IDENTIFICATION AND CHARACTERIZATION OF MICROBIAL POPULATION SPECIES FOUND IN MEAT PIES SOLD IN UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA

BY

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DEPARTMENT OF ANIMAL SCIENCE
FACULTY OF AGRICULTURE
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MAY 2024

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DECLARATION

I hereby declare that this project **"IDENTIFICATION AND CHARACTERIZATION OF MICROBIAL POPULATION SPECIES FOUND IN MEAT PIES SOLD IN UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA"** was carried out by me in the Department of Animal Science, University of Benin, Benin City, Edo State. This project was carried out under the supervision of

_____ _____

Dr. Peter A. Ebabhamiegbegho Date

_____ _____

Uwumarongie Eseosa Benedicta Date

CERTIFICATION

This project work entitled **"IDENTIFICATION AND CHARACTERIZATION OF MICROBIAL POPULATION SPECIES FOUND IN MEAT PIES SOLD IN UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA"** meets the regulation governing the award of degree of Bachelor of Science of University of Benin, Benin-city, Edo State, Nigeria and is approved for its contribution to knowledge and literacy presentation.

_____ _____

Dr. P.A Ebabhamiegbegho Date
Project Supervisor

_____ _____

Prof. J.A Imasuen Date
Head of Department

DEDICATION

This project work is dedicated to God almighty my Heavenly Father who makes all things possible. I am forever grateful to God for his love, mercies, faithfulness, provision, guidance and protection and for His favor and grace that He has continually bestowed on me. All glory and honor belongs to you. I also dedicate this project work to my lovely parents Mr. Cyril U. Uwumarongie and Mrs. Maris I. Uwumarongie, and to my wonderful siblings.

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Uwumarongie, Wealth Uwumarongie, Christabel Uwumarongie, Greatness Uwumarongie and Mirabel Uwumarongie having you all in my life has been and would always be a wonderful blessing to me. May God continue to bless and keep you all for me.

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ABSTRACT

The consumption of snacks such as meat pie has progressively been on the increase. It is assumed that the safety in terms of microbial population may not be guaranteed. Meat pies produced and consumed in University of Benin, Benin City, Edo State, Nigeria were assessed for microbiological population in Areas of Faculty of Arts, Mat-Ice Anatomy gate, Faculty of Agriculture, Faculty of Engineering and Uniben buka. Microbiological quality of meat pies produced and consumed was determined using cultural media and serial dilution for isolation and identification of bacteria and fungi count loads in meat pies sold in the University of Benin. A total of 5 meat pie samples were taken randomly from the five different locations within university of Benin in Benin city. Aliquot of 1ml of the appropriate dilution from each contaminated water was plated in nutrient agar for isolation of bacteria, potato dextrose agar for isolation of fungi. The data obtained were subjected to Analysis of variance (ANOVA) using Genstat. They were all were analyzed for total heterotrophic bacteria count and total fungal count. The 5 samples had a mean total aerobic plate count and coli forming count ranging from 0.914log cfu/g to 1.828log cfu/g for bacteria counts and 0.5log cfu/g to 2.2log cfu/g for fungal counts with. Four different bacterial and four fungal isolate were identified to include Escherichia coli, Salmonella, Shigella, Staphylococcus aureus, *Aspergillus niger, Aspergillus spp*, mold and yeast respectively. The microbial counts showed that there was no significant difference ($p>0.05$), between the mean, bacterial and fungal counts. The presence of Escherichia coli, which is an indicator organism in feces call for concern. Adoption of good manufacturing practices in the meat pie hazard analysis critical' control point (HACCP) are necessary to prevent occurrence of food borne infection. Thus, this study revealed the likelihood of a very high risk associated with the consumption of meat pies within university of Benin, Benin City.

CHAPTER ONE

1.0 INTRODUCTION

Meat pies are a popular and convenient food item enjoyed by students and staff at the University of Benin and across Nigeria. They are typically filled with seasoned meat, vegetables, and spices encased in a pastry crust. While a delicious and affordable option, meat pies can pose a food safety risk if not prepared and handled hygienically (Ikenga *et al.,* 2020). Meat and meat products have been a constant food for man as far back as there has been any evidence of civilization on the face of earth. Meat Pies is one of the numerous ready to eat foods. Ready to eat foods can be described as the status of foods being ready for immediate consumption at the point of sale. Ready to eat foods could be raw or cooked, hot or chilled and can be consumed without further heat treatment (Tsang, 2002). Studies on ready-to-eat (RTE) foods in Nigeria have identified various bacterial contaminants in meat pies, including *Staphylococcus aureus, Escherichia coli*, and *Salmonella spp*. and several others (Oladunmoye and Ayeni, 2014). These bacteria can cause foodborne illnesses with symptoms ranging from mild discomfort to severe diarrhea, vomiting, and even death, depending on the specific pathogen, ingested dose, and individual susceptibility (Adebonyo *et al.,* 2016). Different terms have been used to describe such ready to eat foods. These include convenient, ready, instant and fast foods. Examples of such ready to eat foods include pastries, meat pie, sausage, rolls, burger, moin-moin, salad or coleslaw, fried meat, fried chicken, milk and milk products

(Caserani and Kinston, 1974). There has been a notable and remarkable increase in the consumption of convenience and ready to eat foods by the people in recent times.

Microorganisms play an important role in the quality of meat products before, during and after processing by limiting many undesirable biological changes in it (Ukut *et al*., 2010). Meats and meat products undergo spoilage as a result of microbial action on the fats and proteins (Adesiyun, 1995). Food contamination is the introduction or occurrence of a contaminant (any biological or chemical agent, foreign matter or other substance not internationally added to food which may compromise food safety or suitability) in food or food environment (Omoloya and Adeleke, 2013). Food is prone to contamination at every stage in the food chain. The consumption of food contaminated by micro-organism will result in food borne illnesses.

According to Doyle and Evans (1999), Food borne diseases are diseases resulting from ingestion of bacteria, toxins and cells produced by microorganisms present in food. Data on issues of food borne diseases are well documented worldwide (Hazariwala *et al*., 2002). Food borne illnesses is a major international health problem with consequent economic reduction (Duff *et al.*, 2003). Food borne illnesses have continued to form a significant part of morbidity and mortality of Nigerians and have been on the increase in recent times. The international impact of food borne illnesses is difficult to estimate. Bacteria are the causative agents of food borne illnesses in 60% of cases requiring

hospitalization (Mead *et al.,* 1999). While previous research has investigated the general microbial quality of meat pies in Nigeria, there is a dearth of specific data on the microbial populations present in meat pies sold around the University of Benin. This knowledge gap is crucial for ensuring food safety within the university community. Understanding the specific types of microbes present in meat pies sold in UNIBEN, along with their potential for spoilage and foodborne illness, is very important for developing targeted inventions and promoting safe food handling practices amongst vendors and consumers.

1.1 JUSTIFICATION OF STUDY

Despite the popularity of meat pies which are widely consumed across Nigeria, and particularly within university communities like the University of Benin (UNIBEN), they can pose a significant public health risk if proper hygiene practices are not followed during preparation, handling, and storage. This is due to their inherent characteristics. Meat pies are consumed without further cooking, increasing the risk of ingesting foodborne pathogens that may be present. The warm and moist environment within the pastry crust can create ideal conditions for the growth of certain bacteria, especially during lapses in temperature control. The various stages of preparation, from handling raw ingredients to storage and vending, present numerous opportunities for contamination with foodborne pathogens. This can occur through improper hand

hygiene practices of food handlers, cross-contamination with contaminated surfaces or equipment, and inadequate storage temperatures.

Research on RTE foods in Nigeria has identified a range of concerning bacterial contaminants in meat pies, including; Staphylococcus aureus, Escherichia coli (E. coli), *Salmonella spp*., e.t.c. These pathogens can cause a spectrum of illnesses with varying severity, potentially leading to hospitalization and even death, particularly among vulnerable populations like young children, pregnant women, and the elderly.

Previous studies have investigated the general microbial quality of meat pies in Nigeria. However, there is a critical dearth of research specifically focused on the microbial populations present in meat pies sold in and around UNIBEN. This knowledge gap hinders efforts to ensure optimal food safety practices within the university community. Repeated incidents of foodborne illness could damage the reputation of UNIBEN food vendors, impacting their businesses. Outbreaks can strain the resources of local healthcare facilities. Foodborne illnesses can lead to missed classes, work absences, and decreased productivity for both students and staff. Students experiencing foodborne illness may miss classes, impacting their academic performance while staff illness can also disrupt university operations. Repeated incidents of foodborne illness associated with meat pies can damage the reputation of UNIBEN food vendors and potentially discourage students from patronizing them.

This study, by comprehensively identifying and characterizing the microbial populations present in meat pies sold in UNIBEN will contribute valuable insights to improve food safety practices within the university community. The findings can be used to; develop targeted food safety guidelines for vendors operating on and around the UNIBEN campus, inform educational campaigns aimed at raising awareness among students and staff regarding safe handling practices for RTE foods like meat pies and to provide baseline data for future studies investigating the factors influencing microbial contamination in meat pies sold in the University of Benin (UNIBEN).

