Characterization of Multidrug Resistant Bacterial Flora of Selected Locally Fermented Foods in Ibadan, Oyo State, Nigeria

Kehinde Mary, AKINDE
LCU/PG/000405

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Certification

This is to certify that **Kehinde Mary, AKINDE** with matriculation number LCU/PG/000405 carried out this research work titled "Characterization of Multidrug Resistant Bacterial Flora of Selected Locally Fermented Foods in Ibadan, Oyo State, Nigeria" in the Department of Biological Science, Faculty of Basic Medical and Applied Sciences, Lead City University, Ibadan, Oyo state, for the award of Master of Science (M.Sc) Degree in Medical Microbiology and that this has not been previously submitted.

_____ _____

Dr. Tajudeen Bamidele Date

(Supervisor)

_____ _____

Dr. Felicia Adesina Date

(Head of the Department)

Dedication

This research work is dedicated to God Almighty the Alpha and Omega.

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Abstract

Globally, locally fermented foods have been an important part of the human diet in every culture. In Nigeria, the fermentation process is spontaneous, driven by communities of uncharacterized microflora indigenous to the food substrate. Given the rise in antibiotics resistance, this research was aimed at assessing the antibiotic susceptibility of microroganisms in fermented gruels and condiments to make inferences as to the safety of our locally fermented foods. Samples of locally fermented locust beans, maize and sorghum gruels were collected from different markets in Ibadan, South West Nigeria. Microorganisms present in samples were isolated using different agars (Nutrient Agar, MacConkey Agar, Eosin Methylene Blue (EMB) agar, Mueller-Hinton agar and Salmonella Shigella agar (SSA). Incubation at all times was for 24hrs at 36^0C. The microbial isolates obtained were subjected to various biochemical tests for characterization. Isolates were also screened for their antibiotic sensitivity, and ten that were resistant to at least three classes of antibiotics used were screened for the following antiobiotics resistance genes; β-lactams (*CTX-M, OXA, TEM*) and quinolones (*qnrA, qnrB, qnrC, qnrSM*) resistance gene. The identities of these ten isolates were confirmed using 16S rRNA gene analysis. Out of the ten isolates, half were identified as *Proteus mirabilis*, two were identified as *Providencia vermicola*, while the last two were *Alcaligenes feacalis*. 10% of the isolates showed resistance to the *CTX-M, OXA and* qnrB genes respectively, 60% to qnrA, 80% to qnrSM and 50% to TEM. Isolates one that showed resistance to more than three classes of antibiotics resistance gene used are those that are not normally found in foods but have somehow gotten into the food chain and would seem to be thriving there. Public awareness and sensitization of stake holders should be done on the need to improve hygiene in local fermented foods, especially those sold commercially.

Keywords: Microflora, Antibiotic susceptibility, Fermented foods, Microorganism, Resistance gene.

Word Count: 299

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List of Acronyms

Abbreviation	Meaning
LAB	Lactic Acid Bacteria
cMGEs	Conjugative Mobile Genetic Elements
DNA	Deoxyribonucleic Acid
AR	Antibiotics Resistance
MDR	Multidrug Resistance
IR	Intrinsic Resistance
ESBL	Extended-Spectrum B-Lactamase
NDM-1	New Delhi Metallo-β-lactamase-1
LPS	Lipopolysaccharide
MRSA	Methicillin-Resistant *Staphylococcus aureus*
GIT	Gastrointestinal Tract
PBPs	Penicillin-binding proteins
CPS	Capsular Polysaccharides
VRE	Vancomycin Resistant Enterococci
DAP-R	Daptomycin-Resistant
TVC	Total Viable Count
TVAC	Total Viable Aerobic Count
CFU	Colony Forming Unit
HAIs	Hospital-Acquired Infections
UTIs	Urinary Tract Infections
GMPs	Good Manufacturing Practices
PBPs	Penicillin-Binding Proteins
MLS	Macrolide Lincosamide Streptogramin
rRNA	Ribosoma Ribonucleic Acid
RNA	Ribonucleic Acid
CTX-M	Cefotaxime-Munich
AMR	Antimicrobial Resistant
RBC	Red Blood Cell
NA	Nutrient Agar
EMB	Eosin Methyline
SSA	*Salmonella Shigella* Agar

MR	Methyl Red
VP	Vogues Proskauer
NCCLS	National Committee for Clinical Laboratory Standards
NIMR	Nigeria Institute of Medical Research
PCR	Polimerase Chain Reactioon
TAE	Tris-Acetate
EDTA	Ethylene Diaamine Tetraacetic Acid
NCBI	National Centre for Biotechnology Information
RPM	Rotation Per Minute

Chapter One

Introduction

1.1 Background to the study

Fermentation has been a source of food preservation for thousands of years in human history, long before the science, safety, and nutritional attributes of fermented foods and drinks were fully understood. Fermented foods' health, economic, safety, and alcoholic properties ensure that they will remain a part of the global diet1. Fermented foods are important because they provide and preserve large amounts of nutritious foods in a variety of flavors, aromas, and textures that enrich the human diet [1].

Fermented foods are foods that have been exposed to the metabolic activities of microorganisms or enzymes, resulting in desired biochemical changes and significant food modification. Fermented foods make up about one-third of the global diet[2]. Fermented foods are made in households, villages, and small-scale food businesses, and they have distinct flavors, textures, appearances, and functions that are widely consumed[2].

Fermentation was the most cost-effective method of food preservation in the past[3]. Fermentation not only helps preserve foods by preventing them from spoiling due to microorganisms, but it also improves the nutritional value of the food by synthesizing essential amino acids and vitamins during the fermentation process. Fermentation is also known to improve the nutritional digestibility of foods that are often difficult to assimilate, as well as reducing the volume of material that must be transported and the energy required for the subsequent cooking of preserved products[4]. In some regions, mainly in African countries, fermentation plays important role in the nutrition of infants and young children as it is used for the preparation of complementary foods[5].

Many indigenous fermented foods in Nigeria are fermented before consumption and can be divided into groups based on the substrates or raw materials used. Cassava products (garri, fufu, elubo, abacha, akara akpu), maize products (ogi, agidi, soy-ogi), and millet products are all common fermented foods in Nigeria (ogi-baba, kwunu, tuwo, fura). Dawadawa (African locust bean), ogiri-ugu (fluted pumpkin), ogiri-isi (castor seeds), ogiri-egusi (melon seeds), ugba (African oil bean), ukwa (African breadfruit), daddawa (soybean), eketeke (oil palm nut), and tendered meats and fish are some of the food condiments.

Palmwine, ogogoro, pito, burukutu, maize beer, sorghum beer, and other fermented milk products are available, as are nono, maishanu, and wara. These dishes are made on a modest scale in the home. The majority of these fermentations are natural, involve contact with suitable inocula, and are carried out at tropical temperatures. The advantages of fermentation include enhanced shelf life, toxin elimination, improved texture, taste, and flavor, and higher nutritional content [6].

Another major cereal grain is millet, which is a staple diet all over the world[7]. Sorghum-based fermented dishes, such as Injera, Kisra, and Ogi dominated with *P. acidilactici* and *Lactobacillus paraplantarum*, are consumed in a variety of African countries [8,9,10].

Fermentation is a chemical change in foods brought about by enzymes from living microorganisms. It is a food processing technique practised by man for centuries in various parts of the world, especially Africa and the Orient. The Nigerian indigenous fermented foods constitute a group of foods that are produced in homes, villages and small scale cottage industries at prices within the means of a majority of consumers. Because they began hundreds of years before written records, they are often referred to as "traditional" fermented foods. Fermented foods play an essential role in the

African cuisine, where they can serve as mainstays, supplements, beverages, and condiments. Most fermented African and tropical foods can be served as a main course, a side dish, or a snack, or employed as a flavoring ingredient to enhance the flavor of food, add color and perfume, or modify the physical condition of the substrates[11]. Temperatures favorable for such processes are found in the tropical climate. People in Nigeria who are exposed to such dishes grow accustomed to their pungent odors and flavors.

During harvesting, processing, storage, shipping, and preparation, several distinct bacterial species are responsible for food contamination[12]. Fermented foods, on the other hand, are one of the principal habitats of several indigenous bacteria that have a significant impact on food by changing various dietary substrates[5].

Fermentative microorganisms in foods can help fermented foods last longer[13]. The operations of bacteria during the fermentation process convert the chemical contents of food, resulting in an augmentation of nutritional value in some fermented foods and the transmission of health advantages to consumers[13]. Other bacteria such as *Staphylococcus aureus*, *Klebsiella sp.*, *Escherichia coli*, *Salmonella sp.*, and *Bacillus subtilis* have been isolated from regionally fermented foods in Nigeria, including wara, nono, and ogi[14].

In a different light, the problem of antibiotic resistance has reached a huge global scale, posing a serious threat to public health around the world[15]. Food is a key carrier for successfully transferring antibiotic resistant components straight into the intestinal tract of consumers, particularly humans, beyond the transmission of resistant bacteria between humans[16].

Previously, a review of the microbiology, biochemistry, and nutritional status of fermented foods, as well as the antibiotic characteristics of pathogens discovered in

various food products, including fermented foods, was conducted[5]. As a result of the development of drug-resistant bacteria through contaminated food and water, several countries are now dealing with food-borne infections[13]. There are currently various resources available that demonstrate the probability of antibiotic resistance elements being transmitted to humans via the food chain and cattle. Antibiotic resistance of *Enterococcus*, *Lactobacillus*, and *Lactococcus* species from fermented foods has been evaluated in studies[17].

Enterococci are lactic acid bacteria that are found in many foods, particularly those of animal origin, such as fresh meat, fermented sausages, and cheeses. Enterococci having the ability to thrive at temperatures ranging from 10°C to 45°C, in the presence of 6.5 percent sodium chloride, at a pH range of 4.6–9.6, and to endure heating at 60°C for 30 minutes due to their environment[18]. Antibiotic resistance genetic determinants in enterococci are usually found on conjugative plasmids on transposons. Previous research has found that enterococci's genetic flexibility and promiscuity play a key role in the emergence of problematic lineages[19].

There have been various reports of the succession of different bacteria flora within food matrix and most of the studies have demonstrated LAB as the main surviving group. This is due to the abilities of these bacteria to outcompete others within the fermentation microenvironment. The antibiotics resistant bacteria introduced into fermented foods could be as a result of various factors, ranging from poor harvesting practices to post harvest handling, poor processing/market handling and so on. These bacteria can pose a problem to the gastrointestinal tract of the consumer by transferring antibiotic resistance genes horizontally to the commensals in the gut if the resistance determinants are borne on conjugative mobile genetic elements (cMGEs).

Conjugative mobile genetic elements (cMGEs), Strains of bacteria resistant to antibiotics, particularly those that are multidrug resistant, are an increasing major health care problem around the world. It is now abundantly clear that both Gram-negative and Gram-positive bacteria are able to meet the evolutionary challenge of combating antimicrobial chemotherapy, often by acquiring preexisting resistance determinants from the bacterial gene pool. This is achieved through the concerted activities of mobile genetic elements able to move within or between Deoxyribonucleic acid (DNA) molecules, which include insertion sequences, transposons, and gene cassettes/integrons, and those that are able to transfer between bacterial cells, such as plasmids and integrative conjugative elements. Together these elements play a central role in facilitating horizontal genetic exchange and therefore promote the acquisition and spread of resistance genes[20].

In Nigeria, food surveillance to track the presence of these determinants is almost non-existent. Most of the locally fermented foods are consumed raw and have their plant raw materials grown from the dungs of food animals on which antibiotics have been administered in sub therapeutic doses.

Most previous studies have focused on presenting comparisons of antibiotic resistance profiles of these group of bacteria, depending mainly on the sources of strains. Some studies on enterococci have been carried out using optimal environmental conditions, facilitating the growth of the cells normally. At present, whether these bacteria carry antibiotic resistance genes has become an important indicator for evaluating the safety of a strain and for determining whether it can be used as a safe strain for food. However, there is still vastly a lack of information on the diffusion of antibiotic-resistant, break point and more importantly multidrug resistant bacteria in fermented

foods. Moreover, few data are available on the dynamics of antibiotic resistance diffusion during the fermentation process.

The most serious challenge to antibiotic use is the emergence and spread of resistance in pathogenic bacteria, which can no longer be treated with previously effective regimens. Antibiotic resistance in bacteria is primarily caused by two factors: the presence of resistance genes and antibiotic-induced selective pressure[21]. In this context, the goal of this research was to find out how common multidrug-resistant bacteria are in locally fermented foods and to describe them.

1.2 Statement of the Problem

Fermented foods and condiments are still traditionally produced by spontaneous fermentation which relies on microflora derived from the unprocessed food and the environment. However, changes in the genetic make-up of environmental microflora due to various anthropogenic activities have led to rise of antibiotic resistant organisms. There is still vastly lack of information regarding how organisms involved in spontaneous fermentation of food have been affected by the spread of antibiotic resistance gene in the environment. This has the potential to undermine the safety of foods that are considered safe for consumption.

Due to the derivation of some of the fermenting microorganisms from the environment, there is the need to know whether these organisms have picked up antibiotic resistant genes. if this is the case, fermented foods generally regarded as safe functional foods may pose a problem to the gastro intestinal tract (GIT) of the consumer via horizontal transfer of antibiotic resistant genes to gut commensals[20].

The antibiotics resistant bacteria introduced into fermented foods could be as a result of various factors, ranging from poor harvesting practices to post harvest handling,

poor processing/market handling and so on. These bacteria can pose a problem to the gastrointestinal tract of the consumer by transferring antibiotic resistance (AR) genes horizontally to the commensals in the gut if the resistance determinants are borne on conjugative mobile genetic elements (cMGEs). In Nigeria, food surveillance to track the presence of these determinants is almost non existent. Most of the locally fermented foods are consumed raw and have their plant raw materials grown from the dungs of food animals on which antibiotics have been administered in sub therapeutic doses[22].

1.3 Justification of the study

Investigation into the type and spread of antibiotic resistance gene among the microflora of fermented foods and condiments will help to evaluate the safety of these foods which has before been taken granted. It will also help to see whether there is the need to employ more controlled fermentation methods in preparation of traditional fermented foods by developing strains free of antibiotic resistance genes. Determination of the type and occurrence of antibiotic resistance among the microflora of fermented foods and condiments will help to evaluate the safety of these foods .

1.4 Aim and Objectives of the Study

The study aims at identifying and characterizing multidrug resistant bacterial flora of selected fermented local foods and condiments such as: Locust beans (Woro and Pete), ogiri and fermented gruels (maize and sorghum) consumed in Ibadan, Oyo State.

The specific objectives are as follows:

i. isolation and identification of bacterial morphotypes that are present in selected fermented foods from study sites in Ibadan, Oyo state.
ii. determination of antibiotic susceptibility profiles of isolated microorganism.
iii. molecular identification and phylogenetic analysis of microorganisms
iv. detection of antibiotic-resistant genes.

1.5 Significance of the Study

Resistance bacteria in fermented foods and condiments can be selected simultaneously in fermented foods. The organisms with antibiotic resistance hoped to be isolated will provide adequate data and information on the resistance phenotypes of bacteria that are associated with the selected fermented foods and condiments which are particularly the commonly consumed ones in Ibadan, Nigeria.

Data provided can provide all stakeholders information and data on the possible occurrence of multidrug resistant strains of these bacteria in these foods and condiments.

1.6 Scope of the Study

The scope of this study covers indigenous fermented foods and condiments collected from markets within three local governments namely; Oluyole, Ibadan South West and Ibadan North in Ibadan, Oyo State, Southwestern part of Nigeria. Molete market, Oje market and Orita market precisely.

1.7 Limitation of the Study

This study is limited to investigate the plasmid profiling of the resistant bacteria, genetic locations and transferability of the multidrug resistance. It is also limited to

the determination of plasmid profiling of the resistant bacteria and also investigation of pathogenic traits of the resistance genes.

1.8 Expected Outcomes

The resident bacterial morphotypes in the study fermented food products within the study areas will be identified to species level using molecular methods.

The circulating antibiotics resistance genetic determinants in the fermented food matrix in study locations will be detected

This study will provide all stakeholders including healthcare practitioners, consumers, the general public and relevant agencies with information and data on the possible occurrence of multidrug resistant strains of these bacteria in fermented foods that are vended in selected locations in Ibadan

It is expected the study outcomes will prompt policy shift on antibiotics usage in food animals, stewardship and surveillance

1.9 Operational Definition of Terms

Antibiotics Resistance – This is a phenomenon in which microorganisms proffer opposition to antibiotics which were hitherto effective against them. When this occurs, it brings about a situation where a person continues to take an antibiotic and it proves ineffective and ineffectual.

Multidrug Resistance– A process where a single bacterium is resistant to two or more antibiotics of different pharmacological classes.

Conjugative Mobile Genetic Elements (cMGES) – These are genes that produce a channel (the conjugative pilus) through which the mobile genetic element can be copied between neighbouring bacteria, they do spread traits in bacteria communities,

they have efficiency to transmit large accessory gene cargos between individuals, including those of different species. They also drive rapid evolution and adaptation to environmental change.

Anthropogenic Activities – This is defined as economic and social human activities which can be either agricultural or industrial. This term is also used in the context of pollution from human activities in many ways, pollution,deforestation, and overpopulation.

Lactic Acid Bacteria – They are Gram positive catalase, oxidase negative, heterogenous bacteria which play significant role in a variety of fermentation processes. They have the historic abilities to produce organic acids from sugar fermentation and used as food adjuncts.

Spontaneous Fermentation – This is a process when brewer or distiller leaves the inoculation up to whatever organisms happen to be in the air or on the fruit that they are fermenting.

Natural Fermentation– an uncontrolled fermentation process, occurring without deliberate introduction of known microbial cultures

Gastro Intestinal Tract – The tract or passageway of the digestive system that leads from mouth to the anus.

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Chapter Two

Literature Review

2.1 Human History, Culture and Fermented Foods

Every community and every region on every continent have unique and distinct food and dietary habits based on their own specific cultures and accessibility to edible raw resources of plant/animal origins. Throughout the world, nearly every community has a unique food culture that represents their ethnic, social, and cultural history. In particular, humans on every continent and for thousands of years have included fermented foods and beverages as a major part of their diets. The nutritional and cultural importance of these ancient foods continues in the present era[1].

For fermented foods, in particular, how fermentation is initiated provides yet another way to distinguish between different cultures. For most of human history, fermented foods and alcoholic beverages were produced from plant or animal sources by traditional fermentation (i.e., in the absence of a starter culture). Fermentation relied on either natural or spontaneous fermentation or the back-slopping method where portions of a previously fermented product were added to a fresh substrate[2,3,4]. It has been estimated that majority of traditional fermented foods and alcoholic beverages are still produced at home and rely on these traditional fermentation methods[5].

In Africa, cereal grains endemic to specific regions, including millet, sorghum, maize, and wheat, remain common food fermentation substrates. Cassava and other root crops have long been consumed as staple fermented foods in the dietary cultures of Africa.

In Nigeria more than 80% of the inhabitants consume various fermented foods, beverages depending on their ethnic group or local preferences. The techniques for fermentation of these staple foods are well known by particular ethnic groups are

passed on from generation to generation in certain communities. However they are not properly documented in literature and are probably known only to those Nigerians in the locality where such foods are produced and consumed. Some ethnic groups in Nigeria depend economically on these fermented local foods[6,7,8].

Common fermented foods, food condiments and beverages in Nigeria are derived from the local staples or other foods prevalent in various Nigerian localities. Such staples include cassava *(Mannihot* spp), yams *(Dioscorea* spp), maize *(Zea mays),* sorghum *(Sorghum* spp), millet *(Pennisetum* spp), African bread fruit *(Treculia africana),* etc. Common fermented foods from the staples are garri, fufu (akpu), elubo, abacha, akara-akpu, amala, ogi, agidi, soy-ogi, ogi-baba, kwunu, tuwo, fura, ukwa etc. Fermented beverages include mmanya-ngwo (raffia palm wine), mmanya-nkwu (oil palm wine), ogogoro (distilled palm wine), pito, burukutu, maize beer, sorghum beer, etc. Fermented milk products include nono, maishanu and wara. Most of the food condiments are derived from oil seeds. Such condiments include dawadawa(*Parkia spp*), Ogiri-ugu (*Telfairea occidentalis*), Ogiri-isi (*Ricinus communis*), Ogiri-egusi (*Citrullus vulgaris*), daddawa (*glycine spp*), eketeke (*Elaeis guineensis*), etc. Other condiments are derived from fermented meat or fish products and these include "nsiko" (crab), "afonnama" (beef tripe),"oporo" (shrimp or crayfish) and "azu-okpo" (smoked fish)[9,10].

The wide variety fermented foods is a result of differences in the most represented bacterial species naturally found in raw ingredients of each geographical region, local environmental conditions, and traditional processing procedures. Thus, the wide range of fermented end products is characterized by a diverse microbiota and a variety of organoleptic properties as reported by several authors[11.12,13]. Traditional fermented foods are not only attractive by their nutritional value (proteins, minerals, fats, and

vitamins), distinct flavors, and consistencies, but they also present livelihood opportunities for farmers, processors, and sellers. Microorganisms present in each traditional fermented food could act as resistance superbugs since several reports indicated that fermented foods could be considered as vehicles of antibiotic-resistant bacteria and, thus, antibiotic resistance genes may be transferred to other bacteria including pathogens and commensals through the food chain and into the gastrointestinal tract[14,15].

