BACTERIOLOGICAL QUALITY OF SELECTED READY-TO-EAT FOODS SOLD IN RESTAURANTS WITHIN THE UNIVERSITY OF BENIN, EDO STATE, NIGERIA

BY

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DEPARTMENT OF MICROBIOLOGY

UNIVERSITY OF BENIN

BENIN CITY.

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN MICROBIOLOGY, UNIVERSITY OF BENIN, BENIN CITY.

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CERTIFICATION

This is to certify that this project work was carried out by **Osaivbie Praise OBASOHAN** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

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DR. (MRS). I.B IDEMUDIA **DATE**

(Project Supervisor)

_____ _____

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

(Head of Department)

DEDICATION

This project work is dedicated to God Almighty, for bringing me this far in life. I am truly grateful.

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I would like to begin by expressing my deepest gratitude to God Almighty for His abundant grace, guidance, and blessings, which have been my source of strength and inspiration throughout this academic journey and the completion of this project.

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ABSTRACT

The safety and microbiological quality of ready-to-eat (RTE) foods are essential to ensuring public health, particularly in institutional environments such as universities, where large populations rely on convenient food options. This study evaluated the bacteriological quality of selected RTE foods sold in restaurants within the University of Benin, Edo State, Nigeria. Food samples analyzed over two weeks included Moi Moi (Bean Pudding), Fried Rice, White Rice, Egusi Soup, and Yam and Plantain Sauce. The Total Viable Bacterial Count (TVC) of the food samples ranged from $2.3 \pm 0.30 \times 10^5$ cfu/g to $5.1 \pm 0.28 \times 10^5$ cfu/g in Week 1 and $2.1 \pm 0.18 \times 10^5$ cfu/g to $4.7 \pm 0.30 \times 10^5$ cfu/g in Week 2, indicating a significant microbial load that could pose potential health risks. Cultural, morphological, and biochemical analyses were conducted to identify the bacterial contaminants in the food samples. The bacterial isolates identified included *Escherichia coli*, *Bacillus* sp., *Proteus* sp., *Citrobacter* sp., *Staphylococcus* sp., and *Enterobacter* sp. Among these, *Staphylococcus* sp. was the most prevalent, with the highest percentage occurrence across all food samples. Antibiotic susceptibility testing was performed on the isolates using a panel of commonly used antibiotics, including Cefotaxime, Ampicillin, Ofloxacin, Cefixime, Gentamicin, Levofloxacin, Cefuroxime, Imipenem, Nitrofurantoin, and

Nalidixic Acid. The results showed varying levels of resistance and susceptibility, with certain isolates exhibiting multidrug resistance. This highlights the potential public health threat posed by the consumption of contaminated food, particularly in the context of rising antibiotic resistance. The findings of this study underscore the urgent need for strict adherence to food hygiene and safety standards in restaurants, as well as regular monitoring and enforcement of regulatory guidelines to reduce the risk of foodborne illnesses.

CHAPTER ONE

INTRODUCTION

1.1.Background of the study

The safety of food is fundamental to maintaining health and promoting quality of life. Unsafe food can lead to numerous diseases, disproportionately affecting vulnerable groups such as children, the elderly, and those with weakened immune systems (Azounwu *et al*., 2018). According to the World Health Organization (WHO), approximately 550 million people worldwide fall ill from foodborne diseases each year, with 230,000 deaths, primarily due to diarrheal diseases caused by contaminated food (WHO, 2020). In addition, the World Bank (2018) estimates that foodborne illnesses lead to an overall productivity loss of $95.2 billion annually in developing countries, along with treatment costs amounting to $15 billion per year.

Ready-to-eat (RTE) foods are a category of food that can be consumed immediately without further cooking or preparation. These foods, which may be raw, cooked, hot, or chilled, are particularly popular due to their convenience. Some common examples of RTE foods include pastries, sausage rolls, fried meats, burgers, salads, and milk products (Iwegbue *et al*., 2013). RTE foods have become an integral part of student diets on campuses, including those in the University of Benin, due to their accessibility, convenience, and affordability (Azounwu *et al*., 2018).

However, the increasing reliance on RTE foods raises significant concerns regarding food safety. Poor hygiene practices during the preparation, handling, and serving of these foods pose a considerable risk of contamination by harmful microorganisms. These contamination risks are

especially high in Nigeria, where unregulated food vending is widespread, and foodborne pathogens such as *Escherichia coli O157*, *Salmonella*, *Shigella*, *Vibrio*, *Clostridium* sp., and *Staphylococcus aureus* have been frequently isolated from RTE foods sold in public places (Oje *et al*., 2018; Abebe *et al*., 2020).

Foodborne diseases are a persistent challenge in many parts of the world, with the highest burden observed in Asia and sub-Saharan Africa (Akonor and Akonor, 2010). These diseases are often linked to the contamination of food by microorganisms, which can occur during any stage of food production, from farm to table. The mishandling of food by vendors and food handlers, combined with poor personal hygiene and inadequate food preparation practices, significantly contributes to the spread of foodborne illnesses (Todd *et al*., 2007a; 2007b). In Nigeria, foodborne disease outbreaks are common, and food handlers are frequently implicated as the source of contamination (Akther *et al*., 2021).

One significant outbreak occurred in 2018 when cases of botulism were reported in Abuja, the Nigerian capital, following the consumption of contaminated food (Okunromade *et al*., 2020). This incident underscores the importance of regular monitoring of food hygiene standards and safety practices, particularly in environments such as universities, where a large number of students rely on RTE foods for their daily sustenance.

Foodborne pathogens pose a major public health threat in Nigeria, a country with a growing population and an expanding urban landscape. The sale of RTE foods in public places, such as markets, schools, and food outlets, exposes consumers to potential health risks, particularly when proper hygiene practices are not adhered to by vendors. The economic implications of foodborne

diseases are severe, as they not only result in loss of productivity but also place a significant financial burden on healthcare systems (Abebe *et al*., 2020).

The increasing consumption of ready-to-eat foods, particularly among students and busy professionals, has heightened concerns over the potential contamination of these foods with harmful bacteria. The situation is especially critical in environments like universities, where RTE food vendors often operate under minimal regulation, raising the risk of foodborne illnesses. Several studies have highlighted the role of poor hygiene practices by food vendors as a primary source of contamination (Greig *et al*., 2007).

Most Universities are frequently located far from home, and most hostels throughout the world forbid students from cooking in order to lower the chance of fire outbreak and other potential home accidents. As a result, the bulk of students rely on and frequently patronized the ready-to - eat food outlets dispersed throughout campus for their daily needs. It is firmly held that many students on campus are frequently more concerned with their convenience than with the quality, safety, and hygienic aspects of their food. Thus, it is of utmost importance that the standard of these ready-to eat foods be uncompromised, considering the health implications that could be incurred (Abebe *et al*., 2020). There is a rising concern worldwide on the increased resistance of microbes isolated from ready-to eat foods (Akther *et al*., 2021) due to the lack of adequate treatment options, bacterial related resistant illnesses have become a significant challenge in the management of infectious diseases in health care delivery systems. This study aims to assess the bacterial contamination levels in some ready-to-eat foods sold at some restaurants in the University of Benin.

1.2. Aim of the Study

The aim of this study was to evaluate the bacteriological quality of selected ready-to-eat foods sold in restaurants within the University of Benin, Edo State, Nigeria.

The objectives of this study were to:

1. enumerate , isolates and identify the bacterial isolates .
2. determine the frequency of occurrence of the bacterial isolates.
3. determine the antibiotics susceptibility pattern of the bacterial isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1 OVERVIEW OF READY-TO-EAT FOODS

Ready-to-eat foods, abbreviated RTE foods, are foods intended for direct consumption, without the need for heat treatment before consumption (Manhique *et al*., 2020). The Codex Alimentarius defines ready-to-eat foods as foods (including beverages) that can be raw, cooked, processed, or otherwise prepared and are in a form in which they are consumed directly by the consumer. Global production of Ready-to-eat foods is constantly increasing. The increasing production and consumption of Ready-to-eat foods is due to several factors. One of the factors in the growing popularity of these foods is, for example, the changing lifestyles and preferences of consumers. Due to the fast lifestyle, today's consumers are looking for foods that will be nutritionally rich and at the same time their preparation will be easy and fast (Kotzekidou, 2016). RTE foods represent a large and diverse category of food products that can be divided into different groups according to various criteria. The range of RTE foods varies from country to country according to local production and eating habits. Due to the lack of heat treatment of RTE foods before their consumption, these foods pose a potential health risk to consumers. The risk lies primarily in the possible contamination of RTE foods by pathogenic microorganisms during their production, handling, and storage. Chilled RTE foods, contaminated with pathogenic microorganisms, have already led to many cases of foodborne diseases worldwide. This is one of the reasons why the

issue of microbial food safety has become an important topic in the protection of public health (Mengistu *et al*., 2020).

