µg/g) wet weight.

R=reading of digital scale of AAS.

D= Dilution of prepared sample.

W= Weight of the sample.

3.10 Data Analyses

Data was subjected to descriptive statistics (Tables and Bar charts) using Statistical Package for Social Science (SPSS) version 20.0 (Ieren *et al.*, 2013).

Prevalence was calculated using the formula:

$$\text{Prevalence} = \frac{\text{Number of positive samples}}{\text{Total number of samples collected}} \times 100$$

Levels of Lead and Cadmium were compared with the type of fish and sampling locations using Chi-square.$P \leq 0.05$ was considered statistically significant. Spearman's Rank Correlation was used in determining the association between the levels of lead, cadmium and occurrence of *Listeria* species in frozen, canned and smoked fish.

CHAPTER FOUR

RESULTS

4.1 Determination of Occurrence of *Listeria* Species in Frozen, Smoked and Canned Fish in Kaduna North L.G.A., Nigeria.

Out of 180 samples of fish samples tested 24 yielded positive *Listeria* species.Distribution of the 24 (100%) positive *Listeria* species showed that 9(37.5%) of the positive isolates were *L.grayi*,while 15 (62.5%) were *L. ivanovii* (Table 4.1).

Among the fish samples examined in this study, frequency of isolation of *Listeria*species (31.7%) was from smoked fish when compared with frozen fish which had 5 (8.3%) positive samples whereas none of canned fish 0 (0%) was positive.Distribution of *Listeria* species in Fish samples showed that smoked fish had more *L. ivanovii* isolates 11(73.3%) compared to frozen fish with 4 (26.7%). *L. grayi* 8 (88.9%) was more frequently isolated from smoked fish compared to 1(11.1%) for frozen fish (Table 4.1).

Table 4.1: Distribution of *Listeria* species based on fish sample type in Kaduna North LGA, Nigeria.

Type Of Fish	No. Samples Sample tested	*Listeria* species isolated. *L. ivanovii*	*L. grayi*	Total(%) Positive
Smoked	60	11	8	19 (31.7%)
Fresh	60	4	1	5 (8.3%)
Canned	60	-	-	0
Total	180	15	9	24 (13.3%)

4.2Occurrence of *Listeria* Species in Frozen, Smoked and Canned Fish based on sampling locations in Kaduna North L.G.A., Nigeria.

Distribution of *Listeria* isolates by location showed that samples from Unguwar Rimi market had the highest occurrence of *Listeria* species (25%) followed by Kawo market (8.3%) and the least contamination with *Listeria* species was observed at Abubakar Gumi market (6.6%) (Table 4.2).

Only 2 species of the *Listeria* were recovered from the three markets, of which *Listeria ivanovii*was the most commonly contaminating specie with (8.9%) occurrence (Table 4.2).

Unguwar Rimi market had the highest occurrence of *Listeria ivanovii*isolates (16.6%) and the least occurrence of *Listeria ivanovii* (3.3%) was recorded at Kawo market. Five 5(8.3%) *Listeria grayi*isolates were recovered from Unguwar Rimi market while 3(5%) were recovered from Kawo Market (Table 4.2).

Table 4.2: Distribution of *Listeria* species by Fish sampling locations in Kaduna North L.G.A, Nigeria

Location	No. of samples tested	Number and percentages (%) of positive samples		
		***Listeria* species**	***L. ivanovii***	***L. grayi***
Abubakar Gumi market	60	4 (6.6)	4 (6.6)	0 (0)
Ungwan Rimi market	60	15 (25)	10 (16.6)	5(8.3)
Kawo market	60	5 (8.3)	2 (3.3)	3(5)
Total	**180**	**24 (13.3)**	**16 (8.9)**	**8 (4.4)**

4.3 Antibiotic Resistance Patterns of *Listeria* isolates from Fish obtained in Kaduna North L.G.A, Nigeria.

The percentage susceptibilities of the 24 isolates tested against 9 antibiotics is given in (Figure 4.1).From the percentage distribution of antibiotics susceptibilities of the 24 *Listeria* isolates to 9 antimicrobial agents, all the 24 *Listeria* isolates were susceptible (100%) to gentamicin. The isolates showed susceptibilities to the following antimicrobial agents at various percentages; amoxicillin(87.5%),chloramphenicol(91.7%), ciprofloxacin(58.3%), erythromycin(37.5%), streptomycin(66.7%),tetracycline(66.7%), trimethoprim(70.8%), oxacillin(79.2%) and chloramphenicol(91.7%). The isolates showed least resistance to oxacillin(20.8%), amoxicillin(12.5%) andchloramphenicol(8.3%), (Figure 4.1).

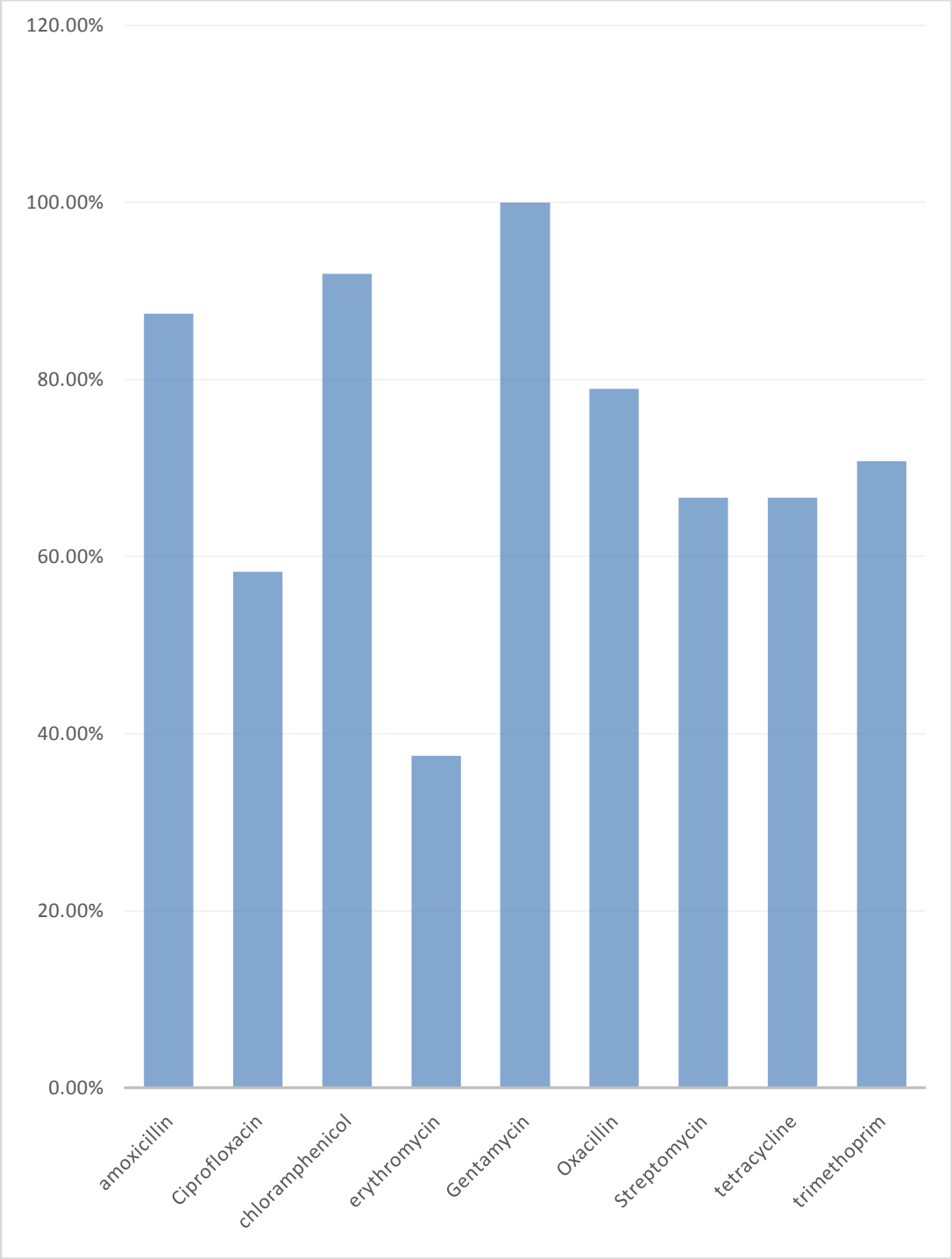


Figure 4.1: Percentage susceptibilities of 24 *Listeria* Isolates from Fishobtained in Kaduna North L.G.A, Nigeria to 9 antimicrobial agents.

