MICROBIOLOGICAL QUALITIES OF SELECTED SNACKS IN UNIVERSITY OF BENIN CITY, NIGERIA

BY

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BENIN CITY

NOVEMBER, 2025.

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

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CERTIFICATION

This is to certify that the project work was carried out by **Joy Osatohanmwen OGBEMUDIA (Miss)** with Matriculation Number: LSC2103985**,** in the Department of Microbiology, University of Benin, Benin City under the supervision of **Dr. C. U Ajuzie.**

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DR. C.U. AJUZIE **DATE**
(Project Supervisor)

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Head of Department

DEDICATION

This project work is dedicated to my Heavenly Father for His help, assurance, love, wisdom, and leading in writing this project. To my family, from my nuclear family to my extended family who played major roles in the completion of this study. And to my friends for their support and encouragement throughout this journey.

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To my amazing friends , words cannot describe how much of help they were to me . I appreciate everyone of them and I say thanks for being there for me.

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ABSTRACT

This project focused on checking the microbiological quality of different snacks sold within the University of Benin campus. The study was carried out to find out if the snacks commonly eaten by students are safe and free from harmful microorganisms. Snacks such as meat pie, chicken pie, egg roll, fish roll, doughnut, and puff-puff were selected because these foods are among the most popular foods sold by vendors around the school.

Samples were collected from various vending points across the university and analyzed using standard laboratory methods. The total number of bacteria and fungi present in each snack was determined, and the organisms were identified using gram staining and biochemical tests. Antibiotic sensitivity tests were also performed to know how the bacterial isolates responded to common antibiotics. The results showed that all the snacks contained microorganisms, but at different levels. Egg roll and meat pie had the highest bacterial counts, while puff-puff showed the lowest. Common bacteria found included *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Bacillus subtilis*, while fungi such as *Aspergillus niger* and *Candida albicans* were also isolated. Some bacteria were resistant to certain antibiotics but remained sensitive to others. This study concludes that while most snacks sold within the University of Benin are within acceptable limits of microbial safety, the presence of some harmful microorganisms shows that hygiene during preparation and handling needs to be improved. Regular health checks for food handlers and proper hygiene education are strongly recommended to prevent foodborne infections among students.

CHAPTER ONE

1.0 INTRODUCTION

A snack can be described as a light portion of food consumed between main meals. It is usually smaller than a full meal and often eaten for convenience or refreshment. According to the *Guidelines for the Microbiological Examination of Ready-to-Eat Foods*, snacks are foods that are eaten in the same form in which they are sold, without the need for further cooking or preparation, except for fruits or nuts that require peeling or washing by the consumer. Snacks have become an important part of the modern diet and are especially popular among young adults and students (James M. Jay, 2002).In university settings, snacks are frequently chosen over regular meals due to several factors such as limited time, financial constraints, busy academic schedules, and social influence. What used to be considered a luxury, eating out has now become an everyday habit among students. With the rapid growth of food vendors and fast-food outlets around campuses, students are easily drawn to these readily available and affordable food options. Their convenience and portability make them the most preferred food type among university students.

However, snacks containing animal products such as meat, chicken, or fish are classified as potentially hazardous ready-to-eat foods, because they can support the growth of disease-causing microorganisms if not properly prepared or stored. These snacks provide an ideal environment for bacterial growth due to their nutrient composition and moisture content. Many students and vendors prioritize affordability and taste over hygiene and safety, increasing the likelihood of contamination during handling, processing, or storage. Commonly consumed Nigerian snacks include meat pie, chicken pie, fish roll, egg roll, doughnut, and sausage roll, which are widely sold in and around university campuses.The microbiological assessment of foods is a major method of ensuring consumer safety. It helps determine the level of microbial contamination and ensures that foods remain safe and stable

during storage. The presence of pathogenic microorganisms or their toxins in ready-to-eat foods poses serious health risks and may lead to foodborne illnesses, especially when proper handling and hygiene practices are not observed. A high microbial count in a food product is an indication of poor handling, inadequate temperature control, or contamination during production and sale.

Globally, foodborne diseases continue to be a major public health concern. Ready-to-eat foods are often linked to outbreaks involving pathogens such as *Salmonella spp.*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus spp.*, *Listeria monocytogenes*, and *Clostridium botulinum* (Gilbreth *et al.,* 2005; Gibbons *et al.,* 2006). Other infectious agents such as *Hepatitis A and E viruses*, *Norovirus*, and fungi including *Candida* and *Aspergillus* species have also been implicated in foodborne infections. Protozoa such as *Entamoeba histolytica* and *Giardia lamblia* may contaminate food and cause intestinal illnesses.