This research aspires to contribute to a safer and healthier food environment for the University of Benin (UNIBEN) community by mitigating the risk of foodborne illnesses associated with meat pie consumption.

1.3 OBJECTIVE OF THE STUDY

The main objective of this study is to identify and characterize the microbial population species found in meat pies sold in the University of Benin, Benin city, Edo state, Nigeria.

1.3.1 Specific Objectives

The specific objectives are to;

(i) determine the microbial population in meat pie sold in the University of Benin.

(ii) identify the microbial species present in meat pies sold at the University of Benin.

(iii) characterize the identified microbial populations.

(iv) access the potential public health risks of the identified microbes.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Composition and Popularity of Meat Pies

Meat pies are a savory staple food enjoyed across various cultures worldwide. They typically consist of a seasoned meat filling encased in a pastry crust (Adebonyo *et al*., 2016). The specific ingredients and preparation methods can vary depending on regional traditions. In Nigeria, meat pies are a popular and affordable choice for people on the go (Ikenga *et al.,* 2020). Their fillings commonly include seasoned ground beef, minced chicken, or a combination of both, along with vegetables like onions, peppers, and peas (Oladunmoye and Ayeni, 2014). Spices like nutmeg, thyme, and curry powder are often used to create a flavorful filling (Ikenga *et al.,* 2020). The pastry crust is typically made with flour, butter, and water, and can be baked or deep-fried (Adebonyo *et al.,* 2016). The portability, affordability, and delicious combination of flavors make meat pies a convenient and widely consumed food item, particularly within university communities like the University of Benin (UNIBEN) (Ikenga *et al*., 2020).

Meat pies have a rich history, dating back to ancient civilizations like the Egyptians and Greeks who used pastry shells to encase various fillings (McGee, 2004). Over time, this culinary concept evolved into diverse regional variations across the globe. Today, meat pies remain a popular and versatile food item enjoyed in many cultures. In Britain, meat pies are a national icon, filled with ingredients like minced beef, lamb, or game birds (Ikenga *et al*., 2020) Eastern European countries like Poland and Russia also have their own variations, often featuring savory fillings like cabbage and mushrooms (Oladunmoye and Ayeni, 2014). Empanadas, a close cousin of the meat pie, are a staple food in many Latin American countries. Fillings can range from seasoned meats like beef and chicken to vegetarian options with vegetables and cheese (Marianella, 2010). Across Africa, savory pies filled with various meats and vegetables are a common street food. In Nigeria, meat pies are a popular choice for breakfast, lunch, or a convenient snack (Aregbesola and Adesoye, 2011). Nigerian meat pies typically consist of two key components:

Filling: The filling is the heart of the meat pie, traditionally made with seasoned ground beef, minced chicken, or a combination of both meats (Udonne and Ikenga, 2014). Some vendors may offer vegetarian options with fillings like lentils or beans (Agu and Udegbunam, 2014). Common vegetables incorporated into the filling include onions, peppers, peas, and sometimes carrots (Oladunmoye and Ayeni, 2014). Spices like

nutmeg, thyme, curry powder, and bouillon cubes add a depth of flavor (Udonne and Ikenga, 2014).

Pastry Crust: The pastry crust serves as the container for the filling and can be made with various flours, including wheat or all-purpose flour (Serventi and Sabban. 2002). Fat, such as butter or vegetable shortening, is added to create a flaky or crumbly texture. Water binds the ingredients together, and sometimes eggs are included for a richer flavor and improved browning (Robertson, 2017). The pastry can be baked or deep-fried, depending on the vendor's preference and desired texture (Oladunmoye and Ayeni, 2014).

Meat pies offer a unique combination of convenience, affordability, and deliciousness. This makes them a popular choice for students and staff at universities like the University of Benin (UNIBEN). Their portability allows for quick consumption on the go, while their affordability caters to student budgets. The savory and satisfying nature of the fillings provides a source of energy between classes or meetings (Ikenga *et al*., 2020).

Plate 1: Nigerian Meat Pie showing the pastry crust.

Plate 2: Nigerian Meat Pie showing the cross section of the warm and moist filling of a Nigerian meat pie

2.1.1 Typical Production Process for Meat Pies

While the specifics may vary depending on the vendor, a general production process for Nigerian meat pies can be outlined (Udonne and Ikenga, 2014, and Oladunmoye and Ayeni, 2014). The following is a breakdown of the key stages:

Dough Preparation

Flour (wheat or all-purpose) is combined with fat (butter, vegetable shortening) using various techniques like rubbing or cutting (Serventi and Sabban, 2002). Water and sometimes eggs are added to bind the ingredients and create a dough with the desired consistency (Robertson, 2017). The dough is then rested to allow the gluten to relax, resulting in a flakier crust (Serventi and Sabban, 2002).

Filling Preparation

Meat (ground beef, minced chicken, or a combination) is seasoned with spices like nutmeg, thyme, curry powder, and bouillon cubes (Udonne and Ikenga, 2014). Vegetables like onions, peppers, and peas are chopped and sauteed (Oladunmoye and Ayeni, 2014). The seasoned meat and vegetables are then combined to create the filling mixture (Udonne and Ikenga, 2014).

Shaping and Filling

The rested dough is rolled out into a sheet. Dough cutters or bowls are used to create circles of the desired size (Ikenga *et al.*, 2020). Each dough circle is placed on a clean

surface, and a portion of the filling is placed in the center (Oladunmoye and Ayeni, 2014). The dough is then folded over the filling to form a semi-circle, and the edges are crimped or sealed to prevent leakage during cooking (Ikenga *et al*., 2020).

Cooking

The filled pies can be cooked using two main methods: baking or deep-frying (Oladunmoye and Ayeni, 2014).

Baking: Pies are placed on baking sheets and cooked in an oven at a moderate temperature (around 180°C) until the pastry crust is golden brown and the filling is cooked through (Adebonyo *et al*., 2016).

Deep-frying: Pies are deep-fried in hot oil (around 170°C) until the pastry is golden brown and cooked through (Oladunmoye and Ayeni, 2014).

Cooling and Storage

Once cooked, the pies are allowed to cool slightly before being displayed for sale or packaged for later consumption (Agu and Udegbunam, 2014). Proper storage practices are essential to maintain food safety. Ideally, pies should be stored below 5°C to minimize bacterial growth (Jay *et al.,* 2005).

2.2 Importance of Meat Pies

Meat pies offer a unique combination of factors that contribute to their popularity:

Convenience: They are portable, self-contained meals that require no preparation before consumption, making them ideal for busy lifestyles (Ikenga *et al*., 2020).

Affordability: Meat pies are a relatively inexpensive food option, particularly suitable for students and individuals on a budget (Aregbesola and Adesoye, 2011).

Deliciousness: The savory fillings and flaky crusts provide a satisfying and flavorful eating experience (Udonne and Ikenga, 2014). They serve as a convenient source of sustenance between classes or meetings, contributing to student well-being and productivity (Agu and Udegbunam, 2014).

Accessibility: Meat pies hold a significant place in Nigerian cuisine, especially within university communities like UNIBEN. They are readily available from street vendors and kiosks, making them a convenient option for students on the go (Ikenga *et al*., 2020).

Affordability: Compared to other protein-based meals, meat pies offer a budget-friendly way to satisfy hunger pangs (Aregbesola and Adesoye, 2011).

Nutritional Value: While the specific composition can vary, meat pies typically provide a source of protein from the meat filling, carbohydrates from the pastry crust, and sometimes vegetables for added vitamins and minerals (Udonne and Ikenga, 2014).

Cultural Significance: Meat pies have become ingrained in Nigerian food culture, representing a familiar and comforting food choice for many university students (Akinbo *et al*., 2012).

2.3 Microbial Contamination Risks in Ready-to-Eat (RTE) Foods

Ready-to-eat (RTE) foods, like meat pies, pose a unique challenge when it comes to food safety. Unlike raw ingredients that undergo further cooking before consumption, RTE foods are consumed in their final form, bypassing an important step that can eliminate or reduce foodborne pathogens (Adebonyo et al., 2016). This inherent risk is compounded by several factors that can contribute to microbial contamination throughout the production chain. Contaminated raw meat, poultry, eggs, or vegetables can introduce pathogenic bacteria like Salmonella, *E. coli,* and *Staphylococcus aureus* into the final product (Jay *et al*., 2005, and Mensah, *et al.*, 2002). Improper hand hygiene practices by food handlers can transfer harmful bacteria from hands to food surfaces or directly contaminate the filling during preparation (Mensah *et al*., 2002). Inadequate cleaning and sanitization of utensils, chopping boards, and other food contact surfaces can harbor and spread bacteria to RTE foods (Jay *et al.,* 2005). Raw ingredients or contaminated surfaces coming into contact with cooked or RTE foods can lead to cross-contamination (Jay *et al.,* 2005).