4.4: Antibiotic Resistance patterns of *Listeria* isolates from Fish obtained in Kaduna North L.G.A., Nigeria.

The antibiogram pattern of resistance to onlyerythromycin (ER) was the most common pattern occurring in 5 of the 24 isolates.(Table 4.3).

Table 4.3:Antimicrobial resistance patterns of24*Listeria* species isolates obtained from Fish in Kaduna North L.G.A., Nigeria.

S/No.	Antibiotic resistance Pattern. (Antibiogram)	Frequency	*Listeria* species
1.	CIP, CL, TE, TM, OX, ER	1	*L. grayi*
2.	CIP, TE, TM, S, OX, ER	1	*L. ivanovii*
3.	CIP, TE, OX, AML, ER	1	*L. ivanovii*
4.	CIP, TE, TM, S, ER	1	*L. ivanovii*
5.	CIP, TM, OX, ER	1	*L. ivanovii*
6.	CIP, CL, S, ER	1	*L. grayi*
7.	CIP, TE, TM	1	*L. ivanovii*
8.	S, AML, ER	1	*L. ivanovii*
9.	TE, TM, ER	1	*L. ivanovii*
10.	CIP, ER	2	*L. grayi*,*L. ivanovii*
11.	CIP, TE	1	*L. grayi*
12.	OX, ER	1	*L. grayi*
13.	S, AML	1	*L. grayi*
14.	ER	5	*L. ivanovii*(3), *L. grayi*(2).
15.	TE	2	*L. ivanovii*(2).
16.	S	3	*L. grayi*,*L. ivanovii*(2).

Key: AML, Amoxicillin; CL, Chloramphenicol; CIP, Ciprofloxacin; ER, Erythromycin; TM, Trimethoprim; S, Streptomycin; TE, Tetracycline; OX, Oxacillin.

4.4: Multiple Antibiotic Resistance (MAR) index of the 24*Listeria* species Isolates Obtainedfrom Fish in Kaduna North L.G.A, Nigeria.

The mean MAR index ranged from 0.22 to 0.67. Fourteen (14) isolates out of the 24 isolates had multiple- antibiotic resistance indices above the bench score of 0.20 (Figure 4.2).

Two *Listeria* isolates showed resistance to six (6), five (5) and four (4) antimicrobial agents respectively. Three *Listeria* isolates showed resistance to 3 antimicrobial agents, while 5 isolates showed resistance to only 2 antimicrobial agents respectively (Figure 4.2).

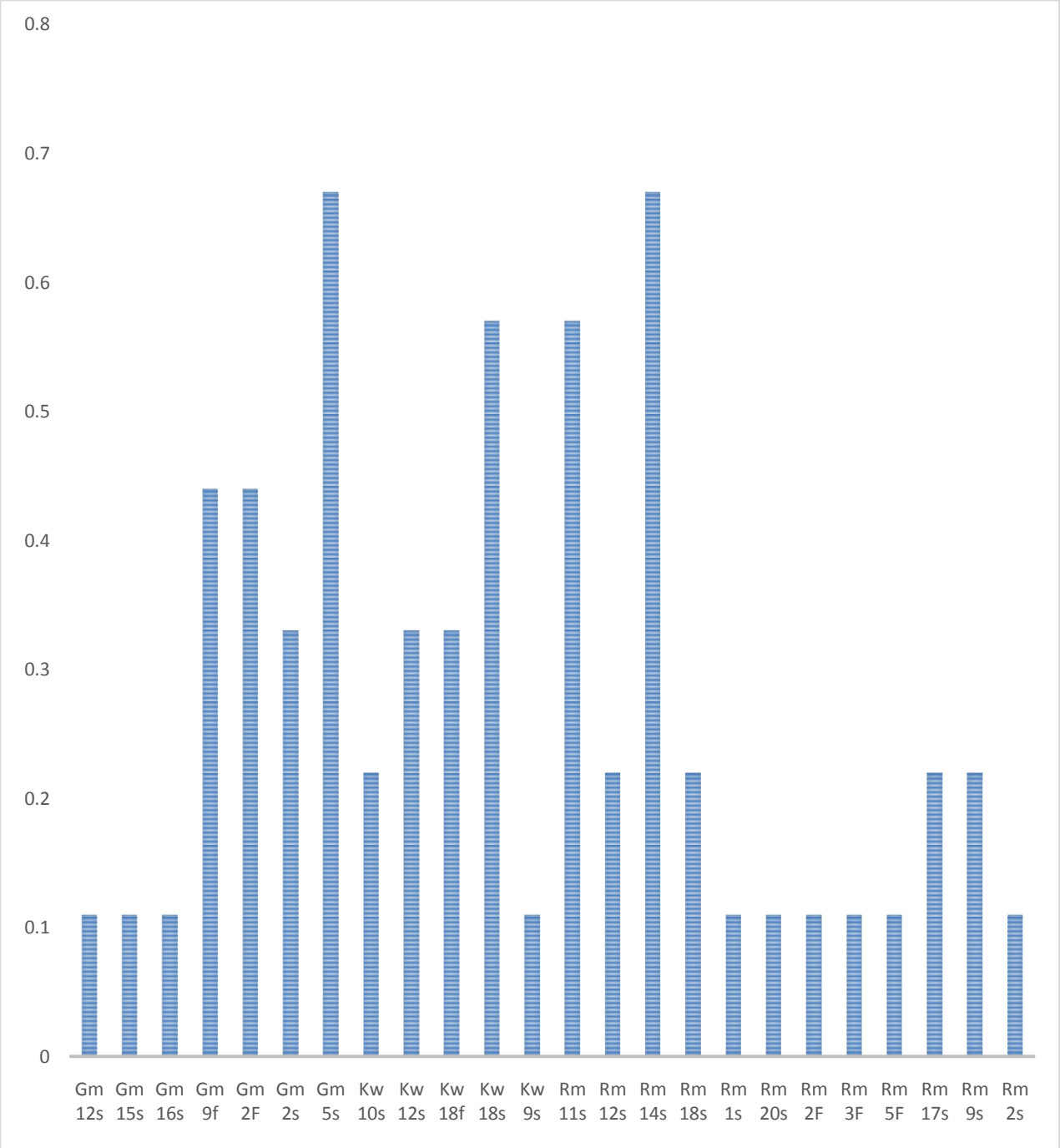


Figure 4.2 Multiple Antibiotic Resistance (MAR) index of the 14*Listeria* species isolates obtainedfrom Fish in Kaduna North L.G.A, Nigeria.

4.4 Determination of Cadmium Concentration (0.05µg/g)inFish Samples Based on Market Location Obtained in Kaduna North L.G.A, Nigeria.

The distribution of cadmium from this study showed that 32(53.3%) 0f the fish samples collected from Abubakar Gumi Market,32(53.3%) Anguwar Rimi market and 38(63.3%) Kawo market were within EuropeanUnion's (EU) allowable limit permitted in fish (0.05µg/g) for Cadmuim (Cd) (E.U., 2005); while 28(46.7%) of Abubakar Gumi Market,28(46.7%) Anguwar Rimi market and 22 (36.7%) Kawo market samples were above the allowable limit permitted in fish (0.05µg/g) by(E.U., 2005)for Cadmuim (Cd), Table 4.4.

Table 4.4: Distribution of Cadmium (Cd) in fish samples based on market locationobtained in Kaduna North L.G.A, Nigeria.

Market locations	No. of samples Tested	No. of samples within allowable limits (%)	No.of samples above allowable limits (%)
Abubakar Gumi	60	32(53.3)	**28(46.7)**
Anguwan Rimi	60	32(53.3)	**28(46.7)**
Kawo	60	38 (63.3)	**22(36.7)**
Total	180	102(56.7)	**78(43.3)**

Chi Square (X^2) = 1.629, Degree of freedom (df) = 2, P-value= 0.443.

*(0.05µg/g)

4.5 Determination of Lead Concentration(0.20µg/g)inFish Samples Based on Market Location Obtained in Kaduna North L.G.A, Nigeria.

