

**BACTERIOLOGICAL ANALYSIS OF SMOKED FISH IN BENIN METROPOLIS**

**BY**

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**FACULTY OF LIFE SCIENCE**

**UNIVERSITY OF BENIN**

**BENIN CITY**

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**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF DEPARTMENT OF  
MICROBIOLOGY, FACULTY OF LIFE SCIENCES IN PARTIAL FULFILLMENT OF  
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**CERTIFICATION**

This is to certify that this project work was carried out by Divine Onomhe OGA (Miss) of the Department of Microbiology, Faculty of Life Science, University of Benin, Benin City.

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## **DEDICATION**

This project work is dedicated to the Almighty God for his grace and mercies throughout my period of study.

## **ACKNOWLEDGEMENTS**

I wish to acknowledge whole heartedly my project supervisor Prof. (Mrs) E.E. AKORTHA for his patience and understanding towards me and the success of this project. May God Almighty richly bless you ma for your efforts.

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## ABSTRACT

Microbiological quality is of importance to public health as it directly relates to spoilage of fish and food poisoning. This study was aimed at evaluating bacteria isolates from smoked fish sold in various location in Ekosodin. The bacterial isolates were characterized and identified using morphological and biochemical methods. The percentage distribution and frequency of the isolates were evaluated using statistical method. From the result obtained in this study, the total heterotrophic bacterial count ranged from  $3.22 \pm 1.5 \log_{10} \text{cfu/g}$  to  $4.550228 \pm 1.00 \log_{10} \text{cfu/g}$ . Using the cultural, morphological and biochemical test results, the bacterial isolates obtained in this study were *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* sp, *Enterobacter* sp, *Pseudomonas* sp and *Shigella* sp. The frequent of the bacterial isolation showed that *E.coil* has the highest percentage of 30%. The bacteria is obtained from this study were all susceptible to few antibiotics namely perfloxacin,, amoxicillin, rocephin and ciprofloxacin but were resistant to ampiclox, zinnacef, sulfamethoxazole and gentamicin. In conclusion, this study has revealed that the level of microbial contamination of dry fish products could either be environmentally dependent. It also revealed fish contamination is a problem and consumption of uncooked smoked fish can be hazardous and should be avoided.

## CHAPTER ONE

### 1.0 INTRODUCTION

#### 1.1 BACKGROUND TO THE STUDY

Fish and fishery products play a significant role as a dietary staple for a considerable portion of the global population, with an average per capita consumption of 20.1 kg (FAO, 2016). Fish is considered to be an affordable and easily obtainable source of protein in developing nations. It is particularly suitable for supplementing the predominantly carbohydrate-based diets of the West African population (Adeyeye *et al.*, 2015; Ikutegbe and Sikoki, 2014). Fish is considered to be the most perishable type of muscle food, as it experiences a decline in freshness following death as a result of autolytic and microbial spoilage (Dehghani *et al.*, 2018; Matak *et al.*, 2015). The preservation of fresh fish in tropical regions continues to be a challenge due to insufficient infrastructure and the adverse effects of environmental and climatic conditions, resulting in rapid spoilage within a short period of time (Anihouvi *et al.*, 2012). In order to mitigate fish spoilage and minimise losses after capture, a range of preservation techniques are employed, such as frying, fermentation, drying, salting, and smoking (Adeyeye *et al.*, 2015; Ikutegbe and Sikoki, 2014).

The process of smoking involves subjecting fish to the direct or indirect effects of smoke, which occurs as a result of the incomplete combustion of specific types of trees used as fuel (Chakroborty and Chakraborty, 2017; Yusuf *et al.*, 2015). The consumption of smoked fish is widely prevalent across the globe due to its distinct flavour, nutritional benefits, and extended shelf life in comparison to fresh fish (Özyurt *et al.*, 2009). According to Kpodékon *et al.* (2014), fish products serve as the primary and crucial source of animal proteins in Benin. Traditional smoking is a commonly employed technique for fish preservation within the country (Dégnon *et*

*al.*, 2013). This method yields two distinct end products, namely smoked fish (SF) and smoked-dried fish (SDF), which are either consumed locally or exported to neighbouring communities.

The smoking process serves the dual purpose of facilitating the dehydration of fish and enhancing its flavour, while concurrently establishing an environment that is less favourable for the proliferation of microorganisms (Goulas and Kontominas, 2005). However, the preservation technique employed, smoked fish is not completely impervious to spoilage. Various microorganisms, including bacteria, yeasts, and molds, have been implicated in the spoilage of smoked fish. However, bacteria are arguably the most significant players due to their ubiquity, diverse metabolic capacities, and rapid growth rates under suitable conditions. In fact, specific bacterial communities have been directly associated with the spoilage process, compromising the product's safety, palatability, and shelf life (Gram and Dalgaard, 2002). The presence of bacterial contamination in smoked fish has been found to result in spoilage, leading to a decrease in quality, safety, and shelf life of the product (Gramme and Huss, 2000).

Fish spoilage is caused by a variety of bacteria, including *Pseudomonas*, *Shewanella*, *Lactic Acid Bacteria*, and *Photobacterium*, which have been identified as some of the predominant microorganisms involved in this process (Dalgaard, 1995; Joffraud *et al.*, 1998). Veld (1996), argued that the presence of these bacteria has the potential to impact various sensory attributes of fish, including its visual appearance, texture, olfactory perception, and gustatory experience. In addition, it has been observed that certain bacterial species have the ability to synthesize toxins that pose a threat to human well-being (Gramme and Dalgaard, 2002). The investigation of the role of bacteria in the spoilage of smoked fish is crucial for the advancement of preservation techniques and the assurance of food safety (Ayeloja *et al.*, 2018; Likongwe *et al.*, 2018;

Udochukwu *et al.*, 2016). However, the aim of this study is to investigate the role of bacteria in the spoilage of smoked dried fish

## **1.2 AIM AND OBJECTIVES:**

The aim of this study was to identify the bacteria associated with spoilage of smoked fish and their antibiotic susceptibility pattern.

The specific objectives of this study were:

1. enumeration of bacterial load associated with spoiled smoked fish
2. phenotypic identification of bacteria associated with spoiled smoked fish
3. antibiotic profiling of bacterial isolates obtained from smoked fish

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 Smoked fish

Smoked fish, a globally appreciated delicacy, is susceptible to spoilage caused by a multitude of factors, one of the most significant being bacterial action. Bacterial spoilage not only impacts the product's sensory attributes flavor, aroma, texture, and visual appeal but also poses a potential risk to human health. Hence, understanding the microbial dynamics during the storage of smoked fish is pivotal for enhancing product shelf-life, quality, and safety. A wealth of research has been dedicated to identifying the predominant spoilage bacteria in smoked fish and elucidating their spoilage mechanisms. This literature review aims to synthesize the current understanding on the role of bacteria in the spoilage of smoked fish, taking into account the critical bacterial species involved, their spoilage pathways, and their interactions within the smoked fish matrix. It encompasses a range of studies, from the fundamental identification of spoilage bacteria to the complex biochemical processes they engage in during spoilage.