Ready-to-eat foods can be raw or cooked, hot or cold foods consumed without further heat treatment (Oje *et al*., 2018). In Nigeria, ready-to-eat foods are usually sold by vendors and hawkers whose hygiene practices and sanitation knowledge are unknown. Others establish selling points where They often cook, package and sell ready-to-eat foods. In most cases, ready-to-eat foods are prepared in environments with compromised sanitation and by individuals who do not possess adequate knowledge of hygiene and sanitation practices. Hence, food handlers may not follow standards thereby creating route for microbial contamination of foods.

Ready-to-eat foods are common and important features of urban centres in many developing countries. Vast changes in the social and cultural milieu and long hours spent away from home, make eating out a necessary part of people's daily life. People who depend on such food are often more interested in its convenience than in questions of its safety, quality and hygiene. All kinds of food are sold by food vendors, presenting the options for variety and choice for customers. The most popular street foods in Nigeria includes Suya, Buns, Eggroll, Doughnuts, Ice creams, Fish rolls and even Zobo (Oje *et al*., 2020). While street vended foods are appreciated for their unique flavours as well as their convenience, they are also important in contributing to the nutritional status of the population to the majority of people, especially the low income group in the developing countries.

In contrast to these potential benefits, it is also recognized that vendors are often poorly educated, unlicensed, untrained in food hygiene, and work under crude unsanitary conditions with little or no knowledge about the causes of food borne disease (Kumar, 2019).The unhygienic conditions in which these foods are prepared, stored and served provide a suitable nutritional and physical environment for the growth and multiplication of microorganisms. The largely unregulated nature of street food vending, and poor hygienic practices as well as lack of running water, toilet, proper storage and waste disposal facilities at preparation and services points has resulted in poor unsanitary conditions, exposure to potential contaminants and an increased risk to public health (Kumar, 2020). Food borne illness of microbial origin is a major international health problem associated to food safety and an important cause of death in developing countries.

Food-borne bacterial pathogens commonly detected in street-vended foods are *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, *Salmonella* sp., *Proteus* sp., *Klebsiella* sp., *Enterobacter* sp. and *Shigella* sp. (Ghosh *et al*.,2007). Vending of street foods is a growing trade Worldwide. The advent of urbanization has led to an Increase in urban population giving rise to the expansion and growth of street food trade in many countries. Vending street food plays an important socio-economic role in meeting the food and nutritional requirements of urban residents at affordable prices to the lower and middle Income groups (Buzby and Roberts, 1997). Further, vending of street foods provides employment and income for many people (Gibbons *et al*., 2006).

Increased urbanization has resulted in a progressively high proportion of urban food consumers particularly those who do not have much time to prepare their own food or go to eating places(Hanson *et al*., 2012). A study in India showed that 42% of working women and men between 25-45 years, and 61% of students between 14-21 years consumed foods from the streets at least once a day. While it is regularly thought that children under-five is fed from home (Buzby and Roberts, 1997 observed that many mothers working at the markets in Accra sometimes fed Their babies on food items from street vendors. Besides providing nutritional requirements, street foods are appreciated for their unique flavors, convenience and Increased reliability to consumers (Muinde and Courier, 2005). It is apparent that consumption of street vended foods is common and plays an essential role in meeting the daily nutritional requirements of urban populations hence; the safety of these foods is of great significance and therefore deserves attention.

2.2 FOOD SAFETY AND EPIDEMIOLOGICAL CONCERNS

Food is essential to all living things hence its safety is not negotiable to ensure the well-being of the consumer is not adversely affected. Food safety is the assurance that upon consumption of food, there is no harm or injury to the consumer (Feed The Future, 2020). The safety of food for consumption cannot be overemphasized. Food safety is however often compromised through contamination of food by various agents which can be living or non-living and can lead to food poisoning. Food poisoning is a situation where someone becomes sick upon consumption of food and is often characterized by vomiting, stomach ache or abdominal pain as well as diarrhoea

(Kumar, 2020). Other symptoms such as fever, headache nausea and discomfort exist with no definite time limit for victims of food poisoning to feel the impact of the poisoning evidenced by the symptoms.

The living organisms that are responsible for food poisoning include bacteria, parasites and viruses. Bacteria such as *Salmonella enterica* serovar *Typhimurium, Vibrio vulnificus, Escherichia coli*, among others have been reported to cause food poisoning. According to Fung *et al*. (2018), about 90% of food-poisoning episodes are caused by species of *Salmonella, Campylobacter, Listeria, Bacillus, Vibrio, Staphylococcus* and *Escherichia coli* O157:H7, a notorious pathogenic strain of *E. coli,* has been found in plant products such as vegetables and fruits as well as animal products like chicken, pork and even milk resulting in severe symptoms (including kidney failure) in victims who ingested it in contaminated food (Fonseca *et al*., 2011; Alum *et al*., 2016). Parasites such as *Toxoplasma* as well as viruses such as rotavirus and astrovirus have also been implicated as agents of food poisoning albeit to a lesser degree than bacterial agents (Kumar, 2020). Non-living agents of food poisoning are toxins and chemicals some of which also serve as allergens in the body. Kassahun and Wongiel (2019) reported 35 food poisoning cases in Ethiopia in 2018 with an attack rate of 25.58 per 10,000 persons and identified risk factors associated with the food-poisoning cases.

In Nigeria, the most populous African country, food-borne illnesses are considered to have high health (resulting in over 200,000 deaths annually) (Ezirigwe, 2018). Salad another major ready to eat food was studied in a research, despite its numerous health benefits, poses a food safety

threats. Salad vegetables are globally considered a major source of nutrients namely: vitamins, minerals, proteins and other relevant nutritional components for the proper functioning of the human body(Amoah, 2014). They are particularly a good source of bioactive phytochemicals with varied potentials such as supplying cancer-fighting agents, providing nourishment for the skin, fiber which aids in digestion and also prevents colon cancer.

Studies (Coulibaly-Kalpy *et al*., 2017) have established that consumption of salad vegetables contributes to a reduction in the incidence of certain diseases like diabetes, coronary heart disease, colon cancer, high blood pressure, obesity, and various digestive disorders in addition to preventing heart disease and skin cancers. The likelihood of the occurrence of foodborne diseases is increased when salad vegetables are consumed without any thermal or chemical treatment, sometimes without washing and peeling(Tambekar and Mundhada, 2006). Mensah *et al*. (2002) implicated wash water used for rinsing the vegetables and sprinkling to keep them fresh as a source of contamination.

Contamination caused by microorganisms according to Udo *et al*. (2009) arises on fresh vegetables and fruits during all processes leading to distribution. Furthermore, processing which includes cutting into desired shapes and sizes with knives or other shredding utensils can be a source of contamination (Ababio and Lovatt, 2014). Although these pathogens cannot be unfortunately detected with the naked eye, felt, tasted or smelled, they have the potential to cause a myriad of diseases of varying severity and ultimately death. The propensity of their encounter is increased especially during processing, handling and storage of these foods which induce the

growth conditions of these microorganisms, for example *Staphylococcus aureus, Escherichia coli* and some others. Their effects on the human body are directly proportional to the degree of infection or levels of contamination. The chances of food contamination increase when food is prepared in bulk (commercial) and is handled by many individuals along the different food processing chains.

The occurrence of unexpected contamination of food during bulk cooking usually leads to foodborne disease outbreaks, which can pose danger to the health of consumers and the national economy(Annor and Baiden, 2011). Again, regardless of their quantity, if not properly handled, ready-to-eat foods may become a channel of transmission of microbes into human bodies as they are not at all processed before consumption. In connection with the consumption of contaminated foods and drinks (including water), report shows that diarrheal diseases are responsible for more than half of the global burden of foodborne diseases, causing 550 million people to fall ill and 230, 000 deaths every year. Children are at particular risk of foodborne diarrheal diseases, with 220 million falling ill and 96 000 dying every year (Kortei *et al*., 2020).

2.3 VARIOUS READY-TO-EAT FOODS

As earlier highlighted, various Ready-to-eat foods commonly vended all over the world include salads, vegetables, junk foods like barbecue, biscuits, egg rolls, buns, sushi, sharwarma, meatpies, doughnuts, Suya, Pap and many others. Each of these food types have high tendency for contamination either resulting from cross contamination from unhygienic and improperly trained handlers, from the food processing material or an unintended process failure overall.

