

**BACTERIOLOGICAL ANALYSIS OF PROCESSED CASSAVA (GARRI) AROUND  
BENIN CITY**

**BY**

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**LSC1806660**

**DEPARTMENT OF MICROBIOLOGY**

**FACULTY OF LIFE SCIENCES**

**UNIVERSITY OF BENIN**

**BENIN CITY**

**SEPTEMBER, 2023.**

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**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY,  
FACULTY OF LIFE SCIENCES IN PARTIAL FULFILLMENT OF THE  
REQUIREMENTS FOR THE UNIVERSITY OF BENIN, BENIN CITY, AWARD OF  
BACHELOR OF SCIENCE (B.Sc HONS) DEGREE**

**SEPTEMBER, 2023.**

**CERTIFICATION**

This is to certify that this project work was carried out by Divine-favour Omonoshi BALOGUN of the Department of Microbiology, Faculty of Life Science, University of Benin, Benin City.

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**Dr. (Mrs.) O. B. Isichei-Ukah**  
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**Prof. (Mrs.) F. I. Akinnibosun**  
(Head of Department)

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**APPROVAL**

This project was carried out by DIVINE-FAVOUR OMONOSHI BALOGUN under the supervision of Dr. (Mrs.) O. B. Isichei-ukah in partial fulfilment for the award of Bachelor of Science (B.SC) degree in Microbiology

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**PROF.(MRS) F.I. AKINNIBOSUN**

Date

(Head of Department)

## **DEDICATION**

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

## ACKNOWLEDGEMENTS

My sincere appreciation goes to the Almighty God for his grace and mercies throughout my period of study.

I wish to acknowledge wholeheartedly my project supervisor Dr. (Mrs.) O. B. Isichei-ukah for her patience and understanding towards me and the success of this project. May God Almighty richly bless you ma for your efforts.

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## ABSTRACT

A bacteriological evaluation of Garri, a cassava product, one of the most popular foods derived from cassava fermentation, is a staple Nigerian food, its raw product Cassava (*Mamihot esculenta* Crantz), is a dicotyledonous perennial plant. It was conducted in five major markets in Benin City, Edo State. The aim and objectives of this study was to isolate bacteria from garri sold at different markets in Benin City, in order to determine the bacterial count of the garri samples, determine the species of bacteria present in the garri samples and gain insights into the susceptibility of the isolated bacteria. Methodology utilized in this research study include: isolation of bacteria utilizing nutrient agar, a total of six isolates (bacteria) with a sample size of five (Olukwu, Uselu, RingRoad, NewBenin, and Mami ) were examined. The mean total heterotrophic bacterial count ranged from (7.00 - 7.91±2.0 log<sub>10</sub>cfu/g) in the five different markets and the pour plate method was employed in achieving the colony count in the Garri samples. The primary bacteria strains identified after observing the cultural, morphological and biochemical test results, included *Pseudomonas spp.*, *Bacillus spp.*, *Escherichia coli.*, *Streptococcus sp.*, *Micrococcus spp.*, and *Staphylococcus spp.* These bacterial strains were prevalent in Garri samples from all five markets, indicating potential contamination during processing or handling. The bacteria obtained from this study were all susceptible to the antibiotic, perfloxacin, but resistant to the others, namely, amoxicillin, rocephin, ciprofloxacin, ampiclox, zinnacef, erythromycin and gentamicin. The high occurrence of bacteria in Garri across these markets could be attributed to suboptimal sanitation conditions, processing techniques, materials used, and inadequate waste management practices. It is recommended that producers and retailers in Benin implement rigorous hygiene measures in preparation and packaging to ensure food safety and the protection of consumers.

# CHAPTER ONE

## INTRODUCTION

### 1.1 BACKGROUND STUDY

#### 1.1.2 Introduction to Garri

Cassava (*Mamihot esculenta* Crantz), a dicotyledonous perennial plant, is a member of the Euphorbiceace family of rubber plants and has white latex flowing from its damaged stem and leaf stalks. It is also a shrub of wood that averages one meter in height and has an edible root.

Brazil and several regions of Central America are where it first emerged. In several parts of the world, it goes by different names. While English-speaking nations in South Asia refer to it as "tapioca," it is known as "mandioca" in Brazil. Spanish-speaking nations in North America, Europe, and Africa refer to it as cassava. The Tiv refer to it as "kyar or nduhar" and the Idomas refer to it as "Logo" in the middle belt region of Nigeria

Depending on regional customs and tastes, the roots of cassava are processed into a variety of goods. Foods with great energy and outstanding quality are the byproducts. However, cassava is impacted by mycoplasmic, bacterial, viral, and virus-like agents. The conditions in which the roots of cassava products remain after processing (or before processing) greatly contributes to the root product's contamination. This food's contamination can be linked to the handlers' hands, clothing, and processing equipment.

Drying cassava chips on an open surface such as a road or floor exposes them to airborne microorganisms, which have a terrible effect on the quality and shelf life of the cassava. Cassava

and its products may become polluted during processing due to the use of unclean techniques, the use of contaminated tools, dust, and domestic animal activity.

Locally, rural women prepare garri by fermenting peeled cassava tubers for 4 to 6 days after steeping them in stream water. The amount of microbial contaminants within the fermented cassava product increases as a result of this processing procedure. It is challenging to regulate and keep an eye on circumstances while using conventional processing techniques. Another significant barrier to traditional garri processing is the excessive time required. This is due to the old dryer's inefficient heat transmission mechanism and maybe the uneven and enormous sizes of the dried balls. As a result, adequate drying is not accomplished, and dried balls have a high moisture content. Which results in the product being vulnerable to mold attack and can lose flavor while stored.

Microorganisms are microscopic forms of life that are organized mostly as single-cell organisms, however some may be multicellular. They are widespread in our natural environment (Soil, Air, Water, etc.) meaning they are ubiquitous and can be found naturally in foods or on food surfaces as contaminants during the production process of foods. One of such common foods is garri, gotten from processed cassava. It is a fermented form of this cassava meal and has gained widespread popularity over the past decades in Benin-city and its environs, and even across the shores of Nigeria. It is consumed by an average of 4 million to 6 million people within Nigeria. (Obadina *et al* 2009)

The process of food fermentation involves employing endogenous organic molecules as both donors of electrons as well as acceptors of electrons to create ATP. In the absence of aerobic respiration, the chemical process of this fermentation produces a small number of ATP

molecules. Cassava flour (Garri) as a staple Nigerian food is essential because it is one of the few foods high in carbohydrate, but the production procedures used and the sales condition involved in its distribution raises its susceptibility to microbial contamination, leaving much more to be desired.

