Molecular identification and characterization of selected food-borne pathogens in imported dried fish sold in informal markets around Gauteng province in South Africa

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DECLARATION

I, Siphiwe Rendy Nkosi, confirm that the work contained in this research dissertation entitled 'Microbial identification and molecular characterization of food borne pathogens in imported dried fish from the informal markets in Gauteng province, South Africa' is my original work submitted for a Degree of Master of Science in Animal Health, working under the supervision of Prof Mulunda Mwanza. This dissertation has not been previously submitted to any University. Each significant contribution to and quotation in this dissertation has been attributed and cited and referenced.

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TABLE OF CONTENTS

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ABSTRACT

Dried fish highly contributes to the demand for nutritional protein in our daily diet. In recent years, dried fish has become more popular in informal markets, which have increased dramatically in size and become a source of income for people. It plays an important role in providing low-cost, easily accessible, and nutritious food to the urban populace. The examination of microbiological quality is therefore needed to ensure health of the general public. The purpose of this study was to assess the microbiological quality of different types of dried fish sold in Pretoria and Johannesburg, South Africa. Based on the instability of the informal markets in Pretoria and Johannesburg and the unknown number of dried fish sellers, a non-probability sampling method was used (convenience sampling).A total of 12 markets were visited, and 140 samples were collected. Three types of dried fish were obtained, which were smoked (40), sun-dried (80), salted (20). During that collection of samples, hygienic measures applied by vender were observed and recorded in a checklist and the results revealed that sellers were not adhering to the most basic hygienic practices and not using proper storage of dried fish. All the sellers did not wash hands before touching the samples, and 83.3% exposed to air outside. The mean total bacterial count, sun-dried fish recorded 2.91×10^{7}cfu/g, smoked fish 2.71×10^{7}cfu/g, and salted fish 2.13×10^{7}cfu/g respectively. According to the standard guideline of bacterial load in seafood, the values obtained in this study reveal that the dried fish sold in the informal markets are not suitable for human consumption as it's above the standard guideline. The bacterial count from the three dried fish bacteria counts differs, which could be attributed to variations in the dried fish samples' preservation methods (smoking, sun, and salting).The identification of the isolated bacteria was based on conventional biochemical tests such as Gram staining, Catalase, Oxidase, Indole and Coagulase. Further the results were confirmed by the amplification of the 16S rDNA genes using the forward primer 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and the reverse 1492R (5'- ACG GCT ACC TTG TTA CGA CTT 3'). BLAST (basic local alignment search tool) from NCBI (National Center for Biotechnology Information) GenBank was used for confirmation and similarities of the isolates. The molecular identification revealed the presence of *Staphylococcus* spp., (11.94%), *Staphylococcus sciuri* (2,98%), *Staphylococcus saprophyticus* (2.98%), *Staphylococcus aureus* (4.47%), *Staphylococcus lentus* (5.97%), *Staphylococcus xylosus* (8.95%), *Klebsiella pneumonia* (7.46%), *Klebsiella* spp.,(4.47%), *Clostridium* spp., (14.92%), *Paraclostridium bifermentaus* (5.97%), *Clostridium bifermentas* (4.47%), *Micrococcus caseolyticus* subsp *homonis* (1.49%), *Micrococcus caseolyticus*

(1.49%), *Enterobacter* spp., (1.49%), *Enterobacter ludwigii* (1.49%), *Lysinibacillus macrolides* (1.49%), *Clostridium botulinum* (2.98%), *Enterococcus faecium* (2.98%), *Enterococcus faecalis* (5.97%), *Corynebacterium variabile* (5.97%) *Planococcaceace bacterium* (2.98%) in the dried fish type collected. Some of these bacteria are important human pathogens responsible to for serious illness. *Staphylococcus aureus, Staphylococcus sciuri, Klebsiella pneumonia* are among the food-borne pathogens.The disc diffusion technique was used to test the antibiotic susceptibility of the isolated bacteria, and the results were interpreted using the Clinical Laboratory Standards Institute (CLSI). *Staphylococcus aureus* (100%), *Staphylococcus xylosus* (100%), *Staphylococcus sciuri* (100%), *Staphylococcus saprophyticus* (100%), and *Staphylococcus lentus* (100%) were all susceptible to Chloramphenicol, according to the findings. In addition, *Enterococcus* species were isolated, and the findings revealed that *Enterococcus faecium* and *Enterococcus faecalis* were completely susceptible to Amoxicillin, Chloramphenicol, and Norfloxacin.The overall results showed that the pathogens were 60.1% susceptible to the antibiotics tested and 29% were resistant; meanwhile, 10.9% of the isolates were intermediate to the tested antibiotics. 73.5% Streptomycin, 58.2% Erythromycin, and Gentamicin had the highest resistance to most pathogens isolated in this study. According to the information acquired, the informal markets continue to pose a risk of antibiotic contamination, and it is one of the major public health issues confronting Africans and the global population. The need to emphasize more caution on microbial hygiene quality of dried fish and more evaluation for public health must be taken into consideration.

LIST OF ABBREVIATIONS AND ACRONYMS

AML10	Ampicillin
ANOVA	Analysis of variance
CFU	Colony-forming units
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standard institute
C30	Chloramphenicol
CN10	Gentamicin
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assays
Et.al.,	And other
E5	Erythromycin
IFTP	Information food trading programme
IZN	Inhibitory zone diameter
FAO	Food and Agriculture Organization
N	Number
NCBI	National Center for biotechnology information
NOR	Norfloxacin
PCR	Polymerase chain reaction
PPE	Personal protective clothing
RTE	Ready to eat
SAMP	Southern Africa Migration Programme
SPSS	Statistical package for the social sciences
S10	Streptomycin
TBC	Total Bacterial count
TCP	Technical Cooperative Programme
WHO	World health organisation

LIST OF SYMBOLS

>	Greater than
<	Less than
%	Percentage
/	Per
°C	Degree Celsius
G	Gram
mL	Milli litre
mm	Millimeter
µL	Micro-litre

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

Fish is one of the most important protein sources on the continent, and it is generally recognized in many African countries as a rich source of protein and other nutrients for keeping a healthy body (Allison *et al.*, 2012; Fisheries, 2006). However, fishery products, especially from informal sectors, have been recognized as significant carriers of food-borne pathogens (Jami *et al.*, 2014). In situations where a state has limited resources, food demand, and the formal economic sector is small, poor people in urban areas often find ways to secure livelihoods to provide for their nutritional needs in the informal food sector (Burchi & De Muro, 2016). The informal food market is the single greatest employer in the informal sector, offering visitors and low-income earners in such locations with affordable and handy dried fish and meat goods (Nwuneli *et al.*, 2014).

In general, it is well documented that fish and fishery products might be contaminated with different food-borne pathogens due to a lack of good hygienic, poor handling, and inappropriate storage practices after fish are harvested (Mbonane & Naicker, 2020; Reilly & Käferstein, 1997). Therefore, questions are raised about food products' safety and microbiological quality sold on the street vendors (Mbonane & Naicker, 2020; Mosupye & Holy, 1999).

Insects, rodents, other infected animals, and air pollution are all factors that aggravate the informal food market setting, according to other studies (Lucca & Torres, 2002; Ngwawe, 2017). Furthermore, fish and fish products are frequently implicated to a disease that can infect people, particularly when customers consume raw or undercooked fish and fish products (Novoslavskij *et al.*, 2016). High bacterial counts have been identified in microbiological tests of street-vendered foods in America, Asia, and Africa. Furthermore, there has been an increase in the occurrence of food-borne bacterial pathogens in food; in certain cases, street-vendered meals have been linked to food-borne disease outbreaks (Rane, 2011).

Moreover, studies have documented the importance of *Staphylococcus aureus*, *Salmonella*, *E. coli*, *Clostridium Perfringens*, *Campylobacter*, and *Listeria Monocytogenes*. Every year, millions of people with illnesses all around the world are linked back to food-borne pathogens, according to public health and food safety authorities (Oliver *et al.*, 2005). Food-borne pathogenic bacteria are, without a doubt, one of the leading causes of sickness and death in

many individuals each year, resulting in enormous economic loss and human misery. For decades, *Salmonella,* Staphylococcus aureus, *Listeria monocytogenes,* and *E. coli* have all been linked to food-borne disease. (Rane, 2011). Food-borne infections are becoming a major public health concern around the world, with outbreaks occurring in both industrialized and poor countries (Osaili *et al.*, 2013; Vos *et al.*, 2013). Several observational studies have discovered that street foods are frequently stored at inappropriate temperatures, handled excessively by food vendors, and served in unsanitary settings (Agbodaze *et al.*, 2005; Barro *et al.*, 2006; Muinde & Kuria, 2005). The microbial load of street food ingredients is critical in the start. However, the microbial load of ready-to-eat (Boss *et al.*) meals at the point of sale may be influenced by handling, processing, storage, and display variables (Angelidis *et al.*, 2006; Beuchat & Ryu, 1997). Various food-borne pathogens have been related to outbreaks of food-borne disease linked to street meals (Gibbons *et al.*, 2006; Gilbreth *et al.*, 2005; Mbonane & Naicker, 2020).

To date, there is limited documentation on participatory risk analysis, neither have any methods been applied to study the food value chain of dried fish (FAO, 2017). As a result, the risk of exposure to food-borne diseases such as *Salmonella, Staphylococcus Aureus, Listeria monocytogenes, Shigella, Clostridium perfringens* and *E. coli* in informal markets in Gauteng province is limited (Bernard & Scott, 2007).The biggest challenge is that most people depending on informal markets foods are often more interested in their convenience than safety, quality, and hygiene (Khuluse, 2016).

Contamination of informal markets foods by microbes has become a major public health concern (Rane, 2011). The increasing danger of food-borne pathogens such as *Staphylococcus Aureus, Escherichia coli, Salmonella spp*., and *Listeria monocytogenes* requires effective detecting and control methods to confirm the absence of these pathogens in fish and fish products. *Salmonella* species are among the most dangerous food-borne pathogens on the planet. *Salmonellosis* is a common food-borne bacterial illness in humans, with symptoms ranging from asymptomatic to life-threatening (Galanis *et al.*, 2006). *Listeria monocytogenes* is a serious public health threat, and the majority of human listeriosis infections are linked to *Listeria monocytogenes*-contaminated food (Kakatkar *et al*., 2010).

Microbiological quality in food products is not the only problem, but antibiotic resistance has become one of the world's principal threats (Acharya & Wilson, 2019). As a result of improper

use of antibiotics in meat products, death rates associated with severe and life-threatening infections have increased, putting human health at risk (Collignon *et al.*, 2016; Guven *et al.*, 2010)

Data about the microbiological safety of fish products in African countries are scarce (Von Holy & Makhoane, 2006). South Africa is also one of the countries that need more evaluation of pathogens in fish as there is a high import of fish and fish products. Therefore, it's critical to look into the microbiological quality and safety of dried fish sold in the Gauteng province's informal market. *Salmonella species, Escherichia coli* and *Listeria monocytogenes* are pathogens recognized as major pathogens that threaten the consumer's health if biosecurity is not implemented when producing the commodity.

1.2 Problem statement

According to the World Health Organization (Kirk *et al.*, 2015), food-borne illnesses remain a severe threat to most developing countries and South Africa due to the lack of proper law enforcement. Food-borne diseases impact the public health and the economy of a country (Helms *et al.*, 2003). Consumption of contaminated food with food-borne pathogens containing resistant strains to different antibiotics exposes consumers to food-borne poisoning. Furthermore, the recent high Listeriosis outbreak South Africa is one of the signs that the country can face any food-borne outbreak with palpable economic consequences (Kaptchouang Tchatchouang *et al.*, 2020). Paudyal *et al.* (2017), in their study, *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus* were shown to be the most prevalent bacteria isolated from various African countries. The researcher's data on bacteria associated with food-borne diseases in dried fish sold in the South African markets is limited. Considering the aforementioned factors, it is necessary to monitor the incidence of Enterobacteriaceae and other harmful bacteria in dried fish sold in Gauteng's informal markets on a regular basis.

1.3 Justification of the study

Lots of dried fish are imported into South Africa from other countries and particularly from African countries. Because of the political instability in their home countries, Africans from neighbouring nations flock to South Africa in search of commercial prospects, economic chances, and tourist attractions (Kalitanyi & Visser, 2010). The emigrating community brings in their traditional food, and some of these foods cross the borders without proper control as required by the Food Safety Act. Meat and fish are among the most common imported products, and most likely the dried fish food has become more popular over the past decades. The demand

increases day by day due to the population growth in South Africa and the increase emigrating populations in the country. Food-borne diseases from meat represent a significant concern in developing countries. However, there is limited data that can be used to establish the control of dried fish sold in the informal markets to ensure their safety (Akhtar *et al.*, 2014). Moreover, studies have indicated that significant meat products sold in the informal markets are not kept hygienically.

Most of the dried fish sold in the informal markets are frequently unhygienic and easily contaminated due to storage practices, transportation, and handling practices. The study addressed all the mentioned factors by looking at the microbial quality and characters of street-vendor of food sold in the informal markets in Johannesburg and Pretoria through collecting samples. Recognizing the threat demands, effective detecting, and control methods to ensure that these pathogens are not present in food and minimize their consequences must be in place. This research demonstrates the occurrence and characteristics of the specific food-borne pathogens of the dried fish in informal food production.

1.4 Research questions

The research question of this study is to respond to the questions

- What is the microbiological quality of these imported dried fishes sold in informal markets in large cities like Johannesburg and Pretoria?
- What are the health hazards linked with the consumption of dried fish and the introduction of other pathogens into the country?

1.5 Hypothesis

The informal markets held by unqualified vendors are the source of food-borne pathogens in food due to poor hygienic practices. Furthermore, some food pathogens isolated from the informal markets might have resistance profiles to different antimicrobial agents used in food-borne contamination. Dried fish can be a great vehicle of transmission of pathogenic bacteria such as *Staphylococcus aureus*, *Klebsiella* spp., and other Enterobacteriaceae that may be of health risk for the country.

1.6 Aim of the study

Identify and characterize pathogens from dried fish imported in South Africa sold in informal markets and establish associated risks for consumers and the country.

1.7 Specific objectives

The objectives of this study were to:

i. Evaluate the microbial quality of dried fish sold in informal markets of Gauteng
ii. Identify and characterization of bacterial isolates in the dried fish
iii. Evaluate the antibacterial resistance profile of isolated bacteria
iv. Establish the possible relatedness between isolates with multiple sequence alignment and construction of phylogenetic trees

CHAPTER TWO

Literature review

2.1 Introduction

In developing countries like South Africa, informal markets has become a common component of city life, because of population cultures, accessibility of particular cuisine not necessarily present in superstores, high joblessness, and restricted labor possibilities or opportunities (Kennedy *et al.*, 2004; Newman & Burnett, 2013). As a result of rapid population expansion, unemployment, deprivation, and the availability of relatively low-cost foodstuffs, food consumption in the informal markets has increased throughout (Kubheka *et al.*, 2001). However, the data show that there is still a dearth of understanding and scientific proof in South Africa about the microbiological quality and safety of street-vended meals. Other emerging countries, particularly those in the African region, already had this knowledge. Food sold on the street could be seen to be hazardous at the time, and it was regarded a practice that should be abolished in communities in South Africa (Von Holy & Makhoane, 2006).

This chapter will explore the literature on street food vendors and their role in the community, as well as measures to enhance food-vendor practices, a wide view of food-borne disease, and food-borne disease diagnosis tools. Relevant literature on the rules and regulation of migrants' movement in South Africa, their food choices, and the causes/ practices that lead to the contamination of the dried fish. Previous research findings on the safety of food made and sold by street vendors, as well as general hygiene practices.

2.2 Informal Markets

2.2.1 Defining and general overview of informal markets

The overview of informal markets linked to the Food and Agriculture Organization (FAO) defines the informal sectors or informal markets are defined as a sector of a country's economy that is not recognized as a traditional source of income, meaning they are neither taxed or regulated by any government (Argenti, 2000). (Bruce, 2000) also mentioned that people who make a living through self-employment are rarely on payrolls and hence are not taxed. In many developing countries, such as South Africa, informal markets have become an accepted part of urban life due to high unemployment and few work opportunities (Newman & Burnett, 2013). Food security is essential to the community, and Food security, as defined by the Food and Agriculture Organization (FAO), is a situation in which all people have physical, social, and economic access to adequate, safe, and nutritious food that always meets their dietary needs

and food preferences in order to live an active and healthy life (Novoslavskij *et al.*, 2016; Schmidhuber & Tubiello, 2007). This concept identifies four key aspects of food security for everyone: availability, access, usage, and stability (Skinner & Haysom, 2016a).

Street food plays an essential economic role in food security. It provides a source of inexpensive, nutritious meals to many construction and office workers and people in transit (Newman & Burnett, 2013). Due to variety, the familiarity, taste, relatively inexpensive, and accessibility, street foods are popular as sources of food (Hanashiro *et al.*, 2005). The experience of many developing countries shows that small and unregulated enterprises in food production, transport, and marketing already play an essential role in food security (Stagl, 2002). Although many municipalities and central governments perceive the sector as a development problem, research from around the world shows that small enterprises are essential sources of income and employment for food sector entrepreneurs (Mbonane & Naicker, 2020). The jobs and revenue gained from the sector thus contribute to their well-being (Carter, 2011). These opportunities can be significant in the empowerment of women and members of marginalized ethnic groups.

Rane (2011) reported that street vendors frequently congregate in overcrowded areas, where many potential customers and a high movement of people (Rane, 2011). Frequently, fundamental hygienic requirements such as running water, garbage pickup and hygienic restrooms are insufficient. waste disposal, and clean bathrooms are insufficient (Hubbard & Onumah, 2001). Large volumes of waste gather in these places, according to Kubheka *et al.* (2001), providing a breeding ground for insects and animal pests. Furthermore, a research by Kubheka *et al.* (2001) found that microbiological tests conducted on street-food vending in numerous developing nations found significant bacterial counts in items sold in street markets.

2.2.2 Participation of migrants in the informal markets industry

According to Peberdy (2000), little is known about the size of South Africa's informal sector and the involvement of migrant and immigrant entrepreneurs, and even less is known about the extent of informal sector cross-border trade between South Africa and the region, as well as its relationship to formal trade patterns and street trading. South Africa attracts a large number of migrants from Southern Africa, who typically engage in informal economic activities (Rogerson, 2018).

The informal markets must be regarded as more than merely a source of revenue. It is strongly recommended that the informal sector facilitates food access, as well as access to affordable and reliable food, which contributes to the FAO's food security definition's utilization dimension (Skinner & Haysom, 2016b). According to statistics, significant changes in South Africa's informal sectors require additional investigation (Cichello & Rogan, 2017). According to Cichello and Rogan (2017), the informal sector remains a major source of employment and income for low-income families. International migrants, particularly illegal migrants, refugee seekers, and refugees, who are mainly excluded from the formal labor market and have no alternative except to create their own jobs, are unlikely to be effectively represented in labor force statistics (Crush & Chikanda, 2015).

According to a 2010 Southern Africa Migration Programme (SAMP) assessment of post-2005 Zimbabwean migrants in Johannesburg and Cape Town, 20% of all migrants worked in the informal economy (Skinner & Haysom, 2016a). Other migrant groups, such as Somalis, have recommended that higher rates of informal sector engagement be implemented (Jinnah, 2010). Although it's difficult to assess on a national scale, smaller-scale research imply that informal food retail is an important source of income for foreign migrants (Crush *et al.*, 2015; Piper & Charman, 2016).

Anti-foreign attitude has been formed, bolstered by a largely punitive approach to the informal sector, which concentrates on regulation and control (Rogan & Skinner, 2017). The parliamentary committee that investigated the 2015 xenophobic attacks that targeted immigrants working informally produced a report in November 2015, recommending that township enterprises be regulated (https://pmg.org.za/tabled-committee-report/2609/). Municipal governments must enhance processes for giving and monitoring business permits and licenses to enterprises operating out of residential residences, according to the report. The municipality's laws (Parliament of South Africa 2015: 38-39) were not followed.

2.2.3 The role played by the state controlling imported food in the informal markets in SA

Dried fish in informal markets places has been studied in many nations. However, there is currently a lack of information about the microbiological quality and safety of dried fish meat marketed in South Africa's informal market. Because of the expanding number of informal food markets and food served on the streets, public health organizations have expressed tremendous worry over food safety (Chakraborty *et al.*, 2011). However, university-based

research conducted the first completely documented scientific examination into the safety of informal markets food in South Africa (Mchiza *et al.*, 2014). According to the study, informal markets on the streets in South Africa have generated plenty of foods with low bacterial counts. However, sufficient hygiene conditions and access to basic sanitary facilities were still required (Skinner, 2016).

According to a study undertaken as part of the Technical Cooperative Programme (TCP) on the economic impact of street food selling in South Africa, informal markets places contributed significantly to the country's economy (Mchiza *et al.*, 2014; Von Holy & Makhoane, 2006). According to Kirsten *et al.* (2002) , in 1994, an estimated R44.7 million was spent on street food shops in Gauteng and R8 million in the Western Cape. In addition, the Durban metropolitan area spent over R18 million on street food in 1998. Furthermore, in 1999, private households in South Africa spent around R4.3 million outside their homes on street food, with more than 47 percent of that amount spent on meals and snacks purchased from hotels, restaurants, and street sellers (Kirsten *et al.*, 2002).

The Gauteng Department of Health launched the Informal Food Trading Programme (IFTP) to encourage safe food handling within the province's informal food-trading sectors as part of the WHO's drive to promote healthy cities among the people of Gauteng (Von Holy & Makhoane, 2006). The program's goals were to disseminate broad information and awareness of appropriate hygiene standards, rules, and by laws to the informal marketplaces. They also encouraged street food vendors to become more responsible and careful about supplying healthy food to their customers, lowering the danger of food-borne disease outbreaks Gauteng (Von Holy & Makhoane, 2006).

2.3 Conservation practice of dried fish products over time

Drying has been a traditional method of preserving meat products for years, and other methods like cooking and salting all work by preventing bacterial growth and delaying food decay (Del Nobile *et al.*, 2012). There are three well-known different processing methods used to produce dried fish, drying, smoking, and salting, and these methods usually rest in the year season as drying depends on the weather condition (Holma & Maalekuu, 2013). Suppose the weather condition is unsuitable for outdoor dryings, such as rain, snow, or high temperatures the fish is stored in a freezing compartment until the weather becomes suitable for drying (Holma & Maalekuu, 2013). Adu-Gyamfi (2006) conducted a study on the microbiological quality of smoked fish in various Accra's informal markets and discovered that the products' safety had been compromised. *Escherichia coli*, *Klebsiella pneumoniae*, *and Proteus mirabiis* were among the bacteria found. Bomfeh (2011) also found *Staphylococcus aureus*, *Escherichia coli*, *Micrococcus* spp., and *Proteus* spp. in smoked fish bought from an Accra street market.

