BACTERIOLOGICAL QUALITY OF KUNU DRINKS SOLD IN ALIMOSHO LOCAL GOVERNMENT AREA, LAGOS STATE, NIGERIA.

BY

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES AND BIOTECHNOLOGY, COLLEGE OF PURE AND APPLIED SCIENCES,

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ABSTRACT

Kunu is a nourishing cereal-based non-alcoholic beverage popular in Nigeria. There is no standardized method for its preparation, so people's production practices vary. Despite its nutritional benefits, kunu consumption has been linked to an increased risk of food-borne diseases. Therefore, this study was aimed to investigate the bacteriological status of kunu. Duplicate kunu samples were obtained from eight vendors in Alimosho and a control sample was prepared in the laboratory. These samples were stored for three days at different temperatures before isolation and identification of isolates using standard biological methods. Susceptibility of the bacterial isolates to antibiotics was determined by disc diffusion method. The results obtained showed that refrigerated samples had lower bacterial counts than those kept at room temperature. Total heterotrophic and total coliform counts ranged from $2.00x10^3$ - $1.42x10^6$ cfu/ml and $5.00x10^3$ - $8.48x10^5$ cfu/ml, respectively. In this study, 11 bacteria genera were isolated in decreasing order of frequency: *Enterobacter* spp, *Klebsiella* spp, *Salmonella* spp., *Escherichia coli*, *Citrobacter* spp., *Acinetobacter* spp., *Aeromonas* spp., *Alcaligenes* spp, *Plesiomonas* spp., *Proteus* spp., and *Providencia* spp. Majority of the microorganisms were found to be susceptible to Gentamycin (80%) and Taravid (76%), while majority were resistant to Chloramphenicol indicating this may not be a good option in treating infections caused by kunu consumption. The bacteria isolated from kunu and their densities could be attributed to kunu producers' poor hygiene and this can lead to variety of infections. Therefore, to ensure that kunu is safe for human consumption, regulatory bodies should enforce the system of monitoring the production, preservation, and packing techniques. Also, manufacturers of kunu should check and improve the quality control process followed for the manufacture of kunu drinks.

DECLARATION

I, IYIOLA COMFORT OLUWAPELUMI, do hereby declare that the project work titled Bacteriological Quality of Kunu Sold in Alimosho Local Government Area, Lagos State, Nigeria is a record of an original work done by me, as a result of my research effort carried out in the Departments of Biological Sciences and Biotechnology, Caleb University, Imota, Lagos. All references made to works of other persons have been duly acknowledged.

_____ _____

Student's Signature Date

CERTIFICATION

This is to certify that this research work was carried out by Iyiola, Comfort Oluwapelumi with matric number 18/4325 in the Department of Biological sciences and Biotechnology, College of Pure and applied science, Caleb University, Imota, Lagos, Nigeria, under the supervision of Dr. Kotun, B. C.

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External examiner Date

DEDICATION

This project is wholeheartedly dedicated to the Almighty God and to my beloved parents, Engineer and Mrs. Iyiola Adeagbo, who continually provide their moral, spiritual, emotional and financial support.

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First and foremost, praises to God, the Almighty, for His blessings and guidance throughout my project work, which enabled me to successfully complete the research.

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CHAPTER ONE

INTRODUCTION

Kunu is a local non-alcoholic, non-carbonated beverage produced and consumed in large quantity in Nigeria, especially in the northern part of the country (Abubakar *et al.*, 2013; Agbo *et al.*, 2013).

As stated by Aseibai *et al.* (2015), kunu is the generic term for all kinds of cereal-based non-alcoholic beverages. Different from zobo, which is produced from only one plant, the preparation of kunu varies among people and can be prepared from several plants, including sorghum (*Sorghum bicolor*), millet (*Penisetum typhoides*), maize (*Zea mays*), rice (*Oryza sativa*), wheat (*Triticum aesstivum*), and acha (*Digitalis exilis*). Depending on the feedstock used for processing, there are various types of Kunu. This includes Kunun zaki, Kunun gyada, Kunun akamu, Kunun tsamiya, Kunun baule, Kunun jiko, Amshau, and Kunun gayamba. Of these, Kunun zaki is the most widely produced and consumed (Aseibai *et al*., 2015).

Agbo *et al*. (2013) have recorded that kunu is rich in carbohydrates, vitamins, and minerals but is generally low in protein and several of the essential amino acids. However, sorghum-made kunu contains starch, protein, fat, fiber, and ash, along with a wide array of amino acids (Antai *et al*., 2017). Both millet and Guinea corn have at least 82% starch each (Adeware &Fapohunda, 2012).

This drink, however, is still manufactured at the local level of technology, although it is also now produced industrially and branded. Wet milling of cereal grains with spices, wet sieving and partial gelatinization of the curry, sugar addition, bottling, and sales are all part of the

preparation process. After 6-24 hours of fermentation, the drink is ready for consumption. Kunu can be prepared using local household equipments and ingredients like ginger (*Zingiber officinale*), aligator pepper (*Aframomum melegueta*), red pepper (*Capsicum* species), black pepper (*Piper guineense*) , Kakandoru or Eru, cloves, and sugar are commonly added as flavor and taste improvers (Abubakar *et al.*, 2013; Agbo *et al*.,2013; Antai *et al*., 2017; Aseibai *et al.*, 2015). The final product is a thin, free-flowing gruel. The drink prepared from sorghum has a milky light brown color, but those made from millet and maize have a whitish color. It is consumed by people of all social backgrounds and ages, particularly in the northern part of Nigeria. Variation in preparation methods can be attributed to cultural differences and individual preferences, which explains the lack of consistency in product taste, quality and specifications (Antai *et al*., 2017; Anumudu and Anumudu, 2019).

Kunu has relatively short shelf-life storage and could serve as a medium for transmission of pathogenic microorganisms (Agbo *et al*., 2013; Antai *et al*., 2017). According to research, Kunu drink has a shelf life of 24 hours at room temperature, which can be extended to days by refrigerating and pasteurizing at 600°C for 1 hour (Agbo *et al*., 2013). Kunu can undergo deterioration due to the presence of microorganisms in the drink that help in the fermentation process. Many organisms can use the carbohydrate content for fermentation processes, causing undesirable changes in Kunu. As stated by Aboh and Oladosu (2014), Kunu is prone to microbial contamination because of its high water content (about 85%) combined with crude methods of production and packaging in unsanitary settings.

Furthermore, although the raw materials used in the production of kunu may contain pathogenic microbes, these microbes can be eliminated through the cooking process, but their

toxins might be heat stable and may not be destroyed. Kotun and Odebode (2019), for example, identified a toxigenic *Penicillium* species from millet grains collected in Southwestern Nigeria. Various studies have revealed that kunu drinks contain lactic acid bacteria like *Lactobacillus* spp., *Streptococcus* spp., and *Leuconostoc* spp., as well as other bacteria such as *Escherichia coli, Enterobacter aerogenes*, *Staphylococcus aureus, Streptococcus* spp., *Bacillus* spp., *Pseudomonas* spp., *Trichoderma* spp., *Salmonella* spp., *Micrococcus* spp. The microbial diversity also includes fungi such as *Saccharomyces* spp., *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., and *Candida* spp. (Abubakar *et al*., 2013; Adamu *et al*., 2014; Agbo *et al*., 2013; Antai *et al*., 2017; Aseibai *et al.*, 2015). The presence of these microbes in the kunu drink could cause the spoilage of the kunu beverage, which in turn causes intoxication in man. Furthermore, the activity of the natural food enzymes could contribute to the spoilage. Temperature, timing, light, and the presence of insects, rodents, or pests in the producing environment are all aspects to consider. These factors, if not adequately controlled, contribute to the spoilage of kunu. Kunu has a very high moisture content, which may encourage growth of strains to dangerous levels during storage at ambient temperature (Agbo *et al*., 2013).

The consumption of this local drink may serve as a source of food-borne diseases posing risks to public health (Antai *et al*., 2017). Though there are lots of literatures on the microbiological and nutritive quality of kunu drinks, there is scarce or no information on the safety and nutritional status of kunu drinks sold in Alimosho. It is therefore necessary for this study to focus on investigating the microbiological characteristics of kunu samples sold in Alimosho, Lagos State, Nigeria. This study is designed to assess the bacteriological quality of

this popular beverage produced locally and sold in Alimosho and the health risks it can pose to its consumers as it will help to improve public health and curb a major means through which pathogens are spread.

1.1 Significance of the Study

The significance of this work is to enable the public to be aware of bacterial species present in kunu hence increasing the health awareness on the dangers of drinking kunu. It is also to enable producers to improve the hygienic condition and handling of kunu during production.

1.2 Statement of Problem

Kunu is already widely consumed by the Nigeria populace because of its low cost, health and nutritional benefits. Despite the economic and nutritional benefits of kunu, its consumption has been suggested to potentially increase the risk of food-borne diseases as it is readily contaminated from different sources.

1.3 Justification for Study

Most kunu vendors lack the adequate knowledge of food processing and adequate handling practices as such, there is likely to be a high risk of chemical and microbial contamination. A large number of lactic acid bacteria, coliforms, molds and yeast have been reportedly implicated in kunu spoilage as they use its carbohydrate content for undesirable fermentation processes. Therefore, it becomes very necessary to conduct this research to determine the bacteriological quality of this non-alcoholic drink called kunu in Alimosho, Lagos State, as there is limited study on the kunu drinks sold in this area. The effect of storage on each drink varies, hence there is a need to know the more appropriate method of

storage, whether ambient or refrigerated, to reduce the incidence of certain diseases. It is also necessary to conduct this study to add to the body of knowledge.

1.4 Aim of Study

To investigate the bacteriological status of kunu drinks sold in Lagos State.

