DETECTION AND IDENTIFICATION OF MICROORGANISMS (FUNGI AND BACTERIA) IN RAW BEEF

BY

RHODA ENIBOKUN IDEHEN (MISS)
PG/LSC2216164

DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY
FACULTY OF LIFE SCIENCES
UNIVERSITY OF BENIN
BENINCITY

JUNE, 2025

DETECTION AND IDENTIFICATION OF MICROORGANISMS (FUNGI AND BACTERIA) IN RAW BEEF

BY

Rhoda Enibokun IDEHEN
PG/LSC2216164

THIS PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE, LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, FOR THE AWARD OF POSTGRADUATE DIPLOMA (PGD) DEGREE OF THE UNIVERSITY OF BENIN, BENIN CITY.

JUNE, 2025

CERTIFICATION

We, the undersign certify that this project work titled **Detection and Identification of Microorganisms (Fungi and Bacteria) in Raw Beef** was carried out by **Rhoda Enibokun Idehen** with **PG/LSC2216164**, to the Department of Science Laboratory Technology, Faculty of Life Science, University of Benin, for the award of Postgraduate Diploma (PGD) of the University of Benin, Benin City.

_____ _____

Prof. E.O. Oshomoh **Date**
Project Supervisor

_____ _____

Prof. J.O. Osarumwense **Date**
Project Coordinator

_____ _____

Prof. E.O. Oshomoh **Date**
Head of Department

_____ _____

External Examiner **Date**

DEDICATION

This seminar report is dedicated to God Almighty, who was, who is and is to come. This seminar report is also dedicated to my Mum Mrs. Fatty Idehen for her love, support and continuous prayers.

ACKNOWLEDGEMENT

I wish to sincerely appreciate God Almighty for the grace and wisdom to accomplish this great height. This acknowledgement will be incomplete if I fail to express my gratitude to the Head of Department/Project Supervisor Prof. E.O. Oshomoh for his guidance and support, also the entire staffs of Science Laboratory Technology. My greatest thanks goes to my mentor Prof. J.O. Ehiorobo for his fatherly support

Finally I want to specially appreciate all my lecturers who imparted new ideals and concept into my intellectual faculty. To my course mate whom we crossbread ideals during the course of studies. I say thank you all.

TABLE OF CONTENTS

ABSTRACT

This study evaluates the microbial contamination in raw meat across four various open market in benin city. Inadequate meat handling and contamination lead to spoilage, waste and reduced shelf life, which negatively affect both producers and vendors. Meat vendors often do not have access to refrigeration, and meat is displayed in unsanitary conditions, making it susceptible to microbial growth. The lack of cold chain infrastructure, where meat products are transported and stored at proper temperatures, exacerbates the situation. In Addition, foodborne illness outbreaks can result in costly hospitalizations, legal liabilities, and loss of consumer trust in the meat supply chain (Wagener *et al.,* 2020). In Benin City, improving microbial detection and control measures would not only enhance public health but also boost the economic sustainability of the local meat industry. As a result, meat often remains at temperatures favorable to bacterial growth for extended periods, leading to foodborne disease outbreaks (Bokhari *et al.,* 2021). In Benin City, as in many Nigerian cities, there is a notable lack of proper monitoring and regulation of food safety standards, making it difficult to address these issues effectively.

The slaughtering process often takes place in poorly regulated slaughterhouses where hygiene and sanitation practices are inadequate. According to a study by Akinmoladun *et al.,* (2020), many slaughterhouses in Edo State lack proper waste disposal systems, hygienic facilities, and pest control measures, which create an environment conducive to microbial growth. Slaughtered meat is often exposed to contamination from unclean surfaces, utensils, and equipment. Moreover, there is often a lack of adequate training in food safety practices for slaughterhouse workers, resulting in improper handling and processing of meat (Ajayi *et al.,* 2020).

The contamination of meat is not limited to slaughterhouses. In Benin City's open-air markets, where meat is sold directly to consumers, poor handling practices and improper storage conditions further contribute to microbial contamination. Meat vendors often do not have access to refrigeration, and meat is displayed in unsanitary conditions, making it susceptible to microbial growth. The lack of cold chain infrastructure, where meat products are transported and stored at proper temperatures, exacerbates the situation. As a result, meat often remains at temperatures favorable to bacterial growth for extended periods, leading to foodborne disease outbreaks (Bokhari *et al.,* 2021). In Benin City, as in many Nigerian cities, there is a notable lack of proper monitoring and regulation of food safety standards, making it difficult to address these issues effectively.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Microbial contamination in raw meat is a significant food safety concern, particularly in developing countries like Nigeria. In Benin City, the capital of Edo State, the situation is particularly critical due to the challenges related to slaughterhouse hygiene, meat handling practices, and the absence of effective food safety regulations. Raw meat is a key protein source in Nigeria, but its contamination with harmful microorganisms is widespread, leading to health risks for consumers, economic losses for producers, and significant public health concerns (Bokhari *et al.,* 2021).

In Benin City, as in other parts of Nigeria, meat is sourced primarily from cattle, goats, sheep, and poultry. However, due to a combination of inadequate infrastructure, poor hygiene, and informal practices, microbial contamination in raw meat is a persistent issue. Factors such as the conditions in which animals are slaughtered, processed, transported, and stored contribute significantly to the prevalence of microbial pathogens in meat products (Ajayi *et al.,* 2020). These microorganisms, including bacteria, fungi, and parasites, can cause foodborne illnesses, spoilage, and a reduction in the shelf life of meat products, posing significant risks to public health (Zhao *et al.,* 2020).

In Benin City, the slaughtering process often takes place in poorly regulated slaughterhouses where hygiene and sanitation practices are inadequate. According to a study by Akinmoladun *et al.,* (2020), many slaughterhouses in Edo State lack proper waste disposal systems, hygienic facilities, and pest control measures, which create an environment conducive to microbial growth. Slaughtered meat is often exposed to contamination from unclean surfaces, utensils, and equipment. Moreover, there is often a lack of adequate training in food safety

practices for slaughterhouse workers, resulting in improper handling and processing of meat (Ajayi *et al.,* 2020).

The contamination of meat is not limited to slaughterhouses. In Benin City's open-air markets, where meat is sold directly to consumers, poor handling practices and improper storage conditions further contribute to microbial contamination. Meat vendors often do not have access to refrigeration, and meat is displayed in unsanitary conditions, making it susceptible to microbial growth. The lack of cold chain infrastructure, where meat products are transported and stored at proper temperatures, exacerbates the situation. As a result, meat often remains at temperatures favorable to bacterial growth for extended periods, leading to foodborne disease outbreaks (Bokhari *et al.,* 2021). In Benin City, as in many Nigerian cities, there is a notable lack of proper monitoring and regulation of food safety standards, making it difficult to address these issues effectively.

Several microorganisms are commonly found in raw meat in Benin City, including *Salmonella*, *Escherichia coli* (E. coli), *Listeria monocytogenes*, *Campylobacter jejuni*, and *Clostridium perfringens* (Wagener *et al.,* 2020). These pathogens, if ingested, can cause severe gastrointestinal illnesses in humans, ranging from mild diarrhea to more severe conditions like kidney failure, particularly in the case of *E. coli* O157:H7 (Bokhari *et al.,* 2021). *Campylobacter* and *Salmonella*, which are prevalent in poultry and cattle, respectively, are major contributors to foodborne infections in Nigeria. Additionally, *Listeria monocytogenes*, which can grow at refrigeration temperatures, presents a significant challenge to meat safety, especially in ready-to-eat products (Beuchat *et al.,* 2020).

In Benin City, the prevalence of these pathogens in raw meat has been the subject of limited but concerning studies. A study by Akinmoladun *et al.,* (2020) found that meat samples from various markets in Benin City were frequently contaminated with *Salmonella*, *E. coli*, and

Staphylococcus aureus, a bacterium associated with foodborne illness. The study highlighted that poor sanitation in slaughterhouses, unclean handling practices by vendors, and inadequate storage facilities were key contributors to the high rates of contamination. Additionally, meat sold in open-air markets was found to have higher microbial loads compared to meat from more regulated retail outlets, further emphasizing the importance of improving hygiene practices at every stage of meat production and distribution (Akinmoladun *et al.,* 2020).

The detection and identification of these microorganisms in raw meat in Benin City is particularly challenging due to the lack of modern laboratory facilities and the absence of a robust food safety monitoring system. Traditional methods, such as microbial culturing and biochemical tests, are commonly used for pathogen detection in Nigeria but are often slow, labor-intensive, and not always capable of identifying all pathogens present in low concentrations. These methods also require well-equipped laboratories and trained personnel, which are often unavailable in rural and semi-urban areas like Benin City (Ranjbar *et al.,* 2020). As a result, there is a growing need for the adoption of more rapid, cost-effective, and efficient detection methods for microbial contamination in raw meat.

In the absence of advanced testing technologies, the role of proper hygiene and sanitation practices in reducing microbial contamination cannot be overemphasized. Improving slaughterhouse conditions, implementing effective cold storage and transport systems, and educating meat vendors on proper food handling practices are critical steps towards reducing the risk of microbial contamination in raw meat in Benin City. Additionally, stronger enforcement of existing food safety regulations by Nigerian authorities such as the National Agency for Food and Drug Administration and Control (NAFDAC) would be instrumental in improving meat safety (Ajayi *et al.,* 2020).

The economic impact of microbial contamination in raw meat is significant. Inadequate meat handling and contamination lead to spoilage, waste, and reduced shelf life, which negatively affect both producers and vendors. Additionally, foodborne illness outbreaks can result in costly hospitalizations, legal liabilities, and loss of consumer trust in the meat supply chain (Wagener *et al.,* 2020). In Benin City, improving microbial detection and control measures would not only enhance public health but also boost the economic sustainability of the local meat industry.