Christison *et al.* (2008) suggested that proper hygiene measures on handlers' hands should be improved to reduce cross-contamination and microorganism spread. Raw dried fish and ready-to-eat products are frequently displayed in informal marketplaces in unsanitary conditions such as open space with no sufficient packaging, no suitable preserving standard temperature, and this has led to certain health issues (Kotzekidou, 2016). Various issues have been predicted prior to the product reaching the market, such as human activity near the dam or lake, contaminated water sources, and inadequate hygiene during capture, handling, and distribution (Sorensen *et al.*, 2015).

The drying process and transportation of fish may have an impact on the presence of bacteria in fish and the water in which they are caught. The growth of bacteria is also due to the fact that fish and fish products are frequently consumed without being heat treated, which has a significant impact on human health (Novoslavskij *et al.*, 2016). Pathogens can infiltrate the food chain through contaminated fish and fish products. Cross-contamination of premises, equipment, and end-product may occur during the processing of fish, allowing pathogenic germs to spread more easily (Novoslavskij *et al.*, 2016). Furthermore, excellent hygienic hygiene is a safeguard against contamination.

2.3.1 Methods of preservation of dried fish

For the most part, fish are dried using the ancient traditional process of sun drying to preserve it (Bala & Mondol, 2001). The different variables used in natural convection dryers are low-cost, locally produced, and do not require any electricity or fossil fuels. However, the drying method is not completely sanitary, and the fish are susceptible to infestation and contamination from insect and larva attacks (Bala & Mondol, 2001). Insecticides are frequently used by farmers to prevent infestation and ensure safe storage. As a result, this fish is severely contaminated, posing a wide range of environmental and health risks to the product's users. Fish is susceptible to a number of food-borne infections that are important to public health (Chukwu & Shaba, 2009).

Fish are one of the most common sources of protein in poor countries. However, due to the quick change in enzymatic and bacterial activity in fish, preservation is difficult (Louka *et al.*, 2004). Fresh fish that is not consumed immediately or processed into finished goods during the early stages of harvest will quickly decompose and become waste. The manner of processing and drying utilized has an impact on the quality of dried fish. The nutritional contents of other species vary based on the kind of fish utilized. Fish drying and smoking methods differ between countries and even within the same country. A processor may additionally adjust salted methods according on the species (Chukwu & Shaba, 2009).

2.3.2 Practice that influences the hygiene of food in the informal market

According to Lambrechts *et al.* (2014), hands of ready-to-eat food handlers are a primary vector in the spread of food-borne disease, accounting for roughly 97 percent of food-borne infections in food service companies and residences, mostly owing to poor personal hygiene. Hand hygiene is insufficient, according to the study, and food contamination from food workers' hands might have major public health effects. Furthermore, the importance of further training for food workers to increase their awareness of proper hand washing practices was stressed.

2.4 Detection and identification methods used for food-borne pathogens

Pathogen detection technologies have been developed and upgraded over time, and their disadvantages and benefits are highlighted. In their study, Priyanka *et al.* (2016) found that an ideal detection method must meet five key criteria: high specificity (only detecting the bacterium of interest), high sensitivity (capable of detecting as few as a single live bacterial cell), short time-to-results (minutes to hours), operational simplicity (no need for lengthy

sampling procedures or specialized equipment), and cost-effectiveness. Nucleic-Acid-Based Methods have the considerable benefit of having a high level of specificity in food pathogen detection assays (Zhao *et al.*, 2014). They detect specific nucleic acid sequences in the target organism by combining them with a short synthetic oligonucleotide that is complementary to that sequence (Zhao *et al.*, 2014) .

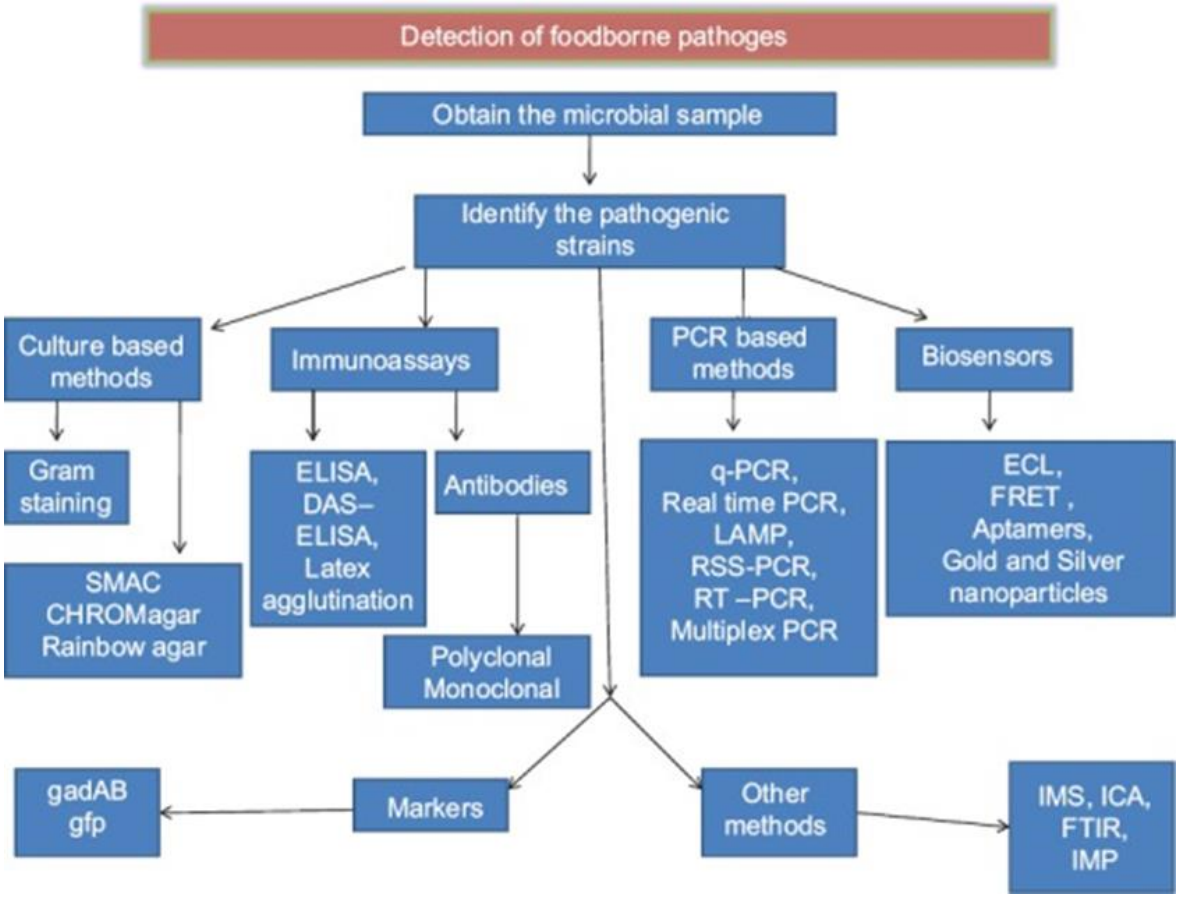

Figure 2.1 Picture taken from (Priyanka *et al.*, 2016). The presented chart shows the procedures that has been followed before for the identification of food pathogens. Schematic representation methods for the detection of pathogens by ELISA, enzyme-linked immuno sorbent assay; DAS, double antibody sandwich; PCR, polymerase chain reaction; LAMP, loop-mediated isothermal amplification; RSS, restriction site-specific; RT, real-time; ECL, electrochemiluminescence; FRET, fluorescence resonance energy transfer; IMS, immunomagnetic assay; ICA, immune chromatic assay; FTIR, Fourier transform infrared spectroscopy.

Food-borne pathogens have been proven to have a significant impact on the economy and causing quite a several setbacks, including diseases outbreaks worldwide and more

significantly in developing countries (Scott, 2003; Todd, 2017). Therefore, establishing the absence of pathogens aids in ensuring the safety of foods, distinguishing the shelf-life stability of the food, and indicating microbial load to monitor the effectiveness of hygienic processing (Hoorfar, 2011).

Food-borne bacteria such as *Escherichia coli* O157:H7, *Salmonella enterica*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Bacillus cereus*, other Shiga-toxin producing *E. coli* strains (non-O157 STEC), and *Vibrio* spp. have been at the top leading list of agents responsible for causing food-borne diseases (Zhao *et al.*, 2014). Research studies have reported the development of rapid, sensitive, specific, easy to use, and cost-effective methods for detecting the pathogens mentioned above. For example, Priyanka *et al.* (2016) reported that advanced culture-based methods, enzyme-linked immunosorbent assays (ELISA), and polymerase chain reaction (PCR) were used for the detection of some of the food-borne diseases.

Multiplex PCR (mPCR), Quantitative PCR (qPCR), and DNA microarray are examples of high-sensitive nucleic acid-based methodologies approaches that have been developed and widely utilized for the identification of food-borne pathogens (Priyanka *et al.*, 2016). Furthermore, the International Organization for Standardization (ISO) certified procedures for detecting food-borne intoxicants and mycotoxins on food products, which are now utilized by food control and reference laboratories from practically every country on the planet (Yeni *et al.*, 2014).

For decades, culture-based conventional procedures such as standard plate count have been employed. However, they necessitate a huge quantity of laboratory equipment, labor, and time, all of which are prohibitively expensive, and the methods' downside is that they require skilled staff to perform (Yeni *et al.*, 2014). Conventional methods, on the other hand, have been deemed more dependable and accurate, and are recommended for confirming emergency results and ongoing epidemiological investigations of food-borne outbreaks (Priyanka *et al.*, 2016).

2.5 Socio-economic factors influencing the use of informal markets by migrants and food safety

Gebre *et al.* (2011) identified various socio-economic aspects related with migrants' mobility, including social networks and improved accessibility and availability of opportunities. In terms

of income generation, street foods have a crucial socio-economic function (Ohiokpehai, 2003). The country's economic state, social problems, and urbanization, among other things, all contribute to the expansion of the informal economy, which includes street food (Ngundu, 2013).

Health is influenced by socioeconomic position through a variety of methods, including psychological variables, health behaviours, and health care (Ngundu, 2013) In developed countries, food-borne disease is a major cause of morbidity and lost productivity (Newman *et al.*, 2015). Every illness has a monetary cost, and the financial impact of health losses associated with food-borne illnesses has not been thoroughly investigated. Diarrhoeal illnesses claim the lives of almost 2.2 million people each year, the majority of whom are children in developing countries (Havelaar *et al.*, 2013; Kuchenmüller *et al.*, 2013; Organization, 2016). Food-borne illnesses have been identified as a multi-sectoral public health hazard associated to agriculture and livestock health (Kuchenmüller *et al.*, 2013)

2.6 Food-borne pathogens of public health importance

2.6.1 Introduction

Food-borne diseases, which impact both developed and developing countries, including South Africa and Africa, are a global public health issue (Scott, 2003). In affluent countries, the World Health Organization (WHO) estimates that up to 30% of the population is affected by food-borne infections or illnesses each year. On the other hand, it is estimated that up to two million people die each year (Hassanain *et al.*, 2013).

There is an introduction of food-borne pathogens and excessive outbreaks in the food industry. (Nsoesie *et al.*, 2014) their study of epidemiology states a change in the epidemiology of food-borne diseases as new pathogens have emerged and reported to the communities. Emphasis on changing the epidemiology of food-borne illnesses is stressed, and the implementation of prevention technologies must be understood and monitored (Nsoesie *et al.*, 2014). Previous research has identified a significant issue. Many food handlers in food service establishments lack basic food safety skills such as temperature control, personal cleanliness, and cross-contamination prevention (Sibanyoni *et al.*, 2017); as a result, many serious food-borne illnesses are introduced.

2.6.2 Incidence of *Listeria monocytogenes* food-borne pathogen in meat products and public health implications

Listeria monocytogenes is gram-positive, facultative intracellular food-borne pathogen that causes food poisoning (Bucur *et al.*, 2018). *Listeria monocytogenes* is the cause of Listeriosis, a serious invasive sickness that affects both humans and animals. Pregnant women, newborns, the elderly, and immune compromised people are all susceptible to Listeriosis (Arslan & Baytur, 2019). Listeriosis has been extremely rare in the general population. However, the infection is linked to a high rate of death, particularly in humans (Bucur *et al.*, 2018).

Meningitis, septicemia, and abortion are all possible symptoms of Listeriosis (Locatelli *et al.*, 2013). Animals infected with *Listeria monocytogenes* are generally asymptomatic carriers, according to (Nwachukwu *et al*., 2010*)* and *Listeria monocytogenes* can be lost through feces, which can contaminate fresh and processed meat products (Oh *et al.*, 2016). According to Norhana *et al.* (2010), *Listeria monocytogenes* is frequently found in fish products such as shrimp and smoked salmon at both the wholesale and retail sectors. The incidence of *Listeria monocytogenes* in aquatic habitats was then found to be related to human activity. The bacterium's virulence level, 10 percent of cleaning cloths from a Johannesburg retail shop that sells a variety of fine, exotic, or foreign prepared meals (Chersich *et al.*, 2018).

Listeria monocytogenes occur extensively in agricultural, aqua culturally atmosphere, and food processing environments and are regarded as the most virulent pathogen (Chersich *et al.*, 2018). A higher prevalence of *Listeria* has been found in soils closer to water, with advanced moisture, recently cultivated, irrigated, or rained upon, and close to pasture. The favourable environment of the bacteria raises an alarm that fish and products are highly exposed to a significant risk of being contaminated with the pathogen (Buchanan *et al.*, 2017).

Fish could be another source of human listeriosis due to the high incidence of *Listeria monocytogenes* and other pathogenic bacteria that can survive in the environment. Because *Listeria monocytogenes* is traditionally connected with the food chain, pre-harvesting, and retail processing, it could constitute a public health risk (Chersich *et al.*, 2018) As a result, contamination of natural goods could be a key contributor to the risk of widespread *Listeria monocytogenes* infection, particularly if products are consumed without prior heat treatment (Novoslavskij *et al.*, 2016).

The National Institute for Communicable Diseases has observed a rise in *Listeria monocytogenes* isolations across the country, particularly in Gauteng Province (NICD). *Listeria monocytogenes* is found in raw or unpasteurized milk, soft cheeses, vegetables, processed meals, ready-to-eat meats, and dried fish items (Gilbreth *et al.*, 2005). Several outbreaks have recently been reported in South Africa, notably the Listeriosis outbreak, which was the largest ever documented globally and was first seen and identified by the public in early 2017. (Chersich *et al.*, 2018). The source of the *Listeria monocytogenes* outbreak in 2018 was food manufactured in South Africa's Limpopo area (Chersich *et al.*, 2018).

2.6.3 Incidence of *Salmonella* food-borne pathogen and public health implications

Salmonella is a Gram-negative, facultative bacterium that causes diarrhea. Symptoms include fever, stomach pains, nausea, and vomiting (Knezev, 2015). These viruses generate significant morbidity, death, and economic burden in South Africa, and are especially dangerous in newborns, the elderly, and patients who are immune-compromised (Mainali *et al.*, 2009). After *Campylobacter*, *Salmonella* is the second most prevalent cause of human gastroenteritis and a prominent cause of food-borne illness globally (Castro Rosas, 2012). *Salmonella* is a mesophilic bacterium that does not live in the aquatic environment naturally. Hygiene problems during production are the major cause of microorganisms in aquaculture settings and products (Budiati *et al.*, 2013). Ready-to-eat food intake is still a well-documented main cause of *Salmonellosis*, with undercooking and cross-contamination being known risk factors (Yang *et al.*, 2016).

More than 2600 serotypes have been identified to date, with an estimated 94 million cases of gastroenteritis and 155.000 fatalities annually. As a result, 85 percent of these instances were linked to diet (Deng *et al.*, 2012; Majowicz *et al.*, 2010). Ingestion of tainted food remained the most common cause of *Salmonellosis*, with undercooking and cross-contamination being identified as significant risks (Carrasco *et al.*, 2012; YU *et al.*, 2010). Serotypes of *Salmonella* can grow and survive in a variety of foods (Castro Rosas, 2012).

Salmonella's behavior in foods is controlled by a variety of environmental and ecological variables, including water activity, pH, chemical composition, natural or added antimicrobial agents, temperature, and processing factors including heat and physical manipulation (Mahmoud, 2012). *Salmonella* infections are spread by consuming infected food or drinking contaminated water (Castro Rosas, 2012). The principal drivers in the salmonellosis outbreak

have been poultry, eggs, beef, and milk products; thus, fruits, vegetables, and seafood are secondary sources (Castro Rosas, 2012).

Non-typhoid Salmonellosis (NTS) is a serious public health disease that affects people all over the world, but especially in Sub-Saharan Africa. It causes gastroenteritis and bloodstream infections in both children and adults (Olobatoke & Mulugeta, 2015). In industrialized countries, the gastroenteritis form is common (Olobatoke & Mulugeta, 2015); immuno-compromised individuals, such as those with HIV, cancer, or diabetes, are at higher risk of NTS bacteremia and are more likely to develop focal infections like meningitis, septic arthritis, pneumonia, and osteomyelitis (Subramoney, 2015). Despite the fact that more than 2500 Salmonella enterica serovars have been identified, *Salmonella typhimurium* and *Salmonella enteritis* are the most common causes of human infection *(Luber, 2009). Salmonella enteritidis* isolates were found in the stool of 18 patients and one food sample in a study done in South Africa's KwaZulu Natal area (Niehaus *et al.*, 2011).

From 2002 to 2006, a retrospective investigation in one of the Democratic Republic of Congo's hospitals found that 59% of children had NTS bacteremia (Vandenberg *et al.*, 2010) and 82% of the cases were caused by *Salmonella typhimurium* and *Salmonella enteritis* (Olobatoke & Mulugeta, 2015). In Mozambique, NTS was said to be responsible for 120 cases of pediatric bacteremia per 100,000 people per year (Vandenberg *et al.*, 2010). Contaminated chicken meat, eggs, and fish, among other things, have been linked to the spread of these tough bacteria (Olobatoke & Mulugeta, 2015). Paudyal *et al.* (2017) *Escherichia coli, Salmonella* spp., *Staphylococcus aureus*, and *Listeria monocytogenes* were the most prevalent bacteria identified from various African countries. In raw and ready-to-eat foods, all of the pathogens mentioned have a two-digit percent prevalence on average. The presence of *Escherichia coli* and *Salmonella* at higher levels in natural and RTE meals indicates a major violation in important control points during food handling.

2.6.4 Prevalence of *Escherichia coli* food-borne pathogen and public health implications

The majority of *Escherichia coli* strains are harmless. However, in many countries, including South Africa, *Escherichia coli* has been identified as amongst the most common causes of food poisoning (Ekici & Dümen, 2019). Furthermore, some strains of Shiga toxin-producing and *Escherichia coli* (Kumar *et al.*, 2009) can cause severe food-borne disease with symptoms such as abdominal pains and diarrhea, which can escalate to bloody diarrhea (hemorrhagic colitis), fever, and vomiting (Ekici & Dümen, 2019). Although *Escherichia coli* O157:H7 is the most

dangerous STEC serotype in terms of public health, other serotypes have been implicated in occasional cases and outbreaks (Law, 2000).

Escherichia coli is a typical indication of fecal contamination in food and is used to evaluate the food hygiene (Ukut *et al.*, 2010). In underdeveloped nations, enterotoxigenic *Escherichia coli* (ETEC) is a prominent cause of traveler's diarrhea (TD) and diarrhea in children in their early years (Fletcher *et al.*, 2013; Rivera *et al.*, 2013). The presence of fecal coliforms in farmed, feral, or processed fish should be tested in every country where a fish inspection program exists to validate the presence of a health concern in the harvest or product (Del Rio-Rodriguez *et al.*, 1997). *Escherichia coli* has been discovered to be contaminating from an external source rather than being present in the fish itself.

2.6.5 Incidence of Staphylococcus food-borne pathogen and public health implications

Under the microscope, *Staphylococci* are gram-positive bacteria appear as clusters of cocci (Ranzani *et al.*, 2020). According to Blaiotta *et al.* (2010), the genus Staphylococcus has around forty species and subspecies that are primarily divided into two categories: CPS (coagulase-positive staphylococci) and CPS (coagulase-negative staphylococci). CNS stands for coagulase-negative staphylococci. CPS *Staphylococcus aureus*, on the other hand, has long been the most pathogenic bacteria of the *Staphylococcus* genus (Chambers, 2001). *Staphylococcus aureus* is known for its dishonourable ability to develop drug resistance. *Staphylococcus aureus* is a neutral and aggressive bacteria that can cause a variety of ailments, from mild skin infections to life-threatening invasive diseases. (Kadariya *et al.*, 2014).

Staphylococcus aureus has been a major source of food poisoning all over the world (Akbar & Anal, 2013). This bacterium can contaminate a variety of foods, including minimally processed ready-to-eat vegetables and processed meat items, all of which create enterotoxins. According to Chambers and DeLeo (2009), direct touch is the most common way for *Staphylococcus aureus* to spread. Skin-to-skin contact with a colonized or infected human is the most common source of infection, however contaminated objects and surfaces can also be a source of infection.

2.6.6 Incidence of multi-drugs resistance

In low-income countries, Antibiotic residence frequently occurs in microorganisms that are likely to be transmitted in the community and some that could cause an outbreak (Ndihokubwayo *et al.*, 2013). Various surveillances on antimicrobial resistance among fish and dried fish products have been published in Africa in recent years; nevertheless, information is

still lacking (FAO, 2012). Growing numbers of the community are concerned about the links between antibiotics in the humans' food chain and antibiotic resistance. Levy and Marshall (2004) also reported that antibiotic use played a significant role in the emerging public health antibiotic resistance crisis. Experts believe that the abuse of medications on farms is to blame for the growth of drug-resistant illnesses in humans, which might have disastrous consequences.

Antibiotic resistance is common in bacteria that are likely to be transmitted in the community and some that could produce an outbreak in low-income countries (Ndihokubwayo *et al.*, 2013). Various surveillance on antimicrobial resistance among fish and dried fish products have been published in Africa in recent years; nevertheless, information is still lacking (FAO, 2012). Antibiotic resistance and its ties to antibiotics in the human food chain are causing increasing concern in the community. Antibiotic use, according to Levy and Marshall (2004), played a substantial influence in the rising public health antibiotic resistance dilemma. Experts believe that the abuse of medications on farms is to blame for the growth of drug-resistant illnesses in humans, which could have disastrous consequences.

Drugs are commonly used in the feed and water of intensively farmed animals to prevent infections that would otherwise be unavoidable in such confined and crowded environments (Mosel, 2001). Unfortunately, the same antibiotics are increasingly used by farmers in human medicine. In some cases, people were negligent and not adhering to environmental health safety through dumping medical waste close to rivers, which resulted in water and fish contamination (Berendonk *et al.*, 2015).