In general, characterization of fermentative bacteria in traditional foods of their AR profile and also their virulence properties has not been routinely accomplished, and in many cases, culture-dependent methods leave 99% of uncultured bacteria unexplored. In fact, the uncharacterized indigenous microbiota present in traditional fermented foods may represent a major risk since many AR genes may be present, but not detectable, in cultivable bacteria; furthermore, their sequences may have been modified by mutation, or even new resistance genes may be present, thus making detection by conventional PCR methods a difficult task. Recently, genome sequencing has provided new information about AR genes in cultivable bacteria, and metagenomic analysis of complex bacterial communities remains a strategy for analyzing the global resistance of each fermented product to inform about its safety[16]

2.2 Occurrence of Multidrug Resistance in Bacteria

Multi drug-resistant (MDR) bacteria are described as bacteria strains that are resistant to at least two antimicrobial agent from three or more classes of antimicrobials when tested using invitro antibiotic susceptibility testing[17]. Micro-organisms, however, already developed different mechanisms that they employ for survival in the presence of naturally occurring antibiotics produced by bacteria and fungi in the environment

many years before the advent of the era of modern antimicrobials. In recent years, there has been a serious increase, globally, in the occurrence of strains of multidrug resistant organisms, leading to more frequent isolation of MDR pathogens both from hospital- and community-acquired infections, ultimately intensifying the problem of antimicrobial resistance[18,19]. These MDR bacteria can infect individuals at any stage of life, they can also affect healthcare, veterinary and agriculture industries, hence, making it one of the world's most urgent global problems. To effectively resist the effect of antibiotics, bacteria have, over many decades, developed various resistance mechanisms among which are: limiting or changing the cell wall structure; using molecular efflux pumps to eliminate drugs; production of enzymes that change the chemical structure of the drug; and altering the target sites for antimicrobial action[20].

2.2.1 Multidrug Resistance: Definition of Terms

Different bacteria species are made up of different genomes that carry genes which code for resistance. Some of these genes are silent, that is, they are not expressed in the absence of antibiotics[21]. This attribute causes a large pool of potential of antibiotic resistance genes to be 'hidden' in various positions in the whole bacteria genome. In defining terms, there is the antibiotic resistome which is a portion of the whole genome that covers the assortment of all antibiotic resistance genes, including those associated with both pathogenic and non-pathogenic bacteria, however, not ones which produce antibiotics[22]. Pál and his colleagues in their study made available a broad characterization of resistance genes, mobile genetic elements (MGEs), and bacterial taxonomic compositions for 864 metagenomes from both humans, animals, and the environment. In the study, it was established that antibiotic-polluted

environments are strong reservoirs and as such significant routes for the transmission of antibiotic resistance[23].

2.2.2 Intrinsic Resistance

Intrinsic Resistance (IR) is defined as the phenotypic expression of a resistance gene that is originally present in the strain, and thus, causing changes in the structure and functions of the gene product[24]. Of all the resistance mechanisms developed by bacteria, the most efficient are the multidrug efflux pumps[25]. There also exists 'acquired resistance' which occurs when phenotypic expression of a resistance-encoding gene comes from outside the bacteria via what is called horizontal gene transfer HGT[24,26]. In such process, the external gene coming from outside the bacterium had been harbored by a plasmid and was taken up by the bacterium from the environment including soil or from the gastrointestinal community[27,28].

2.3 Resistance Problems Related to Significant Bacteria Associated with Locally Fermented Foods

2.3.1 Gram-Negative Bacteria

a) Beta lactams vs Extended-Spectrum B-Lactamase (ESBL) Resistance in *Enterobacteriaceae* and *Klebsiella pneumoniae*

Beta lactams (β-lactams) are a group of antibiotic drugs that were developed to tackle resistance to penicillin by preventing reassembly of peptidoglycan bonds, which ultimately causes the cells to lyse[21]. However, there exist a class of pathogens that are resistant to these β-lactams antibiotics, these pathogens are referred to as Extended-Spectrum β-Lactamase (ESBL) producing Enterobacteriaceae, of which *K. pneumoniae* is a strong member while the extended spectrum β lactamase (ESBL)

producing bacteria are resistant to the cephalosporins. The emergence and occurrence of this group of pathogens has brought serious challenges to treating infections, and the challenges posed by them have been reviewed over the last three decades[29]. Production of β-lactamase by Gram-negative bacteria is usually associated with decrease in the permeability of the outer membrane efflux[30]. However, through biochemistry, the number of β-lactamase enzymes have been increased, with enlarged substrate specificities such as cephalosporins, lactamase inhibitor ampicillin, sulbactam, clavulanic acid, and ureido-penicillins[31,32,33,34]. Furthermore, the prevalence of ESBL production among *E. coli* and *Klebsiella* species is variable[21].

b) New Delhi metallo-β-lactamase-1(NDM-1)

New Delhi metallo-β-lactamase-1 (NDM-1)is a different type of plasmid-encoding carbapenem-resistant metallo-β-lactamase carbapenemase NDM-1 which was identified in 2008 in two Enterobacteriaceae isolates[21]. There emerged a NDM-expressing Klebsiella[38]. Additionally, there are publications that have reported the expression of NDM-1 by *Pseudomonas*[39,40]. It has never been found in Gram-positive bacteria. *Klebsiella pneumoniae,Acinetobacter*, and *Pseudomonas* are spread by clonal dissemination, while *E.coli* and other Enterobacteriaceae spread through polyclonal dissemination[41].

c) Colistin Resistance

The first colistin-resistant mutant was reported in 1981[42]. The advent of colistin resistance, casted a doubt on whether the polymyxins should be considered as the last "life-savers"[43]. Colistin resistance occurs as a result of post-translational modification, or loss, of the lipopolysaccharide (LPS) molecules. The genetic factors behind colistin resistance are the *mcr* gene[44]. Colistin resistance has been evolving under clinical

conditions in *A. baumannii*, *P. aeruginosa*, *K. pneumoniae*, and *Enterobacter cloacae*[45,46,47,48]. The evolution of colistin resistance was more than a one step process, requiring mutation in at least five independent loci synergistically, creating the resistant phenotype.

2.3.2 Gram-Positive Pathogens

Species belonging to *Streptococcus* and *Enterococcus* species are inherently resistant to β-lactams and they typically make use of β-lactamases and/or penicillin-binding polypeptides[21].

a) Methicillin-Resistant *Staphylococcus aureus* (MRSA)

MRSA has been implicated in many diseases worldwide such as nosocomial infections including pneumonia, and endocarditis[49,50]. All these diseases have greatly raised the number of deaths globally[51]. The resistance characteristics of isolates including Methicillin-resistant *S. aureus* (MRSA), and *S. epidermidis* have been a subject of review studies in recent years[52]. The use of vancomycin in clinical settings was previously combined with other antibiotics to tackle poly-resistant clinical isolate[53]. However, these strains evolved resistance to both vancomycin and daptomycin within hours of administration to patients[21].

b) Enterococci

Enterococcus species, particularly *E. faecium, E. faecalis, E. gallinarum,* and *E. cecorum,* are regarded as the Gram-Positive "Vanguards" of the "MDR Movement. They typically inhabit the gastrointestinal tract (GIT) of several of organisms, including humans as commensals[21]. Different penicillin-binding proteins (PBPs) have been identified in clinical isolates of these species, with *E. faecium* identified as the

most resistant to penicillin while *E. bovis* appears as the most sensitive to penicillin, showing the highest affinity for the antibiotic [54]. Initially, streptomycin and penicillin were administered synergistically, showing good activities. Although it worked, a selective condition for MDR enterococci was ensued by that combination[54]. These MDR enterococci strains are adapted to the GIT and as a result can develop to be the dominant flora there[55]. The first epidemic MDR *E. faecium* strain emerged from animal and commensal strains[56]. *E. faecalis* and *E. faecium* remain the most common Enterococcus pathogen species[57]. Because of the rapidly evolving antibiotic resistance characteristics of *Enterococcus faecum*, it has become the most important nosocomial pathogen posing serious problems to clinical practices. The pathogen is resistant to nearly all clinically tested antibiotics, and this could be a result of the many genetic strategies employed by the species[58]. MDR enterococci are known to have combinations of genuine antibiotic resistance mechanisms which include modification of drug targets, inactivation of therapeutic agents, and over expression of efflux pumps[58,59,60]. They use their cell envelopes which contain peptidoglycan, teichoic acids, capsular polysaccharides (CPS), surface proteins, and phospholipids as their first line of defense against antibiotics[61]. Some of these components are well-established target for antibiotics[62]. However, they can undergo different modifications and by that they reduce the susceptibility to antibiotics[63].

c) Vancomycin resistance

Vancomycin was considered the last resort for Gram-positive pathogens when it was first discovered[59]. In the 1980s, there was an emergence of vancomycin-resistant enterococci (VRE) strains, and since then the number of resistances has been increasing steadily, both in Enterococciand Staphylococci[64,65]. As an alternative to the generation of completely new substances, new approaches have focused on structural

modifications of vancomycin to overcome these resistances and to restore its efficacy against vancomycin-resistant enterococci[66].

d) Daptomycin Resistance

Discovering the mechanism of action of daptomycin helps to tackle the problem of daptomycin resistance. Daptomycin-resistant (DAP-R) mutants have also been identified in enterococci[67]. This discovery has resulted in uncovering the mechanisms of DAP-R in different Gram-positive bacteria, including *B. subtilis*, Enterococci, *E. faecalis*, *E. Faecium*, and *S. aureus*[68,69,70]. Additionaly, there were mutations of DNA "mismatch repair" genes in a DAP-R pleiotropic phenotype identified in a clinical isolate of *E. faecium.*

e) *Acinetobacter baumannii* Multidrug resistance

As for the resistance mechanisms of *Acinetobacter baumannii*, tigecycline efflux was described as a mechanism for nonsusceptibility in the organism. Furthermore, a deletion of TnAbaR23 was observed to lead to significant antibiogram changes in a MDR *A. baumannii* strain[71]. Just like other non-glucose-fermenting Gram-negative species like *P. aeruginosa*, *Acinetobacter baumannii* has acquired carbapenem resistance on a greater level[72]. There is an argument that experimentally transferred resistance mutations from other species to *E. coli* have been mostly silent, and frequently yield drug hypersensitivity[73]. This comes against the perceived concerns that *A. baumannii* increases the risk of carbapenemase spread in general, since horizontal resistance gene transfer can occur between Gram-negative species, regardless of their ability to ferment glucose[72].

2.4 Occurrence of Bacteria in Locally Fermented Foods

There has been a great increase in the incidence of food-borne infections globally in the past few years, putting a significant proportion of human population at risk[74]. On a global scale, fermented foods have shown to be very essential for preserving the nutritional status of people, and this is attributed to their availability, richness of nutrients, affordability, and ease of processing. In Africa, fermented foods are almost a daily food for both infants and adults. Substrates such as maize, sorghum, melon, African oil bean, and locust beans are some of the common ones used in the production of these fermented foods. Substrates such as maize, sorghum are consumed as breakfasts and weaning foods while in Nigeria, mahewu and umqombothi are both popularly consumed by black South Africans.

The bacteria involved in the fermentation of these products (Ogi, ogi baba, mahewu, and umqombothi) are mainly LAB[76]. Ogiri, ugba, and iru are condiments that are obtained from the alkaline fermentation of proteinaceous oily seeds commonly found and consumed in Nigeria. The seeds are also exported to other countries. A study showed that 66% of iru, 82.4% of ogiri, and 93.9% of ugba samples gotten from the Middle Belt region of Nigeria had coliforms contamination[77]. In the same light, isolated *Klebsiella*, and *Pseudomonasspp*. from ogiri were discovered. These findings thus make the consumption of these fermented foods dangerous as they may pose a health risk to consumers.

While it is established that Gram-negative bacteria found in fermented foods may cause infections, they can also be toxigenic in nature, producing endotoxins in foods. Endotoxins are pervasive lipopolysaccharide (LPS) complexes which are stable under heat. Adams and colleagues noted that they are seen at the outer cell membranes of Gram-negative bacteria[78]. Different bacterial species have varying potency of

endotoxins, however, *E. coli* produces LPS with extremely high endotoxin activity, making it a model organism. Exposing humans to endotoxins may cause many health problems including septic shock, multiple organ failure, disseminated intravascular coagulation and necropsy[79]. There is generally a poor investigation into the incidences of endotoxin in foods. Majority of the studies carried out on endotoxins cover food categories such as milk. A study by Townsend and colleagues reported endotoxin levels from 40 to 5.5×10^4 endotoxin unit (EU)/g in infant milk manufactured in countries including South Africa, Holland, Spain, Switzerland, USA, Belgium, Ireland, Slovenia, and United Kingdom[80].

There is an urgent need for surveillance of fermented foods in order to ensure the safety of individuals that consume these foods. It is also important to ensure proper interaction of severe factors such as poor handling practices, unstandardized processing methods, the participation of various microbiota and unhygienic display practices that are associated with the production of fermented foods particularly in Africa. These factors may enhance the contamination of these fermented foods with pathogenic microorganisms and their toxins. The fact that endotoxins pose serious threat to human health, it becomes imperative to deeply investigate their presence, as well as that of their causative agents (Gram-negative bacteria) in fermented foods.

2.4.1 Bacterial Flora of Fermented Foods from Nigeria Markets

Fermented foods are very essential to the diet of African populations on the basis of their availability, affordability, and contribution to proffering lasting solution to protein-calorie malnutrition and micronutrient deficiencies. Production of these food products usually follows numerous traditional methods which, however, must be safe for consumption. It has been established that quality of fermented foods from both Nigerian and South African market outlets was found, that means TVC (Total viable

count) of all the samples exceeded the microbiological criteria (>4 Log CFU/g) for food stuffs according to EC(No2073/2005)(EC,2005)[81]. Having these microorganisms in levels beyond tolerable levels is injurious and could facilitate the occurrence of food-borne diseases which could either come in form of food poisoning or intoxication.

As demonstrated on previous research work that Enterobacteriaceae and total viable aerobic count (TVAC) in ogi to be 5.6 and 6.5 Log CFU/g presence respectively[82]. Nonetheless, none of the samples analyzed was positive for V*ibrio spp* which contrasts with the data obtained in the study by Adekoya which isolated *Vibrio spp* from some ogi samples. *E. coli* was the predominant species among the Enterobacteriaceae identified, and that was because its presence in samples such as ogiri and iru points to fecal contamination. Other earlier studies have isolated Enterobacteriaceae from foods during fermentation. For example, a corresponding increase in the population of *Bacillus* species is reported from the *beginning* of the fermentation process of ugb till the end[83]. Other groups of organisms that have been found to be associated with the fermentation of this condiment include *Escherichia* species, *Proteus, Pediococcus, Micrococcus, Staphylococcus, Streptococcus, Alcaligenes, Pseudomonas, Corynebacterium, Enterococcus*[84]. Furthermore, *E. coli, K. pneumoniae, P. mirabilis and P. aeruginosa*, were the Gram-negative bacteria mainly isolated from iru[85].

Majority of the studies on fermented foods in Africa have directed their attention to microbiota involved in the fermentation process, and they have mostly used biochemical analytical methods while some have studied the microbiological status of fermented foods using modern techniques such as DNA sequencing, which enhanced the establishment of previously unreported organisms of public health importance

such as *P. canis* and *V. vulnificus* in iru, and *Aeromonas haemolyticus*, *Aeromonas iwoffii*, *A. salmonicida*, *Sphingomonas paucimobilim*, and *Rhizobium radiobacter* in ugba [86,87,88,89].

Isolated *Serratia marcescens* from 33% of ogi samples obtained from Nigeria. *S. marcescens* is a Gram-negative bacterium that is associated with hospital-acquired infections (HAIs) including urinary tract infections (UTIs), and it is found to be abundant in the environment. While infections induced by *Cronobacter sakazakii* have primarily been among infants and neonates, the occurrence of this causative agent in foods as for infant formula previously reported, raises new concerns, particularly the risk it may pose among immunocompromised consumers[81].

Sphingomonas paucimobilis was found to be persistent in all the samples just like *S. marcescens* it is associated with HAIs and ubiquitous[90]. Additionally, two *Vibrio spp*. including *V. parahaemolyticus* and *V. vul*nificus were isolated from ogiri. *Bacillus spp* was also detected in the samples as part of the Gram positive group. This finding, is in excellent concordance with other reports that fermentation of vegetable proteins (ugba, ogiri and iru) is predominantly caused by proteolytic *Bacillus spp.*, particularly *B. subtilis*[91]. Regarding Bacillus, its incidence was characteristically high in ugba (67%) and iru (74%). *Bacillus altitudinis, B. pumilus, B. halotolerans, B. oleronius, B. safensis, B. xiamenensis and B. toyonensis* were members within the Bacillus genera that were also isolated from the analyzed food samples. When compared to others species, *B. subtilis* appeared to be more adapted and dominant, because of its higher protease, amylase, polyglutamic, pyrazine, and subtiliosin production amongst other antimicrobials[92]. The predominance of *B. cereus* in ogi and umqombothi had an incidence rate of 17% in each of the two sample types, and this might be as a result of its wide distribution in the environment being spore formers[93]. Generally, the lactic

acid fermented foods that were obtained from markets in both countries (ogi, ogibaba, mahewu, and umqombothi) in the study by Adekoya had a better safety record in terms of their bacterial load and in terms of the types of pathogenic microorganisms that were found than those alkaline fermented foods (ugba, iru and ogiri)[94]. While alkaline fermentation entails the hydrolysis of protein and subsequent production of ammonia and amino acids, lactic acid fermentation on the other hand involves the conversion of carbohydrates to alcohol or organic acids. This ultimately creates an antagonistic environment for survival of pathogenic and spoilage organisms, thereby improving the safety of such foods[81]. Poor food safety knowledge, use of contaminated raw materials, utilization of polluted water, inadequate hygienic practices, unstandardized production processes, mixed-culture processing, deplorable hygiene status of processing environments, poor packaging, inadequate preservation techniques, meagre storage habits, and unhygienic hawking activities, are some of the factors that provoke the presence of pathogenic organisms in foods.

2.5 Production of Some Locally Fermented Foods and Condiments in Nigeria

2.5.1 Traditional Production of *Parkia biglobosa* African Locust Beans (Iru)

P. biglobosa has long been widely recognized as an important indigenous multipurpose fruit tree whose uses include food, medicine, manure, tannin, shade, wind breaks, bee food, stabilization of degraded environment, livestock feeds, fuel, fibre, fish poison and several other domestic uses[95].

African fermented locust bean is an important vegetable protein in the Guinea savanna zone of West and Central Africa. It is called 'iru' by the Yorubas of south-westem Nigeria while the Hausas who inhabit most of the northern parts of West

Africa call it 'dawadawa'[96].The locust bean itself is made up of protein, 39-47%; oil, 3140% and carbohydrate 11.7-15.4%[97]. Although 'iru' isa food condiment, it is used in poor rural families in West Africa as a low cost meat substitute and it contributes to their protein and calories intake.

A lot of research work has been done on the production of African locust bean seeds and related aspects such as storage, preservation, processing, time taken to be cooked, packaging and other areas[98]. Also efforts have been made to scientifically study the traditional processing, marketing, physical and chemical changes, and the micro-organisms involved in the processing of African locust bean[97,99,100].

Traditionally, *iru* is prepared by boiling *P. biglobosa* cotyledons for 24 h followed by dehulling in mortar with pestle or foot pressing, to remove the seed coats. The cotyledons obtained are again boiled for 4 h with optional addition of *iku iru*, a softening agent made from ground seeds of sunflower (*Hibiscus sabdariffa*) and native potash (*kaun*)[101]. These are then drained using raffia sieve, spread into wide calabash trays while still hot, covered with jute bags and left to ferment for 3-5 days. Salt may be added at the end of the fermentation process as a temporary means of preservation. Two forms of *iru* are thus produced in Nigeria; *iru pete* obtained as a result of the addition of a softening agent, *iku iru* during the second boiling thereby containing partly mashed beans, and *iru woro* that is devoid of *iku iru* having distinct cotyledons. While *iru pete* is used in soups, *iru woro* is used in cooking leafy vegetables and stews.

Condiments are produced by natural, uncontrolled fermentation conditions, without conscious inoculation of substrates with defined starter cultures. This often leads to batch variations in microbial composition, resulting into products inconsistency, low shelf-life and incidences of safety issues in developing countries[102].

In traditional process, water used for "iru" processing is usually the surface water sources, which can introduce contaminations into the peeled cotyledons. Similarly, the critical hazard points of public health which not usually properly control includes: feet used for de-hulling the seed cotyledons, fermentation trough, addition of salt in open places and wrapping leaves. Different type of leaves are used for wrapping which includes banana, teak (*Tectona grandis*) leaves, etc. these leaves are usually picked from the ground around the shedding trees for wrapping the product and can serve as contaminants. Hence, the microbiological and food safety caution is necessary[103].