The distribution of Lead in this study showed that 15(25%) of the fish samples collected from Abubakar Gumi Market,22(37.6%) Anguwar Rimi market and 18(30%) Kawo market were within EuropeanUnion's (EU) allowable limit permitted in fish (0.20µg/g) for Lead (Pb) (E.C, 2005; Ozturk*et al.,* 2009), while 45(75%) Abubakar Gumi Market,38(63.3%) Anguwar Rimi market and 42 (70%) Kawo market samples were above allowable limit permitted in fish (0.20µg/g) for Lead, (Table 4.5).

Table 4.5: Distribution of Lead (Pb) in fish samples based on market locationobtained in Kaduna North L.G.A, Nigeria.

Market locations	No. of samples Tested	No. of samples Within allowable limits (%)	No. of samples above allowable limits (%)
Abubakar Gumi	60	15(25.0)	**45(75.0)**
Angwan Rimi	60	22(36.7)	**38(63.3)**
Kawo	60	18 (30.0)	**42(70.0)**
Total	180	55(30.6)	**125(69.4)**

Chi Square (X^2) = 1.937, Degree of freedom (df) = 2, P-value= 0.380
*(0.20µg/g)

4.6 Determination of Cadmium Concentration(0.05µg/g) in Fish Samples Based on Fish Type Obtained in Kaduna North L.G.A, Nigeria.

The distribution of cadmium from this study based on fish type showed that 35(58.3%) smoked fish, 23(38.3%) frozen and 20(33.3%) canned fish samples had levels above allowable limit permitted in fish (0.05µg/g) for Cadmuim (Cd), (Table 4.6.). However, smoked fish had 25(41.7%), frozen fish had 37(61.7%) and 40(66.7%) canned fish of the samples collected showed levels within the European Union's (EU) allowable limit permitted in fish (0.05µg/g) for Cadmuim (Cd) (E.U., 2005; Ozturk *et al.,* 2009),Table 4.6.

Table 4.6: Distribution of Cadmium (Cd) based on fish type obtained in Kaduna North L.G.A, Nigeria.

Type of Fish	No. of samples Tested	No. of samples Within allowable limits (%)	No. of samples above allowable limits (%)
Smoked Fish	60	25(41.7)	**35(58.3)**
Frozen Fish	60	37(61.7)	**23(38.3)**
Canned Fish	60	40 (66.7)	**20(33.3)**
Total	180	102(56.7)	**78(43.3)**

Chi Square (X^2) = 8.552, Degree of freedom (df) = 2, P-value= 0.014

*(0.05µg/g)

4.7 Determination of Lead Concentration in Fish Samples Based on Fish Type Obtained in Kaduna North L.G.A, Nigeria.

The distribution of Lead from this study showed that 13(21.7%) of the fish samples collected from smoked fish,24(40%) frozen fish and 18(30%) canned fish were within EuropeanUnion's (EU) allowable limit permitted in fish (0.20µg/g) for Lead (Pb) (E.C, 2005; Ozturk*et al.*, 2009), while 47(78.3%) smoked fish,36(60%) frozen fish and 42 (70%) canned fish samples were above the allowable limit permitted in fish (0.20µg/g) for Lead, Table 4.7.

Table 4.7: Distribution of Pb residue based on fish type obtained in Kaduna North L.G.A, Nigeria.

Type of Fish	No. of samples Tested	No. of samples Within allowable limits (%)	No. of samples above allowable limits (%)
Smoked Fish	60	13(21.7)	**47(78.3)**
Frozen Fish	60	24(40.0)	**36(60.0)**
Canned Fish	60	18 (30.0)	**42(70.0)**
Total	180	55(30.6)	**125(69.4)**

Chi Square (X^2) = 4.765, Degree of freedom (df) = 2, P-value= 0.092

*(0.20µg/g)

4.8:The association between occurrence of *Listeria* species, Lead and Cadmium concentrations in Frozen, Smoked and Canned Fish obtained in Kaduna North L.G.A, Nigeria.

Using Spearman's Rank Correlation at the 0.01significant level, there was a significant association between occurrence of *Listeria* species, Lead and Cadmium concentrations in Frozen, Smoked and canned Fish obtained in Kaduna North L.G.A, Nigeria (Table 4.8).

Table 4.8: The association between occurrence of *Listeria* species, Lead and Cadmium concentrations in frozen, smoked and canned fish obtained in Kaduna North L.G.A, Nigeria.

	Listeria species	Cadmium	Lead
Listeria species	1	0.998***	0.500
Cadmium	0.998***	1	0.500
Lead	0.500	0.500	1

** Correlation is significant at the 0.01 level (2-tailed).

CHAPTER FIVE

DISCUSSION

This study established an overall prevalence of 13.3% of *Listeria* species in frozen and smoked fish in Kaduna North Local Government Area, Nigeria. Similar prevalence of 13.6% was reported in Egypt by Al-Sheikh *et al.* (2012) 12.34% in Belgrade, Serbia by Kuzmanovic Jelena *et al.*(2011); 16.5% in Taiwan by Wang *et al.*(2012) and slightly higher prevalence of 25% was reported on smoked fish in Sokoto, Nigeria by Salihu *et al.*(2008).

From this study, smoked fish showed 10.6% total prevalenceof *Listeria* species; the high occurrence could be due to the method of processing and eviscerationwhichallows ample opportunity for contamination to occur as suggested by Salihu *et al.* (2008). Also,the unhygienic conditions of fishhandling, processing, storage conditions, and environmental factors at different levels could serve asreasons for high occurrence in smoked fish samples.Similar prevalence of 9.2% in smoked fish from Jos, Nigeria was reported by Chukwu *et al.* (2006).

The 8.3% occurrence of *Listeria* species in frozen fish found in this study confirms the ability of the pathogen to survive low temperature conditions and possible recontamination of foods under poor processing and handling conditions as reported by Kuzmanovic Jelena *et al.* (2011). The prevalence in this study is lower than the findings of Kuzmanovic Jelena *et al.* (2011) who reported 9.84% prevalence of *Listeria* species in Belgrade, Serbia.

However the prevalence in canned fish in this study was 0%, which could be as a result of effective canning process and good manufacturing practices observed by the processors.

The absence of *Listeria monocytogenes* in this study agrees with the work of Cabedo*et al.* (2008) who worked on salted herring and anchovies in Spain. Kuzmanovic *et al.* (2011) who worked on

heat-treated products and Ready to Eat food (RTE) in Serbia; Thimothe *et al.* (2002) who worked on fish in USA; Miya*et al.* (2010) who worked on dried fish in Estonia and Okonkwo *et al.* (2014) who reported 0% prevalence in Nigeria. However, Eklund and others (1995) observed that the external surfaces of fish might act as inoculating agents that introduce *Listeria* species into processing plants; thus contamination is extended by further operations such as filleting, rinsing, and brining. Therefore, equipment, personnel, and surfaces might serve as secondary contamination sources of *Listeria* species.

The overall prevalence of *Listeria* species in this study shows that human and environmental factors could be attributed to contamination. It is also an indication of poor hygiene level in the processing and handling of fish viz-a-viz poor sanitary and hygienic practices in the fish market and environmentas reported by Chukwu *et al.* (2006). The implication of this is that, the fish sold to consumers in Kaduna North Local Government Area, Nigeria could be contaminated by pathogenic bacteria which are of public health concern. However, since fish is normally heat treated before consumption, the probability of causing alimentary infections is diminished. Danger of alimentary infection in man arises if such products were thermally sub-treated (less than 70^{o}C) for *Listeria* to be inactivated (Wang *et al.*, 2012).

The findings of this study on drug susceptibility showed that *Listeria* speciesisolates were 100% susceptible to the first drug of choice(Gentamicin) used in the treatment of listeriosis, followed by Chloramphenicol (91.7%), Amoxicillin (87.5%) and then Oxacillin (79.2%).

Although the incidence of antibiotic resistance was high in this study, the range of antibiotics to which resistance has been acquired is wide. It is of concern that this expanding range now includes a number of antibiotics used in the treatment of listeriosis, e.g tetracycline and

streptomycin(Liu, 2006).However, the isolation of resistant strains of *Listeria* specieswashigh, even though evidence of the emergence of resistant strains from various sources has been reported (Liu, 2006).

