

**BACTERIOLOGICAL ANALYSIS OF HONEY SAMPLES OBTAINED FROM
BENIN CITY**

BY

OKOUDO Evelyn (MISS)

LSC2007059

DEPARTMENT OF MICROBIOLOGY

UNIVERSITY OF BENIN

BENIN CITY.

JANUARY, 2025

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

JANUARY, 2025

CERTIFICATION

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

Mr. E.C WEMAMBU

(Project Supervisor)

DATE

APPROVAL

This project work was carried out by OKOUDO EVELYN in partial fulfilment of the award of a Bachelor of Science, B.Sc (Hons) degree in the Department of Microbiology, University of Benin, Benin City.

PROF. (MRS.) F. I. AKINNIBOSUN

(Head of Department)

DATE

DEDICATION

This project work is dedicated to God Almighty, for bringing me this far and for being my solid help. For supporting me every step of the way and loving me despite my shortcomings.

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ABSTRACT

Honey, a natural sweetener and viscous substance produced by honeybees, has been used in human nutrition and traditional medicine for centuries. However, honey can become contaminated by microorganisms during production, processing, and storage, posing potential health risks to consumers. This study aimed to assess the bacteriological quality of honey sold in two markets in Benin City, Edo State.

Honey samples were collected from both markets using sterile containers and transported under controlled conditions to the laboratory. The microbial quality was analyzed by culturing the samples on nutrient agar and MacConkey agar, followed by bacterial isolation, identification, and biochemical characterization (e.g., Gram staining, catalase, oxidase, indole, citrate, and other biochemical tests).

Results showed heterotrophic bacterial counts ranging from 4.0×10^4 to 5.0×10^3 cfu/ml and coliform counts from 1.0×10^3 to 4.0×10^3 cfu/ml.

The highest bacterial contamination was found in Oba Market, while Uselu had the highest coliform count. *Clostridium sp.* was the most frequently occurring bacterium (25%). The presence of potentially harmful bacteria suggests contamination during production, handling, or storage, raising concerns about honey safety. The study highlights the need for improved hygiene practices and stricter quality control measures to ensure consumer safety.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Honey, a natural, sweet, and viscous substance produced by honeybees (*Apis mellifera*), has been a staple in human nutrition and traditional medicine for centuries. Bees produce honey by collecting nectar from flowers, mixing it with enzymatic secretions, and storing it in honeycombs, where it undergoes maturation into the final product. This process results in a complex composition that includes carbohydrates, water, amino acids, vitamins, minerals, and enzymes, making honey a highly nutritious and bioactive food product (Alvarez-Suarez *et al.*, 2010). Honey is widely recognized for its therapeutic applications, including wound healing, antimicrobial activity, and its role in supporting digestive health (Gomes *et al.*, 2010; Mandal and Mandal, 2011).

Honey is primarily composed of carbohydrates, with fructose (38.5%) and glucose (31.0%) being the dominant sugars (Bogdanov *et al.*, 2008). These sugars make honey an excellent source of natural energy. In addition, honey contains trace amounts of proteins, organic acids, flavonoids, and phenolic compounds, which contribute to its antioxidant and anti-inflammatory properties (Gheldof *et al.*, 2002). The presence of bioactive compounds such as polyphenols, hydrogen peroxide, and methylglyoxal further enhances honey's therapeutic potential (Kwakman and Zaat, 2012). Honey has demonstrated efficacy in treating wounds and burns due to its ability to promote tissue regeneration, reduce inflammation, and prevent bacterial infections (Molan, 2001).

In Nigeria, particularly in Benin City, honey is a valued commodity due to its nutritional and medicinal benefits. It is commonly used in traditional medicine for treating burns, infections,

and gastrointestinal disorders, highlighting its importance as a functional food (Bogdanov *et al.*, 2008). The demand for honey in Nigeria is driven by its widespread use in food, cosmetics, and pharmaceuticals. However, despite its numerous benefits, the quality and safety of honey sold in local markets remain critical concerns.

One of the significant challenges associated with honey consumption in Nigeria is the issue of adulteration and contamination. Honey is often subject to adulteration with sugar syrups and other additives, reducing its quality and potential health benefits (Ahmed *et al.*, 2021). Adulteration not only compromises honey's nutritional value but also misleads consumers regarding its authenticity. Additionally, contamination can occur at various stages of production, handling, and storage, leading to the presence of harmful microorganisms and chemical residues (Ajibola *et al.*, 2012).

Microbial contamination is one of the primary concerns associated with honey. Although honey possesses natural antimicrobial properties due to its low pH, high osmotic pressure, and hydrogen peroxide content, contamination can still occur due to poor hygiene practices during harvesting and storage. The most common microbial contaminants in honey include: *Clostridium botulinum* spores: These spores can cause botulism, particularly in infants, making it unsafe for children under one year of age (Seijo *et al.*, 2014). Pathogenic bacteria: Honey can harbor bacteria such as *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*, which pose significant health risks (Snowdon and Cliver, 1996). Yeasts and molds, These spoilage organisms can proliferate in improperly stored honey, leading to fermentation and degradation of quality (Tysset and Rousseau, 1981).

In Benin City, the lack of stringent regulatory frameworks and quality control measures increases the risk of honey adulteration and contamination. Many honey vendors sell their products in open markets without standardized processing and storage conditions, making

them prone to microbial contamination. The absence of routine microbiological testing and certification further exacerbates the problem, leaving consumers vulnerable to potential health hazards (Adebolu and Oladimeji, 2005).

Given the widespread consumption of honey in the region, there is an urgent need for comprehensive microbiological studies to evaluate its safety and quality. Localized research is essential to determine the microbial load of honey sold in Benin City and to ensure that consumers receive a product that meets acceptable safety standards. Studies should focus on assessing the presence of pathogenic microorganisms, antibiotic residues, and heavy metals to provide data that can inform policy decisions on honey production and regulation.

Although honey's antimicrobial properties are well documented, its efficacy as a safe consumable product depends on its quality and purity. The presence of harmful microorganisms in honey sold in local markets highlights the importance of assessing its microbiological safety. This study aims to fill the knowledge gap regarding the microbial quality of honey in Benin City, providing crucial data that can be used to establish regulatory guidelines for ensuring consumer safety. Enhanced regulatory oversight, proper hygiene practices, and routine quality assessments are necessary to protect consumers and maintain the integrity of honey as a functional and medicinal food product

1.2 Aim and Objectives

The aim of this study is to evaluate the microbiological quality of honey sold in Benin City, Nigeria.

The specific objectives of this research are to:

1. Isolate bacterial contaminants present in honey samples sold in Benin City.
2. Identify the bacterial isolates in the honey samples.

CHAPTER TWO

LITERATURE REVIEW

2.1. Honey as a Natural Product

Honey is a natural, sweet, and viscous product synthesized by honeybees (*Apis mellifera*) through the collection, enzymatic processing, and storage of floral nectar. It is composed predominantly of carbohydrates, primarily glucose and fructose, along with water, vitamins, minerals, proteins, enzymes, and an array of bioactive phytochemicals such as flavonoids and phenolic acids (Alvarez-Suarez *et al.*, 2010). These constituents not only determine honey's taste and texture but also confer significant medicinal and functional properties. Historically, honey has been used as both a food and medicine, with ancient civilizations such as the Egyptians and Greeks employing it for wound healing and as an embalming agent due to its antimicrobial properties (Bogdanov *et al.*, 2008).