RTE foods require proper temperature control during storage, display, and transportation. Fluctuations outside the safe temperature zone (typically below 5°C or above 60°C) can allow bacteria to multiply rapidly (Jay *et al.,* 2005). The warm and moist environment within the pastry crust of a meat pie can create ideal conditions for the growth of certain bacteria, particularly if temperature control is inadequate (Jay *et al.,* 2005). This microbial proliferation can lead to spoilage, rendering the food unfit for consumption and causing sensory changes like off-odors, discoloration, and sliminess (Jay *et al.,* 2005). Contamination of RTE foods like meat pies with pathogenic bacteria can pose a serious threat to consumer health.

2.4 Common Foodborne Pathogens Associated with Meat Pies

Meat pies are susceptible to contamination by a variety of foodborne pathogens throughout the production process. The following are the commonly encountered ones;

***Staphylococcus aureus (S. aureus*):** This bacterium can be present on the skin and nasal passages of humans and can contaminate meat pies through poor hand hygiene practices during preparation (Mensah *et al.,* 2002). S. aureus can produce toxins that cause foodborne illness characterized by nausea, vomiting, and cramps, typically with a rapid onset (within a few hours of consumption) (Smeltzer and Holley, 2018). In most countries, the most common food–borne illness is Staphylococcus food intoxication (Talaro *et al.,* 1996). Enterotoxigenic Staphylococcus strains and E. coli strains have

been isolated from foods implicated in illnesses (Adeyiwu, 1995, Firstenberg and Sullivan, 1997; Cencil *et al.,* 2003). S. aureus is a gram positive coccus, resistant to heat, drying and radiation. Its strains can be pathogenic and relatively non pathogenic. They produce disease when the bacteria contaminate food. They produce some enzymes which are implicated with staphylococcal invasiveness and many extracellular substances some of which are heat stable enterotoxins that render the foods dangerous even though it appears normal (Prescott *et al.,* 2005). Once the bacteria have produced toxin, the food can be extensively and properly cooked, killing the bacteria without destroying the toxin. Many of their toxins are gene–based that is carried on plasmids. The intensity of the signs and symptons may vary with the amount of contaminated food ingested and susceptibility of the individuals to the toxin.

***Escherichia coli* (*E. coli*):** E. coli a member of the genus Escherichia with the family Enterobacteriaceae. Members are widely distributed in the environment contaminated food and water are the major sources by which the bacteria are spread. Selected strains can cause a wide variety of infections in hospitals and community setting (Donnenberg, 2005). Most strains of *E. coli* are harmless, certain strains, like *E. coli* 0157:H7, can cause severe foodborne illness. Contaminated meat or improper cross-contamination during preparation can introduce E. coli into meat pies (Rangel et al., 2005). Symptoms of E. coli infection can include severe abdominal cramps, bloody diarrhea, and dehydration (Ryan *et al.,* 2016). A subgroup called Enterohemorrhagic *E. coli* (EHEC)

can cause food borne illness as the *E. coli* 0157:H7 strain which causes severe and potentially fatal illness known as hemorrhagic colitis which is characterized by bloody diarrhea and severe abdominal pain (Dolores and Doyle, 2001). Escherichia coli is commonly used as surrogate indicator, its presence in food generally indicate direct and indirect fecal contamination. However in Nigeria, a number of foods have been reported to have high incidence of bacteria (Adesiyun, 1995, Okonko *et al.,* 2009).

Salmonella spp.: Salmonella bacteria are commonly found in raw poultry and meat and can contaminate meat pies if these ingredients are not handled or cooked properly (Jay *et al.,* 2005). Salmonella infection can cause salmonellosis, a foodborne illness characterized by fever, diarrhea, abdominal cramps, nausea, vomiting, headaches, and muscle aches that can last for several days (Ryan *et al.,* 2016). Symptoms typically begin within 12 to 72 hours of consuming contaminated food and can last for several days (Ryan *et al.,* 2016). Most healthy individuals recover without requiring medical intervention. Salmonellosis can be severe for vulnerable populations like: young children, pregnant women, older adults, and individuals with weakened immune systems. In these cases, dehydration from diarrhea and vomiting can become a serious concern, and hospitalization may be necessary. *Salmonella spp.* are a group of gram-negative, rod-shaped bacteria commonly found in the intestines of animals, including poultry and livestock (Ryan *et al.,* 2016). These bacteria can contaminate meat pies at various stages of production if proper hygiene and handling practices are not followed. During

preparation, if raw meat or contaminated surfaces come into contact with cooked meat or other pie fillings, Salmonella can spread (Mensah *et al.,* 2002). This can occur through unwashed utensils, chopping boards, or even the hands of food handlers if proper hygiene protocols are not strictly enforced. To minimize the risk of Salmonella contamination in meat pies, several key measures can be implemented. Ensure all meat ingredients reach a safe internal temperature during cooking to eliminate Salmonella bacteria (Jay et al., 2005). Food handlers must practice proper hand washing before and after handling raw ingredients, cooked meat, and any other food items (Mensah *et al.,* 2002). Regularly sanitize utensils, chopping boards, and work surfaces to prevent cross-contamination (Jay *et al.,* 2005). Maintain proper storage temperatures throughout the production chain, from receiving raw ingredients to displaying cooked pies for sale. Keep cold foods below 5°C and hot foods above 60°C to inhibit bacterial growth (Jay *et al.,* 2005). By implementing these practices, vendors can significantly reduce the risk of Salmonella contamination in meat pies and protect consumers from the associated health risks.

***Bacillus cereus*:** This spore-forming bacterium can be present in soil and dust, and can contaminate meat pies through improper handling practices. While some strains cause foodborne illness, symptoms are typically milder than those caused by other pathogens, with episodes of vomiting or diarrhea (Granum and Lund, 2002). Bacillus cereus is a gram-positive, rod-shaped bacterium commonly found in soil and various food products,

including meat pies (Granum and Lund, 2002). This bacterium is known for its ability to form heat-resistant endospores that can survive harsh conditions, including cooking processes used in meat pie preparation (Doyle, 2007). Some strains of Bacillus cereus are harmless, while others can produce toxins that cause two distinct types of foodborne illness;

Emetic (Vomiting) Syndrome: This form of illness is caused by a toxin produced by *B. cereus* that accumulates in cooked rice and certain other foods left at room temperature for extended periods (Granum and Lund, 2002). The toxin is heat-stable and survives reheating, leading to symptoms such as nausea and vomiting within a few hours of consumption (Ryan *et al*., 2016).

Diarrhoeal Syndrome: This syndrome is caused by another toxin produced by B. cereus that acts within the intestines. It's more commonly associated with contaminated raw or undercooked meat products, including potentially meat pies if proper cooking temperatures are not reached or the filling is not cooled promptly after cooking (Granum and Lund, 2002). Symptoms of diarrhoeal illness caused by B. cereus typically include abdominal cramps and diarrhea, and usually begin within 6 to 15 hours of consuming contaminated food (Ryan *et al.,* 2016). Inadequate cooking temperatures or uneven heat distribution within the meat pie filling can allow *B. cereus* spores to survive (Granum and Lund, 2002). Holding cooked meat pies within the temperature danger zone

(between 5°C and 60°C) for extended periods can promote spore germination and toxin production by B. cereus (Jay *et al.,* 2005). Improper reheating of leftover meat pies may not eliminate all B. cereus spores, and toxins produced earlier can remain active (Doyle, 2007). In order to minimize the risk of B. cereus contamination and foodborne illness associated with meat pies, several measures can be taken. Ensure all ingredients in the meat pie filling reach a safe internal temperature during initial cooking to eliminate *B. cereus* spores (Jay *et al.,* 2005). Promptly cool cooked meat pies to below 5°C to prevent spore germination and toxin production by B. cereus (Jay *et al.,* 2005). Proper storage temperatures for cooked meat pies should be maintained either below 5°C or above 60°C, to inhibit bacterial growth (Jay *et al.,* 2005). Reheat leftover meat pies thoroughly to an internal temperature exceeding 74°C and consume them immediately after reheating (Jay *et al.,* 2005). By observing these precautions, food vendors and consumers can significantly reduce the risk of Bacillus cereus contamination and foodborne illness associated with meat pies.