Its production is by spontaneous or natural inoculation enhanced by the raw materials, fermentation vessels, processors and environmental microflora, resulting into competitive adaptation and activities of autochthonous, spoilage and pathogenic microorganisms. Processing is carried out using rudimentary household and cottage facilities under varying unhygienic and poor sanitary conditions, devoid of good manufacturing practices (GMPs)[104].

2.5.2 Traditional Production of Ogi

Cereal based foods are the main sources of cheap dietary energy in many developing countries[105,106]. In most developing countries children are weaned with ogi which also is a supplement, breastfeeding between ages of 3-6 months because it is cheap. It is produced from sorghum, maize and millet[106]. Maize is among the oldest cultivated grains worldwide; a common diet of Nigerians for centuries and it constitutes about 90% of the cereals consumed globally[107].

About 20% increase in the linear growth of poor children weaned in communities that maize served as staple food thus contributing to food and nutrition security[108].

The method of traditional Nigerian ogi production was reported by Steinkraus as described by Banigo[109,110]. The cereal grains of interest are steeped in warm water in earthenware, plastic or enamel pots for l-3 days to ferment. The fermented grains are wet-milled and wet-sieved to yield the ogi slurry. In some communities there is a further optional fermentation for l-3 days after wet-sieving. Actually, ogiis a semi-finished food product, but it can be transformed to the finished food by cooking its slurry in hot water. The finished food after cooking is called pap in English language, and local names in Nigeria are '*Eko'*, '*Agidi'*, '*Akamu'*, and '*Koko'*[111]. A report showed that local names in Nigeria could be attributed to some factors like the tribes' names or to the style of its preparation or serving[111]. After preparation to the finished food, it becomes a viscous product, and its final viscosity is attributed to the amount of water added during preparation[112].

The traditional processing of Ogi often employs fermentation techniques that are characterized by the use of simple non-sterile equipment, introduction of natural inoculums, unregulated conditions, sensory fluctuations, poor durability and unattractive packing of the processed products which result in unpredictable quality of the product[113,114].

2.5.3 Traditional Preparation of Ogi-baba (Red Sorghum)

Sorghum cultivation has a special peculiarity with respect to its unique in Africa and Asia continents[115]. Globally, sorghum grains have been observed of increasingly becoming a major food crop in Africa and India, and an important livestock feed in the developed countries[116]. In many parts of Africa, sorghum grains plays an essential role in the attainment of food security for a lot of households while its commercial processing into value-added food and beverage products serves as income generating activities and an important driver for economic development[117].

In West Africa, the traditional soaking method for cereal grains in the course of „ogi" production essentially involves soaking of the grains for up to 72 h at ambient temperature (25 to 35°C) after which the softened grains are wet-milled. However, a modification to the traditional grain soaking method was suggested by Nago which involved initial mild boiling of the grains at 95 to 100°C for 10 min preceding soaking for 12 to 48 h at ambient temperature (25 to 35°C) followed by wet milling[118]. Some research works that are related to soaking of cereal grains during ogi production had also been reported. Sorghum grains play an indispensable role in household food security. Processing of sorghum grains into value added food products on an industrial scale serves as a source of income and an important key to economic development[119]. The agricultural economy of sorghum is ranked the fifth largest for cereals globally[120]. Sorghum has also proven to be a nutritious cereal and an outstanding source of bioavailable iron and zinc; sorghum is also richer in copper and pantothenate than other grains, thus plays an important role in human nutrition[121].

2.5.4 Traditional Production of Ogiri

Oil seeds such as African locust bean, melon seed, castor oil seed, mesquite bean and soybean are also fermented to give condiments. In many cases, fermentation is responsible for the development of taste and aroma, improve digestibility, improvement of nutritional composition, stabilization of the original raw materials and detoxification of antinutrient factors in the products [122].

Ogiri egusi fermentation is still carried out using the traditional method. The traditional preparation of ogiri from melon processed shelled melon seeds will be sorted to remove defective seeds, boiling melon seeds, cooling, wrapping in leaves and fermenting at the prevailing temperature and relative humidity after which they are dehulled (The dehulling process is the separation of the seed coat of the melon

seeds from the cotyledons, and it requires an abrasive action. This abrasive removal of the testa/hulls is carried out manually and because of its tedious nature when done with the hands, the locals have resorted to dehulling the boiled seeds with the aid of the bare feet as this is easier and faster), and then boiled again to soften seeds for fermentation. The softened seeds are wrapped in leaves, kept in sacks and incubated near the earthen pot for a period of three to five days or longer after which the mash is dried and milled to a smooth paste, the ogiri. The number and layer of leaves used in wrapping the seeds before fermentation vary depending on the individual carrying out the fermentation. Thus the rate of oxygen transfer to the fermenting microorganisms will vary from one batch of fermentation to another. Also the prevailing relative humidity and temperature vary from day to day and within regions and seasons. Dehulling is always done manually and with the aid of the feet. The manual dehulling that uses feet, coupled with an unhygienic fermentation and environment of preparation could result in the production of an ogiri with variable quality and unacceptable aroma, short shelf - life and one that can pose health hazards to the consumers.

These variables usually account for the variations in product quality. This chance fermentation as practiced traditionally makes the fermentation difficult to control and results in the contamination of products with pathogens or other microorganisms capable of producing toxins and those that cause off flavours[123].

2.6 Benefits of Some Indigenous Fermented Foods

Ethnic groups have different remedial/therapeutic in fermented products consumption which contains different beneficial microorganisms. Numbers of food especially cereals are poor in their nutritional value which is the main staple diet for low income populations[124].

Traditional fermented protein-rich foods offer excellent opportunities for improving the diets of people in tropical countries. Various attempts have been made to increase the protein level of cassava-based products, particularly gari. Growth of the fungus *Aspergillus niger* for 24 to 30 hours on cassava flour with an initial content of 2% to 3% protein and 80% to 90% carbohydrate resulted in a product containing 18% to 20% protein and 30% to 35% carbohydrate[125]. Another approach is the supplementation of cassava with protein-rich foods, for example, supplementation of gari with soya protein. Soya-ogi, a combination of maize and soya beans, was developed by the Federal Institute of Industrial Research, Oshodi, to increase the protein level of ogi[126].

The germination and fermentation of cereals enhance the availability of elemental iron, the deficiency of which is responsible for the high incidence of anaemia in tropical countries.

2.6.1 Benefits

(a) Fermentation is a cheap means of preserving the local staples for long periods. Perishable substrates, for example, cassava, fish, beans, etc are converted into products with good keeping qualities such as gam, sour fish, fermented bean condiments etc. Fermented food products such as dawadawa that contain spices and salt have an enhanced keeping quality and longer shelf life.

(b) Fermentation eliminates toxic substances like cyanide, protease inhibitors, hemagglutinins, ricin, tannin, phytates, flatus causing oligosaccharides etc[127].

This is important in the case of cassava, soyabeans, castor beans and African locust beans which are very toxic in the raw state but become non-toxic and edible after fermentation. Understandably these toxic compounds which also affect digestibility

are destroyed due to the soaking and cooking steps as well as the discarding of the soak or cook water.

(c) Some foods are converted by fermentation into products with pleasant odour and colours which remove the monotony common among most vegetable diets. In this way some high quality meat flavours are derived from vegetable origin. This leads to increase in acceptability, palatability, better appearance, better flavour and increased nutritional quality.

(d) Fermentation is a low cost method of producing nutritious protein-rich or highvitamin foods. It leads to increase in the protein content of high starch substrates. Digestibility of fermented products is increased by the breakdown of the proteins into amino acids [126]. This is of particular importance in the case of pulses. Because of the limited amount of protein found around the world, there is an increasing need for the poorer countries to be self-sufficient by producing more protein foods via fermentation and other techniques. Some water-soluble B-vitamin levels are increased by fermentation as observed in Ugba, melon seed and castor beans[128]. Some bacteria in fermented foods can also add vitamin B12 to certain vegetable foods.

(e) The bulk of food material is reduced by fermentation. This facilitates transport of the food product especially in developing countries where transportation is poor. Bulk reduction occurs due to the processes (such as peeling, dehulling, grating, soaking, squeezing, drying) involved in the fermentation techniques as observed in cassava fermentation into garri.

(f) Cooking time of most fermented foods is reduced when compared to that of raw unfermented substrates. This may be attributed to processes such as dehulling, soaking, milling, salts addition, etc which are steps involved in the local fermentation techniques.

(g) The fermentation technique can salvage products such as fish bones, waste foods (yams, cassava) that cannot be readily used for food. Such foods become edible and presentable after fermentation.

(h) Most local fermented foods are inexpensive and are therefore within the purchasing power of many individuals in the community. Production of fermented food by small scale food processors will therefore contribute to the economy of Nigeria and to the improvement in the nutrition of consumers.

2.7 Bacteria of Significance in Fermentation

Several bacteria are present in foods, the majority of which are concerned with food spoilage, while some like *Clostridium* are the causative agent for production of toxin like botulin, causing botulism in man[129]. As a result, the important role of bacteria in the food fermentations is often overlooked. Lactic acid bacteria like *Lactobacillus, Pediococcus, Streptococcus, Oenococcus,* etc. are the most important bacteria in fermented foods, followed by *Acetobacter* species, which oxidize alcohol to acetic acid. The acetic acid fermentation has been used extensively to produce fruit vinegars including cider vinegar[130].

A third group of bacteria of signifificance in fermentation are the *Bacillus* species (*Bacillus subtilis*, *B. licheniformis* and *B. pumilus*), which bring about alkaline fermentation. *Bacillus subtilis* is the dominant species causing the hydrolysis of protein to amino acids and peptides and releasing ammonia, which increases the alkalinity and makes the substrate unsuitable for the growth of spoilage organisms. Alkaline fermentations are more common with protein-rich foods such as soybeans and other legumes, although there are few examples utilizing plant seeds. For example, water melon seeds (ogiri in Nigeria) and sesame seeds (ogiri-saro in Sierra Leone) are the substrates for alkaline fermentation[131].

Lactic acid bacteria (LAB) are widely present in many fermented foods and beverages such as *Alkalibacterium*, *Carnobacterium, Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus,* and *Weissella* have been isolated from various globally fermented foods and beverages[132,133].

Bacillus is present in alkaline-fermented foods of Asia and Africa[134]. Species of *Bacillus* that are present, mostly in legume-based fermented foods, are *Bacillus amyloliquefaciens, Bacillus circulans, Bacillus coagulans, Bacillus fifirmus*, *Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus subtilis, Bacillus subtilis* variety *natto,* and *Bacillus thuringiensis* , while strains of *Bacillus cereus* have been isolated from the fermentation of *Prosopis africana* seeds for the production of *okpehe* in Nigeria[135,136].

Some strains of *B*. *subtilis* produce λ-polyglutamic acid (PGA) which is an amino acid polymer commonly present in Asian fermented soybean foods, giving the characteristic of a sticky texture to the product[137].

The association of several species of *Kocuria, Micrococcus* (members of the *Actinobacteria*), and *Staphylococcus* (belonging to the *Firmicutes*) has been reported for fermented milk products, fermented sausages, meat, and fifish products [138]. Species of *Bififidobacterium, Brachybacterium, Brevibacterium,* and *Propionibacterium* are isolated from cheese, and species of *Arthrobacter* and *Hafnia* from fermented meat products[139].

Enterobacter cloacae, *Klebsiella pneumoniae, K. pneumoniae* subsp. *ozaenae, Haloanaerobium, Halobacterium, Halococcus*, *Propionibacterium, Pseudomonas,* etc. are also present in many global fermented foods[132].

Fermented foods are one of the top 10 food trends in 2016 (, continuing the trend over the last few years [140]. Several soy- and cereal-based probiotic products are also in the market in response to the growing prevalence of allergies to dairy proteins, lactose and gluten intolerances, and life style choices such as veganism[141].

2.8 Mechanisms of Antibiotic Resistance

During more than 60 years of global antimicrobial use, several resistance mechanisms have been identified, viz. enzymatic degradation of antibiotics, antibiotic target modification and alternative pathways to escape the activity [142].

In the presence of certain resistance genes, bacteria can avoid antimicrobial agents through any of the three mechanisms:

a) direct inactivation of the active molecule;

b) loss of bacterial susceptibility to the antimicrobial by modification of the target of action; and

c) reduction of the drug concentration that reaches the target molecule without modification of the compound itself (efflux pump)[143].

The antibiotic defence mechanisms of intrinsic resistance are, in most of cases, related to the presence of low affinity targets, absence of targets, innate production of enzymes that inactivate the drug, inaccessibility of the drug into the bacterial cell by decreased drug uptake or extrusion by efflux of drug [144].

Bacteria that have resistance genes located on mobile genetic determinants pose a threat to public health (often referred to as “reservoirs”) and enable the spread of these genes, especially if the environment contains numerous microbiota [145,146]. Today, many researchers have emphasized the hypothesis that commensal bacteria, primarily lactic acid bacteria, can serve as reservoirs of antibiotic resistance genes [147,148]. This is why the population of commensals is highly important in identifying mechanisms of

persistence and spread of resistance genes in the microbial world. Accordingly, the food colonized by bacteria with transmissible antibiotic resistance genes has been specifically addressed. Antibiotics of food borne bacteria has aroused great interest because they may act as reservoirs for antibiotic resistance genes [149].

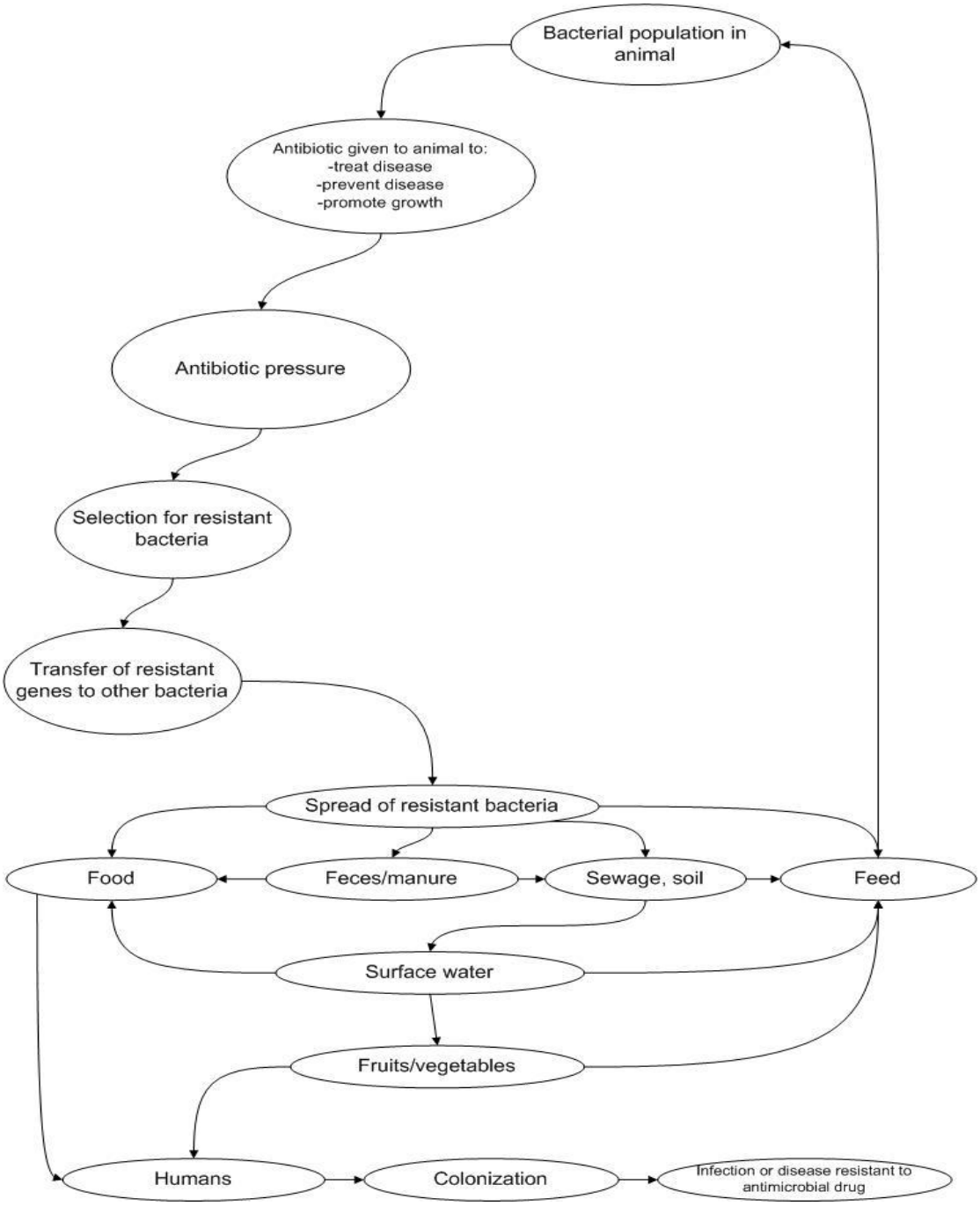

Figure 2.1 Possible transmission routes of antibiotic resistance bacteria from animals to humans. Source adapted from Claycamp et al.[150]

Bacteria have become resistant to antimicrobials through a number of mechanisms; Permeability changes in the bacterial cell wall which restricts antimicrobial access to target sites, Active efflux of the antibiotic from the microbial cell, Enzymatic modification of the antibiotic, degradation of the antimicrobial agent, acquisition of alternative metabolic pathways to those inhibited by the drug, modification of antibiotic targets, overproduction of the target enzyme[151,152].

These AR phenotypes can be achieved in microorganisms by chromosomal DNA mutations, which alter existing bacterial proteins, through transformation which can create mosaic proteins and/or as a result of transfer and acquisition of new genetic material between bacteria of the same or different species or genera[153,154].

Many studies report the high prevalence of multiple drug-resistant (MDR) strains, because MDR infections are often significantly difficult and more expensive to treat, they represent a growing public health threat[155]. However, for different pathogens, different underlying mechanisms are traditionally used to explain these observations, and it is unclear whether each bacterial taxon has its own mechanism(s) for multidrug resistance or whether there are common mechanisms between distantly related pathogens.

The transmission routes of MDR foodborne pathogens along the food chain and how they can reach consumers are not clear at present. The relevance of the mechanisms mentioned above with respect to the possibility that resistant pathogens can be originated at primary production and spread through the food chain is also unknown.

However, it is reasonable to consider that MDR pathogens from a very early food stage (i.e primary production) can enter and remain in the food systems and (re)contaminate, survive, and/or grow in food or food environments resulting in their presence of both raw food and ready-to-eat products at the consumption stage

(**Figure1**)[156]. Even the complete elimination of bacteria by lethal treatments does not assure that AMR is not transmitted, since DNA released from lysed cells can still be transferred, by the process of transformation to living microorganisms (pathogenic or commensal) on foods or in human digestive system.

In addition, environmental stress produced by this production can drive pathogens to adapt to the stressful environment by evoking the expression of the resistance genes, and, as a consequence, enhanced resistance capacity and changes in virulence and infectivity of the populations[157]. In spite of the few studies carried out hitherto, it has been proved that sub lethal stress produced by thermal, acidic, and saline conditions can affect the phenotypic resistance[156]. For example, there are data suggesting that sub lethal high temperatures can reduce the presence of phenotypic resistance while increase in salt concentrations or reduction of pH is rather related to its increase[158].

2.9 Classification of Antibiotics

2.9.1 Beta-lactams

The first antibiotic discovered was a β- lactam, i.e., penicillin. The Scottish scientist Alexander Flemming accidentally noticed the production of a substance with antimicrobial properties by the mold *Penicillium notatum*[159]. Over the last 30 years, many new β-lactam antibiotics have been developed. By definition, all β-lactam antibiotics have a β-lactam nucleus in their molecular structure. The β-lactam antibiotic family includes penicillins and derivatives, cephalosporins, carbapenems, monobactams, and β-lactam inhibitors[160]. They can be grouped in first, second, third, and forth generation cephalosporins according to their spectrum of activity and timing of the agent's introduction. In general, first generation agents have good Grampositive activity and relatively modest coverage for Gram-negative organisms; second

generation cephalosporins have increased Gram-negative and somewhat less Gram-positive activity; third generation antimicrobials have improved Gram-negative and variable Gram-positive activity; fourth generation β-lactams have good true broad spectrum activity against both Gram-negatives and Gram-positives[161]. The second generation cephamycins are sometimes also grouped among the cephalosporins.

The β-lactam antibiotics work by inhibiting the cell wall synthesis by binding to so-called penicillin-binding proteins (PBPs) in bacteria and interfering with the structural cross linking of peptidoglycans and as such preventing terminal trans-peptidation in the bacterial cell wall. As a consequence it weakens the cell wall of the bacterium and finally results in cytolysis or death due to osmotic pressure[162].

The first bacterial enzyme reported to destroy penicillin was an AmpC β-lactamase of *E. coli*[163]. Nowadays, bacterial resistance against β-lactam antibiotics is increasing at a significant rate and has become a common problem. There are several mechanisms of antimicrobial resistance to β-lactam antibiotics. The most common and important mechanism through which bacteria can become resistant against β-lactams is by expressing β-lactamases, for example extended-spectrum β-lactamases (ESBLs), plasmid-mediated AmpC enzymes, and carbapenem-hydrolyzing β-lactamases (carbapenemases)[164].