#### 2.2 Microbes in Dry Fish

According to Busschaert *et al.* (2011), approximately 33% of global food production is lost each year due to microbial spoilage. Microbial activity is the primary cause of spoilage in the majority of fresh seafood and a number of lightly preserved seafood products (Lund *et al.*, 2000). According to Cassin *et al.* (2010), smoked fish and shellfish products have the potential to harbour microbial hazards such as *Listeria monocytogenes*, *Salmonella species*, and *Clostridium botulinum*. According to a study conducted by Omojowo and Ihuahi (2006), the analysis of smoked fish samples obtained from four local markets in the Kainji Lake area of Nigeria

revealed a prevalence of gram-positive bacteria, potential pathogens, coagulase-positive *Staphylococcus*, and *Escherichia coli*.

Fresh fish is considered to be free from microorganisms, however, it is highly vulnerable to deterioration immediately after being caught (Cassin *et al.*, 2010). The process of fish deterioration commences immediately following the death of the fish and continues until complete degradation of the fish occurs. The perishability of deceased fish in the Southern region of Nigeria is estimated to be between 16 and 20 hours, while in the Northern region, it ranges from 20 to 36 hours, primarily as a result of biochemical alterations occurring postmortem (Arvanitoyannis *et al.*, 2009). The rate at which fish deteriorates is influenced by factors such as hygienic conditions, storage temperature, acidity, and the composition of the muscular tissue (Atanassova, *et al.*, 2008).

The rapid spoilage of fish is influenced by the chemical breakdown of protein content, fat content (which acts as an agent of rancidity and off-flavour), and the water content or water activity (Daramola *et al.*, 2007). According to a study conducted by Eklund *et al.* (2014), there is evidence suggesting that the microbial load of fish increases as temperatures rise, leading to accelerated spoilage. The spoilage of a majority of fresh seafood and certain lightly preserved seafoods can be attributed to microbial activity (Lund *et al.*, 2000). The magnitude of fish damage is contingent upon various factors, including the methods employed in processing, the specific species of fish being processed, weather conditions, and the manner in which the fish is stored during transportation (Arvanitoyannis *et al.*, 2009).

According to Eklund *et al.* (2014), fish is characterised by its delicate texture and susceptibility to damage. Consequently, mishandling and the occurrence of bruises can lead to the contamination of fish flesh. Fish will become unsuitable for human consumption within

approximately 24 hours of being caught, unless it undergoes some type of processing or preservation. According to Shewan (2000), the fish may still experience various forms of loss and spoilage even after undergoing processing, especially if traditional methods have been employed. The composition of microbial flora found in freshly harvested fish is primarily determined by the surrounding environment in which the fish are captured, rather than being influenced by the specific fish species. Consequently, the native microbial communities present in fish can exhibit considerable variations (Shewan, 2000). Fish, due to their delicate tissues and the presence of water in their environment, exhibit a high vulnerability to microbial contamination. According to Eklund *et al.* (2014), a significant number of bacteria, including those with the potential to cause spoilage, can be found in the surface slime, gills, and intestines of live fish. However, it is important to note that the flesh of the fish is typically devoid of bacterial presence. The body's innate defence mechanisms serve to inhibit bacterial growth and invasion on fish during their lifespan. However, upon death, the breakdown of these defence systems allows for the proliferation and infiltration of bacteria within the flesh. Eklund *et al.* (2015) opined that, the preservation of fish quality occurs soon after the fish's demise. Nevertheless, spoilage rapidly occurs due to a rise in the surrounding temperature, creating favourable conditions for the proliferation of microorganisms. Aberoumand (2010) asserts that *Escherichia coli* serves as a paradigmatic illustration of enteric bacteria that result in the occurrence of gastroenteritis. *E. coli*, along with other coliforms and bacteria such as *Staphylococcus species* and occasionally *enterococci*, are frequently employed as indicators of potentially unsafe conditions in fish processing. The presence of microorganisms in smoked fish presents a significant risk to the general population due to the potential transmission of these

microorganisms, which can compromise the immune system of the consumer, typically humans, and facilitate the onset of various diseases (Arvanitoyannis *et al.*, 2009).

The composition of microbial communities in fish is influenced by the microbial composition of their aquatic environment. It has been observed that the slime layer present on the surface of fish harbours a diverse range of bacterial genera (Ellouze *et al.*, 2010).

There are numerous hazards associated with the consumption of raw fish harvested from contaminated water. Additionally, due to the considerable microbial presence in freshly harvested fish, it is highly prone to rapid deterioration. Therefore, the preservation of fresh fish assumes significant importance. Various methods can be employed to accomplish this objective, including freezing, smoking-induced drying, sun-drying, and canning (El-Kest *et al.*, 2011).

### **2.3 Contaminants of Dry Fish**

After processing, the goods are packed into jute bags or baskets created locally so they may be transported to the country's numerous markets. The products are frequently not stored and wrapped correctly. As a result, moisture is retained and fish is contaminated after processing. According to Sikoki and Aminigo (2002), the first week of storage was the time when changes in the moisture content of smoked fish were the most noticeable, and the bacterial population also grew during this time.

Microbes have posed a severe hazard to human health by contaminating fish and other fisheries products. Fish spoiling occurs when it leaves its native environment (water), and there are four basic causes of this. These are as follows: The breakdown of 35 proteins and lipids into amino acids and fats by muscular enzymes is known as autolysis, which typically occurs before bacterial deterioration. Microorganism activity, which utilises the amino acid generated by autolysis for proliferation (Hudson *et al.*, 2012), is another contributor. Others include insect

attack and chemical deterioration, both of which significantly worsen things. The majority of microbial action, however, is what leads to the deterioration of fresh and highly preserved fish items. As a result, foods with high sugar/salt concentration are more prone to perish from any type of microbe (Huss *et al.*, 2010). Following the use of some dried fish feed and food, major disease outbreaks have reportedly occurred in both humans and animals (Huss *et al.*, 2010).

This might occur as a result of harmful microorganisms like *Escherichia coli*, *Salmonella typhi*, and *Vibrio cholera* that arise through incorrect handling, cross-contamination, and processing of ready-to-eat "smoked fish" items. *Listeria monocytogenes* and *Clostridium botulinum* are two other pathogens of major significance. If proper care is not taken throughout the smoking process, the chances of other food borne viruses contaminating products increase due to extensive handling (Eklund *et al.*, 2014).