1.2 OBJECTIVES OF THE STUDY

The objectives of this study are to:

1. **Determine the prevalence of microbial contamination** in raw meat samples from various sources of local markets in Benin City, Edo State, Nigeria.
2. **Identify and categorize the types of microorganisms** (bacteria and fungi) commonly found in raw meat sold in Benin City, focusing on foodborne microorganisms such as bacteria and fungi.
3. **Investigate factors contributing to microbial contamination** in raw meat, including local market hygiene, meat handling practices, storage conditions, transportation, and market environments.
4. **Assess the public health risks** associated with microbial contamination of raw meat, examining the potential for foodborne illnesses and the economic impact on meat producers, vendors, and consumers.
5. **Explore the challenges faced by local meat vendors** in ensuring the microbial safety of their products, including limitations in resources, knowledge, and infrastructure for proper food safety practices.

6. **Propose feasible recommendations** for improving meat safety in Benin City, with a focus on enhancing hygiene practices, adopting rapid detection techniques, improving regulatory enforcement, and educating meat handlers and consumers on safe meat handling and storage.

1.3 STATEMENT OF THE PROBLEM

The contamination of raw meat with harmful microorganisms is a significant public health issue in Nigeria, particularly in urban centers such as Benin City, the capital of Edo State. Raw meat from cattle (beef), serves as a major protein source for many Nigerians. However, the safety of meat products has been compromised due to the widespread contamination by microorganisms, including bacteria and fungi. In Benin City, the situation is particularly worrisome due to poor slaughterhouse hygiene, improper meat handling, and inadequate storage conditions. These practices create an ideal environment for microbial growth, leading to the contamination of meat during slaughter, processing, transportation, and retail.

The problem of microbial contamination in raw meat is exacerbated by several critical factors:

1. **Inadequate Sanitation and Hygiene Practices**: In many local markets in Benin City, there are no strict regulations to ensure proper sanitation and hygiene during slaughtering and meat processing. Meat is often exposed to contamination from unclean surfaces, tools, and utensils used during meat handling and processing. Additionally, there is a lack of proper waste disposal systems, which increases the risk of cross-contamination (Akinmoladun *et al.,* 2020).
2. **Poor Storage and Transportation Facilities**: Raw meat is often transported and sold without proper refrigeration or temperature control. In Benin City, meat vendors in local markets often display meat in open-air stalls, where it is exposed to dust, flies,

and environmental contaminants. Without refrigeration or cold storage systems, meat becomes highly susceptible to bacterial growth, leading to spoilage and the proliferation of microorgnisms (Bokhari *et al.,* 2021). This is especially problematic during warm weather, as temperatures favorable for microbial growth are more prevalent.

3. **Lack of Routine Microbial Testing**: One of the major challenges in Benin City is the lack of an efficient system for microbial testing of raw meat. Despite the known risks associated with microbial contamination, there are limited facilities capable of conducting regular microbiological assessments of meat sold in the markets. The cost and complexity of laboratory testing, along with insufficient infrastructure and training, limit the ability of vendors and producers to identify and address microbial contamination in raw meat (Ajayi *et al.,* 2020). The absence of rapid detection methods further hinders efforts to ensure food safety at the point of sale and consumption.
4. **Limited Knowledge of Meat Safety Regulations**: While Nigeria has established food safety regulations through organizations such as the National Agency for Food and Drug Administration and Control (NAFDAC), enforcement of these standards is often weak, particularly in smaller cities and informal market systems. In Benin City, there is limited public awareness among meat vendors and consumers about the importance of microbial testing and safe meat handling practices. This lack of education results in improper handling, processing, and storage of meat, further increasing the likelihood of contamination (Ajayi *et al.,* 2020).
5. **Economic Implications**: The economic impact of microbial contamination is significant in Benin City's meat industry. The spoilage and contamination of raw meat lead to high levels of waste and loss of product quality, which negatively

impacts meat producers and vendors. The costs associated with hospitalizations due to foodborne illness outbreaks also strain the healthcare system and undermine consumer confidence in local meat markets. Furthermore, the inability to guarantee the microbiological safety of meat can limit market opportunities, both domestically and internationally, for meat producers in Benin City (Bokhari *et al.,* 2021).

1.4 AIM OF THE STUDY

The primary aim of this study is to investigate the detection and identification of microorganisms in raw meat in Benin City, Edo State, Nigeria, with a focus on understanding the extent of microbial contamination, identification, and evaluating the effectiveness of current detection methods. The study aims to:

1. **Assess the prevalence and types of microorganisms** (including bacteria and fungi) commonly found in raw beef sold in open markets in Benin City.
2. **Evaluate existing microbial detection techniques** used in raw meat in Benin City, assessing their sensitivity, specificity, and effectiveness in identifying harmful microorganisms responsible for foodborne illnesses and spoilage.
3. **Identify the major sources of contamination** and factors contributing to microbial presence in raw beef in open market hygiene, meat handling, storage, transportation, and market conditions.
4. **Compare current microbial contamination levels** in raw meat from various sources, such as informal market vendors.

1.5 SIGNIFICANCE OF THE STUDY

The significance of this study on the detection and identification of microorganisms in raw meat in Benin City, Edo State, Nigeria lies in its potential to make meaningful contributions

to public health, food safety, the local economy, and regulatory practices. The findings of this study will be pivotal for various stakeholders, including public health authorities, meat vendors, consumers, and policymakers.

1. **Enhancing Public Health and Safety:** One of the most important aspects of this study is its potential to reduce the incidence of foodborne diseases in Benin City. By identifying the types of microorganisms contaminating raw meat, the study will provide critical data that can be used to formulate targeted interventions aimed at preventing foodborne illnesses.
2. **Improvement of Food Safety Practices:** This research will provide valuable insights into the current meat handling, processing, and storage practices in Benin City, highlighting areas where improvements are needed. By identifying the major sources of microbial contamination and recommending appropriate control measures, this study will guide local vendors, meat producers in adopting safer and more hygienic practices.
3. **Economic Benefits to Local Meat Industry:** The findings of this study can help reduce losses due to meat spoilage, contamination, and waste. By promoting safer handling and storage practices, the study will enhance the shelf life of raw meat, thereby reducing economic losses for meat vendors and producers. Additionally, reducing the occurrence of foodborne illnesses will minimize healthcare costs associated with treating foodborne diseases.
4. **Strengthening Regulatory Measures:** This study will provide evidence-based data to inform regulatory bodies such as NAFDAC (National Agency for Food and Drug Administration and Control) and the Federal Ministry of Health on the state of microbial contamination in raw meat in Benin City. It will highlight gaps in the current food safety regulatory framework and offer recommendations for

strengthening enforcement mechanisms. Additionally, the study can help improve the implementation of existing food safety standards and encourage more consistent monitoring and control of microbial contamination in raw meat at both local and national levels.

5. **Contribution to Scientific Knowledge:** The study will contribute to the body of knowledge on microbial contamination in raw meat, particularly in Benin city, Nigeria. While studies have been conducted in other regions of the country, there is a lack of comprehensive research on this topic in Benin City. By documenting the types and prevalence of microorganisms present in raw meat sold in the city, the study will fill a critical research gap. Furthermore, the evaluation of microbial detection methods will add to the scientific understanding of the challenges and innovations in food safety testing. This can be useful for future studies, policy development, and the application of new technologies in food safety.
6. **Education and Public Awareness:** By identifying the key challenges in meat safety and sharing findings with local vendors and the public, this study will increase awareness of the importance of microbial contamination in raw meat. Public health campaigns and educational programs can be developed based on the study's results, guiding both meat vendors and consumers in adopting safe food handling practices.

1.6 SCOPE OF THE STUDY

The scope of this study on the detection and identification of microorganisms in raw meat in Benin City, Edo State, Nigeria encompasses the following key areas:

1. **Geographical Scope:** The study will focus on Benin City, the capital city of Edo State, Nigeria. Benin City is a major urban center with a diverse population and a significant market for meat products. This geographical scope is critical as it allows

for an in-depth examination of the meat supply chain in an urban environment, which has unique challenges in terms of hygiene, regulation, and food safety. The study will consider raw meat from four various local markets in the city.

2. **Microbial Contaminants:** The study will investigate the types of microorganisms that are commonly found in raw meat, including bacteria and fungi. The primary focus will be on bacterial and fungi, although bacterial contamination is expected to be the most common. The study will focus on identifying microorganisms that pose a significant public health risk.
3. **Meat Type:** The study will examine a range of raw beef available in Benin City .Beef is a type of meat commonly consumed by the local population and may have varying contamination levels and microbial profiles. The study will compare microbial contamination across different types of raw meat to identify any significant differences in contamination levels or types of microorganisms present.
4. **Sampling Locations:** Samples will be collected from various sources within Benin City, including four local markets.
    - Uselu market
    - Oba market
    - Ekae market
    - New benin market
5. This wide range of sample sources will provide a comprehensive view of microbial contamination levels in different parts of the meat supply chain.
6. **Microbial Detection Methods:** The study will employ a combination of traditional microbiological techniques to detect and identify microbial contamination in raw meat. These methods will be compared to evaluate their effectiveness in detecting harmful

to understand the advantages and limitations of each approach in the context of meat safety in Benin City.