According to Newell *et al.* (2010), it is reported that overuse of antibiotics in the veterinary field has been the most important source of resistant strains of *Salmonella* and *Campylobacter* bacteria, and *Escherichia coli*, and other bacteria of public importance. Resistant strains of bacteria can pass from animals to humans in numerous ways, mainly through food when people use *Salmonella* bacterium contaminated dried fish as food that may be resistant to antibiotics (Newell *et al.*, 2010). In some cases, resistant bacteria can colonize and multiply in the human gut without causing immediate illness (Guilfoile & Alcamo, 2007).

The presence of antibiotic residence in the food may alarm a significant risk to human health. Health complications are linked to antibiotic-resistant, but more limited therapeutic remedies, especially to developing countries like South Africa that lack excellent access to suitable

quality treatments. As a result, infections continue to be a significant cause of morbidity and mortality (Samie *et al.*, 2009).

2.7 Common food-borne pathogens and antibiotic resistance

2.7.1 Introduction

Antibiotic resistance surveillance and molecular epidemiology research as a means of managing resistance are gaining traction under the "One Health" concept, which aims to regulate clinical, environmental, and veterinary antibiotic resistance (Osei Sekyere, 2016). Antibiotic resistance is mostly caused by ineffective intersectoral national policies, plans, and programs, as well as a lack of education for professionals in key technical positions to assist them in providing effective management.

2.7.2 Antibiotic resistance of *Escherichia coli*

The Resistance to antibiotics in *Escherichia coli* is linked to genetic changes or the acquisition of pre-existing genes that confer resistance in the deoxyribonucleic acid (DNA) of bacterial chromosomes or plasmids (Christopher *et al.*, 2013). For example, *Escherichia coli* was found to be highly resistant to ampicillin, amoxicillin, tetracycline, and trimethoprim-sulfamethoxazole in a study conducted in India (Jiménez-Belenguer *et al.*, 2016). Furthermore, the study found that *Escherichia coli* isolates were more resistant to the drug Amoxycillin (Jiménez-Belenguer *et al.*, 2016).

An increase in the occurrence of extensively drug-resistant gram-negative bacteria was found in 2015, and this was emphasized as a problem for SA (Coetzee *et al.*, 2016). Multidrug resistance (MDR) in *Escherichia coli* bacteria isolated from discharged final effluents of two wastewater treatment plants in the province was discovered in a study conducted in the Eastern Cape in 2015 (Adefisoye & Okoh, 2016). Profile of samples submitted to a polymerase chain reaction (PCR) confirmed *Escherichia coli* isolates against 17 major antibiotics in human and veterinary medicine was determined using the conventional disk diffusion method (Adefisoye & Okoh, 2016). According to Adefisoye and Okoh (2016), wastewater effluents are important reservoirs for the spread of potentially pathogenic *Escherichia coli* and other pathogens in the Eastern Cape's aquatic environment, posing a considerable risk to public health (Adefisoye & Okoh, 2016).

Environmental bacteria communities, such as *Escherichia coli*, have been linked to known antibiotic-resistant gene pools that have been transmitted into normal human and animal flora

(Adefisoye & Okoh, 2016). Because it is often found in a variety of hosts and habitats, and it acquires resistance quickly, *Escherichia coli* has been linked to being used as a proxy for antibiotic resistance surveillance (Adefisoye & Okoh, 2016).

According to Alonso *et al.* (2017), the worldwide usage of antibiotics for animal health and production outnumbers human use. The majority of veterinary medications are closely linked to or belong to the same antimicrobial classes as those used in people (Marshall & Levy, 2011) Because of its medicinal value and prevalence in a wide range of hosts, *Escherichia coli* is widely regarded as a useful indication of antibiotic resistance (Szmolka & Nagy, 2013).

2.7.3 *Salmonella* and antibiotic resistance

Antibiotic-resistant strains of *Salmonella enterica* have emerged in recent years. It has raised a serious concern to the food industry, and Salmonella-resistant strains have also been prevalent in many imported seafood samples (Ravishankar *et al.*, 2010). *Salmonella* has been a leading cause of food-borne disease worldwide, and ready-to-eat (RTE) foods are regarded as a significant source of infection and outbreak of *Salmonella* (Yang *et al.*, 2016). Moreover, antibiotics in animal husbandry and human medicines have created selective pressure that shows favouritism in developing particular resistance among microorganisms (Witte, 2000). Thus, the widespread use of antibiotics in plants and animals has formed a pool of resistance genes in the environment (Doyle *et al.*, 2006).

A study on pasteurized milk documented that contaminated dairy plants were implicated in an outbreak involving 180,000 cases associated with *Salmonella Typhimurium* resistant to five antibiotics (Ravishankar *et al.*, 2010; Ryan *et al.*, 1987). *Salmonella* microbial resistance evolves over time, according to Yang *et al.* (2016), posing major public health consequences.

2.7.4 *Staphylococcus aureus* and antibiotic resistance

Antibiotic-resistant staphylococci have not been routinely investigated in informal markets, and data is only available from a limited number of studies (Nugent *et al.*, 2010). Chajęcka-wierzchowska *et al.* (2014) published a study in which they discovered that *Staphylococcus aureus* was the most common bacteria. Antibiotic-resistant staphylococci have not been routinely investigated in informal markets, and data is only available from a limited number of studies (Nugent *et al.*, 2010). *Staphylococcus aureus* was determined to be the most abundant species in the sample obtained by Chajęcka-wierzchowska *et al.* (2014) followed by *Staphylococcus xylosus*, *Staphylococcus saprophyticus*, and *Staphylococcus epidermidis*. Chajęcka-wierzchowska *et al.* (2014) found that more than 54.9 % of the isolates were resistant

to one class of antibiotic tested, with 35.4% of the strains being classed as multidrug-resistant. Cefoxitin resistance was found in 49.6% of the isolates, followed by clindamycin resistance in 39.3%, tigecycline resistance in 27.4%, quinupristin-dalfopristin resistance in 22.2%, rifampin resistance in 20.5%, tetracycline resistance in 17.9%, and erythromycin resistance in 8.5 percent. Chajęcka-wierzchowska *et al.* (2014) found that more than 54.9% of the isolates were resistant to at least one class of antibiotic tested, with 35.4% of the strains being classed as multidrug-resistant. Cefoxitin resistance was found in 49.6% of the isolates, followed by clindamycin resistance in 39.3%, tirecycline resistance in 27.4%, quinupristin-dalfopristin resistance in 22.2 percent, rifampin resistance in 20.5%, tetracycline resistance in 17.9%, and erythromycin resistance in 8.5%.

Many nations have traditionally treated infections caused by *Staphylococcus aureus* using penicillin, but recent research has found that the bacteria has acquired resistance to the medication (Jamali *et al.*, 2015). In addition, *Staphylococcus aureus* has developed resistance to a range of antibiotics, including methicillin, gentamycin, tetracycline, and others (Chambers, 2001). (Türkyılmaz *et al.*, 2010) discovered that methicillin resistance in Staphylococcus aureus is frequent in hospitals, and that the resistant genes of this bacterium increase slowly, potentially restricting the capacity to treat sickness in the future. Many species of *Staphylococci* have antibiotic resistance (Chajęcka-wierzchowska *et al.*, 2014), which may be passed on to *Staphylococcus aureus*, making it resistant to a wide range of medications.

2.7.5 Antibiotic resistance of *Listeria Monocytogenes*

Conter *et al.* (2009) conducted a study in Italy that found *Listeria monocytogenes* strains originating in the food and food manufacturers are responsive to antibiotics frequently used in the treatment of veterinary and human Listeriosis. However, because *Listeria monocytogenes* is slowly becoming antibiotic-resistant, it was determined that ongoing surveillance of this pathogen's increasing antimicrobial resistance is required to ensure efficient treatment of human listeriosis. More information on antibiotic resistance strains isolated from food and food environments might assist to improve the data that is now available.

Listeria spp., were isolated from raw milk by Jamali *et al.* (2013). Tetracycline resistance was 49.4% and penicillin resistance was 43.4%, however gentamicin, vancomycin, and rifampicin were still susceptible. As a result of the findings, raw milk drinking may pose a risk of human listeriosis.

CHAPTER THREE

METHODOLOGY

3.1 Study area

The selected study areas were Johannesburg and Pretoria, situated in Gauteng province, in South Africa (SA), which attracts most of the migrants or population. Gauteng, which is regarded as the economic powerhouse of South Africa, has long been dependent on immigration to supply its labour requirements, a phenomenon deeply rooted in the province's early economic history (development of mining and heavy industry) (Oosthuizen & Naidoo, 2004). Population in SA was estimated to be 57,73 million mid-year in 2018, representing an overall increase of 1,55% between 2017 and 2018 (www.statssa.gov.za) Gauteng has the highest population density, with 14,7 million people (or 25.4% of the total population) residing in the province. It is significantly urbanized and contains the country's biggest cities. Also, it stands out among the most populated areas in South Africa. Therefore, Gauteng is a significant community of migrants, mostly economic migrants from all over the country and the rest of Africa (http://worldpopulationreview.com/world-cities/pretoria-population). The province attracts most foreign migrants with its energetic environment of wealthy people and artists, musicians, students, and political activists. Pretoria is regarded as the capital city of Gauteng and the second popular after Johannesburg that has similar attributes and shares most of its features as well (Prayag, 2007).

3.2 Study design

A cross-sectional descriptive study was conducted to assess the microbiological quality of dried fish in Gauteng province, SA, from September 2018 to July 2019. Dried fish samples were purchased from different informal markets in Pretoria and Johannesburg. The Selection of vendors was based on the convenience sampling method due to the instability of the informal market.

3.3 Selection of informal markets and samples

The targeted Informal markets were the huge shops where customers were more populated, plus more than one seller was present and selling the same product. Data was collected from Sunnyside, Yeoville, Rosettenville, and MTN taxi rank in Johannesburg markets.

3.4 Ethical consideration

For all experimental and animal care methods, the ethics committee accepted ethical consideration for the North West University study for animal and human. The ethic number is NWU- 01880-19-A5 with risk category 2. All participants involved in the study were kept anonymous for their safety and of the markets. The ethical approval letter is attached (see Appendix 9).

3.5 Data collection

3.5.1 Observation study

The observation was performed using a pre-structured checklist to assess food safety practices of informal markets and vending sites by adapting a variety of checklists that have been utilized in past studies (Tshipamba *et al.*, 2018). The observation was performed during the time of sample collection. The observation sheet was designed to focus on food service management and monitor the following procedures: General management of food vendors, storage, food packaging, holding, wastage, and hygiene practices. In addition, the vending site was assessed for the personal hygiene of food vendors (hands, nails, and hair), protective garments, means of food protection, and the presence of flies, stagnant water, and insalubrious.

- **Vending site and environment**

The vending site was examined for the existence of:

- Stagnant water near the vending site.
- Presence of flies and insects
- Neatness or cleanliness of the vending site

A tick was made on the checklist if one of these aspects was observed on the site of samples collection.

- **Hygienic measures applied by the vendors**

The sanitary standards observed were based on:

- Hand washing before handling the dried fish
- Hand washing with disinfectants

- Packaging of dried fish
- Storage of the dried fish

3.5.2 Data Analysis for the checklist

- **Samples collection**

A total of twelve informal markets from Pretoria (n=50) and Johannesburg (n=90) were conveniently sampled for the laboratory analysis. The samples were collected twice from the same markets. About 70 dried fish samples were collected in the first round, following the other 70 samples after one month.

Dried fish samples (n-140) were collected using the standard aseptic protocol and transported to the Microbiology Laboratory in the Department of Animal Health, North West University, in a sterile container with ice to prevent cross-contamination. For transportation to the Animal Health Microbiology laboratory, the containers were carefully packed, sealed and labelled with random numbers for identification, and put on ice. The summary of samples collection is presented in Table 3.1, and images were taken at the vending sites.

Table 3.1: Summary of sample collection data

Area of collection	Number of large markets	Number of packets of dried fish samples collected
Sunnyside (PTA)	5	50
Yeoville (JHB)	3	30
Rosettenville (JHB)	2	30
MTN taxi Rank (JHB)	2	30
Total	12	140

Keys: PTA- Pretoria, JHB- Johannesburg

Figure 3.1 Dried fish samples photographed taken from the vending site

- **Sample size determination**

Based on the instability of the informal markets in Pretoria and Johannesburg and the unknown number of dried fish sellers, a non-probability sampling method was used (convenience sampling). A total of 12 markets were visited, and 140 samples were collected.

- **Different of samples**

The samples collected were grouped according to the preservation methods used. Three types of dried fish were collected, which were smoked (40), sun-dried (80), salted (20). The fish were packaged in zip plastic and labelled for identification (Figure 3.2).

Figure 3.2 Different types of dried fish sold in the informal markets.

3.6 Bacterial Isolation

A 10 g of fish samples were ground and weighed aseptically and put into an Erlenmeyer flask containing 90 ml of peptone water to obtain a 1-part sample plus nine parts peptone water. The inoculum was homogenized and incubated overnight at 37°C in a shaker incubator (Model, fsies p024, serial C2421, South Africa) for enrichment (Cortese *et al.*, 2016). After incubation, 1ml from the homogenized product was pipetted and dispensed into test tubes containing 9ml of nutrient broth, and a series of fivefold dilutions before spreading on plate count took place. The high dilution 10^{-5} was focused on obtaining bacterial isolates representative density of bacterial (Brook & Frazier, 1995).

One ml of the diluted product was spread on the plate for the bacterial count, and the plates were incubated at 37°C for 24 hours (Apha, 1998). After an incubation period, the plates with the best countable range between 30 and 300 colonies were selected, and colonies were counted manually. Next, a plate was divided into four quarters using a marker, and the colonies were counted, recorded. Finally, the number of colony-forming units was calculated, recorded, and expressed in colony-forming units per ml (CFU/ml) using the following equation:

$$\text{Number of bacteria} = \frac{(\text{Number of colony-forming unit (CFU)} \times \text{Volume plated})}{\text{Total dilution factor}}$$

To get a single bacterial colony, colonies were streaked 2 or 3 times until purity was obtained for Gram staining.

3.7 Phenotypic identification of the isolated bacteria

3.7.1 Gram staining

The Gram staining technique was performed to distinguish Gram-positive from Gram-negative as a preliminary screening method described by Purkayastha *et al.* (2010). A single colony was collected with a sterile wire loop and smeared on the microscope slide with a few drops of running tap water, and fixed on the slide by softly heating for 30-45 seconds. Next, crystal violet was applied and left to stand one minute, followed by washing the slide with sterile water.

The slide was flooded with iodine solution and allowed to remain for 1 minute. The iodine solution was then rinsed off with running distilled water. After, it was flooded with decolourizer (95% alcohol) for 2 seconds and rinsed off with distilled water to decolorize the slide. Finally, a few drops of safranin solution were dropped on the slide as a counterstain for

1 minute, and the slide was then washed with running tap water, blotted, and dried in air. The procedure was done for all the plates with pure colonies.

The slides were examined by light microscope under 100X objective using immersion oil, and the bacteria stain with deep violet-blue colour indicates Gram-positive. In contrast, bacteria stains pink to red indicates Gram-negative. The cells were observed for both colour retained and morphology (cocci, rod, or spiral).

3.7.2 Catalase test

A catalase test was performed to differentiate bacteria that produce the enzyme catalase called catalase. The method was done to aid with the identification of Enterobacteriaceae. Members of the Enterobacteriaceae family are known to be catalase-positive. The catalase test was done following a method described by (Montso & Ateba, 2014a). A single bacterium was handpicked from a pure culture with a sterile wire loop and smeared on the glass slide, and then 2 to 3 drops of 3% hydrogen peroxide were dropped on top of the bacteria. If an isolate was catalase-positive, a bubbler of oxygen was released after 10 seconds, and if the isolates were catalase-negative, no gas was produced (Cheesbrough, 2006).

3.7.3 Oxidase test

An oxidase test was performed following the method Gerth *et al.* (2011) described to identify bacteria that produce cytochrome oxidase. The isolates were subjected to sub-culturing on fresh nutrient agar and incubated at 37˚C for 24 hours. After 24 hours of incubation, a single isolate from the pure culture was smeared over oxidase strips of paper. An intense blue coloration indicated a positive result after 5 to 10 seconds. Delayed positive results appear within 10 to 60 seconds. A negative result was also observed by the absence of coloration after 1 minute.

3.7.4 Indole test

Indole test was done to aid with differentiating Enterobacteriaceae and other general organisms. Indole test was performed as described by Hassan *et al.* (2011). This test was performed using Kovac's reagent. Tryptone broth was inoculated with the test organism and incubated at 37°C for 24−28 hours. After 24 hours, 3−5 drops of Kovac's reagents were added. The red colour ring at the upper layer of the liquid indicated indole positive, while yellow/no colour indicated indole negative.

3.7.5 Coagulase test

Coagulase test was used to differentiate *Staphylococcus aureus*, which produces the enzyme coagulase from other *Staphylococcus* species like S. *epidermis* and *S. saprophyticus,* which do not produce coagulase. The slide test method was used whereby a drop of saline water was placed on each end of a slide, and a portion of the isolated colony was emulsified in each drop to make two thick suspensions using a sterile wire loop. A drop of rabbit plasma was then added to one of the suspensions, well mixed, and the reaction was observed within 10 seconds. Coagulase-positive bacteria formed clots, and coagulase-negative ones had no clumping. The plasma was not added to the second suspension to differentiate false positivity, such as the granular appearance of the organism from the true coagulase clumping.

3.8 Molecular Approach

3.8.1 Extraction of genomic DNA

(D6005 USA supplied by Bio lab, South Africa) According to the manufacturer's instructions, the total genomic DNA of isolates was extracted using a Zymo-Research Fungal/Bacterial DNA kit. A single colony from pure culture was inoculated into 20 mL bottles filled with nutrient broth and incubated aerobically at 37˚C for 24 hours. The inoculum product was then transferred into a 15 mL conical tube and centrifuged at 15 000 rpm for 10 minutes. The supernatant (pellets) obtained was used for genomic DNA extraction using Zymo Research kit as follows: pellets were suspended into ZR Bashing Bead Lysis tubes, and 750 µL lysis solution was added, and the tubes were disrupted with disruptor gene. Following the disruption, the tubes were vortexed at 14.000 rpm for 14 minutes, followed by centrifugation at 10 000 rpm for 1 minute. About 400 µL of the supernatant was transferred to a Zymo-spin III -F filter and centrifuged at 8000 rpm for 1 minute. Then 12 000 µL of Genomic Lysis buffer was added to the filtrate, and 800 µL of the mixture was transferred to a new collection tube (Zymo-spin IIC ^{TM}) Column and centrifuged at 10 000 rpm for 1 minute. The filtered DNA was pre-washed by adding 200 µL DNA pre-wash buffer to the Zymo-Spin IIC Column and centrifuged at 10.000 rpm for 1 minute. A total of 500 µL of gDNA wash buffer was added to the new collection tube (Zymo-spin IIC ^{TM}) and centrifuged at 10.000 rpm for 1 minute. Finally, 100 µL of DNA elution buffer was added to elute the DNA in a sterile 1.5 mL micro-centrifuge tube.

3.8.2 Amplification of 16S rDNA

The identification of the isolated bacteria in this study was based on the amplification of the 16S rDNA gene using a polymerase chain reaction (PCR). Amplification was carried out in reaction volume of 50 µL, containing: 25 µL PCR Master Mix, 2 µL template DNA, 19 µL nuclease-free water, and 4µL of each oligonucleotide primer (25 µM). The forward primer 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and the reverse 1492R (5'- ACG GCT ACC TTG TTA CGA CTT 3') (Oloyede *et al.*, 2017). These primers were synthesized commercially by Inqaba Biotechnical Industrial (Pty) Ltd, Pretoria, South Africa. The reagents were prepared and mixed in the PCR tubes (Ngoma *et al.*, 2013; Tshipamba *et al.*, 2018). The thermal cycling was performed in Bio-Rad T100TM thermal cycler (Singapore). The conditions were as follows: one cycle of 95°C for 30 seconds followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30seconds and extension at 72°C for 2 minutes, followed by the final cycle of extension of 72°C for 10 minutes and incubated at 4°C forever.

3.8.3 Agarose gel electrophoresis

The electrophoresis of the 16S rDNA was performed according to the methods and procedure followed by Ngoma *et al*. (2013). The amplification product were separated in 1% of agarose gel. The agarose gel was prepared as follows: 1g of agarose gel was weighed and mixed to 100ml of TAE buffer, and the agarose was then dissolved by using the microwave for 5 minutes. The gel was then allowed to cool at about 40˚C, and ethidium bromide 0.5ml was added for staining. The gel was cast and allowed to set. After the gel was placed inside the electrophoresis chamber, 5µL DNA and 5µL of loading dye were mixed and transferred to one of the walls in the gel electrophoresis tank. The DNA ladder (100bp) was used.

Electrophoresis conditions were set for 45 minutes at 80 voltages and 400 MA. The gel was then visualized using the Gel Doc imaging system (Bio-Rad Chemi DocTMMP). The presence of a single bright band (DNA fragment) for each sample indicated a successful amplification, and the DNA bands were captured using Image Lab (version 6.00.22) software. Following the electrophoresis, the PCR products were sent to Inqaba Biotechnical Industrial (Pty) Ltd, Pretoria, South Africa, for sequencing.

3.9 DNA Sequencing

The amplified 16S rDNA products were sent to Inqaba Biotechnologies (Pretoria, South Africa) for sequencing. The sequences obtained from Inqaba Biotechnologies were corrected manually where necessary using Finch-TV computer software. Bio-Edit software was then used to align both sequences (forward and reverse), and consensus sequences were used for the identification of the isolates using BLAST (basic local alignment search tool) NCBI (National Center for Biotechnology Information).

3.10 Phylogenetic reconstruction

The homology of partial sequences obtained was compared with the sequences from the NCBI databases. The similarity 95% to 100% was generated by the nucleotide Basic Local Alignment Search Tool (BLAST) program. The Phylogenetic tree was reconstructed by following the neighbor-Joining method's evolutionary history (Saitou & Nei, 1987). The optimal tree with the sum of branch length = 14.33746207 was shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Shimodaira & Hasegawa, 2001). The tree was drawn to scale, with branch lengths in the same units as the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method (Nei & Kumar, 2000) and

the number of base differences per site. This analysis involved 48 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1552 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018).

3.11 GenBank Accession Number

The complete sequences of the 16S rDNA gene of the isolates were deposited in the NCBI and assigned accession numbers.