## **2.4 Fish Spoilage Bacteria**

### **2.4.1 Lactic Acid Bacteria (LAB)**

Lactic acid bacteria (LAB) represent a group of gram-positive, facultative anaerobic or microaerophilic bacteria that are well known for their role in the fermentation and spoilage of many foods, including smoked fish (Cocolin *et al.*, 2001; Vinderola *et al.*, 2019). Some species such as *Lactobacillus sakei* and *Lactococcus piscium* are frequently found in smoked fish and can contribute to the spoilage through various mechanisms including the production of lactic acid, acetic acid, ethanol, carbon dioxide, and biogenic amines (Bjornsdottir *et al.*, 2009; Joffraud *et al.*, 2006).

### **2.4.2 *Photobacterium phosphoreum***

*P. phosphoreum* is a gram-negative bacterium that can be readily obtained from the intestinal tracts of different fish species (Gramme and Huss, 1996). The aforementioned organism exhibits a wide distribution within the marine ecosystem and serves as the primary bacterium responsible

for the deterioration of various fish products derived from both freshwater and saltwater sources. *P. phosphoreum* is known to significantly contribute to the deterioration of freshwater fish, as documented by Gramme and Huss in 1996. According to Leroi's (1998) research, it was determined that the development of *P. phosphoreum* is dependent on an approximate salt concentration of 2.5%. Leroi (2010) further reviewed the topic and found that higher concentrations of salt significantly hindered the growth of *P. phosphoreum*, with no growth observed at concentrations exceeding 6%. According to Kuts and Ismailov (2009), the optimal pH range for various strains of *P. phosphorum* is found to be between 7.0 and 8.5. *P. phosphoreum* has been identified as a microorganism that is capable of converting trimethylamine oxide (TMAO) into trimethylamine (TMA) in fish products during storage. Additionally, this microorganism has been found to play a role in the production of histamine (Gramme and Huss, 1996). According to Leroi's review in 2010, the presence of this bacterium results in the generation of pungent odours in fish products due to the decrease in TMAO levels.

#### **2.4.3 *Vibrio vulnificus***

*Vibrio vulnificus* is a type of bacterium that is classified as gram-negative, halophilic, motile, and rod-shaped. It is commonly found in marine environments, specifically in estuaries, brackish ponds, and coastal areas. This information is supported by studies conducted by Drake *et al.* (2007), Han and Ge (2010), Ji *et al.* (2011), and Quan *et al.* (2010). The microorganism in question is classified as an opportunistic pathogen in humans. It has been successfully identified in various types of seafood, such as shrimps, fish, and shellfish, including oysters, crabs, and clams (Gopal *et al.*, 2005; Ji *et al.*, 2011). *Vibrio vulnificus* possesses the capacity to induce severe and frequently lethal infections, encompassing primary septicemia, gastroenteritis, and wound infections in the human population (Chiang and Chuang, 2003; Ji *et al.*, 2011). Individuals who are susceptible to infection are characterised by chronic illnesses that result in

increased levels of iron in the blood, abnormalities in immune function, and other long-term disorders (Ji *et al.*, 2011; Oliver and Kaper, 2001). The abundance of *V. vulnificus* is significantly impacted by the salinity and temperature conditions of the water in its native environments. The organism exhibits preference for low salinity levels ranging from 0.5% to 2.5% and relatively high temperatures exceeding 20 °C (Motes *et al.*, 1998). *Vibrio* species are believed to be commonly found in CSS, as suggested by previous studies (Joffraud *et al.*, 2006; Matamoros *et al.*, 2009). However, it has been determined that *Vibrio spp.* do not contribute to the production of off-odors (Joffraud *et al.*, 2006).

#### **2.4.4 *Pseudomonas putida***

*Pseudomonas putida* is a bacterium characterised by its rod-shaped morphology and gram-negative cell wall structure. It is commonly distributed in various terrestrial and aquatic environments, particularly those rich in oxygen, such as soil and water habitats. The microorganism exhibits optimal growth within a temperature range of 25-30 °C and can be readily isolated. *Pseudomonas putida* is classified as a saprophytic bacterium, known for its ability to obtain nutrients from decaying organic matter. In contrast, other species within the *Pseudomonas* genus, such as *Pseudomonas aeruginosa* and *Pseudomonas syringae*, are considered opportunistic pathogens, capable of causing infections in certain circumstances (Altinok *et al.*, 2006). *Pseudomonas spp.* are not commonly acknowledged as spoilage bacteria for cold-smoked salmon (CSS); however, they have been identified as particular spoilers of ice-stored tropical freshwater fish (Gramme and Huss, 1996). The spoilage of iced fish by *Pseudomonas spp.* is commonly associated with the presence of odours and flavours characterised by fruity, rotten, and sulfhydryl notes. According to Gramme and Huss (1996), *Pseudomonas spp.* is capable of synthesising various volatile aldehydes, ketones, esters, and sulphides.

#### **2.4.5 *Shewanella putrefaciens***

*Shewanella putrefaciens* is also implicated in the spoilage of smoked fish, particularly in vacuum-packed products (Parlapani *et al.*, 2015). It is capable of producing a wide range of spoilage-associated metabolites, including volatile sulfur compounds such as hydrogen sulfide, methanethiol, and dimethyl disulfide, which contribute to the unpleasant odors of spoiled fish (Gram *et al.*, 2002; Vendrell *et al.*, 2006).

#### **2.4.6 *Enterobacteriaceae***

*Enterobacteriaceae*, including species such as *Escherichia*, *Salmonella*, and *Yersinia*, are commonly found in the marine environment and can be involved in the spoilage of smoked fish under inadequate storage conditions (Ijabadeniyi, 2007; Dalgaard, 1995). Their growth can lead to the production of biogenic amines, gas, off-odors, and slime, thus significantly contributing to the spoilage (Jørgensen *et al.*, 2000).

### **2.5 Spoilage Mechanism**

The spoilage of smoked fish is a multifaceted biochemical process that involves the coordinated activity of bacterial communities, resulting in the degradation of the product's sensory, nutritional, and safety characteristics (Gramme *et al.*, 2002; Huss, 1995). The degradation of fish is predominantly influenced by the proteolytic and lipolytic actions of specific spoilage bacteria. These actions result in the generation of volatile compounds and other degradation products, which subsequently impact the fish's colour, texture, flavour, and odour (Borch *et al.*, 1996; Gramme and Dalgaard, 2002).

Proteolysis, which refers to the enzymatic degradation of proteins into smaller polypeptides and amino acids, constitutes a significant mechanism in bacterial spoilage (Stenström and Molin, 1990; Gramme *et al.*, 1990). Several bacterial species, including *Pseudomonas spp.* and

*Shewanella putrefaciens*, exhibit potent proteolytic activity, playing a substantial role in the breakdown of proteins in smoked fish (Gramme, 1993). The proteolytic actions frequently lead to the liberation of volatile compounds such as ammonia and hydrogen sulphide, which are primarily accountable for the unpleasant odours linked to deteriorated fish (Leisner *et al.*, 1996).