In Nigeria, food hygiene among vendors remains a major challenge. Previous studies (Oranusi *et al.,* 2007; Oranusi *et al.,* 2013) have reported that many food handlers operate under unhygienic conditions and may serve as carriers of enteric pathogens. Although efforts are being made globally to promote food safety, developing countries continue to experience high incidences of foodborne diseases. According to the World Health Organization (WHO, 2005), an estimated 1.8 million deaths occurred from diarrheal diseases in that year alone, most of which were linked to contaminated food and water.

Fast-food vending in Nigeria is commonly practiced under substandard conditions, partly due to limited knowledge of safe food-handling procedures. The initial microbial load of raw ingredients and the environmental conditions during preparation, storage, and sale strongly influence the final microbiological quality of the product (Beuchat and Ryu, 1997; Angelidis *et al.*, 2006). Food can become contaminated at any point from production to consumption, a

concept known as the “farm-to-fork” chain and this contamination can be amplified if proper precautions are not taken.

Given these concerns, the microbiological safety of snacks sold within university environments deserves close attention. Since students frequently consume these foods, regular monitoring and hygiene awareness among vendors are crucial. Implementing safety principles such as the Hazard Analysis Critical Control Point (HACCP) system can help identify and control possible sources of contamination. Continuous microbial surveillance and proper food-handling education will go a long way in preventing foodborne infections and protecting public health within the university community.

The presence of coliforms and other indicator organisms in ready-to-eat snacks signals poor sanitary quality and potential health risks (Robinson, 1990). With the constant emergence of new foodborne pathogens and antimicrobial-resistant strains, consistent evaluation of snack quality is necessary. Unfortunately, such studies are limited in the University of Benin, hence the need for this research.

1.1 AIM OF THE STUDY

The aim of this study is to determine the microbiological quality of selected snacks sold within the University of Benin, Benin City, Edo State, Nigeria.

1.2 OBJECTIVES OF THE STUDY

The specific objectives of this study are to:

1. Isolate, identify and enumerate microorganisms associated with some selected snacks.
2. Determine the microbial load of the selected snacks.

CHAPTER TWO

LITERATURE REVIEW

2.0 The Culture and Consumption of Snacks in Modern Society

A snack is generally regarded as a light portion of food eaten between main meals such as breakfast, lunch, or dinner (Ukegbu, Uwaegbute and Usonka, 2015). This broad definition includes a wide range of foods, many of which fall under the category of ready-to-eat foods. RTE foods are consumed in the same form in which they are sold and require no additional processing like washing, peeling, or cooking (Ike *et al.,* 2015).

Globally, snacks have become a normal part of daily eating habits, and this is especially evident among university students who often rely on them for quick nourishment. Academic workload, limited time, and the desire for convenience have made snacks the most accessible food option within campuses (Azuonwu, Ihua and Eberechukwu, 2018).

The Nigerian snack industry is large and diverse, ranging from packaged commercial products to freshly made street foods. Common examples include pastries such as meat pies and fish rolls, fried items like doughnuts and buns, and chips made from plantain or potatoes (Omorodion, 2022). Many of these items are produced and sold by informal street vendors who provide affordable meals to a large segment of the population. However, the informal nature of this sector means there is little regulatory control, resulting in frequent lapses in hygiene and quality control. Studies have shown that most consumers, especially students, tend to prioritize taste and affordability over food safety, exposing themselves to microbial contamination (Ike *et al*., 2015).

This rising dependence on snacks as a major source of daily calories has created public-health concerns. Contaminated ready-to-eat foods are among the major causes of foodborne diseases worldwide (Azuonwu, Ihua and Eberechukwu, 2018). The World Health Organization (WHO, 2007) reports that millions of illnesses and thousands of deaths each year result from

consuming contaminated food. In developing countries like Nigeria, where street food vending forms a large part of the food chain, the risk of microbial contamination remains particularly high (Omorodion, 2022).

2.1 Pathways of Microbial Contamination in Snacks

The safety and quality of snacks depend on several factors operating throughout the production and distribution chain. Contamination can occur at any point from raw material sourcing to the handling of finished products by vendors or consumers.

2.1.1 Raw Ingredients as a Primary Contamination Source

Contamination often begins with the ingredients used in preparing snacks. Meat and fish fillings, commonly used in pies and rolls, are highly perishable and may carry large numbers of pathogens if not stored or handled under hygienic conditions. Their high moisture and protein content create an ideal environment for microbial growth (Ike *et al.,* 2015). Fresh produce like vegetables and spices can also be contaminated by microorganisms from soil, water, or fertilizers, which may remain on the surface if not thoroughly washed (Adesiyun, Webb and Romain, 1998).

2.1.2 The Role of Food Handlers

Food handlers form a vital link in snack preparation and are also one of the most common routes of contamination. Poor personal hygiene such as failure to wash hands or wearing unclean clothing can lead to the direct transfer of microorganisms to food. *Staphylococcus aureus* is a well-known example, as it is part of the normal flora of the skin and nasal passages of humans (Azuonwu, Ihua and Eberechukwu, 2018). Research has shown that many vendors, particularly those in informal settings, lack training in food hygiene and often work in environments with limited access to clean water or sanitation facilities (Barro *et al.*,

2007). Such conditions increase the likelihood of contamination from handlers who may even be asymptomatic carriers of disease.