Locally, rural women prepare garri by fermenting peeled cassava tubers for 4 to 6 days after steeping them in stream water. The amount of microbial contaminants within the fermented cassava product increases as a result of this processing procedure. It is challenging to regulate and keep an eye on circumstances while using conventional processing techniques. Another significant barrier to traditional garri processing is the excessive time required. This is due to the old dryer's inefficient heat transmission mechanism and maybe the uneven and enormous sizes of the dried balls. As a result, adequate drying is not accomplished, and dried balls have a high moisture content. As a result, the product is vulnerable to mold attack and can lose flavor while stored.

Processed cassava, Garri in particular holds immense popularity in Benin City and throughout Nigeria, owing to its significant economic, social, and geographical significance. However, limited attention has been given to the potential health hazards associated with mishandling, inadequate sterilization of products and packaging, and improper storage and distribution practices of garri. Insufficient research has been conducted locally to identify and isolate pathogenic organisms that may pose risks to consumers' health. Consequently, it becomes crucial to analyze garri products manufactured on a smaller scale, where hygienic conditions might not be given the utmost priority.

As a staple food primarily consumed in the mid-western and western parts of Nigeria, known as Red-garri, white garri, and Ijebu Garri, respectively. The quality of garri is influenced by various factors, including processing and storage conditions. This is so because in Nigeria, specifically, Benin-city, common practices used in the storage of this cassava product are crude methods such as the use of bowls, mats, open buckets, etc. to display this food item and the handling of the garri with their bare hands. such unhygienic processes such as this gives rise to the concern today on the quality of the garri.

Such practices can also give rise to bothersome microbial contamination, arising from several accumulation of bioaerosols deposited on these exposed garri products. (Amadi *et al*, 2008)

Foodborne illnesses are brought on by eating contaminated foods that contain pathogenic bacteria, viruses, or parasites as well as synthetic or organic toxins, including those found in toxic mushrooms. Infections or food poisonings (intoxications) can result by ingesting poisons or microbes. Food contaminating bacteria can enter via a number of different sources. Rodents and arthropods wander among foods carrying microbes on their feet and parts of their bodies, whereas crops transfer soil-borne species of bacteria to the processing plant. An avenue of contamination is also provided by human food handling. (Obadina *et al* 2009)

There are several factors that play are responsible or play a huge role in the contamination of food, one of such sources of contamination is that listed by (Trickett 1992), where he state that the larger the exposed surface area of the food product available, the higher the microbial load of the said food product and the greater the available oxygen ready for use by aerosol organisms for their metabolic activities.

In Nigeria, the consumption of garri just as it is dry or in water has become common practice, to the extent of it being consumed as snacks by students, who make it a quick alternative to having to cook a full meal from scratch. and they do this without taking into account the implications of the microbial quality of the garri product. By conducting this study, this work aims to shed light on the bacteriological aspects of garri processing, focusing on smaller-scale local production. This research will contribute to a better understanding of potential health risks associated with garri consumption and provide insights into the necessary measures to ensure its safe production and distribution.

### **Aim of the Study**

The aim of this study is to isolate bacteria from garri sold at different markets in Benin City.

### **1.2 Objectives**

1. To determine the bacterial count of the garri samples
2. To determine the species of bacteria present in the garri samples.
3. To gain insights into the susceptibility of the isolated bacteria.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 What is Garri?

Garri, a popular food product in Nigeria and other West African countries, is a creamy-white, granular flour derived from fermented and gelatinized fresh cassava tubers (*Manihot esculenta* Crantz). Garri is made from over seventy percent of the cassava that is grown in Nigeria [Adebayo, B.A. et al 2012]. It possesses a distinctive slightly fermented flavor and a mild sour taste. Garri is consumed in various ways, either in its dry form or soaked in cold water. It is often enjoyed with accompaniments such as sugar, coconut, roasted groundnuts, or dry fish. Alternatively, it can be prepared as a paste known as "Eba," which is eaten alongside a variety of African soups. Additionally, garri can be consumed either soaked or dry, without the need for any additional accompaniments. The versatility of garri and its cultural significance make it a widely recognized and cherished food staple within the region.

Cassava roots must first be peeled before being grated, dewatered, fermented into a wet cake, and then roasted to produce gelatinized bits. After that, it is spread out on the ground or a mat to cool before being finally sieved and packaged for sale (Adebayo *et al* 2012), (Ogiehor *et al* 2007). Additionally, it has a moderate to strong sour taste, and particles that range in size from 0.6 to 1.1 millimeters, depending on the production process and user preferences. Garri has been categorized as "street food," a term that describes a wide range of foods and drinks that are served and occasionally cooked in public spaces. Because it is sold and stored in a ready-to-eat state, the product is convenient [Mensah *et al* 2002].

It can therefore be taken in two ways: simply in the dry state by immersing in cold water, occasionally with sugar, milk, and other beverages, or slightly processed by boiling water to create a stiff paste that is eaten with different kinds of African soups.

## **2.2 Historical background of Garri**

Garri holds a significant place in the culinary culture and dietary practices of Nigeria (Umar *et al.*, 2019). Its origins can be traced back to ancient times when cassava was introduced to the West African region by Portuguese traders in the 16th century. Cassava, a starchy tuber crop native to South America, quickly became a staple food due to its adaptability to diverse ecological conditions and high yield potential.

The processing of cassava into garri evolved over centuries, reflecting the cultural and technological advancements of Nigerian societies. Initially, the processing methods were rudimentary, involving manual grating of fresh cassava roots, followed by fermentation and subsequent drying in the sun. This traditional method, known as "wet cassava processing," is still practiced in rural communities today, preserving the authentic flavors and textures of garri.

Over time, as Nigeria witnessed advancements in food processing technologies and increased urbanization, new techniques emerged to meet the growing demand for garri. This led to the introduction of mechanized processing methods, such as the use of mechanical graters and hydraulic presses. These innovations improved efficiency and productivity, enabling larger-scale garri production to cater to the expanding population.

Garri's popularity as a staple food soared across Nigeria due to several factors. Its long shelf life, ease of storage, and versatility in culinary applications made it a reliable and convenient food

source. When stored properly, garri has a shelf – life of greater or equal to six months (Federal Ministry of Agriculture and Rural Development 2010). Additionally, garri became deeply embedded in cultural traditions and social gatherings, being served in various forms and consumed during special occasions, festivals, and everyday meals.

Furthermore, regional variations in garri emerged, reflecting the diverse cultural practices within Nigeria. The mid-western part of Nigeria predominantly consumes red and white garri, while the western region favors Ijebu garri. These regional preferences contribute to the unique culinary landscape and dietary habits across the country.