2.3.1. Ready to cook (RTC) food products

Ready to cook products are the type of food which are processed or prepared with very little extra efforts and it also called as convenience food. Urbanization and globalization change people's lifestyle and living standards. RTC consumers are mostly from urban area including bachelors and busy life peoples in cities (Malik and Kajla, 2020). Demand for ready to cook food are increasing because number of factors are responsible for it such as readily availability of food, culturally acceptable, nutritive and minimally processed, urbanization of domestic labor, dearth of time, convenience of food, increase in per capita income, and affordable by middle class people. Ready to cook meal are can be consider as best alternative for homemade meal and its consumption trends is going increasing due to increasing working women populations (Sathiyabamavathy *et al*., 2020).

2.3.2. Vegetable Salads

Vegetables remain a crucial part of a healthy diet, making the demand for fresh vegetable salads constantly on the increase. Vegetables provide humans with essential vitamins and minerals with enough ability to help reduce the risk of diseases and infections. The World Health Organization (WHO) and Food and Agricultural Organization (FAO) recommend regular intake of vegetables (Toe *et al*., 2017). The health benefits of salad have increased its use for the purposes of weight loss, medical disorders and strokes. A foremost issue associated with the consumption of fresh vegetable salad is their contamination with pathogenic microorganisms (Feng, 2002).

Vegetable salads are consumed uncooked, with washing being the major means of removing

contaminants and microorganisms. This method of decontamination is not often sufficient to remove all pathogenic organisms from the vegetables.

Various factors account for the high risk of microbial contamination associated with vegetable salads. This ranges from environmental conditions (hygiene) where these vegetable salads are prepared, washing of the vegetables using contaminated water, cultivation, post-harvest handling to transportation (Taban and Halkman, 2011). Other ingredients used in the making of the vegetable salad could also be a source of contaminant. Thus, from planting, harvest to processing and consumption, the probability of these vegetable salads coming in contact with pathogenic microorganisms is very high.

Common bacterial isolates linked with contamination of vegetable salads include; *Listeria monocytogenes, Bacillus cereus, Clostridium perfringens, E. coli, Staphylococcus aureus, Shigella sonnei, Salmonella typhi, Pseudomonas aeruginosa* (De Rover, 1998). Studies have shown that these organisms are among the most common foodborne pathogens in Nigeria (Nkere *et al*., 2011).

Vegetable salads are globally produced and commercialized all year and have been accompanied by new food safety threats since they are eaten raw and usually without proper cleansing practices. Thus, these products are recognized as possible vehicles of foodborne diseases, which are highlighted by large and serious national and international outbreaks (Ahmad *et al*., 2018).

The Centre for Disease Control and Prevention has described microbial contamination of ready-to-eat foods as a public health concern and usually in the developed countries due to the presence of bacteria that are of public health concern (Abakari *et al*., 2018).

Occurrences of foodborne disease have shown links between pathogens and RTE vegetable salads which carry the potential risk of microbiological contamination (Park *et al*., 2012; Ahmad *et al*., 2018). Outbreaks related to fresh produce include cases of *Escherichia coli* O157:H7 (lettuce), *Salmonella Typhimurium* (tomatoes, lettuce) and hepatitis A (spring onion) in Lancaster, UK (Heaton and Jones,2008). *Listeria monocytogenes*, *Salmonella*, and *Escherichia coli* O157:H7 are among the most important pathogens of concern to produce food safety (Park *et al*., 2012; Mogren *et al*., 2018) and *Yersinia* spp. (Mogren *et al*., 2018). These are all facultative anaerobic bacteria and the majority are motile. They can grow across a wide pH range, from strongly acidic (pH 2 and for *Cryptosporidium*) to alkaline (pH 9–10). They have an optimum temperature for growth of between 21 and 37◦C, although some are capable of growth at much lower temperatures, close to 0^0C (*Y. enterocolitica* and *L. monocytogenes*).

Some are widespread in the environment (*L. monocytogenes*) and most are found in the gut and faeces of warm-blooded animals (Mogren *et al*., 2018). Outbreaks of salmonellosis and listeriosis due to consumption of vegetables have been reported. Also, *E. coli* O157:H7 and *Salmonella* lead to outbreaks associated with the consumption of pre-cooked leafy green salad vegetables (Qadri *et al*., 2015).

Ready-to-eat salads were implicated in a 2013 outbreak of *E. coli* O157:H7 in the United States of America that resulted in the hospitalization of 33 persons and no fatalities (Jongman and Korsten, 2017). *Salmonella* spp. has been detected regularly in surveys conducted on fresh vegetables at fresh-cut processing companies retail establishments in different countries worldwide. *Salmonella* can pollute fresh produce both during the production through water, soil, insects or other animals contaminated with fecal matter, and during the preparation, through cross contamination (equipment, surfaces, food handlers). A range of fresh vegetable products have been implicated in *Salmonella* infection, most usually lettuce, sprouted seeds and tomatoes (Heaton and Jones, 2008). *L monocytogenes* are ubiquitous in the environment and can be isolated from soil, water, vegetation, the faeces of livestock and vegetation irrigated with contaminated water.

Listeria in the environment has the potential of contaminating fresh produce and lead to enteric infection. L. monocytogenes serotype isolated from salad vegetables has been shown to be serogroup 1 (Heaton and Jones, 2008). These surveys show variation in prevalence on different types of produce and between countries. *L. monocytogenes* from beansprout (85%), leafy vegetables (22%) and cucumber (80%) in Malaysia. In comparison, only 67% of cucumbers sampled in Pakistan yielded L. monocytogenes only from potatoes (50%) and field cress (18%) purchased at farmers' markets. *Escherichia coli* and Enterobacteriaceae were isolated from 35 samples of RTE lettuce salads in Mozambique (Manhique *et al*., 2020).

The incidence of *Bacillus cereus* and *Clostridium perfringens* in 35 salad samples were determined in Johannesburg city and South Africa (Kubheka *et al*., 2001). Abakari *et al*., (2018) observed 30 salads samples vended in the central business district of Tamale, Ghana. *Escherichia coli* was detected in 96.7% of salad samples with levels ranging from 0 to 7.56 log10 cfu/g. *Bacillus cereus* were present in 93.3% of ready-to-eat vegetable salads with counts ranging from 0 to 7.44 log10 cfu/g. Further, *Salmonella* spp. and *Shigella* spp. were present in 73.3% and 76.7% of salads, respectively. *Salmonella* spp. and *Shigella* spp. counts ranged from 0 to 4.54 log10 cfu/g and 0 to 5.54 log10 cfu/g, respectively.

Ajayeoba *et al*., (2015) investigated total of 555 composite samples of vegetables Cucumber (*Cucumis sativas*), Cabbage (*Brassica oleracea*), Carrot (*Daucus carota*) Tomato (*Solanum lycopersicum*) and Lettuce (*Lactuca sativa*) from 30 traditional markets in six states in Southwestern Nigeria in his study on incidence and distribution of *Listeria monocytogenes* in ready- to- eat vegetables in South- Western Nigeria. Lagos state had the highest incidence of *L. monocytogenes* contamination (55%) followed by Ondo (48.89%), Oyo (48.75%), Ogun (44.09%), Osun(34.38%), and Ekiti (33.33%) stated, respectively. Foodborne disease outbreaks of pathogens in developing countries are poorly reported or are not reported at all due to the sub-standard surveillance system in these countries (Jongman and Korsten, 2017).

In Nigeria, vegetables and raw materials for the preparation of vegetable salads are often sold in open unhygienic places. The major means of removing pathogens remains washing and often

times the sources of water are also contaminated. The vendors of these vegetable salads often sell them in open places which are often not germ-free.

2.3.3. Ready-to-eat meat products

The growth of the fast-food chain has brought about a high demand for ready-to-eat meat products. Ready-to-eat meat products also serve as snacks and delicacies during social events or relaxation. However, the lack of appropriate regulations for fast food ventures poses a significant public health risk. There is a very poor or nonexistent reporting system for food-borne illnesses. Hence, most food-borne illnesses or poisoning go unreported, leaving other unsuspecting consumers to go through the same fate (Roobab *et al*., 2020).

Ready-to-eat meat products, prepared as kilichi, tsire, or suya, from either beef, poultry, or mutton and spiced with locally sourced condiments (groundnut oil, pepper, ginger, garlic, groundnut cake, salt, and seasoning) are common local market food products across many African countries (Kim *et al*., 2019, Dawood, 2020, Binda, 2020). Suya is often prepared, staked on sticks, spiced, and roasted on hot charcoal.