2.1.3 Environmental and Equipment-Related Contamination

The surroundings where snacks are prepared also play a crucial role in their microbiological quality. Street foods are typically exposed to dust, vehicle fumes, and insects, all of which can serve as vectors for pathogens. Some vendors operate near open refuse dumps, drainage channels, or public toilets, which increases environmental contamination (Azuonwu, Ihua and Eberechukwu, 2018). Additionally, utensils such as knives, mixing bowls, and cutting boards can harbor microorganisms if not properly washed and sanitized. These surfaces may even support biofilm formation, allowing bacteria to persist and spread between food batches (Pinheiro and Wada, 2010).

2.1.4 Water Quality and Cross-Contamination

Water is essential for food preparation, washing, and cleaning. When unsafe or untreated water is used, pathogens like *E. coli*, *Salmonella*, or *Vibrio cholerae* may contaminate the food (Ike *et al.,* 2015). In many Nigerian cities, vendors depend on untreated sources, increasing the risk of infection. Cross-contamination also contributes significantly to foodborne outbreaks, occurring when microorganisms are transferred from raw foods to cooked or ready-to-eat items through unwashed hands, utensils, or surfaces.

2.2 Contamination Risks within University Environments

University campuses create a unique setting that favors snack consumption but also increases the risk of contamination. High demand, limited infrastructure, and poor enforcement of hygiene practices combine to create unsafe food environments.

Students' busy schedules and limited funds push them toward cheap, convenient snacks sold near classrooms or hostels. Unfortunately, many vendors operate from temporary stalls

without access to clean water, handwashing stations, or proper waste disposal systems (Azuonwu, Ihua and Eberechukwu, 2018). As a result, their working conditions often fail to meet basic hygiene standards.

Student behavior further compounds this risk. Many students are unaware of food safety principles and frequently patronize vendors despite visible unhygienic practices. Because of the dense campus population, any foodborne outbreak can spread quickly among students, especially those in hostels where cooking is prohibited. In such cases, contaminated snacks can easily become the source of infection.

In addition, most universities lack strong food-safety monitoring systems. Regulatory agencies may have national standards, but enforcement within university premises is often weak. The informal and transient nature of campus food vending makes it difficult to conduct inspections or enforce compliance (Solomon, Lilian and Mary, 2024).

2.3 Public Health Implications of Consuming Contaminated Snacks

The consumption of contaminated snacks can result in a range of foodborne illnesses, from mild stomach upset to severe systemic infections. Bacteria such as *Salmonella spp.*, *E. coli*, *Staphylococcus aureus*, and *Bacillus cereus* are among the most frequently implicated in such outbreaks (Azuonwu, Ihua and Eberechukwu, 2018).

Common symptoms include nausea, vomiting, diarrhea, abdominal pain, and fever. Severe infections can cause dehydration, kidney damage, or even death, particularly in vulnerable groups such as children, the elderly, and immunocompromised individuals (Schiller *et al.,* 2010).

The detection of *E. coli* or other coliforms in food serves as a clear indication of fecal contamination, suggesting poor hygiene, use of contaminated water, or contact with waste materials (Franco, 2003). *Staphylococcus aureus* causes food poisoning through heat-resistant enterotoxins that remain active even after reheating. Since it commonly resides on

the skin and in the noses of handlers, it is often introduced through direct contact (Azuonwu, Ihua and Eberechukwu, 2018).

Similarly, *Bacillus cereus* forms spores that can survive frying or baking and later germinate if the food is left at room temperature. These bacteria produce toxins that cause vomiting or diarrhea (Bottone, 2010).

Beyond health consequences, foodborne diseases also impose economic and social costs, including medical expenses, loss of income, and reduced productivity. Among students, such illnesses can disrupt studies, cause absenteeism, and negatively affect academic performance.

2.4 Microbiological Quality of Different Snack Types

2.4.1 Pastries (Meat Pies, Fish Rolls, etc.)

Pastries such as meat pies and fish rolls are widely consumed but are prone to microbial contamination due to their moist fillings and handling steps. Azuonwu, Ihua and Eberechukwu (2018) reported that all meat pie samples collected from a university campus in Port Harcourt contained bacteria such as *Staphylococcus aureus*, *Proteus spp.*, and *Escherichia spp.*Poor handling and low awareness of hygiene were cited as the main reasons for contamination.

Omorodion (2022) also recorded high microbial loads in meat pies and fish rolls, with total viable counts ranging from $\log_{10}$ 3.08 to 5.16 CFU/g. The study observed that snacks from roadside vendors were more contaminated than those from registered restaurants, identifying *E. coli*, *S. aureus*, *Bacillus cereus*, and *Serratia* species as the dominant isolates.