The historical background of garri in Nigeria showcases its evolution from a traditional, manual processing method to modern, mechanized techniques. It highlights the cultural significance and widespread acceptance of garri as a staple food, deeply ingrained in the daily lives of Nigerians. Understanding this historical context provides valuable insights into the cultural, social, and economic importance of garri, setting the stage for further exploration of its nutritional composition, quality factors, and safety concerns in the subsequent sections of this study.

## **2.3 How Is Garri Produced?**

### **2.3.1 Traditional Processing Techniques**

The processing of garri involves a series of traditional and modern techniques that have evolved over time to meet the increasing demand for this staple food. These techniques play a crucial role in transforming fresh cassava tubers into the granular flour known as garri.

In traditional processing methods, fresh cassava tubers are harvested and peeled to remove the outer bark. The peeled tubers are then washed to remove any dirt or impurities. After washing, the tubers are grated manually using traditional grating tools or abrasive surfaces such as rocks or metal graters. The grated cassava is then placed in porous bags or wrapped in thin cloth and pressed to remove excess moisture. This process, known as dewatering, typically involves the application of weights or pressing with the feet. The dewatered cassava is left to ferment for a period ranging from one to several days, depending on the desired flavor and texture. This step is crucial because the fermentation process aids in lowering and detoxifying cassava's high cyanide level (Oke,1969).Fermentation helps to enhance the flavor and nutritional quality of the garri.

After fermentation, the fermented cassava is spread on large trays or mats to dry in the sun. This drying process, which can take several days, reduces the moisture content of the cassava mash and promotes the development of the characteristic granular texture of garri. The dried cassava mash is then sieved or milled to break down any lumps and obtain a fine, homogeneous flour. The resulting garri is typically creamy-white in color, with a slightly fermented flavor and sour taste.

### **2.3.2 Modern Processing Techniques**

In recent years, modern processing techniques have emerged to improve efficiency and increase production capacity. Mechanized graters, which use electric or diesel-powered machines, have replaced manual grating, reducing the labor and time required for processing. Hydraulic presses are now commonly employed to dewater the grated cassava, achieving higher levels of moisture extraction. The use of mechanical dryers has also become prevalent, providing controlled drying

conditions and reducing the reliance on sun drying, which can be unpredictable and time-consuming.

These modern processing techniques have not only enhanced productivity but also contributed to the standardization of garri production, ensuring consistent quality and safety. However, it is important to note that traditional processing methods are still practiced in many rural communities, as they preserve the authenticity and cultural heritage associated with garri production.

Understanding both traditional and modern processing techniques is essential for assessing the impact of processing methods on the quality, nutritional composition, and safety of garri. By exploring these techniques, this study aims to provide insights into the diverse approaches employed in garri production and their implications for ensuring the overall quality of the final product.

## **2.4 Nutritional Composition of Garri**

Garri, as a cassava-based product, possesses a unique nutritional composition that contributes to its importance as a staple food in Nigeria and other West African countries. It serves as a significant source of energy and provides essential nutrients necessary for overall health and well-being.

The primary component of garri is carbohydrates, which make up the majority of its composition. These carbohydrates predominantly exist in the form of starch, a complex carbohydrate that serves as a valuable energy source. Garri is known for its high carbohydrate content, providing a substantial amount of calories per serving.

Apart from carbohydrates, garri also contains small amounts of proteins, vitamins, and minerals. The protein content of garri is relatively low, but it contributes to the overall nutritional profile. Additionally, garri contains essential vitamins, including vitamin C and some B vitamins like thiamine (vitamin B1) and niacin (vitamin B3). (Zekarias *et al* 2019) These vitamins play vital roles in various physiological processes within the body, such as energy production and nerve function.

Minerals present in garri include calcium, iron, phosphorus, and potassium. These minerals are essential for the maintenance of healthy bones, proper oxygen transport, cellular function, and fluid balance. However, the mineral content of garri can vary depending on factors such as soil conditions, cassava variety, and processing methods.

It is important to note that garri is relatively low in dietary fiber compared to whole cassava roots. The fermentation process involved in garri production reduces the fiber content to some extent. Therefore, the consumption of additional fiber-rich foods such as fruits, vegetables, and whole grains is recommended for a balanced diet.

While garri provides a good amount of energy and certain nutrients, it is important to consider portion sizes and overall dietary balance. Excessive consumption of garri, like any other food high in carbohydrates, can contribute to weight gain and potential health issues such as obesity and diabetes if not balanced with other nutrients and a healthy lifestyle.

Understanding the nutritional composition of garri is crucial for assessing its role in the diet and designing balanced meal plans. This knowledge aids in making informed dietary choices and ensuring that garri consumption is part of a well-rounded and diverse nutritional intake.

## 2.4. 1 NUTRITION FACTS ABOUT GARRI

**Serving Size:** 100g

**Amount per Serving:** 360 Calories, 5.4% of which are from fat

0.6 g of total fat 0% Fat Saturated 0g 0% 0% cholesterol at 0 mg 0% sodium, 0 mg 87.3g 29% of total carbohydrates

Nutritional Fiber 0g 0% 0g sugars

1.12g of protein Estimated Calorie Content from Fat: 1.5% 97.2% of the total weight are carbohydrates. 1.2% protein

### 2.4.2. Quality Factors Affecting Garri

The quality of garri is influenced by several factors throughout the production process, from the cultivation of cassava to the final packaging and storage. Understanding these quality factors is crucial for ensuring that garri meets the desired standards of safety, taste, texture, and nutritional composition.

1. **Cassava Variety:** The choice of cassava variety plays a significant role in determining the quality of garri. Different varieties have varying levels of starch content, fiber, and other nutrients, which can impact the final product's texture and nutritional composition.

2. **Harvesting and Freshness:** The timing of cassava harvesting is critical. Delayed harvesting can lead to increased cyanide levels in the tubers, which can negatively affect the safety and taste of garri. Freshness is also important, as deteriorated or spoiled cassava can result in poor-quality garri.
3. **Processing Methods:** The processing techniques employed greatly influence the quality of garri. Factors such as the duration of fermentation, the efficiency of dewatering, and the drying conditions can impact the flavor, texture, and shelf life of the final product. Traditional methods may introduce variations in quality compared to modern mechanized processes.
4. **Fermentation:** The fermentation process is crucial for enhancing the flavor and nutritional quality of garri. Proper fermentation allows for the breakdown of cyanide compounds present in cassava, reducing their toxicity. Insufficient or excessive fermentation can lead to off-flavors, compromised safety, or suboptimal nutritional benefits.
5. **Dewatering:** Efficient dewatering is essential to remove excess moisture from the grated cassava. Inadequate dewatering can result in garri with high moisture content, making it prone to spoilage and mold growth. Proper dewatering ensures a desirable texture and prolongs the shelf life of the product.
6. **Drying:** The drying process affects the texture, color, and shelf stability of garri. Sun drying, the traditional method, is influenced by weather conditions and may lead to inconsistent drying and potential contamination. Modern mechanical dryers offer

controlled drying conditions, ensuring uniformity and reducing the risk of microbial growth.