7. **Factors Affecting Microbial Contamination:** The study will also explore factors that influence microbial contamination, including:
    - **Market hygiene** and meat processing practices.
    - **Handling and transportation** practices in the meat supply chain.
    - **Storage conditions** (temperature, humidity) at various stages of the meat distribution process.
    - **Environmental factors** such as sanitation and cleanliness of market.
8. **Public Health Risk Assessment:** The study will assess the public health implications of microbial contamination in raw meat, considering the potential risks of foodborne diseases to consumers. This includes identifying the most common microorganism and estimating the risk they pose in terms of morbidity and mortality associated with meat consumption.

1.7 LIMITATIONS OF THE STUDY

Limitations: The study will be limited to examining a selected number of microorganisms and may not cover all possible microorganisms found in raw meat. Additionally, logistical constraints may limit the sample size and geographic coverage of the study

Despite the significance and potential impact of this study, several limitations may affect its execution and the interpretation of results. These limitations are important to acknowledge as they provide context for the findings and suggest areas for future research. The limitations of this study include:

1. **Geographical Limitation:** The study is focused on only four major local market in **Benin City**, Edo State, Nigeria, and may not be representative of microbial contamination in raw meat across other regions of Nigeria or Africa. The findings may be specific to this urban area, and generalizing the results to other locations with different environmental conditions, hygiene practices, or market structures should be done with caution.
2. **Sampling Constraints:** While the study will sample meat from various sources within Benin City, it is not possible to capture every possible source of microbial contamination. The sample size, while large enough to provide meaningful data, may still be limited in scope, particularly with regard to seasonal variations or changes in the meat supply chain. Additionally, due to logistical constraints, it may not be feasible to sample meat from all markets in the city.
3. **Microbial Detection Methods:** such as microscopic viewing, pour plate methods (fungi and bacteria), oxidase tests, catalase test and many others.
4. **Financial and Time Constraints:** Given the scope and objectives of the study, there are limitations in terms of the financial resources and time available for sample collection, analysis, and result dissemination. Although the study will aim for a comprehensive approach, the availability of funds and time may restrict the number of samples collected, the breadth of microbial analysis, and the extent of data interpretation.

CHAPTER TWO

LITERATURE REVIEW

Understanding microbial contamination in raw meat is essential for ensuring food safety and public health. Raw meat serves as a potential reservoir for various pathogenic microorganisms such as *Salmonella spp.*, *Escherichia coli*, *Listeria monocytogenes*, and *Campylobacter spp.* which can cause severe foodborne illnesses if consumed (Mead *et al.,* 1999; Sofos, 2008). Knowledge of microbial contamination helps in developing effective strategies for meat handling, storage, and processing to prevent cross-contamination and microbial growth. Proper refrigeration, hygienic slaughter practices, and thorough cooking are crucial in minimizing microbial risks (WHO, 2020). Furthermore, understanding microbial ecology contributes to enhancing shelf life through improved preservation methods like modified atmosphere packaging (Davies and Board, 1998). For food producers, this knowledge ensures compliance with food safety regulations and quality standards, thereby reducing economic losses due to product recalls or legal liabilities. Consumer education also plays a vital role, as informed consumers are more likely to adopt safe food handling and preparation practices at home (Redmond and Griffith, 2003). Overall, a comprehensive understanding of microbial contamination in raw meat supports a safer food supply chain from farm to fork.

2.1 UNDERSTANDING MICROBIAL CONTAMINATION IN RAW MEAT

This is crucial for the following reasons:

1. **Food Safety**: Raw meat can harbor harmful microorganisms like *Salmonella*, *E. coli*, and *Listeria*, which can cause foodborne illnesses. Understanding contamination helps prevent outbreaks and protect public health.
2. **Proper Handling and Storage**: Knowing how microbes grow and spread informs better practices in handling, storing, and preparing meat to minimize risks.

3. **Shelf Life Extension**: Awareness of microbial activity can lead to improved preservation techniques, such as vacuum packaging and refrigeration, to extend meat's shelf life.
4. **Regulatory Compliance**: Food producers and sellers must meet safety standards. Understanding contamination helps them stay compliant with health regulations and avoid legal issues.
5. **Consumer Awareness**: Educating consumers on microbial risks encourages safer food preparation practices at home, such as cooking meat thoroughly and avoiding cross-contamination.

Equally important is the detection and identification of microorganisms, which play a crucial role in food safety and public health. Early and accurate detection methods help in identifying contamination sources, preventing the distribution of unsafe meat products, and enabling timely interventions. Technologies such as polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), and next-generation sequencing (NGS) have significantly enhanced the speed and precision of pathogen identification (Law *et al.,* 2015). These diagnostic tools are essential for monitoring hygiene standards in food processing environments and for conducting epidemiological investigations during foodborne outbreaks (Havelaar *et al.,* 2010). For instance, whole genome sequencing (WGS) has been employed effectively in tracing *Listeria monocytogenes* outbreaks, allowing authorities to implement focused recalls and prevent further illness (Jackson *et al.,* 2016). The ability to detect and characterize specific microbial strains also supports traceability, risk assessment, and the development of targeted control measures, thereby ensuring a safer food supply chain.

For food producers and processors, this knowledge ensures adherence to stringent food safety regulations and quality assurance protocols, helping avoid costly recalls and legal

ramifications. Consumer education is equally important, as awareness of microbial risks empowers individuals to practice safe food handling and preparation at home (Redmond and Griffith, 2003). In essence, understanding microbial contamination in raw meat is a fundamental component in achieving a robust and secure food supply chain from farm to fork.

2.2 IMPACT OF MICROBIAL CONTAMINATION ON HUMAN HEALTH AND THE FOOD INDUSTRY

1. Foodborne Illnesses: Microbial contamination is a leading cause of foodborne diseases worldwide. Pathogens such as *Salmonella*, *E. coli O157:H7*, *Listeria monocytogenes*, and *Campylobacter* are commonly associated with raw meat. These bacteria can cause:

Gastrointestinal infections (vomiting, diarrhea, cramps)

Severe complications like kidney failure (HUS), septicemia, meningitis, or miscarriage

Higher risk for vulnerable groups such as children, the elderly, pregnant women, and immunocompromised individuals

2. Food Spoilage and Shelf Life: Spoilage microorganisms such as *Pseudomonas spp.*, *Brochothrix thermosphacta*, and *lactic acid bacteria* lead to:

Visible spoilage: discoloration, slime, off-odors

Reduced shelf life due to microbial activity

Loss of meat texture and taste, making it unfit for consumption

Increased food waste, which is both economically and environmentally costly

3. Economic Implications for Producers and Vendors: Microbial contamination has serious financial consequences:

Product Recalls: Expensive for companies and damaging to brand reputation

Regulatory Penalties: Non-compliance with food safety standards can lead to legal action or fines

Market Rejection: Export bans or rejected shipments due to contamination

Consumer Confidence: Decline in consumer trust can reduce demand

Increased Costs: More spending on quality control, testing, and improved packaging or refrigeration

2.3 METHODS OF MICROBIAL DETECTION AND IDENTIFICATION IN RAW MEAT

Accurate detection and identification of microorganisms in raw meat are crucial for ensuring food safety, preventing spoilage, and complying with regulatory standards. These methods fall into conventional, biochemical, and modern molecular techniques:

Culture-Based Methods

Plate Count Techniques: Involves culturing samples on nutrient agar to estimate the total viable count (TVC).

Selective Media: Specific pathogens are isolated using media that promote growth of target organisms while inhibiting others (e.g., *MacConkey agar* for Enterobacteriaceae).

Limitations: Time-consuming (24–72 hours), and not all microorganisms are culturable.

Microscopic Examination

Staining Techniques: Gram staining helps differentiate Gram-positive and Gram-negative bacteria.

Morphological Identification: Shape, size, and arrangement under the microscope give preliminary identification clues.

Biochemical Tests

Catalase, Oxidase, Urease Tests: Identify enzymatic activities of bacteria.

Limitations: Require experienced personnel and incubation times.

Global Comparison of Studies on Microbial Contamination in Raw Meat

1. Prevalence of Pathogens

Hygiene Practices and Regional Variations

Hygiene and Processing Practices:

The contamination rates reflect differences in hygiene practices and meat processing conditions. For example, in South Africa, raw beef contamination rates are relatively high, which may be attributed to poor handli

2.4 MICROBIAL CONTAMINATION IN RAW MEAT: AFRICA, NIGERIA, AND EDO STATE

1. Africa (General Overview)

Across Africa, microbial contamination of raw meat is a widespread issue due to several factors including inadequate food safety regulations, lack of infrastructure, poor hygiene practices, and improper meat handling

Prevalence

South Africa: A study by Okoh et al. (2019) reported **45%** of raw beef samples contaminated with *E. coli* and **25%** with *Salmonella*.

Ethiopia: Meat samples from Addis Ababa showed contamination with *S. aureus* (47.5%) and *E. coli* (35%) (Mekonen *et al.,* 2019).

Ghana: Adu-Gyamfi et al. (2021) found *Listeria monocytogenes* in **23%** on beef samples sold in Accra markets.

2. Nigeria (National Level)

In Nigeria, microbial contamination of meat is common in both urban and rural markets, often linked to open-air slaughtering, lack of cold chains, and exposure to flies and dust.

Common Microorganisms:

- *E. coli*
- *Salmonella spp.*
- *Staphylococcus aureus*
- *Pseudomonas spp.*
- *Bacillus spp.*

Prevalence:

Abuja: Oranusi et al. (2017) found *Salmonella* and *E. coli* in 30–35% of raw beef samples sold in open markets.

Lagos: Olatoye and Arinde (2018) reported *S. aureus* in 52.5% and *Salmonella* in 40% of raw meat samples from local markets.

Ibadan: Adebayo-Tayo et al. (2019) found *Listeria monocytogenes* in 22% of beef and poultry samples.