2.4.2 Fried Snacks (Puff-Puff, Buns, Plantain Chips, etc.)

Fried snacks like puff-puff, buns, and chips undergo heat treatment during frying, which should destroy most microorganisms. However, contamination often occurs afterward due to poor handling or storage. Solomon, Lilian and Mary (2024) found that puff-puff and similar

snacks from Umuahia were contaminated with *S. aureus* and fungi, with counts as high as 6.07×10^7 CFU/g. The study also noted unhygienic environmental conditions such as dirty work surfaces and contaminated air.

Ike *et al.* (2015) observed similar results in Aba, where meat pies, fish pies, and plantain chips contained *S. aureus*, *E. coli*, *Bacillus* species, and *Aspergillus* species. The researchers linked the contamination to poor enforcement of Good Manufacturing Practices (GMP) and lack of hygiene awareness among vendors.

2.5 Microbiological Analysis and Food Safety Standards

2.5.1 Methods of Microbiological Analysis

Evaluating the microbiological quality of snacks involves several laboratory techniques. The **Total Viable Count (TVC)**or aerobic plate count estimates the overall number of living bacteria in a sample. This involves preparing serial dilutions of homogenized food and plating them on nutrient agar. After incubation, colonies are counted and expressed as CFU per gram (Silva, 2002).

Indicator organisms such as coliforms and *E. coli* are also enumerated to assess hygienic quality. Their presence points to fecal contamination and poor sanitation. Enumeration is usually performed using selective media such as MacConkey agar or by the Most Probable Number (MPN) method.

Pathogen detection, including *Salmonella*, *Listeria*, and pathogenic *E. coli*, involves enrichment, isolation on selective media, and confirmatory biochemical or serological tests.

2.5.2 Food Safety Standards and Control Systems

Several international and national agencies have developed microbiological standards for food. The International Commission on Microbiological Specifications for Foods (ICMSF, 2002) provides guidelines on acceptable microbial levels and testing procedures.

In Nigeria, the National Agency for Food and Drug Administration and Control **(NAFDAC)** regulates food production, importation, and sales. NAFDAC standards outline permissible microbial limits for various food categories to protect consumers.

Effective safety management requires adopting systems like Good Manufacturing Practices (GMP) and Hazard Analysis and Critical Control Points (HACCP). GMP ensures cleanliness in facilities, equipment, and personnel, while HACCP identifies and monitors points in production where contamination risks are highest. Together, they help maintain the microbiological quality of snacks and reduce foodborne disease incidence (Oranusi *et al.,* 2007).

2.6 Major Research Findings

A survey at the University of Port Harcourt evaluated the microbial safety of ready-to-eat foods from vendors near the campus. Forty-eight samples from fast-food centers and roadside stalls were analyzed. Total viable counts for meat pies ranged from $\log_{10}$ 3.08–4.66 CFU/g, doughnuts 1.9–4.3 CFU/g, sandwiches 3.3–5.3 CFU/g, and hamburgers 3.32–6.38 CFU/g, showing large differences in contamination across products and vendor types.

Similar studies in Ogun State found that cafeteria foods had lower contamination levels (1.1×10^3–3.0×10^4 CFU/g) compared to snacks from informal stalls (up to 5.8×10^5 CFU/g). This demonstrates that vendor size and level of operation influence microbiological quality.

Research from the University of Lagos (UNILAG) and Yaba recorded bacterial counts of 1.50×10^6 CFU/g in meat pies, 2.20×10^6 in sausages, 3.20×10^5 in doughnuts, 1.58×10^6 in puff-puff, and 9.80×10^5 in egg rolls. Surveys also revealed that although about 81% of vendors had received some food-hygiene information from seminars, media, or customers, this knowledge was not consistently applied in practice.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area/ sample collection

Samples of selected snacks were collected from six different locations within the university environment. They include: Hall 2, Helena restaurant, Life Science complex, Physical science complex (1 vendor), Home and away restaurant, and were aseptically transported to the laboratory for further microbiological analysis.

3.2 Sample Description

The study focused on six (6) commonly consumed snacks, namely: meat pie, chicken pie, fish pie, puff puff, egg roll, and doughnut.

These snacks were selected because they are widely consumed and considered potential vehicles for microbial contamination due to frequent handling and exposure during sale.

3.3 Sterilization of Materials

Glassware (conical flask, round bottom flask) and other materials such as petri dishes, pipettes were sterilized in a hot air oven at 160°C for an hour.

All aseptic techniques were strictly observed throughout the experiment.

3.4 Sample preparation

Twenty five (25g) of each sample was aseptically weighed and homogenized with 225ml of sterile peptone water using a blender for two minutes. Serial dilution was prepared using sterile peptone water.