Likewise, the process of lipid degradation, commonly referred to as lipolysis, holds considerable importance in the context of fish spoilage. The process entails the enzymatic breakdown of lipids into free fatty acids and glycerol, which can subsequently be metabolised by bacteria, resulting in the production of undesirable flavours (Venugopal and Shahidi, 1996; Duflos *et al.*, 2005). Gramme (1993) and Dalgaard (2000) have reported that bacterial species, such as *Pseudomonas spp.* and *Shewanella putrefaciens*, exhibit significant lipolytic activities.

*Lactic acid bacteria* (LAB) constitute an additional significant category of spoilage microorganisms found in smoked fish. According to Halász *et al.* (1994), these organisms have the ability to synthesise biogenic amines, such as histamine, by decarboxylating specific amino acids. Histamine, a vasoactive amine, has the potential to cause both spoilage and adverse health effects when consumed in excessive quantities. Scombroid poisoning, a condition characterised by symptoms such as flushing, headaches, and hives, can result from high levels of histamine ingestion (Lehane and Olley, 2000). The production of histamine in smoked fish has been associated with the presence of LAB, such as *Enterococcus*, *Lactobacillus*, and *Lactococcus* (Bjornsdottir *et al.*, 2009).

Furthermore, a number of studies have emphasised that the synthesis of these volatile compounds and biogenic amines can be impacted by multiple factors, such as the initial microbial population, the composition of the bacterial communities, the temperature at which the

fish is stored, and the particular processing techniques employed (Joffraud *et al.*, 2001; Emborg *et al.*, 2005).

In addition to proteolysis and lipolysis, specific bacterial species have the potential to contribute to the deterioration of smoked fish by means of carbohydrate fermentation. The fermentative bacteria, including certain strains of *lactic acid bacteria* (LAB), have the ability to metabolise sugars present in fish, resulting in the production of acidic compounds, alcohols, and gases. These metabolic byproducts contribute to the further degradation of the sensory characteristics of the product (Holzapfel, 1997; Dalgaard, 2000). It is worth mentioning that these fermentation processes can also result in a decrease in pH, thereby potentially impacting the overall microbiological stability of the fish product (Luong, 2015).

An additional intriguing facet of bacterial spoilage in smoked fish pertains to the possibility of synergistic interactions occurring among diverse bacterial species. Several studies have indicated that the coexistence of specific bacteria can augment the growth or spoilage capabilities of other bacteria, leading to a more accelerated and intensified deterioration compared to what would be anticipated based solely on the individual activities of bacterial species (Stohr *et al.*, 2001; Mejholm and Dalgaard, 2009).

The smoking process used in the preparation of fish can result in the formation of specific compounds, including polycyclic aromatic hydrocarbons (PAHs). These PAHs have the potential to cause mutations in the DNA of bacteria, thereby potentially increasing their resistance to different preservation methods and ultimately contributing to the spoilage of the fish (Alomirah *et al.*, 2011).

Furthermore, the presence of spoilage bacteria may be introduced through post-processing contamination, thereby resulting in product deterioration during storage, even subsequent to the smoking process. The occurrence of this phenomenon can be attributed to inadequate handling techniques, substandard hygiene practises, or the utilisation of packaging materials that have been contaminated (Odeyemi and Ahmad, 2017).

The examination of spoilage bacteria in smoked fish encompasses not only the comprehension of the mechanisms that contribute to the degradation of the product, but also encompasses the advancement of techniques for the identification and detection of these bacteria. The utilisation of rapid and precise detection methods, such as polymerase chain reaction (PCR) and other molecular techniques, plays a pivotal role in guaranteeing the safety and quality of smoked fish products (Roth *et al.*, 2005; Parlapani *et al.*, 2015).

## **2.6 Prevention of Smoked Fish from Bacteria Spoilage**

Antimicrobial compounds can be naturally present in food as a result of processing or intentionally added as an ingredient (Ray, 2014). In order for an antimicrobial to be considered a food additive, it must meet the safety requirements outlined in the legislation of the respective country. According to the American Food and Drug Administration (FDA, 2009), it is a requirement in the United States for additives to be listed as GRAS (Generally Recognised as Safe). The classification of antimicrobials can be determined by their properties and activity, resulting in two categories: direct and indirect. The prevention of microbial spoilage in smoked fish commonly involves the utilisation of various antimicrobial compounds such as nitrites, sulphides, and organic acids (Chiple, 2015).

### **2.6.1 Nitrites**

In order to prevent the growth of *Clostridium botulinum* and improve the colour of fish products, nitrites are commonly incorporated as salts, such as sodium nitrite or potassium nitrite, along with NaCl, ascorbate, and erythorbate (Ray, 2014). In addition to their notable antimicrobial properties in the regulation of anaerobic bacteria such as *C. botulinum*, *Staphylococcus aureus*, and *Yersinia enterocolitica*, nitrite salts have been found to be highly efficient in managing issues related to colour, odour, and lipid oxidation (Sindelar and Houser, 2009). Despite the incomplete understanding of its antimicrobial mechanisms, it is established that nitrites can engage in reactions with enzymes within vegetative cells and germinating spores. Additionally, nitrites can restrict the utilisation of iron by bacteria and impede transport processes by interfering with membrane permeability (Ray, 2014).

Previous research has demonstrated that the bactericidal properties observed during fish smoking are not solely attributed to nitrite, but rather to compounds that are derived from nitrite (Cammack *et al.*, 2009). According to Ray (2014), the antibacterial effect of nitrite is intensified under acidic conditions (pH 5-6) and when reducing agents like ascorbate and erythorbate are present. The source of apprehension is linked to the potential development of carcinogenic nitrosamines through extended periods of exposure. Nevertheless, existing epidemiological research has not established a definitive link between the intake of nitrate and nitrite from smoked fish and the development of any particular type of cancer or an increased risk of cancer (Sindelar and Houser, 2009).

### **2.6.2 Sulphites**

The application of sulphiting agents for the purpose of managing undesired microbial growth and activity in smoked fish has a long history, with evidence of its use dating back to ancient Roman times. According to the US Code of Federal Regulations, sulphur dioxide and its salts, including potassium bisulphite, potassium metabisulphite, sodium bisulphite, sodium metabisulphite, and

sodium sulphite, are classified as Generally Recognised as Safe (GRAS), as long as they are not utilised in food products that are acknowledged as significant dietary contributors of thiamine (Walker, 2010). Sodium sulfite serves as an antimicrobial agent in various food products, including soft fruit, fruit juices, wines, beverages, sausages, pickles, and fish. Sulphides have been found to exhibit efficacy against moulds, yeasts, and aerobic Gram-negative bacilli. Microbial activity is generated through the interaction of undissociated sulphurous acid with thiol groups present in proteins, enzymes, and cofactors (Ray, 2014).