3.12 Antibacterial susceptibility test

Antibiotic Sensivity test was performed using the disc diffusion method (Patel *et al.*, 2015). A single colony was inoculated in a 5ml of nutrient broth and incubated overnight. After that, 1ml of the inoculum product was pipetted and spread on the entire surface of Mueller-Hinton agar, and allowed to dry for 3mins. The infused antibiotic discs of different concentrations (Oxoid® Ltd., Basingstoke, Hampshire, England) such as Gentamicin (CN: 10µg/disc); Norfloxacin (NOR: 30µg /disc); Ciprofloxacin (CIP: 30µg/disc); Ampicillin (AML:10 µg /disc); Chloramphenicol (C: 30 g/disc); Streptomycin (S: 10µg /disc) and Erythromycin (E: 5µg /disc) were placed on the Mueller-Hinton agar and incubated at 37℃ for 24hrs. After incubation, the zone of inhibition around each disc was measured with a meter rule and recorded. The results were interpreted following the guideline of the Clinical Laboratory Standards Institute (CLSI) criteria (Patel *et al.*, 2015) (Table 3.2). Standard reference strains of *Staphylococcus aureus* ATCC® 29213 and *Escherichia coli* ATCC® 25922 were used as quality control organisms in antimicrobial susceptibility determination (Boss *et al.*, 2016).

Table 3.2 Guideline of antibiotic resistance according to the Clinical Laboratory Institute (CLSI)

Antibiotics	Abbreviation	Antibiotic concentration (Disc/ μg)	Zone diameter, Breakpoints Nearest whole Mm		
			Susceptible S	Intermediate I	Resistant R
Amoxicillin	AML10	10 μg	≥ 18	14-17	≤ 13
Gentamicin	CN10	10 μg	≥ 15	13-14	≤ 12
Norfloxacin	NOR5	5 μg	≥ 17	13-16	≤ 12
Ciprofloxacin	CIP5	5 μg	≥ 21	16-20	≤ 15
Chloramphenicol	C30	30 μg	≥ 18	13-17	≤ 12
Erythromycin	E5	5 μg	≥ 23	14-22	≤ 13
Streptomycin	S10	10 μg	≥ 25	18-24	≤ 17

3.13 Statistical analysis

The data collected were analysed using descriptive and inferential statistics such as means, frequency distribution, and percentage using IBM SPSS Statistics (ver.27.0). Microbiological counts were transformed into logarithmic values (log10 cfu mL-1). The values were analysed using an Excel worksheet for easy comparison. They were presented as means, standard deviation, and these transformed values were analysed using the General Linear Model for least-square means in SPSS using the fixed-effect model. Analysis of variance (ANOVA) was carried out to measure variability at 5% level of significance.

CHAPTER FOUR

RESULTS

4.1 The worksheet data from the informal markets

The observation checklist had a total number of 12 food vendors which participated in this study (Table 4.1). The estimated age to most of them was 58.3% (n=7/12) aged between 30-49. Remarkably 25 % (n =3/12) were aged 29 years and classified as the youngest. In contrast, 16.6 % (n=2/12) were old and aged over 50 years of age. Furthermore, most food vendors were females, 66.6% (n =8/12), while 33.3% (n = 4/12) were males, as shown in Table 4.1. In addition, all the vendors where the dried fish samples were collected are foreigners.

Regarding the biosecurity on the selling points, the observations from this study revealed that the presence of flies at 91.7% (n=8/12) of vending places. Furthermore, the observation of all selling points 100% (n=12/12 showed that the food handlers/ sellers were not adhering to decontamination of food safety practices and using personal protective equipment (PPE). In addition, other unhygienic practices were observed during this study are presented in Figure 4.1.

Table 4.1 Summary of demographic information of the participants' characteristics

variables	number (n=12)	Percentage (%)
Estimated age		
<29 years	3	25%
30 to 49	7	58.3%
>50	2	16.6%
Gender		
Females	8	66.6%
Males	4	33.3%
Nationality		
None of the vendors were South African nationals		

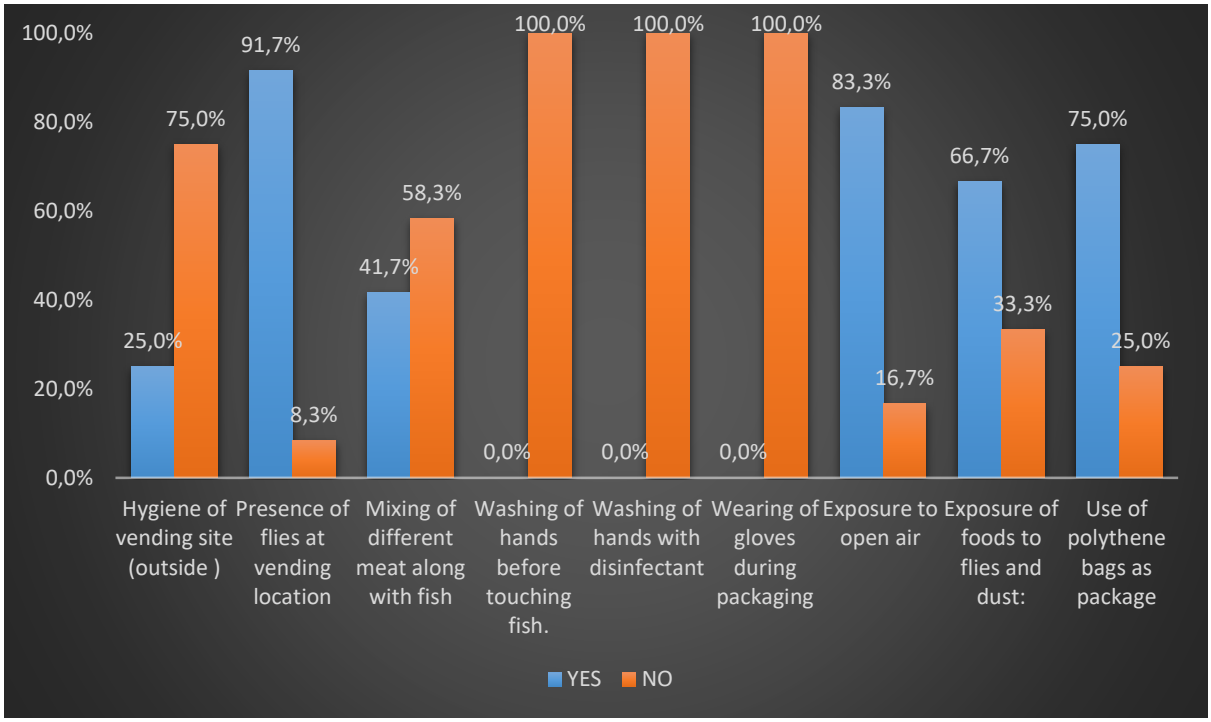

Figure 4.1 a summary of the behaviour and hygiene observation obtained from the informal market.

Results obtained from the observation of the checklist during sample collection disclosed that 25% (n=3/12) of markets were arranged neatly and in order from the street and inside the shop. Seventy-five percent (n=9/12) of the streets were filled with stagnant water with an unpleasant smell, dirt, and the waste was dumped opposite or closer to the markets. All markets 100% (n=12/12) indicated that the food handler/ seller did not adhere to decontamination of food safety practices. Protective equipment (PPE) was not properly used by the personal; all markets were not washing of hands before, also not make use of disinfectant before packaging and wearing gloves during packaging. Among the samples collected, none were stored in the fridge; we assume that since the fish is dried, the sellers didn't worry much about fish spoilage. In addition, no preservation method was observed, and 75% (n=9/12) of vendors used plastic bags to pack, measure, and differentiate their product. Moreover, 66.7% (n=8/12) of markets during purchasing flies, insect, and filthiness was observed, and some dried fish was spoiled although not sold to consumers; they were among those on display. When collecting dried fish, 83.3% (n=10/12) were exposed to an open area even though some were packaged in plastics.

4.2 Bacterial isolation

4.2.1 Total Bacterial Counts (TBC)

A total number of140 dried fish samples were subjected to serial dilution and cultured for the total bacterial count (TBC) method described in Chapter 3 (3.1). The bacterial count from the different dried fish types for this study are presented in Appendix 2, Tables 1, 2, and 3. The total bacterial count in salted fish samples ranged from 0.8×10^7 - 3.2×10^7 cfu/g with an overall mean of 2.13×10^7cfu/g, sun-dried fish samples ranged from 1×10^7 - 4.8×10^7 cfu/g with an overall mean of 2.91×10^7cfu/g, and smoked fish samples ranged from 1×10^7 - 5.34×10^7 cfu/g with an overall mean of 2.72×10^7cfu/g from the four areas sampled. Figures 4.2 represent the overall mean of total bacterial count from the three different dried fish types.

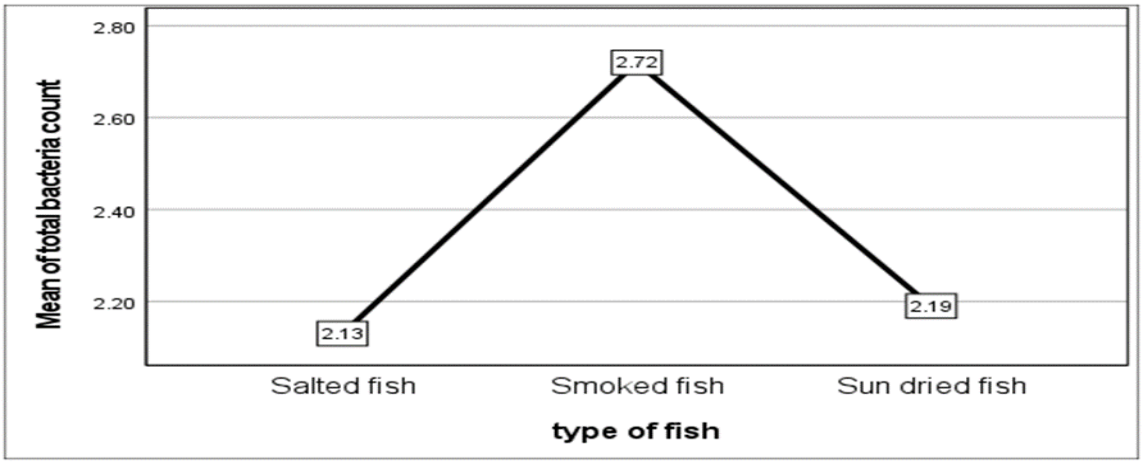

Figure 4.2 Mean of the total bacteria count in all the selected types of fish

4.2.2 ANOVA test results for TBC

The results show that the differences in the mean bacterial count across the fish type presented are statistically significant at 5% ($P < 0.05$). This implies that the mean bacterial counts differ based on the type of fish sampled, and a test of between-subject effects also confirmed this.

Table 4.2 ANOVA test results for TBC

ANOVA					
Total bacteria count ($n\times10^7$)					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	9.563	2	4.781	4.319	0.015
Within Groups	151.663	137	1.107		
Total	161.226	139			

4.3. Comparison of total bacteria count between for the type of fish

The between-subject effects test was implemented to determine whether the independent variable (Type of fish) statistically affects the dependent variable (Total Bacterial Count). The results showed a statistical significance in the mean of Bacterial Count and type of fish because of p-value <0.05. as shown in Table 4.3

Table 4.3 Tests of Between-Subjects Effects

Tests of Between-Subjects Effects					
Dependent Variable: Total bacteria count ($n\times10^7$)					
Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	9.563[a]	2	4.781	4.319	0.015
Intercept	713.772	1	713.772	644.763	0.000
Type of fish	9.563	2	4.781	4.319	0.015
Error	151.663	137	1.107		
Total	1246.785	140			
Corrected Total	161.226	139			
a. R Squared = .059 (Adjusted R Squared = 0.046)					

4.2.2 Mean bacterial count per area

The distribution of total bacterial count between the four areas revealed that the mean bacterial count of samples obtained from Sunnyside was 2.70 (Figure 4.5), Yoeville 3.70 (Figure 4.6), MTN taxi-rank 2.82 (Figure 4.7), and Rosettenville 2.98 (Figure 4.8).

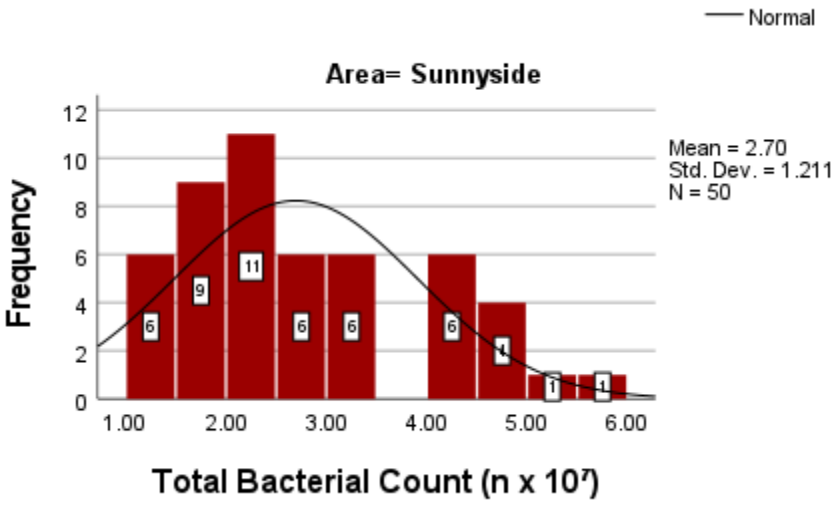

Figure 4.5: Distribution of Total Bacterial Count from Sunnyside

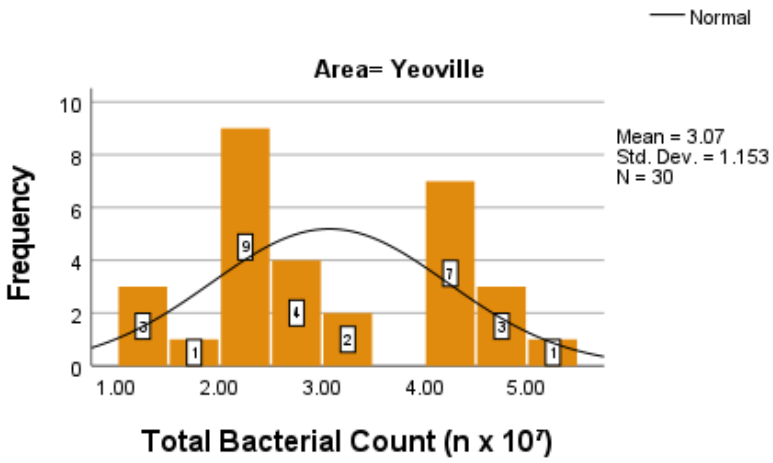

Figure 4.6: Distribution of Total Bacterial Count from Yeoville

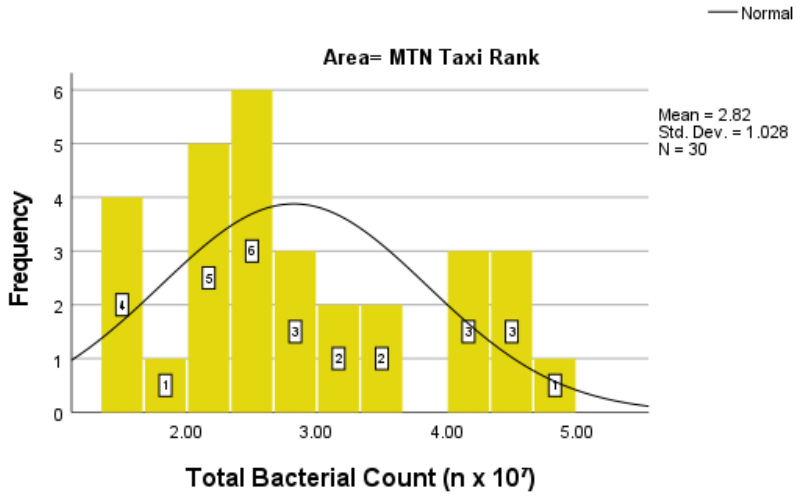

Figure 4.7 Distribution of Total Bacterial Count from MTN Taxi Rank

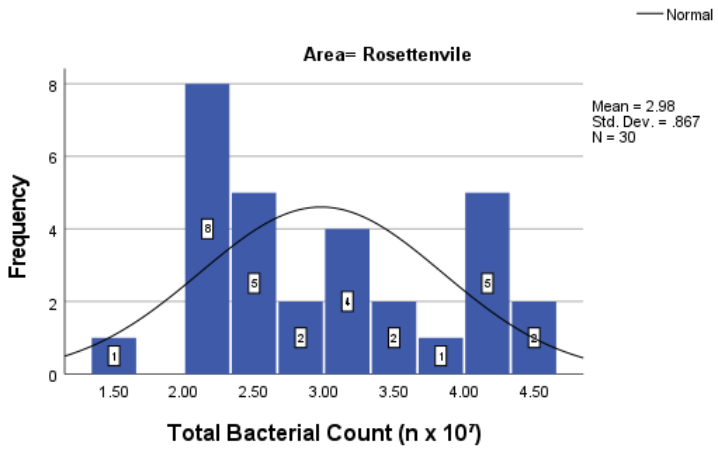

Figure 4.8 Distribution of Total Bacterial Count Rosettenville.

4.2.4 Comparison of bacterial counts between the four areas

The mean comparison of the samples obtained from the different areas reveals no significant difference ($p > 0.05$) between the areas in Table 4.4. In summary, it was observed that the mean range was slightly equal. Furthermore, it was observed that the mean range of samples obtained from Sunnyside was less compared to Yeoville. However, the statistical difference was not established ($p > 0.05$), as shown in Table 4.4.

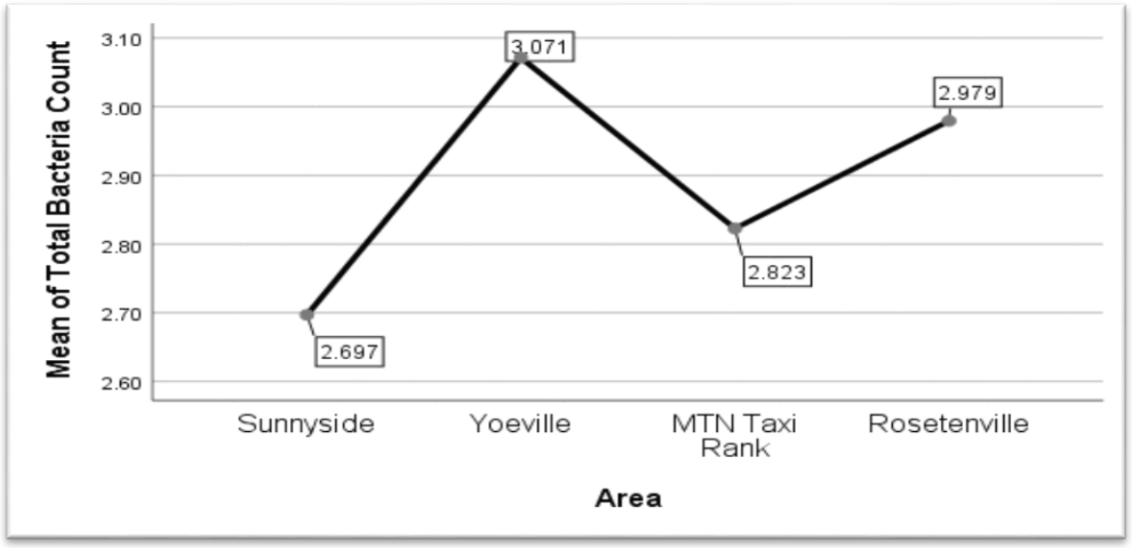

Figure 4.9: Mean of the total bacteria count in all the selected areas

The results show that the differences in the mean bacterial count across the four areas presented are not statistically significant at 5% (P > 0.05). This implies that the mean of bacterial counts does not depend on areas. This was confirmed by a test of between-subject effects and Post Hoc.

Table 4.4: ANOVA test results for TBC

ANOVA					
Total Bacteria Count					
	Sum of Squares	Df	Mean Square	F	p-value
Between Groups	3.136	3	1.045	0.873	0.457
Within Groups	162.898	136	1.198		
Total	166.035	139			

4.2.5 Distribution of bacterial counts between the areas

The test of between-subject effects was aimed to find out whether the independent variable (area) has a statistically significant effect on the dependent variable (bacterial count (n x 10^7)). The results showed no statistical significance in the mean of bacterial count across the areas, and P > 0.05 as presented in Table 4.5. To confirm the results, Turkeys LSD multiple comparisons was implemented as indicated in Table 4.6

Table 4.5 Distribution of bacterial counts between the areas

Tests of Between-Subjects Effects

Dependent Variable: Total Bacterial Count (n x 10^7)

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	3.136[a]	3	1.045	0.873	0.457
Intercept	1115.582	1	1115.582	931.372	0.000
Area	3.136	3	1.045	0.873	0.457
Error	162.898	136	1.198		
Total	1314.865	140			
Corrected Total	166.035	139			

a. R Squared = .019 (Adjusted R Squared = -0.003)

The mean of bacterial count shows all $P > 0.05$, which approves no statistical significance between all four areas. Thus, the results indicate that the bacterial count is not dependent on the sampled area.

Table 4.6 Summary of mean comparison of bacterial count and their significance between areas of collection

Multiple Comparisons

Dependent Variable: Total Bacterial Count (n x 10^7)

LSD

(I) area	(J) area	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval** Lower Bound	Upper Bound
Sunnyside	Yoeville	-.37445	.252748	0.141	-.87428	.12537
	MTN taxi rank	-.12579	.252748	0.620	-.62561	.37404
	Rosettenville	-.28245	.252748	0.266	-.78228	.21737
Yoeville	Sunnyside	.37445	.252748	0.141	-.12537	.87428
	MTN taxi rank	.24867	.282581	0.380	-.31016	.80749
	Rosettenville	.09200	.282581	0.745	-.46682	.65082
MTN taxi rank	Sunnyside	.12579	.252748	0.620	-.37404	.62561
	Yoeville	-.24867	.282581	0.380	-.80749	.31016
	Rosettenville	-.15667	.282581	0.580	-.71549	.40216
Rosettenville	Sunnyside	.28245	.252748	0.266	-.21737	.78228
	Yoeville	-.09200	.282581	0.745	-.65082	.46682
	MTN taxi rank	.15667	.282581	0.580	-.40216	.71549

Based on observed means.

The error term is Mean Square (Error) = 1.198.

4.2 Results based on conventional biochemical test

In this study, out of 140 samples collected, 67 organisms were isolated and selected based on morphological features and subjected to a conventional biochemical test for preliminary screening. The morphological and conventional biochemical test results revealed that 26.86% of isolates were *Clostridium* spp. In comparison, 20.89% of the isolates were *Staphylococcus* spp., a similar occurrence 11.94% was observed for *Klebsiella* spp. and *Staphylococcus aureus,* respectively, as shown in Table 4.7. In addition, the biochemical also reveals different kinds of bacteria at different occurrence levels, as presented in Table 4.7.