Earlier reports have identified the presence of pathogenic microorganisms, heavy metals, and polycyclic aromatic hydrocarbons (PAHs) in suya sold and consumed in Nigeria (Kim *et al*., 2019). The introduction of heavy metals might result from water and contaminated utensils used during processing. While PAHs might have been generated during incomplete combustion during the roasting of the suya meat. In an earlier report of polycyclic aromatic hydrocarbons (PAHs) in four commonly roasted food products (suya, plantain, fish, and yam), roasted plantain had the

highest concentration of PAHs (0.0465mg/kg), followed by suya (0.0372 mg/kg), which were above the permissible limits (Feyereisen *et al*., 2019). Suya is also listed as one of the unsafe ready-to-eat food products to consume in Nigeria due to the unsanitary environment it is prepared and the unhygienic practices of some ofthe vendors . Prepared suya is often served with some slices of onions, cabbage, tomatoes, and/or cucumber to improve its nutritional value, organoleptic and antioxidant properties (James *et al*. 2019).

Most suya vendors who can be seen in various city corners and public places have little or no formal education. Hence, the challenge of knowing about hygienic practices in food handling and sustaining the same (Song *et al*. 2020). There is little or no provision for required storage or cooking facilities, utensils sterilization, quality water access, or waste disposal. The vendors are also fond of handling the meat products with bare hands, no hair covering or apron, talking (whereby some might mistakenly spit, sneeze, or cough), and taking money from consumers while processing the food (James *et al*., 2019). Likewise, it is common practice that consumers also touch suya (spread in an open space) with unwashed hands while making their preferred choice. These food products are often hawked or prepared in unsanitary locations along roadsides or public places where they can be exposed to vehicular emissions, environmental contaminants, or other human activities (Roobab *et al*., 2020). These and many others are public health concerns about suya's nutritional value and unhygienic standards during its preparation, processing, and packaging.

Generally, these varieties of meat products, including "Suya," are subjected to combination of several basic processing steps before reaching their final form, hence get contaminated along the line of production. Micro-organisms that occur in meat and meat products most times are responsible for food borne illness. These micro-organisms include *Bacillus* sp, *Clostridium* sp, *Escherichia coli, Salmonella* sp, *Shigella* sp, *Staphylococcus aureus, Streptococcus pyogenes*, *Proteus* sp, *Pseudomonas aeruginosa, Leuconostoc* sp, *Lactobacillus* sp, *Micrococcus*, *Mycobacterium* sp, *Vibrio* sp, etc (Guimaraes *et al*., 2020; Roobab *et al*., 2020).

Other studies have pointed out that food cross-contamination during preparation contributes remarkably to the occurrence of food-borne diseases 6. In particular, *Salmonella* may be transferred from raw meat to cooked meat by hands, surfaces or utensils. In other food products, outbreaks of salmonellosis have been associated with the consumption of cut watermelon and cantaloupe in the United States of America (Salvetti and O'Toole, 2017; Valero-Cases *et al*., 2020). In general, they all indicate evidences of faecal contamination. Further, this local meat product is often neglected by agencies responsible for food services as having a role in the introduction of potential food borne bacteria or parasites into populations' diet; they concentrate on officially produced licensed products (Feyereisen *et al*., 2019).

Further, as risks of these *E coli* contamination, pathologic syndromes of their various five strains are serious threat to consider: Enteroheamorrhagic *E. coli* (EHEC) causes haemolytic uremic syndrome and sudden kidney failure; Enteroaggregative *E. coli* (EAEC) possess fimbriae which has aggregate tissue culture cells that binds to the intestinal mucosa and produce hemolysin, a

toxin that leads to serious intestinal upset; Enterotoxigenic *E. coli* (ETEC) produces heat-labile or heat stable enterotoxins in the gut which leads to unsuspected hypersecretions in small intestine that causes a sudden, very inconvenient gastroenteritis referred to as traveller's diarrhea; Enteropathogenic *E. coli* (EPEC) attaches to mucosal epithelial cells and produces cyto-skeletal changes, squamous mucosa, and it can invade cells, enter the blood to cause very serious systemic syndromes. Lastly, Enteroinvasive *E. coli* (EIEC) infection causes a syndrome that is identical to shigellosis, with profuse diarrhea and high fever. All of these five pathologies can lead to medical emergencies (Abdelazez *et al*., 2018; Feyereisen *et al*., 2019; Song *et al*., 2020).

2.3.4. Dried foods and Snacks

Dried foods like chips, biscuits, muesli or roasted nuts are very popular snack items all over the world which are available in every grocery shop, super shops as well as in the remote areas. These are enjoyed most during vacation, journey, picnic and even as school tiffin. The principal of production of potato chips are cooked and salted potatoes pieces mixed with different kinds of flavorings and ingredients including herbs, spices, cheeses, natural or artificial flavors and additives. Nuts are a source of protein, fat and minerals. People who are more considerate about their health prefer nuts than junk snacks (Bhat and Vasanthi, 2003).

Dried cereals are best suited for a modern busy life as they can be eaten directly with milk without further processing. Cereals are produced by mixing different cereal grains and processing them together (like roasting, grinding, swelling, shredding, flaking and so on). They are a good source of vitamins, minerals, zinc, phosphorus and calcium (Williams, 2014; Mbaeyi-

Nwaoha *et al*., 2016). Another popular dry food is biscuit which mostly contains carbohydrate, and some protein, gluten and fat content (Adobowale *et al*., 2012).

There are international standards for the microbiological limit of foods and beverages. These standards can be modified slightly by locally. Almost every country has its own regulations and monitoring body to check the quality of the food, exclusively the ready to eat foods due to its high alarm for human health hazard. As a ready to eat foods, chips, biscuits, salted nuts and muesli have a risk for microbial contamination on its production, packaging, transportation or storage (Eze *et al*., 2011; Oladipo *et al*., 2019). *Escherichia coli* O157: H7, *Salmonella* sp, *Shigella* sp., *Bacillus* sp., *Mycobacterium* sp., *Brucella* sp*., Listeria monocytogenes, Yersinia enterocolitis, Pseudomonas* sp*.; Clostridium perfringens, Klebsiella* sp., *Vibrio* sp., *Campylobacter jejuni* and *Staphylococcus aureus* have been reported to be the common food spoilage bacteria (Beuchat *et al*., 1996; Torquato *et al*., 2004; Jay *et al*., 2005; Nordmann *et al*., 2009; Rahman and Noor, 2012; Oladipo *et al*., 2019). Furthermore, it contains toxic and carcinogenic byproducts which are not found in the uncooked foods (Exon, 2006). Potato Chips contaminated by microorganisms(bacteria, yeasts, viruses, and protozoa) can cause health-related issues (Jaykus, 2000). Contaminated nuts with fungus and associated toxin can cause liver damage, cancer, abortion etc. (Abbas *et al*., 2005).

A snack is a portion of food smaller than a regular meal, generally eaten between meals. Snacks comes in a variety of forms including packaged snack foods and other processed food, as well as items made from fresh ingredients at home. The consumption of snacks has become an integral

part of convenient food preparation pattern all over the world including Nigeria. The most popular snacks consumed and sold in Nigeria are meat pie, sausages, doughnut, fish roll, eggroll, hamburger and so on. The consumption of snacks has been reported to be association with serious health problems and hazards (Williams, 2014). “In developing countries such as Nigeria, a large proportion of ready to eat foods are sold on streets” (Adams and Moss, 2013). “Fast food has already become a common feature of urban life with the increasing pace of globalization and tourism “The safety of fast food has become one of the major concerns of public health and a focus for government and scientist to raise public awareness of food” (Mensah *et al*., 2002).

Due to lack of proper knowledge and guidance on fast food vending, vendors prepare this food in explicitly unhygienic and sanitary conditions. Consumers who depend on such food are more interested in its convenience and usually pay little attention to its safety, quality and hygiene. It has been observed that fast food vendors in Nigeria practice minimal hygiene and sanitary practices. They lack knowledge on the epidemiological importance and public awareness of fast foods with hampers precise scientific approach of the food safety problem. However, there were limited studies on specific hazard posed by microorganisms of public health concern in fast food. “Eating out” which has previously been considered a luxury became a common occurrence than a necessity. Workers, working families and students need quick service (Oranusi *et al*., 2013). This need is what drove the phenomenal success of the early fast-food giants, which catered to the family on the go. Fast food became an easy option for a busy family as in the case of many families today. “Also research on the bacteriological safety of some ready to eat food snacks

vended on Onitsha-Owerri highways in south east Nigeria showed the bacterial pollutants to be *Bacillus Cereus, Staphylococcus aureus, Aspergillus niger, Escherichia coli, Shigella* sp., *Salmonella* sp., *Enterococci* sp. and *Pseudomonas* "Ready to eat street foods are most times sold at unclean vicinity exposing these foods to air and dust and often stored at unsuitable temperatures which favour bacteria growth and excessive handling by the food vendors The research also reported "poor unhygienic practices by food handlers and food vendors being healthy carriers serving as potential source of transmission of enteric fevers". (Oranusi and Braided, 2012).