The composition of honey varies depending on its botanical and geographical origin, with factors such as climate, soil type, and floral diversity playing critical roles. This variability accounts for the unique flavors, colors, and therapeutic properties of honey from different regions (Gheldof *et al.*, 2002). In addition to its nutritional value, honey is a rich source of antioxidants, with compounds like flavonoids and phenolic acids contributing to its ability to neutralize free radicals and reduce oxidative stress (Sohaimy *et al.*, 2015).

Honey's antimicrobial properties are attributed to several mechanisms, including its high osmotic pressure, low pH, and the presence of hydrogen peroxide and non-peroxide bioactive compounds. These features make honey effective against a wide range of pathogenic microorganisms, including antibiotic-resistant strains, positioning it as a potential alternative or adjunct to conventional therapies (Mandal and Mandal, 2011).

The quality of honey is a critical factor that determines its safety, nutritional value, and therapeutic efficacy. However, honey is susceptible to contamination and adulteration, which can compromise its integrity. Microbiological analysis is essential for identifying potential contaminants, such as *Clostridium botulinum* spores, *Escherichia coli*, and various yeasts and molds, which may enter honey during processing, handling, or storage (Ahmed *et al.*, 2021). These contaminants can pose health risks, especially to vulnerable populations like infants, the elderly, and immunocompromised individuals.

In addition to ensuring consumer safety, microbiological analyses are crucial for detecting adulteration, a common issue in the honey market. Adulteration often involves the addition of sugar syrups or other sweeteners, which dilute honey's natural composition and diminish its health benefits. Analytical techniques such as high-performance liquid chromatography (HPLC) and mass spectrometry are used to detect such adulterants and verify honey's authenticity (Sohaimy *et al.*, 2015).

In Benin City, Nigeria, honey is widely consumed for its nutritional, medicinal, and functional properties. It is sold in various forms, from locally produced honey sold by roadside vendors to more commercially packaged varieties available in stores. However, the lack of stringent regulatory frameworks and quality control measures has raised concerns about the safety and authenticity of honey sold in the region (Ahmed *et al.*, 2021). Poor handling practices, inadequate packaging, and the potential for adulteration with sugar syrups are common challenges that compromise honey quality in local markets.

2.2. Classification of Honey Based on Packaging and Processing

Honey is classified based on its packaging and processing methods, which influence its physical characteristics, shelf life, and nutritional properties. These classifications cater to

consumer preferences and market demands while ensuring quality and safety. Each type offers unique attributes and processing considerations.

2.2.1 Crystallized Honey

Crystallized honey, also referred to as "granulated" or "candied" honey, occurs when glucose, one of the primary sugars in honey, separates from water and forms crystals. This natural phenomenon is influenced by factors such as the glucose-to-fructose ratio, temperature, and the presence of particles that serve as nucleation sites for crystals (White *et al.*, 1962).

While crystallized honey is often perceived as a sign of adulteration, it is a natural process that does not affect its safety or nutritional quality. Some consumers prefer this texture for spreading on bread or other foods. Crystallization can be reversed by gently warming the honey in a water bath at temperatures below 40°C to preserve its enzymes and bioactive compounds (Flottum, 2010).

2.2.2 Pasteurized Honey

Pasteurized honey undergoes thermal processing at temperatures of at least 61°C. The primary goal of pasteurization is to destroy yeast cells that could lead to fermentation during storage, particularly in honey with high moisture content. Pasteurization also delays crystallization by dissolving microcrystals that act as nucleation sites (Subramanian *et al.*, 2007).

However, excessive heating can degrade honey's quality by increasing hydroxymethylfurfural (HMF) levels, a compound formed when sugars decompose under heat. High HMF levels are considered an indicator of poor honey quality (White, 1975). Moreover, heating can reduce the activity of enzymes such as diastase and invertase, which are essential for honey's biochemical and therapeutic properties. Pasteurized honey is often clearer and smoother,

making it more visually appealing to consumers, but these benefits come at the cost of reduced nutritional value (Subramanian *et al.*, 2007).

2.2.3 Raw Honey

Raw honey is honey that has been extracted and minimally processed, typically involving straining to remove large debris, such as wax and bee parts, without applying heat. This type of honey retains natural components such as pollen, propolis, and small wax particles, which are believed to contribute to its health benefits (Honey.com, 1996).

Raw honey is often marketed as a healthier option due to its higher content of antioxidants and bioactive compounds. Proponents claim that consuming raw honey with pollen can help alleviate seasonal allergies, although scientific evidence on this claim is limited. Raw honey's unprocessed nature also means it may crystallize more quickly and have a cloudier appearance compared to pasteurized or filtered honey (Bogdanov *et al.*, 2008).

2.2.4 Filtered Honey

Filtered honey is processed to remove most impurities, including pollen, small wax particles, and air bubbles, often by heating the honey to 66–77°C to reduce viscosity and facilitate filtration (Damerow, 2011). This type of honey is characterized by its clarity and extended shelf life, as filtration slows down the crystallization process by removing potential nucleation sites.

Filtered honey is popular in commercial markets, particularly in supermarkets, where consumers often prioritize visual appeal. However, the removal of pollen and other natural components has raised concerns about its nutritional and medicinal value. Critics argue that

highly filtered honey may lack some of the beneficial properties associated with raw honey (Codex Alimentarius Commission, 2001).

2.2.5 Ultrasonicated Honey

Ultrasonicated honey is processed using ultrasonic waves, a non-thermal treatment that destroys yeast cells and reduces the rate of fermentation. This method involves exposing honey to high-frequency sound waves that generate localized high temperatures and pressures, breaking down yeast cells and dispersing crystals (David, 2011).

Unlike pasteurization, ultrasonication preserves most of honey's enzymes and bioactive compounds, making it an appealing option for maintaining both quality and safety. Ultrasonicated honey has a smoother texture and resists crystallization for extended periods. This innovative processing method is gaining popularity for its ability to balance consumer demand for clear, long-lasting honey with minimal impact on nutritional and functional properties (Bera *et al.*, 2015).

2.3. Nutritional Benefits of Honey

Honey, a sweet, viscous substance produced by bees from nectar, is not only a popular sweetener but also a powerhouse of nutritional benefits. Rich in carbohydrates, antioxidants, vitamins, and minerals, honey has long been recognized for its therapeutic properties and is frequently utilized in both culinary and medicinal applications. Below are additional nutritional benefits of honey, supported by scientific evidence.

2.3.1. Antioxidant Properties

Honey is a potent source of antioxidants, including phenolic compounds, flavonoids, and organic acids. These antioxidants play a crucial role in neutralizing free radicals in the body,

thus preventing oxidative stress that can lead to various chronic diseases such as cancer, cardiovascular disease, and diabetes. Research has shown that honey, particularly darker varieties, has higher levels of antioxidant activity compared to lighter honeys (Chirumalla *et al.*, 2014). These compounds help protect cells from damage, reduce inflammation, and improve overall health. Additionally, antioxidants in honey may support immune function by enhancing the body's ability to fight infections.