Table 4.7 Summary of suspected organisms after conventional biochemical test

Suspected organism	Number of organisms	Percentage (%)
Enterococcus faecalis	6	8.95
Micrococcus caseolyticus	2	2.98
Clostridium spp.	18	26.86
Klebsiella spp.	8	11.94
Staphylococcus spp.	14	20.89
Staphylococcus xylosus	6	8.95
Staphylococcus aureus	8	11.94
Enterobacter spp.	2	2.98
Enterococcus spp.	3	4.47
Total	67	100

4.4 Confirmatory results based molecular analysis using the 16S rDNA

The 16S rDNA confirmatory results revealed that 14.94% (10/67) of isolates were confirmed as *Clostridium* spp. While 11.94 % (8/67) were confirmed to be *Staphylococcus* spp., and 8.95% (6/67) of isolated were identified as *Staphylococcus xylosus*, as shown in Table 4.8. In addition, the other bacteria revealed different level of occurrence, as presented in Table 4.8.

Table 4.8 Confirmatory results based on 16S rDNA

Confirmed organism	Number of organisms	Percentage (%)
Enterococcus faecalis	4	5.97%
Klebsiella spp.	3	4.47%
Staphylococcus xylosus	6	8.95%
Staphylococcus aureus	3	4.47%
Enterococcus faecium	2	2.98%
Klebsiella pneumonia	5	7.46%
Clostridium botulinum	2	2.98%
Staphylococcus lentus	4	5.97%
Corynebacterium variabile	2	5.97%
Paraclostridium bifermentaus	4	5.97%
Lysinibacillus macrolides	1	1.49%
Staphylococcus spp.	8	11.94%
Micrococcus caseolyticus	1	1.49%
Planococcaceace bacterium	2	2.98%
Clostridium bifermentas	3	4.47%
Staphylococcus saprophyticus	2	2.98%
Micrococcus caseolyticus subsp homonis	1	1.49%
Enterobacter spp.	1	1.49%
Enterobacter ludwigii	1	1.49%
Clostridium spp.	10	14.92%
Staphylococcus sciuri	2	2.98%
Total	67	100%

4.5 Agarose gel amplified 16S rDNA

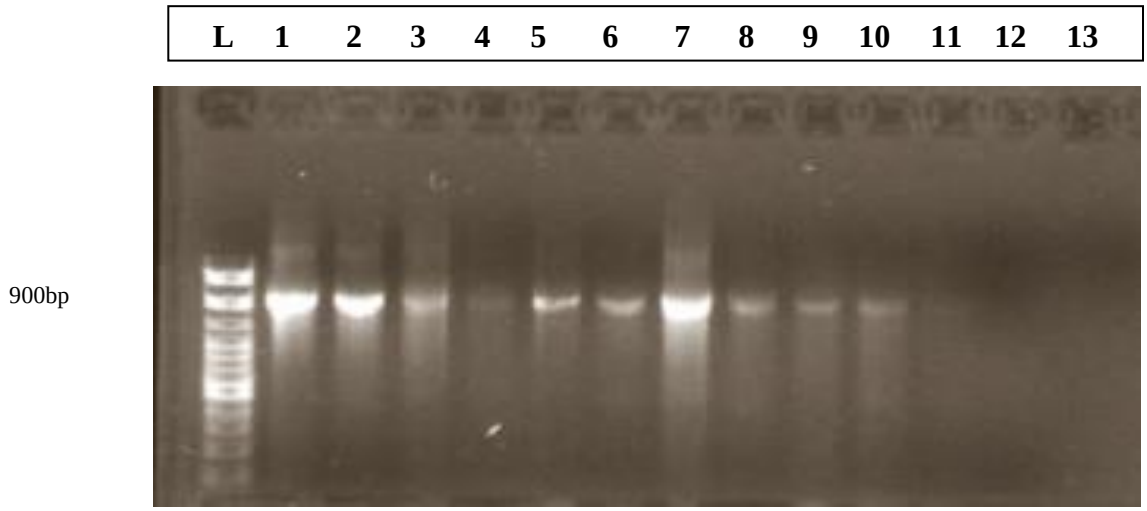

Figure 4.5.1: Image of Agarose gel (1%w/v) of amplified 16S rDNA. From left to right L - ladder marker; 1- *Staphylococcus aureus*; 2- *Klebsiella pneumoniae*; 3- *Staphylococcus xylosus*; 4- *Staphylococcus spp.*; 5 - *Paraclostridium bifermentans*; 6- *Enterococcus faecalis*; 7- *Lysinibacillus macroides*; 8- *Enterococcus faecalis*, 9-10 represents the positive control *Staphylococcus aureus* ATCC® 25923 (JGK, Lab Africa South Africa) and *Escherichia coli* ATCC® 25922 (JGK, Lab Africa South Africa) and 11 -12 represent the negative control as Nuclease DNA free water (Bio concept ltd, Iso 9001, Switzerland).

4.6 Molecular approach of sequences obtained from the GenBank

A total of 67 sequences was submitted to NCBI for accession number. Out of the 67, 30 sequences were assigned the accession number, while others are still pending. The results of assigned sequences are shown in Table 4.9. Phylogenetic tree was contracted and presented in Figure 4.11.

Table 4.9 16S rDNA sequences and their accession number

Sequence_ID	Reference from NCBI database	Accession no. from the Gene Bank	Obtained Accession no.	percentage of similarity (%)
Seq1	*Klebsiella pneumoniae*	MH973164	MW078395	99
Seq2	*Staphylococcus* spp	KT151895	MW078396	99
Seq3	*Staphylococcus xylosus*	MK253321	MW078397	100
Seq4	*Staphylococcus xylosus*	KC456590	MW078398	99
Seq5	*Macrococcus caseolyticus*	NR_159094	MW078399	99
Seq6	*Paraclostridium bifermentans*	MK894870	MW078400	100
Seq7	*Staphylococcus lentus*	MF678888	MW078401	98
Seq8	*Staphylococcus* spp.	HM584794	MW078402	99
Seq9	*Staphylococcus xylosus*	JX035942	MW078403	99
Seq10	*Lysinibacillus macroides*	MG892813	MW078404	100
Seq11	*Enterococcus faecalis*	MK254994	MW078405	99
Seq12	*Enterococcus faecium*	MK748256	MW078406	99
Seq13	*Klebsiella pneumoniae*	CP040363	MW078407	97
Seq14	*Planococcaceae*	LK934680	MW078408	99
Seq15	*Staphylococcus* spp.	KU245713	MW078409	99
Seq16	*Staphylococcus* spp.	KU644384	MW078410	98
Seq17	*Klebsiella* spp	KJ143756	MW078411	99
Seq18	*Staphylococcus* spp.	JX944828	MW078412	98
Seq19	*Corynebacterium variabile*	KP140842	MW078413	99
Seq20	*Staphylococcus* spp	KJ504153	MW078414	99
Seq21	*Clostridium bifermentans*	KP944171	MW078415	99
Seq22	*Staphylococcus aureus*	MK780044	MW078416	97
Seq23	*Paraclostridium bifermentans*	MH346281	MW078417	99
Seq24	*Paraclostridium bifermentans*	MK606081	MW078418	99
Seq25	*Staphylococcus lentus*	MK439492	MW078419	99
Seq26	*Staphylococcus* spp.	KC688883	MW078420	99
Seq27	*Staphylococcus aureus*	LR134268	MW078421	99
Seq28	*Clostridium botulinum*	CP028859	MW078422	97
Seq29	*Clostridium botulinum*	CP013243	MW078423	99
Seq30	*Enterococcus faecalis*	MH250054	MW078424	99

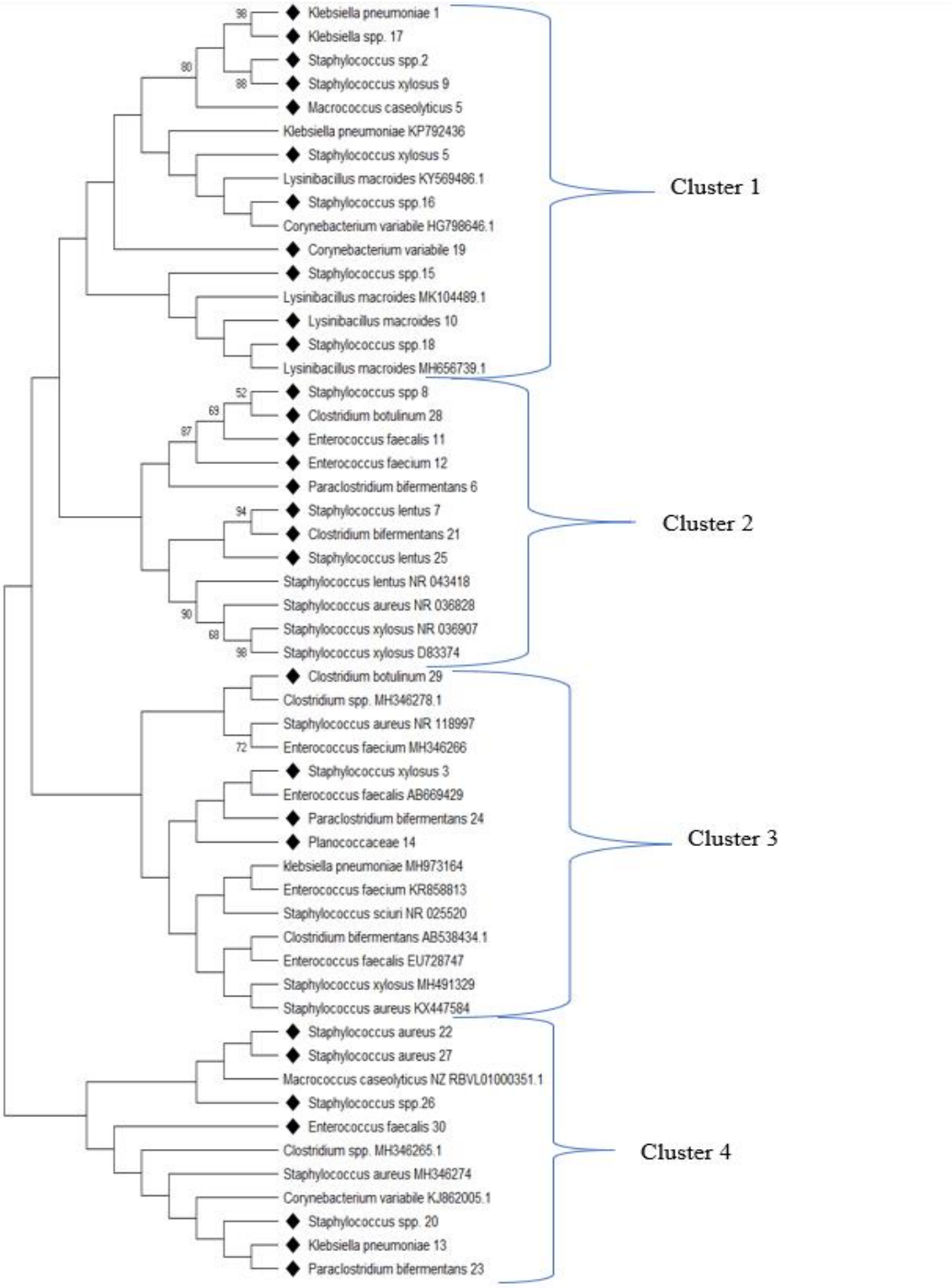

Figure 4.11: Phylogenetic tree showing the relationships among 16S rDNA gene sequences from dried fish sample (representing symbol ♦) with the most similar sequences obtained from the GenBank

4.6.1 The results of pathogens observed based on sampled area

The results reveal that the dried fish samples were contaminated by different pathogens at a different level from the areas obtained. The highest prevalence of bacteria observed was from Rosettenville (35.8%), followed by Sunnyside (32.8%), MTN taxi rank (16.5%), and Yoeville (14.9%), as presented in Table 4.10.

In addition, it was observed that *Lysinibacillus macrolides* (100%) and *Staphylococcus saprophyticus* (100%) were only present in the samples obtained from MTN taxi rank. Meanwhile, *Clostridium botulinum* (100%) and *Staphylococcus lentus* (100%) only occurred in samples obtained from Resettenville. Moreover, the results revealed that *Enterobacter ludwigii* (100%), *Enterobacter* spp. (100%), *Corynebacterium variabile* (100%) was only present from samples obtained from Sunnyside. *Macrococcus caseolyticus* (100%) and *Macrococcus caseolyticus subp. hominis* (100%) occurred in samples obtained from Yoeville, as shown in Table 4.10

Table 10: Bacteria based on the area sampled

Organisms	No of isolated	MTN taxi rank	No of isolated	Rosettenville	No of isolated	Sunnyside	No of isolated	Yoeville
Clostridium bifermentans	0	0%	2	66.7%	0	0%	1	33.3%
Clostridium botulinum	0	0%	2	100%	0	0%	0	0%
Clostridium spp.	1	10%	2	20%	5	50%	2	20%
Corynebacterium variabile	0	0%	0	0%	2	100%	0	0%
Enterobacter ludwigii	0	0%	0	0%	1	100%	0	0%
Enterobacter spp.	0	0%	0	0%	1	100%	0	0%
Enterococcus faecalis	0	0%	2	50%	0	0%	2	50%
Enterococcus faecium	0	0%	1	50%	0	0%	1	50%
Klebsiella pneumonia	0	0%	4	80%	1	20%	0	0%
Klebsiella spp.	0	0%	2	66.7%	1	33.3%	0	0%
Lysinibacillus macrolides	1	100%	0	0%	0	0%	0	0%
Macrococcus caseolyticus	0	0%	0	0%	0	0%	1	100%
Macrococcus caseolyticus subsp. *Hominis*	0	0%	0	0%	0	0%	1	100%
Paraclostridium bifermentans	0	0%	0	0%	3	75%	1	25%
Planococcaceae bacterium	1	50%	0	0%	1	50%	0	0%
Staphylococcus aureus	0	0%	1	33.3%	2	66.7%	0	0%
Staphylococcus lentus	0	0%	4	100%	0	0%	0	0%
Staphylococcus saprophyticus	2	100%	0	0%	0	0%	0	0%
Staphylococcus sciuri	1	50%	1	50%	0	0%	0	0%
Staphylococcus spp.	3	37.5%	2	25%	2	25%	1	12.5%
Staphylococcus xylosus	2	33.3%	1	16.7%	3	50%	0	0%
Total	11	16.5%	24	35.8%	22	32.8%	10	14.9%

4.6.2 Occurrence of pathogens based on the type of samples

The results in Table 4.11 showed that Sun-dried fish samples had the highest contamination of 50.7% (34/67) from the isolated pathogens, namely: *Corynebacterium variabile* 66.7%, *Enterobacter* spp (100%), *Enterobacter ludwigii* (100%), *Planococcaceae bacterium* (100%). Furthermore, Smoked fish isolates were contaminated with 40.3% (27/67), namely, *Enterococcus faecium* (100%), *Klebsiella* spp., (66.7%), *Lysinibacillus macrolides (*100%), *Macrococcus caseolyticus* (100%), *Macrococcus caseolyticus* subsp. *hominis* (100%), *Staphylococcus sciuri* (100%). Salted fish samples showed low contamination at 9% (6/67) overall. In these samples, pathogens such as *Staphylococcus* spp. (66.7%), *Klebsiella* spp (33.3%), *Staphylococcus* spp., (12.5%), and *Staphylococcus xylosus* (16.7%) were identified as indicated in Table 4.11. The chi-square test of the association presented in Table 4.12 shows no statistically significant association between the dried fish samples and the prevalence of organisms ($P > 0.05$).

Table 4.11 Cross-tabulation of the percentage of major isolated pathogens in different dried fish types based on the samples.

Isolated pathogens	N (40)	Smoked fish	N (80)	Sun dried fish	N (20)	Salted fish	Total(%)
Clostridium bifermentans	1	33.3%	3	66.7%	0	0%	100%
Clostridium botulinum	2	100%	0	0%	0	0%	100%
Clostridium spp.,	2	20%	8	80%	0	0%	100%
Corynebacterium variabile	0	0%	2	100%	0	0%	100%
Enterobacter ludwigii	0	0%	1	100%	0	0%	100%
Enterobacter spp.,	0	0%	1	100%	0	0%	100%
Enterococcus faecalis	2	50%	2	50%	0	0%	100%
Enterococcus faecium	2	100%	0	0%	0	0%	100%
Klebsiella pneumonia	1	20%	4	80%	0	0%	100%
Klebsiella spp.	2	66.7%	0	0%	1	33.3%	100%
Lysinibacillus macrolides	1	100%	0	0%	0	0%	100%
Macrococcus caseolyticus	1	100%	0	0%	0	0%	100%
Macrococcus caseolyticus subsp. *Hominis*	1	100%	0	0%	0	0%	100%
Paraclostridium bifermentans	0	0%	2	75.%	1	25%	100%
Planococcaceae bacterium	0	0%	2	100%	0	0%	100%
Staphylococcus aureus	0	0%	1	33.3%	2	66.7%	100%
Staphylococcus lentus	3	75%	1	25%	0	0%	100%
Staphylococcus saprophyticus	1	50%	1	50%	0	0%	100%
Staphylococcus sciuri	2	100%	0	0%	0	0%	100%

Staphylococcus spp.,	3	37.5%	4	50%	1	12.5%	100%
Staphylococcus xylosus	3	50%	2	33.3%	1	16.7%	100%
Total	**27**	**40.3%**	**34**	**50.7%**	**6**	**9%**	**100%**

Table 4.12 Association between the type of fish and percentage of isolated organisms.

Chi-Square Tests			
	Value	Df	Asymp. Sig. (2-sided)
Likelihood Ratio	52.650	40	0.087
N of Valid Cases	67		

Table 4.13 shows the test between subjects aimed to determine whether the independent variable (isolates organism) has a statistically significant effect on the dependent variable (area sampled). Table 4.13 reveals that $P = 0.65$ which shows that $P > 0.05$. Therefore, it concludes that type of fish does not depend on the organism. They are both independent as the p-value is greater than the level of significance.

Table 4.13 Test of between subjects' effects organism based on the area.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	27.744[a]	22	1.261	1.706	0.065
Intercept	170.666	1	170.666	230.877	0.000
Organisms	27.744	22	1.261	1.706	0.065
Error	32.525	44	0.739		
Total	357.000	67			
Corrected Total	60.269	66			

a. R Squared = .460 (Adjusted R Squared = .190)

4.7 Antibiotic resistance profiles of bacteria isolated from Dried fish

The Antibiotic test applied on the isolates revealed the highest resistance to Streptomycin (73.1%). Meanwhile, 58.2 % of isolates were resistant to Erythromycin and 38.8% of isolates were observed to be resistant to Gentamicin. In addition, the isolated bacteria displayed a susceptibility profile to Norfloxacin at 95.5% followed by 94.0 % to Chloramphenicol, 85. 1% Ciprofloxacin and 68.7% Amoxicillin as shown in Table 4.14 and Figure 4.12 revealing examples of the test.

Table 4.14: Overall profile of antimicrobial susceptibility

		No of isolates	Intermediate (%)	No of isolates	Resistant (%)	No of isolates	Susceptible (%)
Antibiotic	AML10	3	4.5%	18	26.9%	46	68.7%
	C30	4	6.0%	0	0.0%	63	94.0%
	CIP 5	9	13.4%	1	1.5%	57	85.1%
	CN10	3	4.5%	26	38.8%	38	56.7%
	E 5	15	22.4%	58	58.2%	13	19.4%
	NOR 5	0	0.0%	3	4.5%	64	95.5%
	S10	17	25.4%	49	73.1%	1	1.5%
Total		**51**	**10.9%**	**155**	**29.0%**	**282**	**60.1%**

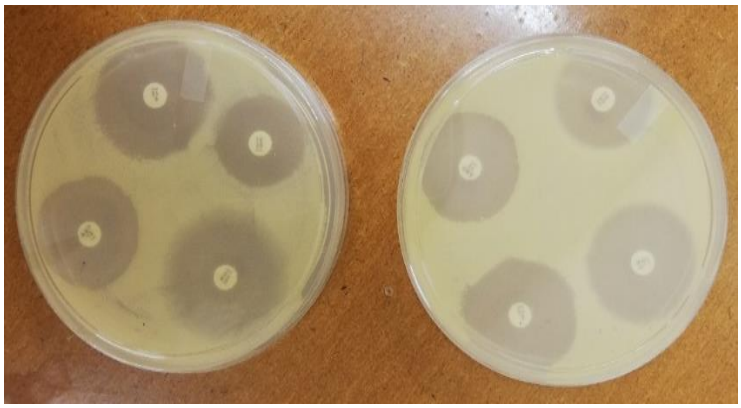

Figure 4.12 The Disc diffusion showing susceptivity to the following antibiotics: Gentamicin (CN: 10 µg), Amoxycillin (AML: 10 µg), Erythromycin (E: 5 µg), and Norfloxacin (NOR: 5 µg).

4.7.1 Antimicrobial resistance profile for selected isolates

In this study, the results revealed that *Clostridium botulinum* (100%), *Clostridium* spp., (50%), *Enterococcus faecium* (50%), *Klebsiella pneumoniae* (20%), *Klebsiella* spp. (66.7%), *Paraclostridium bifermentans* (75%), *Staphylococcus aureus* (100%), *Staphylococcus saprophyticus* (100%), *Staphylococcus lentus* (100%), *Staphylococcus* spp., (87.5%), *Staphylococcus xylosus* (100%) and *Staphylococcus sciuri* (100%) were resistant to Erythromycin (E5). *Planococcaceae bacterium* (50%) was resistant to Ciprofloxacin. While *Clostridium bifermentans* (33.3%), *Clostridium* spp. (50%), *Enterobacter* spp., (100%), *Klebsiella pneumoniae* (80%), *Macrococcus caseolyticus* (100%), *Staphylococcus saprophyticus* (100%), *Staphylococcus* spp., (12.5%), *Staphylococcus xylosus* (50%) were resistant to Amoxicillin.

Clostridium bifermentans (100%), *Clostridium* spp. (70%), *Corynebacterium variabile* (100%), *Enterobacter* spp., (100%), *Enterococcus faecalis* (50%), *Enterococcus faecium* (100%), *Klebsiella pneumoniae* (80%), *Lysinibacillus macrolides* (100%), *M.caseolyticus* (100%), *Macrococcus caseolyticus* subsp. *Hominis* (100%), *Paraclostridium bifermentans* (50%), *Planococcaceae bacterium* (100%), *Stsphylococcus aureus* (100%), *Staphylococcus saprophyticus* (100%), *Staphylococcus lentus* (75%), *Staphylococcus* spp., (87.5%), *Staphylococcus xylosus* (83.3%) were resistant to Streptomycin.