1.5 Objectives of the Study

The main objectives of this study include;

1. To randomly obtain kunu samples
2. To isolate the bacteria species found in kunu drink samples.
3. To observe the effect of storage at different temperatures on the bacterial load of kunu.
4. To characterize isolates obtained using biochemical parameters.
5. To determine the antibiotics reactions of isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1 HISTORY OF KUNU

Kunu is a well-known drink that originates from Northern Nigeria, in the early 1800s, Hajiya Fatima from one of the northern states in Nigeria thought of a beverage to help people combat the hot weather and the idea of cold, iced or chilled kunu came to her mind (Ayo-Omogie & Okorie, 2016). Overtime, it has not only become a common beverage in the northern part of Nigeria but the whole country as well. It can be prepared with millet, rice, groundnuts, maize or sorghum. The principal ingredients used in the production of the drink and groundnut milk, it is called kunu gyada. If the drink is made with millet and potatoes, it is called kunu zaki. If the drink is made from tiger nuts, it is called kunu aya (Ofudje *et al.*, 2016). The choice of ingredients and invariably, the type of drink can be dependent on the producer's interest, availability of raw material and cost (Oluwajoba *et al*., 2014). Kunu is enjoyed in Nigeria today by individuals of different ethnic groups. It is a non-alcoholic beverage and very nutritious as well. The consumption of this beverage cuts across all age groups and social status. The peak period of consumption is usually from February to June. The weather is usually hot at this time so chilled drinks are a welcome relief from the hot weather (Olopade *et al*., 2015).

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2.1.1 METHOD OF PREPARATION AND INGREDIENTS OF KUNU DRINK

The production of kunu is still at village technology level where procedures and materials or ingredients used are always not standardized, although it is now produced industrially and branded. The traditional production process involves steeping of the chosen cereal or mixture of the cereals in water for 24 - 72 hours and wet milling with the aid of a local grinding machine (Edward *et al*., 2019). The steeped and washed grains are usually ground with spices such as ginger (*Zingiber officinale*) and alligator pepper (*Aframomum melegueta*) or red pepper (*Capsicum annuum*), or black pepper (*Piper nigrum*) depending on the taste of the local producer. The slurry obtained is sieved and divided into two unequal portions. A portion (two-third volume) of the slurry is gelatinized with boiling hot water, while to the remaining portion (one-third volume), about an equal volume of sweet potato (*Ipomoea batatas*) tuber paste, malted rice paste or extract of *Cadaba farinosa* stem is added and mixed. The latter mixture is mixed vigorously and thoroughly with the former (the gelatinized portion while still hot) and allowed to ferment for 24 hours at room temperature, and then filtered using a local sieve. The filtrate (kunu-zaki) is consumed as a beverage with or without the addition of

a sweetener, usually sugar. The beverage can be consumed fresh or bottled and stored at refrigeration temperature (Kelechi *et al*., 2020).

2.1.2 NUTRITIONAL VALUES OF KUNU DRINK

The nutritional composition of kunu has been reported to consist of 2.31 - 3.63% (protein), 3.55 - 3.63% (fat), 1.16 - 1.21% (ash), 82.92 - 83.55% (carbohydrate) (Abiodun *et al.*, 2017). The most abundant amino acid in kunu is glutamic acid (4.49-11.66g/100g) with the least being cysteine (0.34-1.45g/100g) (Adelekan *et al*., 2019). The lowest amount of amino acid except for tryptophan was reported to occur when rice was used as substrate to produce kunu beverage (0.44-1.40 g/100g). Also, among the amino acids, cysteine, valine, isoleucine and methionine are present in trace amounts when compared with FAO (Food and Agriculture Organization) and WHO (World Health Organization) reference protein values (Adelekan *et al*., 2019).

2.1.3 HEALTH BENEFITS OF KUNU CONSUMPTION

Kunu drink helps lower blood sugar and control blood glucose in persons living with diabetes. It does this by stimulating the pancreas to produce a reasonable amount of insulin. Drinking a cup of kunu morning and evening helps boosts ovulation in women and increases their chances of getting pregnant. Also, kunu drink relaxes the blood vessels such that it lowers blood pressure. Kunu is good for hypertension and it helps lowers excess blood cholesterol. Too much cholesterol in the blood can cause stroke and coronary heart diseases. Kunu is good for the brain and heart (Abiodun *et al.*, 2017). Kunu is very good and safe in pregnancy. It is rich in vitamins and folic acid. Folic acid is good for pregnant women because it prevents spinal bifida and congenital abnormalities in babies. Kunu stimulates

oxytocin production, a hormone that stimulates the breasts to release milk. Nursing and breastfeeding mothers who take kunu find it easy to breastfeed. Kunu treats indigestion and promotes proper digestion of food. Kunu drink is a high fibre diet that promotes bowel movements and treats constipation (Adelekan *et al*., 2019).

2.2 SOURCES OF MICROORGANISMS IN KUNU

Microorganisms can get into kunu drink from both natural and exogenous sources. The degree of sanitation used in kunu drink handling affects the sources of microbiological kinds. Some of these microbes are common microflora in natural environments; they exist in ecological balance with their hosts, but when food is invaded, they become destructive, causing food deterioration. Natural sources for foods of animal origin include the skin, hair, gastrointestinal tract, plumes, and milk ducts, to name a few. Natural sources for foods of plant origin include the surfaces of fruits, vegetables, and cereals, as well as the pores in some tubers (Akoma *et al*., 2014).

External sources come into touch with microorganisms throughout the creation, harvesting, and consumption of kunu drink. Air, earth, humans (food handlers), equipment, containers, and insects can all be used as external sources of kunu drink. Microorganism sources in kunu drink must be understood in order to develop methods for monitoring microorganism access to food, developing processing procedures for killing microorganisms in foods, determining food microbiological quality, and setting microbiological standards and food ingredient specifications (Adeniji & Keshinro., 2015).

Microorganisms have been used to produce food for thousands of years. Fermented foods are not only perceived as pleasant-tasting but the acids produced as a by-product of microbial metabolism inhibit the growth of many spoilage organisms as well as food- borne pathogens (Nester *et al*, 2004). The microbial isolates found in Kunu zaki sold include *Enterobacter, Shigella, Escherichia coli, Klebsilla, Micrococcus, Proteus, Leuconostoc, Bacillus, Citrobacter* and *Staphylococcus* (Kelechi *et al.*, 2020). Edward *et al*., (2019) reported *Lactobacillus plantarum, Bacillus subtilis, B. cereus, Streptococcus faecium, Streptococcus lactis, Staphylococcus aureus, Micrococcus acidophilus, Escherichia coli, Pseudomonas aeruginosa, Saccharomyces cerevisiae, Candida mycoderma, Aspergillus niger, Penicillium oxalicum* and *Fusarium oxysporum* as microbial diversity found in freshly processed and hawked kunu drinks sold in southwestern (Oyo, Ogun, Lagos and Osun State) Nigeria. Adebayo-Tayo *et al*., (2016) reported microbial isolates found in Kunun drink sold in Calabar, Nigeria as *Staphylococcus, Streptococcus, Bacillus, Pseudomonas species* and *Escherichia coli* (bacteria), and *Fusarium, Aspergillus, Penicillium* and yeast (Fungi), Edward and Ezekiel *et al*. (2019) reported microbial diversity of kunu drink as *Lactobacillus, Bacillus, Staphylococcus, Aspergillus, Penicillium, Fusarium* and *Saccharomyces* species. Musa and Hamza (2013) reported *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Rhizopus nigricans* and *Penicillium* species in Kunu aya sold in Kaduna State University, main campus.

Some of these microbes may have entered kunu drinks through contaminated water used in processing, feedstock (whole cereal used in kunu production) or empty plastic bottles used in the packaging of kunu. Mostly, the plastic bottles used by the processor to package the kunu

drink are picked from ceremony environment where they are found in large quantity. It can sometimes be gotten from individuals who drink bottled water (Abiodun *et al*., 2017).

2.3 SPOILAGE OF KUNU

Kunu has relatively short shelf-life storage. Ezekiel *et al*. (2019) reported that the product has a shelf life of 24 hours at ambient temperature. The shelf life could be extended to days by pasteurization at 60°C for 1 hour and storage under refrigeration conditions (Abiodun *et al*, 2017). Kunu can undergo spoilage as a result of some factors such as microorganisms involved in the fermentation process such as *Lactobacillus sp, Streptococcus sp.* and *Leuconostoc* sp. and these organisms have been reported to cause the spoilage of the beverage. Other organisms such as *Staphylococcus sp, Bacillus sp, Pseudomonas sp, Penicillium sp, Aspergillus sp, Trichoderma sp,* and *Candida* sp could cause the spoilage of kunu beverage if found in large quantity (Ayo-Omogie, 2016).

Other factors such as temperature, light, time and the activities of insects, rodents or pests in the environment during the preparation could contribute to the spoilage of kunu and also, its high moisture content may encourage growth of strains to hazardous levels during storage at ambient temperature (Olasupo *et al*., 2002).

2.4 BACTERIA FOUND IN KUNU

Bacteria are the most common cause of food-borne diseases and exist in a variety of shapes, types and properties. Some pathogenic bacteria are capable of spore formation and thus, highly heat-resistant (e.g. *Clostridium botulinum, C. perfringens, Bacillus subtilus, Bacillus cereus*) (Mbachu *et al*., 2014). Some are capable of producing heat-resistant toxins

(e.g. *Staphylococcus aureus, Clostridium botulinum*). Most pathogens are mesophilic with optimal growth temperature range from 20°C to 45°C. However, certain food-borne pathogens (i.e. psychrotrophs), such as *Listeria monocytogenes*, and *Yersinia enterocolitica* are capable of growth under refrigerated conditions or temperatures less than 10^0C (Mbachu *et al*., 2014).