***Listeria monocytogenes*:** This bacterium can grow at refrigeration temperatures, making it a particular concern for RTE foods like meat pies if not stored properly below 5°C (Jay *et al.,* 2005). Listeriosis, the illness caused by L. monocytogenes, can be severe, especially for pregnant women, newborns, older adults, and immunocompromised individuals (Doyle, 2007). *L. monocytogenes* can survive and even grow in both aerobic (with oxygen) and anaerobic (without oxygen) environments, making it adaptable to

various conditions within a meat pie (Ryan *et al.,* 2016). This bacterium has the ability to grow at low temperatures, including those typically used for refrigerating RTE foods like meat pies (around 4°C) (Jay *et al*., 2005). This characteristic makes it especially problematic, as refrigeration doesn't necessarily stop its growth. *L. monocytogenes* can invade and multiply inside host cells, making it more difficult for the immune system to eliminate (Ryan *et al.,* 2016). Listeriosis can cause miscarriage, stillbirth, or serious infections in newborns if the bacteria are transmitted from mother to fetus (Centers for Disease Control and Prevention. (October 26, 2023). Older adults with weakened immune systems are more susceptible to severe illness from L. monocytogenes infection. People with weakened immune systems due to underlying health conditions or medications are also at higher risk of severe listeriosis. Symptoms of listeriosis can vary depending on the infected individual, but can include: Fever, Muscle aches, Headache, Nausea, Vomiting, Diarrhea. In severe cases, listeriosis can lead to meningitis, an inflammation of the membranes surrounding the brain and spinal cord, or septicemia, a bloodstream infection that can be life-threatening (Ryan et al., 2016). Since L. monocytogenes can grow at refrigeration temperatures, preventing its presence in meat pies is very important. Use ingredients from reputable suppliers who follow strict hygiene protocols to minimize the risk of initial contamination. Ensure all meat ingredients reach a safe internal temperature during cooking to eliminate *L. monocytogenes* (Jay *et al.,* 2005). Maintain proper storage temperatures throughout the

production chain, keeping cooked meat pies below 5°C to inhibit bacterial growth (Jay *et al.,* 2005). Implement rigorous cleaning and sanitation procedures for equipment and surfaces to prevent cross-contamination throughout preparation. Establish clear expiry dates for meat pies to ensure consumption before L. monocytogenes can potentially grow to dangerous levels. By following these practices, food businesses can significantly reduce the risk of Listeria contamination in meat pies and protect consumers from the associated health risks.

2.5 Foodborne Illness Outbreaks Linked to Meat Pies

Foodborne outbreaks occur when two or more people experience similar illness after eating the same food. Foodborne illness outbreaks in the United States are a serious public health concern. Each year, an estimated one in six people (or 48 million people) gets sick, 128,000 persons are hospitalized, and 3,000 deaths occur as a result of foodborne diseases (Centers for Disease Control and Prevention, 2016). The World Health Organization estimates that as many as 600 million (or almost 1 in 10 people) fall ill and 420,000 deaths occur each year from eating contaminated food (WHO, 2017). In the United States, hundreds of foodborne outbreaks are reported each year. In 2015 alone, the Centers for Disease Control and Prevention (CDC) identified that more than 900 foodborne illness outbreaks occurred and were associated with bacterial, chemical, toxin, parasitic, or viral etiology (Centers for Disease Control and Prevention, 2015).

The primary reason for investigating a foodborne illness outbreak is to identify the source(s) of the exposure so that public health action can be taken to establish control measures that will prevent continued episodes of illness and the spread of disease (Centers for Disease Control and Prevention, 2012). By objectively identifying detailed data and information gathered from the outbreak, investigators should be able to identify what went wrong to ensure that future similar events can be prevented. Meat pies, a delicious and convenient food, can sometimes be associated with outbreaks of foodborne illness. A well-documented case involved an outbreak of Salmonella braenderup gastroenteritis in Switzerland (Schmid *et al.,* 1996) linked to commercially produced meat pies. The pies were found to be heavily contaminated with the bacteria, likely due to mishandling and potentially the reuse of contaminated jelly used for glazing (Schmid *et al.,* 1996). This case highlights the critical role of proper hygiene practices and careful handling of ingredients at every stage of production.

Another study focused on the presence of bacteria in meat pies sold by street vendors in Yenagoa metropolis, Nigeria (Okonko and Adebayo-Tayo, 2009). The study found Staphylococcus aureus, Bacillus cereus, and Escherichia coli among the bacteria present, suggesting potential risks associated with inadequate sanitation and temperature control during preparation and vending (Okonko and Adebayo-Tayo, 2009). This emphasizes the need for stricter regulations, particularly for street vended foods, to ensure proper hygiene protocols are followed throughout the process.

2.6 Microbiological Studies on Meat Pies in Nigeria

Several Nigerian studies have investigated the microbiological quality of meat pies, highlighting potential food safety concerns. Research published in the African Journal of Microbiology Research examined the bacterial contamination of meat pies from standard eateries and local kiosks in Benin City, Nigeria (Oboh *et al.,* 2020). The study found that the mean total bacterial count in the pies varied depending on the source and storage conditions (Oboh et al., 2020). Fresh pies generally had lower bacterial loads compared to those stored for two days at room temperature (Oboh *et al.,* 2020). This indicates the importance of proper storage and consumption within a recommended timeframe.

Another study, published in the International Journal of Pharmaceutical and Bio Medical Science, investigated meat pies sold in Owerri, Nigeria (Ikenebomeh and Ekpo, 2012). The study found evidence of coliform bacteria in all the samples analyzed (Ikenebomeh and Ekpo, 2012). Coliforms are a group of bacteria commonly associated with fecal contamination and can indicate poor hygiene practices during preparation or handling (Ikenebomeh and Ekpo, 2012). This finding emphasizes the need for stricter hygiene protocols throughout the meat pie production chain. While the studies mentioned above focused on general bacterial loads and coliform presence, other Nigerian research has delved deeper. A study published in ResearchGate investigated the presence of Staphylococcus aureus and Escherichia coli in meat pies from Lagos

(Adebayo et al., 2020). This study found a high prevalence of both these bacteria, which can cause foodborne illness if present in sufficient quantities (Adebayo *et al*., 2020). These Nigerian studies highlight the potential presence of harmful bacteria in meat pies sold in the country.

2.6.1 Techniques Employed for Microbial Identification in Nigerian Meat Pie Studies

Microbiological studies on Nigerian meat pies, as discussed earlier (section 2.6), play a crucial role in ensuring food safety. These studies rely on various techniques to identify the specific types of bacteria present. Some commonly employed techniques for microbial identification are;

Culturing and Colony Morphology: This traditional technique involves growing bacteria on specific culture media that favor their growth. By observing colony characteristics like size, shape, color, and hemolytic properties (red blood cell lysis), scientists can get initial clues about the types of bacteria present (Cappuccino and Natalie Sherman, 2014). However, further tests are often needed for definitive identification.

Biochemical Tests: These tests assess the metabolic capabilities of bacterial isolates. Different bacteria have unique enzyme profiles, allowing them to utilize various substrates in specific ways. Biochemical tests like catalase, oxidase, and sugar

fermentation tests provide additional information to narrow down the identification of bacterial isolates obtained from culture plates (Koneman *et al.,* 1997).

Gram Staining: This staining technique differentiates bacteria based on their cell wall structure. Gram-positive bacteria retain a blue or purple stain, while Gram-negative bacteria appear pink (Tortora *et al.,* 2009). This basic yet valuable test helps categorize bacteria into broad groups, guiding further identification steps.

Molecular Techniques: Advancements in technology have introduced more sophisticated identification methods. Techniques like Polymerase Chain Reaction (PCR) and DNA sequencing can target specific genes unique to certain bacterial species. This allows for highly accurate identification and differentiation of even closely related bacteria (Janda and Abbott, 2007).

The specific techniques employed in a Nigerian meat pie study will depend on several factors, including:

Scope of the Study: Is the study aiming for a general assessment of bacterial load or a more detailed identification of specific pathogens?

Available Resources: Laboratories may have varying access to sophisticated equipment needed for molecular techniques.

Cost Considerations: Some techniques can be more expensive than others.

Accurately identifying bacteria present in meat pies is necessary for several reasons:

Targeted Interventions: Knowing the specific types of bacteria allows for targeted interventions to prevent their growth or eliminate them.

Risk Assessment: Certain bacteria pose greater health risks than others. Identification helps assess the potential severity of foodborne illness risks associated with the contaminated meat pies.

Public Health Monitoring: Data from these studies can be used for public health surveillance to track trends in foodborne illness outbreaks and identify areas requiring stricter regulations.

By employing these techniques, researchers in Nigeria can gain valuable insights into the microbial landscape of meat pies, ultimately contributing to improved food safety standards.

2.6.2 Reported Microbial Contaminants in Nigerian Meat Pies

As highlighted earlier (section 2.6), Nigerian studies have identified a concerning range of microbial contaminants in meat pies. Some commonly reported bacteria are:

Gram-Positive Bacteria

***Staphylococcus aureus*:** This bacterium can cause a variety of foodborne illnesses. Studies from Lagos and Lafia, Nigeria, have found a high prevalence of S. aureus in meat pies (Ikenebomeh and Ekpo, 2012 and Adebayo *et al.,* 2020).

***Bacillus cereus*:** This bacterium can produce two types of toxins, causing either diarrheal or vomiting foodborne illness. Research from Lafia, Nigeria, identified Bacillus cereus among the microbial isolates in meat pies (Adebayo *et al.,* 2020).

Gram-Negative Bacteria

***Escherichia coli* (*E. coli*):** The presence of E. coli in food is often an indicator of fecal contamination. Studies from Owerri and Lagos, Nigeria, have found E. coli in meat pie samples (Ikenebomeh and Ekpo, 2012 and Adebayo *et al.,* 2020). This suggests potential hygiene issues during preparation or handling.