2.4. MICROBIA CONTAMINATION OF READY-TO-EAT FOOD

The microorganisms in food products do not arise by spontaneous generation; they must contaminate the food at some stage of production, harvesting, handling, processing, storage, distribution, or preparation for consumption. Microbiological contamination is the most frequent cause for spread of most foodborne diseases. European legislation stipulate that food shall not contain microorganisms, toxins or metabolites thereof in amounts that cause risk for human health. Food may not be distributed if not safe, including food that is microbiologically contaminated (Marèenkova, 2010).

Listeria monocytogenes is considered to be the highest risk factor for ready-to-eat products, because of its ubiquity in the environment, tolerance to unfavourable environmental conditions, and ability to survive on equipment that results in contamination of end-products. The disease primarily affects older adults, pregnant women, newborns, and adults with weakened immune

systems. There was a recent documented outbreak in Switzerland caused by consumption of ready-to-eat salads (Mataragas, 2010; Stephan *et al*., 2015).

Salmonella species cause salmonellosis. Mortality from this infection disease for the population as a whole is low (< 1%), but there is raised risk for the elderly and infants. Meat and poultry area the main sources of *Salmonella*. Rates of contamination from this bacteria vary, but it is predicted that the highest contamination is in poultry products. Human salmonellosis is normally seen in the form of small family outbreaks and one of the final stages in the contamination chain is usually the cross-contamination of cooked food by raw food or by dirty working surfaces, the cooked food being left at room temperature for a number of hours (Forsythe and Hayes, 1998).

Symptoms of ingestion of food contaminated with *Staphylococcus aureus* appear quickly, within 1–6 hours. The most typical symptoms are vomiting, cramps and diarrhoea and mortality recorded is extremely low. An important source of *S. aureus* is human body, the main reservoir being the nose. There is a risk from cooked foods that have been handled by a *S. aureus* carrier and stored under warm conditions for a long period, for example, cured cooked meat, ham and cold meat and poultry products (Forsythe and Hayes, 1998)

Non-pathogenic *Escherichia coli* strains are typical for intestines of warm blooded animals; however, there are six groups of *E.coli* strains pathogenic to humans in different degrees (Forsythe and Hayes, 1998). Pathogenic *E. coli* strains cause diverse intestinal and extra intestinal diseases by means of virulence factors that affect a wide range of cellular processes (Kaper *et al*., 2004).

There are no available data on clinically diagnosed cases of lysteriosis and poisoning with *S. aureus* in humans in Latvia, but in many cases they may remain undiagnosed. Other foodborne illnesses are more likely to be diagnosed and the potential pathogen can be found. The incidence of salmonellosis cases has decreased during the last three years, while incidence of undifferentiated gastrointestinal disease cases have stayed almost the same. There are a number of unknown cases of bacterial disease, particularly when clinical symptoms of food borne diseases are not severe.

2.5. FOODBORNE PATHOGENS

Foodborne diseases can have a variety of causes, e.g., overeating; allergies; nutritional deficiencies; actual poisoning by chemicals; toxic plants or animals; toxins produced by bacteria; infestation by animal parasites; and infection by microorganisms. The term "food poisoning" is applied to diseases caused by microorganisms, which include both illness caused by the ingestion of toxins elaborated by the organisms and those resulting from 20 infection of the host through the intestinal tract. All food borne diseases are subdivided into poisonings and infections. *Escherichia coli, Salmonella, Campylobacter jejuni, Shigella* sp*., Yersinia enterocolitica, Vibrio parahaemolyticus, Vibrio cholera, Aeromonas* sp*., Clostridium perfringens* and *Listeria monocytogens* are responsible for food borne infections. *Staphylococcus aureus, Bacillus* spp., *Clostridium botulinum* and other protozoa and viruses are responsible for food borne intoxications. The majority of outbreaks and causes are attributed to Staphylococcal intoxication, Salmonellosis and *Clostridium perfringens* gastroenteritis (Fraizer and Westhoff, 2008e).

2.5.1 *Salmonella* sp.

With a few important exceptions, e.g. *Samonella enterica* sub sp. *Samonella typhi*, *Samonella dublin* and *Samonells choleraesuis*, salmonellae show little host specificity and most can cause gastroenteritis when ingested by humans. *Salmonella typhi* and *S. paratyphi* A, B and C are worthy of special mention. These serotypes are host adapted to humans, but can be transmitted in food. The usual source of these organisms in food is by contamination from an infected food worker or by direct contamination from human sewage (Roberts and Greenwood, 2003).

All members of the genus *Salmonella* are potentially pathogenic for humans as well as for vertebrate animals. The transmission of the disease is usually from animals to humans by the ingestion of food of animal origin. Direct transmission is also possible from human to human, from human to animal and from animal to human (Banwart, 2000c).

Salmonella occurs worldwide and it is recognized as a zoonotic agent. The primary habitat is the intestinal tract of animals including humans. Ingestion of certain strains of Salmonella can result in foodborne disease. Foods that are commonly identified as vehicles of salmonellosis to humans include eggs, poultry, meat and meat products. The food poisoning syndrome is generally due to the ingestion of foods that contain significant numbers of certain serotypes of Salmonella. Normally levels necessary to cause salmonellosis range from 10^7-10^9 cells/g. Levels of 10^5/g is highly suggestive of the possibility of food poisoning occurring.

2.5.2. *Clostridium perfringens*

Clostridium perfringens has been called ubiquitous, due to its widespread distribution in nature. It is found in soil, dust, air, water, sewage, human and animal feces, and on many food products. Good growth occurs between pH 5.5 and 8.0. The optimum range for enterotoxin 21 production is pH 6.5 to 7.3. *C. perfringens* grows rapidly at temperatures between 20o and 50^{o}C, with maximum growth between 37o and 47^{o}C.

These organisms produce spores that are relatively heat stable which influences their survival during and after cooking. Those that survive will grow and multiply especially during poor storage conditions and cause food poisoning. Food poisoning caused by this organism is relatively mild. Normally large numbers of cells have to be ingested to cause illness. Counts of 105/g are highly suggestive of the possibility of food poisoning occurring. Foods commonly associated with *Clostridium perfringens* contamination include dairy products, pasta, flour, poultry and vegetables, which have been exposed to soil, dust and faecal material (Banwart, 2000c).

2.5.3 *Escherichia coli*

Escherichia coli is an important organism in the microbiology of foods. An important reservoir of this organism is the intestinal tract of human, cattle and other food animals. However, fecal contamination causes it to spread to other environments, especially soil and water. It is widely distributed in food environments in low numbers. The infectious dose is low (as low as 10^1 - 10^2 /g). Low doses cause illness in young children, the elderly and immunocompromised persons.

Foods implicated include undercooked hamburger patties and other fast foods and cheese made from unpasteurized milk.

2.5.4 *Listeria monocytogens*

The organism is a small Gram-positive rod with a tendency towards a diplobacillary form. Although, it is aerobic, it grows better at reduced O_2 and increased CO_2 levels. It is ubiquitous in nature (i.e. widespread in soil, food-processing environments, raw meats and faeces of healthy humans and animals). It is an opportunistic pathogen affecting mainly the elderly, immuno-compromised persons, pregnant women and young children. The minimal infectious dose is estimated to be $>10^2$/g. It has the ability to grow even at refrigeration temperatures, which makes it a problem in refrigerated foods. Foods normally implicated in outbreaks include soft cheeses, fermented sausages and coleslaw and other salads .

2.5.5 *Shigella* spp.

The genus *Shigella* includes four species: *S. dysenteriae, S. flexneri, S. boydii,* and *S. sonnei*. These are not the natural inhabitants of the environment. The normal habitat is the intestinal tract of human beings and other primates. Isolation from other animals is rare. These are host adapted organisms and only infect humans and other primates. Foods, which serve as vehicles include milk, vegetable salads, orange juice and cooked rice. The infective dose is small (10^1 - 10^2 /g). The main source of *Shigella* involved in outbreaks is people who are symptomless carriers, or ambulant cases. Foodborne outbreaks of shigellosis are caused by the mishandling of food.

2.5.6. *Yersinia enterocolitica*

Yersinia enterocolitica is a member of the family *Enterobacteriaceae*. *Y. enterocolitica* can be found almost everywhere in nature, but only certain serotypes are involved in human infections. Pigs are believed to be the principal reservoir of serotypes pathogenic to man. It is sensitive to heat while resistant to other adverse storage conditions. The ability of the organism to grow at refrigeration temperatures makes refrigerated animal foods, such as milk, a potential hazard. The minimum infectious dose is uncertain. Foods implicated in outbreaks include unpasteurized milk, chocolate milk and raw pork.