2.4.1 *Lactiplantibacillus plantarum*

Lactiplantibacillus plantarum (formerly *Lactobacillus arabinosus*) (Oluwajoba *et al*., 2014) is a widespread member of the genus *Lactiplantibacillus* and commonly found in many fermented food products as well as anaerobic plant matter (Seddik *et al*., 2017). *L. plantarum* was first isolated from saliva, and based on its ability to temporarily persist in plants, the insect intestine and in the intestinal tract of vertebrate animals, it was designated as nomadic organism (Shao *et al*., 2015). *L. plantarum* is Gram positive, bacilli shaped bacterium. *L. plantarum* cells are rods with rounded ends, straight, generally 0.9–1.2 μm wide and 3–8 μm long, occurring singly, in pairs or in short chains (Behera et al., 2018). *L. plantarum* has one of the largest genomes known among the lactic acid bacteria and is a very flexible and versatile species. It is estimated to grow between pH 3.4 and 8.8 (Shao *et al*., 2015). *Lactiplantibacillus plantarum* can grow in the temperature range 12 °C to 40 °C (Mbachu *et al*., 2014). The viable counts of the *L. plantarum* stored at refrigerated condition (4^0C) remained high, while a considerable reduction in the counts was observed stored at room temperature (25 ± 1^0C) (Oluwajoba *et al*., 2014).

2.4.2 *Bacillus subtilis*

Bacillus subtilis is a ubiquitous organism. In the laboratory, *B. subtilis* is easy to grow and manipulate. In nature, *B. subtilis* inhabits the soil, roots of plants, and aquatic environments. Although *B. subtilis* can grow in the gastrointestinal (GI) tract of animals, it is not considered a human pathogen. In fact, *B. subtilis* along with other species of *Bacillus* is considered a GRAS (Generally Regarded as Safe) organism by the Food and Drug Administration (FDA) (Seddik *et al*., 2017). Moreover, *B. subtilis* has been widely used in biotechnology. Recently, improved expression and secretion of proteins by *B. subtilis* has become an efficient tool for enzyme production. It is estimated that *Bacillus* species, including *B. subtilis*, produce 60% of commercially available enzymes (Seddik *et al*., 2017). Additionally, *B. subtilis* plays a major role in the production of food (fermented products, flavor enhancers, sweeteners, and animal feed additive), household detergents, antibiotics, and vitamins and in the development of vaccines; it serves as a model organism for the development of sporicides, which are chemical agents that kill spores. The biotechnology industry has helped drive the research of molecular genetics and cell biology forward, using *B. subtilis* as one of its greatest workhorses (Oluwajoba *et al*., 2014).

2.4.3 *Bacillus cereus*

Bacillus cereus is a member of the family *Bacillaceae*. They are Gram-positive, motile rods, and they have the ability to form spores (Mbachu *et al*., 2014). Most *Bacillus* spp. are found throughout the environment, including soils, fresh and marine water environments. Spores produced by *B. cereus* possess appendages and/or pili and are more hydrophobic than any other *Bacillus* spores. These properties enable the spores to adhere to many different types of

surfaces and to resist removal during cleaning and sanitation (Behera *et al*., 2018). Vegetative cells of *B. cereus* grow at temperatures ranging from 4–15°C to 35–55 °C but prefer 30–40°C, depending on the strain (Peng *et al*., 2021). The organism grows at pH 4.9–9.3, but the inhibitory effect of pH is reduced in foods as evidenced by limited growth on meat at pH 4.35 (Mbachu *et al*., 2014). The minimum a_w, for growth has been established at 0.93, but it has been suggested to use 0.912 as the minimum required for growth, because fried rice tends to have a_w values ranging from 0.912 to 0.961 and readily supports *B. cereus* growth (Peng *et al*., 2021).

B. cereus produces two types of toxins, the emetic (vomiting) and the diarrheal one, causing two types of illness. The emetic syndrome is caused by emetic toxin produced by the bacteria during the growth phase in the food. The diarrheal syndrome is caused by diarrheal toxins produced during growth of the bacteria in the small intestine. The rapid onset of the emetic type is characterized by nausea and vomiting while the late onset of the diarrheal type is characterized by diarrhea and abdominal pain. Both syndromes (i.e., diarrheal and emetic) are a result of *B. cereus* endospores surviving the cooking process, after which germination and subsequent proliferation of vegetative cells occurs at some point during storage. Foods that are frequently implicated in *B. cereus* diarrheic food poisoning include meat products, soups, vegetables, puddings, sauces, milk and milk products (Behera *et al*., 2018). Symptoms are characterized by abdominal pain, nausea, and diarrhea after an incubation period of approximately 8–16 h. Diarrheal syndrome symptoms generally persist no longer than 12–24 h. After a 1–5 h incubation period, emetic syndrome symptoms include primarily nausea and vomiting and persist for 6–24 h. Foods implicated in *B. cereus* emetic food poisoning include

fried and cooked rice, pasta, noodles, and pastry (Behera *et al*., 2018). The diarrheal syndrome type of food poisoning results from the action of a thermolabile enterotoxic complex, whereas the emetic syndrome type involves the action of a thermostable toxin (Seddik *et al*., 2017).

2.4.4 *Lactococcus lactis*

Lactococcus lactis has been used for centuries in the fermentation of food especially cheese, yoghurt, sauerkraut and the like, thereby rendering it's generally recognized as safe (GRAS) status by the Food and Drug Administration (FDA). Apart from imparting flavour, *L. lactis* being a lactic acid bacteria (LAB) also produces acid which preserves food (Seddik *et al*., 2017). Some strains further enhances this preservation property with the production of bacteriocins, thus reinforcing its role in the food industry. Other than its important function in food, *L. lactis* has become the model LAB when it comes to genetic engineering. Several factors including its small-sized fully sequenced genome (2.3 Mbp) (Seddik *et al*., 2017), and the development of successfully compatible genetic engineering tools such as cloning and expression systems with customizable options, have rendered it a desirable model. Over the past two decades, *L. lactis* has vastly extended its application from food to being a successful microbial cell factory, and on many occasions, acting as a gram-positive alternative to *Bacillus subtilis* and *Lactobacillus plantarum*, or its gram-negative counterpart, *Escherichia coli* (Oluwajoba *et al*., 2014).

2.4.5 *Staphylococcus aureus*

Staphylococcus aureus are non-motile, gram-positive cocci that appear singly or in pairs, tetrads, short chains, or characteristic "grapelike" clusters. Staphylococci are facultative

anaerobes that, with the exception of *Staphylococcus saccharolyticus* and *Staph. aureus* subsp. *anaerobius*, grow more rapidly under aerobic conditions (Mbachu *et al*., 2014). *Staphylococcus* spp. are widespread throughout nature and can be found on the skin and skin glands of mammals and birds, in addition to the mouth, blood, mammary glands, and intestinal, genitourinary, and upper respiratory tracts of infected hosts (Mbachu *et al*., 2014). Outside the body, *Staph. aureus* can survive for long periods of time in a dry state, and have been isolated from air, dust, sewage, and water, making it one of the most resistant non-spore-forming pathogens (Behera *et al*., 2018). In addition to environmental sources of infection, some reported *Staph. aureus* containing foods include ground beef, pork sausage, ground turkey, salmon steaks, oysters, shrimp, cream pies, milk, and delicatessen salads (Mbachu *et al*., 2014).

Staphylococcus aureus grow, depending on the strain, at temperatures ranging from 7 to 47.8°C and produce enterotoxins between 10 and 46 °C but prefer an optimum temperature between 40 and 45 °C. The bacterium grows between pH 4.5 and 9.3, with an optimum between 7.0 and 7.5, and is very tolerant to high levels of salt (>10% sodium chloride); enterotoxin production requires a minimum a_w of 0.86, whereas growth has been demonstrated at a_w of 0.83 (Aoudia *et al*., 2016).

The presence of *Staphylococcus aureus* in food may be considered a public health hazard because of its ability to produce enterotoxin and the risk of subsequent food poisoning. Although there are nine identified staphylococcal enterotoxins, designated as A, B, C1, C2, C3, D, E, F, and G, types A and D are responsible for the majority of the outbreaks (Seddik *et al*., 2017). *Staphylococcal* enterotoxins are included in a larger family of toxins, known as

pyrogenic toxins, which have the unique ability to act as super antigens, thereby stimulating an extraordinarily high percentage of T cells. They are difficult to inactivate with heat, because temperatures required to inactivate them are higher than those needed to kill the organism (Mbachu *et al*., 2014). *Staphylococca l*enterotoxin A is more heat sensitive than enterotoxins B or C and requires heating at 80 or 100°C for 180 or 60s, respectively, to cause a loss in serological reactivity (Mbachu *et al*., 2014).

2.4.6 *Lactobacillus acidophilus*

Lactobacillus acidophilus, non-pathogenic and a member of the normal intestinal microflora is widely used in fermented dairy products and is of considerable industrial and medical interest because it has been reported to aid in the reduction of the levels of harmful bacteria and yeasts in the small intestine and to produce lactase, an enzyme which is important for the digestion of milk (Deraz *et al*., 2007). Therefore *L. acidophilus* group of lactic acid bacteria (LAB) is added as dietary adjuncts to commercial fermented milk products and the intake of these bacteria may have beneficial effects on human health (Kawai *et al*., 2001). The properties of *L. acidophilus* have been investigated in order to establish its specific role in the complex microbial intestinal equilibrium, both of man and higher animals (Oluwajoba *et al*., 2014).

2.4.7 *Escherichia coli*

Escherichia coli is a Gram-negative, non-spore forming rod. It may or may not be mobile; some rods are flagellated and some are not (Peng *et al*., 2021). The organism is a facultative anaerobe and ferments simple sugars such as glucose to form lactic, acetic, and formic acids;

the optimum pH for growth is 6.0 to 8.0; however, growth can occur as low as pH 4.3 and as high as 9 to 10 pH (Peng *et al*., 2021).

E. coli comprise a large and diverse group of bacteria. Most strains of *E. coli* are harmless; other strains have acquired characteristics, such as the production of toxins, which make them pathogenic to humans (Aoudia *et al*., 2016). 5351 genomes have been completed up to now according to the data retrieved from NCBI. The median total length of the genome is 5.171 Mb (Oluwajoba *et al*., 2014). Pathogenic variants of *E. coli* (pathovars or pathotypes) cause much morbidity and mortality worldwide; many of these pathotypes are a major public health concern as they have low infectious doses and are transmitted through ubiquitous mediums, including food and water (Seddik *et al*., 2017). Transmission of *E. coli* occurs when food or water that is contaminated with feces of infected humans or animals is consumed. Contamination of animal products often occurs during the slaughter and processing of animals. The use of manure from cattle or other animals as fertilizer for agricultural crops can contaminate produce and irrigation water. *E. coli* can survive for long periods in the environment and can proliferate in vegetables and other foods (Aoudia *et al*., 2016).