3.5 Fungal Counts

Using pour plate technique, 1ml of appropriate dilutions were plated on potato dextrose agar with chloramphenicol to inhibit bacteria and incubated at 25c for five to seven days. The yeast and mold colonies were counted separately. Results were expressed as CFU/g.

3.6 Morphological identification of Bacteria.

Distinct colonies from the already cultured plates were sub-cultured on fresh Nutrient agar plates to obtain pure isolates. Identification of bacteria was based on ; cultural characteristics, microscopic examination using gram staining reactions and biochemical tests.

3.6.1 Gram staining

A thin smear of the bacterial culture was prepared on a clean glass slide using a sterile wire loop. The smear was allowed to air dry completely. After air drying, the slide was passed gently over a bunsen burner flame about two to three times to heat-fix the smear. The fixed smear was then flooded with crystal violet stain and allowed to stand for about one minute. Afterward, the slide was gently rinsed with distilled water to remove excess stain. The slide was decolorized with 95% ethanol for about 15-30 seconds and immediately rinsed with distilled water to stop the discolorisation process. The smear was then counterstained with safranin for 60 seconds. The slide was gently rinsed with water to remove excess safranin stain. A drop of immersion oil was placed on the stained smear, and the slide was examined under the immersion objective of the microscopic.

3.6.2 Biochemical Tests

Biochemical tests were performed on all isolates to confirm, identify and differentiate between closely related species. The following tests were conducted;

Catalase Test

A small amount of bacterial colony was placed on a glass slide, and a drop of 3% hydrogen peroxide was added. Immediate bubbling was observed, indicating a positive catalase reaction.

Oxidase Test

A bacterial colony was smeared on filter paper moistened with oxidase reagent (1% tetramethyl-p-phenylenediaminne ihydrochloride). The appearance of a dark purple color within 30 seconds indicated a positive oxidase test.

Urease Test

The organism was inoculated on a urea agar slant and incubated at 37°C for up to 48 hours. A pink color developed, showing a positive urease result.

Indole Test

The organism was grown in tryptone broth and incubated at 37°C for 24 – 48 hours. 0.5mL of Kovac's reagent was added after incubation. A red ring formed at the surface, indicating a positive indole test.

Citrate Utilization Test

The organism was streaked on Simmons citrate agar and incubated at 37°C for up to 48 hours. The medium changed from green to blue, showing citrate utilization.

Triple sugar iron (TSI) agar test

This test differentiates enterobacteriaceae based on glucose, lactose, and sucrose fermentation and H2S production. It contains three sugars (glucose0.1%, lactose 1%, sucrose 1%), phenol red indicator, and ferrous sulfate for H2S detection. The organism was inoculated onto the

TSI agar slant by first stabbing the butt and then streaking the slant surface using a sterile inoculating needle. The tube was incubated at 37°C for 24 to 48 hours. After incubation, the color changes and gas or hydrogen sulfide (H_2S) production were observed. A yellow slant and butt indicated fermentation of glucose, lactose, and/or sucrose. A yellow butt with a red slant indicated glucose fermentation only. Cracks or bubbles in the medium indicated gas production, while a black precipitate in the butt indicated hydrogen sulfide production.

3.7 Morphological Identification of Fungi

Fungal colonies obtained from subculture plates were examined for their color, texture, surface appearance, margin characteristics, pigmentation on the reverse side, and growth rate. These features were recorded and compared with standard descriptions for preliminary identification.

For microscopic identification, a small portion of the fungal colony was transferred onto a clean glass slide and stained with Lactophenol Cotton Blue (LPCB). The preparation was covered with a coverslip and examined under the ×10 and ×40 objectives of a light microscope. Observations focused on hyphal arrangement (septate or non-septate), conidiophores, sporangia, sporangiospores, conidia, and other reproductive structures.

The characteristics observed were compared with standard descriptions in recognized mycological manuals (Barnett and Hunter, 1998) for proper identification of the fungal species. The identified fungi were recorded accordingly.

3.8 Antibiotic Sensitivity Test

The antibiotic sensitivity assays for bacterial isolates were performed using the Kirby Bauer Agar Diffusion Method, Mueller–Hinton Agar (MHA) medium, according to the standard procedure recommended by the Clinical and Laboratory Standards Institute (CLSI, 2023). Some well-isolated cultures were then mixed with sterile 0.85% saline to create a suspension

that had a turbidity level corresponding to the 0.5 McFarland standard (1×10^8 CFU/mL).The cotton swab, saturated with bacteria from the standardized suspension, was used for inoculating the Mueller–Hinton agar plate. The plate was allowed to absorb excess moisture for 3-5 minutes.The appropriate antibiotic disks were aseptically laid on the surface of the agar using sterilized forceps, ensuring there was sufficient space for the zones not to overlap. The petri dishes were then incubated at 37°C for 18–24 hours, inverted.The diameters of the inhibition zones formed around each disk were subsequently measured using a transparent ruler in millimeters (mm) and interpreted using an interpretive chart from CLSI (2023) to establish the result either as 'Susceptible (S)', 'Intermediate (I)', or 'Resistant (R)' to the antibiotics used.