3. Edo State (Regional Focus): Few but significant studies have been conducted in Edo State, especially in Benin City and surrounding towns. These studies show that local slaughter slabs and market handling conditions contribute significantly to contamination.

Common Microorganisms:

- *E. coli*
- *Salmonella spp.*

- *S. aureus*
- *Proteus spp.*
- *Klebsiella spp.*

Prevalence:

Benin City: Eniola et al. (2020) reported that 34% of raw beef samples were positive for *E. coli*, and 28% for *Staphylococcus aureus*. The study linked contamination to water used in processing and inadequate refrigeration.

Ekpoma: A local study by Ehigiator et al. (2021) identified *Salmonella* in 18% and *Klebsiella* in 21% of meat samples.

Auchi: Research by Uwadiae et al. (2022) found that meat sold in open markets had *Proteus* and *S. aureus* contamination levels ranging from 20% to 40%.

2.4 FACTORS AFFECTING MICROBIAL CONTAMINATION AND IDENTIFICATION IN RAW MEAT

Factors Affecting Microbial Contamination in Raw Meat

1. Slaughtering and Processing Practices

Poor hygienic practices at slaughterhouses (e.g., dirty surfaces, tools, or hands) significantly contribute to contamination.

Cross-contamination between clean and dirty zones, and between organs and carcasses, is common.

Use of contaminated water during processing (washing carcasses, cleaning equipment) introduces or spreads microbes.

2. Environmental and Handling Conditions

- Ambient temperatures in open-air markets favor bacterial growth.
- Exposure to dust, flies, and rodents increases the risk of contamination.
- Poor personal hygiene among meat handlers further worsens contamination levels.

3. Storage and Transportation

- Lack of refrigeration during meat storage and transport allows microbial proliferation.
- Extended storage time increases bacterial load, especially in tropical climates.

4. Meat Type and Animal Health

Red meat (beef, lamb) tends to carry more surface contamination due to larger carcasses and handling.

Factors Affecting Identification of Microorganisms in Raw Meat

1. Detection Method Used

Conventional methods (culture, biochemical tests) are inexpensive but slower and less

Slaughter and Processing Hygiene in Relation to Microbial Contamination in Raw Meat

Slaughter and processing hygiene refers to the sanitary conditions and practices followed during the slaughtering of animals and the processing of meat. Poor hygiene at this stage is a major contributor to microbial contamination, which directly affects meat safety, shelf life, and public health.

Hygiene Factors Affecting Contamination

Sanitation of Slaughter Facilities: Unclean floors, tables, tools, and cutting boards can harbor and spread pathogens like *E. coli*, *Salmonella*, and *Listeria*.

Inadequate waste disposal and poor drainage encourage the proliferation of flies and rodents.

1. **Personal Hygiene of Workers**
    - Handlers with unwashed hands, dirty clothing, or infected wounds can introduce *Staphylococcus aureus* and other microbes.
    - Lack of hand washing stations and gloves increases contamination risk.
2. **Cross-Contamination Practices**
    - Using the same knives and surfaces for different animal parts without cleaning promotes cross-contamination.
    - Contamination of carcasses with intestinal contents during evisceration is common, especially if the animal is improperly stunned or handled.
3. **Water Quality**
    - Contaminated water used for washing carcasses or equipment introduces bacteria.
    - Re-use of dirty water further increases microbial load.
4. **Air and Environmental Exposure**
    - Open-air slaughter exposes meat to dust, insects, and airborne bacteria.
    - Absence of protective coverings or enclosures allows pathogens to settle on exposed surfaces.
5. **Inadequate Cooling/Storage**
    - Failure to rapidly cool meat after slaughter allows bacteria to multiply.
    - Poor cold-chain systems are especially problematic in tropical regions.

Evidence from Research

Found that carcasses processed in Nigerian open-air markets had significantly higher microbial counts than those from controlled abattoirs.

High prevalence of *S. aureus* and *E. coli* in Benin City meat samples due to improper dressing, washing with contaminated water, and lack of refrigeration.

WHO (2015) emphasized that many foodborne outbreaks globally are linked to contaminated meat resulting from unhygienic slaughter and processing conditions.

2.5 RECOMMENDATIONS FOR IMPROVEMENT

- Implement Hazard Analysis Critical Control Point (HACCP) systems at abattoirs.
- Train workers in basic food safety and hygiene.
- Ensure clean water supply and sanitary tools/equipment.
- Upgrade facilities to allow closed-environment processing and proper refrigeration.

Temperature and Storage

Temperature and storage conditions are critical in determining the microbial safety and shelf life of raw meat. Improper temperature control promotes rapid microbial growth, increasing the risk of foodborne illnesses, spoilage, and economic loss.

1. Temperature Control

Optimal Storage Temperatures

- Chilling (0–4°C): Slows microbial growth but doesn't eliminate pathogens.
- Freezing (-18°C or lower): Halts microbial activity but may not kill all microorganisms (especially spores and viruses).
- Room temperature (25–35°C): Ideal for rapid bacterial growth, especially in tropical climates.

Impacts of Poor Temperature Control

- At ambient temperatures, bacteria like *E. coli*, *Salmonella*, and *Listeria monocytogenes* can double in number every 20–30 minutes.

- Studies show that raw meat kept above 5°C for over 2 hours develops significantly higher microbial loads.

2. Storage Conditions

Cold Chain Integrity

- Maintaining a continuous cold chain from slaughter to sale is essential. Interruption at any point (e.g., power outages, delays in transport) can allow microbial proliferation.

Packaging

- Vacuum packaging and modified atmosphere packaging (MAP) slow down aerobic spoilage bacteria but may still allow anaerobic pathogens to persist.
- Uncovered or loosely wrapped meat, especially in open-air markets, is vulnerable to contamination from dust, insects, and human contact.

Storage Duration

- Even under refrigeration, extended storage (beyond 3–5 days) increases the risk of spoilage and the growth of psychrotrophic bacteria like *Pseudomonas spp.*

Handling and Transportation:

Handling and transportation practices are critical control points in the meat supply chain. Inadequate hygiene, temperature abuse, and improper packaging during these stages can significantly increase microbial contamination and compromise meat safety and quality.

1. Meat Handling Practices

Poor Hygiene by Handlers

- Unwashed hands, dirty clothing, and use of contaminated tools lead to the introduction and spread of pathogens such as *Staphylococcus aureus*, *Salmonella*, and *E. coli*.
- Lack of Personal Protective Equipment (PPE) like gloves or aprons increases the chance of direct contamination.

Study Insight: Eniola *et al.,* (2020) reported high levels of *E. coli* and *S. aureus* on meat handled in open markets in Benin City due to direct hand contact and lack of sanitation.

Cross-contamination

- Using the same cutting boards and knives for multiple carcasses without cleaning between uses spreads bacteria.
- Meat often comes into contact with contaminated surfaces, dirty market stalls, or wooden tables that absorb blood and fluids, fostering bacterial growth.

2. Transportation Conditions

Lack of Refrigeration during Transport

- In many regions, especially rural and urban markets in Nigeria, raw meat is transported without cold storage, often in open vans, on motorbikes, or in baskets.
- Exposure to high ambient temperatures (often exceeding 30°C) allows rapid microbial multiplication.

Case Study: Uwadiae and Osamudiamen (2022) observed that meat samples transported from rural slaughterhouses to Auchi town had bacterial loads up to 10^6 CFU/g within 2 hours of transit without refrigeration.

Packaging and Exposure

- Meat transported without proper wrapping or packaging is exposed to dust, flies, and pollutants, which introduce spoilage organisms like *Pseudomonas spp.* and *Proteus spp.*

2.6 ENVIRONMENTAL SANITATION: ROLE IN MICROBIAL CONTAMINATION OF RAW MEAT

Environmental sanitation refers to the cleanliness of the physical surroundings where meat is slaughtered, processed, handled, and sold. Poor environmental conditions can significantly contribute to microbial contamination, posing serious risks to public health and reducing the quality and shelf life of meat.

1. Cleanliness of Slaughterhouses and Meat Markets

- Dirty floors, walls, and surfaces harbor pathogenic and spoilage microorganisms such as *Salmonella spp.*, *E. coli*, and *Listeria monocytogenes*.
- Blood, waste, and animal feces left uncleaned create a breeding ground for bacteria, flies, and rodents.

2. Waste Management Practices

- Improper disposal of animal waste (blood, bones, entrails) attracts vectors like flies and vermin.
- Wastewater pooling around slaughter or processing areas can contaminate meat directly or indirectly via tools and worker footwear.

4. Air Quality and Dust

- In open markets, dust particles can settle on exposed meat, carrying airborne pathogens.

- Lack of proper enclosures or protective barriers increases risk, especially in dusty, windy environments.

5. Water Source and Drainage

- Contaminated or untreated water used for washing carcasses or cleaning surfaces can introduce bacteria.
- Poorly designed drainage systems can lead to backflow of contaminated water into clean zones.

Supporting Evidence: Adu-Gyamfi et al. (2021) emphasized the role of unhygienic water and improper drainage as key contributors to microbial contamination in meat processing areas in Ghana and parts of West Africa.

Recommendations

- Maintain routine cleaning and disinfection of slaughter and processing areas.
- Install effective drainage and waste disposal systems.
- Use screened enclosures to reduce fly and rodent access.
- Provide access to clean, potable water for all stages of meat processing.

2.7 CONTROL AND PREVENTION MEASURES

Control and Prevention Measures for Microbial Contamination in Raw Meat

Controlling microbial contamination in raw meat requires a multi-point strategy spanning from animal slaughter to final retail. Effective prevention reduces foodborne illnesses, improves meat shelf life, and ensures consumer safety.