Pathogenic *E. coli* have been categorized into six groups according to the pathogenic mechanism: (1) Enteropathogenic *E. coli* (EPEC); (2) Enterohemorrhagic *E. coli* (EHEC, also known as Shiga toxin-producing *E. coli* [STEC] and formerly referred to as verotoxin-producing *E. coli* [VTEC]); (3) Enterotoxigenic *E. coli* (ETEC); (4) Enteroaggregative *E. coli* (EAggEC); (5) Enteroinvasive *E. coli* (EIEC); and (6) Attaching and Effacing *E. coli* (A/EEC) (Oluwajoba et al., 2014).

2.5 CHARACTERISTICS USEFUL IN CLASSIFICATION AND IDENTIFICATION OF BACTERIA FROM KUNU.

2.5.1 Morphological Characteristics

Both wet-mounted and appropriately stained bacterial cell suspensions can yield a lot of data. These basic tests can show the Gram response of the organic entity; whether it is corrosive quick; its motility; the plan of its flagella; the presence of spores, cases, and consideration bodies; and, obviously, its shape. This data frequently can permit recognizable proof of a life form to the sort level, or can limit the likelihood that it has a place with some gathering. Settlement attributes and pigmentation are likewise very accommodating. For instance, settlements of a few Porphyromonas animal categories autofluoresce under lengthy frequency bright light, and *Proteus* species swarm on fitting media.

2.5.2 Growth Characteristics

An essential distinctive trademark is whether an organic entity develops vigorously, anaerobically, facultatively (i.e., in either the presence or nonappearance of oxygen), or microaerobically (i.e., within the sight of a not exactly environmental fractional strain of oxygen). The legitimate environmental circumstances are fundamental for segregating and distinguishing microbes. Other significant development evaluations incorporate the brooding temperature, pH, supplements required, and protection from anti-infection agents. For instance, one diarrheal illness specialist, *Campylobacter jejuni*, develops well at 42^0C within the sight of a few anti-infection agents; another *Y. enterocolitica*, develops better compared to most different microscopic organisms at 4^0C. *Legionella*, *Haemophilus*, and a few different

microorganisms require explicit development factors, though *E. coli* and most other *Enterobacteriaceae* can develop on negligible media.

2.5.3 Antigens and Phage Susceptibility

Cell divider (O), flagellar (H), and capsular (K) antigens are utilized to support ordering specific life forms at the species level, to serotype strains of medicinally significant species for epidemiologic purposes, or to distinguish serotypes of general wellbeing significance. Serotyping is likewise here and there used to recognize strains of outstanding harmfulness or general wellbeing significance, for instance with *V. cholerae* (O1 is the pandemic strain) and E. coli (enterotoxigenic, enteroinvasive, enterohemorrhagic, and enteropathogenic serotypes).

Phage composing (deciding the helplessness example of a segregate to a bunch of explicit bacteriophages) has been utilized fundamentally as a guide in epidemiologic observation of infections brought about by *Staphylococcus aureus*, M*ycobacteria*, *Pseudomonas aeruginos*a, *Vibro cholerae*, and *Salmonella typhi*. Defenselessness to bacteriocins has additionally been utilized as an epidemiologic strain marker. Much of the time as of late, phage and bacteriocin composing have been displaced by atomic techniques.

2.5.4 Biochemical Characteristics

The reaction of most microorganisms in a series of biochemical tests are used to differentiate and classify them to a great extent. Some tests are used on a regular basis for some groups of microscopic organisms (oxidase, nitrate reduction, amino acid degrading enzymes, starch utilization); others are confined to a single family, sort, or species (coagulase test for *Staphylococci*, pyrrolidonyl arylamidase test for Gram-positive cocci).

Starting with one group of living organisms and moving on to the next, both the number of tests required and the actual tests used for identifiable proof shift. Hence, the lengths to which a lab ought to go in recognizing and distinguishing life forms should be chosen in every lab based on its capacity, the sort of populace it serves, and its assets. Clinical research centers today base the degree of their work on the clinical pertinence of a detach to the specific patient from which it started, the general wellbeing meaning of complete ID, and the general money saving advantage examination of their systems. For instance, the Centers for Disease Control and Prevention (CDC) reference lab utilizes something like 46 tests to distinguish individuals from the *Enterobacteriaceae*, while most clinical labs, utilizing business recognizable proof packs or basic quick tests, recognize disconnects with far less measures.

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY AREA

The study area is Alimosho [6.5744° N, 3.2570° E], a local government area in the Ikeja division, Lagos State, Nigeria. It is the largest local government area in Lagos and consists of: Agbado/Oke-odo LCDA, Alimosho LG, Egbe/Idimu LCDA, Ikotun/Igando LCDA and Mosan Okunola LCDA. The LGA contains the urban area of Egbeda/Akowonjo.

3.2 SAMPLE COLLECTION

Eight (8) samples of kunu drink were collected in duplicates from different locations where Kunu is sold in Alimosho, Lagos state; one sample each was gotten from Olugbede market, Ikotun market, Abesan market, Egbeda market, Igando market, Isheri-Olofin Bus-stop, and two supermarkets in Alimosho. The samples were labeled and stored in separate plastic bags, and transported in an ice-packed cooler to the microbiology laboratory for analysis.

3.2.1 Questionnaire Survey

A face-to-face questionnaire survey was used to obtain information from kunu vendors regarding factors such as manufacturer of the kunu, the source of the raw ingredients and packaging materials, the individuals who patronize the most, the target customers, and the sanitary condition of the preparation area.

3.3 PREPARATION OF CONTROL SAMPLE

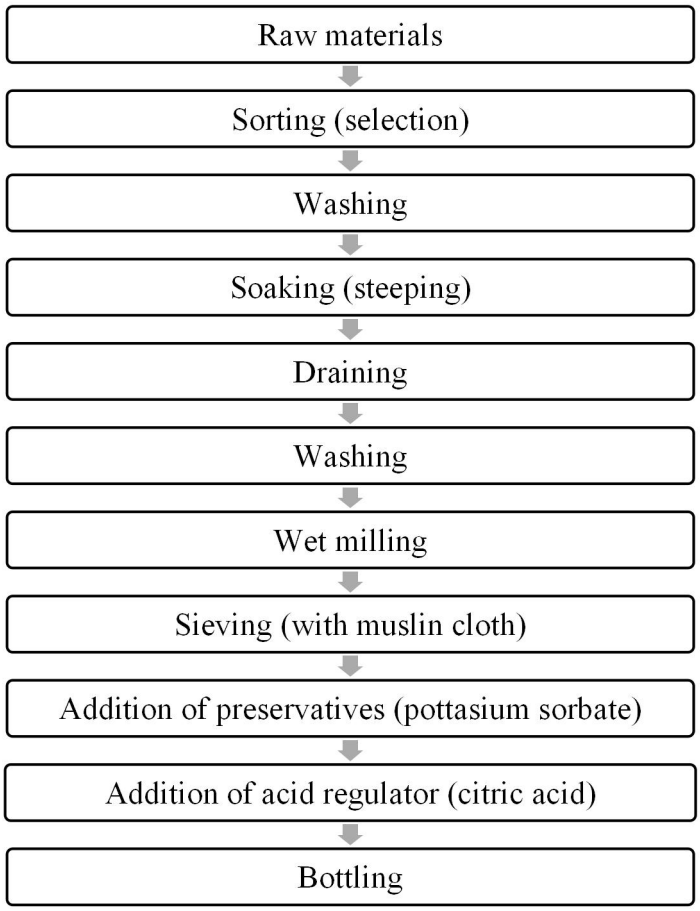

Fig 3.1: Method of control sample preparation in the laboratory

A 500ml trial batch was prepared for sensory evaluation before the bulk production of 2 liters using; 1 liter of Kunu slurry paste, 1 liter of water, 60ml of clove, 1g of pepper, 0.4g of citric acid, 6g of Ginger, and 60g of Sugar.

3.4 MATERIALS USED

3.4.1 Equipment and apparatus used

The equipment and apparatus used include; Autoclave, Incubator, Dry heat oven, Water bath, Bunsen burner, Refrigerator, Beaker, Petri dishes, Test tubes, Inoculation loop, Bijou bottle, Weighing balance, Microscope, Test tubes, Durham tubes, Spatula, Slides, Micropipette and

tips, Laminar air flow hood, Analytical balance, pH meter, Vortexer.

3.4.2 Culture media and reagents

The media that were used in this work are; Nutrient agar, Nutrient Broth, MacConkey agar, Gelatin Agar, Blood Agar, Mueller Hinton Agar, Motility test Media, Simmon's Citrate Media, Urea Agar, Methyl-Red Voges-Proskaurer (MRVP) Medium, Starch Agar, Casein Agar, and Phenol Red Broth Base. They were prepared in accordance to manufacturer's instructions.

Reagents used in this work are; Crystal violet, Iodine, Ethanol, Safranin, Oil immersion, Oxidase test strips, Nitrate reagent 1 and 2, Saint James reagent, Methyl Red indicator, APHA reagent (Sodium hydroxide + Potassium hydroxide solution), Mercuric chloride solution, Antibiotic disks, Sugars (Glucose, Fructose, Maltose, Sucrose, Lactose and Mannitol).

3.5 DETERMINATION OF pH OF THE SAMPLES

This was done within 3 hours of collection. The pH meter was first standardized with a buffer solution of pH 7.0 to calibrate it. The samples were then measured out in a beaker and the electrode was rinsed with distilled water before being dipped into the kunu samples. The results obtained were recorded.