7. **Packaging and Storage:** Proper packaging is crucial for maintaining the quality of garri. Inadequate packaging can lead to moisture absorption, insect infestation, or contamination. Storage conditions, including temperature, humidity, and exposure to light, also play a role in preserving the quality and extending the shelf life of garri.
8. **Hygiene and Sanitation:** Maintaining hygienic practices throughout the production process is vital to prevent contamination and ensure the safety of garri. Proper cleaning of equipment, adherence to personal hygiene, and effective sanitation protocols contribute to the overall quality of the final product.

By considering these quality factors and implementing appropriate measures at each stage of garri production, producers can ensure consistent quality, safety, and consumer satisfaction. Regular monitoring, quality control checks, and adherence to food safety standards are essential for maintaining the desired attributes of garri and meeting consumer expectations.

## **2.5 Microbiological Hazards in Garri**

Microbiological hazards in garri pose significant concerns for public health and food safety. Garri, being a processed cassava product, is susceptible to contamination by various microorganisms that can lead to foodborne illnesses if not properly controlled and managed. This section of the literature review explores the microbiological hazards associated with garri and their potential impact on consumer health.

### **2.5.1 Microorganisms Associated with Cassava**

Cassava, as the primary raw material for garri production, can harbor various microorganisms during cultivation, harvesting, and processing. The outer surfaces of cassava tubers can come into contact with soil, water, and other environmental sources, leading to contamination with bacteria, fungi, and other microorganisms.

Some of the common microorganisms associated with cassava include bacteria like *Bacillus cereus*, *Clostridium perfringens*, and *Listeria monocytogenes*, as well as fungi like *Aspergillus* spp. and *Fusarium* spp. *Bacillus cereus*, for instance, is known for producing heat-stable toxins that can cause food poisoning, while *Listeria monocytogenes* can cause severe infections, especially in vulnerable populations.

### **2.5.2 Foodborne Pathogens and Garri Contamination**

Foodborne pathogens can find their way into garri during various stages of production and processing. Contamination can occur through contact with contaminated water, utensils, surfaces, or through improper handling practices by food handlers.

Pathogens commonly associated with foodborne illnesses, such as *Salmonella*, *Escherichia coli* (*E. coli*), and *Staphylococcus aureus*, are of particular concern when it comes to garri safety. These pathogens can cause a range of gastrointestinal symptoms, from mild to severe, and may pose significant health risks, especially for vulnerable individuals such as children, the elderly, and those with compromised immune systems.

### **2.5.3 Impact of Unhygienic Practices on Garri Safety**

Unhygienic practices during garri processing, storage, and distribution can exacerbate the risk of microbiological hazards. Factors such as poor sanitation of processing equipment, inadequate personal hygiene of food handlers, and unsanitary storage conditions can lead to contamination and microbial growth in garri.

In regions where traditional processing methods are still prevalent, the lack of proper sanitation facilities and limited access to clean water may contribute to higher levels of microbial contamination. Additionally, the use of contaminated water during processing or poor drying practices can further compromise the safety and quality of the final product.

Furthermore, the absence of proper quality control measures and regulatory oversight can increase the likelihood of garri products with high microbial loads reaching consumers, potentially leading to outbreaks of foodborne illnesses.

To ensure the safety of garri, it is crucial to implement and enforce hygienic practices at all stages of production, processing, and distribution. Regular monitoring and testing for microbial contaminants, adherence to good manufacturing practices (GMP), and proper hygiene training for food handlers are essential measures to mitigate microbiological hazards and safeguard public health. By addressing these challenges, the safety and quality of garri can be significantly improved, making it a safer and more reliable food source for consumers in Nigeria and beyond.

Note: While the information provided here is based on general knowledge, for a comprehensive literature review, it is essential to refer to scientific research papers, studies, and relevant publications to support and substantiate the discussed microbiological hazards in garri.

A Study on Microbial Evaluation Of Garri Sold Within Ahmadu Bello University Main Campus,  
Samaru - Zaria, Kaduna State

The findings of this study revealed substantial variations in the proximate composition of garri samples, indicating diverse nutritional profiles across different sampling sites. Moisture content ranged from 2.46% to 2.88%, ash content from 1.35% to 1.62%, lipid content from 6.85% to 8.20%, protein content from 2.47% to 3.45%, fiber content from 1.32% to 1.54%, and carbohydrate content from 84.14% to 86.36%. The documented garri specifications recommend enrichment with essential nutrients, such as vitamins and proteins, to improve its nutritional value.

The relatively low protein content observed in this study underscores the necessity for protein enrichment strategies before garri consumption. Consumers commonly supplement garri with protein sources like smoked fish, beans, and milk, aligning with the recommended enrichment practices. To optimize nutritional intake, consumers are encouraged to continue these practices, enhancing the protein content of their garri-based meals.

Moisture and ash content were generally within the specified limits recommended for garri, except for one location, which slightly exceeded the recommended ash content. The relatively low moisture levels observed are conducive to microbial spoilage prevention, aligning with prior research suggesting that well-processed low-moisture garri can be stored for extended periods without microbial deterioration.

Variations in moisture content could be attributed to temperature variations, drying practices, and storage conditions. Additionally, ash content, an indicator of mineral quality, was relatively lower than values reported by other studies, signifying potentially diverse mineral compositions.

The levels of carbohydrate, crude fiber, and lipid content were consistent with prior research, validating the reproducibility of these values. Statistical analysis indicated no significant variation in crude fiber, carbohydrate, moisture, and ash content across the garri samples.

The functional properties of garri, measured by swelling capacity and water absorption capacity, revealed no significant differences among sampling locations. These properties influence water absorption and retention rates, with temperature influencing their variability.

The pH values of the garri samples indicated an acidic range, potentially due to the presence of fermentation bacteria and fungi. Microbial analysis revealed that while total bacterial counts exceeded fungal counts, both were within acceptable limits recommended by international standards.

However, the presence of *E. coli*, a key indicator of fecal contamination, is concerning. Other bacterial isolates included *Micrococcus* sp., *Klebsiella* sp., *Lactobacillus* sp., *Enterobacter* sp., *Staphylococcus aureus*, and more, while fungal isolates comprised *Rhizomucor* sp., *Aspergillus* sp., *Trichophyton* sp., and *Candida* sp. This microbial landscape highlights the need for improved hygiene practices throughout the garri production, handling, and retailing process.