1. Good Hygienic Practices (GHP)

Description: Basic sanitation and hygiene protocols at every stage of meat production.

Measures:

- Regular cleaning and disinfection of equipment a nd surfaces.
- Personal hygiene training and use of protective clothing by workers.
- Preventing cross-contamination through tool separation and sanitation.

2. Good Manufacturing Practices (GMP)

Description: Systematic approach to meat processing ensuring controlled conditions.

Measures:

- Standard Operating Procedures (SOPs) for handling, cutting, and packaging meat.
- Adequate facility layout to separate clean and dirty zones.
- Maintenance of clean water supply and pest control systems.

5. Inspection and Surveillance

Description: Governmental or third-party checks to ensure compliance with safety regulations.

Measures:

- Regular microbial testing of meat and surfaces.
- Certification of abattoirs and retail outlets.
- Enforcement of penalties for non-compliance.

6. Public and Worker Education

Description: Awareness campaigns to encourage food safety culture.

Measures:

- Community training on safe meat handling and cooking.
- Capacity-building programs for butchers and vendors.
- Labeling and consumer information on meat safety.

7. Proper Packaging and Labeling

Description: Protection from contamination during storage and sale.

Measures:

- Use of vacuum-sealing or Modified Atmosphere Packaging (MAP).
- Clear labeling with production and expiry dates.
- Tamper-proof and hygienic containers for transport and retail.

1. Hygiene During Slaughtering

- Healthy animals should be selected for slaughter to minimize the introduction of zoonotic pathogens.
- Slaughter should be done in sanitary, controlled environments (e.g., approved abattoirs).
- Tools and surfaces must be disinfected regularly to avoid cross-contamination.
- Prevent spillage of intestinal content, which can introduce E. coli, Salmonella, and Campylobacter.

2. Personal Hygiene of Workers

- Meat handlers must wash hands with soap before and after handling meat.

- **Use of clean protective gear**: aprons, gloves, masks, and hairnets.
- Avoid eating, smoking, or touching the face during meat handling.
- Sick workers should not be allowed to handle meat.

3. Clean Equipment and Surfaces

- Knives, boards, and other utensils must be sanitized before and after use.
- Use non-porous, easy-to-clean materials for tables and equipment.
- Routine cleaning schedules should be in place using approved disinfectants.

4. Safe **Water Use**

- Use clean, potable water for washing carcasses and cleaning surfaces.
- Change water regularly to prevent it from becoming a contamination source.

5. Environmental Cleanliness

- Maintain clean slaughter and processing environments (walls, floors, drains).
- Keep the area free of pests, dust, and waste.
- Properly manage animal by-products and blood to avoid attraction of flies and rodents.

6. Proper Meat Handling

- Avoid direct hand contact with meat as much as possible.
- Use clean containers for transporting meat, and avoid stacking uncovered meat.
- Separate raw meat from cooked or ready-to-eat items.

7. Consumer Education

- Teach consumers about safe handling, cooking, and storage of raw meat at home.
- Encourage thorough cooking of meat to kill pathogens.

CHAPTER THREE

MATERIALS AND METHOD

3.1 MICROBIAL LOAD ANALYSIS OF RAW BEEF SAMPLES

Sample collection

Beef samples were collected from four different markets at New Benin, Uselu, Ekae and Oba Market, Benin City, Edo State. Each sample (approximately 50g) was aseptically placed in sterile polyethylene bags, labeled and transported to the laboratory for microbiological analysis within 2 hours of collection.

Preparation of Media

Nutrient Agar (NA): Used for the isolation and enumeration of bacteria. Prepared according to manufacturer's instructions. Sterilized by autoclaving at 121^{o}C for 15 minutes.

Sabourand Dextrose Agar (SDA): Employed for fungal isolation, the medium was supplemented with ampicillin (50mg/L) to inhibit bacterial growth. Sterilized similarly and poured into sterile Petri dishes under aseptic condition.

Sample Processing and Inoculation

2g of each beef samples were aseptically homogenized in 9mL of sterile distilled water to form a 1:10 dilution. Serial dilutions up to 10^{-5} were prepared. One milliliter (1mL) from each dilution was inoculated onto:

Nutrient Agar plates for bacterial isolation

Sabouraud Dextrose Agar plates for fungal isolation

The plates were incubated as follows

- NA plates: 30^{o}C for 24 – 48 hours
- SDA plates: 25^{o}C for 3 – 5 days
- Colony-Forming Units (CFU): were counted and expressed as CFU/g of beef sample
- Microscopic identification

Bacteria:

Gram Staining: A thin smear from isolated colonies was heat-fixed onto a glass slide, stained using the standard gram staining procedure:

- ⇨ Crystal violet (orunart staun) – 1 minute
- ⇨ Iodine (Mordant) – 1 minute
- ⇨ Alcohol (decolorizer) – 15 – 30 seconds
- ⇨ Safraining (counterstain) – 1 minute

Slides were examined under oil immersion (100 x objective) using a light microscope. Bacteria were classified as Gram-positive (purple) or Gram-negative (pink/red) based on cell wall compassion (Prescott *et al.,* 2022)

FUNGI:

Lactophenol Cotton Blue (LPCB) staining: Fungal colonies were mounted on slides with a drop of Lactophenol Cotton Blue stain. Coverslips were placed gently to avoid trapping air bubbles. Slides were examined under a light microscope to observe spore structure, hyphae and conidiophores for identification (Alexopoulos *et al.,* 2021)

3.2 MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF BACTERIA

Gram Stain

The isolates were prepared as thin smears on glass slides using a wire loop, and subsequently heat-fixed and allowed to cool. To stain the smears, crystal violet stain was applied for one minute, followed by immediate washing with potable water. Lugol's iodine was then added to the smears for the duration of 30 to 60 seconds, after which it was promptly washed off with water. Acetone or alcohol was used for rapid decolorization of the smears, followed by a swift rinse with clean water after 5 seconds. Subsequently, the smears were stained with safranine for the duration of 60 seconds and immediately washed off. Once stained, the

smears were left to air-dry, and a few drops of oil immersion were added. Finally, the stained smears were observed under an optical microscope using the 100x objective lens. Gram-positive organisms appeared as purple cells, while Gram-negative organisms were visualized as pink or red cells.

Biochemical Tests

The purpose of these tests was to assess the enzymatic capabilities of the bacterial isolates, specifically their ability to produce catalase, oxidase, and urease enzymes. Additionally, various biochemical tests were performed to evaluate the bacteria's capacity to utilize different sugar or substrate sources.

Catalase (Hydrogen peroxide; H_2O_2) Test

The biochemical test was conducted to evaluate the presence of the catalase enzyme. Catalase is an enzyme that facilitates the release of oxygen from hydrogen peroxide, leading to the production of bubbles or effervescence. This enzymatic reaction plays a crucial role in breaking down harmful hydrogen peroxide into harmless water and oxygen. As catalase is produced by all aerobic organisms, this test proves valuable in distinguishing between aerobic and anaerobic organisms.

Methodology: A grease-free slide is prepared, and a drop of 3% hydrogen peroxide (H2O2) is placed on it. A loopful of the bacterial isolate is then added to the hydrogen peroxide. The presence of catalase is indicated by the occurrence of effervescence, while the absence of effervescence suggests the absence of the enzyme.

Oxidase Test

The biochemical test aims to determine the presence of the cytochrome-c-oxidase enzyme, which acts as an artificial electron acceptor capable of reducing oxygen. This test is employed to detect the presence of this specific enzyme in bacteria. It involves assessing whether the bacteria possess certain oxidases necessary for the transfer of electrons between

the redox dye, tetramethyl-p-phenylene-diamine, and the electron donors within the bacterial cells.

Procedure: A Whatman filter paper is saturated with a 1% solution of tetramethlyphenylene diamine hydrochloride. A culture of the test isolate(s), grown for 24 hours, is then smeared onto the filter paper impregnated with the solution. The development of a purple color on the filter paper indicates a positive result, signifying the presence of the cytochrome-c-oxidase enzyme.

Test for Urea Hydrolysis (Urease Test)

This test was conducted to determine the ability of certain bacteria to produce an alkaline product, namely ammonia, by hydrolyzing urea through the action of the enzyme urease.

Procedure: Urea was incorporated into a urease agar base, which was then inoculated with the test organism on a slant. The inoculated agar was incubated at the optimal temperature of 37°C for a period of 24 to 48 hours. A positive result was indicated by the development of a vibrant pink or red color, while a negative result showed no color change.

Coagulase Test:

Slide coagulase Test: A smear of the isolates was made on the a slide and a drop of pooled plasma was added with the aid of a pasteur pipette and rocked, negative control was done without the organism observed for about 20seconds, the presence of clumps is indicative of positive slide coagulase test.

Citrate Utilization test (Simmons Citrate Agar (SCA) Slant)

SCA (Simmons Citrate Agar) slants were employed for this biochemical test. The objective of this test was to assess the bacteriums ability to utilize citrate as its sole carbon source.

Procedure: The medium was prepared in the form of slants using test tubes. The bacterial isolate under investigation were inoculated into agar slant and incubate for 24 hours in an incubator. A positive reaction, indicating the utilization of citrate, was indicated by the

development of a blue color. In contrast, a negative reaction was observed if there was no color change or if the medium retained its original green

3.3 SAMPLING TECHNIQUES

A stratified random sampling technique will be used to ensure adequate representation across meat types and sources. The stratification will be based on:

- **Type of raw meat** (beef)
- **Point of sale** (four open markets)

Within each stratum, simple random sampling will be used to select individual meat vendors or stalls. This minimizes selection bias and ensures that the samples reflect typical microbial conditions in Benin City's meat distribution chain.