2.3.2. Skin Health and Healing Properties

Honey has long been used for its wound-healing properties, and recent studies have provided scientific backing for this traditional use. Honey contains natural enzymes, such as glucose oxidase, which produce hydrogen peroxide, an antimicrobial agent that can help cleanse wounds and prevent infection. It also creates a moist healing environment, which is essential for tissue repair. Furthermore, honey is rich in trace elements such as zinc and copper, which are vital for skin regeneration and healing. Several studies have demonstrated that honey promotes faster healing of burns, cuts, and ulcers, reducing inflammation and scarring (Duncan *et al.*, 2006). Its anti-inflammatory and antimicrobial properties make it a valuable tool in treating various skin conditions, including acne, eczema, and psoriasis.

2.3.3. Digestive Health and Anti-inflammatory Effects

Honey has been shown to possess digestive benefits due to its prebiotic properties. It helps promote the growth of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*, which are essential for maintaining a healthy gut microbiome. These beneficial bacteria improve digestion, support the immune system, and contribute to overall gut health. Furthermore, honey is often used as a remedy for indigestion, acid reflux, and stomach ulcers. The

antimicrobial and anti-inflammatory effects of honey help soothe the digestive tract, reduce irritation, and promote healing (Al-Waili, 2003).

2.3.4. Cardiovascular Health

Emerging research suggests that honey may have cardiovascular benefits, primarily due to its antioxidant content and its ability to reduce inflammation and improve lipid profiles. Honey has been shown to reduce total cholesterol levels, lower triglycerides, and improve the ratio of high-density lipoprotein (HDL) to low-density lipoprotein (LDL), which are important markers of cardiovascular health (Singh *et al.*, 2008). Additionally, honey's ability to lower blood pressure has been demonstrated in animal studies, potentially contributing to a reduced risk of heart disease. The polyphenols and other bioactive compounds in honey may help prevent the oxidation of LDL cholesterol, which is a key factor in the development of atherosclerosis and other cardiovascular diseases.

2.3.5 Immune System Support

Honey contains a variety of bioactive compounds, including vitamins (such as vitamin C) and minerals (such as zinc and selenium), which are essential for optimal immune function. Studies have shown that honey can boost the immune system by enhancing the body's ability to fight infections and inflammation. Its antibacterial, antifungal, and antiviral properties make it an effective natural remedy for preventing and treating respiratory infections, colds, and sore throats. Honey has been used traditionally in cough syrups due to its ability to soothe the throat, suppress coughing, and ease irritation (Oduwale *et al.*, 2008).

2.3.6 Weight Management

While honey is a calorie-dense food, it has been suggested that it may play a role in weight management when used in moderation. Research indicates that honey, when consumed in place of refined sugars, may help reduce body weight and body fat percentage. This is because honey has a lower glycemic index (GI) than regular sugar, meaning it causes a slower rise in blood sugar levels. It may also aid in curbing hunger by promoting feelings of fullness, leading to reduced calorie intake overall (Mandal *et al.*, 2015). Additionally, honey has been shown to promote fat oxidation and improve metabolism, further supporting its potential role in weight management.

2.3.7 Rich Source of Vitamins and Minerals

Honey contains small amounts of a variety of essential vitamins and minerals that contribute to overall health. These include B-vitamins (such as riboflavin, niacin, and pantothenic acid), vitamin C, and minerals like calcium, iron, magnesium, and potassium. Although the concentrations are relatively low, these micronutrients still contribute to honey's nutritional profile, supporting functions such as bone health, red blood cell formation, and nerve function. The mineral content of honey, especially trace minerals like zinc and copper, is particularly beneficial for enzymatic processes, immune function, and antioxidant defense.

2.4 Medicinal Uses of Honey

2.4.1. Antibacterial Agent

Honey's antibacterial properties stem from its high osmotic pressure, acidity, hydrogen peroxide content, and other phytochemicals (Molan, 1992). It has been used to treat various infections, including ear and respiratory infections, meningitis, and urinary tract infections.

Honey is also effective in wound care, helping with tissue regeneration, pain reduction, and odor control. It demonstrates both bactericidal and bacteriostatic effects against a range of bacteria, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus pyogenes*, and *Salmonella typhi* (Namias, 2003).

Recent studies show that unheated, natural honey has broad-spectrum antibacterial activity against several pathogens, oral bacteria, and food spoilage organisms (Lusdy *et al.*, 2005; Mundo *et al.*, 2004). Honey's medicinal use has a long history, with traditional and alternative medicine—like apitherapy—emphasizing its nutritional and healing properties. Today, honey is used in a variety of medicinal products with standardized antibacterial properties. Ancient cultures regarded honey as both food and medicine, a belief that persists today (Onyeji, 2008).

Honey's sweetness comes from fructose and glucose, which have a similar sweetness level to granulated sugar. Honey's low water activity (0.6) prevents most microorganisms from growing. However, it can contain dormant endospores of *Clostridium botulinum*, which can pose a risk to infants if the spores transform into toxin-producing bacteria in their immature intestinal tracts. Honey also serves as a source of energy and is used in various medicinal practices. For long-term storage, honey should be kept in airtight containers at temperatures below 52°F (11°C) or within the range of 70-130°F (21-27°C) (National Honey Board, 1996).

Honey's antibacterial properties are attributed to several factors. Its high osmotic pressure, naturally low pH of around 3.9 (acidic), and the production of hydrogen peroxide, a critical element in its antimicrobial action, are key contributors (Kwakman and Zaat, 2012; Kacaniova *et al.*, 2011; Wahdam, 1998). Additionally, honey contains various phytochemicals that enhance its antibacterial potency. Its low water activity (A_w) of 0.5 to 0.62 inhibits bacterial growth, while the presence of non-peroxidase flavonoids also plays a significant role (Molan, 1992).

2.4.2 Wound Healing

Honey is widely regarded for its wound healing properties (Lusby *et al.*, 2005). It has been used to treat various types of wounds such as abrasions, abscesses, diabetic ulcers, burns, and ulcers caused by leprosy or varicose veins. Honey promotes healing by cleansing wounds, stimulating tissue regeneration, and reducing inflammation. Its application as a wound dressing also minimizes infection, and honey-soaked dressings act as non-stick bandages (Efem, 1998). Although the precise mechanism by which honey facilitates healing remains unclear, its role in providing a moist environment and preventing bacterial growth is well documented. A specific case involving a knee amputation heavily infected with *Pseudomonas* and *Staphylococcus aureus* responded positively to honey treatment, with complete healing in ten weeks (Dunford *et al.*, 2000).