In addition to the production of β-lactamases resistance can also be due to possession of altered PBPs. Since β-lactams cannot bind as effectively to these altered PBPs, the antibiotic is less effective at disrupting cell wall synthesis. The PBPs are thought to be the ancestors of the naturally occurring chromosomally mediated β-lactamase in many bacterial genera[165].

2.9.2 Macrolide Lincosamide Streptogramin b

The first macrolide, erythromycin A, was discovered in the early 1950s[166.] The main structural component of this molecule is a large lactone ring to which amino and/or neutral sugars are attached by glycosidic bonds. To address the limitations of erythromycin, like chemical instability, poor absorbance, and bitter taste, newer 14-, 15-, and 16-membered ring macrolides such as clarithromycin and the azalide, azithromycin, have been developed[167].

Macrolides have a similar mode of antibacterial action and comparable antibacterial spectra as two other antibiotic classes, i.e., lincosamides and streptogramins B. Consequently, these antibiotics, although chemically distinct, have been clustered together as Macrolide–Lincosamide–Streptogramin B (MLS) antibiotics[167]. Nowadays this class of antibiotics should even be extended due to the development of various synthetic drugs. The ketolides and oxazolidinones can be grouped together with the MLS antimicrobial agents which results in the MLSKO family of antibiotics[168].

Macrolides, lincosamides, and streptogramins B all inhibit protein synthesis by binding to the 50S ribosomal subunit of bacteria[167].

The most common mechanism of MLS resistance is due to the presence of rRNA methylases, encoded by the *erm* genes. These enzymes methylate the adenine residue(s) resulting in MLS resistance. The methylated adenine prevents the binding of the drugs from binding to the 50S ribosomal subunit. The other two mechanisms efflux pumps and inactivating genes are encoded by *msr* and *ere* determinants, respectively.

2.9.3 Quinolone

In 1962, during the process of synthesis and purification of chloroquine (an antimalarial agent), a quinolone derivative, nalidixic acid, was discovered which possessed bactericidal activity against Gram-negatives[169]. The second generation quinolones arose when it became clear that the addition of a fluoride atom at position 6 of a quinolone molecule, creating a fluoroquinolone, greatly enhanced its biological activity. During the 1980s, various fluoroquinolones were developed, e.g., ciprofloxacin, norfloxacin, and ofloxacin. These fluoroquinolones demonstrated a broadened antimicrobial spectrum, including some Gram-positives[170].

Quinolones inhibit the action of DNA gyrase and topoisomerase IV, two enzymes essential for bacterial DNA replication and as a result the microbes are killed[171]. DNA gyrase is a tetrameric enzyme composed of 2 GyrA and 2 GyrB subunits. The topoisomerase IV has a similar structure, comprised of 2 A and 2 B subunits, encoded by *parC* and *parE*, respectively. The four genes coding for the subunits of these enzymes are the targets for resistance mutations.

2.9.4 Aminoglycoside

Aminoglycoside antibiotics are low-molecular-weight molecules of approximately 300–600 daltons. All natural and semisynthetic aminoglycosides share a similar structure consisting of several, usually three, rings. Aminoglycoside antibiotics are highly efficacious drugs, particularly against gram-negative bacteria[172]. Piepersberg.

Aminoglycoside antibiotics are widely used in hospitals for treatment of very severe human infection by Gram-negative and Gram-positive of bacteria and in veterinary medicine[173]. Their antimicrobial action is by the inhibition of microorganism protein synthesis [174].

Their outstanding value comes from their broad antibacterial spectrum and their bactericidal properties, i.e. their ability to kill bacteria and not only to prevent their growth. Their original indication against *Mycobacterium tuberculosis* is still one of their uses today: streptomycin and amikacin are commonly part of a regimen against the recently emerging multidrug-resistant tuberculous bacteria. Infections by *Pseudomonas* are another major indication for aminoglycosides[175].

The actions of aminoglycosides on bacteria are a multistep process beginning at the plasma membrane, followed by internalization and by effects on various intracellular processes[176]. Binding of the drugs to the 16S ribosomal RNA leads to inhibition of protein synthesis[177]. This inhibition is specifically directed against bacteria since mammalian ribosomal RNA has a different structure. Whether this step causes the bactericidal effects remains to be established[178].

2.9.5 Nitrofuran

Nitrofurans are a class of drugs typically used as antibiotics or antimicrobials[179]. The defining structural component is a furan ring with a nitro group[180].Nitrofuran compounds are prodrugs. The antibacterial mechanism of the nitrofuran derivatives, once activated by nitroreductases, is ill defined. Multiple effects have been observed, including DNA lesions and oxidative stress and inhibition of the RNA and protein biosynthesis[181,182]. However, it remains to be clarified which of these affected targets (DNA, RNA, and protein) are directly attacked by the nitroreductase-activated nitrofuran derivatives and which are simply downstream effects of the interaction between these derivatives and bacterial essential targets. Of great concern is the reported nitrofurantoin resistance in *E. coli* uropathogenic clinical isolates in one hospital in North Wales (United Kingdom) in 2020[183]. These isolates were found to

have a mutated version of the extended spectrum β-lactamase CTX-M-14, which differs from the wild-type protein by 3 nonsynonymous changes (T55A, A273P, and R277C). There have been very few attempts to develop next-generation nitrofuran antibacterial drugs in several decades. Over the past few years, however, work on nitrofurans has been revived, in parallel with their resurgence as an effective treatment option in the context of widespread resistance to other antibiotics.

2.10 The Impact of the Food Chain on the Antimicrobial Resistance Transmissions

The food chain is one of the main driving forces in the transmission of MDR due to the fact that foods are not sterile and usually present microbiological contamination or can become contaminated at specific stages along the food chain (i.e., cross contamination, recontamination)[184]. Indeed, the food chain can act as a booster for MDR dissemination, allowing for survival or even increase of MDR pathogens. MDR transmission is not exclusive for food of animal origin or fish, and plant foods may also harbor MDR microorganisms as vegetables can be contaminated during primary production, through water contaminated with fecal material from effluent of surrounding farms. In general, pathogens are not frequent in foods, and the major risk arises from the high prevalence of non-pathogenic microorganisms that can transfer AMR genes to other microbial species, including foodborne pathogens. It is well documented that genes encoding resistance are transmissible between different bacteria in FPA and also from them to bacteria in foods and in humans by horizontal gene transfer (conjugation, and transduction)[156].

In a study, identical gentamicin resistance gene was found in *Enterococcus* spp. isolated from FPA, retail foods, and humans from geographically different areas

which can also be as a supporting result of the dissemination of gentamicin-resistant *Enterococcus* spp. from FPA to humans *via* the food chain[185]. Liu also showed that mobile elements harboring *mcr-1* and *bla*NDM acquired by FPA strains are the ways of their transmission to our foods and then from foods to humans, after finding structurally similar *mcr-1* and *bla*NDM bearing plasmids both in foods and in clinical isolates. In addition, identical *mcr-1* genes were reported in 21% healthy swine at slaughter, 15% marketed pork and chicken meat, and 1% patients in China in 2016[186,187].

On the other hand, AMR gene transfer is not only specific to live cells; conversely, stressed or partially inactivated cells are able to confer resistance to other microorganisms, including pathogens, or microbiota in general and, after ingestion, mobilized to intestinal microbiota in humans through the gene transfer mentioned above[156].

The pressure exerted by the wide use of biocides for food production such as disinfectants, preservatives, and other chemicals or even environmental and process conditions applied through the food distribution chain has been proved to trigger the adaption of microbial populations by developing transient resistances [188,189].

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Chapter Three

Methodology

3.1 Collection Of Samples

A total of fifteen (15) samples were bought from three different markets purposively selected in Ibadan, South West Nigeria (Orita market, Molete market, Oje market) each of Locust beans (woro and pete), Ogiri and Ogi (Surghum and maize) aseptically.

Samples was transported in an ice-box and directly analyzed in the microbiology lab at the University upon arrival.

3.1.1 Equipment

Beakers (Pyrex), Conical flasks (Pyrex), Autoclave, Petri-dishes, Forceps, Cotton wool, Spirit lamp, McCartney bottles, Filter paper, Measuring Cylinder, Refrigerator, Spatula, Test tubes(Pyrex), Weighing Balance SUN-5202, Incubator, Inoculating Loop, Antibiotic discs, Cock borer, Hand gloves, Nose masks, Compound Microscope, Slides, Pippete, Forcepts, Spatula, Durham tubes, Water bath (CB.2Sq2) (Gallenkampe England), Oven (Gallenkampe England), Pasteur Pipettes (Pyrex), Spin Column and Collection tubes, Electrophoresis machine, DNA Engine (PTC-200 Peltier Thermal Cycler), Dry Bath Incubator (Biobase).

3.1.2 Chemicals/ Reagents

Crystal Violet, Lugol's Iodine, Immersion Oil, Safranin, Hydrogen peroxide, Amyl alcohol, Starch, Cysteine, P-Dimethylaminobenzaldehyde, Hydrochloric acid, Potassium hydroxide, Sodium hydroxide, Methyl red, Lead acetate, Ethidium bromide,

TAE buffer, Agarose powder, NIMR Kit contains Lysis Buffer, Wash Buffer 1, Wash Buffer 2, Elution Buffer.

3.1.3 Media used

Nutrient Agar, Agar Agar, Nutrient Broth, Peptone Water, MacConkey Agar, Eosin Methylene Blue (EMB) agar, Mueller-Hinton agar and Salmonella Shigella agar (SSA). The media were prepared according to manufacturer's instructions.

3.1.3 Isolation of Bacteria

A serial dilution is a series of sequential dilutions used to reduce a dense culture of cells to a more countable concentration. Each dilution will reduce the concentration of bacteria by a specific amount. Serial dilution was carried out to 10^{-6} for all the samples. In brief, six serial dilutions were made of 1 gram of sample in 9 ml sterile distilled water (10^{-1} – 10^{-6}). Each dilution was inoculated into four different agar plates by pour plate techniques, allowed to set before being incubated in air at 37^{o}C for 24 hrs.

The bacteria isolated, counted and reported as CFU/ml (colony forming units). Pure culture was isolated from this plates by streak plate method using inoculating loop on nutrient agar[1].

3.1.4 Characterization of Bacterial isolates

The bacterial were characterized based on morphological tests such as shape, colour, appearance and Gram reaction. In addition to morphological characterization the isolated bacterial culture were cultivated in selective media and certain biochemical test were performed[1].

3.2 Gram Staining

3.2.1 Gram's Staining Procedure

Thin smear of pure bacterial culture were made on clean glass slide air dried and heat fixed. Smear was covered with crystal violet for 30 seconds, washed with distilled water and smear was flooded with Grams iodine solution for 60 seconds, washed with 95% ethyl alcohol and then distilled water again, the smear will be covered with safranin for 30 seconds, wash with distilled water and blot dried. Air dried and observed under microscope[2].

3.3 Biochemical Identification of the Isolates

The biochemical properties of identified isolates were confirmed through standard biochemical methods[3].

The used biochemical tests are Catalase test, Indole test, Citrate test, Hydrolysis of starch, Production of hydrogen sulphide, Gas production, Acid production, MR, VP, Hemolysis test.

3.3.1 Catalase Test

Catalase test is used to identify microrganisms that produce the enzym catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into oxygen and water.

$2H_2O_2 \rightarrow 2H_2O+O_2$

This test was carried out in a lamina flow hood. Small inoculum of bacteria isolate was mixed into two to three drops of hydrogen peroxide solution (3%) and observed for the rapid elaboration of oxygen bubbles occurs. The lack of catalase was observed by a lack or weak bubbles production[4].

3.3.2 Indole Test

This test is important in the grouping and identification of anaerobic bacteria. The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole. Tryptophan is an amino acid that can undergo deamination and hydroysis by bacteria that express tryptophanse enzyme.

24hrs young culture media was inoculated into 10ml of tryptophan broth which was already sterilizes at 121^0C for 15 minutes and allow to cool before the inoculation, incubated at 37^0 for 24-48 hours. After 48 hours of incubation 5 drops of 0.5mL of Kovac's reagent was added to the broth culture, shaken gently allow to stand for 20 minutes. Formation of a deep red colour at the top layer indicate a positive and yellow coloration indicates negative result[4].

3.3.3 Citrate Test

This test was carried out to study the ability of an organism to use citrate as a sole source of carbon and energy. 24g of citrate agar was dissolved in 500ml of distilled water followed by sterilization in autoclave. Dispense into the petri dishes allow to cool, aseptically inoculate by streaking the organisms once across the surface. The inoculated medium was incubated for 24 to 48 hours, the colour of the medium indicates the result. A change from green to blue indicates utilization of the citrate which is positive but if the media retain the green color after incubation period then the bacteria is citrate negative[4].

3.3.4 Hydrolysis of Starch Test

This test was done to identify bacteria that can hydrolyse starch (amylose and amylopectin). Starch agar plates were inoculated with the selected isolates and

incubated at 35°C for 2 days. After incubation each plate was flooded with aqueous iodine fo 30 seconds. The iodine reacts with the starch to give a dark brown colour. Hence a clear zone around the bacteria growth indicates positive results, while blue black colouration indicates a negative result[4].

3.3.5 Hydrogen Sulphide Utilization Test

An iron compound and a sulfur compound are included in the test medium to test for the production of hydrogen sulfide gas. Indicator paper was used (filter paper) cut in strips and sterilised, soaked in saturated lead acetate solution, the soaked paper was dried.

The strip was inserted inside the inoculated sample bottles between the plug with the lower end above the medium. Incubated at optimum temperature for 2-7days, together with an uninoculated control. Production and liberation of hydrogen sulphide causes blackening which occured by the end of the incubation period given a positive result, when there are no changes on the filter paper it indicate a negative result[4].

3.3.6 Hemolysis Test

Blood agar is an enriched bacterial growth medium used to culture those bacteria that do not grow easily. Preparation of Blood Agar was done as instructed by the manufacturer. Sterilize by autoclaving at 121°C for 15 minutes. Transfer thus prepared blood agar base to a 50°C water bath.

When the agar base was cooled to 50°C, sterile human blood aseptically was added and mix well gently. Formation of air bubbles was avoid, the blood was warmed to room temperature at the time of dispensing to the molten agar base. 15 ml was dispense to sterile Petri plates aseptically. Inoculated the isolates on the plates.

Incubate at 35-37°C overnight. Growth were checked for characteristics of each species.

To know the type of hemolysis, the blood agar plate must be held up to a light source and observed with the light coming from behind (transmitted light). If either type of hemolysis is present, then one will observe a zone of hemolysis surrounding a growing colony.

Alpha (α) hemolysis: Partial lysis of the RBC (red blood cell) to produce a greenish-grey or brownish discoloration around the colony.

Beta (β) Hemolysis: Beta-hemolysis is the complete lysis of RBCs, resulting in a distinct, clear,colorless zone surrounding and under the colony.

Gamma (γ) or non-hemolysis: Gamma-hemolysis indicates no hemolysis of RBCs. There is no change in the medium under and surrounding the colonies[5].

3.3.7 Methyl Red Test

5mililiter of glucose phosphate broth (1g glucose 0.5% KH_2PO_4, 0.5% peptone) and 100ml of distilled water were dispensed in clean test tubes and sterilized. Tubes were inoculated with the test organisms and incubated at 37^0C for 48hrs. At the end of incubation, few drops of methyl red solution were added to each test tubes and colour change was observed. A red colour indicates a positive reaction[6].

3.3.8 Voge's - Proskaeur Test

5mililiter of glucose phosphate broth (1g glucose 0.5% peptone and 100ml of distilled water) were dispensed in clean test tubes and sterilized. The tubes were then inoculated with the test organisms and incubated at 37^0c for 48hrs.

After incubation, 6% α-naphtol and 6% sodium hydroxide were added to about 1ml of the broth culture. A strong red colouration formed within 30mins indicates positive reaction[6].

3.3.9 Gas Production Test

It is a test on organism's ability to ferment the sugar glucose as well as its ability to convert the end product of glycolysis, pyruvic acid into gaseous byproducts. 4-5 ml of glucose phosphate broth (1g glucose 0.5% peptone and 100ml of distilled water) were dispensed in clean test tubes, Durham tubes are inserted upside down in the test tubes and Autoclave the prepared test media (at 121°C for 15 minutes) to sterilize. The sterilization process will also drive the broth into the inverted Durham tube. Aseptically inoculate each test tube with the test microorganism using an inoculating loop. Tubes was Incubated at 35-37°C for 7days. Longer incubation periods to confirm a negative result. Positive result will produce a bubble (small or big depending up the amount of gas produced) the inverted Durham tube[7].

3.4 Antibiotic Susceptibility Testing of Isolated Bacteria

Nutrient Agar (NA) was the culture medium that was used for this test and microbial sample preparations were done according to Mc Farland standard (1.5 x 10^8 cfu/ml). A sterile swab stick was used to streak the culture on the whole surface of the NA plate , allowed to dry, after which the antibiotic discs were placed on the NA plate with the aid of a sterile forcep. Incubation was subsequently done for 24 hours. Single disc diffusion method (NCCLS) was used to examine bacterial susceptibility to antimicrobial agents. The antibiotic sensitivity discs utilized and their concentrations were; Imipenem(IMP; 10/10µg), Ceftriaxone (CRO; 10µg), amoxicillin clavulanic (AUG; 30µg), ciprofloxacine (CIP; 5µg), Ampiclox (ACX; 10µg), Ofloxacin (OFX;

5µg), ampiclox (ACX; 10µg), Cefotaxime (CTX; 25µg), Levofloxacin (LBC; 5µg), Gentamycin (GN; 10µg), Nalidixic acid (NA; 30µg), Nitrofurantoin (NF; 30µg), Cefexime (ZEM; 10µg), Levofloxacin (LBC; 5µg), Erythromycin (ERY; 15µg), Azithromycin(AZN; 15µg). The diameter of the inhibitory zones exhibited by the exposed isolated microorganisms against the respective antibiotics was then measured with the aid of a meter ruler[8].

3.5 Molecular Identification of Bacteria

3.5.1 DNA Extraction using Genomic DNA Extraction Kit

The strains were identified using 16SRNA amplification.

Step by step Procedure for extraction is as described: 100µl of sterile water was added into a well labelled eppendorf tube containing harvested cells. 500µl of the lysis buffer was added to each tube containing the samples, the tubes were vortexed and incubated at 56ºC for 10 min, after which they were centrifuged at 10,000 RPM for 1 min. This was followed by the addition of 200µl of Absolute ethanol to the centrifuged tube, the mixture was transfered into the spin column placed inside a collection tube and then centrifuged at 10,000 RPM for 30 sec. The flow-through was discarded while the collection tube was blotted on a tissue paper. Each of the spin column was returned into their respective collection tubes and then 500µl of Wash Buffer 1 was added to each spin column containing the samples and then centrifuged at 10,000 RPM for 30 sec. The flow-through was again discarded and the collection tube was blotted on a tissue paper. 500µl of Wash Buffer 2 was added to each of the spin column and then centrifuged at 10,000 RPM for 1 min. The flow-through was again discarded and the collection tube was blotted on a tissue paper. The spin column in the collection tube was Centrifuged again at 12,000 to 14,000rmp for 3 minutes to

remove all traces of ethanol. The Spin column was placed in a well labelled 1.5mL Microcentrifuge tubes and then 50µl of Elution Buffer was added to the centre of each Spin column, this was incubated at room temperature for 1 to 2 minutes, centrifuged at 10,000 RPM for 1 min to elute the DNA. The eluted DNA was stored at -20.

3.5.2 DNA Quantification

3.5.2.1 Spectrofluoremetry

Qubit fluorometer was used to measure DNA quantity. 199µl aliquot of the elution buffer was added into a sterile Qubit tube followed by addition of 1µl of the extracted DNA, this was voltexed and placed in the Qubit fluorometer to read the DNA concentration. The readings were measured in ng/µl and it ranges from 5-150ng/µl to 10-200ng/µl .

3.5.2.2. Gel Electrophoresis for Extracted DNA

It is a technique used to separate DNA, RNA or protein molecules based on their size and electric charge. 1gram of agarose powder was measured for the electrophoresis was used for DNA quantification, 1g agarose powder was mixed with 100mL 0.5xTAE(Buffer TAE and EDTA) in a microwavable flask and microwaved for 3 minute until the agarose was completely dissolved, 30µl of ethidium bromide was added. The agarose solution was left to cool for 5 minutes, the agarose was poured into a gel tray with the well comb in place and kept at 4ºC for 15 minutes until it has completely solidified.

3.5.2.3 Loading Samples and Running an Agarose Gel

Solidified agarose gel tray was placed into the gel box (electrophoresis unit) and filled with 0.5xTAE until the gel was covered. A 3µl molecular weight ladder was carefully loaded into the first lane of the gel followed by negative control, continued with 3µl of each PCR product into the wells that was created. The gel was ran at 80-120 V for about 1 hour, then was carefully removed from the gel box. To visualize the DNA fragments, the gel was viewed under UV transillumination (Edvotek MD 20827-1232)

3.5.3 Polymerase Chain Reaction

3.5.3.1 Polymerase Chain Reaction Mix Components

The PCR mix was made up of 20µl of Taq 5X FIREPol® Master Mix from Solis BioDyne Estonia. 0.5µl each of forward(799F-5'AACACGGATTAGATACCC-3') and reverse (1193R-5'ACGTCATCCCCACCTTCC-3') primer, 4µl of the Master mix, 2µl of DNA template and then made up with 13µl Nuclease free water, the mixture was mixed gently with the aid of micropipete tip and vortexed briefly. The tubes were placed in the PCR machine.