3.6 SERIAL DILUTION

One milliliter (1ml) of each sampled kunu drink was put into 9ml of sterile distilled water in sterile test tubes. To homogenize the samples, they were vortexed vigorously. From the initial dilution, 10-fold serial dilutions were carried out in clean sterile test tubes containing 9ml of sterile distilled water. From the third dilution, 1ml was inoculated separately onto sterile Petri dishes. Each experiment was carried out in duplicates to get the mean standard value of the

colony forming unit per milliliter (cfu/ml) and coliform, respectively (Ekanem *et al., 2018).*

3.7 PLATING PROCEDURES

1ml of the 10^3 dilution was pour plated onto Nutrient agar, and MacConkey agar each. The Nutrient agar and MacConkey agar plates were then incubated at 35°C for 24 hours for bacterial and coliform counts.

3.8 BACTERIAL COUNT

After the period of incubation, the colonies on the plates were counted and recorded as colony forming unit per milliliter (cfu/ml).

3.9 PURIFICATION AND MAINTENANCE OF BACTERIAL ISOLATES

Following enumeration of total bacterial and coliform counts, each of the colonies on the MacConkey agar plates were sub-cultured and the pure culture obtained. The isolates were sub-cultured on Nutrient agar. Purified isolates were stored for further studies.

3.10 CHARACTERIZATION AND IDENTIFICATION OF BACTERIAL ISOLATES

Using Bergey's manual of determinative bacteriology, purified isolates were characterized on the basis of their morphology and biochemical characteristics (Anumudu & Anumudu, 2019). Gram staining, spore staining, and biochemical tests such as catalase, coagulase, oxidase, citrate utilization, nitrate reduction, gelatin hydrolysis, starch hydrolysis, casein hydrolysis, indole production, methyl red, urease, Voges-Proskauer, hemolysis, antibiotic susceptibility, and sugar fermentation were used to identify isolates.

3.10.1 COLONIAL MORPHOLOGY

The shape, size, color, and elevation characteristics of the bacterial species were examined on nutrient agar plates after incubation.

3.10.2 GRAM STAIN

Smears of 18-24 hours old cultures of bacterial isolates were heat fixed and stained with crystal violet for 30 seconds. The dye was drained and then fixed with iodine for 30 seconds. The slides were rinsed with tap water, decolorized with 95% ethanol for about 10-15 seconds and again washed with tap water. The slides were counter stained with safranin for 30 seconds, then rinsed, air dried and examined under the microscope using the oil immersion lens for Gram reaction and cellular morphology. Gram-positive organisms stained blue to purple while Gram-negative stained pink to red.

3.10.3 BIOCHEMICAL TESTS

The following tests were performed on each isolate;

3.10.3.1 GROWTH ON MACCONKEY AGAR

MacConkey agar is a selective medium for the isolation of *Salmonella*, *Shigella* and coliform organisms. Gram positive microbial flora are suppressed to a large extent by the bile salts and the crystal violet present in the medium. The degradation of lactose is established by the pH indicator neutral red. From the third dilution of the kunu sample, 1ml was inoculated separately onto sterile Petri dishes and then the MacConkey Agar was added and vortexed. The plates were then allowed to dry and were incubated at 35^0C for 48 hours.

3.10.3.2 MOTILITY TEST

This test was carried out using Edwards and Wing motility test medium. The semi-solid medium was inoculated with the different bacterial isolates by stabbing with a sterile inoculating needle at the center of the medium column to over half the depth. The motile organisms grew and spread out from the line of puncture while the non-motile organisms grew only along the line of puncture.

3.10.3.3 CATALASE TEST

The microorganisms were mixed with hydrogen peroxide (H_20_2) on a clean glass slide. Most aerobic microorganisms are capable of producing the enzyme catalase although to different extents. The principle of this test is that when organisms containing catalase enzyme are mixed with hydrogen peroxide (H_20_2), gaseous oxygen is released, causing the appearance of bubbles or effervescence.

3.10.3.4 OXIDASE TEST

This was carried out for the detection of cytochrome oxidase in the microorganisms. The organisms were smeared on oxidase test strips and left for 10 minutes for color change. Color change from yellow to dark purple confirmed the presence of oxidase.

3.10.3.5 NITRATE REDUCTION TEST

The test organisms were inoculated separately into test tubes containing nutrient broth and incubated at 35^0C for 3 days. Test for nitrate reduction was determined by addition of 1ml each of nitrate reagents 1 and 2 (Nit1 and Nit2). The presence of nitrite was indicated by the development of a pink or purple color within a few minutes while the negative remained

yellow.

3.10.3.6 INDOLE PRODUCTION TEST

Some microbes are capable of hydrolyzing the amino acid tryptophan and one of the end products is indole. Indole reacts with 4-dimethylaminobenzaldehyde to form a dark red dye color. This procedure involved growing the isolates in nutrient broth for 48 hours at 35^0C, after which about 2ml of Saint James Reagent were added, shaken gently and allowed to stand for 20 minutes. A cherry-red color at the reagent layer indicated indole production.

3.10.3.7 CITRATE UTILIZATION TEST

Simmon's citrate medium is a nutrient substrate that offers ammonium salts as the only source of nitrogen and citrate as the only carbon source. The degradation of citrate leads to alkalinisation of the medium which is indicated by the pH indicator bromothymol blue changing color from green to deep blue. Simmon's citrate agar plates were inoculated with the isolates and incubated at 35^0C for 3 days. Color change from green to blue indicated a positive result. The presence of the original blue color observed in some agar plates indicated citrate was not utilized and this means a negative reaction.

3.10.3.8 UREASE ACTIVITY TEST

Urea, a common organic nitrogen source for many microbes, can be hydrolyzed to ammonia and carbondioxide. The latter produces an alkaline condition in the medium which is indicated by a color change of the pH indicator. Slants of urea agar were inoculated with the isolates and incubated at 35^0C for 5 days, watching daily for any color changes. The development of color change from yellow to pink showed a positive urease activity.

3.10.3.9 METHYL-RED VOGES PROSKAURER TEST

These are actually two tests in one. In the methyl red test, a medium that contains a little carbohydrate fermentable by microorganisms is used. Some microorganisms normally ferment carbohydrates accompanied with acid production and hence the color of methyl red retains its red (acid) color while others ferment carbohydrates without acid production and hence the methyl red changes to yellow. The isolates were inoculated into 10ml of MRVP medium and incubated at 35^0C for 3 days.

After incubation, the tests were performed in the following way;

MR TEST - Five drops of methyl red indicator were added to the culture. A red color indicated a positive reaction.

VP TEST - 5ml of APHA reagent (mixture of lg of copper sulphate (blue) dissolved in 40ml of saturated sodium hydroxide solution plus 960ml of 10% potassium hydroxide solution) were added to the culture. A pink to red color indicated a positive reaction.

3.10.3.10 GELATIN HYDROLYSIS TEST

Plates of gelatin agar were inoculated with test organisms and incubated at 35^0C for 3 days. Following incubation, the plates were flooded with 5ml acid mercuric chloride solution. Clear zones indicated areas of gelatin hydrolysis.

3.10.3.11 STARCH HYDROLYSIS TEST

Starch is a polysaccharide. Many bacteria possess enzymes called amylases, which can hydrolyze complex molecules of starch to sugars. Starch agar plates were inoculated with different bacterial isolates and incubated at 35^0C for 2 days. After incubation, each plate was

flooded with aqueous iodine and left for 30 seconds. A clear zone surrounding the colonies indicated a positive test, while a blue-black coloration indicated the presence of starch meaning the latter had not been hydrolyzed.

3.10.3.12 CASEIN HYDROLYSIS TEST

Nutrient agar was prepared and 1% casein powder was added to the nutrient agar, homogenized on hot plate magnetic stirrer. The medium was sterilized in an autoclave at 115^0C for 10 minutes, cooled to about 45^0C and was aseptically distributed into Petri dishes. The plates were allowed to dry. Fresh cultures of isolates of 24 hours were inoculated onto the plates of casein agar, incubated at 35^0C for 3 days. Plates were examined for clearing zones around the bacterial growth with aid of 20% mercuric chloride solution ($HgCl_2$).

3.10.3.13 HEMOLYSIS TEST

Hemolysis was determined by streaking for isolation on a blood agar plate. After incubation overnight, the medium was inspected for tellable signs of alpha- or beta- hemolysis. If the medium is discolored or darkened after growth, the organism has demonstrated alpha-hemolysis, if the medium has been cleared under growth, the organism is beta-hemolytic and no discernible change in the color of the medium constitutes gamma-hemolysis.

3.10.3.14 SUGAR FERMENTATION TEST

The fermentation of sugars is demonstrated by the production of acid or acid and gas (carbondioxide and/or hydrogen). The ability of an organism to ferment several sugars can be demonstrated by incorporating the sugars into a basal medium (phenol red broth base) and

testing for acid and gas production. The sugars used were glucose, fructose, maltose, sucrose, lactose and mannitol. Each of the isolates were inoculated into test tubes containing each of the sugars and incubated at 35^0C for 3 days watching daily for color change. Acid production was indicated by the appearance of a yellow color in the medium and gas production by the presence of an air space in the inserted Durham tubes.

3.10.3.15 ANTIBIOTIC SUSCEPTIBILITY TEST

This was done using the Mueller Hinton agar. 38g of Mueller Hinton agar was weighed into 1000ml of distilled water, homogenized on hot plate magnetic stirrer and subsequently sterilized at 121^0C for 15 minutes in an autoclave. The cool molten Mueller Hinton agar was poured into sterile Petri dishes. The plates were allowed to set and surface dried in an oven at 45^0C. Suspension of the organisms were made by inoculating the test organisms of about 24 hours into 1ml of water each to give a concentration of about 10^5 cells/ml and 0.1ml aliquot test organism suspension was placed on the agar surface and was spread using a glass spreader. The plates were allowed to aseptically spread on the agar surface. The plates were allowed to dry for 1hr at room temperature.

Multi-disk containing the antibiotics was placed onto the inoculated plates using (diffusion disc method). The plates were incubated at 35^0C for 24 hours. After incubation period, the culture plates were examined for areas of no growth around the disc (zone of inhibition). Bacterial strains resistant to antimicrobial grow up to the edges of the disc as against the sensitive strains which are inhibited at a distance from their disc. Disk containing septrin, chloramphenicol, sparfloxacin, ciprofloxacin, amoxicillin, augmentin, gentamicin, pefloxacin, tarivid, and streptomycin were used.