2.4.3 Gastrointestinal Disorder

Honey has been shown to effectively treat gastrointestinal issues such as diarrhea and gastroenteritis. At a 5% (v/v) concentration, honey significantly reduced the duration of diarrhea in bacterial gastroenteritis cases, though it had no effect on viral gastroenteritis (Obi *et al.*, 1994; Haffejee and Moosa, 1985). Additionally, honey enhances potassium and water absorption without increasing sodium intake in rehydration fluids, and it aids in repairing damaged intestinal mucosa, promoting tissue growth, and acting as an anti-inflammatory agent. *Helicobacter pylori*, the bacteria associated with ulcers, is also sensitive to honey, and the antibacterial action of honey may explain its potential anti-ulcer effects (Al-Somal *et al.*, 1994).

2.4.4 Honey as a Wound Dressing

Honey creates a moist healing environment that effectively inhibits bacterial growth in infected wounds. Its viscosity helps shield wounds from infection, and it provides glucose to immune cells, enabling them to produce hydrogen peroxide and combat bacteria. The acidity of honey also contributes to its antibacterial activity (Efem, 1998). Furthermore, honey contains essential nutrients such as amino acids and vitamins that promote tissue regeneration. Honey's osmotic effect lifts debris from the wound bed, and its application allows for pain-free dressing changes. While some patients experience discomfort due to the acidic nature of honey, its benefits, including faster healing and reduced hospital costs, are significant (Asadi Pooya *et al.*, 2003).

2.4.5 Honey in Diabetic Infections

In clinical trials, honey has shown promise in managing infections in diabetic patients. It has a lower glycemic index than glucose or sucrose, which is beneficial for individuals with type I and type II diabetes. Honey also reduces blood lipids, homocysteine levels, and C-reactive proteins (CRP) in normal and hyperlipidemic individuals (Al-Waili, 2004).

2.4.6 Ophthalmic Applications of Honey

Honey is utilized globally in treating various eye conditions, including blepharitis, keratitis, conjunctivitis, and corneal injuries. In one study, honey ointment improved symptoms in 85% of patients with resistant eye disorders (Al-Waili, 2004). It also helps reduce redness, swelling, and discharge in cases of infective conjunctivitis, speeding up bacterial eradication.

2.5. Microbiological Aspects of Honey

Honey is a highly valued natural product known for its unique composition, including sugars, enzymes, organic acids, and natural antioxidants, which collectively contribute to its health benefits and preservation properties. Its microbiological profile, however, is shaped by a combination of intrinsic factors such as low water activity (a_w), acidic pH (approximately 3.2–4.5), and the presence of antimicrobial compounds like hydrogen peroxide, as well as extrinsic factors related to environmental contamination and handling practices. These microbial contaminants may impact honey's quality, safety, and shelf life under certain conditions.

2.5.1. Microbial Contaminants in Honey

Honey's microbiological load is generally low due to its natural antimicrobial properties. However, various contaminants can still be introduced from the environment, bees, flowers, equipment, and during storage. While most microorganisms found in honey are harmless, a few pose potential health risks, especially under conditions conducive to microbial growth, such as high humidity or dilution.

2.5.1.1. Common Microbial Contaminants

2.5.1.1.1. Bacteria in Honey

Honey, despite its antimicrobial properties, can contain a variety of bacteria, particularly spore-forming species. These bacteria are often resilient to extreme environmental conditions, allowing them to survive in honey for extended periods. The presence of these bacteria can be attributed to factors such as contamination during honey collection, handling, and storage. *Bacillus* species are among the most commonly isolated bacteria from honey due to their

environmental ubiquity and their ability to form spores that are resistant to environmental stresses. *Bacillus cereus*, a well-known pathogenic species, is often found in honey samples. This bacterium is capable of surviving in dry conditions and can proliferate in foods that are improperly stored or contaminated (Olaitan *et al.*, 2007). Although *B. cereus* is typically not pathogenic under normal circumstances, it can pose a risk to human health by producing enterotoxins responsible for foodborne illnesses, especially when honey is consumed in large quantities or stored improperly. *Bacillus* species are also known for their ability to degrade organic matter in honey, which can lead to changes in flavor, texture, and overall quality, thus compromising honey's integrity as a food product.

A particularly concerning bacterial contaminant, although rare, is *Clostridium botulinum*. This bacterium produces botulinum toxin, one of the most potent neurotoxins known. *C. botulinum* spores can survive in honey due to its low water activity (aw) and acidic pH, conditions that generally inhibit microbial growth. However, when consumed by infants under one year of age, these spores can germinate in the immature gut and produce botulinum toxin, potentially causing infant botulism, a life-threatening illness characterized by paralysis (Codex Alimentarius, 2021). The fact that *C. botulinum* spores are inert in honey but can pose a significant risk to infants underscores the importance of not feeding honey to children under one year of age. While *C. botulinum* contamination in honey is rare, the risk it presents has led to rigorous quality control measures in honey production and storage.

The presence of *Salmonella* and *Escherichia coli* in honey is typically associated with contamination during the handling and processing stages. These bacteria are often introduced through poor sanitary practices, such as contact with fecal matter, contaminated surfaces, or inadequate cleaning of equipment (Efem, 1988). Although honey's antimicrobial properties generally limit the survival of these bacteria, their presence is still concerning. *Salmonella*

species can cause foodborne illness, leading to symptoms such as diarrhea, fever, and abdominal cramps. Similarly, *E. coli* contamination may cause gastroenteritis, particularly if pathogenic strains like *E. coli* O157:H7 are present. In most cases, these pathogens are unable to survive in honey for prolonged periods due to its high sugar concentration and low moisture content. However, their detection in honey signals lapses in hygiene during production, storage, or handling, raising concerns about food safety.

2.5.1.1.2. Fungi

Fungal contamination in honey is typically associated with improper storage and handling. Fungi, particularly yeasts and molds, thrive in conditions where honey has been exposed to high humidity or fluctuating temperatures. These microorganisms can alter the quality of honey, leading to spoilage or the production of harmful metabolites.

Zygosaccharomyces rouxii is one of the most common osmophilic yeasts found in honey. Osmophilic yeasts are particularly resilient to high sugar concentrations and can ferment honey in humid storage environments. This fermentation leads to the breakdown of sugars, producing alcohol and carbon dioxide, which impairs honey's flavor, aroma, and texture, contributing to spoilage. The ability of these yeasts to survive and proliferate in honey makes them a significant concern, especially in poorly sealed containers or honey that has been exposed to air and moisture (Alvarez-Suarez *et al.*, 2014). Other yeasts such as *Saccharomyces cerevisiae* have also been isolated from honey, particularly in cases where storage conditions are suboptimal. These yeasts may contribute to spoilage by fermenting honey's sugars, further affecting its quality.

Molds, such as *Aspergillus* and *Penicillium* species, are common fungal contaminants found in honey, especially when proper post-harvest handling is not adhered to or when honey is stored

in humid conditions. These molds can produce mycotoxins, such as aflatoxins, which are potent carcinogens that pose a health risk when consumed. *Aspergillus* species, in particular, are known to produce aflatoxins and ochratoxin A, which are harmful to human health (Alvarez-Suarez *et al.*, 2014). Molds generally enter honey during improper storage or when the honey is exposed to contaminated surfaces or air. The presence of mold in honey is a sign that the product has been exposed to conditions that are not conducive to maintaining its antimicrobial properties, leading to quality degradation.