***Salmonella spp*.:** Salmonella bacteria can cause severe foodborne illness with symptoms like typhoid fever. Though not as frequently reported as other bacteria, some Nigerian studies have identified Salmonella presence in meat pies (Okoli and Nwaneme, 2005).

Other Microorganisms

Fungi: Mold and yeast, although not typically associated with acute foodborne illness, can affect the quality and safety of meat pies by causing spoilage and potentially

producing mycotoxins (Samson *et al*., 2000). Microbial studies on Nigerian meat pies have occasionally identified fungal contaminants (Adebayo *et al*., 2020).

2.7 Knowledge Gap Regarding Meat Pies sold in UNIBEN

Given the potential risks associated with Nigerian meat pies in general, investigating meat pies sold in UNIBEN specifically would be valuable. Understanding the microbial content of meat pies sold in UNIBEN would help identify any unique hazards associated with their preparation, handling, or vending practices. If specific contaminants are prevalent, targeted interventions could be implemented to improve hygiene, storage, or handling protocols at UNIBEN. Knowledge about the specific risks associated with meat pies sold in UNIBEN would empower students and consumers to make informed choices about consumption and handling practices.

There are several possible areas for investigation of meat pies sold in UNIBEN;

Microbiological analysis of UNIBEN meat pies: Studies could analyze samples from vendors around UNIBEN to identify the types of bacteria present.

Assessment of hygiene practices: Investigating hygiene protocols during preparation, vending, and storage of meat pies sold in UNIBEN could reveal areas for improvement.

Consumer awareness and practices: Studies could assess student awareness of foodborne illness risks associated with meat pies and their storage and handling practices.

2.8 Significance of Studying Microbial Populations in meat Pies sold in UNIBEN

Understanding the microbial populations present in meat pies sold in UNIBEN holds significant value for ensuring students safety and improving food quality on campus. By analyzing the microbial population, researchers can identify the presence of harmful bacteria like Staphylococcus aureus, E. coli, or Salmonella spp. These pathogens can cause foodborne illness with symptoms ranging from mild discomfort to severe gastroenteritis (Centers for Disease Control and Prevention; October 26, 2023 and Centers for Disease Control and Prevention; April 11, 2023). Early detection allows for interventions to prevent outbreaks and protect student health. Understanding the types and quantities of bacteria present allows for a risk assessment of the potential severity of foodborne illness associated with meat pies sold in UNIBEN. Knowing the specific microbial populations allows for targeted interventions to address them. Study results can be used to educate vendors about the types of bacteria commonly found in meat pies and strategies to prevent their growth or contamination. Understanding the microbial populations can guide the development of improved storage and handling protocols to extend the shelf life and maintain the quality of meat pies sold in UNIBEN. Microbial growth can lead to spoilage, making the pies unappetizing and unfit for consumption. Studying the microbial populations can help identify ways to minimize spoilage and ensure students get a good quality product. Data generated from studies on meat pies

sold in UNIBEN can contribute to a broader knowledge base for public health agencies. This information can be used for surveillance of foodborne illness trends and informing public health regulations. These studies can lay the groundwork for future research on food safety practices specific to the UNIBEN campus environment. This can lead to the development of more targeted interventions and continuous improvement of food safety standards.

2.8.1 Public Health Implications of Microbial Populations in UNIBEN Meat Pies

By understanding the public health implications, universities can take proactive steps to mitigate risks. Study results can inform the development and implementation of stricter food safety regulations on campus, particularly regarding hygiene practices, storage temperatures, and vendor licensing. Universities can establish training programs for vendors on proper food handling practices, including hygiene protocols, safe temperatures for storage and transport, and proper handwashing techniques. Raising student awareness about foodborne illness risks associated with meat pies can empower them to make informed choices about purchasing and consuming these foods. Educational campaigns can focus on identifying safe vendors, proper storage practices at home, and recognizing signs of spoilage.

2.8.2 Potential Economic and Social Impact of Microbial Populations in Meat Pies sold in UNIBEN

The primary concern surrounding microbial populations in meat pies sold in UNIBEN is public health (addressed in section 2.8.1), there are also potential economic and social consequences to consider. Foodborne illness outbreaks or even individual cases of illness caused by contaminated meat pies can lead to decreased student productivity (Nguyen and Schwartz, 2016). Studies have shown that absenteeism due to illness can negatively impact student academic performance (Nguyen and Schwartz, 2016). Students who are sick may miss classes, fall behind on coursework, or be unable to concentrate during lectures. This can lead to lower grades, extended semesters, or even delayed graduation, all of which can have financial implications for students and the university. Outbreaks of foodborne illness associated with meat pies sold in UNIBEN can damage the university's reputation. Negative publicity can deter prospective students from enrolling, impacting the university's applicant pool and potentially leading to a decline in student enrollment. A tarnished reputation can also lead to decreased morale and trust among current students, faculty, and staff. If students become sick and have to leave the university due to foodborne illness, the university can experience a loss of revenue from tuition fees and housing costs. Additionally, negative publicity may lead to decreased enrollment, further impacting revenue streams. Foodborne illness outbreaks can create anxiety and fear among students, impacting their

overall morale and sense of well-being on campus. Students may be worried about getting sick and avoid purchasing food from vendors, leading to a less vibrant campus dining atmosphere. Social interaction over shared meals is an important aspect of campus life, and limitations due to fear of illness can negatively impact this aspect of student life. If students lose trust in the safety of meat pies sold in UNIBEN they may be hesitant to purchase them at all. This can negatively impact vendors' livelihoods and restrict students' food options on campus. A lack of trust can strain the relationship between students and vendors, affecting the overall campus community. A strong commitment to food safety can improve the university's image and attract more students, leading to positive social and economic outcomes. A good reputation attracts better faculty and staff as well, creating a more well-rounded academic environment. By promoting safe food handling practices, the university can create a healthier and more enjoyable environment for students and staff. This can lead to increased student satisfaction and a more positive overall campus experience.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MICROBIAL ANALYSIS

3.1.1 COLLECTION OF SAMPLES

Five (5) meat pies samples were purchased from different vendors or sellers within University of Benin (UNIBEN) premises, Benin city; Edo state, Nigeria. The samples were carried with the aid of sterilized polyethylene bags and they were labelled properly and taken aseptically to the laboratory for microbial analysis. The microbial analysis was carried out in three (3) replicates.

3.2. STUDY AREA

The study was carried out in Central Research Laboratory inside University of Benin, Benin city; Edo state, Nigeria.

3.3 MATERIALS

Materials used include; Weighing balance, Spatula, Conical flasks (1000mls, 500mls, and 250mls), Alumnium foil paper, Glass pipette and micropipette, Mortal and Pestle, Test tubes and test tube racks, Distilled water, Vortex mixer, Nutrient Agar, Cotton wool, Measuring cylinder, Peptone water, Hand gloves, Face masks, Potato Dextrose Agar, Petri Dishes, Mac Conkey Agar, Colony Counter, Standard inoculation wire loops, Bursen burner, Disinfectants (ethanol), Autoclave, Incubators, Masking tape, Gram stain

reagents, Beakers, Marker pens, Microscope glass slides, Microscope, Pasture pipette, Immersion oil.

3.4 STERILIZATION OF WORK BENCH AND MATERIALS

All laboratory work was carried out under aseptic condition while following laboratory rules and regulations. All glass wares such as pipettes, beakers, conical flasks etc. used in this study were thoroughly washed with detergent and rinsed in clean water to ensure that they were grease-free. The glass wares were properly washed and sterilized in an autoclave at 121°C at 15 mmHg for 15 minutes before use. The work bench was disinfected with 70% ethanol. The inoculating wire loops were sterilized by the red heat method with the aid of the Bursen burner flame before and after use. Commercial Petri dishes which had already been sterilized were used. Laboratory coat was worn throughout the practical procedures and good hygiene was maintained during the procedure.

3.5 PREPARATION OF SAMPLE

A portion of the meat pie sample (20g) was weighed into sterile laboratory mortal and homogenized with the aid of a sterile laboratory pestle into 180 ml peptone water. The solution made from homogenizing the meat pie sample into the peptone water is known as the stock solution.

3.5.1 PREPARATION AND STERILIZATION OF CULTURE MEDIA

All culture media were prepared from commercially available products and made up according to the manufacturers' instructions. The sterilization of the media was carried out in a conical flask and sealed with foil paper to prevent contamination. They were sterilized by autoclaving at 121°C for 15 minutes at 15 psi after that it was allowed to cool before dispensing into the sterile petri dishes. The culture media used were: Nutrient Agar (NA) for the general culture of bacteria and some fungi resulting in a mixed culture of several microorganisms before sub culturing, Potato Dextrose Agar (PDA) for the culture of fungi, Mac Conkey Agar (MCA) which is designed to selectively isolate Gram-negative and enteric (normally found in the intestinal tract) bacteria and differentiate them based on lactose fermentation, and Mannitol Salt Agar (MSA) used as a selective and differential medium for the isolation and identification of Staphylococcus aureus from clinical and non-clinical specimens. It is recommended for the detection and enumeration of coagulase-positive Staphylococci in milk, food, and other specimens and encourages the growth of a group of certain bacteria while inhibiting the growth of others and Salmonella Shigella Agar (SSA) which is a moderately selective and differential medium for the isolation, cultivation, and differentiation of Salmonella spp. and some strains of *Shigella spp*.