Clostridium bifermentans (66.7%), *Clostridium* spp., (40%), *Enterobacter* spp., (100%), *Enterococcus faecalis* (50%), *Enterrococcus faecium* (100%), *Lysinibacillus macrolides* (100%), *Macrococcus caseolyticus* (100%), *Macrococcus caseolyticus* subsp. *Hominis* (100%), *Paraclostridium bifermentans* (25%), *Staphylococcus saprophyticus* (100%), *Staphylococcus lentus* (50%), *Staphylococcus sciuri* (50%) *Staphylococcus* spp., (25%), *Staphylococcus xylosus* (66.7%) were resistant to Gentamicin. *Klebsiella pneumoniae* (20%), *Planococcaceae bacterium* (50%), *Staphylococcus* spp., (12.5%) were resistant to Norfloxacin as presented in Table 4.15

Table 4.15: Descriptive data of antimicrobial resistance profile for selected isolates

Isolates	Antibiotics	E 5	CIP 5	AML10	S10	CN10	C30	NOR 5
Clostridium bifermentans	R	33.3%	0.0%	33.3%	100%	66.7%	0.0%	0.0%
	I	66.7%	66.7%	33.3%	0.0%	0.0%	0.0%	0.0%
	S	0.0%	33.3%	33.3%	0.0%	33.3%	100%	100%
Clostridium botulinum	R	100%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	I	0.0%	0.00%	0.0%	100%	0.0%	0.0%	0.0%
	S	0.0%	100%	100%	0.0%	100%	100%	100%
Clostridium spp.	R	50%	0.0%	50%	70%	40%	0.0%	0.0%
	I	10%	0.0%	10%	30%	0.0%	30%	0.0%
	S	40%	100%	40%	0.0%	60%	70%	100%
Corynebacterium variabile	R	0.0%	0.0%	0.0%	100%	0.0%	0.0%	0.0%
	I	100%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	S	0.0%	100%	100%	0.0%	100%	100%	100%
Enterobacter ludwigii	R	0.0%	0.0%	100%	100%	0.0%	0.0%	0.0%
	I	100%	100%	0.0%	0.0%	100%	0.0%	0.0%
	S	0.0%	0.0%	0.0%	0.0%	0.0%	100%	100%
Enterobacter spp.,	R	0.0%	0.0%	100%	100%	100%	0.0%	0.0%
	I	100%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	S	0.0%	100%	0.0%	0.0%	0.0%	100%	100%
Enterococcus faecalis	R	0.0%	0.0%	0.0%	50%	50%	0.0%	0.0%
	I	100%	0.0%	0.0%	50%	0.0%	0.0%	0.0%
	S	0.0%	100%	100%	0.0%	50%	100%	100%
Enterococcus faecium	R	50%	0.0%	0.0%	100%	100%	0.0%	0.0%
	I	50%	50%	0.0%	0.0%	0.0%	0.0%	0.0%
	S	0%	50%	100%	0.0%	0.0%	100%	100%
Klebsiella pneumoniae	R	20%	0.0%	60%	80%	0.0%	0.0%	20%
	I	20%	0.0%	0.0%	20%	20%	20%	0.0%
	S	60%	100%	40%	0.0%	80%	80%	80%
Klebsiella spp.,	R	66.7%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	I	0.0%	0.0%	0.0%	66.7%	0.0%	0.0%	0.0%
	S	33.3%	100%	100%	33.3%	100%	100%	100%
Lysinibacillus macrolides	R	0.0%	0.0%	0.0%	100%	100%	0.0%	0.0%
	I	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	S	100%	100%	100%	0.0%	0.0%	100%	100%
Macrococcus caseolyticus	R	0.0%	0.0%	100%	100%	100%	0.0%	0.0%
	I	0.0%	100%	0.0%	0.0%	0.0%	0.0%	0.0%
	S	100%	0.0%	0.0%	0.0%	0.0%	100%	100%
Macrococcus caseolyticus subsp. *Hominis*	R	0.0%	0.0%	0.0%	100%	100%	0.0%	0.0%
	I	0.0%	100%	0.0%	0.0%	0.0%	0.0%	0.0%
	S	100%	0.0%	100%	0.0%	0.0%	100%	100%
Paraclostridium bifermentans	R	75%	0.0%	0.0%	50%	25%	0.0%	0.0%
	I	25%	0.0%	0.0%	50%	0.0%	0.0%	0.0%

	S	0.0%	100%	100%	0.0%	75%	100%	100%
Planococcaceae bacterium	R	0.0%	50%	0.0%	100%	0.0%	0.0%	50%
	I	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	S	100%	50%	100%	0.0%	100%	100%	50%
Staphylococcus aureus	R	100%	0.0%	0.0%	33.3%	0.0%	0.0%	0.0%
	I	0.0%	0.0%	0.0%	66.7%	0.0%	0.0%	0.0%
	S	0.0%	100%	100%	0.0%	100%	100%	100%
Staphylococcus lentus	R	100%	0.0%	0.0%	75%	50%	0.0%	0.0%
	I	0.0%	25%	25%	25%	0.0%	0.0%	0.0%
	S	0.0%	75%	75%	0.0%	50%	100%	100%
Staphylococcus saprophyticus	R	100%	0.0%	100%	100%	100%	0.0%	0.0%
	I	0.0%	50%	0.0%	0.0%	0.0%	0.0%	0.0%
	S	0.0%	50%	0.0%	0.0%	0.0%	100%	100%
Staphylococcus sciuri	R	100%	0.0%	0.0%	100%	50%	0.0%	0.0%
	I	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	S	0.0%	100%	100%	0.0%	50%	100%	100%
Staphylococcus spp.,	R	87.5%	0.0%	12.5%	87.5%	25%	0.0%	12.5%
	I	12.5%	0.0%	0.0%	12.5%	0.0%	0.0%	0.0%
	S	0.0%	100%	87.5%	0.0%	75%	100%	87.5%
Staphylococcus xylosus	R	100%	0.0%	50%	83.3%	66.7%	0.0%	0.0%
	I	0.0%	16.7%	0.0%	16.7%	16.7%	0.0%	0.0%
	S	0.0%	83.3%	50%	0.0%	16.7%	100%	100%

Keys R= Resistance; I = Intermediate; S = Susceptible

E5= Erythromycin; NOR 5= Norfloxacin; C30 = Chloramphenicol ; AML10 = Amoxicillin ;CN10 = Gentamicin; S10 =Streptomytocin and CIP5 =Ciprofloxaxacin

Table 4.16 reveals the chi-square test of association was significant at 5%, implying a significant association between antibiotic and antibiotic resistance of the isolated pathogens. This association is described in detail in the following Table 4.16

Table 4.16: Association between resistance and antibiotic

Chi-Square Tests			
	Value	Df	p-value
Pearson Chi-Square	244.619[a]	12	0.000

Kruskal Wallis non- parametric test aims to assess the statistical significance of resistance on each antibiotic. Kruskal Wallis test applied was because assumptions of the one-way ANOVA have not been met. The p-value for the Kruskal-Wallis test is less than 0.05 for all the Antibiotics at all categories of resistances. Thus, the antibiotics are significantly different across the resistance levels. The post-hoc test has been analyzed to confirm where these differences are present and the direction of such.

Table 4.17: Kruskal-Wallis Non-parametric Test for Antibiotic distribution.

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of E5 is the same across categories of resistance.	Independent-Samples Kruskal-Wallis Test	0.008	Reject the null hypothesis.
2	The distribution of CIP 5 is the same across categories of resistance.	Independent-Samples Kruskal-Wallis Test	0.013	Reject the null hypothesis.
3	The distribution of AML10 is the same across categories of resistance.	Independent-Samples Kruskal-Wallis Test	0.033	Reject the null hypothesis.
4	The distribution of S10 is the same across categories of resistance.	Independent-Samples Kruskal-Wallis Test	0.001	Reject the null hypothesis.
5	The distribution of CN10 is the same across categories of resistance.	Independent-Samples Kruskal-Wallis Test	0.018	Reject the null hypothesis.
6	The distribution of C30 is the same across categories of resistance.	Independent-Samples Kruskal-Wallis Test	0.007	Reject the null hypothesis.
7	The distribution of NOR 5 is the same across categories of resistance.	Independent-Samples Kruskal-Wallis Test	0.027	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .050.

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is 0.005. The Bonferroni correction for multiple tests has adjusted asymptotic Significance values.

Table 4.18: Post-hoc test resistance and antibiotics

Pairwise Comparisons

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
resistance-S10	-1.231	0.373	-3.299	0.001	0.020
resistance-E 5	-2.485	0.373	-6.658	0.000	0.000
resistance-CN10	-2.679	0.373	-7.178	0.000	0.000
resistance-AML10	.731	0.373	1.959	0.050	1.000
resistance-CIP 5	-1.664	0.373	-4.459	0.000	0.000

resistance-C30	-2.649	0.373	-7.098	0.000	0.000
S10-E 5	1.254	0.373	3.359	0.001	0.016
S10-CN10	-1.448	0.373	-3.879	0.000	0.002
S10-AML10	-1.963	0.373	-5.259	0.000	0.000
S10-C30	-1.418	0.373	-3.799	0.000	0.003
E5-AML10	3.216	0.373	8.618	0.000	0.000
E5-CIP 5	.821	0.373	2.199	0.028	0.585
CN10-AML10	-3.410	0.373	-9.138	0.000	0.000
CN10-CIP 5	-1.015	0.373	-2.719	0.007	0.137
AML10-CIP 5	2.396	0.373	6.418	0.000	0.000
AML10-C30	-3.381	0.373	-9.058	0.000	0.000
CIP 5-C30	-.985	0.373	-2.639	0.008	0.174

CHAPTER 5

DISCUSSION

5.1. Analysis of the observation worksheet

The observation results based on the checklist questions (Appendix 8) as well as the observation of the researcher showed that most of the participants (66.6%) were found to be females (8 out of 12) (Table 4.1). Although the study has no association of gender and food safety, other previous studies found that females had better scores in food hygiene than males (Baluka *et al.*, 2015; Cortese *et al.*, 2016). The observation results also revealed that the majority of the samples collected from markets were from people the age of 30- 49 years (58.3%) compared to the overall number, Çakıroğlu and Uçar (2008) in their study discovered that older people had better experience and knowledge of food hygiene than young labours. Furthermore, in their research, Egan *et al.* (2007) mentioned that inappropriate food handling might be implicated in 97% of all food-borne illnesses associated with food preparation outlets. Therefore, knowledge and education in food handling is imperative factor.

Furthermore, the study revealed also that the food handlers were all from foreign countries based on their language (accent), physical and personal appearances (attires, choice of food sold, and looks). The dried fish is not originally from South Africa. Nevertheless, finding out the country of origin without interviewing the sellers' person was a challenge.

The observations on food handlers revealed that many did not have a good vending site safe for food and products safety. An inappropriate vending site is the first risk for consumers, as confirmed by Adesiyun (1984), who in their study emphasized that consumption of food products that have been repeatedly exposed to human handling in the market for extended periods may be a health hazard.

The results from the observation checklist revealed several interesting results (Figure 4.1). First, the vending setups were on the streets (open areas), where there was a lot of movement of people that increased the chance of contamination. In a study by Bhowmik and Saha (2012), these were their main complaints against street vending. Secondly, they caused congestion on the roads, and lastly, there was a lack of quality and hygiene among them. This was found to be a concern raised by the consumers of the street markets.

Additionally, the dried fish sampled were displayed on a table /bench that was surrounded by a dusty ground where some were closer to the dustbin, and in other instances, the dried fish

were layered without any packaging (exposed to air or dust); this lead to be touched by many customers during examination for purchase. Sani *et al.* (2016) on a study based on smoked fish emphasized that the environment where the dried fish is displayed in the market is a contributing factor to the microbial contamination. It was also discovered that they did not wash their hands before making and packing the food samples, and they were not dressed in protective equipment (gloves, hair cover, apron). These results are in line with Egan *et al.* (2007), who in their study also noted that the vending sites food handlers may correspondingly be asymptomatic carriers of food poisoning organisms.

The dried fish samples collected were 100% not kept in the refrigerator or used any other preservation method to maintain shelf life and prevent the growth of any microorganism. As a result, some samples were detected to be having moulds and a bad smell. In addition, 66.7% of the samples were exposed to a lot of dirt; dust; different insects were observed, and 91.7% of the markets visited for sampling had flies.

Environmental hygiene and mishandling of food are considered significant for food safety (Baluka *et al.*, 2015); this includes the enforcement use of good facilities and tools (PPE) for the protection of food as they play a significant role in the occurrence of food-borne illness (Egan *et al.*, 2007). Yet, all the food handlers (100%) were not adhering or participating in washing hands and using PPE. Furthermore, 83.3% of the informal markets exposed their dried fish products to the environment; that practice reveals a lack of knowledge and ignorance. In addition, this is a disturbing practice to be observed in a human food market.

5.2 The microbial quality of dried fish

One of the goals of this study was to assess the microbial quality of the different dried fish from the sampled areas. The values obtained in this study reveal that the dried fish sold in the informal markets are not suitable for human consumption as it's above the standard guideline. According to the standard guideline of bacterial load in seafood followed by Surendran *et al.* (2006), the acceptable limit of bacterial load in dried fish is 1×10^5cfu/g at 37°C.

The results (Figure 4.2) of this study revealed that the salted fish ranged between 0.8×10^7 - 3.2×10^7 cfu/g and the overall mean of bacterial counts was 2.13×10^7cfu/g. Meanwhile, the mean contamination load in sun-dried fish was 2.91×10^7cfu/g with a range from 1×10^7 - 4.8×10^7 cfu/g, and in smoked fish, the range was between 1×10^7 - 5.34×10^7 cfu/g and mean as 2.71×10^7cfu/g (Appendix 2). The variation observed in the mean bacteria count of the sample obtained shows that the product's quality standard varies (Table 4.2.). This could be due to the

differences in the processing (method of preservation), storage, distribution, and handling of the dried fish samples. The bacterial species isolated from the collected samples in this study are associated with poor hygiene which could be from the processing and handling of fish from the point of harvesting to the final product. Huss (1995) isolated similar bacteria and concluded that the handling process have the all the capacity to maintain food safety and good quality from the time fish is caught until it is consumed.

It was found that in the smoked fish, the bacterial load was higher. The value ranged from 1×10^7 - 5.34×10^7 cfu/g with an overall mean of 2.72×10^7cfu/g, which was not found not acceptable for consumption based on the standard guideline that states the bacterial load should not exceed the regulatory limit 1×10^5cfu/g (Aliya *et al.,* 2018) which was the highest as compared to the other two fish types sampled dried fish, this is in line with the results obtained in the smoked fish sold in Owerri by Ohalete *et al.* (2012).

The overall mean results revealed that the total bacterial count in smoked fish samples ranged from 1×10^7- 5.34×10^7 cfu/g with an overall mean of 2.72×10^7cfu/g, which was the highest as compared to the other two fish types sampled, the results are in line with the findings obtained in the smoked fish sold in Owerri by Ohalete *et al.* (2012) who disclosed that the variations in microbial counts of smoked fish samples from markets might be due to improper smoking process, improper hygienic and handling procedures adopted by the dried fish sellers.

In addition, a study done by Dutta *et al.* (2018) mentions that smoking or drying is not a 100% effective means of preserving and preventing microbial proliferation in dried fish. In another study, Jakhar *et al.* (2015) mentioned that the smoking process is not standard in most cases. Parameters like temperature, quality of smoke, relative humidity, and smoke temperature remain uncontrolled, leading to poor nutritional quality and high microbial load in the smoked fish.

The mean contamination load in Sun-dried fish was 2.91×107cfu/g with a range from 1×10^7 - 4.8×10^7 cfu/g, and the results exceeded the regulatory limit. Sun-drying depends on the weather conditions. When the natural conditions are not favourable such as humidity is high, during the monsoon season, traditional methods as Sun-drying cannot achieve drying. In such cases, the dried fish can reabsorb the air's moisture and serve as a habitat for the microbial population such as bacteria, fungi, and others (Shamsan & Al-Jobory, 2018).

Salted fish had the least count of bacterial count compared to the other type of fish. 0.8×10^7–3.2×10^7 cfu/g with an overall mean of 2.13×10^7cfu/g detected. Depending on the kind of fish used and the type of product required, salting and drying of fish differs between nations, as well as within the same country (Chukwu & Shaba, 2009). The results presented in Figure 2 reveal that Salted fish was observed to have a low bacterial count. The salting method as a preservative destroys the growth of microorganisms Martinez *et al.* (2011), and salt can reduce the water activity on food. Therefore, play a good role in preserving (Ginigaddarage *et al.*, 2018).

The differences in the mean bacterial count across the fish type presented are statistically significant at 5% ($P < 0.05$), as illustrated in Table 4.2. This suggests that the mean bacterial counts differ based on the type of fish sampled, and a test of between-subject effects also confirmed the difference. The between-subject effects test confirmed a statistically significant impact on the dependent variable (Total Bacterial Count).

The present study also looked at bacterial count from the different areas (Figure 4.5- 4.8), and the results disclosed that the distribution of bacterial is not entirely different from each other. Figure 4.9 presented a precise distribution of the bacterial count mean distribution in all the selected areas. The mean bacterial count from Sunnyside was 2.697×10^7, Yeoville 3. 071×10^7 2.823, MTN taxi rank 2.823×10^7, and Rosettenville 2.979×10^7, the overall is similar when compared to each other. There is no significant difference between the markets, meaning that where the samples are collected had no impact on the contamination of the samples. ANOVA test, Tests of Between-Subjects Effects, and Multiple comparisons (see Table 4.4, 4.5, and 4.6) were completed to confirm whether the similarity observed from the histograms carries some statistical significance. It is concluded that there is a probability. However, it could be occurring by chance. That disguised that the mean of the bacterial count does not depend on the area.

5.3 Occurrence of isolated bacteria from the dried fish

Table 10 gives a summary of the results from the four areas sampled compared to each other. It was found that Rosettenville had the highest prevalence of pathogens (35.8%) with bacteria like *Clostridium botulinum*, *Enterobacter ludwigii*, *Enterobacter* spp., *Lysinibacillus macrolides*, *Staphylococcus saprophyticus*, and *Staphylococcus lentus*, all the mentioned isolated showed 100% contamination only to one sampled area. On the other hand, Sunnyside showed (32.8%) of isolated pathogens mentioned as *Corynebacterium variabile*, *Enterobacter ludwigii*, *Enterobacter* spp., *Enterococcus faecalis*, Enterococcus *faecium*, *Paraclostridium*

bifermentans, *Planococcaceae bacterium*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. Meanwhile, MTN taxi rank (16.5%) was highly contaminated with *Lysinibacillus macrolides* and *Staphylococcus saprophyticus.* Yoeville had the lowest contamination (14.9%), with some pathogens identified as *Macrococcus caseolyticus* subsp. *Hominis*; *Enterococcus faecalis, Enterococcus faecium*, *Staphylococcus* spp., from the samples collected respectively.

Samples collected in Rosettenville were highly exposed to unfavourable conditions. The dried fish was displayed outside and might have contact with dust and flies, which could be the reason for the high contamination rate. In most situations, according to Rasul *et al.* (2020) sufficient hygiene and sanitation are not maintained during the drying and harvesting of fish; as a result, insect infestation, the presence of filth, dust, and pesticide residue are very prevalent concerns with dried fish products. In MTN Taxi Rank had similar conditions as Rosettenville. Meanwhile, Sunnyside informal markets were primarily situated in better structure markets even though some samples were purchased from markets selling outside the streets but had the advantage that is packaged in plastics to prevent exposure to dust and flies. Contamination was viewed as limited compared to other sampled areas, and that could be the reason for less contamination to different organisms.

In this study, several different species of *Staphylococcus* spp., were found, and they were present in all the types of fish sampled. The prevalence of *Staphylococcus aureus* in dried fish is of public health significance because it has been implicated with food-borne intoxication and infection (Yusuf & Hamid, 2017). Its survival in the dried fish may be related to its ability to survive high salt levels, and it can grow well at a temperature ranging from 30-37℃ (Yusuf & Hamid, 2012); hence it was found in all the types of dried fish sampled. The findings are consistent with those of (Mensah *et al.*, 2002; Sina *et al.*, 2011), who discovered that inadequate hygiene procedures and insufficient food handling might lead to the spread of *Staphylococcus aureus*.

Staphylococci are not normally found in fish microflora, and their presence on them appears to be the consequence of post-harvest contamination caused by poor personal hygiene or fish illness (Huss, 1995). *Staphylococcus aureus* is a typical body microorganism, could have also been introduced into dried fish through the unclean hands and mouth of the vendor, where they attempt to open the packaging material by blowing air into it in order to open it (Akusu *et al.*, 2016). Yusuf and Hamid (2017) mentioned that during the handling and preparation of fish, the natural flora of the fish environment could be contaminated with organisms such as

members of the *Enterobacteriaceae* and *Staphylococcus aureus*. Furthermore, dried fish products are quickly implicated in contamination by *Staphylococcus aureus* (Seo *et al*., 2010). The pathogens may play a significant role in causing illnesses to humans by acting together with lactic acid bacteria in the fermentation process of fish or fermented meat products Sergelidis *et al.* (2014).

Other Staphylococcus species, such as *Staphylococcus sciuri, Staphylococcus lentus, Staphylococcus Saprophyticus,* and *Staphylococcus xylosus,* were found in smoked fish and sun-dried fish in this investigation (Table 11). *Staphylococcus sciuri* are important human pathogens responsible for endocarditis, peritonitis, septic shock, urinary tract infection, pelvic inflammatory disease and wound infections. Furthermore, Bhutia *et al.* (2021) detected many many pathogenic bacteria such as *Staphylococcus* spp., *Enterobacter* spp., etc. and figured that most of these pathogenic bacteria are salt-tolerant.

Macrococcus caseolyticus and *Macrococcus caseolyticus* subsp. *Hominis* (100%) was found to be prevalent (Table 10). *Micrococcus* species are well-known for causing food deterioration. According to Abolagba *et al.* (2011) the prevalence of *Macrococcus caseolyticus* could be linked to the vendors' poor handling procedures and food exposure to dust.

Clostridium botulinum was found to be more present in the smoked. *Clostridium botulinum* is more likely to be found in an aquatic environment (Davies *et al.*, 2001). In addition, a high incidence of *Clostridium* spp. was detected in Sun-dried fish in Kumar (2018) and further published that *Clostridium botulinum* is a biological hazard linked with the storage of Sun-dried fish. Therefore, storage is another fact that contributed to the contamination of the samples. The study conducted by Heinitz and Johnson (1998) scrutinized the occurrence of *Listeria* spp., *Salmonella* spp., and *Clostridium botulinum* isolation from smoked fish and shellfish products. Seafood like fish is accountable for many food-borne diseases and represents a significant concern from a public health perspective (Onmaz *et al.*, 2015).