Saccharomyces cerevisiae is another yeast that has been isolated from honey. Although typically associated with fermented products like bread and wine, *S. cerevisiae* can also contaminate honey if it is stored improperly or contaminated during the processing phase (Bogdanov, 2011). Like other yeasts, *S. cerevisiae* can ferment honey's sugars, leading to a change in its texture, taste, and overall quality. While *S. cerevisiae* is not usually pathogenic, its presence in honey indicates that the storage conditions may have allowed for the growth of undesirable microorganisms, reducing the honey's shelf life and commercial value.

2.5.2. Sources of Microbial Contamination

Environmental dust and soil represent major sources of microbial contamination in honey. Honeybees inadvertently carry microorganisms from the floral nectar, pollen, and their surroundings. Spore-forming bacteria and yeasts are common contaminants in these environments, highlighting the unavoidable interaction between bees and their ecosystem (Snowdon and Cliver, 1996). Additionally, rainwater and airborne particulates can introduce contaminants during nectar collection or honey processing, further contributing to microbial presence.

The handling and processing of honey are critical stages where contamination can be introduced. During harvesting, improper hygiene or the use of unsterilized equipment can lead

to microbial contamination. Honeycombs and extraction tools, if not adequately sanitized, may harbor bacteria and fungi. In cases of extreme negligence, microorganisms such as fecal coliforms, including *Escherichia coli*, can be introduced, typically as a result of unclean processing conditions (Kacániová *et al.*, 2012). Storage practices significantly influence the microbial quality of honey. High humidity and fluctuating temperatures during storage create favorable conditions for the growth of osmophilic yeasts, which can ferment the sugars in honey and lead to spoilage. Inadequately sealed containers can also allow airborne fungal spores to colonize the honey over time.

2.6. Control of Microbial Contaminants in Honey

Honey, a natural sweetener with a rich history of medicinal and nutritional use, is susceptible to microbial contamination primarily due to environmental exposure and improper handling during processing and storage. The control and preservation of microbial contaminants in honey are essential to ensure its safety and quality for consumption. This section explores various methods and strategies employed to achieve this, supported by relevant scientific references.

Honey possesses inherent antimicrobial properties that inhibit the growth of most microorganisms. Factors contributing to these properties include its low water activity (A_w), acidic pH (typically 3.2–4.5), and the presence of hydrogen peroxide and other bioactive compounds. These properties create an unfavorable environment for microbial survival, particularly for bacteria and fungi (Bogdanov, 2017; Mondragón-Cortez *et al.*, 2013). Pasteurization is a common method used to control microbial contaminants in honey. This process involves heating honey to temperatures typically between 60°C and 70°C for a specific duration. It effectively reduces the microbial load, particularly yeasts and molds, which are responsible for fermentation (Alvarez-Suarez *et al.*, 2014). However, care must be

taken to avoid excessive heating, as this can degrade honey's nutritional and antimicrobial properties.

Filtration is employed to remove particulate matter, including wax, pollen, and dead bees, which can harbor microorganisms. Fine mesh filters or microfiltration techniques are used to ensure the removal of microbial contaminants while maintaining honey's quality (Buba *et al.*, 2013). Gamma irradiation has been explored as a non-thermal method for decontaminating honey. It is effective against a wide range of microorganisms, including bacterial spores. Research indicates that irradiation does not significantly affect the physicochemical properties of honey, making it a viable preservation method (Finola *et al.*, 2007).

Proper storage conditions are critical in preserving honey's quality and preventing microbial growth. Honey should be stored in airtight containers to minimize moisture absorption and exposure to contaminants. The storage temperature should ideally be below 25°C to inhibit yeast growth and fermentation (FAO, 2009). The incorporation of natural antimicrobial agents, such as propolis and essential oils, has been investigated to enhance honey's preservative properties. These substances act synergistically with honey's inherent antimicrobial components to control microbial contaminants (Eteraf-Oskouei and Najafi, 2013). Maintaining hygiene during honey harvesting and processing is vital in controlling microbial contamination. Equipment and containers used should be sterilized, and contact with contaminants such as dust, insects, and dirty surfaces should be minimized (Codex Alimentarius, 2020).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Sample Collection

A total of two (2) samples were collected from Uselu and Oba market, Benin City, Edo State, Nigeria. The samples were obtained using pre-sterilized, labeled 100 mL capacity bottles with perforated screw caps. Each sample was transported to the laboratory in a cooler packed with a freezing mixture of salt and ice blocks to minimize fermentation. Analysis was carried out within one hour of collection to ensure the reliability of results.

3.2 Preparation of Culture Media

The media used were prepared according to the manufacturer's instructions. The media used were MacConkey agar and Nutrient Agar.

3.2.1 Preparation of Nutrient Agar

Twenty eight (28 g) 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 min. The medium was cooled to 45-50°C and then dispensed aseptically into sterile petri dishes in the laminar flow.

3.2.2 Preparation of MacConkey agar

MacConkey agar (MCA) was prepared by dissolving 51.55 g 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 min. The medium was allowed to cool to 45°C and then dispensed aseptically into sterile Petri dishes in a laminar flow chamber.

3.3 Isolation of Bacteria

1ml of sample was dispensed into 9ml sterile water and allowed to stand for 30 minutes. The aliquot was then transferred aseptically to sterile petri plates. The prepared agar (for bacteria growth) was poured in aseptically and incubated at 37°C for 24 hrs. 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:

$$\text{Cfu/g} = \text{CFU/g} = \frac{\text{number of colonies}}{\text{volume plated} \times \text{dilution factor}}$$

3.3.1. Cultural Characteristics

Each colony morphology e.g. size, shape, margin, elevation, consistency, color, transparency was determined by physical examination.

3.3.2. Preparation of Pure Cultures

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

3.5 Morphological test on the bacterial isolates

3.5.1 Gram staining

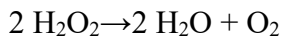
Smears of the bacterial isolates were prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal violet. This was washed out with distilled water. The slides were flooded with dilute Grams' iodine solution for one minute. This was washed off with distilled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

safranin solution for one minute. Finally, the slides were washed off with distilled water, air dried and observed under oil immersion objective.

3.6. Biochemical Test

3.5.2 Catalase Test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses 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.



3.5.3 Oxidase Test

A piece of filter paper was wet with a few drops of the dilute (1%) solution of oxidase reagent (tetramethyl-pphenylenediamine-dihydrochloride) which was prepared by standard procedure. A bit of growth from the nutrient agar slant was obtained using sterilized platinum wire loop and smeared on the wet piece of paper. Development of an intense purple color by the cells within 30 seconds indicates a positive oxidase test.

3.5.4 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 hr. Urease positive cultures produced a red-pink color due to changes in the color of the indicator.



3.5.5 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 hr. The development of deep blue colour after incubation indicates a positive result.

3.5.6 Hydrogen Sulphide (H₂S) Test

Hydrogen sulphide production can be detected by incorporating a heavy metal salt containing (Fe²⁺) or lead (Pb²⁺) 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 sulphate) forming a visible insoluble black sulphide precipitate.

3.5.7 Indole Test

Indole test is performed to determine the ability of the organism to split tryptophan molecule into indole. This test is 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 hr 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.