In their study, Omojowo *et al.* (2009) conducted a comparative analysis of the effects of metabisulphite and sorbate salts, at concentrations ranging from 1% to 3%, on the safety and shelf life of smoked catfish. The researchers indicated that both interventions demonstrate efficacy in reducing the presence of *E. coli* and *Streptococcus* sp. Additionally, it was observed that the presence of potassium sorbate did not result in any significant impact on the count of *Staphylococcus* bacteria during the entire storage period. However, it was found that only a 5% concentration of sodium metabisulphite was effective in reducing the count of *Staphylococcus* bacteria to zero, and this reduction was maintained until the conclusion of the eighth week of storage.

### **2.6.3 Lactic acid/lactates**

The application of lactic acid as a rinse for smoked fish has demonstrated certain antimicrobial properties. The ability to inhibit bacterial growth is attributed to the decrease in pH levels that prevent the bacteria from initiating their growth process. In a study conducted by Sallam (2007), the objective was to assess the shelf life, chemical quality, and sensory attributes of smoked fish that underwent treatment by immersion in a 2.5% aqueous solution of sodium acetate (NaA), sodium lactate (NaL), or sodium citrate (NaC). The evaluation was performed under refrigerated

storage conditions at a temperature of 1°C. The author observed a noteworthy decrease in the K value, Hypoxanthine (Hx) concentration, Total Volatile Base Nitrogen (TVBN), and Trimethylamine (TMA) levels in the processed smoked fish, as indicated by the results of the chemical analyses. The estimated shelf life for the control group was 8 days, while the groups treated with Sodium Lactate (NaL), Sodium Chloride (NaC), and Sodium Acetate (NaA) had estimated shelf lives of 12, 12, and 15 days, respectively.

According to Matamoros *et al.* (2009), lactic acid bacteria demonstrate efficacy in the suppression of undesirable microorganisms in food through the production of lactic acid and other organic acids. In their study, Matamoros *et al.* (2009) successfully isolated multiple species of psychrotrophic lactic acid bacteria from a diverse range of smoked fish samples. In their study, Tome *et al.* (2008) effectively managed the growth of the pathogenic bacterium *Listeria monocytogenes* on cold-smoked salmon by employing various strains of lactic acid bacteria. The strain *Enterococcus faecium*, belonging to the group of lactic acid bacteria, exhibited the most effective outcomes in terms of control, as observed in the conducted study.

#### **2.6.4 Ascorbic acid**

Studies have demonstrated that ascorbic acid (commonly known as vitamin C), sodium ascorbate, and D-isoascorbate (also known as erythorbate) possess the ability to augment the antimicrobial properties of sulfites and nitrites. The increased level of activity can be attributed to both the antioxidant properties and the sequestration of iron (Tompkin *et al.*, 2007).

#### **2.6.5 Benzoic acid**

Benzoic acid and sodium benzoate serve as preservatives in acidic products. A study was conducted by Efluvwev and Ajiboye (2006) to investigate the efficacy of various chemical preservatives in controlling microbial spoilage of smoked catfish. According to their findings,

the efficacy of sodium benzoate-treated samples was observed to be limited to the initial four-day period following treatment in a tropical environment. According to a study conducted by Van Spreekens (2007), it was found that the growth of Pseudomonad-like bacteria, which are known to spoil smoked fish, could be effectively inhibited by the addition of a minimum concentration of 0.8% benzoic acid as a preservative.

In their study, Dąbrowski *et al.* (2012) employed both qualitative and quantitative research methods to investigate the microflora present in low-salt smoked fish samples. These samples were further supplemented with varying concentrations of sodium benzoate, specifically 0%, 0.2%, and 0.3%. The researchers noted that the presence of sodium benzoate resulted in a decrease in the overall diversity of bacteria and yeasts within the tested product, while having no impact on the total quantity of bacteria and yeasts present. The evidence presented indicates that the elimination of certain species by the preservative resulted in the creation of an unoccupied ecological niche on the smoked fish, which was subsequently filled by other species.

## **2.7 Empirical Review**

The presence of bacteria associated with smoked fish in Nigeria has been documented in various regions across the nation. A study was conducted by Ibrahim *et al.* (2014) in the urban area of Minna to perform an in-vitro assay aimed at determining and identifying the primary bacterial contaminants. Samples of smoked fish were obtained from three distinct markets. The identification and characterization of different isolates were conducted using the gram-staining technique and biochemical tests. The species selected for the study was *Clarias gariepinus*. The results of the in-vitro assay indicated that the samples were contaminated with six bacterial species, namely *Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus epidermis*, *Salmonella epidermis*, *Salmonella typhi*, *Streptococcus spp*, and *Shigella spp*.

In the study conducted by Omojowo *et al.* (2010) in New Bussa, Niger State, the authors examined the microbial quality and safety of smoked catfish (*Clarias gariepinus*) that had been treated with sodium chloride (table salt) and stored at room temperature for a duration of 8 weeks. Thirteen bacterial isolates were acquired from samples of smoked fish. There were a total of eleven (11) rod-shaped specimens and two (2) cocci-shaped specimens. All isolates, except for one, exhibited positive catalase activity. The isolates were classified into six genera, specifically *Bacillus*, *Klebsiella*, *Staphylococcus*, *Pseudomonas*, *Streptococcus*, and *Proteus*, with *Bacillus* being the most predominant.

In the urban area of Yenagoa, Oku and Amakoromo (2013) successfully isolated a total of 12 bacterial strains from raw samples. All of the isolates exhibited positive catalase activity and negative indole production. The bacterial strains were classified into five genera, specifically *Bacillus subtilis*, *Corynebacterium*, *Lactobacillus*, *Pseudomonas*, and *Staphylococcus aureus*.

The present study focuses on the geographical regions of Oyan Lake and Ogun Waterside, located within the Ogun State of Nigeria. In their study, Adelaja *et al.* (2013) conducted a comparison of the microbial load present in smoked fish (*Chrysichthys nigrodigitatus*) obtained from two different locations, namely Oyan Lake and Ogun Waterside in Ogun State. A total of six samples were acquired, with three samples obtained from Lafenwa market and three samples obtained from Makunomi market. The microbial burden present in the skin, intestine, and gills was evaluated through the utilisation of MacConkey agar and Nutrient agar as culture media for the isolation and enumeration of bacteria. The microorganisms that were isolated and identified in the markets encompassed several bacterial families, namely *Bacillus spp*, *Micrococcus spp*, *Staphylococcus saprophyticus*, *Escherichia coli*, and *Staphylococcus aureus*. Among these,

*Staphylococcus saprophyticus*, *Escherichia coli*, and *Staphylococcus aureus* exhibited a higher occurrence rate in Lafenwa market.