Plate 3: Culture media sterilized in the Autoclave at 121°C for 15 minutes at 15 psi

3.5.2 PREPARATION OF NUTRIENT AGAR (NA)

Twenty-eight (28) grams of nutrient agar (NA) powder was dissolved in 1 liter of distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 minutes. The medium was cooled to 45-50°C and then dispensed aseptically into sterile petri dishes in the laminar flow.

3.5.3 PREPARATION OF MAC CONKEY AGAR (MCA)

MacConkey Agar (MCA) was prepared by dissolving 51.55 grams of agar powder in 1000 ml distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 minutes. The medium was allowed to cool to 45°C and then dispensed aseptically into sterile Petri dishes in a laminar flow chamber.

3.5.4 PREPARATION OF POTATO DEXTROSE AGAR (PDA)

Potato Dextrose Agar (PDA) was prepared by dissolving 39 grams of PDA powder in to 1000 ml distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 minutes. The medium was allowed to cool to 45°C and then dispensed aseptically into sterile Petri dishes in a laminar flow chamber.

3.5.5 PREPARATION OF MANNITOL SALT AGAR (MSA)

111 grams of Mannitol Salt Agar (MSA) was dissolved in 1000ml of distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 minutes. The medium was allowed to cool to 45°C and then dispensed aseptically into sterile Petri dishes in a laminar flow chamber.

3.5.6 PREPARATION OF SALMONELLA SHIGELLA AGAR

Salmonella Shigella Agar was prepared by dissolving 60 grams in 1000ml of distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 minutes. The medium was allowed to cool to about 45°C- 50°C and then dispensed aseptically into sterile Petri dishes in a laminar flow chamber.

3.5.7 SERIAL DILUTION

The serial dilution method of Harrigan and McCance (1976) was aseptically carried out in test tubes. Sterile test tubes were used for the ten-fold dilution (Slaby et al., 1981). Test tubes labelled 10-1, 10-2, 10-3, 10-4, 10-5, 10-6, and 10-7 were prepared and used for each of the samples. 1g of each samples from the stock solution was transferred was into 9ml of sterile water in a test tube. Serial dilution was carried out to 10-7 dilution by

transferring from the first test tube using a sterile pipette and transferred to the second test tube containing 9ml of distilled water to get 10^{-1}. 1ml was picked from the test tube containing 10-1 dilution by the pipette and transferred to the third tube containing 9ml sterile diluted water to get 10-2. The process continued until the 10-7 serial dilution.

3.6 INOCULATION AND INCUBATION

1ml aliquot of 10-7 diluted sample was inoculated into 7 sterile petri dishes. Prepared and sterilized nutrient agar was poured into the inoculated petri dishes and mixed gently to evenly spread the organisms and then allowed to solidify. The petri dishes were incubated at 37 degrees Celsius for 24 hrs.

3.7 COLONY COUNTING

After successful growth of microorganisms, the colonies were counted with a colony counter and the results per dilution count were recorded. The number of colony forming unit per milliliter was calculated with the formula: Cfu/g = CFU/g.

Plate 4: A colony counter and petri dish containing microorganisms in Nutrient Agar.

3.8 SUBCULTURING

The bacterial colonies were streaked on fresh Nutrient agar, MacConkey agar, Manitol salt agar, Potato dextrose agar, and Salmonella Shigella agar respectively to obtain the pure culture. Pure cultures were checked from the various culture media. After achieving a pure culture, the same colony was streaked onto a nutrient agar slant. These cultures were incubated at 37°C for 24 hours.

3.9 BACTERIAL IDENTIFICATION

The bacterial isolates were characterized based on colonial morphological characteristics such as colony shape, size, elevation, optical activity or density, margination and pigmentation on Nutrient agar, MacConkey agar, Manitol salt agar and Salmonella Shigella agar. Biochemical tests were also carried out to further identify the bacterial isolates. The fungal isolates were identified using colonial morphological characteristics such as size, texture colour and reverse colour. These parameters were evaluated by physical examination. Microscopy was also carried out using lactophenol cotton blue staining and a bright field microscope.

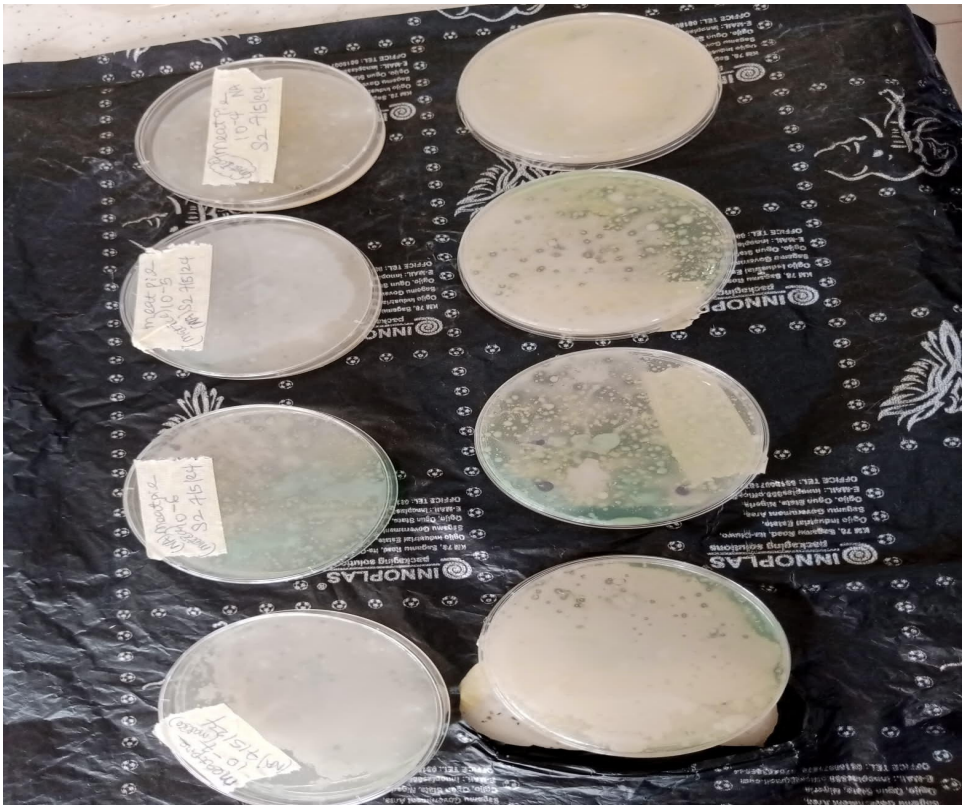

Plate 5: Bacteria growing on the Nutrient Agar Culture media in the sterile petri dishes

Plate 6: Labelling of the sterile petri dishes before inoculation for identification under aseptic conditions.

3.10 GRAM STAINING TEST FOR BACTERIAL ISOLATE

The smear of each of the isolates were prepared by picking a small portion of microbial growth from the plates with the aid of a sterilized standard inoculation wire loop and placed on the slide, then the slides were heated and fixed carefully. The heat fixed smears were stained with crystal violet for 60s, washed off with water and drained, then flooded with Lugol's iodine for about 60s and wash off gently with water and drained. The slides were rinsed with 50-50 alcohol-acetone for 3s and were rinsed with water and drained. The slides were then counter stained with safranin for 1min after then, the stains were washed off with water. The slides were air dried; immersion oil was dropped on the smears and the smears were examined for cell morphology and arrangement, presence of capsule and staining reaction.

3.11 BIOCHEMICAL TESTS FOR IDENTIFICATION OF ISOLATES

The selected bacterial isolates were subjected to Biochemical and Staining techniques as described and key provided in the Bergy's Manual of Determinative Bacteriology. The following biochemical test were carried out catalase test, oxidase test, motility test), methyl red test, indole test, citrate test, and triple sugar iron agar test (Tsi).

3.11.1 CATALASE TEST

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyzes the breakdown of hydrogen peroxide to release free oxygen gas and the formation of water. 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.

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

3.11.2. OXIDASE TEST

A piece of filter paper was wet with a few drops of the dilute (1%) solution of oxidase reagent (tetramethyl-phenylenediamine-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.

3.11.3. UREASE TEST

The urease test is used to determine the ability of an organism to split urea in the presence of the enzyme urease. The bacterial isolates were inoculated into slants of urea medium and incubated at 37°C for 24 - 48 hours. Urease positive cultures produced a red-pink color due to changes in the color of the indicator.