CHAPTER FOUR

RESULTS

Kunu, a cereal-based product widely consumed in Nigeria has been discovered to be a source of transmission of pathogenic microorganisms. This study therefore using standard microbiological methods isolated and identified the bacteria present in kunu sold in Alimosho and studied their susceptibility to antibiotics. The results show the following;

The location where kunu samples used for analysis were obtained and the frequency with which the samples from each location were obtained, the pH values of the samples, and the total bacteria and coliform count of the kunu samples at room and refrigerator temperatures, respectively. The results also presents the bacterial species isolated in the study, their morphological characteristics, their reaction to specific antibiotics, and their frequency of occurrence.

Firstly, the locations where kunu samples used for analysis were obtained, as well as the frequency with which samples from each location were obtained were represented in Table 4.1. Two samples each were collected from each of the location indicating equal distribution of samples through the sampling locations (Table 4.1).

The pH values of the kunu samples used for analysis ranged from 3.89 to 4.45 (Table 4.2) showing all the kunu samples were acidic. This could be attributed to the formation of lactic acid by lactic acid bacteria.

The bacteria load of the kunu samples were high and ranged from $1.00x10^3$ to $2.00x10^5$ cfu/ml and $1.20x10^4$ to $6.00x10^5$ cfu/ml for ambient and refrigeration temperatures

respectively. Overall, samples KC, KD and KF had very high microbial loads while sample KD had a relatively lesser microbial load (Fig 4.1). Sample KCD which was prepared in the laboratory under controlled environment had the least microbial load.

In a comparison of the total coliform count, samples kept at room temperature had significantly higher colony count than those kept in the refrigerator (Fig 4.2).

The morphological characteristics of isolates from kunu drink were shown in Table 4.3. All isolates were gram negative rods, circular in form, had convex elevation and grayish-white in colour (Table 4.3).

The bacterial isolates were either susceptible, intermediate, or resistant to all tested antibiotics which are; Septrin, Chloramphenicol, Sparfloxacin, Ciproflaxin, Amoxicillin, Augmentin, Gentamycin, Perfloxacin, Taravid and Streptomycin. Most isolates were susceptible to Gentamycin (80%) and Taravid (76%) while majority of the isolates were resistant to Chloramphenicol (60%) (Table 4.4).

The biochemical characteristics of the isolates showed most of the isolates were seen to be catalase positive and oxidase negative. Most of the isolates fermented glucose, sucrose, maltose, mannitol, fructose and, lactose (Table 4.5). A total of 11 bacterial genera were identified and sample KA had the highest bacterial diversity with 5 genera, followed by Samples KB, KC and KD having 3 genera each. Sample KCD which was prepared in the laboratory had the lowest number of isolates (2) (Table 4.5).

The most occurring species were the *Enterobacter* species and the *Klebsiella* species both occurring as 23%. (Fig4.3)

Table 4.1 Samples by locations

LOCATION	NUMBER OF SAMPLES	FREQUENCY (%)
Egbeda Market	2	12.5
Olugbede Market	2	12.5
Igando Market	2	12.5
Igando Market	2	12.5
Isheri-Olofin Bus-stop	2	12.5
Abesan Market	2	12.5
Superstore A, Igando	2	12.5
Superstore B, Iyana-Ipaja	2	12.5
TOTAL	**16**	**100**

Table 4.2 pH values of Kunu samples

S/N	Sample Code	pH Value
1	KA	3.96
2	KB	4.15
3	KC	4.06
4	KD	4.13
5	KE	4.09
6	KF	3.89
7	KG	4.22
8	KH	4.25
9	KCD	4.45

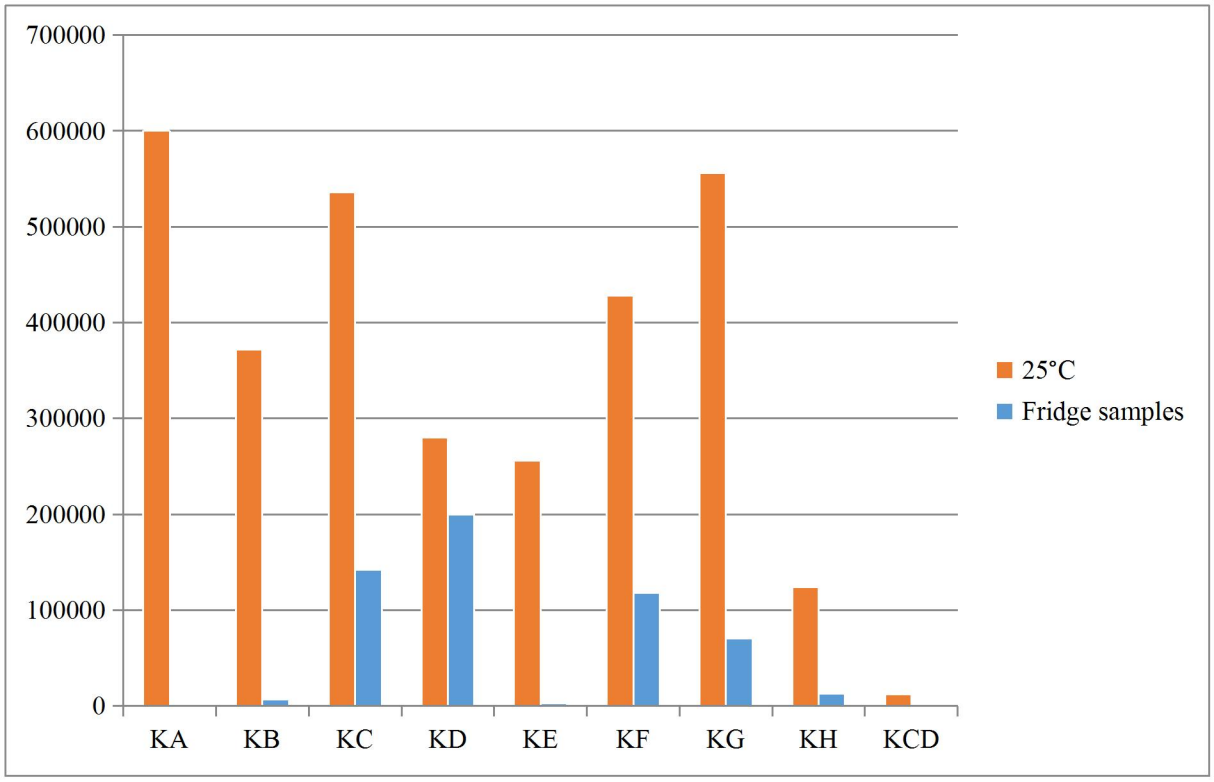

Fig 4.1: Comparison of the bacterial load of kunu samples stored at different temperatures.

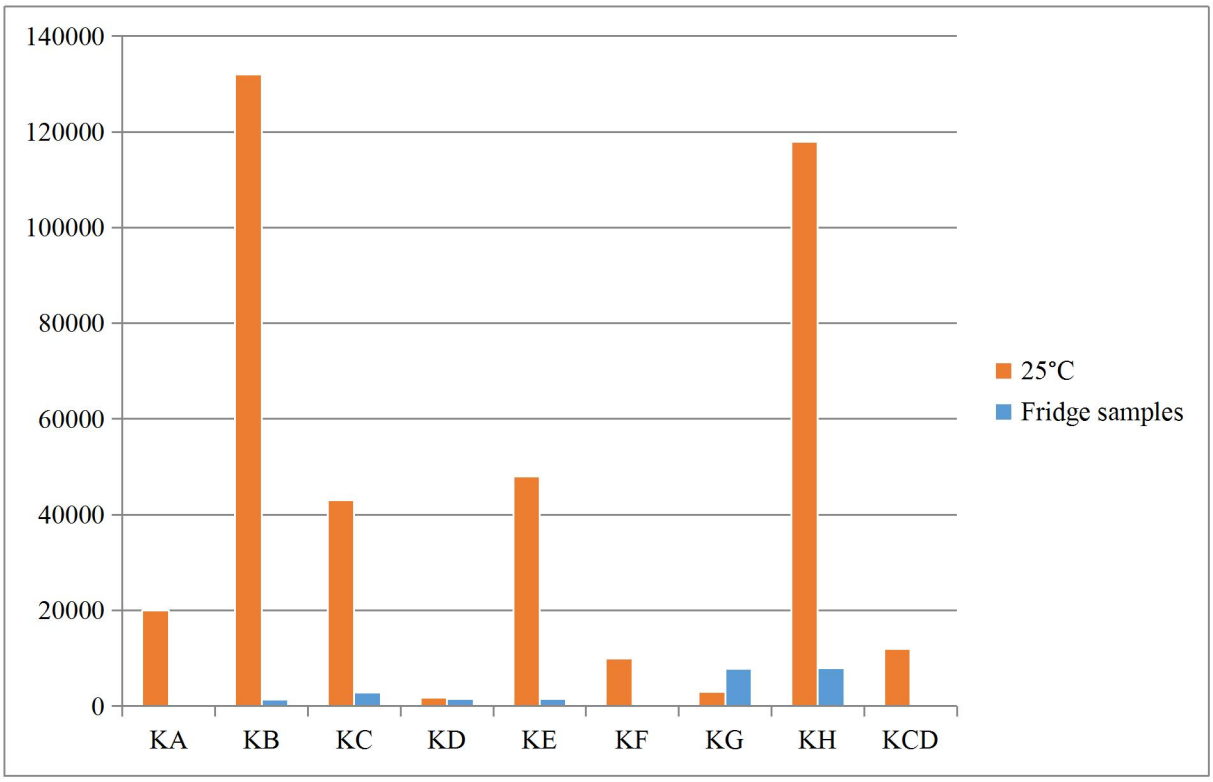

Fig 4.1: Comparison of the colony count of kunu samples stored at different temperatures.