3.5.8 Sugar Fermentation Test

Each bacterial isolate was tested for its ability to ferment specific sugars, resulting in the production of acid and gas, or acid alone. This test serves as an essential tool for

differentiating bacterial species, particularly Gram-negative bacteria, which typically utilize various sugars as carbon and energy sources, leading to acid and/or gas production. The growth medium used for the test was peptone water. To prepare the medium, peptone water was measured into a conical flask, and bromocresol purple was added as an indicator. The prepared medium was distributed into test tubes, each containing a Durham tube to capture any gas produced during fermentation. The test tubes and their contents were sterilized by autoclaving at 121°C for 15 minutes. A 1% solution of the sugar to be tested was prepared and sterilized separately at 115°C for 10 minutes. Once cooled, 5 ml of the sterile sugar solution was aseptically dispensed into each test tube containing the peptone water and indicator. Each tube was inoculated with a young culture of the bacterial isolate and incubated at 37°C for 24 hours. After incubation, acid and gas production were assessed. A color change in the medium from light green to yellow indicated acid production, while the presence of gas bubbles in the Durham tube confirmed gas production.

3.6 Statistical analysis

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

CHAPTER FOUR

RESULTS

Figure 4.1. Present the result obtained for the mean heterotrophic bacterial and coliform counts (cfu/ml) in honey samples from various locations in Edo State. The mean heterotrophic bacterial counts ranged from $4.0 \times 10^4 \pm 0.58$ to $5.0 \times 10^3 \pm 1.15$ (cfu/ml), while the mean coliform counts ranged from $1.0 \times 10^3 \pm 0.58$ to $4.0 \times 10^3 \pm 1.15$ (cfu/ml). The highest mean heterotrophic bacterial count was observed in Oba market (OM), while the lowest was observed in Uselu (U). The highest mean coliform count was also observed in Uselu (U), while the lowest was observed in Oba market (OM).

Table 4.2 and 4.3. Present the results for the cultural, morphological and biochemical characteristics of the bacterial isolates in honey samples from various locations in Edo State. Morphological characteristics such as colony shape, size, elevation, optical activity, margination and pigmentation were taken into account. Biochemical tests were also carried out to further identify the bacterial isolates. Among the biochemical tests used were catalase, oxidase, indole and citrate tests. The bacteria isolated from the honey samples include, *Staphylococcus* sp., *Enterobacter* sp., *Shigella* sp., *Samonella* sp., *Clostridium* sp., *Lactobacillus* sp. and *Bacillus* sp,

Table 4.5 Presents the distribution pattern of bacterial isolates in honey samples collected from various locations in Edo State. The table shows that honey samples from Oba Market contained *Enterobacter* sp., *Clostridium* sp., *Lactobacillus* sp., *Salmonella* sp., and *Staphylococcus* sp., while *Bacillus* sp. and *Shigella* sp. were absent. In contrast, honey samples from Uselu showed the presence of *Enterobacter* sp., *Bacillus* sp., *Lactobacillus* sp., and *Staphylococcus* sp., whereas *Clostridium* sp., *Salmonella* sp., and *Shigella* sp. were not detected.

Figure 4.2. Illustrates the percentage frequency of occurrence of bacterial isolates in honey samples from various locations in Edo State. The most frequently occurring isolate was *Clostridium* sp., accounting for 25% of the total bacterial population, followed by *Lactobacillus* sp. at 21%. *Enterobacter* sp. and *Staphylococcus* sp. were also prevalent, with percentages of 14% and 13%, respectively. *Bacillus* sp. represented 10% of the isolates, while *Shigella* sp. and *Salmonella* sp. were the least detected, with occurrences of 8% and 6%, respectively.

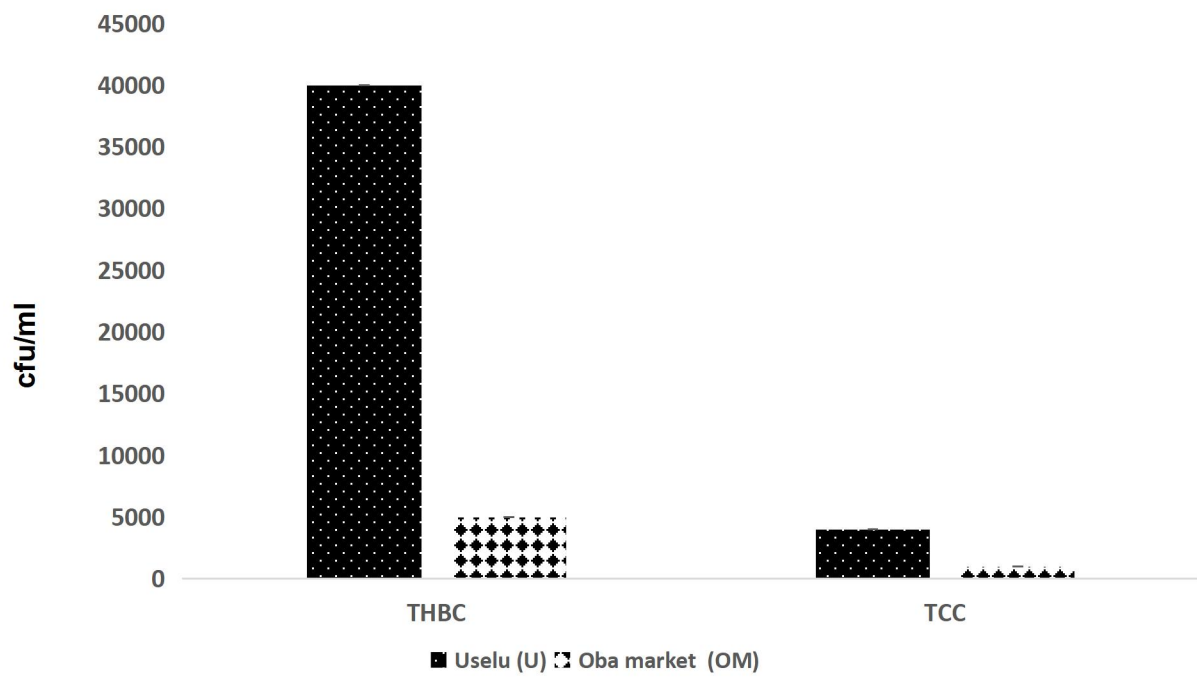


Figure. 4.1: Mean heterotrophic bacterial and coliform counts of the respective honey samples

Table 4.1: Cultural and Morphological Characteristics of the Bacterial Isolates in Honey Samples from Various Locations in Edo State.

Characteristics	B1	B2	B3	B4	B5	B6	B7
Shape	Irregular	Round	Irregular	Irregular	Irregular	Irregular	Irregular
Size	Large	Small	Large	Medium	Large	Small	Medium
Elevation	Slightly raised	Flat	Flat	Flat	Flat	Slightly raised	Flat
Transparency	Opaque	Opaque	Opaque	Transparent	Opaque	Opaque	Transparent
Margin	Lobate	Entire	Entire	Lobate	Entire	Entire	Lobate
Color (Agar 1 - NA)	-	Cream	Cream	Cream	-	-	Cream
Color (Agar 2 - MCA)	Cream	-	-	-	-	-	-
Gram Stain	-	+	+	+	-	-	+
Cell Type	Rod	Rod	Rod	Rod	Rod	Rod	Cocci
Cell Arrangement	Pairs	Clusters	Chains	Clusters	Single	Pairs	Clusters

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

Table 4.2: Biochemical Characteristics of the Bacterial Isolates in Honey Samples from Various Locations in Edo State.