The isolation of *Enterococci* and *Klebsiella* spp., are indications of faecal contamination, in agreement with the study of Dike-Ndudim *et al.* (2014), which stated that microbial flora of fish depends on the microbial contents of the waters in which they lived. Again the tested samples were purchased at retail, and faecal indicators such as *Enterococci* might be a sign of animal and/or human faecal pollution of the aquaculture environment or could be acquired during processing because these bacteria are not part of the normal flora (Boss *et al.*, 2016). *Enterococcus* spp., are opportunist and commensal microbiota found in the oral cavity,

genitourinary and gastrointestinal tract of humans and animals. They are found in soil, plants, and water and are widely spread in the environment (Araújo *et al.*, 2020). Furthermore, Sun drying might reduce the microbial load in fish flesh but does not necessarily eliminate contaminants, hence the presence of these pathogens. Toxigenic strains as *Enterococcus* spp., *Staphylococcus aureus*, and *Clostridium perfringens* were found to be dangerous and possibly be transmitted through the process of handling and processing (Normanno *et al.*, 2005; Onmaz *et al.*, 2015).

The occurrence of *Klebsiella pneumoniae* is found in sun-dried and smoked fish samples. The highest contamination is observed in sun-dried fish. *Klebsiella pneumoniae* is a common opportunistic pathogen that causes human infections (Guo *et al.*, 2016), and it's not only a deadly pathogen, but it can cause septicemia, liver abscesses, and diarrhea in humans (Zhang *et al.*, 2018). The occurrence of *Klebsiella pneumonia* from the informal markets in dried fish and all of the reports by other researchers indicated potential faecal contamination, possible cross-contamination between food handlers, food preparation, surfaces, and the food itself. This study found 66.7% of *Klebsiella* spp., in samples collected in Rosettenville, followed by 33.3% in Sunnyside (Piper & Charman, 2016).

Clostridium bifermentans and *Paraclostridium bifermentaus* are usually isolated from marine sediments. They have been reported to be non-pathogenic (Jyothsna *et al.*, 2016) making them a rare and potentially lethal cause of infection in humans. However, in association with *Clostridium perfringens*, it becomes capable of making people ill (Rai *et al.*, 2015). A case study by Edagiz *et al.* (2015) presented *Clostridium bifermentans* as the causal agent of empyema, an infection where the pleural space is filled with purulent fluid (Yu, 2011). Therefore, the presence of pathogens in the dried fish needs to be taken seriously.

Smoking is the traditional method of preserving foodstuffs, improves organoleptic food characteristics, induces water loss, and reduces the microbial load (Anihouvi *et al.*, 2019). However, the organisms in the Smoked fish samples might be due to increased moisture content of the fish product during storage and increase in temperature, favouring the growth of these organisms. Dried fish consumed in Nigeria is mainly smoked fish, according to Dike-Ndudim *et al.* (2014). However, in their study, it is cited that smoking does not achieve complete elimination of microbes and pathogenic bacterial of fresh fish. In this study, smoked fish was 40.3 % contaminated with different microorganisms than the other dried fish samples, as presented in Table 11. Furthermore, these organisms indicate faecal contamination, implying

that the vendors handled the product improperly and hygiene was not properly implemented (Oudiz *et al.*, 2004).

In addition, Moon *et al*. (2017) recommended that dried fish be stored at a refrigerated temperature. Storage was shown to be compromised in this study. fish is extremely perishable, as a result, a concerted effort must be made to increase the shelf life of fish using preservation and processing procedures such as chilling, freezing, canning, smoking, salting, and drying (Adeyeye *et al.*, 2015).

5.4 Neighbor-Joining tree based on the 16s rDNA

The dried fish isolates used in the multiple alignments were divided into four clusters (Figure 4.11). Among the four clusters, different bacterial species were obtained from other countries were retrieve from the GenBank. Cluster I, the concatenated Neighbor-joining showed *Klebsiella pneumoniae* 1 and *Klebsiella* spp., 17, highly have a homology of 98% and the closest relationship between *Staphylococcus* spp., 2, *Staphylococcus xylosus* 9, which belongs to the *Enterobacteriaceae* family. In the sub-group, *Macrococcus caseolyticus* 5 obtained in this study demonstrates to be an outliner from this sub-group. *Klebsiella pneumoniae* KP792436.1, which is isolated in South Africa from red cabbage vegetables (Aremu & Babalola, 2015), was clustered among the mentioned sequences obtained from this study.

The isolate showed close relatedness to *Staphylococcus* spp., 5 and *Lysinibacillus macrolides* KY569486.1 obtained in Nigeria by Ademola *et al.* (2018). *Staphylococcus* spp 16 and *Corynebacterium variabile* HG798646.1 isolate were significantly related, as seen in the same sub-group. *Corynebacterium variabile* 19 from this study branched alone, showing a relationship with isolates in Cluster 1 even though it is outlined. *Staphylococcus* spp., 15, *Lysinibacillus macrolides* MK104489.1 (South Africa), *Lysinibacillus macrolides* 10, *Staphylococcus* spp., 18, and *Lysinibacillus macrolides* MH656739.1 isolated in Nigeria are in the collective cluster with isolates of this study.

It was also discovered that the second cluster was divided into two sub-groups, as shown in the diagram (Figure 4.11).The similarity is observed from *Staphylococcus* spp., 8, *Clostridium botulinum* 28, *Enterococcus Faecalis* 11, *Enterococcus faecium* 12, *Paraclostridium bifermentans* 6. The second sub-group was combined with *Staphylococcus lentus* 7; *Clostridium bifermentans* 21, *Staphylococcus lentus* 25 isolated from the dried fish sampled, which were closely related to *Staphylococcus* species, namely: *Staphyloccocus lentus*

NR_043418.1 (unpublished), *Staphylococcus aureus* NR036828 (Japan), *Staphylococcus xylosus* NR036907, *Staphylococcus xylosus* D83374 (Japan) attained from the GenBank.

The third cluster (Cluster 3) was formed of primary species acquired from the GenBank. The organism isolated in this study was *Clostridium botulinum* 29, clustered with *Clostridium* spp., MH346278.1. Isolated in milk samples in South Africa. The organism seems to be from the same ancestor as they show close relations and genetic similarities. *Staphylococcus aureus* 3 and *Enterococcus faecalis* AB669429 are closely related with *Paraclostridium bifermentans* 24 and *planococcaceae* 14 isolated in this study. The second sub-group in Cluster 3 demonstrates the high similarity between the organisms obtained from GenBank.

The phylogenetic tree also revealed the existence of a fourth cluster (Cluster 4), which is made up of two sub-groups descending from a common ancestor. The first sub-group shows the variety of organisms belonging to the *Staphylococcic* family. *Staphylococcus aureus* 22 and 27 are closely related to *Macrococcus caseolyticus* NZ_RBVL01000351.1 isolated in Sudan and *Staphylococcus* spp. 26 isolated from this study; these isolates show to be having a common ancestor. Among various bacterial genera, *Macrococcus* is the most closely related to the genus *Staphylococcus*, and this can be justified by the relatedness of *Staphylococcus aureus* to *Macrococcus* species. Historically, it was included in the staphylococcal family until it was reassigned to an independent genus (Baba *et al.*, 2009). The second sub-group showed a great diversity of different species clustered in one group. *Staphylococcus* spp. 20, *Klebsiella pneumoniae* 13 and *Paraclostridium bifermentans* 23 from this study displayed similarities to *Clostridium* spp., MH346265.1, *Staphylococcus aureus* MH346274, and *Corynebacterium variable* KJ862005.1 obtained from GenBank, as shown in Figure 4.11. However, there was no close percentage of relatedness observed as they branched out differently.

5.5 Antibacterial resistance profile from isolated bacteria

To the various drugs examined, the bacterial isolates showed varying levels of phenotypic resistance. Table 4.15 shows that 100 percent of *Staphylococcus aureus* strains were resistant to Erythromycin. This result aligns with the study conducted by Rağbetli *et al.* (2016), who also reported the resistance of *Staphylococcus aureus* to erythromycin. Erythromycin was determined to be antibiotics with the highest resistance among the ones tested. Furthermore, other *Staphylococci* species like *Staphylococcus xylosus*, *Staphylococcus sciuri*, *Staphylococcus saprophyticus,* and *Staphylococcus lentus* revealed 100% resistance to Erythromycin. Comparable studies that examined resistance of *Staphylococcus* isolates to

Erythromycin found that 40 isolates out of a total of 1 235 isolates of *Staphylococcus* spp., were found to from diverse sources were resistant or intermediate to Erythromycin (Schlegelova *et al.*, 2008; Shittu & Lin, 2006).

Nevertheless, the results from this study indicate that *Staphylococcus* isolates showed a highly sensitive occurrence to Chloramphenicol and Norfloxacin. In our study, we found high percentage of 94.0 susceptibility to Chloramphenicol (see table 4.7). *Staphylococcus aureus* (100%), *Staphylococcus xylosus* (100%), *Staphylococcus sciuri* (100%), *Staphylococcus saprophyticus* (100%) and *Staphylococcus lentus* (100%) were all susceptible Chloramphenicol. Chloramphenicol is a drug that other studies found their isolates more susceptible to (Montso & Ateba, 2014b). Chloramphenicol is very effective against most gram-positive (Fayyaz *et al.*, 2013). Moreover, the gram-positive isolates in the study, like *Enterococci* spp., *Staphylococcus aureus*, *Corynebacterium*, and *Clostridium* spp., showed great susceptibility to Chloramphenicol.

Enterococcus species were isolated, and the results of this study show that *Enterococcus faecium* was 100% susceptible to Amoxicillin, Chloramphenicol, and Norfloxacin Furthermore, *Enterococcus faecalis* had similar results, including being 100% sensitive to Ciprofloxacin. Other research has found that *Enterococcus* spp. are generally nontoxic bacteria, however recently, they have been linked with intra-peritoneal and urinary tract infections (Kajihara *et al.*, 2015). *Enterococcus faecalis* was highly prevalent compared to *Enterococcus faecium* and it shown common resistance against streptomycin and gentamicin. *Enterococcus faecalis* and *Enterococcus faecium* were intermediate to Erythromycin; in addition, few isolates of *Enterococcus faecium* were resistant to Erythromycin.

Rahman *et al.* (2017), isolated *Enterococcus* species from the infected Tilapia and Catfish, found that it was surprising that all *Enterococcus faecalis* isolates displayed resistance to multiple antibiotics like Amoxycillin and Ampicillin. However, in this study it was susceptible to Amoxicillin. Similarly, ChajÄ *et al.* (2012)found that the total percentages of antimicrobial resistance isolates of ready-to-eat meat products were 1.1 percent to ampicillin, penicillin, and linezolid in their study.

The susceptibility of *Klebsiella* spp., and *Klebsiella pneumoniae* was found more prominent to Ciprofloxacin, Gentamicin, and Norfloxacin. The results show that (see Table 4.14) *Klebsiella* spp. was highly susceptible to five antibiotics used and least susceptible to Erythromycin and Streptomycin. *Klebsiella pneumoniae* was more resistant to Streptomycin. In humans,

antimicrobials have long been used to treat *Klebsiella pneumoniae* infections. Antimicrobial resistance has been on the rise in recent years, particularly that caused by extended-spectrum -lactamases (ESBL), plasmid-borne AmpCs, and carbapenemases (Guo *et al.*, 2016).

Haryani *et al.* (2007) used susceptibility testing in their research. All *Klebsiella pneumoniae* isolates were shown to be extremely resistant to ampicillin, erythromycin, rifampicin, streptomycin, and sulfamethoxazole, but responsive to chloramphenicol. In addition, the isolated organism in this study also showed high susceptibility to chloramphenicol, which is aligned to other previous findings of other researchers (Haryani *et al.*, 2007; Kumar, 2008). Therefore, Chloramphenicol is still adequate to use.

Our study observed a high prevalence of *Staphylococcus* spp., Resistant to Erythromycin, Streptomycin, and gentamicin (see Table 4.14). Furthermore, other *Staphylococci* species were *Staphylococcus xylosus, Staphylococcus sciuri,* and *Staphylococcus lentus* isolates found to be resistant to Erythromycin and Streptomycin. Comparable studies that examined resistance of *Staphylococcus* isolates to Erythromycin found that 40 isolates out of a total of 1 235 isolates of *Staphylococcus* spp., of various origins were found to be resistant or intermediate to Erythromycin (Schlegelova *et al.*, 2008; Shittu & Lin, 2006). Nevertheless, the results from this study indicate that *Staphylococcus* isolates showed a highly sensitive occurrence to Chloramphenicol and Norfloxacin.

Macrococcus caseolyticus isolated from the smoked fish sample was resistant to Amoxicillin, Streptomycin, and Gentamicin (Table 4.14). *Macrococcus caseolyticus* subsp. *Hominis* was resistant to Streptomycin and Gentamicin, and they presented no intermediate profile to any of the antibiotics selected. Food infected with multidrug-resistant microbes has the potential to spread food-borne diseases to humans, posing major public health concerns. The findings revealed that the grade of dried fish offered on the informal marketsis declining. If microorganisms that are resistant to antibiotics are added to this type of food, it could represent a risk to consumers in the future.

Because of the relatively high, changing incidence and multi-drug resistance in bacterial isolates, considerable intervention measures such as monitoring and education are required to avoid cross-contamination at all stages of meat processing. Nonetheless, as compared to earlier investigations, the percentages observed in this study are slightly lower. The reason can be due to differences in the methods used. Studies of Kumar (2008) mentioned that infectious

organisms are observed to develop forms resistant to specific antibiotics. Therefore, newer antibiotics must be originated.

One of the most severe hazards to public health that Africans and the rest of the world face is antibiotic contamination (Darwish *et al.*, 2013). According to Organization (2008), travelers, refugees, and immigrants may be exposed to unknown food-borne dangers in new locations; nonetheless, changes in microorganisms result in the continual evolution of novel diseases, antibiotic resistance, and variances in virulence of recognized infections. Furthermore, as people consume more food prepared outside the home, such as in informal markets, the food is exposed to a significant risk of contamination in many nations' informal foodservice settings (Khairuzzaman *et al.*, 2014).The study displayed a comparable susceptibility to most antibiotics, including Norfloxacin, Chloramphenicol, and Ciprofloxacin. The results in Table 4.14 show that the pathogens were 60.1% susceptible to the bacterial tested and 29% were resistant; meanwhile, 10.9% was intermediate

CHAPTER 6

CONCLUSION

Our study aimed to identify pathogens from dried fish imported in South Africa sold in informal markets and establish associated risks for consumers and the country. The results demonstrated that contamination could have resulted from harvest, drying, and production stages based on the isolated organisms. Packaging the fish products in dirty containers and displaying the fish uncovered in the markets is not suitable for public health. Therefore, enhanced hygienic practices in handling and processing dried fish during storage and transportation to the retail outlet are advised to be considered by the street vendors. According to the standard guideline of bacterial load (Surendran *et al.*, 2006), values obtained in this study reveal that the dried fish sold in the informal markets are not accepted as it's above the standard guideline of dried fish followed by fellow researchers. The variation observed in the mean bacteria count results implies that the dried fish sold in the informal markets is unsuitable for human consumption. Along with Immaculate *et al.* (2013), many authors argued that determining the microbial quality of such processed fishes is very important for guarding consumers' health and hygiene. According to the findings, food handlers should follow correct sanitary measures while handling foods to avoid pathogenic microorganism infection and ensure food safety.

The increasing use of antimicrobial agents in food animal production has been linked to an increase in multi-antimicrobial-resistant bacteria identified from the samples. Because of the rising concern that excessive antibiotic usage in aquaculture is increasing the incidence of antibiotic-resistant bacteria in aquatic food items, it is recommended that fish be treated properly before eating. Furthermore, quality control procedures for export-oriented and locally consumed fish should be implemented, as opposed to present practices in which quality control measures for locally consumed fish are not accessible; this is a public health problem.

RECOMMENDATION

There is a need for further molecular studies on antibiotics among bacterial isolates, and this could assist in terms of determining the presence of antibiotic resistance genes and their spread in the environment. The government should establish inter-sectoral national strategies and action plans on antibiotic resistance and guidelines that are accessible and affordable meanwhile maintaining good quality results; this approach could include food safety programs and could promote the prudent use of antibiotics in all sectors. Otherwise, the government could establish a platform with a specific interaction mechanism between the health ministry and other relevant ministries and authorities to address antibiotic resistance in the food chain. As significant numbers of unemployed individuals from South Africa or different African countries earn income from street vending food, the government should give attention to that and provide the necessary infrastructure to the vendors to improve the safety of street vended food. Caution is advised concerning the origin of isolated bacteria.

LIMITATION OF THE STUDY

The following limitations were encountered in the duration of the project

i. The samples were collected in shops and markets from foreign countries, where French is the principal spoken language. Since the researcher does not speak French herself, a translator was needed to assist with communication.

ii. Financial constraints due to the unavailability of funds to conduct the research delayed the project to be completed on record time.

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APPENDICES

Appendix 1

Preparation of the samples and determination of microbial in the dried fish

All growth media, instruments, and applicators (bottles, beakers, test tubes, etc.) used in this study were sterilized by autoclaving at 121°C in an electrically operated autoclave. Upon arrival, Samples were grinded aseptically at the laboratory and labelled for identification. In situations where the samples were not analysed immediately after collection, they were stored at - 80°C until analyses.

3.6.1 Peptone Water (Sorensen *et al.*)

The medium Peptone Water powder (Biolab, Merck-Modderfontein-1645-South Africa, HG000C134.500, Batch 104667) was prepared by measuring 20 g of the powdered medium and dissolved in 1 litre of distilled water. The culture medium was mixed well, and 10 ml were dispensed into capped test tubes. The test tubes were then sterilized by autoclaving at 121°C for 15 minutes and cooled to 25°C before use. All the unused prepared media plates were being stored under refrigeration temperature.

3.6.2 Mannitol Salt Agar (Shamsan & Al-Jobory)

Mannitol Salt Agar selective medium, PCA powder, (Biolab, Merck-Modderfontein-1645-South Africa, HG000C26.500, Batch 10444408) was prepared for the isolation of presumptive pathogenic staphylococci. The medium was prepared according to the manufacturer's instructions by suspending 111g in 1 liter of distilled water, mixed well, and boiled to dissolve completely. The medium was sterilized by autoclaving at 121°C for 15 minutes, cooled to below 45°C, and poured into sterile petri dishes.

3.6.3 Eosin Methylene Blue (EMB) Agar

Eosin Methylene Blue is an isolation medium (EMB powder, Biolab, Merck, KGaA-Darmstadt -16 27-Germany, Lot VM693347518) for the differentiation of the Enterobacteriaceae. The medium was prepared according to the manufacturer's instructions by suspending 37.5g in 1 liter of distilled water and bringing it to boil to dissolve completely. It was then sterilized by autoclaving at 121°C for 15 minutes, cooled to 60°C, and shaken in order to oxidise the methylene blue (i.e., restore its blue colour) and to suspend the precipitate, which was an essential part of the medium.

3.6.4 Xylose Lysine Deoxycholate (XLD) Agar

Xylose Lysine Deoxycholate is a selective medium (XLD powder, Biolab, Merck-Modderfontein-1645-South Africa, HG000C21.500, Batch 1041192) for the isolation of Salmonella and Shigella from clinical specimens and foods. The media is composed of Yeast extract 3g, L- Lysine HCL 5g, Xylose 3.75g, Lactose 7.5, Sucrose 7.5g Sodium deoxycholate 1g, Sodium chloride 5g, Sodium thiosulphate 6.8g, Ferric ammonium citrate 0.8g, Phenol red 0.08g, agar 12.5g and pH 7.4 ± 0.2 at 25℃. The medium was prepared according to the manufacturer's instructions whereby was suspended in 1 litre of distilled water, heated with frequent agitation until the medium-boiled. The medium was transferred immediately to a water bath at 50°C, poured into sterile Petri dishes as soon as the medium cooled.

3.6.5 Nutrient Agar (NA)

The medium Nutrient Agar (NA powder, Biolab, Merck-Modderfontein-1645-South Africa, HG0000C1.500, Batch 1048588. The medium was prepared according to the manufacturer's instructions, whereby 23 g of the powdered medium was suspended into 1 litre of distilled water, mixed well, and left on the bench to stand until the mixture was uniform. The mixed solution was then heated with gentle agitation and boiled until it was completely dissolved. The medium solution was sterilized in the autoclave at 121°C for 15 minutes, then allowed to cool to 45 ℃ and poured onto sterile Petri dishes. The plates were left at room temperature for two hours for the media to solidify, then put upside down in the incubator for 24 hours at 37°C to check for sterility and to dry the condensed vapor on the plate cover.