3.4 METHODS OF DATA COLLECTION

1. Sample Collection

- Meat samples (raw beef) were aseptically collected using sterile gloves, scalpel blades, and sample bags.
- Approximately 10g of each raw meat sample will be cut from the surface and deep tissues, placed in sterile polyethylene bags, labeled, and transported in an icebox at 4°C to the microbiology laboratory for analysis within 2 hours of collection.
- Each sample was documented by:
    - Date and time of collection
    - Location (market)
    - Meat type
    - Observations on storage and hygiene conditions

2. Microbiological Analysis

Standard microbiological procedures will be used to isolate and identify microorganisms in raw meat samples, including:

- Pre-enrichment and enrichment sterile water and selective broths
- Culture plating on selective media such as:
    - Nutrient Agar (for bacteria)
    - Sabouraud Dextrose Agar (for fungi)

Nutrient agar

15ml agar for each plate (40p)

$$300 \times 65 = \frac{600\text{ml dro} \times 28}{100} = 16.8\text{g}$$

Sabouraud Dextrose agar

15ml agar for each plate (20p)

$$\frac{19{,}500 \times 19.5\text{g sda}}{300}$$

Sabouraud Dextrose Agar (SDA)

Nutrient Age (NA)

$15 \times 40 = 600\text{ml} \times 28.0\text{g} = 16.8\text{g}$

$$20 \times 15\text{ml of agar} = \frac{300 \times 65\text{g}}{1000} = 19.5\text{g}$$

Incubation at appropriate temperatures (35–37°C for bacteria, 25°C for fungi) for 24–48 hours

Identification using:

- Colony morphology
- Gram staining
- Biochemical tests (e.g., catalase, coagulase, oxidase)

CHAPTER FOUR

RESULTS

4.1 RESULTS

This chapter covers the morphology of microorganism the microbial (bachertia & fungi) analysis and biochemical tests form the all the beef collected from the four open markets. Uselu Market, Oba Market, Ekae market and New Benin Market respectively

Table 4.1: Bacteria morphology

The Bacteria morphology describe the shape, colour and sizes of bacteria present in the samples

Samples	Bacterial	Abbreviation	Color	Shape
Nbm4a1	Gram positive bacilli	Gpb	Bluish purple	Rod shape
Em4b2	Gram positive cocci	Gpc	Bluish purple	Round shape
Om2a	Gram positive bacilli	Gpb	Bluish purple	Rod shape
Nbm4a2	Gram positive bacilli	Gpb	Bluish purple	Rod shape
Um42	Gram positive bacilli	Gpb	Bluish purple	Rod shape
Om2b	Gram positive cocci	Gpc	Bluish purple	Round shape
Um41	Gram negative bacilli	Gnb	Pink	Round shape

Table 4.2: Biochemical Test result

This table shows the results (positive & negative) gotten from the oxidare, atrase, indole, catalase, urease and capsule tests respectively

Sample	Gram	Ox	Cit	Indole	Cat	Urease	Coag
Um4i	Gpc	-	-	-	+	+	+
Um4 ii	Gnb	+	+	-	+	-	-
Em4bi	Gpb	-	+	-	+	-	-
Em4bii	Gpc	-	-	-	+	+	+
Om2i	Gpb	-	+	-	+	-	-
Om2ii	Gpc	-	-	-	+	+	+
Nbn4i	Gpb	+	-	-	+	-	-
Nbn4ii	Gnb	-	-	-	+	+	-

KEY:

OX = Oxidase

CIT = Citrate

CAT = Catalase

GPC = Gram Positive Cocci

GPB = Gram Positive Bacilli

GNB = Gram Negative Bacilli

NK = No Reaction

COA = Coagulase

Table 4.3: Carbohydrate Fermentation

This table shows the reaction of bacteria to various media containing carbohydrate (lactose, sucrose, glucose and manitol)

Lactose	**Sucrose**	**Glucose**	**Manitol**	**Isolates**
Acid	Acid	Acid	NR	Staphylococcus aureus
NK	NK	NK	Acid	Pseudomonu aeruginose
NK	NK	NK	NK	Bacillus cereus
Acid	Acid	Acid	NK	Staphylococcus aureus
NK	Acid	Acid	Acid	Bacillus subtilis
Acid	Acid	Acid	NK	Staphylococus aureus
NK	NK	NK	NK	Bacillus cereus
NK	Acid-gas	Acid	Acid	Proteus spp (gnb)

Sample Fungal Isolates

M4 Cephalosporium Acremonium

EM5 Cephalosporium Acremonium

Table 4.4: Bacteria counts in cfu/m

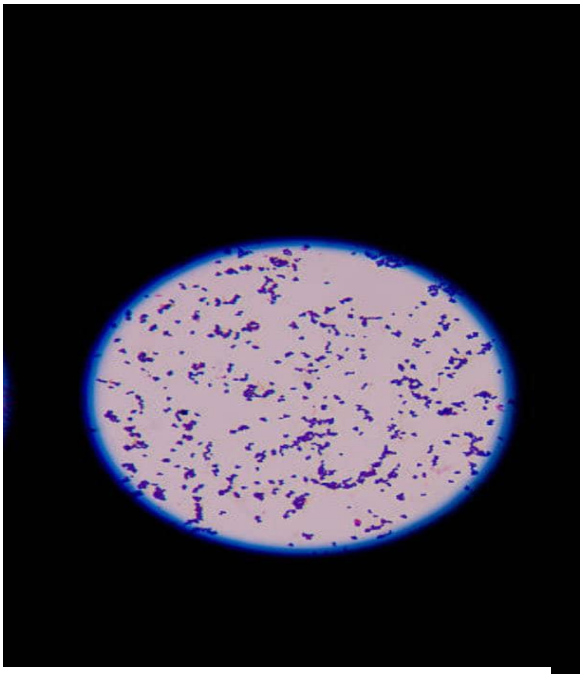
This describes the bacteria counts in colony forming units per volume of culture plates

Sample	P1	P2	Average	Total
10_{-1}	10^{-2}	10^{-3}	10^{-4}	
Om2	10	10	10	1 x 10^{4}cfu/m
Om1	10	10	10	1 x 10^{4} cfu/m
Om3	3	2	3.5	1 x 10^{5}cfu/m
Em2	27	16	21.5	1 x 10^{3}cfu/m
Em3	13	14	13.5	1 x 10^{3}cfu/m
Um1	12	5	8.5	1 x 10^{2}cfu/m

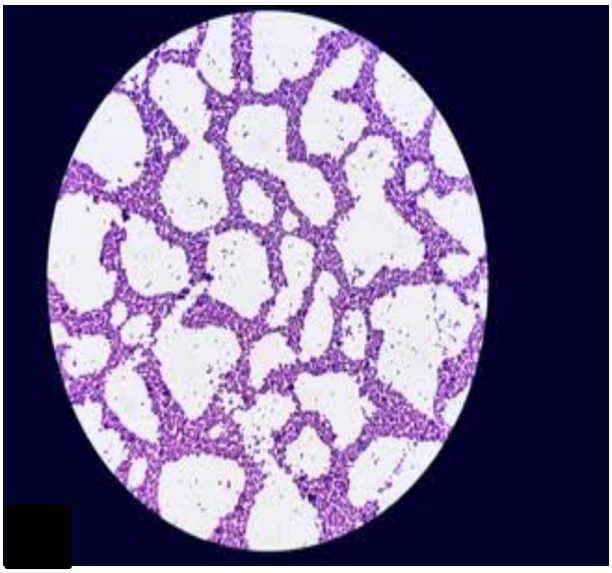
Table 4. 5: Fungal counts in cfu/m

This describes the fungal counts in colony forming units per volume of culture plates

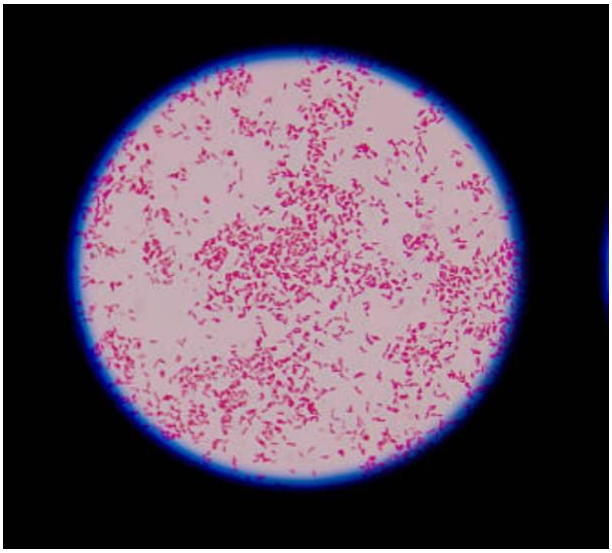
Sample	P1	P2	Average	Total
	10_{-1}	10^{-2}		10^{-4}
Om4	2	1	1.5	1 x 10^{-2}cfu/m
Em5	2	2	2	2.5 x 10^{-3}cfu/m