Amoxicillin or penicillin and gentamicin remain the drugs of choice for most manifestations of listeriosis. Trimethoprim is considered to be a second choice therapy; but erythromycin is also used respectively to treat bacteremia and pregnant women diagnosed with listeriosis (Charpentier and Courvalin, 1999). The majority of *Listeria* species isolated in this study were susceptible to the antibiotics commonly used in veterinary and human listeriosis, even though more than one isolate were resistant to Amoxicillin, Erythromycin and Trimethoprim. A similar pattern of resistance has also been found by other authors (Aurelli *et al*., 2003) suggesting the worldwide increase in antibiotic resistance.

Most of the *Listeria* speciesisolates in this study showed high frequency of resistance to Erythromycin and Ciprofloxacin.This suggests the extent of distribution of resistance of *Listeria* species to commonly used antimicrobial agents in the study area.The widespread use of antibiotics in human and veterinary therapy, alongside the length of time over which it has been available in Nigeria and other countries of the world could account for this trend. *Listeria* speciescan either acquire or transfer antibiotic resistances' genes from plasmids and transposons of other bacterial species including *Enterococcus* spp. either *in vivo* or *in vitro* in the intestinal tract (Pourshaban *et al*., 2002).

However,(Akano *et al.*, 2009) reported that the rate of abuse of antibiotics could promote the acquisition of resistance genes by bacteria in developing nations like Nigeria. This finding is also at variance with findings in previous studies conducted where high resistance levels were observed among food bacterial isolates (Ebigwei and Olukoya, 1991; Olasupo *et al.*, 2002a). The

high resistance level among food bacterial isolates have been partly attributed to possible transfer of resistance trait from indigenous micro flora associated with the sources of the raw materials used in the preparation and processing of foods (Olasupo *et al.*, 2002b). Since water is one of such materials used, contamination from environmental isolates is quite a possibility(Olasupo *et al.*, 2002b).

The resistance shown to erythromycin (62.7%) in this studyindicates that, thiswidely used drug may be ineffective in the treatment of listeriosis. The resistance to antibiotics can be due to selective antibiotic pressure (Hanchung *et al*., 2004) or intergons and other insertion elements (Didier *et al.,* 2000).The emergence of antibiotic resistant isolates from fish samples is of medical and public health importance (Hanchun *et al.,* 2004), which will defy almost all the antibiotic chemotherapy.

The Maximum Residue Limit (MRL) for cadmium based on fish type in this study showed 78(43.3%) samples were contaminated with levels above allowable limitspermitted in fish for Cadmuim. The highest cadmium (Cd) levels was observed in smoked fish in which 35 out of 60 samples had levels above allowable limits permitted in fish for Cadmuim, these highlevels is alarming as this has a major public health implication with possible adverse health hazards. The findings in study is in line with the work of Celik *et al.* (2004) and Tuzen, (2003)who reported high Cadmium contents in fish.

The levelof contaminants from this study could be influenced by the durationof exposure of fish to contaminants in water, feedinghabit of fish, concentrations of contaminants in watercolumn, water chemistry, contamination of fish duringhandling and processing, quality of canned fish andshelf life of canned fish as suggested by Iwegbue, (2015).

In this study,125(69.4%), fish sampleswere contaminated with lead (Pb)above allowable limits (0.02µg/g) permitted in fish (E.U., 2005). The highest Pb contamination was observed in smoked fish 47(78.3%) and canned fish 42(70%) samples.The levels of lead in this study(0.01–3.915 µg/g) is higher when compared to the findings of Doherty *et al.,* 2010 (0.395 – 0.62µg/g), Obasohan *et al.,* 2006 (0.10 – 0.83µg/g) in some fishes from Ogba river, Daka *et al.* (2008) who obtained 0.01-0.06µg/g in fish species from Azuabie Creek in the BonnyEstuary, Nigeria.

Although most peoplereceive the bulk of their lead intake from seafood, inspecific populations other sources may be moreimportant, such as water in areas with lead piping andplumb solvent water, air near point of sourceemissions, soil, dust, paint flakes in old houses orcontaminated land(Cesar *et al.,* 2003). Lead in the air contributes to leadlevels in food through deposition of dust and raincontaining the metal, on crops and the soil (Cesar *et al.,* 2003).

The relationship of metal contents in canned food over the storage time is well established in the work of Bigelow that showed tin concentration in canned food increased over periods of a few months to two years (Bigelow, 1916). This was subsequently supported by other investigators who reported that canned food accumulated heavy metals when stored for longer periods (Cichon, 1995). Contamination of Pb in canned food may be attributed to two sources: the solder used in fabrication of can seams and the Pb contained in the tin coating on tinplate.The risk of lead contamination remains, although technology of pure tin solder had been introduced as an alternative to lead solder(Iwegbue, 2015). Higher material cost due to pure tin solder has hindered most of the canned industry from employing it (Iwegbue, 2015). Soldering is a source of lead contamination in the canning process. Therefore, the monitoring of lead concentration in canned fish is important for human health (Voegborlo *et al.,* 1999).

Cans stored for longer periods are likely to be subjected to physical damage such as corrosion and dent. The iron in the mild steel base of tinplatemay dissolve into foods especially when the lacquer coating is damaged. According to Taha´n*et al.*(1995), the pH of the canned product, the quality ofthe lacquer coatings of canned products, oxygenconcentration in the headspace, quality of coatingand storage place may also control metal levels incanned fishes.

The correlation between the occurrence of *Listeria* species and presence of cadmium in this study gave a value of 0.998, which implies a very high positive correlation suggesting that cadmiumcontaminationhas a strong positive influence on *Listeria* species contamination which is in concordance with the work of Elhanafi *et al.* (2010), Gilmour *et al.* (2010) and Ratani *et al.* (2012) who reported that several outbreaks of listeriosis have involved cadmium-tolerant strains.

On the other hand, correlation between the occurrence of *Listeria* species and presence of lead in this study was 0.500, which also implies a fairly positive correlation, but not as strong as for*Listeria* and cadmium. However, between cadmium and lead, the correlation was 0.500, which is a fairly positive correlation, suggesting a relationship between the levels of the two metal ions in fish.

In this study, statistical correlation of the occurrence of heavy metals (cadmium and lead), and *Listeria* species,could have arisen from the contamination of heavy metals in fish. This agrees with previous findings of(Alonso *et al.,* 2001; Baker-Austin *et al.,* 2006; Stepanauskas *et al.,* 2006; summers, 2002)which suggested that metal contamination in natural environments could have an important role in the maintenance and proliferation of bacteria. There is concern regarding the persistence and proliferation of co-resistance to antibiotics and heavy metals in bacterial pathogens in different environments (Baker-Austin *et al.,* 2006; Langsrud *et al.,* 2003).

Resistance to antibiotics and disinfectants, including heavy metals, is important for bacterial survival in contaminated environments (He Yan*et al*., 2014).

Furtherstudies are also needed to identify the possible association between *Listeria* species and heavy metals.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The study established an overall prevalence of 13.3% for *Listeria* species in frozen, canned and smoked fish which could be attributed to poor sanitary and hygienic practices in the fish market and environment. The study showed that all *Listeria* speciesisolates were susceptible (100%) to gentamicin and high frequency of 62.5% resistance to erythromycin.The study also, showed 125(69.4%) and 78(43.3%) fish were contaminated with lead (Pb) and cadmium (Cd) above allowable limits permitted in fish (0.20µg/g for Pb, and 0.05µg/g for Cd (E.U., 2005).

The correlation between the occurrence of *Listeria* species and presence of cadmium in this study was 0.998, which implies a positive correlation.This finding has provided evidence of potential threat to health and safety of the public because a long term consumption of fish containing high levels of heavy metals has deleterious impact on human health.

6.2 Recommendations

From the results of this study, the following are recommended.

1. Fish mongers should always ensure good sanitary conditions within and around the fish processing environments and also maintain a good personal hygiene.
2. Consumers should properly cook fish before consumption and re-heat refrigerated fish to limit the possibility of contamination with *Listeria* species.
3. Public health education, enlightenment and awareness campaigns are needed in fish markets and processing plants.

4. There is need to establish standards for the levels of heavy metals in fish and put in place systems for monitoring.
5. Further studies should involve other indigenous fish species such as cat fish and *Tilapia*, etc, to ascertain their levels of contamination with *Listeria* species and heavy metals.

6.3Limitations of the study

1. Non-probability sampling method was employed in collecting fish samples, therefore the results of this study may not be generalized.