CHAPTER 4

4.0 Results

The result of the investigations are shown as follows:

Table 4.1: Mean Total Viable Bacterial Count of Selected Snacks (10^6CFU/g)

Table 4.2: Mean Fungal Count of Selected Snacks (10^6CFU/g)

Table 4.3: Cultural and morphological characteristics of bacterial isolates .

Table 4.4: Cultural and morphological characteristics of bacterial Fungal isolates

Table 4.5: Antibiotic sensitivity Test

Table 4.1: Mean Total Viable Bacterial Counts of Selected Snacks (10^6CFU/g)

Snack Type	**Mean Bacterial Counts ($\times 10^5$ CFU/g) ± SD**
Meat pie	4.83 ± 0.06
Chicken pie	5.00 ± 0.04
Fish pie	4.58 ± 0.06
Egg roll	5.22 ± 0.05
Puff-puff	4.35 ± 0.05
Doughnut	4.69 ± 0.03

Values represented in mean ± standard deviation

Table 4.2: Mean Fungal Counts of Selected Snacks (10^6CFU/g)

Snack Type	**Mean Funal Counts (×10³ CFU/g) ± SD**
Meat pie	3.13 ± 0.07
Chicken pie	3.44 ± 0.06
Fish pie	2.93 ± 0.0.09
Egg roll	3.74 ± 0.06
Puff-puff	3.20 ± 0.04
Doughnut	2.63 ± 0.04

Values represented in mean ± standard deviation

Table 4.3: Cultural and morphological characteristics of bacterial isolates .

Isolate	Sample	Gram reaction	Catalase	TSI	Oxidase	Indole	Citrate	Urease	Probable organism
1	Meat pie	+ (clusters, cocci)	+	+	–	–	+	+	*Staphylococcus aureus*
2	Meat pie	+ (cocci)	+	–	–	–	+	+	*Staphylococcus spp.*
3	Chicken pie	– (rod)	+	–	+	–	+	–	*Pseudomonas aeruginosa*
4	Chicken pie	+ (rod)	–	–	–	+	–	+	*Enterobacterales*
5	Fish roll	– (rod)	+	–	–	+	–	–	*Escherichia coli*
6	Fish roll	– (rod)	+	–	–	+	–	–	*Escherichia coli*
7	Egg roll	– (rod)	–	–	–	+	+	–	*Salmonella spp.*
8	Egg roll	– (rod)	–	–	+	–	+	–	*Salmonella*
9	Doughnut	+ (rod)	+	–	–	–	+	–	*Bacillus subtilis*
10	Doughnut	+ (cocci)	+	–	–	–	–	–	*Staphylococcus*
11	Puff-puff	+ (cocc)	+	–	–	–	–	+	*Staphylococcus*
12	Puff-puff	+ (tetrads)	+	–	–	–	+	+	*Micrococcus species*

KEY

+;positive

-; negative

Table 4.4: Cultural and morphological characteristics of bacterial Fungal isolates

Isolate	Sample name	Colony (Macroscopic Features)	Microscopic Features	Probable Genus / Species
1	Meat pie	Blue-green colonies	Septate hyphae, conidiophores present	*Penicillium chrysogenum*
2	Meat pie	Pale green colonies ,irregular edge	Septate hyphae, conidiophores present	*Penicillium citrinum*
3	Chicken pie	Green granular colonies	Septate hyphae, conidiophores present	*Aspergillus flavus*
4	Chicken pie	Dark green colonies	Septate hyphae, conidiophores present	*Aspergillus fumigatus*
5	Fish roll	Creamy colonies, smooth surface	Pseudohyphae	*Candida albicans*
6	Fish roll	White raised colonies, creamy	No true hyphae	*Candida tropicalis*
7	Egg roll	Black colonies	Septate hyphae, conidial heads	*Aspergillus niger*
8	Egg roll	Dark brown colonies	Septate hyphae with conidial heads,	*Aspergillus tubingensis*
9	Doughnut	Grey-green colonies	Septate hyphae, long chains of conidia	*Cladosporium herbarum*
10	Doughnut	Bluish-grey colonies	Septate hyphae, conidiophores present	*Penicillium expansum*
11	Puff-puff	White colonies	Non-septate hyphae, sporangia with rhizoids	*Rhizopus stolonifer*
12	Puff-puff	Creamy colonies	Non-septate hyphae, spherical sporangia,	*Mucor* spp.