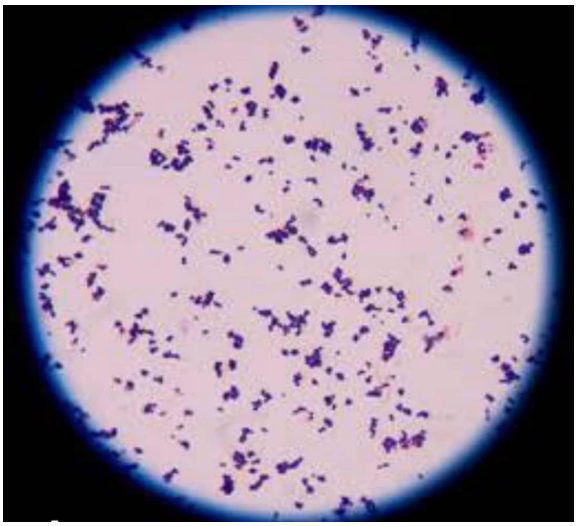
Slide 4.1: Bacilli cereus gram position and shaped anaerobic bacteria



Slide 4.2: Staphylococcus Aureus, Gram Positive Cocci-Shaped Bacteria Arranged in Cluster, Purple Colour



Slide 4.3: Gram Negative Bacilli, a Pseudomonas Spp, an Aerobic Non-Spare Forming Rod Bacteria Pink in Colour



Slide 4.4: Gram Positive Cocci, Streptococcus (Catalase Negative)

The above slides 4.1 to 4.4 shows the microscopic view of bacteria isolates on slides after gram staining procedures

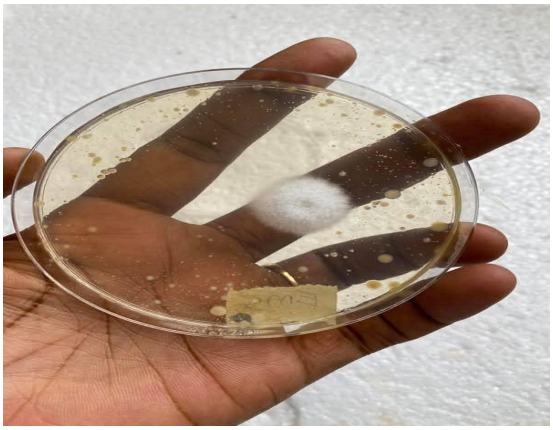


Plate 4.1 Cephalosporium Acremonium fungi seen in sample of NBM plates

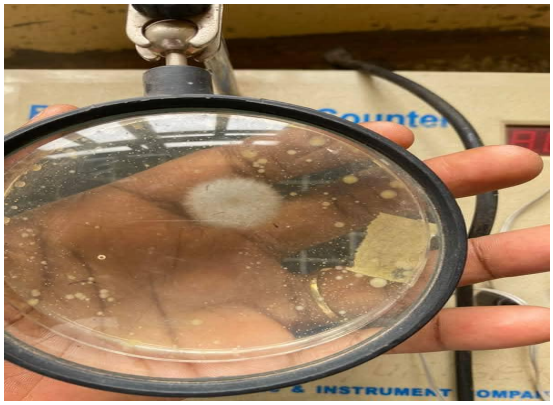


Plate 4.2: Cephalosporium Acremonium seen in sample UM plates

Plate 4.1 and 4.2 shows the fungal growth as Cephalosporium Acremonium presented I samples of NUM and UM

Plate 4.2 shows the fungal growth of Cephalosporium Acremonium present in samples from NBM and UM plate.

Key:

Om - Oba Market

NUB - New Benin Maeket

UM - Uselu Market

EM - Ekae Market

CHAPTER FIVE

DISCUSSION

This study shows the presence of bacterial and fungi in raw beef samples collected at four different markets in benin city, edo state, Nigeria at the early hours of the, these markets were visited between 7;40 am to 9;00am in the morning time. It was observed that these meat were the first set to be brought to the market for the day and the appeared very fresh abd reddish. Upon the collection and transportation to the laboratory where work comensed almost immediately, below are the obvious and visible observation of the results gotten after all the tests were carried out in the laboratory, although the tests lasted a period of time.

5.1 BACTERIA

Table 4.1 carefully details the morphological and biochemical characteristics of bacteria found in the different raw beef collected across the city of benin. This discussion highlights the microbial knowledge and research and also detailing their importance in a postgraduate study.

5.2 MORPHOLOGY OF ISOLATES

The table consistently categorizes bacteria based on their gram stain reaction (positive or negative) and shape (rod or round)

Gram positive and gram negative rods:

Bacillus cereus: this is a gram positive bacterium seen in slide 4.1. They contaminate raw meat and are found in the environment, it can quickly multiply at room temperature, the can cause gastrointestinal illnesses like vomiting and diarrhoeal syndrome, these were found in samples plates gotten from uselu, ekae and oba market.

Staphylococcus aureus: this is a gram positive spherically shaped bacterium seen in slide 4.2 , they can multiply in raw meat and produce toxins, but however, thorough cooking can kill them, if not killed they can cause illnesses. These were observed and seen in the plate samples of uselu and new benin market.

Proteus species: they are gram negative rod shaped bacterium found in plate samples of oba market.

Pseudomonas species: this is a gram negative, straight or curved rod motile by one or more polar flagella, seen in slide 4.3 these were found in plate samples gotten from Ekae market.

5.3 FUNGI

Table 5.1 clearly shows and describes the visible creamy color of the fungi called Acremonium chrysogenum formerly called Cephalosporium Acremonium which was found present in all the plates.

There is a pressing need to improve hygiene practices at all levels of the meat supply chain—from slaughter to retail. Meat handlers, butchers, and vendors should be trained regularly on proper meat handling, sanitation, and personal hygiene to minimize contamination (FAO, 2017). Hand washing facilities, clean utensils, protective clothing, and access to potable water should be made available in slaughterhouses and markets.

Regulatory bodies such as NAFDAC, the Nigeria Centre for Disease Control (NCDC), and local environmental health departments should intensify routine inspections of slaughterhouses and meat markets. Enforcement of existing food safety laws and implementation of Hazard Analysis.

Consumer education campaigns on the risks of consuming contaminated or undercooked meat can empower the public to make safer food choices. This can be done through community outreach, media programs, and school curricula

More localized studies should be conducted to monitor microbial trends in meat, antibiotic resistance patterns, and the effectiveness of implemented control measures. Data-driven policies.

REFERENCES

Afolabi, C. G. and Akinyosoye, F. A. (2017). Bacteriological quality of meat and meat contact surfaces in selected abattoirs in Lagos State, Nigeria. *African Journal of Food Science* **11**(5): 134–140.

Ajayi, O. I., Ogunbanwo, S. T. and Oluwaseun, O. (2020). The Role of NAFDAC in Ensuring Meat Safety in Nigeria. *Food Quality Assurance*, **4**(2): 45-53.

Akinmoladun, O. S., Olayemi, S. O. and Aruna, T. (2020). Microbial Contamination in Nigerian Meat Markets: An Overview of Challenges and Control Measures. *African Journal of Food Science*, **14**(7): 121-129.

Akinro, A. O. and Olowofela, A. T. (2017). *Microbial evaluation of meat-contact surfaces in Nigerian slaughterhouses*. *Nigerian Journal of Microbiology*, **31**(1): 3881–3888. – Demonstrates use of ANOVA and chi-square in meat microbiology research.

Akinro, A. O., Oloruntoba, E. O. and Ajayi, D. D. (2020). Microbiological quality of fresh meat from open markets in Ibadan, Nigeria. *African Journal of Environmental Science* and *Technology*, **14**(2): 51–59.

Aman, R., Mahas, A. and Mahfouz, M. (2020). Nucleic acid detection using CRISPR/Cas biosensing technologies. *ACS Synthetic Biology,* **9**(6): 1226–1233. DOI: 10.1021/acssynbio.0c00143

Barbut, S. (2015). The Science of Meat Quality. Wiley-Blackwell. ISBN: **978(**1): 118-3817-6.

Beuchat, L. R., Cousin, M. A. and Mahon, B. E. (2020). Detection and Identification of Foodborne Pathogens in Raw Meat. *Journal of Food Protection*, **83**(8): 1342-1351.

Bhandari, D. and Nepali D. B. (2021). Microbiological quality of raw meat in local markets: A cross-sectional study. *Journal of Food Safety and Hygiene*, **7**(1): 42–48 – Supports standard lab methods and observational checklists.

Bokhari, H., Hussain, S. I. and Alam, S. (2021). Microbial Contamination of Meat and Its Public Health Implications. *Food Safety* and *Hygiene*, 45(2), 111-121.

Centers for Disease Control and Prevention (CDC). (2022). *Surveillance for Foodborne* Disease Outbreaks–United States.

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries (Part 2). *Cambridge University Press.*

CLSI (2020). Performance Standards for Antimicrobial Susceptibility Testing, 30th Edition. *Clinical and Laboratory Standards Institute*, Wayne, PA.

Codex Alimentarius Commission. (2003). HACCP: Hazard Analysis and Critical Control Point (HACCP). *System* and *Guidelines for its Application. FAO/WHO.*

Davies, A. R. and Board, R. G. (1998). The Microbiology of Meat and Poultry. *Springer*.

Dwivedi, H. P. and Jaykus, L. A. (2011). Advances in Detection Methods For Foodborne Pathogens. *Journal of Microbiological Methods,* **86**(2): 232–254. DOI: 10.1016/j.mimet.2011.05.003

Edo State Ministry of Agriculture and Natural Resources. (2020). Annual report on food production and meat supply. Benin City, Nigeria. *Provides official data on meat production and the structure of slaughterhouses in the state.*

EFSA and ECDC (2022). The European Union One Health 2021 Zoonoses Report. *EFSA Journa*l 2022; **20**(12):7666. https://www.efsa.europa.eu/en/efsajournal/pub/7666

Ehigie, B. O. and Ekundayo, T. F. (2018). Assessment of hygienic practices in meat handling among meat vendors in Benin City, Nigeria. *African Journal of Environmental Science and Technology*, **12**(7): 262–268. – Provides insight into meat handling and hygiene challenges specific to Benin City.