$NH_2CO.NH_2 + H_2O \rightarrow 2NH_3 + CO_2$

3.11.4. CITRATE UTILIZATION TEST

This test is based on the ability of some organisms to utilize citrate as a sole source of carbon. This was carried out by inoculating the test organism in test tube containing Simon's citrate medium and this was incubated at 37°C for 24 – 48 hours. The development of deep blue colour after incubation indicates a positive result.

3.11.5. HYDROGEN SULPHIDE TEST

Hydrogen sulphide production can be detected by incorporating a heavy metal salt containing (Fe^{2+}) or lead (Pb^{2+}) ion as H2S indicator to a nutrient culture medium containing cysteine and sodium thiosulfate as the sulphur substrates. Hydrogen sulphide, a colourless gas, when produced reacts with sulphur metal salt (ferrous sulfate) forming a visible insoluble black sulphide precipitate.

3.11.6 INDOLE TEST

Indole test is performed to determine the ability of the organism to split tryptophan molecule into indole. This test was performed to help differentiate species of the family Enterobacteriaceae. Kovac's reagent which contains hydrochloric acid, dimethylaminobenzaldehyde and amyl alcohol is used. The broth was inoculated with the test organism and incubated for 18 hours at 37°C. 5ml of Kovac's reagent was then added down the inner wall of the tube. Development of bright red colour at the interface

of the reagent and the broth within seconds after adding the reagent was indicative of the presence of indole and a positive result.

3.11.7 SUGAR FERMENTATION TEST

Each of the isolates was tested for its ability to ferment a given sugar with the production of acid and gas or acid only. The growth medium comprised of peptone water, sugar (1%) and the indicator (bromocresol purple). The mixture was dispensed into test tubes and sterilized by autoclaving at 121°C for 15 minutes. After sterilizing, the test tubes were allowed to cool and then inoculated with the isolates and incubated at 37°C for 24hrs. Acid and gas production or acid only were observed after about 24 hours of incubation. Acid production was indicated by the change of the medium from purple to yellow colour indicated a positive test. Sugars used were lactose, sucrose, glucose, fructose, maltose, starch and sorbitol.

3.12. IDENTIFICATION OF FUNGAL ISOLATES

A drop of lactophenol blue stain was dropped on a clean grease free sterilized glass slide and after this a sterile standard inoculating wire loop was used to pick the mycelium unto the glass slide from the mould culture. The mycelium was spread evenly on the slide. Teasing was carried out to separate the mycelium in order to get a homogeneous mixture and the mixture was then covered with cover slips gently and then allowed to stay for some seconds before observing under x40 under the microscope. The

microscope examination of actively growing mould was on the basis of structures bearing spores, presence or absence of septate.

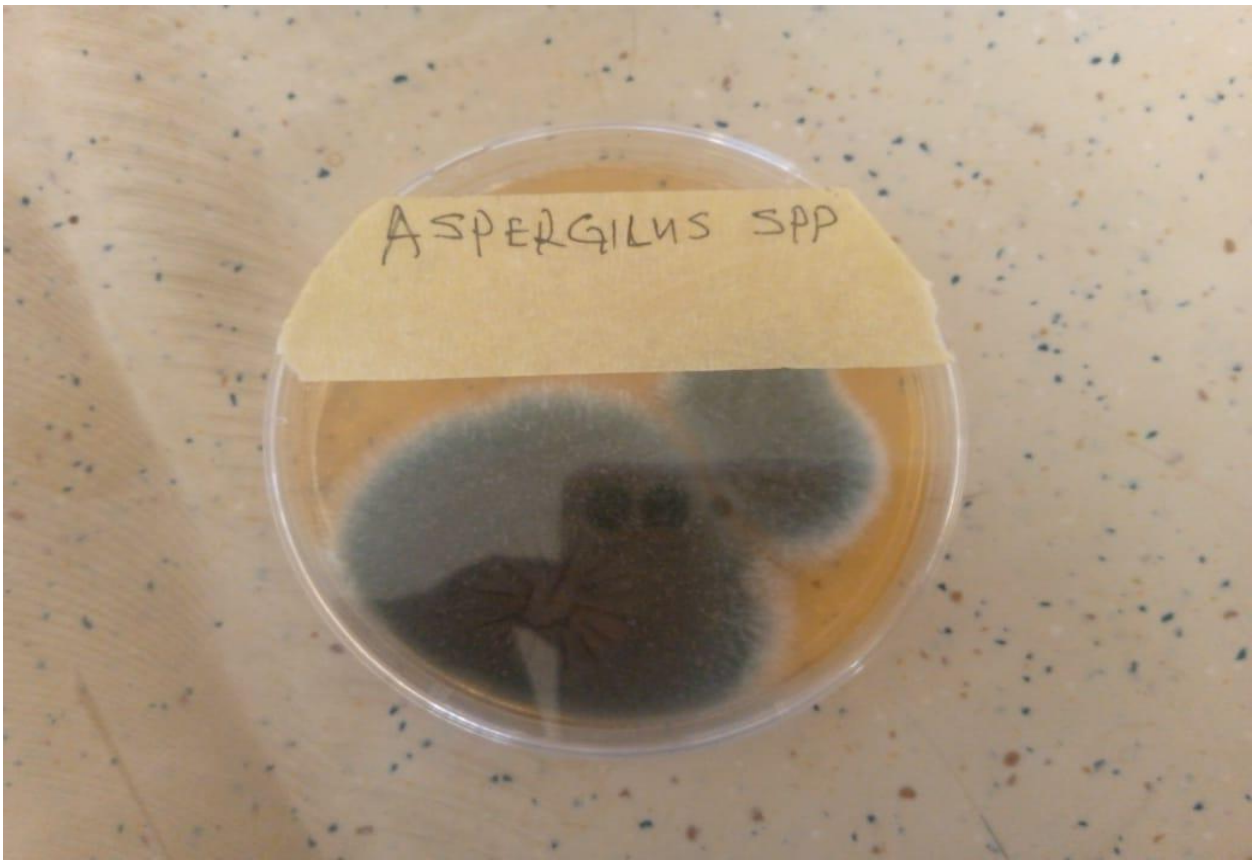

Plate 7: Showing the growth of *Aspergillus spp*. on Potato Dextrose Agar

3.13. STATISTICAL ANALYSIS

Data was analyzed by ANOVA appropriate to each experiment using Micro Excel programme and any statistical significance of difference between means were tested at 5% confidence level ($P<0.05$).

CHAPTER FOUR

4.0 RESULTS

Microbial population of meat pies obtained from five locations within University of Benin are presented below in:

Table 4:1: Heterotrophic Bacterial colony count (cfu/g) of meat pies

Dilution factor(df)	Sample 1 (Faculty of Arts)	Sample 2 (Mat- Ice Anatomy gate)	Sample 3 (Faculty of Agriculture)	Sample 4 (Faculty of Engineering)	Sample 5 (Uniben buka)
10^1	291	271	283	170	315
10^2	254	213	180	115	290
10^3	111	85	93	75	188
10^4	93	55	63	66	79
10^5	55	33	42	31	42

Heterotrophic Bacterial colony count (cfu/g) of meat pies for each dilution factor all five samples as indicated by the location name.

Table 4:2: Fungal colony count (cfu/g) for all five samples

Samples	Total Heterotrophic Fungi Count(cfu/g)
Faculty of Arts	15
Mat-Ice Anatomy gate	5
Faculty of Agriculture	7
Faculty of Engineering	11
Uniben buka	22

Fungal colony count (cfu/g) for all five samples as indicated by the location name for the 5^{th} dilution factor

Table 4:3: Mean Microbial Aerobic Plate Counts (cfu/g) of Meat pies obtained from 5 different locations within University of Benin, Benin city

Location/samples	Mean Total Heterotrophic Bacteria Count MTHBC (cfu/g)	Mean Total Fungi Count MTFC (cfu/g)
Faculty of Arts	1.608×10^6	1.5×10^6
Mat-Ice Anatomy gate	1.312×10^6	0.5×10^6
Faculty of Agriculture	1.322×10^6	0.7×10^6
Faculty of Engineering	0.914×10^6	1.1×10^6
Uniben buka	1.828×10^6	2.2×10^6

Results presented in Table 4.3 above showed that the highest mean total heterotrophic bacteria count (1.828log cfu/g) were obtained from Uniben buka, and the lowest mean total heterotrophic bacteria count (0.914log cfu/g) were obtained from Faculty of Engineering.

There was a significant difference ($P > 0.05$) in the mean heterotrophic bacterial counts among the five meat pie samples. However, when Tukey's HSD post-hoc test was performed to identify the specific pairs of samples with significant differences in their means, no significant pairwise differences were found at the 0.05 significance level. While that of mean total heterotrophic fungal count, we cannot reject the null hypothesis that all location means are equal. This suggests that there is no significant difference in fungal counts among the five locations based on the fifth dilution factor.