3.5.3.2 Cycling Conditions

The PCR thermal cycler used was Gene Amp PCR system 9700. The cycling parameters include: Initial denaturing at 94ºC for 2 minutes, followed by 35 cycles of denaturing at 94ºC for 2 minutes , annealing at 53ºC for 1 minutes and elongation at 72ºC for 1 minutes. Followed by a final elongation step at 72ºC for 10 minutes and hold temperature at 4ºC forever. Amplified fragments were visualized on safe view stained 0.5g agarose electrophoresis gels.

3.5.3.3 Gel Electrophoresis for PCR Product

1.5gram of agarose powder was measured for the electrophoresis was used for polymerase chain reaction (PCR) product, 1.5g agarose powder was mixed with 100mL 0.5xTAE(Buffer TA and EDTA) in a microwavable flask and microwaved for 3 minutes until the agarose was completely dissolved, 30µl of ethidium bromide was added. The agarose solution was left to cool for 5 minutes, the agarose was poured into a gel tray with the well comb in place and kept at 4ºC for 15 minutes until it has completely solidified.

3.5.5 Loading Samples and Running an Agarose Gel

Solidified agarose gel tray was added into the gel box (electrophoresis unit) and filled with 0.5xTAE until the gel was covered. A 3µl molecular weight ladder was carefully loaded into the first lane of the gel followed by negative control, continued with 3µl of each PCR product into the wells that was created. The gel was ran at 80-120 V for about 1 hour, then was carefully removed from the gel box. To visualize the DNA fragments, the gel was viewed under UV transillumination (Edvotek MD 20827-1232)

3.5.6 PCR for resistance gene

The occurrence of antibiotic resistance genes among those that showed resistance to the antibiotics was determined using PCR mix which is made up of 12.5µL of Taq 2X Master Mix from New England Biolabs (M0270); 1µL each of 10µM forward and reverse primer; 2µL of DNA template and then made up with 8.5µL Nuclease free water The primer sets, targeted genes, Oligonucleotide Sequence are all listed in table 4.9.

3.5.6.1 Cycling Conditions

Initial denaturation at 94˚C for 5mins, followed by 36 cycles of denaturation at 94˚C for 30sec, annealing at 55˚C for 30secs and elongation at 72˚C for 45sec. Followed by a final elongation step at 72˚C for 7 minutes and hold temperature at 10 ˚C forever.

3.5.7 Gene Sequencing

The Isolate sequences gotten after the polymerase chain reaction were opened with Bio-Edit and the sequences obtained were compared with data available in nBLAST on GenBank database using the Mega Blast network service of the National Centre for Biotechnology Information (NCBI).

Endnotes

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Chapter Four

Result and Discussion of Findings

4.1 Results

Table 4.1 shows the morphological and biochemical results of the tests done on the isolates gotten from fermented melon seeds (Ogiri). Out of 14 isolates 9 were Gram positive 5 where Gram negative. Out of Gram positive 3 were rod, 6 were cocci. Out of Gram negative 2 were cocci and 1 positive cocci. The result of the various biochemical test shows that 10 were catalase positive, 4 were catalase negative. 11 were able to hydrolyse starch, 3 were unable to hydrolyse. 11 produced hydrogen sulphide, 3 were negative. 12 were indole positive, 2 were indole negative.

Fig 4.1 shows the result of the antibiotic change to antibiotic susceptibility pattern done on the isolates gotten from fermented melon seeds (Ogiri). All the isolates showed varying degrees of resistance to the antibiotics used with isolates $3A^3$ and $12A^5$ been the highest recorded both in negative and positive disc. Isolates $1A^6$,$7A^1$,$8A^4$,$11A^6$,$13A^6$ showed resistance to only one or two of the antibiotics used making them the least resistant to the antibiotics. Organisms isolated from fermented melon seeds showed the highest resistance to the antibiotic Ceftriaxone.

Table 4.1: Morphological and Biochemical Characteristics of Isolates Obtained from Fermented Melon Seeds (Ogiri).

Isolates Code	Gram's Result	Catalase Result	S/H Result	H_2S Result	Gas Production Result	Acid Production Result	MR Result	VP Result	Indole Result	Blood Agar Result	Simon Citrate Result
$1A^6$	+ve Cocco bacilli	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	β	+ve
$2A^4$	+ve Cocci	+ve	-ve	++	-ve	+ve	+ve	+ve	-ve	γ	-ve
$3A^3$	+ve Cocci	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	β	+ve
$4A^5$	+ve Cocci	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	α	+ve
$5A^2$	+ve Cocci	+ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	α	-ve
$6A^2$	-ve Cocci	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	β	-ve
$7A^1$	-ve Cocco bacilli	-ve	-ve	-ve	++	+ve	+ve	+ve	-ve	α	-ve
$8A^4$	+ve Rod	+ve	-ve	++	-ve	-ve	-ve	+ve	-ve	α	+ve
$9A^5$	+ve Cocci	+ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	α	-ve
$10A^1$	+ve Cocco bacilli	+ve	+ve	-ve	+ve	+ve	+ve	-ve	-ve	γ	+ve
$11A^6$	+ve Cocci	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	γ	+ve
$12A^5$	-ve Cocci	+ve	-ve	-ve	++++	-ve	-ve	+ve	-ve	γ	+ve
$13A^6$	+ve Rod	+ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	α	+ve
$14A^1$	+ve Cocco bacilli	-ve	-ve	-ve	+++	+ve	+ve	+ve	+ve	α	+ve

Source: Author's Field Work, 2022

Key: -ve – Negative, α – Alpha, γ – Gamma, β – Beta, +ve – Positive,+++ – fast positive reaction

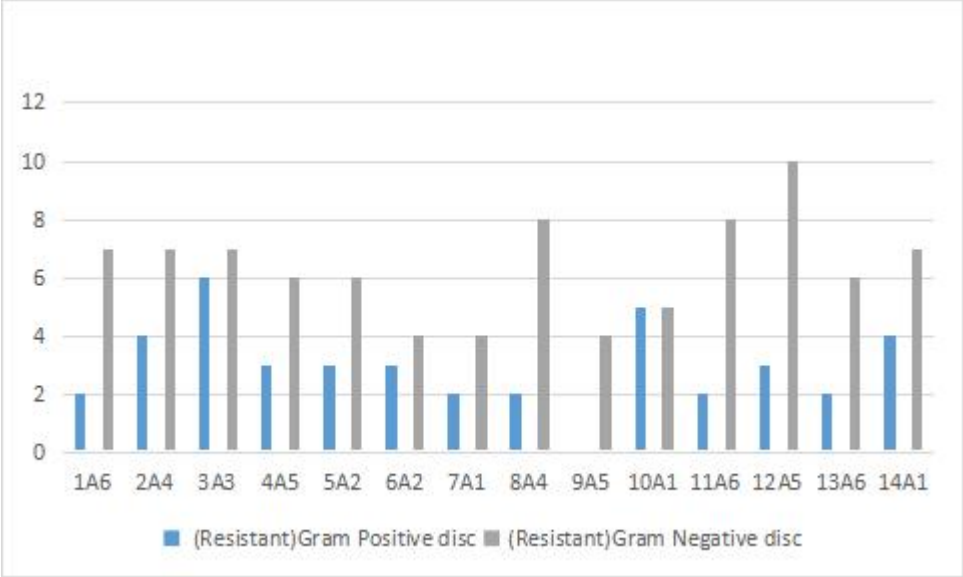

Fig 4.1: Antibiotic Susceptibility Pattern of Bacteria Isolated from Fermented Melon Seeds (Ogiri)

Source: Author's Field Work, 2022

Table 4.2 shows the morphological and biochemical results of the tests done on the isolates gotten from locust beans (Iru woro). Out of 6 isolates 2 were Gram positive, 4 where Gram negative. Out of Gram positive 4 were rod, 2 were cocci. Out of Gram negative 2 were cocci and 2 rod. The result of the various biochemical test shows that 2 were catalase positive, 4 were catalase negative. 5 were able to hydrolyse starch, 1 were unable to hydrolyse. 5 produced hydrogen sulphide, 1 was negative. 3 were indole positive, 3 were indole negative.

Fig 4.2 shows the result of the antibiotic susceptiility test done on the isolates gotten from locust beans (Iru woro). All the isolates varying degrees of resistance to the antibiotics used with isolates $2B^5$ been the highest recorded in positive disc and $6B^6$ in negative disc. Isolates$1B^3$ and $4B^6$ showed resistance to only one or two of the antibiotics used making them the least resistant to the antibiotics. Levofloxacin has the highest resistance to the isolates gotten from locust beans (Iru woro).

Table 4.2: Morphological and Biochemical Characteristics of Isolates Obtained from Locust Beans (Iru Woro)

Isolates Code	Grams Result	Catalase Result	S/H Result	H_2S Result	Gas Production Result	Acid Production Result	MR Result	VP Result	Indole Result	Blood Agar Result	Simon Citrate Result
$1B^4$	-ve rod	-ve	-ve	++	-ve	-ve	-ve	-ve	+ve	α	+ve
$2B^5$	+ve Cocci	+ve	+ve	++	-ve	-ve	-ve	+ve	+ve	α	+ve
$3B^5$	+ve Cocci	+ve	+ve	++	+ve	+ve	+ve	+ve	+ve	α	+ve
$4B^6$	-ve Cocci	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	α	+ve
$5B^3$	-ve Cocci	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	α	+ve
$6B^6$	-ve Cocci	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	α	+ve

Key: -ve – Negative, α – Alpha, +ve – Positive,– -ve Negative.

Source: Author's Field Work, 2022

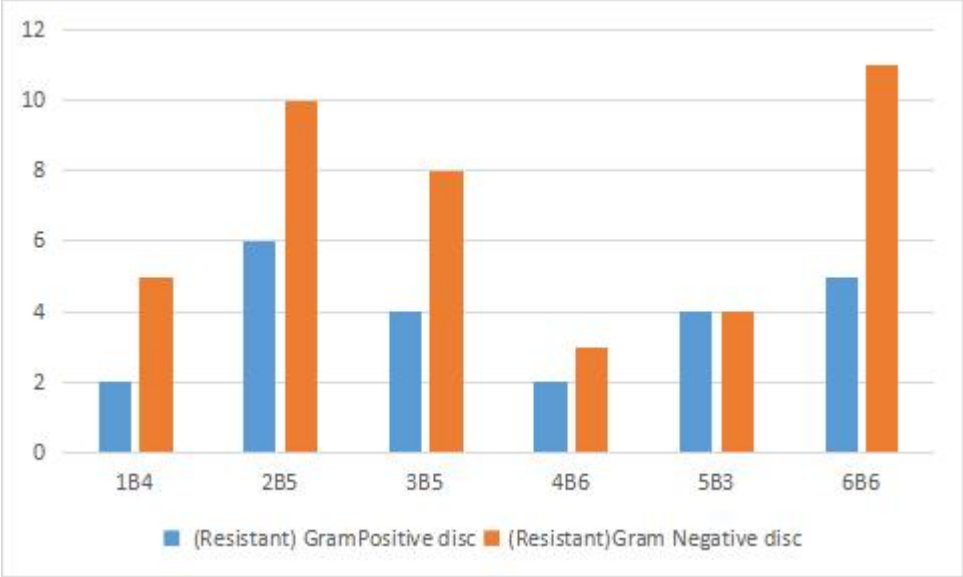

Fig 4.2: Antibiotic Susceptibility Pattern of Bacteria Isolated from Locust Beans (Iru Woro)

Source: Author's Field Work, 2022

Table 4.3 shows the morphological and biochemical results of the tests done on the isolates gotten from locust beans (Iru pete). Out of 12 isolates 11 were Gram positive, 1 where Gram negative. Out of Gram positive 5 were rod, 7 were cocci. Out of Gram negative 1 was cocci and 0 rod. The result of the various biochemical test shows that 6 were catalase positive, 6 were catalase negative. 6 were able to hydrolyse starch, 6 were unable to hydrolyse. 4 produced hydrogen sulphide, 8 were negative. 2 were indole positive, 10 were indole negative. 10 were citrate positive, 2 were negative.

Fig 4.3 shows the result of the antibiotic susceptiility test done on the isolates gotten from locust beans (Iru pete). All the isolates varying degrees of resistance to the antibiotics used with isolates $5C^6$ been the highest recorded in positive disc and $7C^4$ in negative disc. Isolates $8C^6$ and $6C^2$ showed resistance to only one or two of the antibiotics used making them the least resistant to the antibiotics. Imipenem has the highest resistance to the isolates gotten from locust beans (Iru pete) on the positive disc while Ampiclox has the highest level of resistance on the negative disc.

Table 4.3: Morphological and Biochemical Characteristics of Isolates Obtained from Locust Beans (Iru Pete)

Isolates Code	Grams Result	Catalase Result	S/H Result	H_2S Result	Gas Production Result	Acid Production Result	MR Result	VP Result	Indole Result	Blood Agar Result	Simon Citrate Result
$1C^2$	+ve Cocci	-ve	-ve	-ve	+++	+ve	+ve	-ve	-ve	γ	+ve
$2C^3$	+ve Cocci	+ve	+ve	-ve	++	+ve	+ve	+ve	-ve	α	+ve
$3C^5$	+ve rod	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	α	+ve
$4C^6$	+ve Cocci/rod	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	α	+ve
$5C^6$	+ve rods	+ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	α	+ve
$6C^2$	+ve Cocci	-ve	+ve	+ve	++	+ve	+ve	-ve	-ve	α	+ve
$7C^4$	+ve Cocci	-ve	+ve	+ve	++	+ve	+ve	-ve	-ve	α	+ve
$8C^6$	+ve Cocci	-ve	+ve	+ve	++	+ve	+ve	-ve	+ve	α	+ve
$9C^5$	+ve Cocci	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	α	+ve
$10C^1$	+ve rod	-ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	α	-ve
$11C^2$	+ve rod	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	α	-ve
$12C^1$	+ve rod	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	α	+ve

Source: Author's Field Work, 2022

Key: -ve – Negative, α – Alpha, γ – Gamma, +ve – Positive,+++ – fast positive reaction

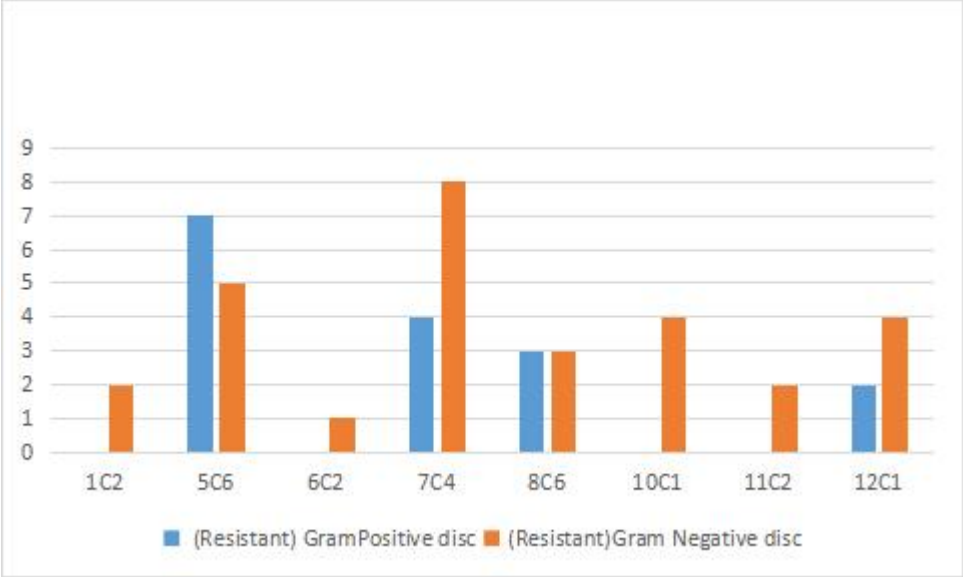

Fig 4.3: Antibiotic Susceptibility Pattern of Bacteria Isolated from Locust Beans (Iru Pete)

Source: Author's Field Work, 2022

Table 4.4 shows the morphological and biochemical results of the tests done on the isolates gotten from fermented Ogi (maize) sample. Out of 14 isolates 9 were Gram positive, 5 where Gram negative. Out of Gram positive 2 were rod, 7 were cocci. Out of Gram negative 4 were cocci and 1 rod. The result of the various biochemical test shows that 4 were catalase positive, 10 were catalase negative. 9 were able to hydrolyse starch, 5 were unable to hydrolyse. 2 produced hydrogen sulphide, 12 were negative. 2 were indole positive, 12 were indole negative. 12 were citrate positive, 2 were negative.

Fig 4.4 shows the result of the antibiotic susceptiility test done on the isolates gotten from fermented Ogi (Surghum) . All the isolates varying degrees of resistance to the antibiotics used with isolates $8D^5$ been the highest recorded in positive disc and $8D^5$ in negative disc. Isolates $2D^3$ and $10D^3$ showed resistance to only one or two of the antibiotics used making them the least resistant to the antibiotics. Levofloxacin, Ciprofloxacine, Ofloxacin, Nalidixic acid and Gentamycin has the highest resistance to the isolates gotten from fermented Ogi (maize) sample.

Table 4.4: Morphological and Biochemical Characteristics of Isolates Obtained from Fermented Ogi (Maize)

Isolates Code	Gram's Result	Catalase Result	S/H Result	H_2S Result	Gas Production Result	Acid Production Result	MR Result	VP Result	Indole Result	Blood Agar Result	Simon Citrate Result
$1D^5$	-ve Cocci	-ve	+ve	-ve	++++	+ve	+ve	-ve	-ve	γ	+ve
$2D^3$	-ve Rod	+ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	α	+ve
$3D^1$	+ve Rod	+ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	α	+ve
$4D^2$	+ve Rod	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	α	+ve
$5D^4$	-ve Cocci	-ve	-ve	-ve	+++	-ve	-ve	-ve	-ve	α	+ve
$6D^3$	+ve Cocci	+ve	-ve	-ve	++	-ve	-ve	-ve	-ve	α	+ve
$7D^3$	+ve Cocci	-ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	γ	+ve
$8D^1$	+ve Cocci	-ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	β	+ve
$9D^5$	+ve Cocci	-ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	α	+ve
$10D^3$	+ve Cocci	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	α	-ve
$11D^3$	-ve Cocci	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	γ	+ve
$12D^3$	-ve Cocci	-ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	α	+ve
$13D^3$	+ve Rod	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	α	-ve
$14D^2$	+ve Cocci	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	β	+ve

Source: Author's Field Work, 2022

Key: -ve – Negative, α – Alpha, γ – Gamma, +ve – Positive, β – Beta, +++ – fast positive reaction.

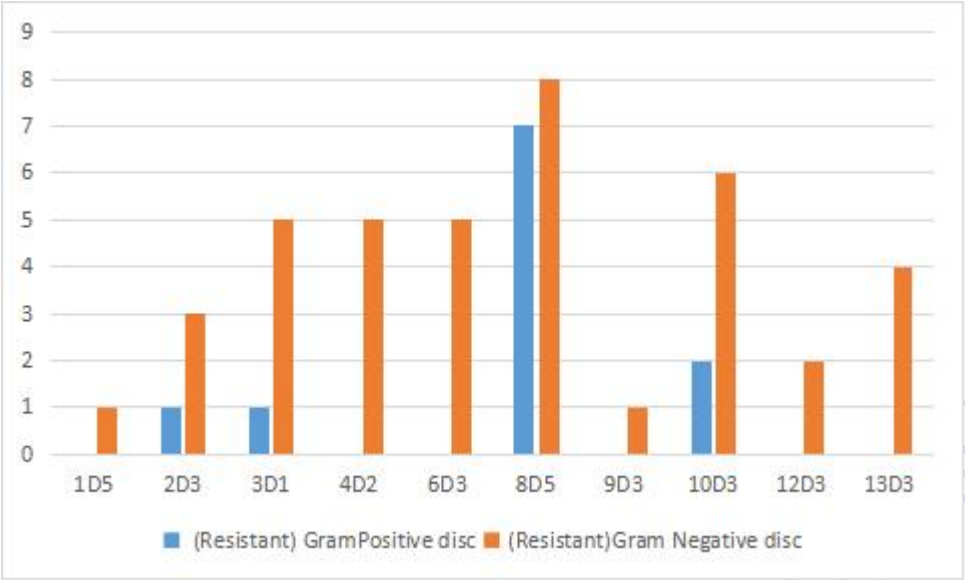

Fig 4.4: Antibiotic Susceptibility Pattern of Bacteria Isolated from Fermented Ogi (Maize)

Source: Author's Field Work, 2022

Table 4.5 shows the morphological and biochemical results of the tests done on the isolates gotten from fermented Ogi (Sorghum gruel) sample. Out of 15 isolates 0 was Gram positive, 15 where Gram negative. Out of Gram negative 12 were cocci and 3 were rod. The result of the various biochemical test shows that 9 were catalase positive, 6 were catalase negative. 15 were able to hydrolyse starch. 7 produced hydrogen sulphide, 8 were negative. 4 were indole positive, 11 were indole negative. 15 were citrate positive.