2. Frozen and canned fish samples may be derived from different of countries. The study did not investigate the occurrence of *Listeria* species and heavy metals based on country of origin.

3. Correlation between Cadmium and occurrence of *Listeria* species did not determine the critical level of concentration of Cadmium that could influence the survival of *Listeria* species.

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APPENDIX I

In this study the mean concentrations of cadmium detected in the fish samples were 0.10840±0.05392µg/g in smoked fish; 0.12528±0.01346µg/g in frozen fish and 0.09350±0.01256µg/g in canned fish is nearly 2 times higher than European Union's (EU) maximum residue limit (MRL) permitted in fish 0.05µg/g for Cadmuim (Cd) (E.C, 2005; Ozturk *et al.*, 2009). This study is in agreement with Shareef, (2011) who reported 0.090±0.039 µg/g average concentration of. Cd in *M. furnieri* fish species in Egypt.

Mean (± SEM) - Values of Cadmium (Cd) µg/g and Lead (Pb) µg/g concentrations based onType of Fish samples obtained in Kaduna North L.G.A, Nigeria.

S/N	Heavy metals	Smoked n=60	Frozen n=60	Canned n=60	p- value	
	Cadmium (Cd)	0.10840± 0.05392	0.12528±0.01346	0.09350±0.01256	0.792	NS
	Lead (Pb)	0.17730± 0.03801	0.63705± 0.12101	0.20168±0.36737	0.00	S

P >0.05

Key

NS: Not significant

S: Significant

APPENDIX II

Mean (± SEM) - Values of Cadmium (Cd) µg/g and Lead (Pb) µg/g concentrations in Fish obtained in (3) different markets in Kaduna North L.G.A, Nigeria.

Heavy metals	GM	RM	KW	p- value	Significance
	n=60	n=60	n=60		
Cadmium (Cd)	0.09689± 0.014364	0.14012±0.05393	0.09018±0.01074	0.507	NS
Lead (Pb)	0.27508± 0.06175	0.49572± 0.10687	0.24523±0.61957	0.055	NS

P >0.05

Key

NS: Not significant

RM: Ungwan Rimi market.

GM: Abubakar Gumi market.

KW: Kawo market.

APPENDIX III

Results of lead and cadmium in fish samples obtained from atomic absorption spectrophotometry (AAS).

sample no.	Conc. Of cadmium (µg/g)	Maximum Residue Limit (MRL)
GM 1s	0.066	1
GM 2s	0	2
GM 3s	0.046	2
GM 4s	0.093	1
GM 5s	0.037	2
GM 6s	0.047	2
GM 7s	0	2
GM 8s	0.037	2
GM 9s	0.161	1
GM 10s	0.049	2
GM 11s	0.051	1

GM 12s	0.155	1
GM 13s	0	2
GM 14s	0.226	1
GM 15s	0.064	1
GM 16s	0	2
GM 17s	0.016	2
GM 18s	0.098	1
GM 19s	0.136	1
GM 20s	0.092	1
GM 1 F	0.318	1
GM 2F	0	2
GM 3F	0	2
GM 4F	0	2
GM 5F	0	2
GM 6F	0	2
GM 7F	0	2
GM 8F	0	2
GM 9F	0	2
GM 10F	0	2
GM 11F	0.212	1
GM 12F	0.197	1
GM 13F	0.24	1
GM 14F	0.185	1
GM 15F	0.223	1
GM 16F	0.24	1
GM 17F	0.192	1
GM 18F	0.228	1
GM 19F	0.184	1
GM 20F	0.116	1
GM 1C	0.129	1
GM 2C	0.167	1
GM 3C	0	2
GM 4C	0	2
GM 5C	0	2
GM 6C	0	2
GM 7C	0	2
GM 8C	0	2
GM 9C	0.227	1
GM 10C	0.199	1
GM 11C	0.611	1
GM 12C	0.136	1

GM 13C	0.125	1
GM 14C	0.051	1
GM 15C	0	2
GM 16C	0.1	1
GM 17C	0.158	1
GM 18C	0.016	2
GM 19C	0.157	1
GM 20C	0.028	2

S/No.	sample no.	Conc. Of Lead (µg/g)	MRL
1	GM 1 s	0.105	2
2	GM 2 s	0.157	2
3	GM 3s	0.193	2
4	GM 4 s	0.115	2
5	GM 5 s	0.495	1
6	GM 6s	0.155	2
7	GM 7s	1.066	1
8	GM 8s	0.327	1
9	GM 9s	0	2
10	GM 10s	0.19	2
11	GM 11s	0.118	2
12	GM 12s	0.014	2
13	GM 13s	0.221	1
14	GM 14s	0.089	2
15	GM 15s	0.171	2
16	GM 16s	0.156	2
17	GM 17s	0.175	2
18	GM 18s	0.094	2
19	GM 19s	0.123	2
20	GM 20s	0	2
21	GM 1 F	0.024	2
22	GM 2F	0	2
23	GM 3F	0.102	2
24	GM 4F	0.176	2
25	GM 5F	0.121	2
26	GM 6F	0.363	1
27	GM 7F	0	2
28	GM 8F	0.11	2

29	GM 9F	0.147	2
30	GM 10F	0.046	2
31	GM 11F	0.099	2
32	GM 12F	3.136	1
33	GM 13F	0.891	1
34	GM 14F	0.078	2
35	GM 15F	0.843	1
36	GM 16F	0.851	1
37	GM 17F	0.042	2
38	GM 18F	0.805	1
39	GM 19F	0.057	2
40	GM 20F	0.04	2
41	GM 1C	0.581	1
42	GM 2C	0.041	2
43	GM 3C	0.128	2
44	GM 4C	0.14	2
45	GM 5C	0.103	2
46	GM 6C	0.137	2
47	GM 7C	0.142	2
48	GM 8C	0.108	2
49	GM 9C	0.777	1
50	GM 10C	0.758	1
51	GM 11C	0.101	2
52	GM 12C	0	2
53	GM 13C	0.262	1
54	GM 14C	0	2
55	GM 15C	0	2
56	GM 16C	1.252	1
57	GM 17C	0	2
58	GM 18C	0	2
59	GM 19C	0	2
60	GM 20C	0.08	2

S/No.	sample no.	Conc. Of cadmium (µg/g)	MRL
1	RM 1 s	0	2
2	RM 2 s	0.024	2
3	RM 3s	0.132	1
4	RM 4 s	0.163	1
5	RM 5 s	0	2

6	RM 6s	0	2
7	RM 7s	0	2
8	RM 8s	0	2
9	RM 9s	0	2
10	RM 10s	0	2
11	RM 11s	0	2
12	RM 12s	3.251	1
13	RM 13s	0	2
14	RM 14s	0	2
15	RM 15s	0	2
16	RM 16s	0	2
17	RM 17s	0	2
18	RM 18s	0.002	2
19	RM 19s	0	2
20	RM 20s	0	2
21	RM 1 F	0	2
22	RM 2F	0	2
23	RM 3F	0	2
24	RM 4F	0	2
25	RM 5F	0	2
26	RM 6F	0.19	1
27	RM 7F	0.19	1
28	RM 8F	0.209	1
29	RM 9F	0.235	1
30	RM 10F	0.212	1
31	RM 11F	0.221	1
32	RM 12F	0.179	1
33	RM 13F	0.168	1
34	RM 14F	0.209	1
35	RM 15F	0.167	1
36	RM 16F	0.151	1
37	RM 17F	0.148	1
38	RM 18F	0.205	1
39	RM 19F	0.19	1
40	RM 20F	0.225	1
41	RM 1C	0	2
42	RM 2C	0	2
43	RM 3C	0.209	1
44	RM 4C	0	2
45	RM 5C	0	2
46	RM 6C	0	2

47	RM 7C	0.154	1
48	RM 8C	0.175	1
49	RM 9C	0.122	1
50	RM 10C	0.179	1
51	RM 11C	0.062	1
52	RM 12C	0.223	1
53	RM 13C	0.085	1
54	RM 14C	0.047	2
55	RM 15C	0.139	1
56	RM 16C	0.067	1
57	RM 17C	0.128	1
58	RM 18C	0.115	1
59	RM 19C	0.139	1
60	RM 20C	0.092	1