4.1:1 Bacterial Identification on the samples

In identification of bacteria, cultural characteristics are the use of color, shape, size, elevation and opacity to suspect microorganism that might be present in the sample before undergoing further tests. Morphology is testing how the organism will behave to gram stain before final testing. Biochemical test is used to confirm that the organism is present in the sample.

Table 4:4 Identified Bacteria from meat pies sold in Uniben

CULTURAL CHARACTERISTICS	**A**	**B**	**C**	**D**
Shape	Round	Round	Round	Round
Color	Milky	Milky	Milky	Milky
Size	Large	Large	Large	Large
MORPHOLOGY				
Gram stain	-	-	-	+
Cell type	Rod	Rod	Rod	Cocci
Cell Arrangement	Single, Pair	Pair	Single	Cluster
BIOCHEMICAL TEST				
Citrate utilization	+	+	-	+
Spore forming	-	-	-	-
Catalase production	+	+	+	+
H_2S(Hydrogen Sulphide)	+	-	-	-
Urease	-	-	-	+
Indole	+	+	-	-
Motility	+	+	-	-
Methyl red	+	+	+	+
Oxidase test	-	-	-	-
SUGAR FERMENTATION TEST				
Lactose	-	+	-	-
Glucose	+	+	+	+
Galactose	+	+	-	+
Maltose	+	+	-	+
Probable Identity	***Salmonella***	***Escherichia Coli***	***Shigella***	***Staphylococcus Aureus***

Key:
+ = Positive to test
- = Negative to test

4.1:2 Identified Fungi isolated from meat pies

Table 4:5 Characterization of the fungi

Isolate	Cultural	Microscopic Examination	Fungal isolates
F1	White, fluffy and velvety smooth cottony surface	Thread-like hyphae with septate conidiophores	**Mold**
F2	Black fluffy colony with reverse side yellow	Simple septate and branched conidia in chain	**Aspergillus Niger**
F3	Light-green colony with reverse side pink	Simple septate and branched conidia in chain	*Aspergillus spp.*
F4	Cream coloured smooth and moist surface	Oval single cells that often reproduce by budding	Yeast

CHAPTER FIVE

5.0 DISCUSSION

5.1:1 Microbial counts in the Salads samples

The microorganisms present in these meat pies are a direct reflection of the sanitary conditions of the processing site of meat pies produced in Uniben. One of the main reasons for such patronage of the product is the well reported nutritional and health benefit and the flavorful taste of the meat pies enjoyed by both staff and students of the University of Benin community. However, it is also well reported that meats and meat products undergo spoilage as a result of microbial action on the fats and proteins (Adesiyun, 1995). And meat pie is one of the meat products enjoyed by everyone. All the bacteria and fungi isolated in this study have previously been isolated from other meat pies in other studies (Adesiyun, 1995; Okonko *et al.,* 2009, Chelea and Jideani, 1996), Oranusi *et al*., 2011 and Bickert 2010). The microbial contamination observed in the meat pies sold in these five locations may be as a result of the poor processing and poor facility used in storing the product.

The isolated bacteria were: Escherichia coli., Salmonella, Shigella, and Staphylococcus aureus. These microorganisms are known to cause infection in the human bodies, especially in an accumulated form (Adebolu *et al.,* 2001).

The total heterotrophic bacteria count in the five locations which ranges from 0.914log (cfu/g) to 1.828log (cfu/g) was found to be lower than some of the studies carried elsewhere. For example, a study carried out in the Dominican Republic reported aerobic mesophilic counts of between 5 and 9log cfu/g in street-vended fried fish, chicken, beef, meat, stew and rice (Alvarez 1988). In Pakistan, aerobic mesophilic as high as 8log cfu/g were reported for street- vended rice and mesophilic counts as high as 7log cfu/g for cooked ground meat sold on the street (Bryan *et al.,* 1997).

In Zambia, aerobic mesophilic counts of between 3 and 9log cfu/g were reported for street-vended meatball (Bryan et al 1997). A study carried in Zaria, Nigeria bacteria count of street vended food samples were lower and did not exceed 4.7log cfu/g (Umoh *et al.,* 1999). However, the above bacterial counts were still higher in relation to counts reported in the study.

The fungi species isolated were: Yeast, Mold, *Aspergillus spp*, and *Aspergillus niger*. The facts that these fungi were detected may be an indication that they were poorly processed and stored. The total fungal counts of the meat pie samples ranges from 0.5log (cfu/g) to 2.2log (cfu/g) This study, showed that the presence of contaminating pathogen (bacteria and fungi) in the meat pies can be attributed to cross-contamination from environmental sources and handling by the sellers (Alvarez *et al.,* 1988).

5.1:2 Microorganism Identified and their distribution

The results, showed that the environmental conditions under which street sellers worked as well as their handling practices were no different from those observed in other studies (Bryan *et al.,* 1997). Restaurants in Benin City had limited access to clean running water. As a result, the water that was collected in the morning was frequently used until the end of the day activity. This water were usually boiled much at the beginning of the day for the major day activities and will be used till dawn. However, by the time samples were collected, this water had been standing at ambient temperature for several hours. The water is not only used for meat pie preparation but also for cleaning of food preparation areas and for hand washing by the vendors and their customers before and after eating. Vendors also commonly washed their hands in dishwater when they returned from toilets. This was reflected by high bacterial counts as well as by relatively high incidence of *E.coli*. The predominance of Yeast, Mold, *Aspergillus spp*. and *Aspergillus niger* isolate on aerobic counts was possible due to the presence of spore in the raw materials. These heat resistant spores have survived because meat pies are rarely heat treated before consumption.

Staphylococcus aureus are common environmental bacteria and could thus have been introduced into the food after cooking through cross-contamination. For instance, from utensils used by the vendors to serve food. Uniben buka was found to contain highest

bacteria population when compared with other locations reasons being to dirt areas where the meat pie ingredients are being sold, followed by Faculty of Arts meat pies Samples. This shows that the students who regularly patronize Uniben buka have a very high chances of being infected with these bacteria when compared with the other four locations. The study, showed that there was a significant different ($P<0.05$) of fungal organism Yeast, Mold, *Aspergillus spp, Aspergillus niger* when compared with control but was not significantly different ($P>0.05$) when compared among the five locations. This could be due to the fact that these organisms are spore formers and are known common environmental contaminants: However they have been known as food borne pathogens (Peraica *et al*., 2001, Aboloma *et al.,* 2008). Salmonella, *Escharichia coli,* Staphylococcus, Shigella, are environment contaminants. They have been isolated from plant, human skin, animals and dairy products.

The presence of *E.coli, Staphylococcus spp*. seeks for attention due to these organism are frequently associated with poor sanitary practices and could be a pointer to danger of possible food borne infection. Escherichia coli are especially of faecal origin and have been implicated in numerous food borne disease (Oranusi *et al*., 2007).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

The warm and moist environment within the pastry crust of meat pies creates ideal conditions for the growth of certain bacteria especially during lapses in temperature control. The various stages of preparation, from handling raw ingredients to storage and vending, present numerous opportunities for contamination with foodborne pathogens. This occurs through improper hand hygiene practices of food handlers, cross-contamination with contaminated surfaces or equipment, and in adequate storage temperatures. Microbial proliferation can lead to spoilage, rendering the meat pies unfit for consumption and causing sensory changes like off-odors, discoloration, and sliminess.

The aerobic plate count (APC) of meat pies analyzed from different sources of risk range between 0.9 to 1.8 which were all within both the International Common for Microbiological Specification in Foods (ICMSF) Escherichia coli and Staphylococcus aureus were highly detected in the meat pies making the meat pies sold in the above specified five locations a potential source of food poisoning to consumers. From the study, majority of the vendors stored meat pies at room temperature which are consumed frequently.

Storage condition of meat pies during sales and the frequency of consumption respectively represent the post processing handling practices and consumption pattern that contribute significantly to the microbiological quality of meat pies from these locations within the University of Benin, Benin city, and the risk of microbial contamination.

6.2 RECOMMENDATION

It is recommended that more research be conducted in other parts of the University of Benin community using other pathogen, other types of foods and other services establishment to establish a comprehensive profile of microbial risk and safety of staff and students. It is highly recommended that the meat pies should always be heated up before eating and there should be adequate storage temperatures during storage or display within the safe temperature zone typically not below 5°C or above 60°C as fluctuations outside the safe temperature zone can allow bacteria to multiply rapidly. Raw ingredients should undergo further cooking to reach a safe internal temperature as contaminated raw meat, poultry, eggs or vegetables can introduce pathogenic bacteria like Salmonella, E. coli, and Staphylococcus aureus in to the final product. All work surfaces and cooking utensils must be properly washed and sterilized to avoid cross contamination of the food products. All workers must properly wash their hands regularly at intervals during the production chain and must be properly dressed with hair

covered and apron on to prevent fecal contamination as improper hand hygiene practices by food handlers can transfer harmful bacteria from hands to food surfaces or directly contaminate the filling during preparation. There is therefore the need for the government to mount educational campaigns among vendors regarding safe handling practices to prevent microbial contamination throughout the production chain to ensure a reduced microbial count.

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