A study conducted by Agu *et al.* (2013) aimed to assess the occurrence and prevalence of pathogenic bacteria in smoked fish that is commercially available in four prominent markets located in Benin City, which is situated in the South Western region of Nigeria. The markets included in the study were Oba market (OBM), New Benin market (NBM), Ohila market (OLM), and Ekiosa market (EM). A total of 124 samples were analysed. Human pathogens were identified in all of the samples examined. The identification of the isolates involved the analysis of cultural, morphological, physiological characteristics, as well as biochemical reactions and enzymatic activities. The organisms that were isolated from the samples consisted of *Salmonella typhi*, which accounted for 25 cases (20%), *Staphylococcus aureus*, with 51 cases (41%), and *Shigella sonnei*, with 10 cases (8%). The bacterium *Staphylococcus aureus* was found to have a higher isolation rate of 41% compared to other microorganisms, with *Salmonella typhi* at 20% and *Shigella sonnei* at 8%. The bacterium *Salmonella typhi* exhibited the highest isolation rate at the OLM market, accounting for 12 cases (9.7%). Conversely, the OBM and NBM markets had the lowest isolation rates, with each market recording 4 cases (3.2%) of *Salmonella typhi*. The bacterium *Staphylococcus aureus* exhibited the highest isolation frequency at the NBM market, with a rate of 18 isolates (14.5%). *Shigella sonnei* was exclusively isolated from the OBM and EM markets, exhibiting isolation rates of 4 (3.2%) and 6 (4.8%) respectively. The findings of this study indicate that smoked fish available in the urban area of Benin contains potential pathogens capable of causing foodborne illnesses and various gastrointestinal issues commonly associated with bacterial infections.

The study conducted by Dike-Ndudin *et al.* (2014) in Owerri aimed to determine the microbial quality of the environment and assess the presence and prevalence of microorganisms that are of public health significance. A total of 108 samples were obtained from three distinct sources: smoking factory, open market, and hawkers. The samples underwent microbiological analysis to determine the presence of viable heterotrophic bacteria. The bacteria that have been identified in this study are *Staphylococcus aureus*, *Escherichia coli*, *Bacillus spp.*, and *Klebsiella spp.* The significant level of contamination can be attributed to the individuals responsible for handling the fish, as well as the environment in which it is exposed during the processes of smoking and selling. Given the potential risks it poses to human health, it is imperative for authorities in public health and food safety to enhance their monitoring endeavours in order to effectively control such instances of contamination.

According to Ray (2014), bacterial populations exhibit an initial increase following the filleting process, followed by a subsequent decrease after smoking. However, these populations tend to increase once again during the subsequent stages of packaging, storage, and marketing. Multiple bacterial species have been identified in smoked fish. The bacterial species that fall under this category consist of *Acinetobacter*, *Alcaligenes*, *Pseudomonas*, and *Vibrio*, along with coryneforms (Chipley, 2005).

The occurrence of fish contaminated with *L. Monocytogenes* is relatively low, but it becomes more evident during the processes of brining and post-brining. In addition to the process of brining, it has been determined that slicing is a significant contributor to contamination. It is crucial to ensure that smoked fish does not harbour any microorganisms, as numerous countries have implemented a zero tolerance policy for defects in ready-to-eat food products. In order to mitigate the presence of *L. monocytogenes* and *Salmonella species* in smoked products, it is

imperative for processors to establish and execute Hazard Analysis and Critical Control Point (HACCP) plans, in conjunction with thorough cleaning and sanitation protocols (George, 2010).

## **CHAPTER THREE**

### **3.0 MATERIALS AND METHODS**

#### **3.1 Study Area/sample collection**

Samples were collected from five different locations in Ekosodin and was transported to the laboratory for microbiological analysis. Duplicate samples were collected in sterile containers.

#### **3.2 Sterilization of Materials**

Materials such as Petri-dishes, pipette, glass containers (conical flask, round bottom flask) and bottles were washed, drained and dried. They were wrapped with aluminum foil and sterilized in a hot-air oven at 160°C for an hour. They were allowed to cool after sterilization before usage. An aseptic working environment was achieved with the use of Bunsen burner flame and disinfection of work surfaces with alcohol.

#### **3.3 Preparation and Sterilization of media**

##### **3.3.1 Nutrient agar**

Twenty-eight grams (28 g) of nutrient agar was dissolved in 1000 ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000 ml of distilled water in a conical flask. The medium will be the placed in an autoclave to sterilize it for 15 minutes at 121 °C. After sterilization, the flask was allowed to cool.

##### **3.3.2 McConkey Agar**

A 52.0g of MacConkey agar (MAC) was weighed and poured into a serial conical flask. 1000ml of distilled water will be added and the mixture was sterilized at 120°C for 15 minutes at 15 pis in the autoclave for even distribution of the ager. It will be poured in to petridishes in 20ml amounts and allowed to set at room temperature.

### **3.4 Enumeration and isolation of total heterotrophic bacterial**

Hundred fold serial dilution of the fish samples were prepared aseptically in sterile physiological saline. An aliquot of 1ml was inoculated using the pour plating technique. Appropriate media were used for fungal and bacterial enumeration. Nutrient agar (supplemented with fluconazole) for bacteria. Plates were cultured at  $37\pm 2^{\circ}\text{C}$  for 24 hours. The number of colony forming unit per milliliter (cfu/ml) was calculated using the formula below:

$$\frac{cfu}{ml} = \frac{\text{number of colonies} \times \text{dilution fold/series}}{\text{volume of inoculum}}$$

(Willey *et al.*, 2008)

### **3.5 Phenotypic Identification of Bacteria from Samples**

Following successful pour plate technique, isolation and culture was made from a single colony and characterized using cultural, morphological and biochemical methods using the Bergey's manual. Several tests such as Gram reaction, catalase, urease, indole, oxidase, sugar fermentation, citrate utilization, respective reaction on triple sugar iron agar tests were carried out to presumptively identify bacterial isolates (Holt *et al.*, 1994).

### **3.6 Morphology identification**

The morphological identity of each bacteria isolate was obtained by Gram staining so as to know the gram reaction, cell morphology and arrangement by viewing under the microscope. The Gram stain procedure is as follows:

A smear of the bacteria isolate was made on grease free slide and heat fix by passing over flame. The smear was flooded with crystal violet which is the primary stain for 1min then washed with distilled water.

Subsequently the slides were flooded with Lugol's iodine solution for 30sec and then washed off with distilled water.