Fig 4.5 shows the result of the antibiotic susceptiility test done on the isolates gotten from fermented Ogi (Sorghum). All the isolates varying degrees of resistance to the antibiotics used with isolates $4E^5$ been the highest recorded in positive disc and $4E^5$ in negative disc. Isolates $9E^6$ and $11E^3$ showed resistance to only one or two of the antibiotics used making them the least resistant to the antibiotics. Levofloxacin, Ciprofloxacine, Ceftriaxone and Gentamycin has the highest resistance to the isolates gotten from fermented Ogi (Sorghum) sample.

Table 4.5: Morphological and Biochemical Characteristics of Isolates Obtained from Fermented Ogi (Sorghum Gruel)

Isolates Code	Grams Result	Catalase Result	S/H Result	H_2S Result	Gas Production Result	Acid Production Result	MR Result	VP Result	Indole Result	Blood Agar Result	Simon Citrate Result
$1E^6$	-ve Cocci/rod	-ve	-ve	-ve	++	+ve	+ve	+ve	-ve	α	+ve
$2E^5$	-ve Cocci	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	α	+ve
$3E^4$	-ve Cocci/rod	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	γ	+ve
$4E^5$	-ve Cocci/rod	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	α	+ve
$5E^1$	-ve Cocci	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	α	+ve
$6E^3$	-ve Cocci	+ve	-ve	++	++	+ve	+ve	-ve	-ve	γ	+ve
$7E^2$	-ve Cocci	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	α	+ve
$8E^6$	-ve Cocci	-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	α	+ve
$9E^3$	-ve Cocci/rod	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	α	+ve
$10E^3$	-ve Cocci	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	α	+ve
$11E^6$	-ve Cocci	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	α	+ve
$12E^5$	-ve Cocci	+ve	-ve	++	-ve	++	+ve	-ve	+ve	α	+ve
$13E^4$	-ve Cocci	-ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	α	+ve
$14E^5$	-ve Cocci	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	α	+ve
$15E^3$	-ve Cocci	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	α	+ve

Source: Author's Field Work, 2022

Key: -ve – Negative, α – Alpha, γ – Gamma, + – Positive.

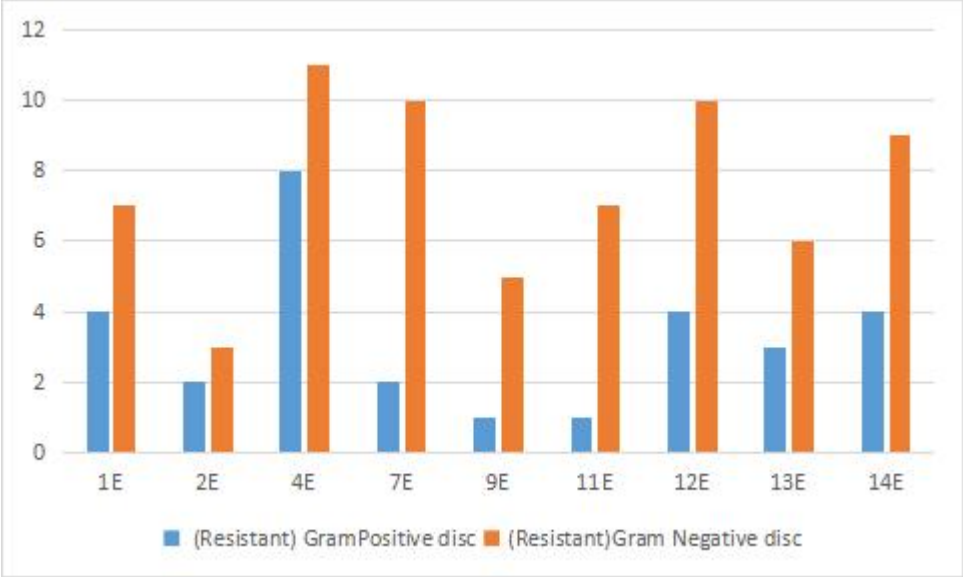

Fig 4.5: Antibiotic Susceptibility Pattern of Bacteria Isolated from Fermented Sorghum Gruel

Source: Author's Field Work, 2022

Table 4.6: Shows the Isolate Code and the Probable Identity based on the Biochemical Tests that was done.

Isolate Codes	Gram's Result	Probable identity
$1A^6$	+ve Cocco bacilli	*Staph. aureus*
$3A^3$	+ve Cocci	*Staph. aureus*
$8A^4$	+ve Rod	*Bacillus subtilis*
$11A^6$	+ve Cocci	*Shigella*
$12A^5$	-ve Cocci	*Shigella*
$1B^4$	-ve Rod	*Salmonella*
$6B^6$	-ve Cocci	*Pseudomonas aurogeninosa*
$3C^5$	+ve Rod	*Lactobacillus*
$4C^6$	+ve Cocci/rod	*Microbacterium flavum*
$5C^6$	+ve Rods	*Microbacterium flavum*
$10C^1$	+ve Rod	*Lactobacillus*
$11C^2$	+ve Rod	*Lactobacillus*
$12C^1$	+ve Rod	*Pediococcus*
$2D^3$	-ve Rod	*Enterobacter aerogene*
$3D^1$	+ve Rod	*Bacillus cereus*
$5D^4$	-ve Cocci	*Pediococcus*
$8D^1$	+ve Cocci	*Streptococcus*
$1E^6$	-ve Cocci/Rod	*Acinetobacter*
$2E^5$	-ve Cocci	*Shigella*
$3E^4$	-ve Cocci/Rod	*Salmonella*

Source: Author's Field Work, 2022

Table 4.7 Shows the selected isolates used for molecular, criteria for selection was based on microorganism that are resistant to at least 2 of the antibiotics used. The isolate that has the highest number of resistance pattern to the antibiotics used. Different probable microorganism was identified phenotypically and molecular identification was done.

Table 4.7: Suspected Microorganisms and Resistant Pattern of Isolates Obtained for Molecular Identification.

Isolate Codes	Gram's Result	Probable identity	Positive resistant pattern	Negative resistant pattern	Molecular identity
$3A^3$	+ve Cocci	*Staph. aureus*	6	7	*Proteus mirabilis*
$11E^6$	-ve Cocci	*Moraxella*	4	10	*Providencia vermicola*
$3B^5$	+ve Cocci	ND	6	10	*Proteus mirabilis*
$6B^6$	-ve cocci/rod	*Pseudomonas aurogeninosa*	5	11	*Proteus mirabilis*
$5C^6$	+ve Rod	*Microbacterium flavum*	7	5	*Proteus mirabilis*
$7C^4$	+ve Cocci	*Pediococcus*	4	8	*Proteus mirabilis*
$9D^5$	-ve Cocci	*Streptococcus*	7	8	*Providencia vermicola*
$11D^3$	-ve Cocci	ND	3	6	ND
$4E^5$	-ve Cocci/rod	*Salmonella*	8	11	ND
$8A^4$	+ve Rod	*Bacillus subtilis*	3	8	*Alcaligenes faecalis*

Source: Author's Field Work, 2022

Key: --ve – Negative, +ve – Positive, ND - non determined.

Fig 4.6 shows the result of the antibiotic susceptiility pattern done on the isolates gotten from all the locally fermented foods and condiments. The antibiotics used were Imipenem(IMP; 10/10 µg), Ceftriaxone (CRO; 10 µg), amoxicillin clavulanic (AUG; 30µg), ciprofloxacine (CIP; 5 µg), Ampiclox (ACX; 10µg), Ofloxacin (OFX; 5 µg), ampiclox (ACX; 10 µg), Cefotaxime (CTX; 25 µg), Levofloxacin (LBC; 5 µg), Gentamycin (GN; 10 µg), Nalidixic acid (NA; 30 µg), Nitrofurantoin (NF; 30 µg), Cefexime (ZEM; 10 µg), Levofloxacin (LBC; 5µg), Erythromycin (ERY; 15µg), Azithromycin(AZN; 15µg), Cefuroxime (CXM; 30 µg). All the isolates varying degrees of resistance to the antibiotics used with Cefexime (ZEM)been the highest recorded in all the isolates used. Isolates Cefuroxime (CXM)showed lowest resistance making it the least resistant of the antibiotic used.

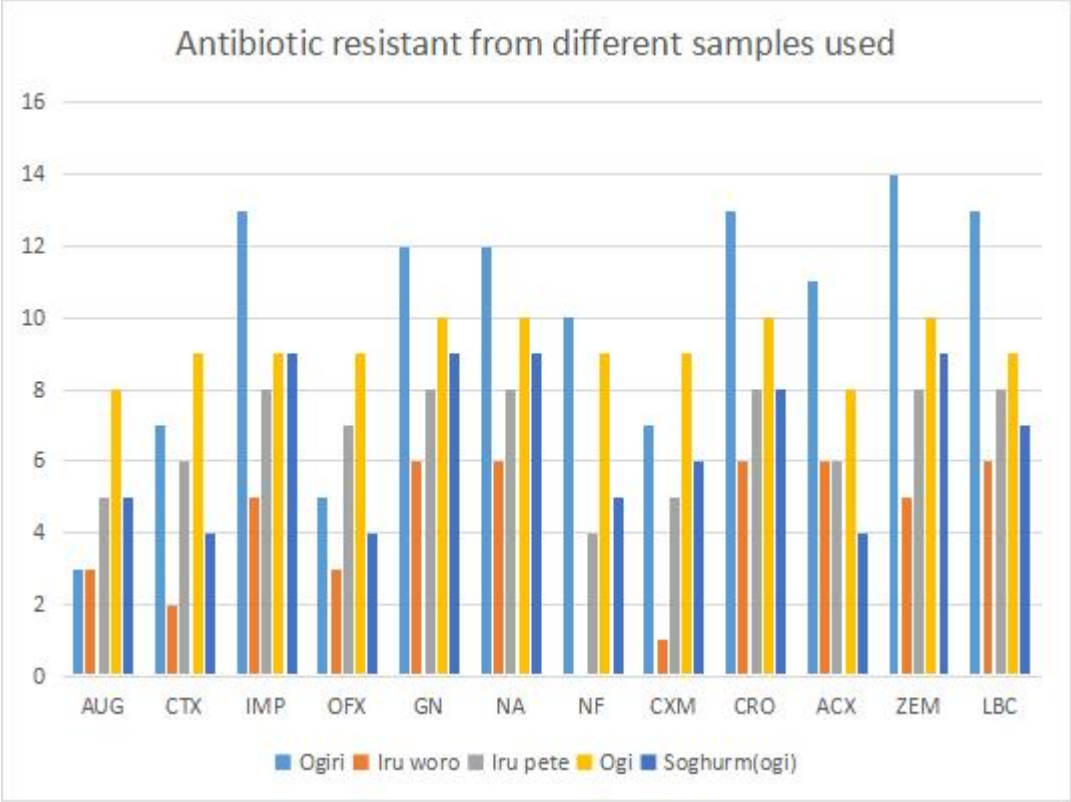

Fig: 4.6: Shows Antibiotic Pattern of all Samples Used in Various Degree.

Source: Author's Field Work, 2022

4.8 Molecular Identification and Phylogenetic Analysis of Bacteria

4.8.1 Molecular Identification of Bacteria

Isolates coded $3A^3$, $11E^6$, $3B^5$, $6B^6$, $5C^6$, $7C^4$, $9D^5$ and $8A^4$ were identified as *Proteus mirabilis, Providencia vermicola* and *Alcaligenes faecalis* srespectively after subjecting their isolated DNA to 16S ribosomal RNA gene amplification by polymerase chain reaction (PCR), also confirming it by subjecting it to gel electrophoresis and analyzing the base sequences of the amplified strand by Sanger sequencing following the manufacturer's instruction.

Proteus mirabilis, Providencia vermicola and *Alcaligenes faecalis* has a 100%, 98.67% and 100% identity respectively using BLAST 2.10.0N+. The 16S ribosomal RNA gene of each of the isolates was submitted to GenBank and each was assigned the following accession numbers: OL762479 *Proteus mirabilis*, OL762480 *Providencia vermicola*, OL762481 *Proteus mirabilis,* OL762482 *Proteus mirabilis* ,OL762483 *Proteus mirabilis*, OL762484 *Proteus mirabilis*, OL762485 *Providencia vermicola*,OL762486 *Alcaligenes faecalis*

The gel image obtained after running the amplified 16SrRNA gene of the ten isolates with the molecular marker is shown in Fig 4.8.

Table 4.8: Characteristics and Properties of Partial 16S Ribosomal RNA Sequences from Ibadan Markets, Oyo State using nBLAST on GenBank

Accession Number	Properties	Identified species	Details	E-value	Alignment score	Highest query coverage (%)
OL762479	347 bp	*Proteus mirabilis*	100% similarity using BLAST 2.10.0N+	0.0	>200	100
OL762480	375 bp	*Providencia vermicola*	99.20% similarity using BLAST 2.10.0N+	0.0	>200	100
OL762481	375 bp	*Proteus mirabilis*	99.46% similarity using BLAST 2.10.0N+	0.0	>200	100
OL762482	375 bp	*Proteus mirabilis*	100% similarity using BLAST 2.10.0N+	0.0	>200	100
OL762483	378 bp	*Proteus mirabilis*	100% similarity using BLAST 2.10.0N+	0.0	>200	100
OL762484	379 bp	*Proteus mirabilis*	99.74% similarity using BLAST 2.10.0N+	0.0	>200	100
OL762485	379 bp	*Providencia vermicola*	98.67% similarity using BLAST 2.10.0N+	0.0	>200	100
OL762486	368 bp	*Alcaligenes faecalis*	100% similarity using BLAST 2.10.0N+	0.0	>200	100

Source: Author's Field Work, 2022

Table 4.9: Oligonucleotide primers used to identify resistance genes.

Target Gene	Primer name	Internal number	Sequence	Denaturation	Annealing	Extension	No. Of cycle	final elongation	References
β-Lactams resistance associated genes									
blaCTX-M	CTX-M U1	Primer 1354	5'ACCAATG CTTAATCA GTGAG3'	94˚C for 5mins	60	72˚C for 45sec	36	72^{O}C/7 min	B.R Pribul et al 2016
OXA48	OXA-48	Primer 2641 Primer 2642	F-5'GCGTGGT TAAGGATG AACAC3' R-5'CATCAAG TTCAACCC AACCG3'	94˚C for 5mins	55	72˚C for 45sec	36	72^{O}C/7 min	B.R Pribul et al 2016
blaTEM	TEM front P1	Primer 757 Primer 686	F-5'GCGGAAC CCCTATTTG 3' R-5'ACC AAT GCT TAA TCA GTG AG3'	94˚C for 5mins	55	72˚C for 45sec	36	72^{O}C/7 min	B.R Pribul et al 2016
Quinolone-resistance associated genes									
qnrA	QnrA	Primer 1685 Primer 1686	F-5'GGATGCC AGTTTCGA GGA-3' R-5'-TGCCAGGC ACAGATCT TG-3'	94˚C for 5mins	59	72˚C for 45sec	36	72^{O}C/7 min	B.R Pribul et al 2016
qnrB	QnrB	Primer 1831 Primer 1832	F-5'GGMATHG AAATTCGC CACTG-3' R-5'TTTGCYG YYCGCCAG TCGAA3'	94˚C for 5mins	57	72˚C for 45sec	36	72^{O}C/7 min	B.R Pribul et al 2016
qnrC	QnrC	Primer 2196 Primer 2197	F-5'GGGTTGT ACATTTATT GAATC3' R-5' TCCACTTTA CGAGGTTC T 3'	94˚C for 5mins	55	72˚C for 45sec	36	72^{O}C/7 min	B.R Pribul et al 2016
qnrSM	QnrSM	Primer 1829 Primer 1830	F-5' TCGACGTG CTAACTTGC G 3' R-5'GATCTAA ACCGTCGA GTTCGG3'	94˚C for 5mins	50	72˚C for 45sec	36	72^{O}C/7 min	B.R Pribul et al 2016

Source: Author's Field Work, 2022

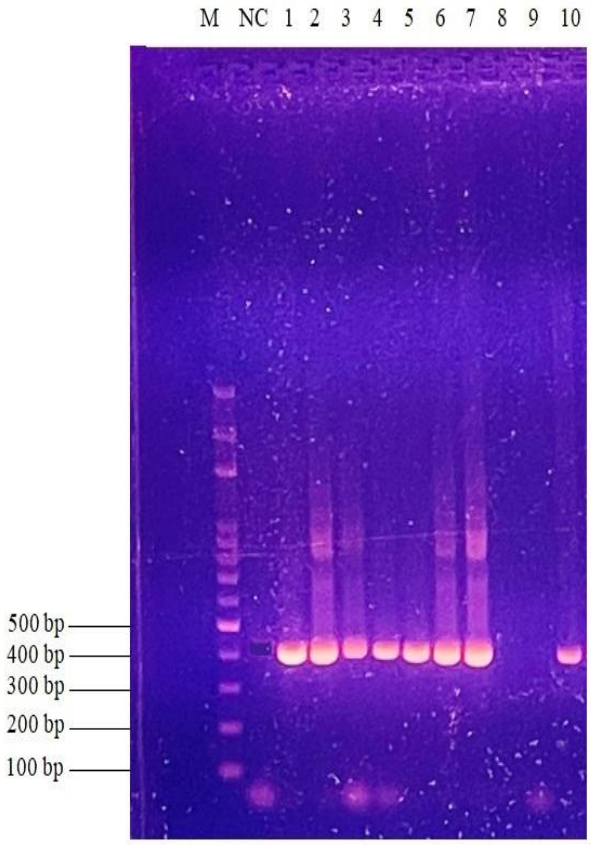

Fig. 4.7: Shows the outcome of the gel electrophoresis done after genomic DNA isolation from the selected samples.Gel image for PCR of the ten isolates with molecular marker/ladder. The DNA samples loaded in the gel showing amplification of the amplicon in 400bp. Sample $4E^5$ which was labelled as 8 was not amplified. Suspected reason might be fungi sample.

Source: Author's Field Work, 2022

4.8.2 Phylogenetic Analysis of Bacteria

Fig 4.8 Isolate 3A[3] *Proteus mirabilis* LCU-MS-001 16S strain 16S ribosomal RNA gene, partial sequence Accession number OL762479.