Table 4.3 Morphological Characteristics of Bacterial Isolates from Kunu Drink

S/N	SAMPLE	ISOLATE CODE	FORM	ELEVATION	TEXTURE	COLOR	GRAM
1	KA	K1	Circular	Convex	Smooth	Grey-white	Negative
2	KA	K2	Circular	Convex	Smooth	Grey-white	Negative
3	KA	K3	Circular	Convex	Smooth	Grey-white	Negative
4	KrA	K4	Circular	Convex	Mucoid	Grey-white	Negative
5	KrA	K5	Circular	Convex	Mucoid	Grey-white	Negative
6	KrA	K6	Circular	Convex	Mucoid	Grey-white	Negative
7	KB	K7	Circular	Convex	Mucoid	Grey-white	Negative
8	KB	K8	Circular	Convex	Smooth	Grey-white	Negative
9	KB	K9	Circular	Convex	Smooth	Grey-white	Negative
10	KrB	K10	Circular	Convex	Mucoid	Grey-white	Negative
11	KrB	K11	Circular	Convex	Mucoid	Grey-white	Negative
12	KrB	K12	Circular	Convex	Smooth	Grey-white	Negative
13	KC	K13	Circular	Convex	Smooth	Grey-white	Negative
14	KC	K15	Circular	Convex	Mucoid	Grey-white	Negative
15	KrC	K14	Circular	Convex	Smooth	Grey-white	Negative
16	KrC	K16	Circular	Convex	Mucoid	Grey-white	Negative
17	KrC	K17	Circular	Convex	Mucoid	Grey-white	Negative
18	KD	K18	Circular	Convex	Smooth	Grey-white	Negative
19	KD	K19	Circular	Convex	Smooth	Grey-white	Negative

20	KrD	K20	Circular	Convex	Smooth	Grey-white	Negative
21	KE	K21	Circular	Convex	Mucoid	Grey-white	Negative
22	KrE	K22	Circular	Convex	Mucoid	Grey-white	Negative
23	KrE	K23	Circular	Convex	Smooth	Grey-white	Negative
24	KF	K24	Circular	Convex	Smooth	Grey-white	Negative
25	KF	K25	Circular	Convex	Mucoid	Grey-white	Negative
26	KrF	K26	Circular	Convex	Smooth	Grey-white	Negative
27	KrF	K27	Circular	Convex	Mucoid	Grey-white	Negative
28	KrF	K28	Circular	Convex	Mucoid	Grey-white	Negative
29	KrG	K29	Circular	Convex	Mucoid	Grey-white	Negative
30	KrG	K30	Circular	Convex	Mucoid	Grey-white	Negative
31	KG	K31	Circular	Convex	Smooth	Grey-white	Negative
32	KH	K32	Circular	Convex	Mucoid	Grey-white	Negative
33	KH	K33	Circular	Convex	Mucoid	Grey-white	Negative
34	KrH	K34	Circular	Convex	Smooth	Grey-white	Negative

Table 4.4 Antibiotic Susceptibility Testing

S/N	ANTIBIOTIC	SUSCEPTIBLE (%)	INTERMIDIATE (%)	RESISTANT (%)
1	**Septrin**	44	-	56
2	**Chloramphenicol**	36	4	60
3	**Sparfloxacin**	65	-	35
4	**Ciprofloxaxin**	24	52	24
5	**Amoxicillin**	64	-	36
6	**Augmentin**	64	-	36
7	**Gentamycin**	80	-	20
8	**Perfloxacin**	52	-	48
9	**Taravid**	76	-	24
10	**Streptomycin**	56	4	40

Table 4.5 Biochemical Characterization of Bacterial Isolates from Kunu

Isolate code	Kunu sample	CATALASE	OXIDASE	INDOLE	MOTILITY	MR	VP	CITRATE	UREASE	CASEIN	GELATIN	STARCH	NITRATE	HEMOLYSIS	GLUCOSE	SUCROSE	MALTOSE	MANNITOL	LACTOSE	FRUCTOSE	PROBABLE ORGANISM
K1	KA	+	+	+	-	+	-	-	-	-	+	+	-	β	+	+	+	+	+	+	*Aeromonas* spp.
K2	KA	+	+	+	-	+	-	+	-	-	-	+	+	α	+	+	+	+	+	-	*Alcaligenes* spp.
K3	KA	+	+	-	-	+	+	+	-	+	-	+	-	γ	+	+	+	+	+	+	*Proteus* spp.
K4	KrA	+	+	-	-	+	+	+	-	+	+	+	-	γ	+	+	+	+	+	+	*Klebsiella* spp.
K5	KrA	+	+	-	-	+	+	-	-	+	-	+	-	γ	+	+	+	+	+	+	*Klebsiella* spp.
K6	KrA	+	+	+	+	+	-	-	-	+	+	+	+	γ	+	+	+	+	+	+	*Escherichia coli*
K7	KB	+	+	-	-	+	-	+	-	+	+	+	-	γ	+	+	+	+	+	+	*Klebsiella* spp.
K8	KB	+	+	+	-	+	-	+	-	+	-	+	+	γ	+	+	+	+	+	+	*Providencia* spp.
K9	KB	+	+	+	-	-	-	+	-	+	-	+	+	γ	+	+	+	+	+	+	*Plesiomonas* spp.
K10	KrB	+	+	+	-	-	-	-	-	+	-	+	+	γ	+	+	+	+	+	+	*Escherichia coli*
K11	KrB	+	-	-	-	+	+	-	-	+	+	+	-	γ	+	+	+	+	+	+	*Klebsiella* spp.
K12	KrB	+	+	-	-	-	-	-	-	+	+	+	-	β	+	+	+	+	+	+	*Acinetobacter* spp.
K13	KC	+	-	-	-	-	-	-	-	-	+	+	-	γ	+	+	+	+	+	+	*Salmonella* spp.
K14	KC	+	+	-	+	-	-	-	-	-	-	-	-	α	+	+	+	+	+	+	*Enterobacter* spp.
K15	KC	+	+	+	-	-	-	-	-	+	+	-	+	γ	+	+	+	+	+	+	*Escherichia coli*
K16	KrC	+	+	-	-	-	+	+	-	-	-	+	-	α	+	+	+	+	+	+	*Enterobacter* spp.
K17	KrC	+	+	-	-	-	+	+	-	-	-	+	-	α	+	+	+	+	+	+	*Enterobacter* spp.
K18	KD	+	-	-	-	-	-	+	-	+	-	+	-	γ	+	+	+	+	+	+	*Salmonella* spp.

K19	KD	+	-	-	-	-	-	+	-	+	-	+	-	γ	+	+	+	+	+	+	*Salmonella* spp.
K20	KrD	+	+	-	-	-	-	+	-	+	-	+	-	γ	+	+	+	+	+	+	*Salmonella* spp.
K21	KE	+	-	-	+	+	-	-	-	-	+	+	-	α	+	+	+	+	+	+	*Enterobacter* spp.
K22	KrE	+	-	-	+	-	+	-	-	-	-	+	-	α	+	+	+	+	+	+	*Enterobacter* spp.
K23	KrE	+	-	-	-	-	+	+	-	-	+	+	-	γ	+	+		+	+	+	*Citrobacter* spp.
K24	KF	+	-	-	-	-	+	+	-	+	-	+	-	γ	+	+	+	+	+	+	*Citrobacter* spp.
K25	KF	+	+	-	-	-	+	+	-	-	-	+	-	α	+	+	+	-	+	+	*Enterobacter* spp.
K26	KrF	+	-	-	-	-	-	-	-	-	+	+	-	γ	+	+	+	+	+	+	*Salmonella* spp.
K27	KrF	+	-	-	+	-	-	-	-	-	+	-	-	α	+	+	+	+	+	+	*Enterobacter* spp.
K28	KrF	+	+	-	-	-	-	+	-	-	+	-	-	α	+	+	+	+	+	+	*Enterobacter* spp.
K29	KrG	+	-	-	-	+	+	-	-	-	+	+	-	γ	+	+	+	+	+	+	*Klebsiella* spp.
K30	KG	+	-	-	-	+	+	+	-	-	+	-	-	γ	+	+	+	+	+	+	*Klebsiella* spp.
K31	KG	+	-	-	-	+	-	+	-	-	+	-	-	γ	+	+	+	+	+	+	*Salmonella* spp.
K32	KH	+	-	-	-	-	-	+	-	-	+	+	-	γ	+	+	+	+	+	+	*Klebsiella* spp.
K33	KH	+	-	-	-	-	-	+	-	-	-	+	-	γ	+	+	+	+	+	+	*Klebsiella* spp.
K34	KrH	+	-	-	-	+	+	-	-	+	+	-	-	γ	+	+	+	+	+	+	*Salmonella* spp.

Key: + = Positive, - = Negative, MR = Methyl Red Test, VP = Voges-Proskaurer Test.