Biochemical Test	B1	B2	B3	B4	B5	B6	B7	
Urease	-	-	-	-	-	-	-	
Indole	-	-	-	+	-	-	-	
Citrate	+	-	+	-	+	-	+	
Catalase	+	-	+	-	+	-	+	
H ₂ S	-	+	+	+	+	+	-	
Coagulase	-	-	-	-	-	-	+	
Oxidase	-	-	-	-	-	-	-	
Lactose	+	-	+	+	+	+	+	
Sucrose	+	-	+	+	+	+	+	
Glucose	+	+	+	+	+	+	+	
Fructose	+	+	+	+	+	+	+	
Maltose	+	+	+	-	+	+	+	
Starch	-	-	+	-	-	-	-	
Sorbitol	+	-	+	-	+	+	+	
Isolates	<i>Enterobacter sp.</i>		<i>Clostridium sp.</i>	<i>Bacillus sp.</i>	<i>Lactobacillus sp.</i>	<i>Salmonella sp.</i>	<i>Shigella sp.</i>	<i>Staphylococcus sp.</i>

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

Table 4.3: Distribution pattern of bacteria isolates in honey samples from various locations in Edo State.

SOURCE	PROBABLE ORGANISM						
	<i>Enterobacter</i> sp.	<i>Clostridium</i> sp.	<i>Bacillus</i> sp.	<i>Lactobacillus</i> sp.	<i>Salmonella</i> sp.	<i>Shigella</i> sp.	<i>Staphylococcus</i> sp.
OBA MARKET	+	+	-	+	+	-	+
USELU	+	-	+	+	-	+	+

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

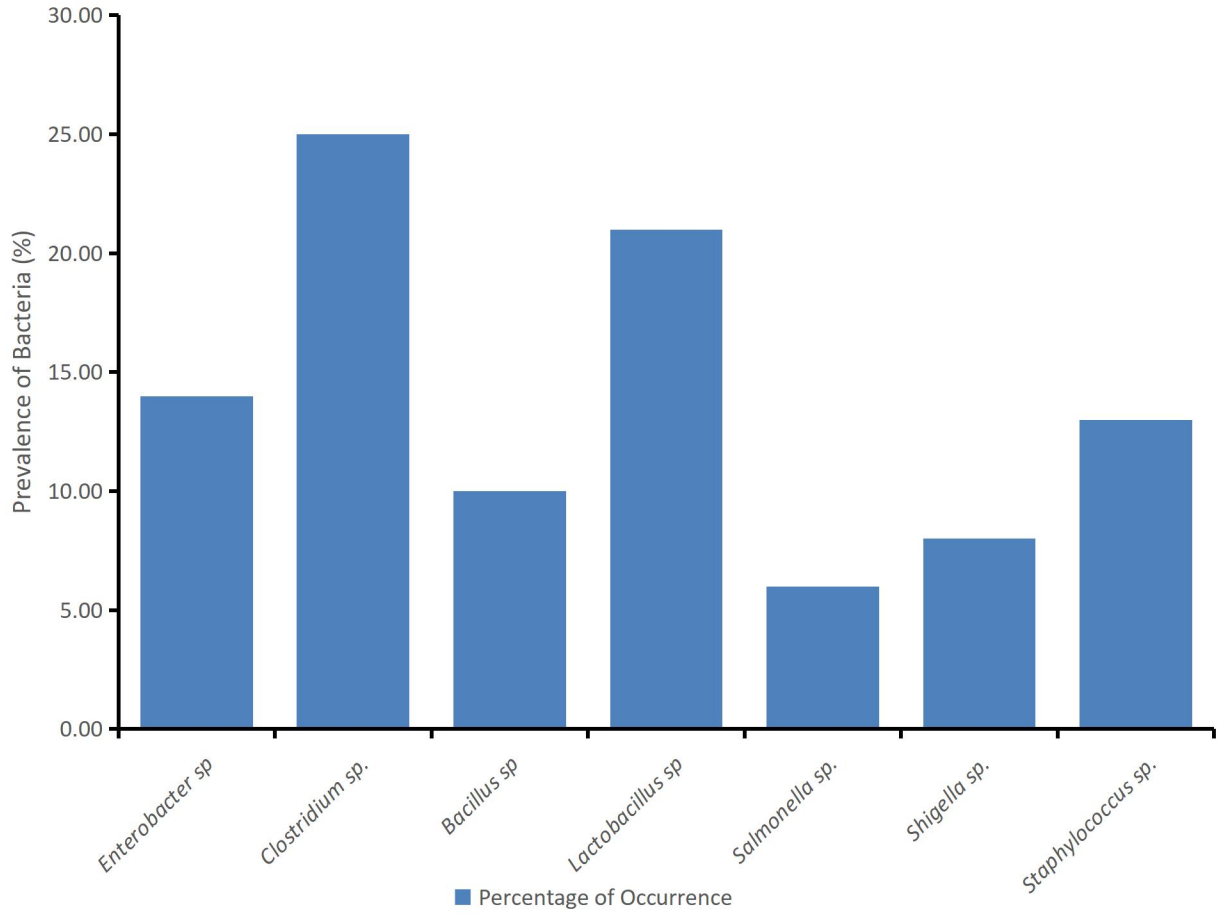


Figure 4.2: Percentage frequency of occurrence of bacterial isolates

CHAPTER FIVE

5.0. Discussion

Honey, a natural product produced by bees, has long been considered an antimicrobial agent due to its low moisture content, acidic pH, and high sugar concentration, all of which inhibit the growth of most microorganisms (Almasaudi, 2021, Mandala and Mandal, 2011). However, as shown in this study, bacterial contamination is still prevalent in honey, and the results raise concerns about the safety of honey sold in the Benin City market. .

The mean heterotrophic bacterial counts in the honey samples ranged from $4.0 \times 10^4 \pm 0.58$ to $5.0 \times 10^3 \pm 1.15$ Cfu/mL, with the highest count observed in Oba Market and the lowest in Uselu. These bacterial counts suggest that honey from certain locations may have been exposed to conditions that favor bacterial growth, such as improper storage or handling practices, or contamination during collection (Abdi *et al.*, 2024).