95% alcohol was used for decolorization for 10sec and immediately washed off with distilled water.

Finally, the smear was counter stained with saffranin for 1min and washed off.

The slides were allowed to air dry before observing under the microscope using an oil immersion objective lens of  $\times 100$  magnifications to view the slides.

### **3.7 Biochemical identification**

Biochemical test was carried out so as to help in the identification of the bacteria isolates as phenotypic (cultural) characteristics is not sufficient. The various biochemical test carried out are shown below;

#### **3.7.1 Oxidase test**

This is mainly used to differentiate between *pseudomonas* from other gram-negative rod bacteria. Oxidase test was carried out to identify bacteria species that will produce cytochrome oxidase enzyme. *Staphylococcus aureus* and *Escherichia coli* which are gram positive and gram negative respectively were employed as control. A piece of filter paper using sterilized wire loop 2-3 drops of freshly prepared oxidase reagent (1% aqueous tetramethyl-3-phenyl nediamine dichloride) was added. A positive oxidase test is indicated by purple colouration within 10 seconds.

#### **3.7.2 Urease test**

This is used to test organisms that have the abilities to produce the enzyme urease which catalyzes the breakdown of urea to produce ammonia. The test is usually used to differentiate organisms like *Proteus mirabilis* from other non-urease positive organism. A sterilized medium was dispensed into test tubes aseptically and the test bacteria isolated were inoculated into the

medium and incubated at 37 degree centigrade for 24 hours. A change in colour from yellow to red-pink confirmed the presence of urease.

### **3.7.3 Indole production test**

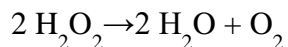
This test was used to determine which of the isolates has the ability to split indole from tryptophan present in peptone water. The best is usually used in differentiating gram-negative bacilli especially those of enterobacteriaceae. Five grams of commercially available peptone broth was dissolved in 1litre of distilled water. The medium was then sterilized by autoclaving at 121 degree centigrade for 15 minutes. The 4 ml of the medium was dispensed into sterile test tube and each of the bacteria isolates was inoculated into the peptone broth. The inoculated media was incubated 37 degree centigrade for 24 hours after which few drops of KOVAC reagent was added. KOVAC reagents consist of 150ml of amylalcohol, 10g dimethylamino benzaldehyde and 150ml of concentrated hydrochloric acid. Positive test was indicated by the red colouration that occurs immediately at the upper part of the test tube.

### **3.7.4 Citrate utilization test**

This test is used to identify which of the isolate can utilize citrate as the sole source of carbon for metabolism. The medium used for this test is simon`s citrate agar. In the preparation, 22 grams of commercially available simon`s citrate agar was dissolved in litre of distilled water and sterilized by autoclaving at 121 degree centigrade for 15 minutes. The medium is dispensed into test tubes and the test organism was inoculated by stablign the medium on the tubes using sterile straight inoculation wire containing culture. The tubes were incubated at 37 degree centigrade for about 24 hours. Positive result is indicated by a change in colour from green to bright blue colouration.

### **3.7.5 Catalase test**

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdowns of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive.



### **3.7.6 Sugar fermentation and production of gases using Triple sugar iron agar (TSI)**

TSI was prepared following manufacturer's instruction and the prepared media was placed in a test tube and kept in a slant position for it to solidify. The slant and butt of the medium was inoculated with the test bacterium using a sterile loop and it was incubated for 18- 24 hr. The results were read on the basis of acid or alkaline production in the slant or butt region of the tube and gas production was confirmed by the presence of crack or air bubbles in the slant or butt region. More so, production of hydrogen sulphide was confirmed by the blackening of the medium. A prepared laboratory chart was used for result interpretation in line with microbiological standard protocol as well as other biochemical tests carried out on the isolates to confirm or ascertain their identity.

### **3.8 Antibiotic susceptibility test**

The bacterial isolates were subjected to commonly used antibiotics using Kirby Bauer agar disc diffusion technique as described by Aromolaran and Badejo (2014) and Akinyemi *et al.* (2005). Pure colonies of bacterial isolates were streaked on sterile Muller Hinton agar (MHA) plates and incubated at 37 °C for 24 h. The bacterial cells were harvested into sterile normal saline solution and standardized using 0.5 McFarland standards. The cultured cells were introduced on the surface of sterile MHA using sterile swab sticks and multi-disc antibiotics were placed on the

culture media aseptically and incubated at 37 °C for 24 h. The antibiotic discs used were ceftazidime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), nitrofurantoin (300 µg), ampicillin (10 µg), and augmentin (30 µg). The diameter of the zones of inhibition around each disc was measured after the incubation period and recorded.

## CHAPTER FOUR

### 4.0 RESULT

Table 1 shows the heterotrophic bacterial count of smoked fish and the result ranged from  $3.22 \pm 1.5 (\log_{10} \text{cfu/g})$  to  $4.55022 \pm 5.0 (\log_{10} \text{cfu/g})$ .

Table 2 represents the cultural, morphological and biochemical test of the bacterial isolates *Staphylococcus aureus*, *E-coli*, *Salmonella*, *shigella*, *Enterobacter* sp. and *Pseudomonas* sp.

Table 3 represents the percentage occurrence of the bacterial isolates with *E. coli* having the highest percentage of 30%.

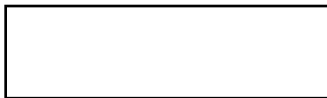
Table 4 shows the antibiotic sensitivity test bacteria isolates.

**Table 1: Total heterotrophic bacterial count**

SAMPLE	BACTERIA COUNT
SAMPLE 1	3.22±1.5(log <sub>10</sub> cfu/g)
SAMPLE 2	4.51851±2.5(log <sub>10</sub> cfu/g)
SAMPLE 3	4.21748±0.5(log <sub>10</sub> cfu/g)
SAMPLE 4	4.41497±4.0(log <sub>10</sub> cfu/g)
SAMPLE 5	4.55022± 5.0(log <sub>10</sub> cfu/g)

KEY:

- SAMPLE 1: EDO STREET
- SAMPLE 2: NEWTON STREET
- SAMPLE 3: BOUNDARY
- SAMPLE 4: JB STREET
- SAMPLE 5: EKOSODIN BUS STOP