S/No.	sample no.	Conc. Of Lead (µg/g)	MRL
1	RM 1 s	0.211	1
2	RM 2 s	0.29	1
3	RM 3s	0.031	2
4	RM 4 s	0	2
5	RM 5 s	0.124	2
6	RM 6s	0.036	2
7	RM 7s	0	2
8	RM 8s	0.056	2
9	RM 9s	0.19	2
10	RM 10s	0	2
11	RM 11s	0.137	2
12	RM 12s	0.101	2
13	RM 13s	0.066	2
14	RM 14s	0.071	2
15	RM 15s	0.172	2
16	RM 16s	0.178	2
17	RM 17s	0.124	2
18	RM 18s	0.169	2
19	RM 19s	0	2
20	RM 20s	0.145	2
21	RM 1 F	0.133	2
22	RM 2F	0.101	2
23	RM 3F	0.048	2
24	RM 4F	0.169	2

25	RM 5F	0.697	1
26	RM 6F	2.184	1
27	RM 7F	0.707	1
28	RM 8F	3.915	1
29	RM 9F	0.914	1
30	RM 10F	1.411	1
31	RM 11F	1.873	1
32	RM 12F	0	2
33	RM 13F	0	2
34	RM 14F	1.777	1
35	RM 15F	0.113	2
36	RM 16F	0	2
37	RM 17F	3.506	1
38	RM 18F	1.918	1
39	RM 19F	1.698	1
40	RM 20F	1.509	1
41	RM 1C	0.142	2
42	RM 2C	0.135	2
43	RM 3C	0.799	1
44	RM 4C	0.222	1
45	RM 5C	0.17	2
46	RM 6C	0.18	2
47	RM 7C	0.609	1
48	RM 8C	0.651	1
49	RM 9C	0.268	1
50	RM 10C	0.774	1
51	RM 11C	0.006	2
52	RM 12C	0.88	1
53	RM 13C	0	2
54	RM 14C	0.133	1
55	RM 15C	0	2
56	RM 16C	0	2
57	RM 17C	0	2
58	RM 18C	0	2
59	RM 19C	0	2
60	RM 20C	0	2

S/No.	sample no.	Conc. Of Cadmium (µg/g)	MRL
1	KW 1 s	0.117	1

2	KW 2 s	0.171	1
3	KW 3s	0	2
4	KW 4 s	0.114	1
5	KW 5 s	0	2
6	KW 6s	0	2
7	KW 7s	0.038	2
8	KW 8s	0.153	1
9	KW 9s	0	2
10	KW 10s	0.2	1
11	KW 11s	0.109	1
12	KW 12s	0.078	1
13	KW 13s	0.208	1
14	KW 14s	0.136	1
15	KW 15s	0.011	2
16	KW 16s	0.054	1
17	KW 17s	0	2
18	KW 18s	0.081	1
19	KW 19s	0.011	2
20	KW 20s	0.077	1
21	KW 1 F	0	2
22	KW 2F	0	2
23	KW 3F	0.112	1
24	KW 4F	0	2
25	KW 5F	0	2
26	KW 6F	0	2
27	KW 7F	0	2
28	KW 8F	0	2
29	KW 9F	0	2
30	KW 10F	0	2
31	KW 11F	0.165	1
32	KW 12F	0.217	1
33	KW 13F	0.237	1
34	KW 14F	0.197	1
35	KW 15F	0.22	1
36	KW 16F	0.227	1
37	KW 17F	0.28	1
38	KW 18F	0.192	1
39	KW 19F	0.241	1
40	KW 20F	0.195	1
41	KW 1C	0.068	1
42	KW 2C	0.091	1

43	KW 3C	0	2
44	KW 4C	0	2
45	KW 5C	0.171	1
46	KW 6C	0.054	1
47	KW 7C	0	2
48	KW 8C	0	2
49	KW 9C	0	2
50	KW 10C	0.08	1
51	KW 11C	0.052	1
52	KW 12C	0.087	1
53	KW 13C	0.162	1
54	KW 14C	0.095	1
55	KW 15C	0.155	1
56	KW 16C	0.084	1
57	KW 17C	0.147	1
58	KW 18C	0.114	1
59	KW 19C	0.139	1
60	KW 20C	0.071	1

S/No.	sample no.	Conc. Of Lead (µg/g)	MRL
1	KW 1 s	0.106	2
2	KW 2 s	0.015	2
3	KW 3s	0	2
4	KW 4 s	0	2
5	KW 5 s	0.189	2
6	KW 6s	0.211	1
7	KW 7s	0.122	2
8	KW 8s	0.038	2
9	KW 9s	0.153	2
10	KW 10s	0	2
11	KW 11s	0.006	2
12	KW 12s	0.348	1
13	KW 13s	2.06	1
14	KW 14s	0.111	2
15	KW 15s	0.203	1
16	KW 16s	0.185	2
17	KW 17s	0.216	1
18	KW 18s	0	2
19	KW 19s	0.203	1

20	KW 20s	0.407	1
21	KW 1 F	0	2
22	KW 2F	0	2
23	KW 3F	0.117	2
24	KW 4F	0	2
25	KW 5F	0.045	2
26	KW 6F	0	2
27	KW 7F	0	2
28	KW 8F	0.222	1
29	KW 9F	0.043	2
30	KW 10F	0	2
31	KW 11F	0.031	2
32	KW 12F	0.05	2
33	KW 13F	2.06	1
34	KW 14F	0.91	1
35	KW 15F	0	2
36	KW 16F	1.677	1
37	KW 17F	0	2
38	KW 18F	0.018	2
39	KW 19F	0.719	1
40	KW 20F	1.727	1
41	KW 1C	0.295	1
42	KW 2C	0.507	1
43	KW 3C	0.229	1
44	KW 4C	0.127	2
45	KW 5C	0.558	1
46	KW 6C	0.343	1
47	KW 7C	0.147	2
48	KW 8C	0.116	2
49	KW 9C	0.167	2
50	KW 10C	0	2
51	KW 11C	0	2
52	KW 12C	0	2
53	KW 13C	0.033	2
54	KW 14C	0	2
55	KW 15C	0	2
56	KW 16C	0	2
57	KW 17C	0	2
58	KW 18C	0	2
59	KW 19C	0	2
60	KW 20C	0	2

S/No.	sample no.	Conc. Of cadmium (µg/g)	MRL
1	GM 1s	0.066	1
2	GM 2s	0	2
3	GM 3s	0.046	2
4	GM 4s	0.093	1
5	GM 5s	0.037	2
6	GM 6s	0.047	2
7	GM 7s	0	2
8	GM 8s	0.037	2
9	GM 9s	0.161	1
10	GM 10s	0.049	2
11	GM 11s	0.051	1
12	GM 12s	0.155	1
13	GM 13s	0	2
14	GM 14s	0.226	1
15	GM 15s	0.064	1
16	GM 16s	0	2
17	GM 17s	0.016	2
18	GM 18s	0.098	1
19	GM 19s	0.136	1
20	GM 20s	0.092	1
21	RM 1 s	0	2
22	RM 2 s	0.024	2
23	RM 3s	0.132	1
24	RM 4 s	0.163	1
25	RM 5 s	0	2
26	RM 6s	0	2
27	RM 7s	0	2
28	RM 8s	0	2
29	RM 9s	0	2
30	RM 10s	0	2
31	RM 11s	0	2
32	RM 12s	3.251	1
33	RM 13s	0	2
34	RM 14s	0	2
35	RM 15s	0	2
36	RM 16s	0	2
37	RM 17s	0	2

38	RM 18s	0.002	2
39	RM 19s	0	2
40	RM 20s	0	2
41	KW 1 s	0.117	1
42	KW 2 s	0.171	1
43	KW 3s	0	2
44	KW 4 s	0.114	1
45	KW 5 s	0	2
46	KW 6s	0	2
47	KW 7s	0.038	2
48	KW 8s	0.153	1
49	KW 9s	0	2
50	KW 10s	0.2	2
51	KW 11s	0.109	1
52	KW 12s	0.078	1
53	KW 13s	0.208	1
54	KW 14s	0.136	1
55	KW 15s	0.011	2
56	KW 16s	0.054	1
57	KW 17s	0	2
58	KW 18s	0.081	1
59	KW 19s	0.011	2
60	KW 20s	0.077	1