Notably, some of the identified bacteria, like *Lactobacillus* sp. and *Enterococcus* sp., can serve as beneficial probiotics. However, the presence of pathogenic bacteria and mycotoxin-producing fungi underscores the importance of stringent hygiene measures to mitigate potential health risks.

The identification of similar microorganisms by other researchers supports the consistency and validity of these findings, emphasizing the urgent need to address poor sanitary practices in garri production and distribution.

In conclusion, this study highlights the need for consistent and proper garri processing practices to ensure its safety and nutritional value. Addressing the challenges related to microbial contamination and adopting recommended enrichment practices can contribute to enhancing garri's quality and its role as a staple food in Nigeria.

## **CHAPTER THREE**

### **MATERIALS AND METHODS**

#### **3.1 Collection of Garri Samples**

Garri samples were collected aseptically from the sellers at 10 different points from five (5) different markets within Benin City, Ringroad (Oba) Market, New Benin Market, Uselu Market, Olukwu Market, Mami Market. These markets were selected because they are the main markets within Benin and are the major points for the supply chain of this product in Benin City and Edo state. The samples after collection in sterile plastic bags were labeled and taken to the Microbiology laboratory for analysis which is usually not later than 12 hours after collection.

#### **3.1 Media and Apparatus Used**

##### **3.1.1 Media**

The reagents utilized in this experiment included peptone water, Nutrient Agar.

##### **3.1.2 Apparatus**

The equipment employed in the experiment included syringes of varying capacities (2ml and 10ml), single-use petri dishes, a rack for holding test tubes, conical flasks, beakers, a digital weighing balance, aluminum foil, masking tape, a spatula, cotton wool, a spirit lamp, a marker, and a measuring cylinder.

### **3.2 Sterilization of Materials**

Glass-wares such as test tubes, measuring cylinder, conical flasks needed for analysis were properly sterilized.

#### **Glassware Sterilization Procedure**

The glassware and slides underwent a rigorous sterilization process to ensure contamination-free experimentation. They were meticulously cleansed using OMO detergent, followed by thorough rinsing in multiple changes of tap water and concluding with distilled water. After rinsing, they were carefully air-dried.

To achieve a state of complete sterility, these items were subjected to an electric oven, maintained at a temperature of 60°C, for an extensive duration of 24 hours. This procedure ensured that any potential microbial contaminant, was eliminated.

#### **Flame Sterilization of Cover Slips**

Prior to usage, cover slips were subjected to flame sterilization. This involved a controlled exposure to an open flame, ensuring their surface integrity and sterility.

#### **Enhanced Protection Measures for Utensils**

For droppers, pipettes, cotton wool, and plugs, an additional layer of safeguarding was implemented. Aluminum foil was carefully wrapped around these items. This strategic measure was enacted to prevent the infiltration of condensed water vapor into the working media.

## **Preparation of Sterile Water Blank**

A meticulous process was undertaken to create a sterile water blank. Using precise pipetting techniques, ten milliliters of distilled water were accurately measured and dispensed into McCartney bottles. Each McCartney bottle corresponded to a specific sample collected from the market. Each McCartney bottle was meticulously labeled to correspond to its respective stock solution and sample. This stringent process ensured the establishment of a contamination-free baseline for further experimentation.

## **3.3 Preparation of Media**

**Nutrient Agar:** This is a versatile medium supporting the growth of a wide spectrum of non-demanding microorganisms, was meticulously prepared adhering to the manufacturer's guidelines. It was meticulously prepared, carefully dispensed into sterilized petri dishes bearing appropriate labels.

For the formulation, 7g of Nutrient Agar was meticulously dissolved in 250ml of distilled water within a sanitized conical flask, immediately sealed with a stopper, and subsequently subjected to sterilization at 121°C for 15 minutes in an autoclave.

**Peptone water:** In the creation of the peptone water component, 0.9g was meticulously blended with 60ml of distilled water in a pristine conical flask, ensuring thorough mixing to avoid any particulate matter. Utilizing a 10ml syringe, precisely 9ml of the prepared peptone water was accurately pipetted and filled into clean test tubes, then subjected to autoclaving at 121°C for 15 minutes.

Following sterilization, a gram of each sample was meticulously weighed and dispensed

from the stock. Subsequently, tenfold serial dilutions were meticulously executed. In duplicates, 1ml of each dilution (ranging from  $10^1$  to  $10^6$ ) was meticulously dispensed onto separate petri dishes. The procedure was replicated for Nutrient Agar.

## **Serial Dilution Technique**

The McCartney bottles underwent meticulous division into five distinct groups. Pre-sterilized McCartney bottles were thoughtfully labeled as  $10^1$  to  $10^6$ , each corresponding to its respective stock bottle and sample. Aseptically, using sterile pipettes, 1ml from the stock bottles was meticulously transferred into McCartney bottles labeled  $10^1$  to  $10^6$ . These bottles contained a precise 9 milliliters of previously sterilized distilled water, forming a sequence of serial dilutions.

## **Inoculation Methodology**

For the isolation of microorganisms present in the samples, the pour plate technique was employed. This method involves the precise introduction of microorganism-containing samples into appropriate culture media. Utilizing a syringe, 1ml from each serial dilution-prepared sample was meticulously pipetted into the corresponding and pre-labeled Petri dishes. Subsequently, 9ml of molten nutrient agar (NA) was meticulously poured into their respective Petri dishes. These dishes were then incubated at room temperature for a duration of 24 hours.

## **Microbial Load Determination**

The quantification of microbial load within the samples was achieved through visible colony counting. Following an incubation period of 24 hours for bacteria and 72 hours for fungi, the colony-forming units were enumerated. To calculate the microbial load per milliliter, the following formula was employed:

$$\text{Count/ml} = \frac{\text{No of colonies on plate}}{\text{Amount plated}} * \frac{1}{\text{dilution factor}}$$

This process ensured an accurate assessment of the concentration of microorganisms present in the samples.

### **Determination of Percentage Occurrence**

This was successfully carried out using the formula below.

$$\% \text{Occurrence} = \frac{\text{No of particulate isolate on plate} * 100}{\text{Total number of isolates on same} * 1}$$

### **Subculturing of Microorganisms**

Newly prepared nutrient agar (NA) was meticulously poured into sterilized Petri dishes, creating a solidified medium. Subsequently, the streaking technique was employed to transfer microorganisms from previous plates onto the freshly prepared nutrient agar surface.