TCGATTTAGAGGTTGTGGTCTTGAACCGTGGCTTCTGGAGCTAACGCGTTA
AATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTG
ACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGC
GAAGAACCTTACCTACTCTTGACATCCAGCGAATCCTTTAGAGATAGAGG
AGTGCCTTCGGGAACGCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCG
TGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTT
GTTGCCAGCACGTAATGGTGGGAACTCAAAGGAGACTGCCGGTGATAAAC
CGGAGGAAGGTGGGGATGACGT

OL762481 *Proteus mirabilis* strain LCU-MS-003 16S ribosomal RNA gene, partial sequence

GTCGATTTAGAGGTGGGGGTCTTGAACCGTGGCTTCTGGAGCTAACGCGT
TAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAAT
TGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAAC
GCGAAGAACCTTACCTACTCTTGACATCCAGCGAATCCTTTAAAGATAGA
GGAGTGCCTTCGGGAACGCTGAGACAGGTGCTGCATGGCTGTCGTCAGCT
CGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCT
TTGTTGCCAGCACGTAATGGTGGGAACTCAAAGGAGACTGCCGGTGATAA
ACCGGAGGAAGGTGGGGATGACTT

OL762482 *Proteus mirabilis* strain LCU-MS-004 16S ribosomal RNA gene, partial sequence

AGTCGATTTAGAGGTTGTGGTCTTGAACCGTGGCTTCTGGAGCTAACGCGT
TAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAAT

TGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAAC

GCGAAGAACCTTACCTACTCTTGACATCCAGCGAATCCTTTAGAGATAGA

GGAGTGCCTTCGGGAACGCTGAGACAGGTGCTGCATGGCTGTCGTCAGCT

CGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCT

TTGTTGCCAGCACGTAATGGTGGGAACTCAAAGGAGACTGCCGGTGATAA

ACCGGAGGAAGGTGGGGATGACG

OL762483*Proteus mirabilis* strain LCU-MS-005 16S ribosomal RNA gene, partial sequence

GATGTCGATTTAGAGGTTGTGGTCTTGAACCGTGGCTTCTGGAGCTAACGC

GTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGA

ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCA

ACGCGAAGAACCTTACCTACTCTTGACATCCAGCGAATCCTTTAGAGATA

GAGGAGTGCCTTCGGGAACGCTGAGACAGGTGCTGCATGGCTGTCGTCAG

CTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATC

CTTTGTTGCCAGCACGTAATGGTGGGAACTCAAAGGAGACTGCCGGTGAT

AAACCGGAGGAAGGTGGGGATGACGT

OL762484 *Proteus mirabilis* strain LCU-MS-006 16S ribosomal RNA gene, partial sequence

GATGTCGATTTATAGGTTGTGGTCTTGAACCGTGGCTTCTGGAGCTAACGC

GTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGA

ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCA

ACGCGAAGAACCTTACCTACTCTTGACATCCAGCGAATCCTTTAGAGATA

GAGGAGTGCCTTCGGGAACGCTGAGACAGGTGCTGCATGGCTGTCGTCAG

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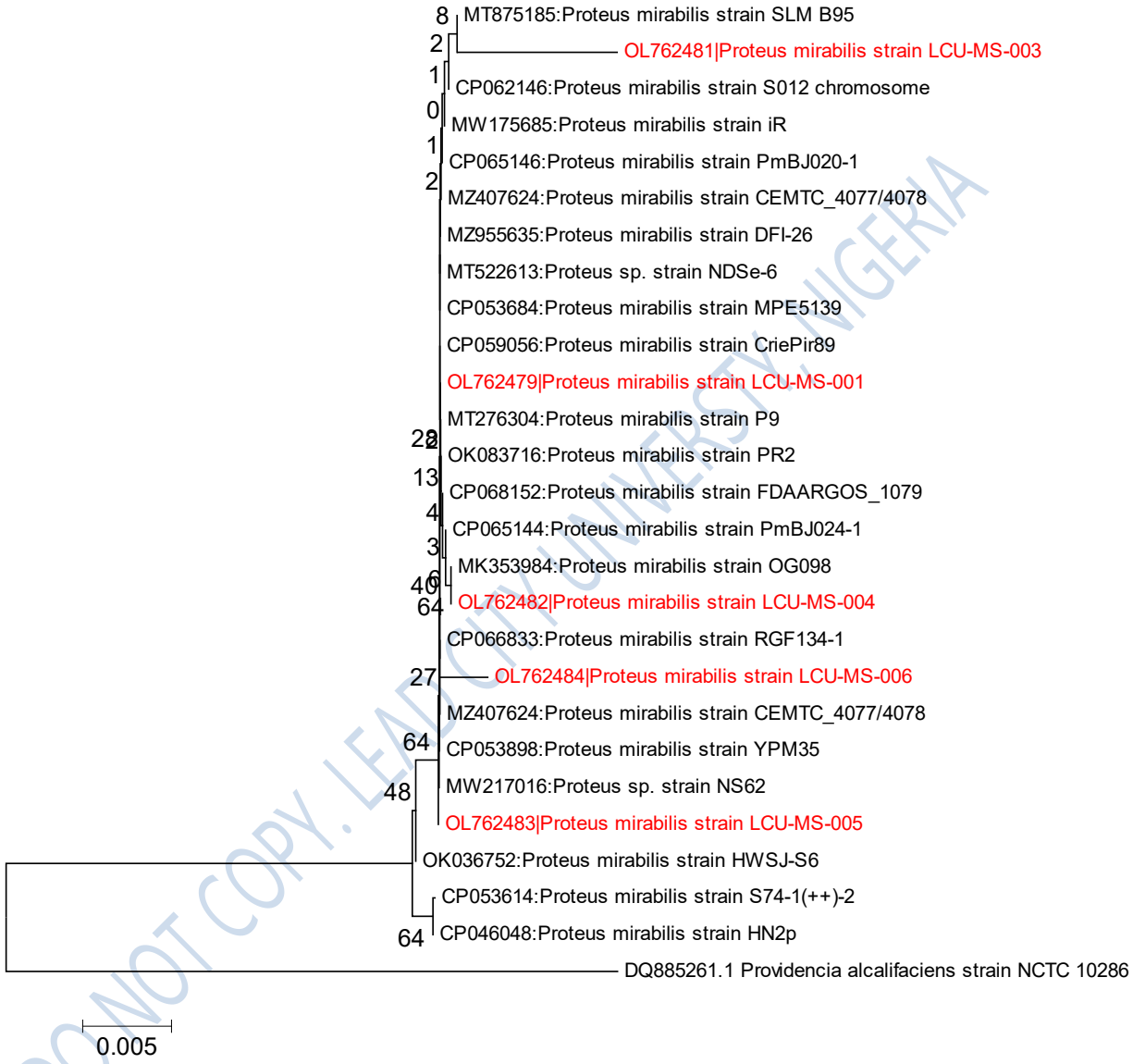


Fig. 4.8: Phylogenetic Tree of *Proteus mirabilis*

Source: Author's Field Work, 2022

Fig. 4.8 Evolutionary Relationships of Taxa for *Proteus mirabilis*

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 0.06909394 is 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 [2]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method [3] and are in the units of the number of base substitutions per site. The analysis involved 27 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 1479 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [4].

Fig 4.9 Isolate 9D[5] *Providencia vermicola*LCU-MS-002 16S strain 16S ribosomal RNA gene, partial sequence Accession number OL762480.

GTCGCTTTGTAGGGTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTT
AAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATT
GACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACG
CGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTAGCAGAGATGCTT
TGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTC
GTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTT
TGTTGCCAGCGATTCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAAC
CGGAGGAAGGTGGGGATGACGTA

OL762485 *Providencia vermicola* strain LCU-MS-007 16S ribosomal RNA gene, partial sequence

GGATGTCGCTTTGTGGGGTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAAC
GCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAAT
GAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATG
CAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTAGCAAAGA
TGCTTTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCA
GCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTA
TCCTTTGTTGCCAGCGATTCGGTCGGGAACTCAAAGGAGACTGCCGGTGA
TAAACCGGAGGAAGGTGGGGATGACGTA

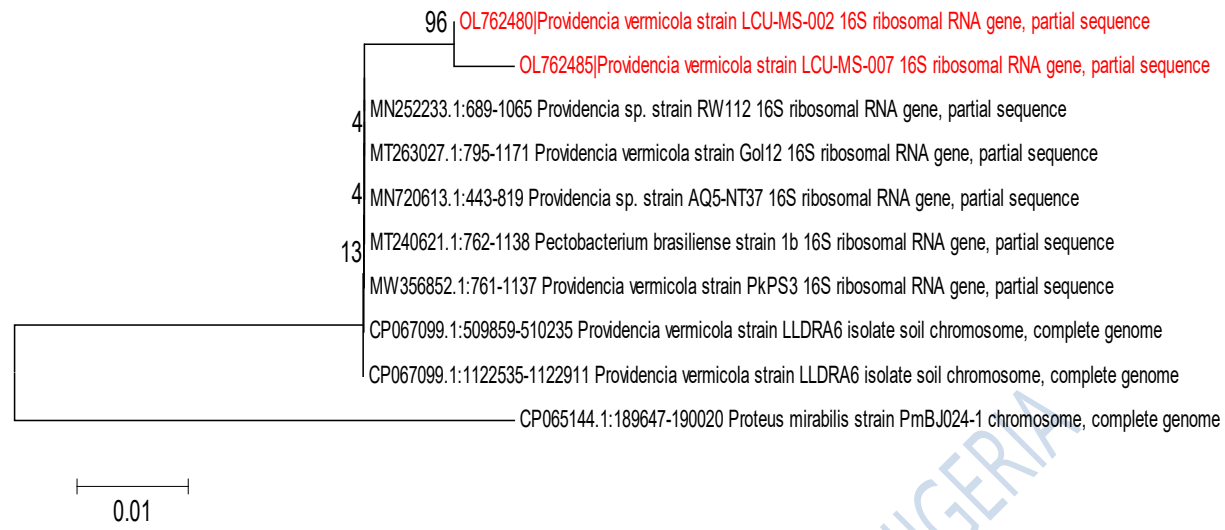


Figure 4.9: Phylogenetic Tree for *Providencia vermicola*

Source: Author's Field Work, 2022

Fig. 4.9 Evolutionary Relationships of Taxa for *Providencia vermicola*

Phylogenetic tree showing evolutionary relationship of *Providencia vermicola* obtained from in Ibadan, Nigeria (in red) with others stains available in the GenBank database Fig. 4.9

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 0.08965343 is 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 [2]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method [3] and are in the units of the number of base substitutions per site. The analysis involved 10 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 380 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [4].

Fig. 4.10: Isolate 9D[5] *Providencia vermicola*LCU-MS-008 16S strain 16S ribosomal RNA gene, partial sequence Accession number OL762486.

TCAACTAGCTGTTGGGGCCGTTAGGCCTTAGTAGCGCAGCTAACGCGTGA

AGTTGACCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTG

ACGGGGACCCGCACAAGCGGTGGATGATGTGGATTAATTCGATGCAACGC

GAAAAACCTTACCTACCCTTGACATGTCTGGAAAGCCGAAGAGATTTGGC

CGTGCTCGCAAGAGAACCGGAACACAGGTGCTGCATGGCTGTCGTCAGCT

CGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCA

TTAGTTGCTACGCAAGAGCACTCTAATGAGACTGCCGGTGACAAACCGGA

GGAAGGTGGGGATGACGT

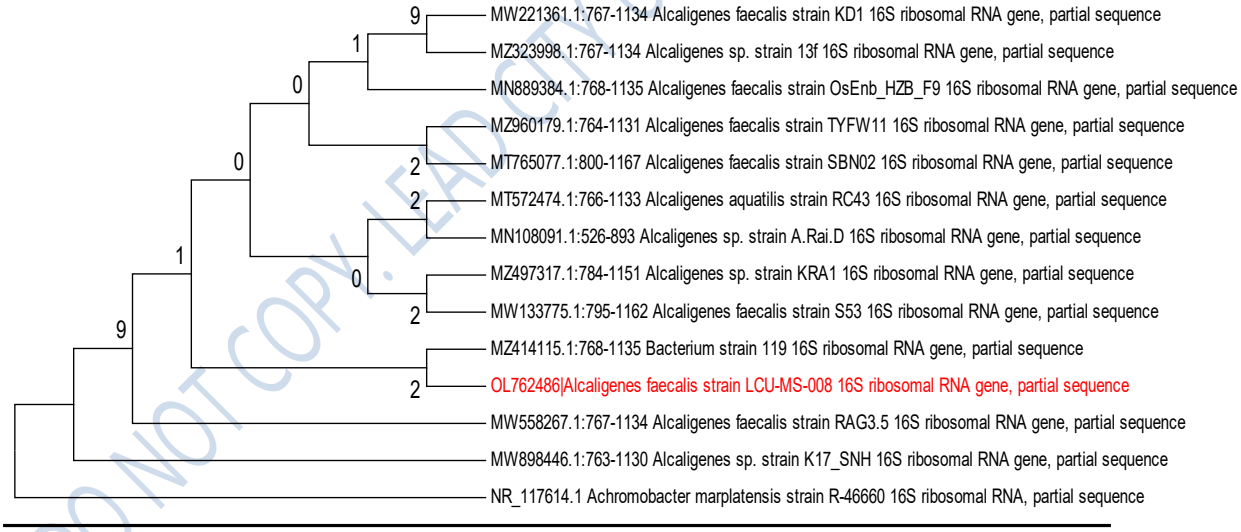


Figure 4.10: Phylogenetic Tree for *Alcaligenes faecalis*

Source: Author's Field Work, 2022

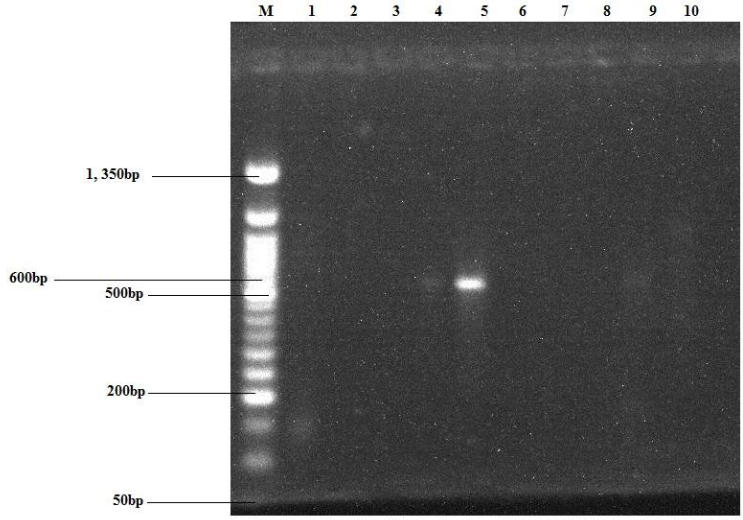
Fig. 4.10:Evolutionary Relationships of Taxa for *Alcaligenes faecalis*

Phylogenetic tree showing evolutionary relationship of *Alcaligenes faecalis* obtained from in Ibadan, Nigeria (in red) with others stains available in the GenBank database Fig. 4.10

The evolutionary history was inferred using the Neighbor-Joining method [1]. The bootstrap consensus tree inferred from 1000 replicates [2] is taken to represent the evolutionary history of the taxa analyzed [2]. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [2]. The evolutionary distances were computed using the Kimura 2-parameter method [3] and are in the units of the number of base substitutions per site. The analysis involved 14 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 1509 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 [4].

4.9 Detection of Antibiotic Resistant Genes

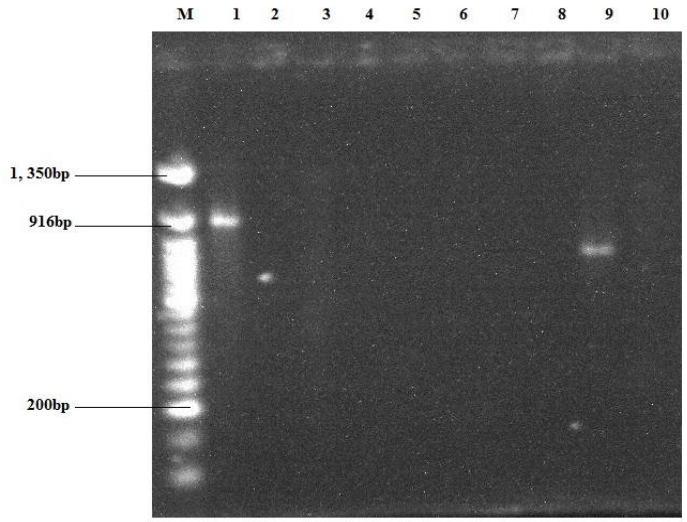
Different antibiotic resistant genes was used (*CTXM, OXA*-48*, qnrA, qnrB, qnrC, qnrSM and TEM*). β-lactams (*CTX-M, OXA*-48*, TEM*)andquinolones (*qnrA, qnrB, qnrC, qnrSM*)resistance gene. The isolates were screened for the seven (7) genes coding for resistance to antimicrobials in two families (beta-lactams and quinolones). In quinolones *qnrC* genes were not amplified on all the isolates (Fig. 4.15). But *qnrSM* resistance genesshows 80% on all the isolates which makes it highest gene resistance identified (Fig. 4.16). *TEM* in beta-lactams gene has 50% of resistance gene on the isolates (Fig. 4.17). Figure4.11 to 4.17show images of Gel electrophoresis of detected antimicrobial resistance genes.



Gel image showing amplification of CTXM at about 600bp.
M is 50bp ladder.

Fig. 4.11: A gel electrophoresis showing the CTXM (500 bp) gene. M = Molecular ladder (50 bp), lane 5 positive isolates. Lane M; Molecular ladder

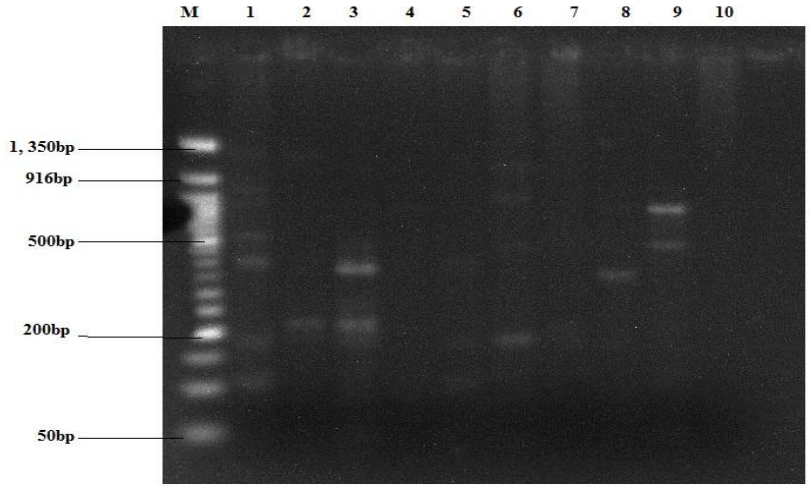
Source: Author's Field Work, 2022



Gel image showing PCR amplification of OXA-48
M is 50bp ladder.

Fig. 4.12:A gel electrophoresis showing the OXA-48 (600 bp) gene. M = Molecular ladder (50 bp), lane 1and 9 positive isolates. Lane M; Molecular ladder

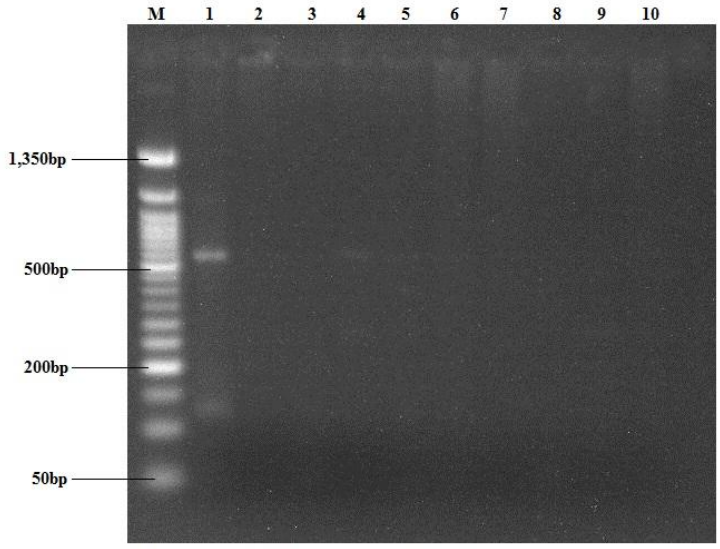
Source: Author's Field Work, 2022



Gel image showing PCR amplification of qnrA
M is 50bp ladder.

Fig. 4.13: A gel electrophoresis showing the qnrA (200 bp) gene. M = Molecular ladder (50 bp), lane 1-3, 5-6 and 8-9 positive isolates. Lane M; Molecular ladder

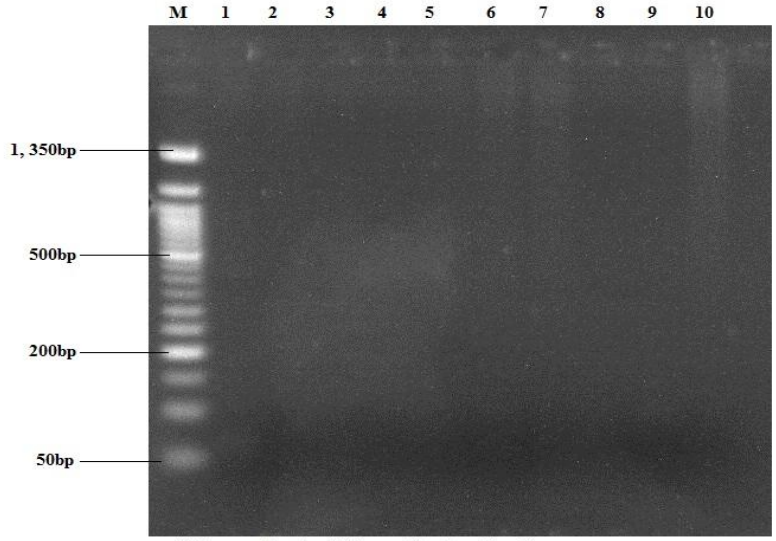
Source: Author's Field Work, 2022



Gel image showing PCR amplification of qnrB.
M is 50bp ladder.

Fig. 4.14: A gel electrophoresis showing the qnrB (550 bp) gene. M = Molecular ladder (50 bp), lane 1 positive isolates. Lane M; Molecular ladder

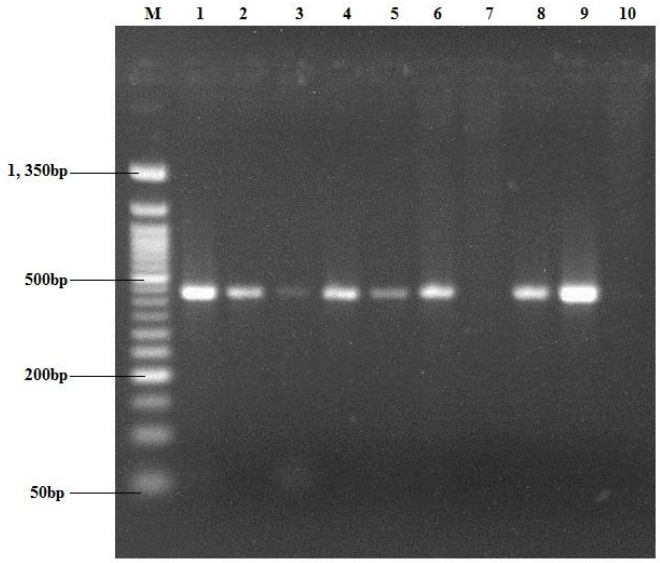
Source: Author's Field Work, 2022



Gel image showing PCR amplification of qnrC
M is 50bp ladder.