2.5.7 *Staphylococcus aureus*

Staphylococcus aureus is found in the mucous membranes and skin of most warm-blooded animals, including humans. Unless heat processing steps have been applied, this opportunistic pathogen may be expected to exist in low numbers in many food products that are of animal origin or in those that are handled directly by humans. It does not compete well with other bacteria. It is seldom linked to food poisoning outbreaks from consumption of raw products. It can be readily killed by cooking, but toxins are heat stable and will survive. It is resistant to freezing and thawing, and survives well in foods stored at –20^{o}C, but at higher temperatures ranging from –10^{o}C to 0^{o}C the viability of these cells decreases markedly during frozen storage.

The minimum number of cells of *S. aureus* required to produce the minimum level of enterotoxin considered necessary to cause the gastroenteritis syndrome in humans depends on the

substrates. The minimum quantity of enterotoxin needed to cause illness in humans is about 200 ng. Counts of 105/g are highly suggestive of the possibility of food poisoning occurring.

2.7. ANTIMICROBIAL SUSCEPTIBILITY OF COMMON PATHOGENS ASSOCIATED WITH READY-TO-EAT FOODS

Due to the nature of it's processing and sales, ready-to-eat foods are often colonized by a plethora of pathogenic and highly resistant organisms .The following are organisms that are commonly encountered include: *Staphylococcus aureus, Escherichia coli, Clostridium botulinum, Listeria monocytogenes, Vibrio vulnificus, Clostridum perfringes, Bacillus cereus, Vibrio cholera, Streptococcus* sp, *Enterococcus* sp, *Shigella dysenteriae, Klebsiella pseudomonas, Micrococcus* sp, *Flavobacterium, Mucor, Penicillium* sp, *Aspergillus niger, Aspergillus flavus, Fusarium* sp, *Proteus vulgaris, Salmonella* sp (Asiegbu, *et al*.,2020)

Staphylococcus aureus (*S. aureus*), i.e. a Gram-positive and catalase-positive bacterium, is a major cause of foodborne diseases with a short incubation period as well as symptoms, such as weakness, vomiting, nausea, and abdominal cramps in people (Wang and Ruan, 2017). Contaminated foodstuffs, particularly ready-to-eat food samples, are considered reservoirs of *S. aureus* (Islam *et al*., 2019). This bacterium develops resistance to diverse kinds of antibiotic agents. Resistant *S. aureus* bacteria are responsible for about 100,000 infectious disease cases, with about an annual mortality rate of 20–30% in the United States. Resistant *S. aureus* bacteria cause complicated diseases for a long period (Li and Webster, 2019). Research reports that *S. aureus* bacteria harbor high resistance to diverse kinds of antibiotic drugs, particularly penicillins,

cephalosporins, tetracyclines, aminoglycosides, macrolides, and fuoroquinolones (Mahadi *et al*., 2014).

Some antibiotic resistance genes are responsible for development of antibiotic resistance in *S. aureus* strains (Abdulmaleki *et al*., 2019). TetK and tetM (tetracycline resistance genes), ermA and msrA (macrolide resistance genes), gyrA and grlA (fuoroquinolone resistance genes), blaZ (penicillin resistance gene), dfrA (folate inhibitor resistance gene), rpoB (ansamycin resistance gene), aacA-D (aminoglycoside resistance gene), linA (lincosamide resistance gene), and cat1 (phenicol resistance gene) are the major resistance genes among *S. aureus* bacteria (Abdulmeleki *et al*., 2019). Given the high consumption rate of ready-to-eat foodstufs in Iran and the high importance of *S. aureus* as a food-borne pathogen, the present survey was performed to assess the prevalence as well as phenotypic and genotypic patterns of antibiotic resistance in *S. aureus* bacteria isolated from diverse kinds of ready-to-eat food samples.

Asides Staphylococcus aureus, newer strains of Gram negatives rods such as *Escherichia coli* and *Klebsiella pneumoniae,* have proven to be intrinsically resistant to conventional available antibiotics such as Cephalosporins, Methicillin, Carbapenems, Tetracyclines, Aminoglycosides and a host of others. This poses a serious health concern and public health emergency, seeing the proliferation of roadside ready-to-eat foods and drinks. This of course shows a huge gap between research and implementation of research findings.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was conducted in a restaurant located in the University of Benin (UNIBEN), Benin City, Edo State, Nigeria. The University of Benin has a vibrant food service sector that caters to the diverse population of students and staff. The selected restaurant was chosen based on its popularity and the high level of patronage by the university community.

3.2. Sample Collection

Sample collection was conducted over a two-week period. A total of six food samples were collected from a restaurant located within the University of Benin campus. The selected foods for this study were fried rice, plantain sauce, yam, moi moi (bean pudding), egusi soup, and white rice. Samples were aseptically collected in sterile plastic bags and transported to the laboratory under refrigeration (2°C to 8°C) for bacteriological quality assessment. Upon arrival, the samples were immediately analyzed in the laboratory.

3.3.STERILIZATION OF MATERIALS

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

3.3.1. PREPARATION AND STERILIZATION OF MEDIA

Materials used include; Glass wares such as test tubes, beakers, conical flasks, Petri-dishes, McCartney bottles, stirring glass rod and measuring cylinder. Media and Biochemical test reagents and Gram's staining kit . All glassware which include MacCartney bottles, Petri dishes, test tubes, conical flasks, measuring cylinders and pipettes, were sterilized at 160 ^{o}C for 1 hr in a hot-air-oven before use. The media used in this study were sterilized at 121 ^{o}C for 15 min in an autoclave. Agar media, agar slant and biochemical reagents were prepared freshly and refrigerated at 3-4 ^{o}C. Aseptic conditions were ensured during inoculation and subculturing.

3.3.1.1. Preparation of Nutrient agar

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

3.4. ENUMERATION AND ISOLATION OF BACTERIA

The food samples were processed within 24 hours of collection. Each sample (10 g) was homogenized in 90 mL of sterile peptone water, This mixture was considered to be a 10^{-1} dilution. The mixture (1 ml) was transferred to a tube containing 9 ml of saline diluent to make 10^{-2} dilution. Further dilutions were made by transferring 1 ml of the succeeding dilutions to the tubes containing 9 ml diluent to achieve six-fold dilutions. Aliquots (0.1 mL) from each dilution were plated on nutrient agar, using the pour plate technique. The plates were incubated at 37°C for 24–48 hours. After incubation, bacterial colonies were counted and expressed as colony-forming units per gram (cfu/g),

3.4.1. PREPARATION OF PURE CULTURES

One single colony was identified and re-streaked as a primary inoculant on the surface of a nutrient agar plate medium. Pure cultures were checked from nutrient agar plates. After achieving a pure culture, the same colony was streaked onto a nutrient agar slant. These cultures were incubated at 37°C for 24 hr.

3.5. PHENOTYPIC IDENTIFICATION OF BACTERIA FROM SAMPLES

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

3.5.1. Morphology identification

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

Smears of the bacterial isolates were prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal violet. This was washed out with distilled water. The slides were flooded with dilute Grams' iodine solution for one minute. This was washed off with distilled water and the smears were decolorized with 95% alcohol for 30 seconds and rinsed off with distilled water. The smears were then counter stained with safranin solution for one minute. Finally, the slides were washed off with distilled water, air dried and observed under oil immersion objective .

3.5.2. Potassium Hydroxide (KOH) test

Two drops of 3% solution of KOH were applied on a clean glass slide and a loopful of pure bacterial growth was stirred in a circular motion in the slide. The loop was occasionally raised and observed for the presence of a string of the mixture. The solution was observed to be of a viscous and mucoid consistency indicating a Gram-negative bacterium.No reaction (absence of stringing) indicates a Gram-positive bacterium (Roberts and Sandle, 2008).

3.6. Biochemical identification

3.6.1. Catalase Test

A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive.

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

3.7.2 Indole production test

Several drops of Kovac's indole reagent were placed on a filter paper. A portion of a pure isolated colony picked from the nutrient agar pure culture with an inoculating loop was smeared onto the reagent-saturated area of the filter paper. It was allowed and examined to observe for colour development within 2 - 3 minutes. In this spot test, indole combined with the reagents in the filter paper matrix to produce a blue-to-blue-green colour change on the bacterial smear and adverse reactions remained colourless or light pink.