### **3.4 Characterization and Identification of Bacterial Isolates**

Identification and characterization of the bacterial isolates was performed on the basis of cultural characteristics, morphological characteristics and biochemical characteristics.

### **3.4.1 Cultural Characteristics**

For the bacterial isolates, cultural characteristics were observed on Nutrient agar plates. The cultural characteristics include. Size, shape, surface, opacity, texture, elevation and pigmentation were determined by visual observation.

### **3.4.2 Morphological Characteristics**

Morphological characterization was carried out using Gram staining technique. Gram stain or Gram staining, also called Gram's method, is a method of staining used to classify bacterial species into two large groups: gram-positive bacteria and gram-negative bacteria

#### **Gram Staining Technique**

Smears were prepared from the cultured plates aseptically on clean properly labelled glass slides. A drop of sterile water was emulsified on the smear and dried, heat. The smear was then stained with crystal violet for 1 min and then washed off with water. Iodine was applied to the smear for 1 min and then washed off with water. The smear was decolourized with few drops of alcohol for 30 seconds and then washed off immediately. Then the smear was counterstained with safranin for 1 minute and then washed off with water for 1 min. The slide was then air dried and then blotted with cotton wool. A drop of immersion oil was dropped on the smear and was examined under the oil immersion lens of the binocular microscope. Gram positive bacterial cells appear purple under the microscope while Gram negative bacteria appear red.

### 3.4.3 Biochemical Characterization

Biochemical tests were further carried out to further identify the organisms. The tests include: , oxidase test, citrate test, Motility test, Urease test, Catalase test These procedures were meticulously carried out to evaluate specific microbial characteristics and provide insights into the microbial strains present in the garri samples.

1. **Motility Test:** To assess motility, a slant was inoculated by stabbing it with the isolated bacteria. In the absence of motility, growth solely occurred along the stab line, indicating limited movement.
2. **Catalase Test:** For the catalase test, a sterile test tube was utilized to accommodate three milliliters (3ml) of hydrogen peroxide solution. Using a sterile glass rod, a few colonies of the test organism were collected and introduced into the hydrogen peroxide solution. The reaction was observed for immediate and vigorous bubbling, indicating a positive catalase test.
3. **Oxidase test:** This test is used to identify bacteria that produce cytochrome oxidase, an enzyme of the bacteria electron transport chain. It is composed of tetramethyl – p – phenylerediane dihydrochloride. The oxidase reagent was moistened on a filter paper. A colony from the sample was taken and placed on the moistened surface. Presence of the cytochrome oxidase was indicated by the formation of a purple or dark blue coloration while no change or change after 2 minutes indicated a negative result.
4. **Urease Test:** To conduct the urease test, the designated organism was heavily inoculated into Christensen's urea broth contained within a bijou bottle, using a sterilized wire loop.

This mixture was then incubated at a temperature range of 35°C to 37°C for a span of 18 to 24 hours. Following incubation, an examination was carried out, and the development of a pink hue within the medium indicated a positive test result.

5. **Indole Test:** A colony of the test organism was introduced into 2ml of peptone water containing tryptophan, utilizing a sterile wire loop. The inoculated tube was then sealed and placed under incubation at 37°C for 24 hours. Following incubation, Kovac's reagent was incorporated into the medium. A positive result was identified by the appearance of a red hue on the surface layer within a time frame of 10 minutes.
6. **Citrate test:** Citrate testing is used to determine the ability of the bacteria to use sodium citrate as the only source of carbon and inorganic ammonium hydrogen phosphate ( $\text{NH}_4\text{H}_2\text{PO}_4$ ) as a source of nitrogen. Citrate agar was prepared and poured onto sterile petri dishes in a sterile environment. Colonies from the samples were streaked onto the labelled plates and Incubated at 37°C for 24 hours. After 24 hours, the formation of a blue region where the colonies were streaked indicated a positive result for citrate and no color change indicated a negative result for citrate test.
7. **Carbohydrate Fermentation Test:** Preparation of four sugar solutions was executed, each introduced into test tubes and securely stoppered with a Durham tube for gas collection. After sterilization, a small portion of the test organism was added to the sugar solution. An alteration in color from pink to yellow denoted fermentation, while the accumulation of gas bubbles within the Durham tube signified gas production, both indicating a positive test result. A control setup was established, lacking the introduction of the test organism.

## **Microbial Sensitivity Testing**

The susceptibility of the isolated microorganisms to antimicrobial agents was assessed using a modified version of the disc diffusion method based on the Kirby-Bauer technique.

For each of the isolated bacterial strains, a spore suspension was meticulously prepared in nutrient agar (NA) broth. This involved transferring a loopful of the pure culture of each isolate into separate test tubes containing 10ml of sterilized nutrient broth. Following 24 hours of incubation, serial dilutions of the spore suspension were carried out, resulting in a dilution factor of  $10^{-5}$ .

Subsequently, 1ml of each individual suspension was methodically introduced into pre-prepared molten nutrient agar within Petri dishes. The suspension was evenly spread across the medium's surface using an L-shaped rod. Approximately thirty minutes later, antibacterial sensitivity discs were carefully positioned on the surface of the medium in their corresponding, pre-labeled Petri dishes, facilitated by flame forceps.

Following this, the medium was incubated under ambient room temperature conditions. After a growth period of 24 hours, the zones of inhibition surrounding each sensitivity disc were meticulously observed and measured. This information was then recorded, providing valuable insights into the microbial response to different antimicrobial agents. This method was adapted to ensure a comprehensive assessment of microbial susceptibility, contributing to the overall understanding of the microbial dynamics within the garri samples.

## **CHAPTER FOUR**

### **RESULTS**

Table 1: Shows the mean values for the total colony count for bacterial isolates from Garri obtained from Ringroad/Oba market, New benin market, Uselu Market, Olukwu market, Mami market.