Appendix 2: Total bacterial count of dried fish sample sold in the informal markets in Gauteng

Table 1 Total bacterial count of Salted fish

Sample ID	Number of colonies counted	Total bacteria count (n×10^7)
S6	90	1.8
S16	130	2.6
r48	126	2.52
S5	50	1
S3	80	1.6
s9	100	2
M4	150	5
Y14	130	2.6
Y30	80	1.6
Y5	50	1
R14	160	3.2
S45	80	1.6
R69	110	2.2
R9	40	0.8
M15	100	2
M21	160	3.2
S45	70	1.4
S44	120	2.4
M63	135	2.7
M6	90	1.8

Table 2 Total bacterial count of Sun-dried fish

Sample ID	Number of colonies counted	Total bacteria count (n×10^7)
S3	220	4.4
R28	140	2.8

R46	120	2.4
R46	230	4.6
R48	122	2.44
R48	210	4.2
R49	240	4.8
Y1	100	2
S15	50	1
R10	120	2.4
S16	120	2.4
S45	200	4
R13	70	1.4
R30	120	2.4
Y2	140	2.8
S53	120	2.4
R43	200	4
M30	90	1.8
S16	160	3.2
S17	200	4
R9	120	2.4
S45	220	4.4
M63	170	3.4
S9	135	2.7
R33	110	2.2
S42	150	3
Y30	90	1.8
M4	100	2
519	200	4
S1	214	4.24
M12	100	2
R19	130	2.6
S4	120	2.4
R54	140	2.8
R1	120	2.4
S41	180	3.6
R31	100	2
Y5	140	2.8
S69	130	2.6
Y41	200	4
R7	120	2.4
R10	75	1.5
S10	240	4.8
S14	80	1.6
Y28	150	3
R49	230	4.6
M21	67	1.34
R48	230	4.6

R28	100	2
R69	75	1.5
M1	155	2.3
S40	200	4
R8	130	2.6
S44	100	2
Y2	120	2.4
S40	130	2.6
S16	111	2.22
M6	200	4
M40	145	2.9
R13	100	2
R19	70	1.4
R24	100	2
R3	133	2.66
S4	100	2
S22	200	4
R33	100	2
M25	200	4
M8	232	4.64
R9	162	3.24
S15	178	3.56
Y31	130	2.6
M4	165	3.3
R1	178	3.56
Y17	190	3.8
S2	220	4.4
S44	145	2.9
R21	200	4
Y40	100	2
M10	150	3
S36	165	3.3

Table 3 Total bacterial count of smoked fish

Sample ID	Number of colonies counted	Total bacteria count ($n \times 10^7$)
S17	139	2.784

Y10	200	4
Y15	70	1.4
S3	160	3.2
Y2	150	3
R33	110	2.2
M63	220	4.4
S45	84	1.68
M9	80	1.6
Y9	50	1
S16	110	2.2
R16	115	2.3
M30	167	3.34
48	200	4
M1	84	1.68
S53	150	3
Y2	75	1.5
S30	205	4.1
M31	88	1.78
S18	200	4
M6	65	1.3
S16	100	2
S45	130	2.6
M9	80	1.6
S40	70	1.4
30	248	4.96
S18	110	2.2
S58	100	2
S21	240	4.8
Y10	267	5.34
YI	100	2
Y2	131	2.62
s9	145	2.9
S9	151	3.02
R49	240	4.8
R24	211	4.22
R24	200	4
M15	240	2.40
S5	70	1.4
S40	230	4.6

Appendix 3: Preliminary results based on morphological and biochemical characteristics

Sample ID	Morphology	Gr	Cat	Ox	Ind	CT	Suspected organisms
M9	straight cocci	+	–	–	–	+	*Staphylococcus spp.*
S6	straight rods	–	–	–	+	–	*Enterobacter spp.*
S6	Paired rods	–	+	–	+	–	*Enterobacter spp.*
r48	long rod chain	–	+	–	–	–	*Klebsiella spp.*
S5	cocci chain	+	–	–	–	+	*Staphylococcus spp.*
S5	cocci chain	+	–	–	–	+	*Staphylococcus xylosus*
s9	cocci chain	+	–	–	–	+	*Staphylococcus xylosus*
M4	rod chain	+	–	–	–	+	*Staphylococcus spp.*
M4	long cocci chain	+	–	–	–	+	*Staphylococcus xylosus*
Y30	cluster cocci	+	+	–	–	–	*Micrococcus caseolyticus*
Y5	cluster cocci	+	+	–	–	–	*Micrococcus caseolyticus*
R14	cocci chain	+	+	–	–	–	*Clostridium spp.*
S45	rods cluster	+	+	–	–	–	*Clostridium spp.*
R69	rod straight	+	+	–	–	–	*Clostridium spp*
R69	cocci cluster	+	+	–	–	+	*Staphylococcus spp.*
M21	cocci cluster	+	+	–	–	+	*Staphylococcus spp.*
M21	cocci cluster	+	+	–	–	+	*Staphylococcus spp.*
S45	cocci chain	+	+	–	–	+	*Staphylococcus spp.*
S44	cocci crusted	+	+	–	–	+	*Staphylococcus xylosus*
M6	cocci clustered	+	+	–	–	+	*Staphylococcus xylosus*
M6	rods clustered	+	+	–	–	–	*Staphylococcus spp.*
S17	rods clustered	+	+	–	–	–	*Clostridium spp.*
S17	rods cluster	+	+	–	–	–	*Clostridium spp.*
Y10	cocci parried	–	–	–	–	–	*Enterococcus faecalis*
Y10	cocci parried	–	–	–	+	–	*Enterococcus faecalis*
R3	cocci chain	–	–	–	+	–	*Enterococcus faecalis*
Y2	cocci chain	–	–	–	+	–	*Enterococcus faecalis*
R33	rods cluster	–	–	–	–	–	*Enterococcus spp*
M63	rods cluster	+	–	–	–	–	*Clostridium spp*
S45	rods cluster	+	–	–	–	–	*Clostridium spp*
R9	rods cluster	+	–	–	–	–	*Clostridium spp*
R9	rods cluster	–	+	–	–	–	*Klebsiella spp.*
S16	rods cluster	–	+	–	–	–	*Klebsiella spp.*
S16	cocci	+	+	–	–	–	*Staphylococcus aureus*
M30	Cocci	+	–	–	–	–	*Staphylococcus aureus*
R48	cocci chain	+	–	–	–	+	*Staphylococcus aureus*
M1	cocci chain	+	–	–	–	+	*Staphylococcus aureus*
S53	cluster cocci	+	–	–	–	+	*Staphylococcus spp*
Y2	cluster cocci	+	–	–	–	+	*Staphylococcus spp*
R30	cluster cocci	+	–	–	–	+	*Staphylococcus spp*
R31	rods cluster	+	–	–	–	–	*Klebsiella spp.*
S18	rods cluster	+	–	–	–	–	*Klebsiella spp.*
M6	Cocci	+	+	–	–	+	*Staphylococcus spp.*

S16	Rod	+	+	–	–	–	*Enterococcus spp*
S45	rods cluster	+	+	–	–	–	*Enterococcus spp*
M9	Cocci	+	+	–	–	+	*Staphylococcus spp*
S40	rods cluster	+	+	–	–	+	*Clostridium spp*
R30	rods cluster	+	+	–	–	+	*Clostridium botulinum*
S16	Cocci	+	+	–	–	–	*Staphylococcus aureus*
S16	rods cluster	–	–	–	–	–	*Clostridium spp.*
S16	rods cluster	–	+	–	–	–	*Clostridium spp.*
R10	rods cluster	+	+	–	–	–	*Klebsiella spp*
YI	rods cluster	+	+	–	–	–	*Clostridium spp*
Y1	rods cluster	+	+	–	–	–	*Clostridium spp.*
s9	rods cluster	+	+	–	–	–	*Clostridium spp*
S9	cocci chain	+	+	–	–	–	*Clostridium spp*
R49	Cocci	+	–	–	–	–	*Staphylococcus lentus*
R24	rods cluster	–	+	–	–	–	*Klebsiella* spp
R24	rods cluster	–	+	–	–	–	*Klebsiella* spp.
M15	cocci chain	+	–	–	–	–	*Staphylococcus* spp.
S5	cocci chain	+	–	–	–	+	*Staphylococcus xylosus*
S3	cocci chain	+	–	–	–	+	*Staphylococcus aureus*
R28	rods cluster	+	–	–	–	+	*Staphylococcus aureus*
R46	rods cluster	+	–	–	–	–	*Clostridium botulinum*
R46	rods cluster	+	–	–	–	–	*Clostridium botulinum*
r48	cocci chain	–	+	–	+	–	*Enterococcus faecalis*
R48	cocci chain	–	+	–	+	–	*Enterococcus faecalis*

Keys: Positive = (+); Negative = (-); Gram stain = Gr; Oxidase test= (Ox); Catalase test= (Cat).

CT = coagulase test; Ind = Indole test

Appendix 4: Sample identification based on the type of fish

Sample ID	Organisms	Identification
M9	*Staphylococcus saprophyticus*	M
S6	*Enterobacter* spp.	S
S6	*Enterobacter ludwigii*	S
R48	*Klebsiella pneumoniae*	M
S5	*Staphylococcus* spp.	M
S5	*Staphylococcus xylosus*	M
s9	*Staphylococcus xylosus*	S
M4	*Staphylococcus saprophyticus*	S
M4	*Staphylococcus xylosus*	S
Y30	*Macrococcus caseolyticus*	M
Y5	*Macrococcus caseolyticus subsp. hominis strain*	M
R14	*Clostridium bifermentans*	S
S45	*Paraclostridium bifermentans*	D
R69	*Clostridium* spp.	M
R69	*Staphylococcus lentus*	M
M21	*Staphylococcus lentus*	M
M21	*Staphylococcus sciuri*	M
S45	*Staphylococcus* spp.	S
S44	*Staphylococcus xylosus*	M
M6	*Staphylococcus xylosus*	M
M6	*Lysinibacillus macrolides*	M
S17	*Clostridium* spp.	S
S17	*Clostridium* spp.	S
Y10	*Enterococcus faecalis*	S
Y10	*Enterococcus faecalis*	S
R3	*Enterococcus faecium*	M
Y2	*Enterococcus faecium*	M
R33	*Clostridium bifermentans*	S
M63	*Clostridium* spp.	S
S45	*Clostridium* spp.	S
R9	*Clostridium* spp.	S
R9	*Klebsiella pneumoniae*	S
S16	*Klebsiella pneumoniae*	S
S16	*Planococcaceae bacterium*	S
M30	*Planococcaceae bacterium*	S
R48	*Staphylococcus lentus*	S
M1	*Staphylococcus sciuri*	M
S53	*Staphylococcus* spp.	S
Y2	*Staphylococcus* spp.	S
R30	*Staphylococcus* spp.	S
R31	*Klebsiella pneumoniae strain*	S
S18	*Klebsiella* spp.	D
M6	*Staphylococcus* spp.	M
S16	*Corynebacterium variabile*	S
S45	*Corynebacterium variabile*	S

M9	*Staphylococcus* spp.	M
S40	*Clostridium* spp.	M
R30	*Clostridium bifermentans*	M
S16	*Staphylococcus aureus*	S
S16	*Paraclostridium bifermentans*	S
S16	*Paraclostridium bifermentans*	S
R10	*Klebsiella pneumoniae*	S
YI	*Clostridium* spp.	S
Y1	*Paraclostridium bifermentans*	S
S9	*Clostridium* spp.	S
S9	*Clostridium* spp.	S
R49	*Staphylococcus lentus*	M
R24	*Klebsiella* spp.	M
R24	*Klebsiella* spp.	M
M15	*Staphylococcus* spp.	D
S5	*Staphylococcus xylosus*	D
S3	*Staphylococcus aureus*	D
R28	*Staphylococcus aureus*	D
R46	*Clostridium botulinum*	M
R46	*Clostridium botulinum*	M
R48	*Enterococcus faecalis*	M
R48	*Enterococcus faecalis*	M

keys: M- Smoked fish; D - Salted fish; S - Sun-dried fish

Appendix 5: Summary of isolates sent to GenBank

Sequenced	Organism	Strain	Isolation-Source	Country	Collection-Date	Specimen-Voucher	Isolate
Seq1	*Klebsiella pneumoniae*	MH973164	Dried fish	South Africa	2018	FISH2	1
Seq2	*Staphylococcus*	KT151895	Dried fish	South Africa	2018	FISH3	2
Seq3	*Staphylococcus xylosus*	MK253321	Dried fish	South Africa	2018	FISH4	3
Seq4	*Staphylococcus xylosus*	KC456590	Dried fish	South Africa	2018	FISH5	4
Seq5	*Macrococcus caseolyticus*	NR_159094	Dried fish	South Africa	2018	FISH6	5
Seq6	*Paraclostridium bifermentans*	MK894870	Dried fish	South Africa	2018	FISH7	6
Seq7	*Staphylococcus lentus*	MF678888	Dried fish	South Africa	2018	FISH9	7
Seq8	*Staphylococcus*	HM584794	Dried fish	South Africa	2018	FISH10	8
Seq9	*Staphylococcus xylosus*	JX035942	Dried fish	South Africa	2018	FISH11	9
Seq10	*Lysinibacillus macrolides*	MG892813	Dried fish	South Africa	2018	FISH12	10
Seq11	*Enterococcus faecalis*	MK254994	Dried fish	South Africa	2018	FISH14	11
Seq12	*Enterococcus faecium*	MK748256	Dried fish	South Africa	2018	FISH15	12
Seq13	*Klebsiella pneumoniae*	CP040363	Dried fish	South Africa	2018	FISH18	13
Seq14	*Planococcaceae*	LK934680	Dried fish	South Africa	2018	FISH19	14
Seq15	*Staphylococcus*	KU245713	Dried fish	South Africa	2018	FISH21	15
Seq16	*Staphylococcus*	KU644384	Dried fish	South Africa	2018	FISH22	16
Seq17	*Klebsiella*	KJ143756	Dried fish	South Africa	2018	FISH23	17
Seq18	*Staphylococcus*	JX944828	Dried fish	South Africa	2018	FISH24	18
Seq19	*Corynebacterium variabile*	KP140842	Dried fish	South Africa	2018	FISH25	19
Seq20	*Staphylococcus*	KJ504153	Dried fish	South Africa	2018	FISH26	20
Seq21	*Clostridium bifermentans*	KP944171	Dried fish	South Africa	2018	FISH28	21
Seq22	*Staphylococcus aureus*	MK780044	Dried fish	South Africa	2018	FISH29	22
Seq23	*Paraclostridium bifermentans*	MH346281	Dried fish	South Africa	2018	FISH30	23
Seq24	*Paraclostridium bifermentans*	MK606081	Dried fish	South Africa	2018	FISH32	24
Seq25	*Staphylococcus lentus*	MK439492	Dried fish	South Africa	2018	FISH34	25
Seq26	*Staphylococcus*	KC688883	Dried fish	South Africa	2018	FISH36	26
Seq27	*Staphylococcus aureus*	LR134268	Dried fish	South Africa	2018	FISH37	27
Seq28	*Clostridium botulinum*	CP028859	Dried fish	South Africa	2018	FISH38	28
Seq29	*Clostridium botulinum*	CP013243	Dried fish	South Africa	2018	FISH39	29
Seq30	*Enterococcus faecalis*	MH250054	Dried fish	South Africa	2018	FISH40	30

Appendix 6: GenBank Obtained Accession numbers

isolates	Isolates name	Accession number
1	*Staphylococcus xylosus*	NR_036907.1
2	*Staphylococcus xylosus*	MH491329.1
3	*Staphylococcus aureus*	NR_118997.2
4	*Staphylococcus aureus*	NR_036828.1
5	*Staphylococcus aureus*	MH346274.1
6	*Staphylococcus sciuri*	NR_025520.1
7	*Staphylococcus aureus*	KX447584.1
8	*Staphylococcus xylosus*	D83374.1
9	*Staphylococcus lentus*	NR_043418.1
10	*Planococcaceae*	KM349955.1
11	*Planococcaceae*	KM349897.1
12	*Lysinibacillus macroides*	MK104489.1
13	*Lysinibacillus macroides*	KY569486.1
14	*Lysinibacillus macroides*	MH656739.1
15	*Enterococcus faecalis*	AB669429.1
16	*Enterococcus faecium*	KR858813.1
17	*Enterococcus faecium*	MH346266.1
18	*Enterococcus faecalis*	EU728747.1
19	*Klebsiella pneumoniae*	KP792436.1
20	*Klebsiella pneumoniae*	MH973164.1
21	*Corynebacterium variabile*	HG798646.1
22	*Corynebacterium variabile*	KJ862005.1
23	*Clostridium* spp	MH346278.1
24	*Macrococcus caseolyticus*	NZ_RBVL01000351.1

Appendix 7: Antibiotic residence profile

Isolates	**E 5**	**CIP 5**	**AML10**	**S10**	**CN10**	**C30**	**NOR 5**
Staphylococcus saprophyticus	1	2	1	1	1	3	3
Enterobacter spp.	2	3	1	1	1	3	3
Enterobacter ludwigii	2	2	1	1	2	3	3
Klebsiella pneumoniae	2	3	1	1	2	3	3
Staphylococcus spp.	1	3	1	1	1	3	3
Staphylococcus xylosus	1	3	1	1	2	3	3
Staphylococcus xylosus	1	3	1	1	1	3	3
Staphylococcus saprophyticus	1	3	1	1	1	3	3
Staphylococcus xylosus	1	3	1	1	1	3	3
Micrococcus caseolyticus	3	2	1	1	1	3	3
Micrococcus caseolyticus subsp. hominis	3	2	3	1	1	3	3
Clostridium bifermentans	1	2	3	1	1	3	3
Paraclostridum bifermentans	1	3	3	1	1	3	3
Clostridium spp.	1	3	2	1	1	3	3
Staphylococcus lentus	1	3	3	1	1	3	3
Staphylococcus lentus	1	2	2	1	1	3	3
Staphylococcus sciuri	1	3	3	1	1	3	3
Staphylococcus spp.	1	3	3	1	1	3	3
Staphylococcus xylosus	1	3	3	1	1	3	3
Staphylococcus xylosus	1	2	3	1	1	3	3
Lysin bacillus macrolides	3	3	3	1	1	3	3
Clostridium spp.	3	3	3	1	1	3	3
Clostridium spp.	3	3	3	1	1	3	3
Enterococcus faecalis	2	3	3	1	1	3	3
Enterococcus faecalis	2	3	3	1	1	3	3
Enterococcus faecium	2	3	3	1	1	3	3
Enterococcus faecium	1	2	3	1	1	3	3
[Clostridium] bifermentans	2	2	2	1	1	3	3
Clostridium spp.	3	3	1	1	3	2	3
Clostridium spp.	3	3	1	1	3	2	3
Clostridium spp.	1	3	1	1	3	2	3
Klebsiella pneumoniae	3	3	1	1	3	3	3
Klebsiella pneumoniae	3	3	1	1	3	2	3
Planococcaceae bacterium	3	3	3	1	3	3	3
Planococcaceae bacterium	3	1	3	1	3	3	1
Staphylococcus lentus	1	3	3	1	3	3	3
Staphylococcus sciuri	1	3	3	1	3	3	3
Staphylococcus spp.	1	3	3	1	3	3	3
Staphylococcus spp.	1	3	3	1	3	3	3
Staphylococcus spp.	1	3	3	1	3	3	3
Klebsiella pneumoniae	3	3	3	1	3	3	3
Klebsiella spp.	3	3	3	3	3	3	3
Staphylococcus spp.	2	3	3	1	3	3	1
Corynebacterium variabile	2	3	3	1	3	3	3
Corynebacterium variabile	2	3	3	1	3	3	3

Staphylococcus spp.	1	3	3	1	3	3	3
Clostridium spp.	2	3	1	1	3	3	3
Clostridium bifermentans	2	3	1	1	3	3	3
Staphylococcus aureus	1	3	3	1	3	3	3
Paraclostridum bifermentans	1	3	3	2	3	3	3
Paraclostridum bifermentans	1	3	3	2	3	3	3
Klebsiella pneumoniae	1	3	3	2	3	3	1
Clostridium spp.	1	3	1	2	1	3	3
Paraclostridum bifermentans	2	3	3	1	3	3	3
Clostridium spp.	1	3	3	2	3	3	3
Clostridium spp.	1	3	3	2	3	3	3
Staphylococcus lentus	1	3	3	2	3	3	3
Klebsiella spp.	1	3	3	2	3	3	3
Klebsiella spp.	1	3	3	2	3	3	3
Staphylococcus spp.	1	3	3	2	3	3	3
Staphylococcus xylosus	1	3	3	2	3	3	3
Staphylococcus aureus	1	3	3	2	3	3	3
Staphylococcus aureus	1	3	3	2	3	3	3
Clostridium botulinum	1	3	3	2	3	3	3
Clostridium botulinum	1	3	3	2	3	3	3
Enterococcus faecalis	2	3	3	2	3	3	3
Enterococcus faecalis	2	3	3	2	3	3	3

Keys: Susceptible- 3**,** Intermediate – 2**,** Resistance – 1

E 5- Erythromycin, CIP 5- Ciprofloxacin, AML10-Amoxicillin, S10- Streptomycin, CN10-Gentamicin, C30-Chloramphenicol, NOR5- Norfloxacin

Appendix 8: Survey on hygiene practices - observation form

Starting time: Ending time:

Date: Gender:

Location: Estimated Age:

	YES	NO	Comment
Hygiene of vending site (outside)			
Presence of flies at the vending location			
Mixing o different meat along with fish			
Washing of hands before touching fish.			
Washing of hands with disinfectant			
Wearing of gloves during packaging			
Exposure to open air			
Exposure of foods to flies and dust:			
Use of polythene bags as the package			

Appendix 9: Ethical clearance letter

Private Bag X1290, Potchefstroom
South Africa 2520

Tel: 018 299-1111/2222
Fax: 018 299-4910
Web: http://www.nwu.ac.za

Research Ethics Regulatory Committee
Tel: 018 299-4849
Email: nkosinathi.machine@nwu.ac.za

ETHICS APPROVAL LETTER OF STUDY

Based on approval by the **North-West University Animal Production Sciences Research Ethics Committee (NWU-AnimProdREC)** on 07/08/2019, the NWU Animal Production Sciences Research Ethics Committee hereby **approves** your study as indicated below. This implies that the North-West University Senate Committee for Research Ethics (NWU-SCRE) grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Molecular identification and characterization of selected food borne pathogens in imported dried fish sold in informal market around Gauteng province in South Africa

Study Leader/Supervisor (Principal Investigator)/Researcher: Prof M Mwanza

Student: Nkosi SR

Ethics number:

N	W	U	-	0	1	8	8	0	-	1	9	-	A	5
Institution				Study Number						Year			Status	

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Application Type: Single Study
Commencement date: 2020/08/01 **Risk Category:** 2
Expiry date: 2021/12/31
Approval of the study is initially provided for a year, after which continuation of the study is dependent on receipt and review of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.

Special in process conditions of the research for approval (if applicable):

- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the NWU-AnimProdREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:

- *The study leader/supervisor (principle investigator)/researcher must report in the prescribed format to the NWU-AnimProdREC:*
  - *annually (or as otherwise requested) on the monitoring of the study, whereby a letter of continuation will be provided, and upon completion of the study; and*
  - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the study leader/researcher must apply for approval of these amendments at the NWU-AnimProdREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *Annually a number of studies may be randomly selected for an external audit.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility, the NWU-SCRE and NWU-AnimProdREC reserves the right to:*

- *request access to any information or data at any time during the course or after completion of the study;*
- *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;*
- *withdraw or postpone approval if:*
  - *any unethical principles or practices of the study are revealed or suspected;*
  - *it becomes apparent that any relevant information was withheld from the NWU-AnimProdREC or that information has been false or misrepresented;*
  - *submission of the annual (or otherwise stipulated) monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and / or*
  - *new institutional rules, national legislation or international conventions deem it necessary.*

- *NWU-AnimProdREC can be contacted for further information or any report templates via upenyu.marume@nwu.ac.za or 018 389 2725.*

The NWU-AnimProdREC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the NWU-AnimProdREC or the NWU-SCRE for any further enquiries or requests for assistance.

Yours sincerely

Prof Upenyu Marume
Chairperson NWU Animal Production Sciences Research Ethics Committee

Original details: (22351930) C:\Users\22351930\Desktop\ETHICS APPROVAL LETTER OF STUDY.docm
8 November 2018

Current details: (22351930) M:\DSS1\8533\Monitoring and Reporting Cluster\Ethics\Certificates\Templates\Research Ethics Approval Letters\9.1.5.4.1 NWU-AnimProdREC Ethical Approval Letter.docm
5 December 2018

File reference: 9.1.5.4.2