3.7.3 Oxidase Test

A piece of filter paper was wet with a few drops of the dilute (1%) solution of oxidase reagent (tetramethyl-pphenylenediamine-dihydrochloride) which was prepared by standard procedure. A bit of growth from the nutrient agar slant was obtained using sterilized platinum wire loop and

smeared on the wet piece of paper. Development of an intense purple color by the cells within 30 seconds indicates a positive oxidase test.

3.7.4 Citrate utilization test

Well-prepared and sterilized citrate agar plates were inoculated from the pure isolated culture by streaking the surface with a sterilized loop. The plates were then incubated at 37°C for 24 hours. There were changes in colour due to bacterial growth of the organisms on the medium due to citrate metabolism, which gave a positive citrate test. The shift in pH turns the bromothymol blue indicator in the medium from green to blue (positive result). A negative test was demonstrated with no growth, no colour change, or the colour of the medium remains green

3.7.5. Triple sugar iron (TSI) agar test

An agar slant prepared of a TSI agar was used in carrying out this test in a sterile test tube at a slanted angle. The slanted medium was inoculated with TSA pure culture using a straight inoculation needle by stabbing first through the center to the bottom of the tube and streaking the agar slant's surface. After inoculations, the test tubes were covered with foil paper and left at an ambient temperature of 36°C to incubate for 24 hours. Reactions on test tubes were examined, and sugar fermentations were indicated by the production of H_2S, gas and a change in colours from red (alkaline) to yellow (acid). When an alkaline/acid (red top/yellow bottom) slant reaction appeared, it only indicated dextrose (glucose) fermentation. When an acid/acid (yellow top/yellow bottom) slant reaction appeared, it showed the fermentation of dextrose, lactose and/or sucrose. The appearance of an alkaline/alkaline (red top/red bottom) slant reaction represented the absence of sugar fermentation. The blackening of the medium in the slant

indicated H_2S production. Bubbles, cracks, or bottom-raised space in the slanted agar indicated gas production (formation of CO_2 and H_2)

3.8 Antibiotic susceptibility test

The identified colonies of bacteria were used to determine the susceptibility and resistance of bacterial isolates, which were subjected to standard antibacterial susceptibility testing (AST) to decipher their resistance or susceptibility to common antibiotics used for treatment within the locality. The standard discs were produced by Oxoid, UK, which was used to execute the disc diffusion method employed in this study. For this assay, a fully grown bacterial culture (from 18-24 hours) was cultured on MHA. The inoculum corresponding to 1.5 x 10^8 cells/ml McFarland standard was streaked using a sterile loop onto the MHA plates before the introduction of antibiotic discs and were added with extreme care to the plates with the aid of sterile forceps. The susceptibility results were recorded after incubation for 24 hours at 37 ºC. Following the standard or rules of AST established in 2017 by CLSI (Clinical Laboratory Standards Institute). The inhibition zone around each disc (measured using a meter rule in diameter) was assessed and interpreted based on the 2020 CLSI standard as Resistant (R), Intermediate resistant (I) and Sensitive (S).

3.9. Multiple Antibiotic Resistance (MAR) Index

This index is obviously a good tool which identifies the region where the isolates were obtained. Whether they are from places of high or low risks or from arears where antibiotics are abused. This tool becomes necessary for health risk assessment. According to Davis and Brown (2016), an index of ≥ 0.2 and above is indicative of a ‘high-risk’ contamination source. In his study the

MAR index was determined by employing the methods delineated by Chitanand *et al.* (2010). The formula below was used to decipher MAR index of bacterial isolates.

$$MAR\ index = \frac{y}{nx}$$

$$where\ y = number\ of\ resistance\ scored,$$

$$n = number\ of\ isolates\ and$$

$$x = total\ number\ of\ antibioitics$$

It is a general established rule that MAR index greater than 0.2 is indicative of the fact that the bacterium originates from areas where antibiotics have been abused (or regularly used) or worse still from areas of high-risk source of contamination.

3.10. Statistical analysis

Data obtained in this study were collected and analysed using Microsoft excel and by statistical package for social scientist (SPSS) version 22.0 (SPSS Inc., Chicago, IL, USA). Normal distributed data was expressed as mean ± standard deviation and means were compared by analysis of variance.

CHAPTER FOUR

4.0. RESULTS

Table 4.1. Presents the Total Viable Bacterial Count (TVC) in colony-forming units per gram (CFU/g) for various ready-to-eat food samples collected from a restaurant in University of Benin over two weeks. The food samples include Moi Moi (Bean Pudding), Fried Rice, White Rice, Egusi soup and Yam and Plantain sauce. Total viable bacterial count range from 2.3 ± 0.30 to 5.1 ± 0.28 for the food sample in week 1 to 2.1 ± 0.18 to $4.7 \pm 0.3 \times 10^5$ (cfu/g) week 2.

Table 4.2. Shows the result of the cultural, morphological and biochemical identification of the bacterial isolates. The characteristics were used for morphological identification of the isolates and these includes; shape, size, arrangement, cell type, colour and surface appearance of each isolates. The biochemical test conducted were Gram stain, Urease test, Citrate test, Indole test, Catalase, Lactose and gas formation test etc. Possible organism identified include, *Escherichia coli, Bacillus* sp., *Proteus* sp.,*Citrobacter* sp., *Staphylococus* sp. and *Enterobacter* sp.

Table 4.3: Present the prevalence bacterial isolates across the food samples collected, along with their corresponding percentage occurrence, with *Staphylococcus* sp. exhibiting the most percentage of occurrence.

Table 4.4: Show the resistance and susceptibility of the various isolates to the specific antibiotics used which include: Cefotaxime, Ampicillin, Ofloxacin, Cefixime, Gentamicin, Levofloxacin, Cefuroxime, Imipenem, Nitrofurantoin and Nalidixic acid.

Table 4.1: Total Viable Bacterial Count x 10^5 (cfu/g) of Ready-to-Eat Food Samples from the restaurant

Food Samples	Week 1	Week 2
Moi Moi (Bean Pudding)	2.3 ± 0.30	2.1 ± 0.18
Fried Rice	3.8 ± 0.18	4.2 ± 0.22
White Rice	3.9 ± 0.20	4.7 ± 0.3
Egusi soup	5.1 ± 0.28	3.9 ± 0.81
Yam and Plantain sauce,	3.6 ± 0.12	2.5 ± 0.32

Table 4.2: Cultural and morphological, biochemical tests of the bacterial isolates

Shape	Irregular	Circular	Circular	Circular	Irregular	Circular	Circular
Size	Medium	Small	Large	Medium	Medium	Medium	Medium
Colour	Golden yellow	Cream	Red	Cream	Cream	Red	Milky
Cell type	Rod	Rod	Rod	Cocci	Rod	Rod	Rod
Cell arrangement	Disperse	Clusters	Cluster	Disperse	Disperse	Disperse	Disperse
Gram	-	+	-	+	-	-	-
KOH	+	-	+	-	+	+	+
Gas formation	+	-	+	-	+	+	+
Indole	+	-	-	-	-	-	+
Citrate	-	+	-	-	+	+	+
Oxidase	-	-	-	-	-	-	+
Catalase	+	+	+	+	+	+	+
H_2S formation	-	+	+	-	+	-	+
Sucrose	+	+	-	+	+	+	+
Glucose	+	+	+	+	+	+	+
Lactose	+	-	-	+	+	+	-
TSI reaction (slant/butt)	A/A	K/A	K/A	A/A	A/A	A/A	K/A
Identity	*Escherichia coli*	*Bacillus* sp.	*Proteus* sp.	*Staphylococcus* sp.	*Salmonella* sp.	*Enterobacter* sp.	*Citrobacter* sp.

KEY:
+: Positive to test, -: Negative to test.

A: Acid; K: Alkaline; G: Gas production (bubble); H_2 S: Hydrogen sulphide (black precipitate); KOH: Potassium hydroxide test; TSI: Triple sugar iron test.