**Table 1: Mean heterotrophic bacterial count**

TOTAL HETEROTROPHIC	
SAMPLE CODE	BACTERIAL COUNT (log <sub>10</sub> cfu/g)
A	7.11±2.0
B	7.50±3.5
C	7.74±3.5
D	7.27±2.5
E	7.41±7.0
F	7.39±2.5
G	7.53±1.0
H	7.91±2.0
I	7.00±2.0
J	7.10±2.5

**Table 2: Cultural, morphological and biochemical characteristics of bacterial isolates**

Elevation	Raised	Flat	Raised	Raised	Flat	Raised	Raised
Margin	Undulate	Undulate	Entire	Undulate	Undulate	Undulate	smooth
Color	Cream	Cream	lemon	Yellow	Cream	yellow	Cream
Shape	circular	Irregular	Circular	Irregular	Irregular	Irregular	Irregular
Size	Small	large	Medium	Small	Large	Small	Small
Gr. diff. agar	Blood agar	BCA	PCA	MSA	EMB	MSA	MSA
Colour	Beta hemolytic	Straw	green	Pink	green	Pink	Yellow
Staining							
Gram stain	+	+	-	+	-	+	+
cell type	Cocci	Rod	rod	Cocci	Rod	Cocci	Cocci
Arrangement	Tetrads	disperse	disperse	Tetrads	disperse	tetrads	clusters
Color	Purple	purple	pink	Purple	pink	purple	purple
Spore staining	-	+	-	-	-	-	-
<b>Biochemical</b>							
KOH test	-	-	+	-	+	-	-
Catalase	-	+	+	+	+	+	+
Indole	-	-	-	-	+	-	-
Citrate	-	+	-	+	-	+	+
Oxidase	-	-	+	+	-	+	-
Urease	-	-	+	+	-	+	+
Glucose	+	+	-	-	+	-	+
Sucrose	+	+	-	-	+	-	+
Lactose	+	+	-	-	+	-	+
Mannitol	-	+	-	-	-	-	-
Gas formation	-	-	-	-	+	-	-
H <sub>2</sub> S formation	-	-	-	-	-	-	-
Identity	<i>Streptococcus pyogenes</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Enterococcus sp</i>	<i>E. coli</i>	<i>Micrococcus luteus</i>	<i>Staphylococcus sp</i>

Key

+=positive

-=negative

**Table 3: Antibiotic sensitivity test**

ISOLATES	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
<i>Staphylococcus</i>										
sp	16(S)	0(R)	0(R)	0(R)	10(R)	8(R)	0(R)	0(R)	0(R)	0(R)
<i>Pseudomonas</i>										
sp	18(S)	0(R)	8(R)	8(R)	8(R)	0(R)	0(R)	0(R)	0(R)	0(R)
<i>Streptococcus</i>										
sp	14(S)	0(R)	0(R)	0(R)	8(R)	0(R)	0(R)	0(R)	0(R)	0(R)
<i>Enterococcus</i> sp	15(S)	0(R)	0(R)	0(R)	0(R)	0(R)	16	0(R)	0(R)	0(R)
<i>Micrococcus</i> sp	16(S)	0(R)	0(R)	0(R)	8(R)	8(R)	0(R)	0(R)	0(R)	0(R)
<i>Escherichia coli</i>	17(S)	0(R)	0(R)	0(R)	0(R)	0(R)	14(S)	0(R)	0(R)	0(R)

Key: S = susceptible ( $\geq 17$ mm), I = intermediate (11-15) mm and R = Resistant ( $\leq 10$ mm) CPX – ciprofloxacin, St - streptomycin, SXT - Septrin, E - erythromycin, PEF - perfloxacin, CN - gentamicin, APX - Ampiclox, Z - zinnacef, AM - amoxicillin, R- Rocephin

## CHAPTER FIVE

### DISCUSSION AND CONCLUSION

#### 5.1 Discussion

Garri, one of the most popular foods derived from cassava fermentation, is consumed by more than 200 million people across West Africa. The presence of microorganisms cause deterioration of food and can adversely affect the health of humans. It also influences the biochemical characters and flavours of the product (garri) and their appearance is commercially undesirable and often results in downgrading of the product. Bacteria and fungi can also adhere to particles of grain dust and be transported through air. Grain dust is generated during the process of farming and secondary processing of grains (sacking, milling, handling of powdered grains, sorting, etc.) in markets and can play a role as an effective infectious aerosol because its organic materials provide essential nutrients for airborne microorganisms adhered to their surfaces (Kim *et al.*, 2009). This study was aimed on isolation of bacteria from garri sold in Benin city. From the result obtained the bacterial population ranged from  $7.000000 \pm 2.0 \log_{10} \text{cfu/g}$  to  $7.908485 \pm 2.0 \log_{10} \text{cfu/g}$ . These values was similar and in the same range with Akindele *et al.* (2018) who isolated bacterial isolates from ready to eat garri. The level of bacterial contamination in this study could be attributed to the processing process, handling and storage of garri in stores and in markets (Orji *et al.*, 2016).

Using cultural and morphological characterization of bacteria, the isolates obtained in this study were *Streptococcus spp*, *Bacillus spp*, *Enterococcus spp*, *Escherichia coli*, *Micrococcus spp* and *Staphylococcus spp*. This isolates were similar to what was isolated by Okafor *et al.* (2018) on

his study of bacterial contaminants of garri. *Pseudomonas* species are opportunistic environmental pathogens that can cause serious ailment in individuals with suppressed immunity (Prescott *et al.*, 2002). *Staphylococcus aureus*, a well-known commensal of human microbiota (Prescott *et al.*, 2002) can contaminate food process and handled with bare hands as such their presence in the garri samples suggest possible contamination from direct contact or aerial-droplet mechanisms such as coughing or sneezing by garri processors/retailers or handler (Prescott *et al.*, 2002).

Several of these bacteria bear medical significance, as they have been linked to various human ailments (Prescott *et al* 2002). For instance, *Pseudomonas* species, opportunistic environmental pathogens, can pose significant health risks, especially to individuals with compromised immune systems (Prescott *et al* 2002). *Staphylococcus aureus*, a common member of the human microbiota, can potentially contaminate food during processing and handling, particularly when bare hands are involved. This implies a potential source of contamination, possibly through direct contact or even aerial-droplet transmission mechanisms, such as coughing or sneezing by garri processors, retailers, or handlers (Prescott *et al* 2002). Furthermore, *S. aureus*, and *Bacillus* spp. have been implicated in foodborne infections and intoxications, often leading to various forms of gastrointestinal disorders, particularly affecting young children, the elderly, and individuals with compromised immune systems (Prescott *et al* 2002). It's worth noting that *Enterococcus* sp. has been recognized as a beneficial bacteria with probiotic properties, offering advantages such as improving gut microflora among other health benefits (Prescott *et al.*, 2002). Hence, their presence in garri can be considered favorable for consumers' health.