Ekunwe, P. A. and Emokaro, C. O. (2009). Technical efficiency of commercial livestock farmers in Edo State, Nigeria. *Research Journal of Agriculture and Biological Sciences,* **5**(2): 150–155. – Discusses the livestock sector and meat demand within Edo State, justifying the study location.

European Food Safety Authority (EFSA) and ECDC. (2023). The European Union One Health 2022. Zoonoses Report.

Ezenduka, E. V., *et al.,* (2019). Surveillance of antimicrobial residues in food animals and products in Nigeria: A review. *Tropical Animal Health* and *Production*, **51**(4), 1019–1033

FAO. (2017). Food safety and quality control in food production. Food and Agriculture Organization of the United Nations. *Retrieved from FAO website.*

FAO/WHO (2020). Codex Alimentarius: General Principles of Food Hygiene (CXC 1-1969). *Available: FAO Codex*

Fasanmi, O. G., et al. (2018). Public health concerns of antimicrobial residues in meat and ilk in Nigeria. *J. Food Qual.*, 1–6.

Field, A. (2013). Discovering Statistics Using IBM SPSS Statistics (4th ed.). Sage Publications. *A comprehensive guide for applying statistical tests in SPSS.*

Grace, D. (2015). Food safety in low and middle income countries. *International Journal of Environmental Research and Public Health*, **12**(9): 10490–10507. DOI: 10.3390/ijerph120910490

Havelaar, A. H., Brul, S., de Jong, A., de Jonge, R., Zwietering, M. H. and ter Kuile, B. H. (2010). Future challenges to microbial food safety. *International Journal of Food Microbiology*, **139**, S79-S94.

Iroha, I. R., *et al.,* (2011). Bacteria contamination of raw meat sold in Abakaliki, Ebonyi State Nigeria. *J. Public Health Epidemiol.*, **3**(2): 49–53.

Jackson, B. R., Tarr, C., Strain, E., Jackson, K. A., Conrad, A., Carleton, H. and Silk, B. J. (2016). Implementation of nationwide real-time whole-genome sequencing to enhance listeriosis outbreak detection and investigation. *Clinical Infectious Diseases*, **63**(3): 380-386.

Kim, S. A., *et al.,* (2018). Prevalence and characterization of *Listeria monocytogenes* isolated from raw meat in retail markets. *Korean Journal for Food Science of Animal Resources*, **38**(3): 592–598.

Law, J. W.-F., Ab Mutalib, N.-S., Chan, K.-G., and Lee, L.-H. (2015). Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations. *Frontiers in Microbiology*, 5, 770.

Mead, G. C. (2004). Microbiological analysis of red meat, poultry and eggs. In Managing Risks in Meat Processing (pp. 143–175). *Woodhead Publishing.*

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C. and Tauxe, R. V. (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases*, **5**(5): 607-625.

Mhone, T. A., Matope, G. and Saidi, P. T. (2011). Aerobic bacterial, coliforms and *Escherichia coli* counts of raw and processed meat from selected outlets in Zimbabwe. *Meat Science*, **89**(1): 41–45.

NAFDAC. (2017). National Agency for Food and Drug Administration and Control (NAFDAC) regulations and guidelines for food safety. *Abuja, Nigeria*.

National Population Commission (NPC). (2022). Population statistics of Nigeria. Abuja, Nigeria: NPC. *Provides updated demographic data on Benin City and Edo State.*

Newell, D. G., *et al*., (2010). Food-borne diseases—the challenges of 20 years ago still persist while new ones continue to emerge. *International Journal of Food Microbiology*, 139(Supplement 1), S3–S15. DOI: 10.1016/j.ijfoodmicro.2010.01.021

Notomi, T., *et al.,* (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research,* **28**(12), e63.DOI: 10.1093/nar/28.12.e63

Ogunbanwo, S. T. and Joseph, S. A. (2021). *Microbiological quality of raw beef sold in major markets of southwestern Nigeria*. *Nigerian Journal of Microbiology*, **35**(1), 2472–2480. – Supports the need for regional studies on microbial contamination of meat.

Ogundipe, O. O. and Adetunji, V. O. (2020). Occurrence of antibiotic-resistant bacteria in meat from slaughterhouses and markets in Nigeria. *African Journal of Microbiology Research*, **14**(3): 120–129.

Olatoye, I. O. and Basiru, A. (2013). Antibiotic resistance in Escherichia coli and Salmonella spp. isolated from retail raw meat in Ibadan, Nigeria. *Journal of Infectious Diseases and Immunity,* **5**(1): 8–14.

Omemu, A. M., *et al.* (2014). Microbiological hazard and critical control points of ready-to-eat roasted beef (Suya) sold in Ilorin, Nigeria. *Food Control*, **28**(2): 434–438.

Onyeze, G. O. C. and Obiakor, O. E. (2020). *Assessment of microbial load of raw meat from local markets in southeastern Nigeria*. *Nigerian Journal of Microbiology,* **34**(1): 2334–2342.

Oranusi, S., Oguoma, O. I. and Agusi, E. (2013). Microbiological quality assessment of meat and meat products sold in open markets in Nsukka, Enugu State, Nigeria. *International Journal of Current Microbiology* and *Applied Sciences*, **2**(3): 347–353.

Priyanka, B., Krishnan, V., and Palaniyandi, S. A. (2016). Current trends in detection of foodborne pathogens: a review. *Critical Reviews in Food Science and Nutrition,* **56**(3): 364–379.
DOI: [10.1080/10408398.2012.654874](https://doi.org/10.1080/10408398.2012.

Quick, J., *et al.*, (2017). Real-time, portable genome sequencing for Ebola surveillance. Nature, **530**(7589): 228–232. DOI: 10.1038/nature16996

Ranjbar, R., Dastgheib, S. M. M. and Haghshenas, M. (2020). Foodborne Pathogens in Raw Meat: A Review of Detection Methods and Recent Advances. *Journal of Food Science* and *Technology*, **57**(7): 2405-2418.

Redmond, E. C. and Griffith, C. J. (2003). Consumer food handling in the home: a review of food safety studies. *Journal of Food Protection*, **66**(1), 130-161.

Scallan, E., Hoekstra, R. M., Angulo, F. J., *et al*., (2011). *Foodborne illness acquired in the United States—major pathogens. Emerging Infectious Diseases,* **17**(1): 7–15.
DOI: 10.3201/eid1701.P11101

Sofos, J. N. (2008). Challenges to meat safety in the 21st century. *Meat Science,* **78**(1-2): 3–13. DOI: 10.1016/j.meatsci.2007.07.027

Tada, M., Kuwahara, T. and Kimura, T. (2019). Emerging Technologies in Food Microbial Detection: PCR and Biosensors. *Food Research International*, **121:**, 57-68.

UNIBEN Research Ethics Policy. (2021). University of Benin Guidelines for Ethical Conduct Scientific Research, *Institutional framework for ethical clearance and compliance.*

Van den Broeck, E. *et al.* (2009). *Microbial spoilage of meat during storage.* Advances in Meat, *Poultry and Seafood Packaging*, **95**–111.DOI: 10.1533/9781845696573.95

Velusamy, V., Arshak, K., Korostynska, O., Oliwa, K. and Adley, C. (2010). An overview of foodborne pathogen detection: In the perspective of biosensors. *Biotechnology*

Advances, **28**(2): 232–254. DOI: 10.1016/j.biotechadv.2009.12.004

Wagener, S., Von Ah, U., and Riegel, R. (2020). Role of Meat in Foodborne Pathogen Transmission and Detection. *Foodborne Pathogens* and *Disease*, **17**(6): 417-425.

Wang, Y., Ye, Z., and Ying, Y. (2012). New trends in impedimetric biosensors for the detection of foodborne pathogenic bacteria. *Sensors*, **12**(3): 3449–3471. DOI: 10.3390/s120303449

WHO and FAO. (2020). Food Safety Risk Management: *Evidence-informed policies* and *decisions*. Geneva: World Health Organization.

WHO (2015). Estimates of the Global Burden of Foodborne Diseases. Geneva: World Health Organization.

WHO (2015). Foodborne Disease Burden Epidemiology Reference Group 2007–2015. *Available: WHO Food Safety*

WHO. (2017). Antibacterial Agents in Clinical Development: *An Analysis of the Antibacterial Clinical Development Pipeline*. Geneva: World Health Organization.

WHO/FAO. (2006). Food safety risk analysis: A guide for national food safety authorities. *Food and Agriculture Organization of the United Nations*.

World Health Organization (WHO). (2015). Estimates of the global burden of foodborne diseases. https://www.who.int/publications/i/item/9789241565165

World Health Organization (WHO). (2020). Five keys to safer food manual. *Geneva*:

Yamaguchi, N., and Nasu, M. (2012). Development of portable and rapid microbial detection systems using microfluidic and nanotechnology. *Biosensors and Bioelectronics*, **34**(1): 1–9. DOI: 10.1016/j.bios.2012.01.034

Zhang, G., Ma, L., Zhang, Y. and Zhang, Y. (2020). Recent advances in biosensors for detection of foodborne pathogens and toxins. TrAC Trends in Analytical Chemistry, 127, 115895. DOI: 10.1016/j.trac.2020.115895

Zhao, T., Xia, X., and Zhang, W. (2020). Microbial Contamination in Raw Meat and Meat Products: Detection and Control Strategies. *Food Control*, **109**: 106903.

Zweifel, C., and Stephan, R. (2012). Microbiological monitoring of meat and meat products in Switzerland. *International Journal of Food Microbiology,* **154**(3): 225–232.