S/No.	sample no.	Conc. Of cadmium (µg/g)	MRL
1	GM 1 F	0.318	1
2	GM 2F	0	2
3	GM 3F	0	2
4	GM 4F	0	2
5	GM 5F	0	2
6	GM 6F	0	2
7	GM 7F	0	2
8	GM 8F	0	2
9	GM 9F	0	2
10	GM 10F	0	2
11	GM 11F	0.212	1
12	GM	0.197	1

	12F		
13	GM 13F	0.24	1
14	GM 14F	0.185	1
15	GM 15F	0.223	1
16	GM 16F	0.24	1
17	GM 17F	0.192	1
18	GM 18F	0.228	1
19	GM 19F	0.184	1
20	GM 20F	0.116	1
21	RM 1 F	0	2
22	RM 2F	0	2
23	RM 3F	0	2
24	RM 4F	0	2
25	RM 5F	0	2
26	RM 6F	0.19	1
27	RM 7F	0.19	1
28	RM 8F	0.209	1
29	RM 9F	0.235	1
30	RM 10F	0.212	1
31	RM 11F	0.221	1
32	RM 12F	0.179	1
33	RM 13F	0.168	1
34	RM 14F	0.209	1
35	RM 15F	0.167	1
36	RM 16F	0.151	1
37	RM 17F	0.148	1
38	RM 18F	0.205	1
39	RM 19F	0.19	1
40	RM 20F	0.225	1
41	KW 1 F	0	2
42	KW 2F	0	2
43	KW 3F	0.112	1
44	KW 4F	0	2

45	KW 5F	0	2
46	KW 6F	0	2
47	KW 7F	0	2
48	KW 8F	0	2
49	KW 9F	0	2
50	KW 10F	0	2
51	KW 11F	0.165	1
52	KW 12F	0.217	1
53	KW 13F	0.237	1
54	KW 14F	0.197	1
55	KW 15F	0.22	1
56	KW 16F	0.227	1
57	KW 17F	0.28	1
58	KW 18F	0.192	1
59	KW 19F	0.241	1
60	KW 20F	0.195	1

S/No.	**sample no.**	**Conc. Of Lead (µg/g)**	**MRL**
1	GM 1 F	0.024	2
2	GM 2F	0	2
3	GM 3F	0.102	2
4	GM 4F	0.176	2
5	GM 5F	0.121	2
6	GM 6F	0.363	1
7	GM 7F	0	2
8	GM 8F	0.11	2
9	GM 9F	0.147	2
10	GM 10F	0.046	2
11	GM 11F	0.099	2

12	GM 12F	3.136	1
13	GM 13F	0.891	1
14	GM 14F	0.078	2
15	GM 15F	0.843	1
16	GM 16F	0.851	1
17	GM 17F	0.042	2
18	GM 18F	0.805	1
19	GM 19F	0.057	2
20	GM 20F	0.04	2
21	RM 1 F	0.133	2
22	RM 2F	0.101	2
23	RM 3F	0.048	2
24	RM 4F	0.169	2
25	RM 5F	0.697	1
26	RM 6F	2.184	1
27	RM 7F	0.707	1
28	RM 8F	3.915	1
29	RM 9F	0.914	1
30	RM 10F	1.411	1
31	RM 11F	1.873	1
32	RM 12F	0	2
33	RM 13F	0	2
34	RM 14F	1.777	1
35	RM 15F	0.113	2
36	RM 16F	0	2
37	RM 17F	3.506	1
38	RM 18F	1.918	1

39	RM 19F	1.698	1
40	RM 20F	1.509	1
41	KW 1 F	0	2
42	KW 2F	0	2
43	KW 3F	0.117	2
44	KW 4F	0	2
45	KW 5F	0.045	2
46	KW 6F	0	2
47	KW 7F	0	2
48	KW 8F	0.222	1
49	KW 9F	0.043	2
50	KW 10F	0	2
51	KW 11F	0.031	2
52	KW 12F	0.05	2
53	KW 13F	2.06	1
54	KW 14F	0.91	1
55	KW 15F	0	2
56	KW 16F	1.677	1
57	KW 17F	0	2
58	KW 18F	0.018	2
59	KW 19F	0.719	1
60	KW 20F	1.727	1

S/No.	sample no.	Conc. Of cadmium (µg/g)	MRL
1	GM 1C	0.129	1
2	GM 2C	0.167	1
3	GM 3C	0	2
4	GM 4C	0	2
5	GM 5C	0	2
6	GM 6C	0	2
7	GM 7C	0	2
8	GM 8C	0	2
9	GM 9C	0.227	1
10	GM 10C	0.199	1
11	GM 11C	0.611	1
12	GM 12C	0.136	1
13	GM 13C	0.125	1

14	GM 14C	0.051	2
15	GM 15C	0	2
16	GM 16C	0.1	1
17	GM 17C	0.158	1
18	GM 18C	0.016	2
19	GM 19C	0.157	1
20	GM 20C	0.028	1
21	RM 1C	0	2
22	RM 2C	0	2
23	RM 3C	0.209	1
24	RM 4C	0	2
25	RM 5C	0	2
26	RM 6C	0	2
27	RM 7C	0.154	1
28	RM 8C	0.175	1
29	RM 9C	0.122	1
30	RM 10C	0.179	1
31	RM 11C	0.062	1
32	RM 12C	0.223	1
33	RM 13C	0.085	1
34	RM 14C	0.047	2
35	RM 15C	0.139	1
36	RM 16C	0.067	1
37	RM 17C	0.128	1
38	RM 18C	0.115	1
39	RM 19C	0.139	1
40	RM 20C	0.092	1
41	KW 1C	0.068	1
42	KW 2C	0.091	1
43	KW 3C	0	2
44	KW 4C	0	2
45	KW 5C	0.171	1
46	KW 6C	0.054	1
47	KW 7C	0	2
48	KW 8C	0	2
49	KW 9C	0	2
50	KW 10C	0.08	1
51	KW 11C	0.052	1
52	KW 12C	0.087	1
53	KW 13C	0.162	1
54	KW 14C	0.095	1

55	KW 15C	0.155	1
56	KW 16C	0.084	1
57	KW 17C	0.147	1
58	KW 18C	0.114	1
59	KW 19C	0.139	1
60	KW 20C	0.071	1

S/No.	sample no.	Conc. Of Lead (µg/g)	MRL
1	GM 1C	0.581	1
2	GM 2C	0.041	2
3	GM 3C	0.128	2
4	GM 4C	0.14	2
5	GM 5C	0.103	2
6	GM 6C	0.137	2
7	GM 7C	0.142	2
8	GM 8C	0.108	2
9	GM 9C	0.777	1
10	GM 10C	0.758	1
11	GM 11C	0.101	2
12	GM 12C	0	2
13	GM 13C	0.262	1
14	GM 14C	0	2
15	GM 15C	0	2
16	GM 16C	1.252	1
17	GM 17C	0	2
18	GM 18C	0	2
19	GM 19C	0	2
20	GM 20C	0.08	2
21	RM 1C	0.142	2
22	RM 2C	0.135	2
23	RM 3C	0.799	1
24	RM 4C	0.222	11
25	RM 5C	0.17	2
26	RM 6C	0.18	2
27	RM 7C	0.609	1
28	RM 8C	0.651	1
29	RM 9C	0.268	1
30	RM 10C	0.774	1
31	RM 11C	0.006	2

32	RM 12C	0.88	1
33	RM 13C	0	2
34	RM 14C	0.133	2
35	RM 15C	0	2
36	RM 16C	0	2
37	RM 17C	0	2
38	RM 18C	0	2
39	RM 19C	0	2
40	RM 20C	0	2
41	KW 1C	0.295	1
42	KW 2C	0.507	1
43	KW 3C	0.229	1
44	KW 4C	0.127	2
45	KW 5C	0.558	1
46	KW 6C	0.343	1
47	KW 7C	0.147	2
48	KW 8C	0.116	2
49	KW 9C	0.167	2
50	KW 10C	0	2
51	KW 11C	0	2
52	KW 12C	0	2
53	KW 13C	0.033	2
54	KW 14C	0	2
55	KW 15C	0	2
56	KW 16C	0	2
57	KW 17C	0	2
58	KW 18C	0	2
59	KW 19C	0	2
60	KW 20C	0	2

Key

RM: Ungwan Rimi market.

GM: Abubakar Gumi market.

KW: Kawo market.