The presence of coliforms, which are used as indicators of fecal contamination, further supports the notion that the honey samples may have been contaminated through unsanitary handling during the production, storage, or transportation processes (Mandal *et al.*, 2011). The mean coliform counts ranged from $1.0 \times 10^3 \pm 0.58$ to $4.0 \times 10^3 \pm 1.15$ cfu/ml, with Uselu exhibiting the highest coliform contamination. This is consistent with studies that have identified significant bacterial contamination in honey, highlighting the need for better hygiene practices to prevent microbial contamination (Ayansola and Banjo, 2002; Nzeh *et al.*, 2020; Tchoumboue *et al.* 2007). The results are also in agreement with findings by Nzeh *et al.*, (2020), who reported similar bacterial contamination in honey samples in Ghana. They found that bacterial counts varied

significantly between different honey samples, depending on factors such as the source of honey, collection methods, and storage conditions. In their study, bacterial contamination was higher in honey samples that were improperly handled or stored at inadequate temperatures, similar to the findings in the present study. However the results differ from the work of Adenekan *et al.* (2010) and Malika *et al.* (2005) where coliforms were not detected in any sample of the honey samples.

A wide variety of bacterial species were isolated from the honey samples, as shown in Table 4.2, including both pathogenic and non-pathogenic organisms. The bacterial isolates identified in the honey samples include *Staphylococcus* sp., *Enterobacter* sp., *Shigella* sp., *Salmonella* sp., *Clostridium* sp., *Lactobacillus* sp., and *Bacillus* sp., The presence of these bacteria, particularly in honey consumed by vulnerable populations such as infants, raises important public health concerns. Ingestion of honey contaminated with harmful bacteria can lead to gastrointestinal illnesses, and in some cases, more severe health issues like botulism, especially if the honey is not stored properly (Al-Waili *et al.*, 2012).

Staphylococcus sp., a common inhabitant of the human skin and mucous membranes, is often implicated in foodborne illnesses such as staphylococcal food poisoning. This pathogen is capable of producing enterotoxins that can cause severe gastrointestinal symptoms (Pinchuk *et al.*, 2010). The presence of *Bacillus* sp., particularly species such as *Bacillus cereus*, in honey is concerning due to its ability to produce heat-resistant spores and enterotoxins that can lead to foodborne illnesses such as vomiting and diarrhea (Osimani *et al.*, 2018).

Similarly, *Enterobacter* sp., a member of the Enterobacteriaceae family, is commonly found in the gastrointestinal tracts of humans and animals, and its presence in honey suggests fecal contamination during handling or storage. *Salmonella* sp. and *Shigella* sp., both highly

pathogenic bacteria, can cause severe gastrointestinal diseases, including diarrhea, dysentery, and even systemic infections in severe cases (Sell and Dolan, 2016). The detection of *Clostridium* sp., a spore-forming bacterium, further underscores the need for proper hygiene, as these bacteria can survive in harsh environmental conditions and pose significant health risks if consumed in contaminated food products (Ruwandeeepika *et al.*, 2023).

Lactobacillus sp., while generally regarded as beneficial in the context of probiotics, was also found in some of the honey samples. *Lactobacillus* species are known for their ability to ferment sugars into lactic acid, which may contribute to the preservation of honey, but they can also grow in honey under certain conditions (Silva *et al.*, 2017). Their presence may reflect the ability of honey to support the growth of certain microorganisms under favorable conditions, such as in honey that is exposed to higher humidity or improper storage conditions. The variety of bacterial species identified in the present study highlights the complex microbiota present in honey and the potential for contamination with both harmful and benign microorganisms, depending on various factors such as handling, environmental exposure, and the geographical origin of the honey.

Table 4.5 presents the distribution of bacterial isolates across different honey samples collected from various locations in Benin City. Honey samples from Oba Market contained a wider diversity of bacterial species, including *Enterobacter* sp., *Clostridium* sp., *Lactobacillus* sp., *Salmonella* sp., and *Staphylococcus* sp., while *Bacillus* sp. and *Shigella* sp. were absent. On the other hand, honey samples from Uselu were less diverse and contained only *Enterobacter* sp., *Bacillus* sp., *Lactobacillus* sp., and *Staphylococcus* sp., with the absence of *Clostridium* sp., *Salmonella* sp., and *Shigella* sp..

The variation in the types of bacteria detected between the different locations may reflect differences in the hygienic practices during honey collection and handling. For instance, honey collected in Oba Market may have been exposed to more contamination due to the higher diversity of bacterial species present. In contrast, honey samples from Uselu appear to have been less contaminated. This distribution pattern aligns with research by Gashu *et al.* (2021), who found that honey samples from different geographical regions exhibited varying bacterial profiles, with higher contamination levels observed in samples from regions with less stringent sanitation and handling practices.

Figure 4.2 illustrates the frequency of occurrence of the bacterial isolates identified in honey samples. *Clostridium* sp. was the most frequently occurring bacterium, accounting for 25% of the total bacterial population, followed by *Lactobacillus* sp. at 21%. *Enterobacter* sp. and *Staphylococcus* sp. were present in 14% and 13% of the samples, respectively, while *Bacillus* sp., *Shigella* sp., and *Salmonella* sp. occurred less frequently at 10%, 8%, and 6%, respectively. The high prevalence of *Clostridium* sp. in honey samples is particularly concerning due to its potential to form spores that can survive in unfavorable conditions and lead to contamination (Salaria *et al.*, 2024). This aligns with the study of Nzeh *et al.* (2020) who also recorded *Clostridium* spp. and *Lactobacillus* spp. As the most dominant isolates in locally produced honey in the Tamale metropolis of the northern region of Ghana. *Clostridium* spp. and *Lactobacillus* spp. constituted 28 (93 %) and 27 (90 %) of the 30 honey samples analysed. The presence of *Lactobacillus* sp., although not harmful in many cases, raises questions about the conditions that support the growth of microorganisms in honey, highlighting the need for further investigation into the storage and handling conditions that allow for microbial proliferation.

The relative abundance of *Enterobacter* sp. and *Staphylococcus* sp. underscores the need for improved hygiene practices in the collection and storage of honey. These bacteria are commonly associated with human and animal sources, and their presence in honey suggests potential contamination from improper handling or exposure to unclean environments. The presence of pathogenic bacteria such as *Salmonella* sp., *Shigella* sp., and *Staphylococcus* sp. in honey poses significant public health risks. These microorganisms can cause foodborne illnesses, particularly gastrointestinal diseases. *Salmonella* sp. and *Shigella* sp. are responsible for a large number of foodborne outbreaks worldwide, often resulting in severe symptoms such as diarrhea, vomiting, and abdominal cramps (Bintsis, 2017).

While honey is known for its natural antimicrobial properties, these findings highlight the importance of proper handling, storage, and sanitation practices to prevent bacterial contamination. Honey sold in markets must be processed and stored under conditions that minimize the risk of contamination. It is essential that vendors, beekeepers, and regulatory bodies ensure that honey is collected and stored in clean environments and at appropriate temperatures to prevent the growth of harmful microorganisms.

5.1 Conclusion

The microbiological analysis of honey samples sold in Benin City, Edo State, revealed widespread bacterial contamination, including both pathogenic and non-pathogenic microorganisms. The diversity of bacterial species isolated from the samples suggests that honey may be susceptible to contamination during production, handling, or storage. The findings of this study emphasize the need for improved hygiene practices and regulatory oversight to ensure the

safety of honey sold to consumers. Further research is necessary to explore the factors contributing to microbial contamination and to develop effective strategies to mitigate these risks.

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