Fig. 4.15: A gel electrophoresis showing the qnrC (0 bp) gene. M = Molecular ladder (50 bp), No resistant gene on all the lanes. Lane M; Molecular ladder qnrC

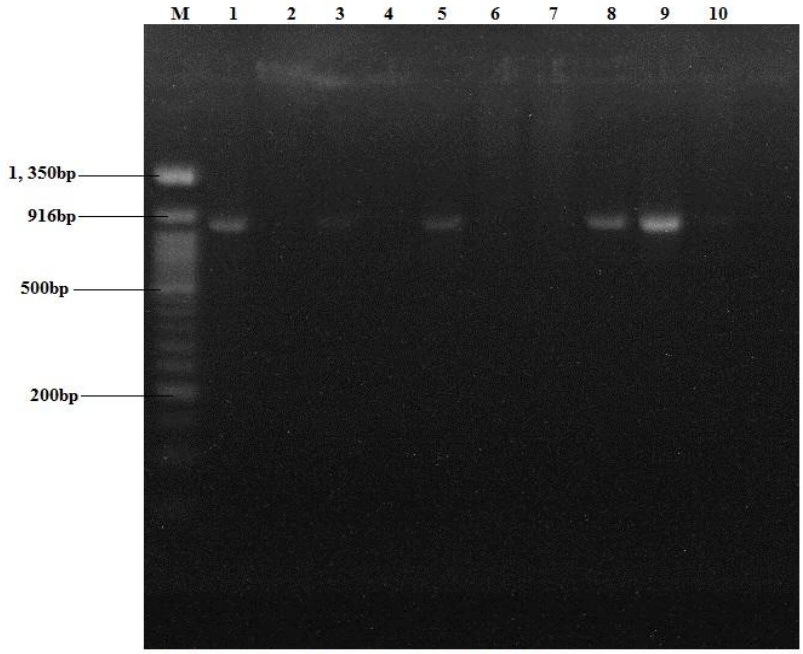
Source: Author's Field Work, 2022



Gel image showing PCR amplification of qnrSM
M is 50bp ladder.

Fig. 4.16: A gel electrophoresis showing the qnrSM (450 bp) gene. M = Molecular ladder (50 bp), lane 1-6 and 8-9 positive isolates. Lane M; Molecular ladder.

Source: Author's Field Work, 2022



Gel image showing PCR amplification of TEM.
M is 50bp ladder.

Fig. 4.17: A gel electrophoresis showing the TEM (500 bp) gene. M = Molecular ladder (50 bp), lane 1,3,5,8 and 9 positive isolates. Lane M; Molecular ladder

Source: Author's Field Work, 2022

4.2 Discusssion of Findings

Traditional Nigerian fermented foods and condiments are made mostly on a small scale in households under highly varied conditions, resulting in commodities of variable quality. The fermented condiments are used to improve the flavour of foods and the ones (fermented Iru, Ogi and Ogiri) utilized in this study are used by families on almost daily basis to prepare several savoury dishes with appealing taste and flavour and could also result in dishes with higher nutritional quality[5]. Ogi remains a staple in many Nigerian households and are made from a variety of starchy grains such as maize, sorghum and millet. The gruel made from maize and sorghum is still the most consumed out of the three and are now being sold in many markets and by neighbourhood hawkers forming small cottage businesses engaged in by individuals[6].

Attempts were made to identify organisms isolated phenotypically first, and selected ones based on their antibiotics resistance profile molecularly. Phenotypic identification was based on tests like catalase, indole, hydrogen sulphide and Acid production tests, citrate utilization, MR-VP test, Starch hydrolysis, Haemolysis test[7]. It was however observed that for those selected and subjected to molecular identification, the phenotypic identification didn't tally with the molecular identification and it may be due to the fact that the basis of phenotypic characteristics is generally not always accurate as identification based on genotypic methods [8].

The 10 isolates that was selected for molecular identification were also identified phenotypically, the suspected microorganisms were *Staph. aureus, Pseudomonas aureginosa, Microbacterium flavum, Pediococcus, Streptococcus, Moraxella and Salmonella.* Using the molecular techniques the identified organism was different from phenotypic identification. P*roteus mirabilis, Providencia vermicola* and *Alcaligenes faecalis* were the organism from molecular identification.

Isolation and phenotypic identification of 2 strains of *Lactobacillus brevis* and 2 isolates of lactic acid fungi (*Aspergillus flavus and A niger*) has been reported as the predominant lactic acid organisms isolated from the spontaneous fermentation of yam using traditional (biochemical) methods. These same organisms were identified as *Bacillus subtilis, B. pumilus, Aspergillus flavus* and *Aspergillus niger* respectively using molecular methods [9]. Data from the present study showing 50% disparity in identification results when the results from the phenotypic (biochemical) and genotypic (molecular) methods were compared confirms earlier reports that phenotypic identification of microorganisms carries a high risk of misidentification and that genotypic identification using molecular methods are superior to biochemical methods [10].

Despite the fact that the American food supply is among the safest in the world in terms of antibiotic resistance, people can still suffer food poisoning by eating tainted foods. Antibiotic-resistant bacteria are responsible for some cases of food poisoning. Foods, particularly fermented foods, are ecosystems that favor bacterial development, both for human health and as a food safety issue[11].Antibiotic-resistant bacteria infection symptoms are similar to those of other types of food poisoning, which range from moderate to life-threatening and include diarrhea, nausea, and vomiting[12].

Antibiotics resistance test was carried out on all isolated microorganisms and most of the organisms showed multidrug resistance to the tested antibiotics. The antibiotic resistant pattern from organisms found in food. Conclusion was made on other studies that multidrug resistance bacteria in foods are still very scarce and need to be further investigated [13].

Previous research on multidrug resistance organisms in fermented foods and condiments had limitations in terms of methodology, reproducibility, and the possible

organisms found in fermented foods. Traditional microbiological cultivation methods and phenotypic tests were also used to generate the data, which only provided limited information on taxonomic, genetic, and uncultivable microbes[14].

As a result, this study was carried out in order to provide adequate information on multidrug resistance in isolates from fermented foods and condiments using culture-independent molecular techniques for characterization and identification.

In total, 61 isolates were obtained from fermented foods and condiments as discussed in table 4.1, table 4.2, table 4.3, table 4.4, and table 4.5 in the results, isolates along with the results of the various biochemical tests. Each fermented foods and condiments revealed multiple isolates of diverse types in the majority of cases.

As suggested by Abriouel there is need for closer investigations on antibiotics-in-food safety. Tremendous resistance to the test antibiotics, by associated bacterial flora of the local fermented food and condiments were also indicated in this study[15].

So, in this study, narrow and broad-spectrum test antibiotics (belonging to the *β*-lactams, aminoglycosides, macrolides, ampicillins, quinolones) were used for 50 isolates out of 61.

Worldwide, the contribution of resistant microbes from various sources seems to be the major base of resistance in the environment[16]. The results from this study revealed that various fermented foods and condiments get contaminated with bacteria, which can be highly pathogenic to human. In the study, *Proteus mirabilis* was the most common bacterial isolated with an occurrence of 50%, 7 antibiotic resistant genes was used (*CTX-M, OXA, qnrA, qnrB, qnrC, qnrSM and TEM*), *P .Mirabilis* was not resistance to qnrC. . According to Huang 2017 *P .Mirabilis* carried a *bla CTX-M* gene (44.74%) and product size (424 bp), Manar 2019 also discover in their study that *P .Mirabilis* has no qnrC resistance gene on *P .Mirabilis*[17,18].

Emerging resistant strains of *A. faecalis* to imipenem and meropenem have been found since 2018, high resistance rate of many antibiotics was also found in 2019. The best sensitivity rate to *A. faecalis* was 66.7% for three antibiotics (imipenem, meropenem, and ceftazidime). Four articles mentioned that *A. faecalis* is susceptible to colistin [19,20,21]. In this study *A. faecalis* was not resistance to all the antibiotic genes (*CTX-M, OXA, qnrA, qnrB, qnrC, qnrSM and TEM*) that was tested on all the isolates. In this present study, 10 isolates were screened for β- lactams *CTX-M, OXA, TEM* andquinolones *qnrA, qnrB, qnrC, qnrSM* resistance gene. 10%of the isolates showed resistance to the *CTX-M, OXA and* qnrB genes respectively, 60% to qnrA, 80% to qnrSM and 50% to TEM.

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Chapter Five

Conclusion

5.1 Summary of Findings

The Nigerian indigenous fermented foods constitute a group of foods that are produced in homes, villages and small scale cottage industries at prices within the means of a majority of consumers. During harvesting, processing, storage, shipping, and preparation, several distinct bacterial species are responsible for food contamination. Fermented foods, on the other hand, are one of the principal habitats of several indigenous bacteria that have a significant impact on food by changing various dietary substrates

The finding from this present study showed that each fermented foods and condiments revealed multiple isolates of diverse types in the majority of cases. The results from this study revealed that various fermented foods and condiments get contaminated with bacteria, which can be highly pathogenic to human. Using the molecular techniques method these organisms were identified, P*roteus mirabilis, Providencia vermicola* and *Alcaligenes faecalis.* Unique accession numbers for these isolates were generated on NCBI Genebank database. 10 isolates were screened for β- lactams *CTX-M, OXA, TEM* andquinolones *qnrA, qnrB, qnrC, qnrSM* resistance gene. 10%of the isolates showed resistance to the *CTX-M, OXA and* qnrB genes respectively, 60% to qnrA, 80% to qnrSM and 50% to TEM In the study, *Proteus mirabilis* was the most common bacterial isolated with an occurrence of 50%, 7 antibiotic resistant genes was used (*CTX-M, OXA, qnrA, qnrB, qnrC, qnrSM and TEM*), *P .Mirabilis* was not resistance to qnrC.

5.2 Conclusion

Although the extent of which the food sector is contributing to the creation of antibiotic resistance is still largely unknown, this research however has demonstrated the need to further investigate this seeming problem so that safe and high-quality foods are assured.

The isolates obtained showed that more than one gene was linked with a given phenotypic resistance. This is of great concern and demands caution in the production of locally fermented foods.

The points of entry of antibiotic resistant bacteria and their resistant genes must be identified and suitable actions must be taken to prevent their continuous transmission in primary food production and food processing, so that the safety of foods can be assured.

5.3 Contribution to Knowledge

The high resistance to antibiotics is noteworthy in view of its health significance and impact on food safety. The consumption of foods containing high resistant strains could serve as a means of spreading resistance to antibiotics among the consumers and further promote the perennial problem of disease treatment by antibiotics.

5.4 Area of Further Research

There is the need for other researcher to investigate the genetic locations and transferability of the multidrug resistance. They can also determine whether the resistance is intrinsic or acquired.

5.5 Recommendation

It is recommended that multidrug resistance bacteria in foods are still very scarce and need to be further investigated.

Public awareness and sensitization of stake holders should be done on the need to improve hygiene in local fermented foods, especially those sold commercially.

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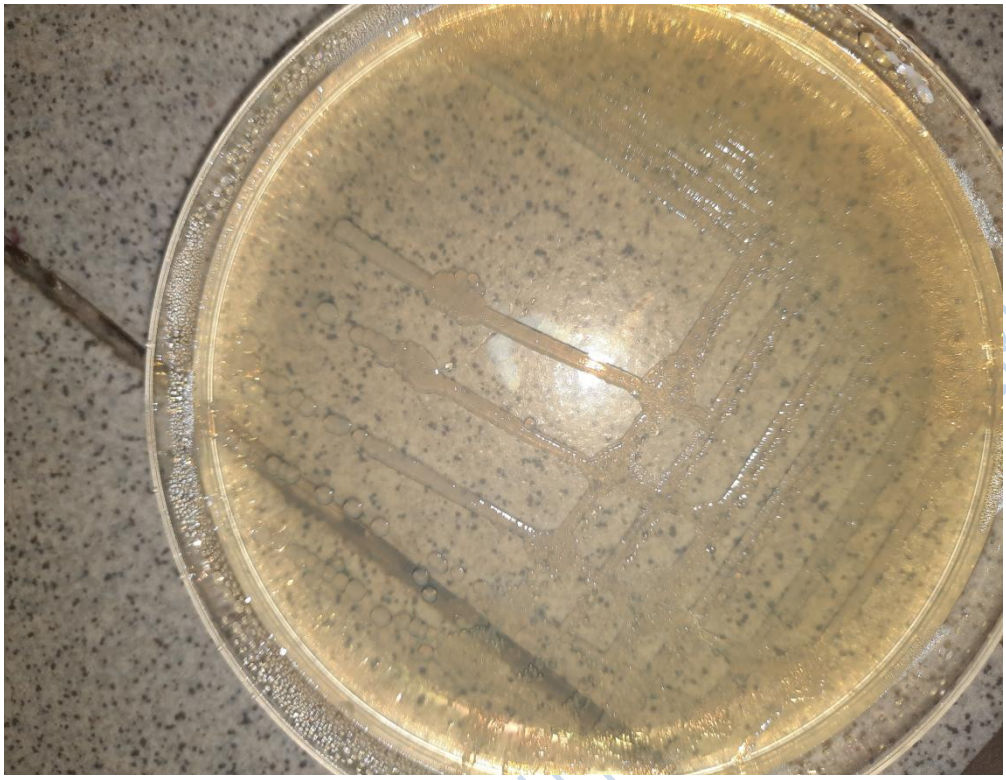
Appendix i

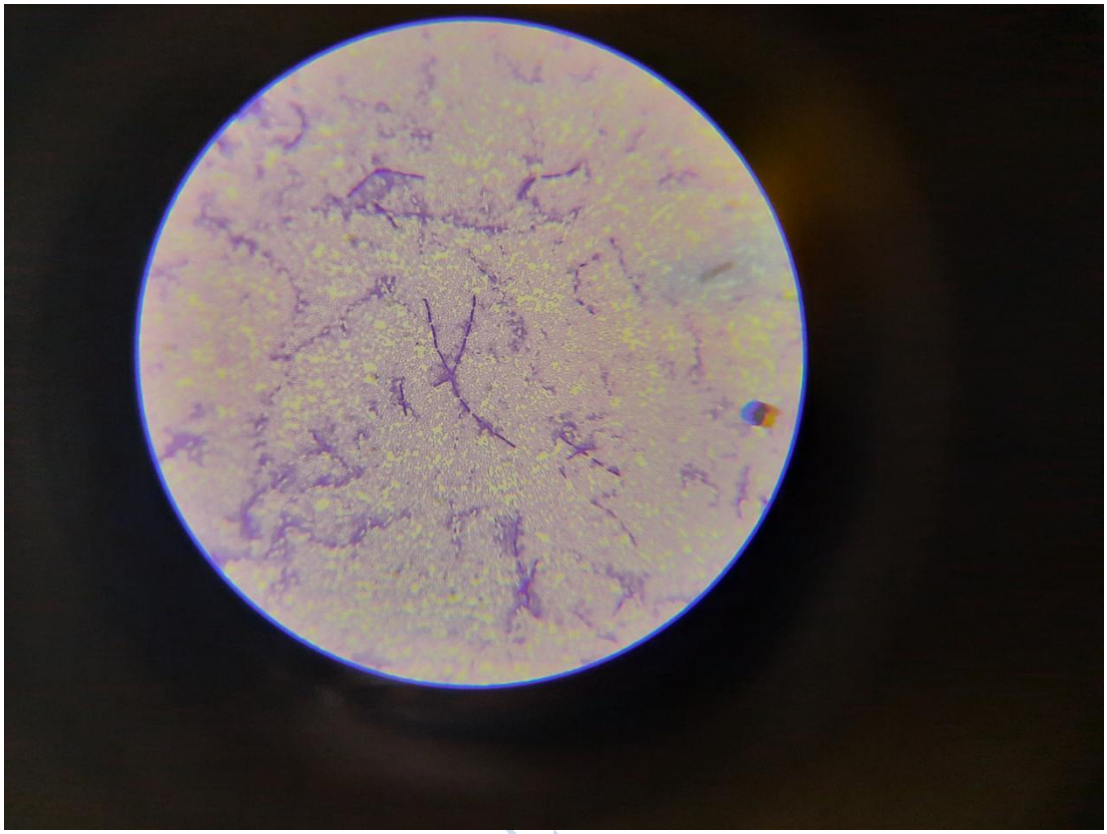
Isolate Subcultured on Nutrient Agar Plate



Source: Author's Field Work, 2022

Appendix ii

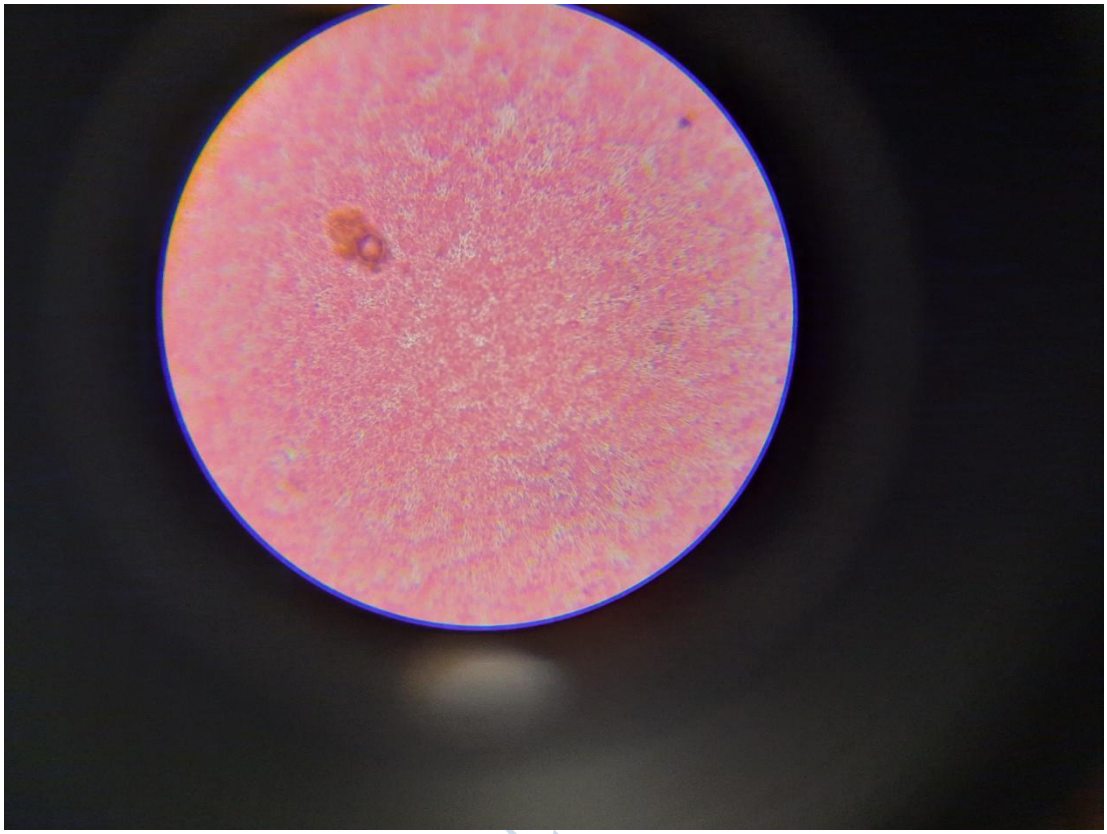
Gram Negative Rod viewed on 100x Immersion Oil Microscope



Source: Author's Field Work, 2022

Appendix iii

Gram Positive Cocci viewed on 100x Immersion Oil Microscope



Source: Author's Field Work, 2022

Appendix iv

Antibiotic Susceptibility Plate

Source: Author's Field Work, 2022

Appendix v

Antibiotic Susceptibility Plate

Source: Author's Field Work, 2022

Bio-data

A. Personal Data:

1. Full Name:	Kehinde Mary, AKINDE Plot 6, Lagelu Estate, Felele, Ibadan, Oyo State. ololadeakinde@gmail.com 07068477598
2. Date and Place of Birth:	18th November, 1989; Ijale Ketu
3. Nationality:	Nigerian
4. Name and Address of Next of Kin:	Akinde Taiwo David 12 Paul Street, United Estate, Sangotedo Lagos State.

B. Educational Background:

School Attended	Date	Qualifications
❖ Y.N.L.G. School II, Oja-Odan.	1995-2001	First Leaving Sch. Cert.
❖ Comprehensive High Sch. Ayetoro.	2001-2007	S.S.C.E
❖ Lead City University, Ibadan.	2008-2012	B.Sc Microbiology
❖ Lead City University, Ibadan.	2019-2022	M.Sc in view

C. Working Experience with Dates

Unofficial research assistant 2019-Till Date

D. Awards and Fellowship Nill

E. Membrship of Academi Professional Bodies Nill

F. Publication: 3rd FASCON Conference 2022 Poster Presentation

Date Signature

University Compliance Certificate

This is to certify that the thesis by **Kehinde Mary, AKINDE** with Matric no LCU/PG/000405 in the department of **Biological Science**, Faculty of Natural and Applied Science, Lead City University, is in full compliance with the approved university format and style.

_____ _____

Signature Date