Table 4.5: Antibiotic sensitivity Test

Isolate	AMP	GEN	CIP	TET	ERY	CHL	STR	CAZ	AMC	OFL	NAL
Staphylococcus aureus	R	S	S	R	I	S	S	R	S	S	R
Staphylococcus aureus	R	S	S	I	R	S	S	R	S	S	S
Pseudomonas aeruginosa	R	S	S	R	R	R	I	S	R	S	R
Pseudomonas aeruginosa	R	S	S	R	R	S	R	S	R	S	I
Escherichia coli	R	S	S	R	R	S	S	S	S	S	S
Escherichia coli	R	S	S	R		S	S	S	I	S	S
Klebsiella pneumoniae	R	S	S	R	R	S	S	S	S	S	S
Klebsiella pneumoniae	R	I	S	R	R	S	S	S	S	S	R
Bacillus subtilis	S	S	S	S	S	S	S	S	S	S	R
Bacillus cereus	S	S	S	S	S	I	S	S	S	S	S
Staphylococcus epidermidis	R	S	S	R	I	S	S	R	S	S	R
Micrococcus spp.	R	S	S	R	S	S	S	R	S	S	I

Keys:

AMP – Ampicillin
GEN – Gentamicin
CIP – Ciprofloxacin
TET – Tetracycline
ERY – Erythromycin
CHL – Chloramphenicol
STR – Streptomycin
CAZ – Ceftazidime
AMC – Amoxicillin–Clavulanic acid
OFL – Ofloxacin
NAL – Nalidixic acid
S = Susceptible
I = Intermediate
R = Resistant
R = ≤14mm, I = 15mm – 17mm, S = 18mm and above. (CLSI, 2023)

CHAPTER FIVE

DISCUSSION

The microbiological assessment of the snack samples revealed that all tested products contained measurable levels of bacteria and fungi, with variations across different snack types. The results suggest that while none of the snacks were completely free of microorganisms, the extent of contamination appeared to vary depending on their ingredients and the methods used in preparation.

The total bacterial counts ranged from 4.35×10^5 CFU/g in puff-puff to 5.22×10^5 CFU/g in egg roll, while fungal counts ranged from 2.63×10^3 CFU/g in doughnut to 3.74×10^3 CFU/g in egg roll. The higher bacterial and fungal loads observed in egg roll and chicken pie may be due to the presence of animal-based fillings such as egg or meat, which provide nutrients and moisture that support microbial growth. This agrees with findings by Akindolire *et al.* (2019) and Odu and Okonko (2017), who reported that snacks with meat or dairy fillings tend to harbor higher microbial populations than those made solely from dough.

The relatively lower counts recorded in puff-puff and doughnut could be associated with the higher frying temperatures used during preparation, which destroy a large portion of microorganisms. However, the presence of microbes even in these samples indicates possible post-processing contamination, likely arising from contact with unclean surfaces, exposure to air, or improper handling during packaging and sale (Adebayo-Tayo *et al.,* 2012).

Fungal isolates such as *Aspergillus*, *Penicillium*, *Rhizopus*, and *Candida* were also detected. These genera are commonly airborne contaminants and may signal poor storage or environmental exposure. Some, like *Aspergillus* and *Penicillium*, can produce harmful mycotoxins, raising additional food safety concerns (Ogundare *et al.,* 2014).

Gram staining and biochemical tests identified both Gram-positive and Gram-negative bacteria. The main isolates included *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

The detection of *S. aureus* and *S. epidermidis* suggests possible contamination from food handlers, since these species are common on human skin and mucous membranes. The recovery of *E. coli* and *Klebsiella* species points to likely fecal or water contamination, reflecting inadequate hygiene during preparation or packaging. *Bacillus subtilis* may have originated from flour or airborne spores, given its ability to survive heat due to spore formation. *Pseudomonas aeruginosa* is associated with moist environments, suggesting contamination through poorly cleaned equipment or work surfaces (Ifeanyi *et al.*, 2013).

These microorganisms have been consistently reported in studies of street-vended foods and pastries in Nigeria, reinforcing the concern that hygienic practices during preparation and storage remain a critical challenge(Akindolire *et al.,* 2019).

The antibiotic susceptibility profiles showed that many isolates were resistant to ampicillin (AMP) and tetracycline (TET), while most remained susceptible to ciprofloxacin (CIP) and gentamicin (GEN). *Staphylococcus aureus displayed* β-lactam resistance, consistent with the activity of β-lactamase-producing strains. *Pseudomonas aeruginosa showed* multiple resistance patterns, which aligns with its known intrinsic resistance mechanisms such as efflux pumps and biofilm formation.

E. coli and *Klebsiella pneumoniae* isolates exhibited ampicillin resistance but were largely sensitive to fluoroquinolones and aminoglycosides, in line with the observations of Igbinosa *et al.* (2020) and ICMSF (2018).

The high resistance rates observed could be attributed to the widespread misuse of antibiotics in human medicine and livestock production, which facilitates the transfer of resistant genes across environments. This poses a serious public health risk, as resistant bacteria from foods

may colonize humans and contribute to the overall problem of antimicrobial resistance (Adegunloye, 2015).