**Table 2: Cultural, Morphological and Biochemical characteristics of Bacterial Isolates**

PARAMETERS	B1	B2	B3	B4	B5	B6
<b>CULTURAL</b>						
Shape	Circular	Circular	Irregular	Circular	Circular	Irregular
Colour	White	Yellow	Cream	Cream	Green	Creamy
Size	Large	Medium	Large	Medium	Small	Medium
Elevation	Flat	Raised	Flat	Flat	Flat	Raised
Transparency	Opaque	Translucent	Opaque	Transparent	Transparent	Opaque
<b>MORPHOLOGICAL</b>						
Gram stain	+	-	-	-	-	-
Cell type	Cocci	Rod	Rod	Rod	Rod	Rod
Cell arrangement	Chains	Cluster	Single	Scattered	Single	Singly
<b>BIOCHEMICAL</b>						
Urease	-	-	-	-	-	-
Indole	-	+	-	-	-	-
Citrate	-	-	-	-	+	-
Catalase	+	+	+	+	+	+
Motility	-	+	+	-	+	-
Coagulase	+	-	-	-	-	-
Oxidase	-	-	-	-	+	-
H <sub>2</sub> S	-	-	+	-	-	-
<b>FERMENTATION TEST</b>						
Glucose	+	+	+	+	+	AG
Sucrose	-	+	-	+	+	AG
Lactose	+	+	-	+	+	A
Isolates identity	<i>Staphylococcus aureus</i>	<i>E. coli</i>	<i>Salmonella</i>	<i>Enterobacter</i> sp.	<i>Pseudomonas</i> Sp.	<i>Shigella</i>

Key: + = positive and - = negative

**B= Bacteria isolates**

**Table 3: percentage occurrence of isolates**

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<i>Staphylococcus</i> sp.	25%
<i>Pseudomonas</i> sp.	10%
<i>E. coli</i>	30%
<i>Enterobacter</i> sp.	10%
<i>Salmonella</i> sp.	10%
<i>Shigella</i> sp.	15%

---

**Table 4: Antibiotics susceptibility pattern of bacteria isolates**

	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
<i>Staphylococcus</i>	S	S	R	R	S	S	R	S	R	I
<i>Pseudomonas</i>	S	I	R	R	S	S	S	I	I	I
<i>E. coli</i>	S	R	R	R	I	S	S	R	S	R
<i>Enterobacter</i>	S	R	R	R	S	S	S	S	I	S
<i>Salmonella</i>	S	S	S	I	S	S	S	S	I	S
<i>Shigella</i>	S	S	R	R	S	S	S	I	S	S

Key: S = susceptible ( $\geq 17$ mm), I = intermediate (11-16) mm and R = Resistant ( $\leq 10$ mm)

CPX – ciprofloxacin, St - streptomycin, SXT - sulfamethoxazole, E - erythromycin, PEF - perfloxacin, CN - gentamicin, APX - ampicillin, Z - zinnacef, AM - amoxicillin, Ro – rocephin



## CHAPTER FIVE

### 5.0 DISCUSSION

Microbiological quality is of importance to public health as it directly relates to spoilage of fish and food poisoning (Oramadike *et al.*, 2012). From the result obtained in this study, the total heterotrophic bacterial count ranged from  $3.22 \pm 1.5 \log_{10} \text{cfu/g}$  to  $4.550228 \pm 1.00 \log_{10} \text{cfu/g}$ . This was similar to the study conducted by Ebeniro and Nwosu (2020) on isolation of microorganism from smoked fish where he obtained bacteria count ranged at  $4 \log_{10} \text{cfu/g}$ . Most farmers especially those living close to large number of consumers deliberately limit the time of heat exposure of the fish to avoid excessive loss of weight, size and the consequent low price tag of small sized fish products. High moisture content of dried fish products are reported to be an indication of retention of all the essential growth factors that favour microbial growth. In an earlier work, Frazier and Westhoff (2010) reported that the availability of nutrients is crucial to increase or decrease microbial numbers in any food during spoilage. In this study, the microbial count and coliform were high at  $4 \log_{10} \text{cfu/g}$ . This could be as a result of the anthropogenic activities in the environment or the duration of fish storage before sale. According to the rule of International Association of Microbiology Society, fish should possess neither *Vibrio* spp. nor *Salmonella* spp. The investigated samples were free from these pathogenic organisms. The presence of coliforms in the fish is a clear indication of environmental and faecal pollution, either from humans and /or from animals and poor handling practices (Eze *et al.*, 2011). The frequency of the bacteria isolation showed that *E. coli* has the highest percentage of 30%. Most outbreaks of food poisoning associated with fish are derived from the consumption of raw or insufficiently heat-treated fish, which may be contaminated with bacteria from water environment.

Using the cultural, morphological and biochemical test results, the bacterial isolates obtained in this study were *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* sp, *Enterobacter* sp, *Pseudomonas* sp and *Shigella* sp. This isolates corresponded with what was isolated by Anihouvi *et al.*(2019) in his evaluation of bacterial and antibiotic profiling of dry fish. Food borne illness is a major international health problem with consequent economic reduction (Duff *et al.*, 2003). Street vended foods which are prepared under poor hygiene pose risk to public health. However, it was recognized from this study that some vendors prepared and smoked in poor hygiene conditions, the study confirmed that the sanitary level in the sale places are deteriorated, leading to the occurrence of severe public health hazards (Amusan *et al.*, 2010).

Isolating *Staphylococcus* sp from the majority of samples, a well-known food-borne pathogen, is also an implication of cross contamination (Amusan *et al.*, 2010). This bacterium may be contributed through human handling of the raw products. Nevertheless, adequate precautions can prevent *S .aureus* contamination, growth and enterotoxin production from occurring in roasted products (Himelbloom *et al.*, 2008; Amusan *et al.*, 2010). Contamination of ready-to-eat products can be prevented through the use of latex gloves to reduce excessive human hand contact (ICMSF, 2000; Amusan *et al.*, 2010).

*Staphylococcus aureus* is a Gram positive *coccus* resistant to heat, drying and radiation. Its strains can be pathogenic and relatively non pathogenic. They produce some enzymes which are implicated with Staphylococcal invasiveness and many extracellular substances some of which are heat stable enterotoxins that render the foods dangerous even though it appears normal (Prescott *et al.*, 2005). Once the bacteria have produced toxin, the food can be extensively and properly cooked, killing the bacteria without destroying the toxin. Many of their toxins are gene-

based that is carried on plasmids. The intensity of the signs and symptoms may vary with the amount of contaminated food ingested and susceptibility of the individuals to the toxin. Some signs and symptoms of Staphylococcal food poisoning include nausea, vomiting, abdominal cramp and diarrhoea (Amusan *et al.*, 2010). The bacteria obtained from this study were all susceptible to few antibiotics namely perfloxacin,, amoxicillin, rocephin and ciprofloxacin but were resistant to ampiclox, zinnacef, sulfamethoxazole and gentamicin. This was similar to the antibiotic resistance profiling done by Anihouvi *et al.* (2019).

## **CONCLUSION**

In conclusion, this study has revealed that the level of microbial contamination of dry fish products could either be environmentally dependent. It also revealed fish contamination as a public health problem. Eating uncooked smoked fish could be hazardous and should be avoided.

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