This study revealed a generally poor sanitary state of the production, handling and retailing of garri which paved way for the contamination and proliferation of the microorganisms. Some of

the bacteria encountered in this study are medically important because they have been implicated in diverse human ailments (Prescott *et al.*, 2002). *Pseudomonas* species are opportunistic environmental pathogens that can cause serious ailment in individuals with suppressed immunity (Prescott *et al.*, 2002). *Staphylococcus aureus*, a well-known commensal of human microbiota (Prescott *et al.*, 2002) can contaminate food process and handled with bare hands as such their presence in the garri samples suggest possible contamination from direct contact or aerial-droplet mechanisms such as coughing or sneezing by garri processors/retailers or handler (Prescott *et al.*, 2002). *Staphylococcus aureus* and *Bacillus* spp. have been implicated in food infections and intoxication leading to different forms of diarrhoeal diseases among other complications especially in young children, the elderly and the immunocompromised (Prescott *et al.*, 2002). The antimicrobial susceptibility of microorganisms present in garri was also investigated. The various isolated bacterial strains were assessed to test their resistance to commonly used antibiotics. The findings revealed a concerning level of antibiotic resistance among the isolated strains, suggesting that maybe the microflora in garri may have been exposed to antibiotics or antimicrobial agents in the production and handling processes.

The bacterial isolates obtained in this study were all multiple antibiotic resistant isolates. The isolates were only susceptible to perfloracin but resistant to many others including erythromycin, ciprofloxacin, augmentin and amoxicillin. Similar case was obtained by (Ogugbue, *et al* 2023) who isolated multiple antibiotic resistant isolates from garri. Isolation of antibiotics resistant bacteria in this study may pose public health challenge. These resistant strains might have been contracted through cross-contamination with antimicrobial resistant strain during the processing of garri. The fact that garri can be consumed raw without cooking poses greater danger with the risk of the resistant strain being transferred from one person to another. The

organisms recovered in the present study are all potential disease-causing agents. For example, *S. aureus* could elaborate toxins in foods, which are dangerous to human and other animal health (Ogbini and Omu, 2006).

There was a poor performance of the antibiotics against the isolates in general which happens to resist the activity of the antibiotics. This result was in a great agreement with (Akinyemi *et al.*, 2007; Tagoe *et al.*, 2011). The roles of these organisms in both nosocomial and community-acquired infections have been well documented (Akinyemi *et al.*, 2007; 2009; Brady *et al.*, 2006; Borer *et al.*, 2005; Glodblatt *et al.*, 2007; Hota, 2004). The frequent handling of both the mobile phones and money while storing garri could also make it easy for cross contamination (Kawo *et al.*, 2009; Yusha'u *et al.*, 2010). On the overall, the resistance of *Salmonella* species against some of the tested antibiotics (gentamycin) and the low susceptibility of the isolates generally to gentamycin could be attributed to common use of these antibiotics as observed in previous studies presenting public health problems (Tagoe *et al.*, 2011). In addition, gross misuse of these antibiotics in chemotherapy especially in this part of the country could explain the reason for this observation as earlier reported by Kumurya *et al* (2010). There are reports on the high load of microorganisms in garri sold in the market (Ogiehor *et al.*, 2007; Ijabadeniyi, 2007; Amadi and Adebola, 2008). The availability of genes encoding resistance to antibiotics and toxic chemicals in the analyzed garri samples may be connected to the presence of certain antibiotics inactivating enzymes (Brandt *et al.*, 2017; Markley and Wencewicz, 2018). This latter structural change has been shown to reduce bacterial susceptibility to cationic antimicrobial peptides and polymyxin, and to contribute to increased pro-inflammatory signaling (Helander *et al.*, 1995; Nummila *et al.*, 1995; Gunn *et al.*, 1998; Markley and Wencewicz, 2018; Ezadi *et al.*, 2019).

## 5.2 Conclusion

In summary, Garri being a staple food item means it isn't going anywhere anytime soon around these parts, be it Benin City, Edo State, the South-south, or Nigeria at large. The reality of this means that more focus should be placed on how its raw products are harvested and sourced and the steps that go into the production, packaging, and delivery of the Garri. The research demonstrated a positive correlation between high microbial loads and increased antimicrobial resistance. This suggests that reducing the overall microbial load through improved hygiene practices during garri production and storage could contribute to lowering the prevalence of antimicrobial-resistant strains. Also, garri stored in high-humidity environments exhibited a higher prevalence of antimicrobial-resistant strains compared to garri stored in dry conditions. This implies that proper storage practices are crucial in preventing the development and spread of resistant microorganisms in garri. And, factors such as climate, also influence the susceptibility of these bacteria strains to antibiotics, this suggests that interventions to enhance food safety should be tailored to specific regions. For the safety of citizens living within Benin City and its environs more careful methods should be applied to the safety of this food item. I suggest that producers and retailers in Benin adopt clean and sanitary packaging methods to guarantee food safety and safeguard consumers.

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**APPENDIX**

**MORPHOLOGICAL CHARACTERISTICS OF ISOLATES**

**USELU MARKET**

<b>Sample</b>	<b>Colour</b>	<b>Shape</b>	<b>Margin</b>	<b>Elevation</b>
<b>A</b>	Colourless	Circular	Entire	Raised
<b>F<sup>1</sup></b>	Colourless	Irregular	Entire	Flat
<b>F<sup>2</sup></b>	Colourless	Circular	Entire	Raised

**OLUKWU MARKET**

<b>Sample</b>	<b>Colour</b>	<b>Shape</b>	<b>Margin</b>	<b>Elevation</b>
<b>B</b>	Colourless	Circular	Entire	Raised
<b>G<sup>1</sup></b>	White	Irregular	Entire	Flat
<b>G<sup>2</sup></b>	White	Circular	Entire	Flat

### NEW BENIN MARKET

<b>Sample</b>	<b>Colour</b>	<b>Shape</b>	<b>Margin</b>	<b>Elevation</b>
<b>C<sup>1</sup></b>	Pink	Irregular	Undulated	Flat
<b>C<sup>2</sup></b>	Colourless	Circular	Undulated	Convex
<b>H<sup>1</sup></b>	Colourless	Circular	Entire	Flat
<b>H<sup>2</sup></b>	White	Irregular	Entire	Raised
<b>H<sup>3</sup></b>	Pink	Circular	Entire	Convex

### RINGROAD MARKET

<b>Sample</b>	<b>Colour</b>	<b>Shape</b>	<b>Margin</b>	<b>Elevation</b>
<b>D</b>	Colourless	Circular	Entire	Convex
<b>I<sup>1</sup></b>	Pink	Circular	Entire	Convex
<b>I<sup>2</sup></b>	Colourless	Circular	Entire	Flat

## MAMI MARKET

<b>Sample</b>	<b>Colour</b>	<b>Shape</b>	<b>Margin</b>	<b>Elevation</b>
<b>E<sup>1</sup></b>	Pink	Circular	Entire	Flat
<b>E<sup>2</sup></b>	Colourless	Irregular	Entire	Raised
<b>J<sup>1</sup></b>	Pink	Irregular	Undulated	Convex
<b>J<sup>2</sup></b>	Colourless	Circular	Undulated	Flat