F: Frozen fish sample

C: Canned fish sample

S: Smoked fish sample

1: above maximum residue limit

2: below maximum residue limit



Plate 1: A 48-hour culture of *Listeria* isolate on Oxford Listeria Agar plate showing typical diagnostic features of aesculin hydrolysis to aesculitin (chocolate brown) around the colonies.



Plate 2: Result of Microbact 12L test on *Listeria* Isolate showing biochemical reaction.

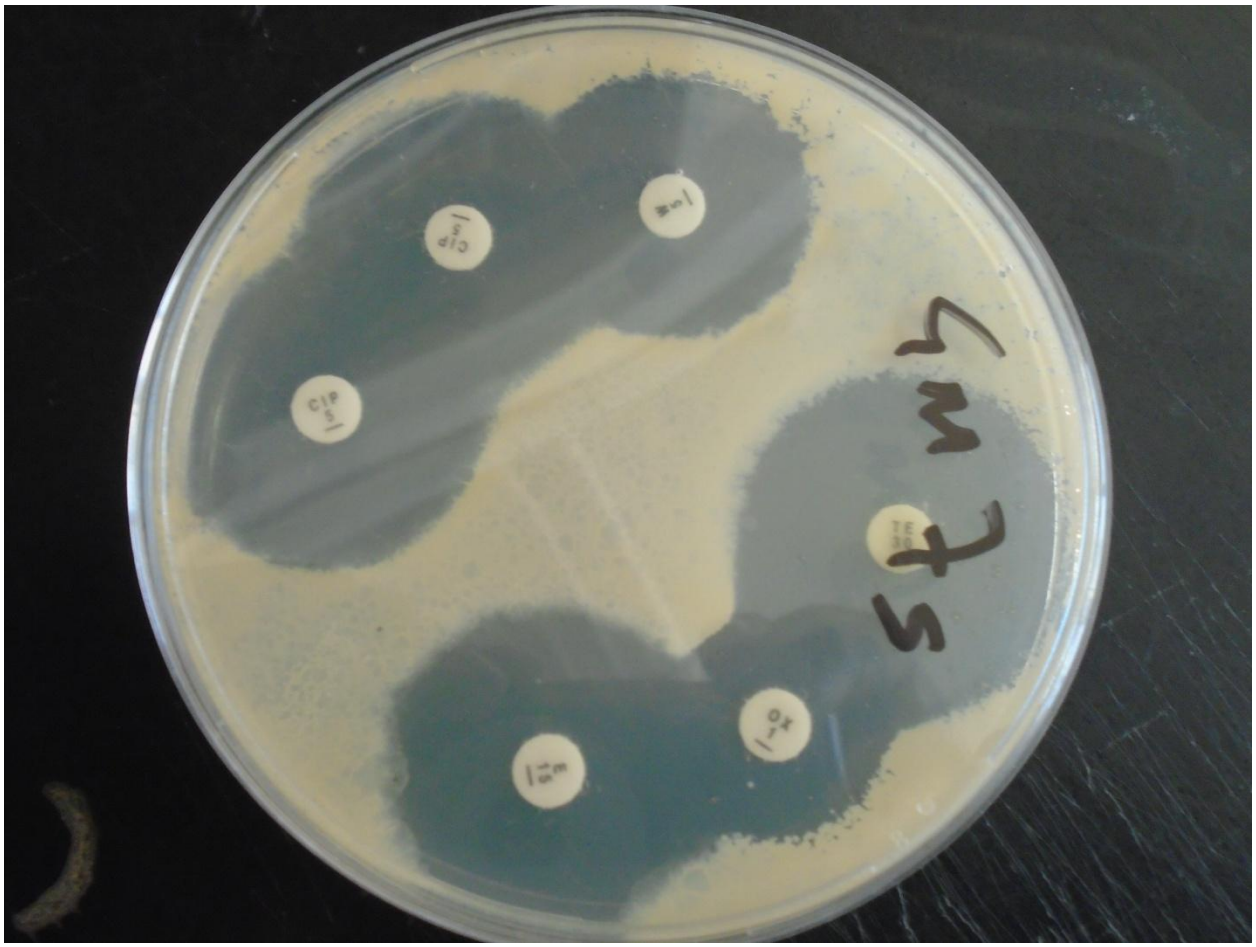


Plate 3: The Antibiotic Susceptibility of *Listeria* specie against each antibiotic on a disc showing potency by inhibition zones.

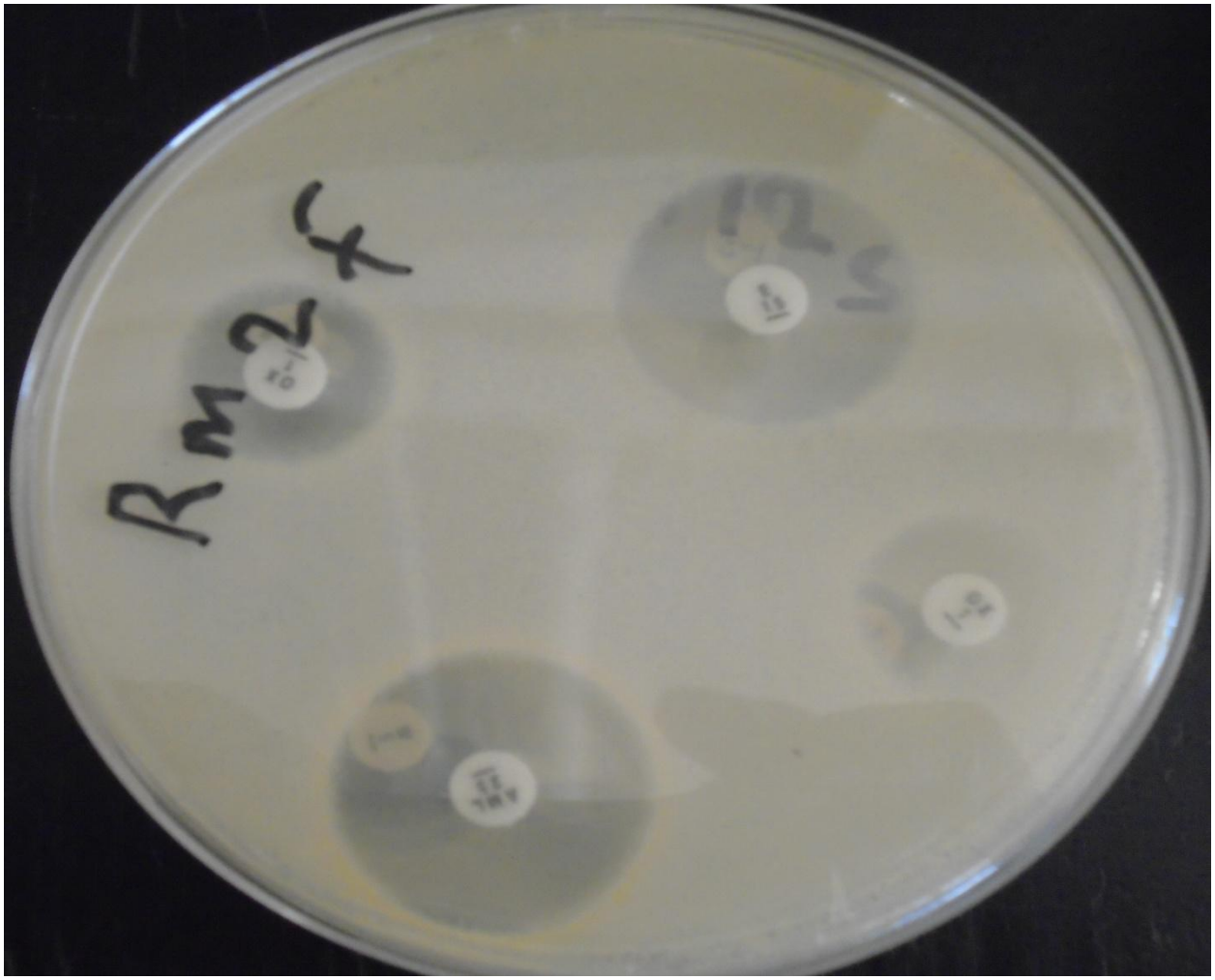


Plate 4: The Antibiotic Susceptibility of *Listeria* specie against each antibiotic on a disc showing drugs potency by inhibition zones.