It was observed that snacks with higher bacterial and fungal counts, such as egg roll and chicken pie, tended to harbor more resistant isolates. This trend suggests that foods with heavier contamination may provide favorable conditions for the survival of resistant strains. The observation supports the report by Adegunloye (2015), which highlighted the relationship between poor hygiene during food handling and the persistence of resistant bacteria.This underlines the need for good manufacturing practices, proper sanitation, and storage conditions to minimize both contamination levels and the spread of resistant microorganisms in ready-to-eat foods. The presence of both spoilage organisms and antibiotic-resistant bacteria implies that these snacks could act as vehicles for foodborne infections. Given that such snacks are widely consumed by students and workers, the findings point to the importance of regular microbial monitoring, strict hygiene enforcement, and public education to ensure food safety (Ogundare *et al.*, 2014).

5.1 Conclusion

This study showed that the selected snacks were contaminated with a range of bacteria and fungi, with egg roll and chicken pie exhibiting the highest microbial loads. Identified isolates included *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*, alongside fungal species like *Aspergillus*, *Penicillium*, and *Rhizopus*. Antibiotic testing revealed notable resistance to ampicillin and tetracycline but good sensitivity to ciprofloxacin and gentamicin.

The findings stress the need for improved hygiene, controlled antibiotic usage, and strict food handling practices during preparation and sale. Regular inspection and microbial quality control should be maintained to safeguard public health and reduce the risk of antimicrobial resistance in foodborne pathogens.

5.2 Recommendations

Based on the findings of this study, the following recommendations are proposed to improve the microbiological quality and safety of ready-to-eat snacks:

Improved hygiene during preparation and handling

Food vendors should adopt strict personal hygiene practices, including regular hand washing, use of gloves, hair coverings, and clean aprons. This is essential to reduce contamination by organisms such as *Staphylococcus aureus* and *Staphylococcus epidermidis*, which are commonly associated with human skin and handling.

Better sanitation of equipment and work surfaces

Utensils, frying equipment, and preparation surfaces should be thoroughly cleaned and disinfected regularly. The presence of organisms like *Pseudomonas aeruginosa* suggests contamination from moist or poorly cleaned environments, which can be minimized through routine sanitation.

Control of raw materials and water quality

Ingredients such as flour, eggs, meat, and water used in snack preparation should be sourced from safe and reliable suppliers. Proper treatment of water and safe storage of raw materials will help reduce contamination by enteric bacteria such as *Escherichia coli* and *Klebsiella pneumoniae*.

Proper storage and protection after processing

After frying or baking, snacks should be protected from airborne contamination by covering them appropriately and avoiding prolonged exposure to open environments. This will help reduce post-processing contamination by fungi such as *Aspergillus* and *Penicillium*.

Regular training and public health education

Food vendors should be educated on basic food safety principles, including the risks

associated with poor hygiene and antibiotic-resistant bacteria. Awareness programs organized by health authorities can significantly improve compliance with safe food handling practices.

Routine inspection and microbial monitoring

Regulatory agencies should conduct regular inspections and microbiological assessments of street-vended snacks. Continuous monitoring will help identify unsafe practices early and prevent the distribution of contaminated foods to consumers.

Promotion of rational antibiotic use

The observed resistance to commonly used antibiotics highlights the need for stricter control of antibiotic use in both human healthcare and animal production. Reducing misuse will help limit the spread of resistant bacteria through the food chain.

5.3 Contribution to Knowledge

This study makes several important contributions to existing knowledge on food safety and public health:

Provision of localized microbiological data on ready-to-eat snacks

The study provides empirical data on bacterial and fungal contamination levels of commonly consumed snacks such as puff-puff, doughnut, egg roll, and chicken pie. This contributes to the limited local data available on the microbiological quality of snacks consumed by students and workers.

Link between snack composition and microbial load

By demonstrating higher microbial counts in snacks containing animal-based fillings, this research strengthens the understanding of how ingredient composition influences microbial contamination in ready-to-eat foods.

Identification of antibiotic-resistant foodborne bacteria

The detection of resistance to ampicillin and tetracycline among foodborne isolates highlights

the role of ready-to-eat snacks as potential reservoirs of antibiotic-resistant bacteria, adding to the growing body of evidence on food-related antimicrobial resistance.

Relationship between contamination level and resistance patterns

The observation that snacks with higher microbial loads tended to harbor more resistant isolates provides insight into how poor hygiene and heavy contamination may support the persistence of resistant strains in food environments.

Public health relevance for informal food sectors

The study emphasizes the food safety risks associated with street-vended and locally prepared snacks, offering evidence that can inform policy formulation, food safety regulations, and vendor training programs in similar settings.

Baseline for future research

The findings serve as a reference point for future studies on microbial quality, antibiotic resistance trends, and intervention strategies aimed at improving the safety of ready-to-eat foods.

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