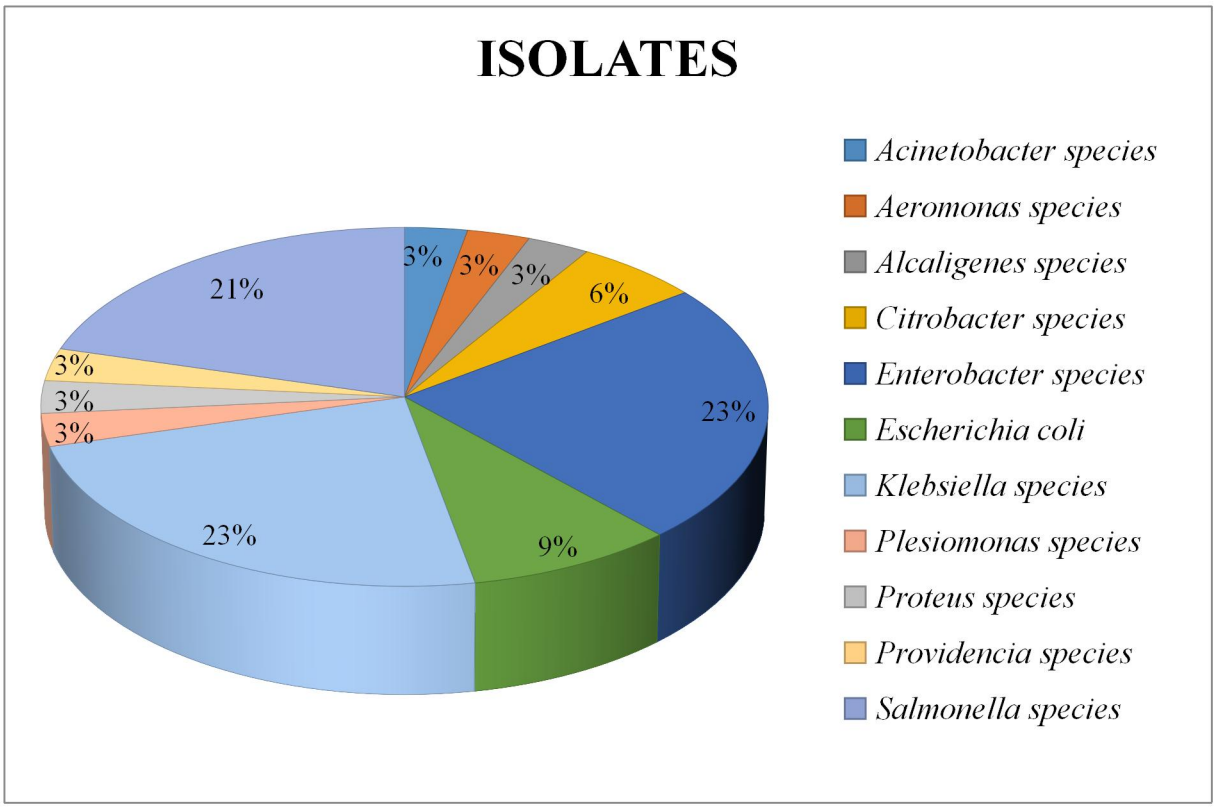

Fig 4.3: Frequency of occurrence of bacterial isolates from kunu.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Kunu is a local non-alcoholic, non-carbonated beverage produced and consumed in large quantity in Nigeria, especially in the northern part of the country. Despite its economic and nutritional benefits, kunu consumption has been linked to an increased risk of food-borne diseases making it necessary for this study to investigate the bacteriological status of kunu.

The results from this study confirm the presence of bacteria in high concentrations in kunu drink sold in Alimosho, Lagos state. Several researchers also have reported the isolation of several bacteria including *Stapyloccus* sp., *Streptococcus* sp., *Bacillus* sp., *Pseudomonas* sp., *Escherichia coli, Listeria* sp., *Corynebacterium* sp., *Listeria innocua*, *Kurthia* sp., *Salmonella* sp., and *Lactobacillus* sp. in kunu drink in high concentrations (Agbo *et al*., 2013; Mbanchu *et al*., 2014; Ekanem *et al*., 2018; Sule *et al*., 2016; Chukwuma *et al*., 2020).

Total heterotrophic and total coliform counts from this study are lower than the reports of Abubakar *et al*. (2013), who reported total viable counts of 9.1×10^7 to 2.6×10^8 cfu/ml in kunu drinks sold in Keffi metropolis. This could be linked to the poor sanitary practices in the north, as a survey by Nurudeen *et* al. (2014) has shown that only 2.7% of the food vendors in the north had formal training on food preparation in the north. Similarly, the coliform counts are lesser than that of 1.1×10^6 to 1.6×10^6 cfu/ml reported by Chukuma *et al*. (2020), but higher than 1.0×10^2 to 8.0×10^3 cfu/ml reported by Sule *et al*. (2016) in locally produced kunu drinks sold in Ilorin, Kwara State, Nigeria. The difference in microbial load could be

due to the fact that the kunu samples were gotten from different locations.

The bacterial and colony count from kunu stored ranged from $1.00x10^3$ to $2.00x10^5$ cfu/ml and $1.20x10^4$ to $6.00x10^5$ cfu/ml for room and refrigeration temperatures, respectively. The findings were similar to that of Mbachu *et al*. (2014) as it was observed that the bacterial count in kunu samples stored at room temperature was higher than that stored at refrigeration temperature; .3 $x10^3$ cfu/ml to TNC and 2.1 x 10^3 to 6.5 x 10^3 cfu/ml for room and refrigeration temperatures respectively. Thus, refrigeration storage hindered microbial growth while room temperature storage encourages microbial growth and proliferation. Agbo *et al*. (2013) reported that kunu drink has a shelf life of 24 hours at room temperature, which can be extended to days by refrigerating and pasteurizing at 600°C for 1 hour.

As shown in the results of the bacterial counts, samples KC, KD and KF had very high microbial loads and the constant consumption of kunu drinks from these markets can possibly lead to a case of food poisoning. Sample KD had a relatively lesser microbial load indicating better sanitary practices. Sample KCD which was prepared in the laboratory under controlled environment had the least microbial load indicating that proper control of the fermentation process, use of potable water, addition of preservatives, and proper hygiene will result in the production of kunu drinks with minimal microbial load.

Acinetobacter sp. and *E. coli* are the organisms that were found in kunu after 3 days of storage at room temperature that was not found in those stored in the refrigerator. This may be because these organisms have the ability to grow at elevated temperatures. *Acinetobacter* sp. isolated from human specimens has been discovered to grow readily at 37^{o}C. Also, Guyot

et al. (2014) has confirmed that *E. coli* can grow at elevated temperatures as high as 50^{o}C.

The frequency and percentage occurrence of the bacterial isolates presented indicates that *Enterobacter* species and *Klebsiella* species were the organisms of importance in kunu as revealed in this study, by its numerical predominance. These bacterial species are both members of the family *Enterobacteriaceae*. Due to their superior fermentative ability, *Enterobacter* and *Klebsiella* may have adapted to growth in the special condition of kunu. The *Enterobacteriaceae* are lactose-fermenters. This explains to large extent their dominance in the kunu drink. These organisms can cause the spoilage of the beverage if not eliminated during the heating process.

Part of kunu preparation involves cooking, a process that would eliminate all the isolates reported in this work so the presence of these organisms suggests that it must have been contaminated after the cooking process when the drink must have cooled down. Contamination could come from the syrup, fermentation vessels, storage containers, sieves used for filtration, hands of the handlers, and even the bottles or polyethene bags in which it was packaged for sale. (Mbachu *et al*., 2014). There is therefore the need for high degree of sanitation during the processing (collection, dilution) and post-processing (bottling, storage, transportation) of the beverage as microbial contamination can result in spoilage of kunu and can sometimes influence the stability of the product and its hygienic quality.

The presence of *Escherichia coli*, *Klebsiella* sp., *Citrobacter* sp., *Enterobacter* sp., and *Salmonella* sp. which are coliforms indicates possible fecal contamination of the drink, most likely due to the use of unclean water in the fermentation process which may have serious

health implications. For example, although most types of *E. coli* are harmless or cause relatively brief diarrhea, a few strains, such as *E. coli* O157:H7 can cause severe stomach cramps, bloody diarrhea, fever, and vomiting (Mayo Clinic, 2020). In severe cases, *E. coli* infection could lead to bloody urine, decreased urine output, pale skin, bruising and dehydration (Pietrangelo, 2021). Similarly, *Salmonella* infection can cause salmonellosis which also characterized by an upset stomach, diarrhea, fever, vomiting, and pain and cramping in the belly. In severe cases, it can be life-threatening (WebMD, 2022). *Klebsiella* species have been implicated in spoilage of food and beverages (Mbachu *et al*., 2014).

Finally, the antimicrobial susceptibility pattern of the isolates indicate the antibiotic which most of the organisms were resistant to is Chloramphenicol (60%) implying this may not be a good option in treating infections caused by kunu consumption.

The organisms isolated from kunu retailed in Alimosho, Lagos State are of public health concern as these bacteria species are known to cause a variety of human illnesses and have been implicated in cases of food intoxication. There is therefore the need to maintain adequate hygienic conditions during and after processing of the beverage to eliminate these microbial contaminants and to improve on the quality of the final product. There is also the need to employ adequate preservative measures to improve the shelf life of the beverage. From the data obtained, it is recommended that kunu drink should be consumed within 24 hours of preparation or preserved using chemical preservatives rather than the refrigerator

Limitation of the study: Although the generalizability of the results is limited by the sample size, the results of this study could still indicate the bacteriological quality of kunu drinks

sold in Alimosho.

5.2 CONCLUSION AND RECOMMENDATION

Of all the samples analyzed, the lowest microbial load was recorded for Sample KCD, which was prepared under controlled environment in the laboratory indicates that with correct control of the fermentation process, the rate of microbial contamination of street vended kunu drink will be drastically reduced. Therefore, there is the need to maintain adequate hygienic conditions during and after processing of the beverage to eliminate these microbial contaminants and to improve on the quality of the final product. Also, apart from sanitary measures, to ensure that kunu is safe for human consumption, regulatory bodies like the State and Federal Ministries of Health should enforce the system of monitoring the production, preservation, and packing techniques. There is also the need to employ adequate preservative measures to improve the shelf life of the beverage. From the data obtained, it is recommended that kunu drink should be consumed within 24 hours of preparation or preserved using chemical preservatives rather than the refrigerator. Also, manufacturers of branded kunu samples should check and improve the quality control process followed for the manufacture of kunu drinks.

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APPENDIX A

	Variable	Frequency	Percentage (%)
Manufacturer	Self-made	4	66.67
	Purchased	2	33.33
Target Customers	Students	1	16.67
	Northerners in the area	3	50
	Shop owners	1	16.67
	Passerby	1	16.67
Customer population per	1-20	0	0.00
day	20-50	2	33.33
	50-100	3	50
	More than 100	1	16.67
Sanitary practices	Poor	2	33.33
	Medium	2	33.33
	Good	2	33.33
Source of ingredients	Cultivated, Self-grown	0	0.00
	Purchased	6	100
Source of bottles	New, purchased	2	33.33
	Used, purchased	2	33.33
	Hand-picked from	2	33.33
	streets		
Addition of flavor	Yes	3	50

	No	3	50
Total		**6**	**100**