Table 4.3: Percentage occurrence of Bacterial Isolates in Food Samples from Different Locations

Bacterial Isolates	Total Occurrence	Percentage occurrence
***Escherichia* coli**	9	14.3
***Bacillus* sp.**	9	14.3
***Proteus* sp.**	9	14.3
***Staphylococcus* sp.**	12	19.0
***Salmonella* sp.**	8	12.7
***Enterobacter* sp.**	7	11.1
***Citrobacter* sp.**	9	14.3
Total Isolates	63	100

Table 4.4: Antibiotic Sensitivity Test on the Bacterial Isolates Represented as Zones of Inhibition Measured in Millimeters

ISOLATES	CTX	OFX	GEN	CFX	AMP	NIT	CFM	LEV	IPM	NAI
Escherichia coli	9	20	18	10	12	0	9	12	4	16
Bacillus sp.	6	14	18	8	0	12	4	22	24	19
Proteus sp.	10	18	16	0	8	0	10	17	0	9
Staphylococcus sp.	3	17	20	14	9	7	11	14	16	14
Salmonella sp.	7	15	17	13	6	10	6	18	19	11
Enterobacter sp.	12	19	21	11	12	9	5	16	14	10
Citrobacter sp.	8	14	20	12	6	8	4	20	15	12

Key:

CTX = Cefotaxime, **OFX** = Ofloxacin, **GEN** = Gentamicin, **CFX** = Cefuroxime, **AMP** = Ampicillin, **NIT** = Nitrofurantoin, **CFM** = Cefixime, **LEV** = Levofloxacin, **IPM** = Imipenem, **NAI** = Nalidixic Acid

R = Resistant (0-10 mm)

I = Intermediate (11-16 mm)

S = Susceptible (17 mm and above)

CHAPTER FIVE

5.0. DISCUSSION

Ready-to-eat (RTE) foods are food items that require no additional cooking or preparation before consumption, making them highly convenient, especially in fast-paced environments such as university campuses. However, their convenience comes with potential health risks, primarily due to their susceptibility to bacterial contamination. In many settings, particularly in informal or small-scale food service operations, RTE foods may not undergo rigorous processing or temperature controls, leaving them vulnerable to microbial growth and contamination. Given these risks, assessing the bacteriological safety and antibiotic resistance profiles of bacteria found in RTE foods is crucial. This study specifically addresses these concerns by evaluating selected RTE foods sold within the University of Benin.

Total viable bacterial count range from 2.3 ± 0.30 - 5.1 ± 0.28 for the food sample in week 1 to 2.1 ± 0.18 - 4.7 ± 0.3 x 10^5 (cfu/g) week 2. The high Total Viable Bacterial Count (TVC) observed in RTE foods such as Fried Rice, White Rice, Egusi soup and Yam and Plantain sauce, indicates that contamination is widespread. These findings align with studies by Mensah *et al*. (2012) and Asiegbu *et al*. (2016), which highlight that RTE foods are particularly susceptible to contamination due to extensive handling during preparation and limited or no reheating before consumption. The highest bacterial counts 5.2 ± 0.28 x 10^5 cfu/g was observed in Egusi soup, with values ranging from. This is consistent with research by Aycicek *et al*. (2005), who noted that moist foods are more prone to bacterial growth because of their water activity, which facilitates microbial proliferation.

The report on total bacterial count on fried Rice in the present study disagree by the findings of Odu and Assor, (2013) who reported a range of $2.45x10^5$ cfu/g - $1.78x10^6$ cfu/g on cooked rice in Port Harcourt, Nigeria. Our report on bacterial load of Moimoi (a derivative of beans) is comparable to that reported by Odu and Assor (2013) on cooked beans in Port Harcourt. Moreover findings of the present study was higher than a range of 1.0 x 10^2 cfu/g to 8.7 x 10^4 cfu/g reported by Monday *et al*. (2014) on Rice but comparable with the report on Moimoi in a higher institution of learning in Taraba State, Nigeria. Generally lower bacterial count of microorganisms on Moimoi might be due to its preparation and packaging as it is often wrapped and boiled in paper foil and exposure is minimal. The specification of International Commission for Microbiological Specification for Foods (ICMSF, 1996) states that ready-to-eat foods with plate count between 0-10^3 is acceptable, between 10^4 and $\leq 10^5$ is tolerable and 10^6 and above is unacceptable. Rating the findings of the present study therefore, the food samples in this study appears generally tolerable.

The five RTE foods (Moimoi, Fried Rice, White Rice, Egusi soup and Yam and Plantain sauce,) evaluated in the present study were grossly contaminated with different bacteria. Bacteria isolated from the foods: *Proteus* sp, *Citrobacter* sp, *Enterobacter* sp., *Escherichia coli, Salmonella* sp., *Bacillus* sp. and *Staphylococcus* sp. are consistent with the previous reports (Oluyege *et al*., 2009; Oladipo and Adejumobi, 2010 ; Majolagbe *et al*., 2011).

Of the total of 63 bacteria isolated, *Staphylococcus aureus* was the highest prevailing bacteria with a prevalence of 19.0 % followed by *Escherichia coli* (14.3%), *Bacillus* sp. (14.3%), *Citrobacter* sp. (14.3%), Proteus sp. (14.3%), *Salmonella* sp. (12.7%) and *Enterobacter* species (11.1%). This is consistent with the report of Oluwapelumi *et al*., (2020) who Evaluated

Bacterial Contamination of Ready-To-Eat Foods Sold in Ado-Ekiti, Ekiti State, Nigeria. *E. coli, Citrobacter* sp., *Proteus* sp. and *Enterobacter* sp. are environmental microorganisms generally found on soils, garden vegetables, water or sewage (Mezzatesta *et al*., 2012). The presence of the microorganisms on RTE foods indicate poor hygiene in food handling processes, insufficient heating of food, or failure to adherence to standard protocols by the staff working in the eateries. *E. coli* is an enteric microorganism and its presence on RTE foods could be an indication of direct or indirect fecal contamination from the hands of food handlers (Lombaert *et al*., 2003). *Salmonella* sp. is also an enteric microorganism. Contamination of drinking water or food by this organism is a major cause of typhoid fever. *E. coli* could pose serious challenges to food safety most particularly in cases of enterotoxigenic *E. coli* serogroup 0:157; the main causative agent of hemorrhagic colitis (Moro *et al*., 2000; Mosupye *et al*., 1999).

Bacillus sp. and *Pseudomonas* sp. are known environmental contaminants. Their presence on RTE foods could be as a result of undue exposure of foods to air thereby allowing airborne and dust contamination of foods. *B. cereus* is a common soil saprophyte and is effortlessly being transmitted to many types of foods, especially of plant origin, but is also commonly isolated from meat, eggs and dairy products (Schlegelova *et al*., 2003). *Bacillus* sp. is found in uncooked Rice and as an aerobic spore former is often reported in fried Rice food poisoning (Sarrias *et al*., 2000; Blackburn and McClure, 2005). The vegetative cells may be destroyed by heat during processing but the heat resistant spores grow and release toxins on store processed food under favorable condition (Ryu and Beuchat, 2005). Contaminated equipment and utensils, inappropriate processing or inadequate heating as in pre-heating of food before serving-could cause contamination of RTE foods (Hedberg *et al*., 2006).

The antibiotic susceptibility tests conducted on isolates revealed significant resistance patterns, particularly against commonly used antibiotics such as Ampicillin and Cefixime. This aligns with findings by Odonkor and Addo (2018), who observed high resistance rates to these antibiotics in foodborne pathogens isolated from RTE foods. The widespread resistance observed poses a risk not only to public health but also to clinical treatment options, as infections from resistant strains are harder to manage and treat. Of particular concern is the high susceptibility of isolates to Gentamicin and Imipenem, indicating that these antibiotics remain effective. However, the resistance to commonly used antibiotics such as Ampicillin is problematic, as these drugs are often first-line treatments in clinical settings. According to a report by the Center for Disease Dynamics, Economics and Policy (2019), antibiotic resistance in foodborne pathogens can be traced back to improper usage of antibiotics in agricultural practices and inadequate regulation of antibiotics in food production. The current study's findings underscore the need for regulated antibiotic use in the food industry and more stringent guidelines to mitigate resistance.

The findings from this study strongly suggest the need for reinforced food safety practices, particularly for RTE foods that are more susceptible to contamination. Effective interventions may include training food handlers on proper hygiene, regular microbial monitoring of food outlets, and stricter enforcement of food safety regulations. Studies by Muinde and Kuria (2005) have shown that educational programs targeting food vendors can significantly reduce bacterial contamination in RTE foods.

Additionally, regulatory agencies need to focus on stricter monitoring of antibiotic usage within food supply chains to prevent the propagation of resistant strains. The implementation of antibiotic stewardship programs in agricultural and food production settings could be a step

towards reducing the selective pressure that drives resistance. According to the European Food Safety Authority (EFSA, 2021), such measures can significantly reduce the occurrence of MDR bacteria in food.

5.1. CONCLUSION

The results from this study reveal the prevalence of bacterial contamination in RTE foods and the disturbing trend of antibiotic resistance among isolates. By aligning with similar studies, it becomes evident that there is an urgent need for improved food safety practices and stringent regulatory measures to manage contamination risks and mitigate antibiotic resistance. The presence of MDR organisms in foods not only poses a direct risk to consumers but also contributes to the global health challenge of combating antibiotic-resistant infections.

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