MICROBIOLOGICAL ASSESSMENT OF SELECTED READY-TO-EAT FRUITS SOLD IN AN OPEN MARKET IN BENIN CITY, NIGERIA.

BY

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UNIVERSITY OF BENIN
BENIN CITY, EDO STATE.

NOVEMBER 2025.

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

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CERTIFICATION

This is to certify that this project work was successfully carried out by **PERPETUAL OLUCHI IMOH** with the matriculation number of **LSC2103950** of the Department of Microbiology Faculty of Life Sciences, University of Benin, Benin city in Edo state under my supervision.

_____ _____

DR. CHRIS AJUZIE
(Project supervisor). Date

_____ _____

PROF. (Mr.) E.O. IGBINOSA
(Head of Department) Date

DEDICATION

I dedicate this report to God almighty for everything he has done for me, for making me attain this height so far. Also dedicating this project to my beloved parents (MR&MRS IMOH), for their great love, encouragement, and financial support towards me.

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The potential public health risk associated with ready-to-eat (RTE) fruits sold in open markets was investigated through a microbiological assessment of three popular fruits: Apple, Pineapple, and Watermelon. A total of three samples were purchased from a market in Benin City and analyzed using standard microbiological techniques, including serial dilution, plating on selective media, and biochemical analysis. The mean total bacterial count was highest in Pineapple and lowest in Apple, while the mean total fungi count was highest in Apple and lowest in Watermelon. Bacterial isolates included *Staphylococcus aureus*, *Bacillus* spp, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterobacter*. Fungal isolates included *Aspergillus niger*, Yeasts, *Penicillium* species, and *Mucor*. Antibiotic susceptibility testing revealed isolates with resistance to multiple drugs, including Rocephin, Pefloxacin, and Ampliclox (for Gram-positive bacteria), while showing susceptibility to agents like Ciprofloxacin, Gentamicin, Levofloxacin, Ofloxacin, and Amoxicillin. The presence of these pathogenic and opportunistic micro-organisms confirms the contamination of the RTE fruits, which poses a significant public health hazard. This poor microbiological quality is likely due to unhygienic preparation environments, contaminated water, improper handling, and environmental exposure. Regulatory bodies must enhance surveillance, and vendors should strictly adhere to safe food handling practices. Consumers are advised to ensure proper washing of fruits before consumption.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Ready-to-eat foods (RTEF) typically include pre-prepared fruits and vegetables that are displayed by street vendors or market sellers and are consumed instantly without requiring additional processing (Oranusi and Olorunfemi, 2011; Mengistu and Tolera, 2020). These ready-to-eat fruits (RTEF) are fruits that have been cut open and carried around by hawkers on the street, and are eaten immediately without washing or having to cut or peel them before consuming, because they have been packaged already by vendors (Kaplan and Compbell, 1982; Lund, 1992; De Rover, 1998). They are wrapped in small transparent nylon for sale.(Orji *et al*., 2016). Fruits are a vital component of a healthy diet, providing essential nutrients, micronutrients, vitamins, and dietary fiber that promote overall well-being (Jerry *et al*., 2016). A balanced diet rich in fruits has been shown to prevent hypovitaminosis and certain diseases, particularly those related to Vitamin A and C deficiencies (Jerry *et al*., 2016). A diet rich in ready-to-eat fruits reduces the chances of getting chronic illnesses including cardiovascular, cerebrovascular and skin diseases, and also helps in preventing diabetes, high blood pressure,

cataract and certain cancers. (Mercanoglu Taban and Hallman 2011; September Malaterre *et al*., 2018). Fruits such as Pineapple, Apple, Watermelon, Orange, Banana, have become known due to their high nutritional value and convenience. Consuming these ready-to-eat fruits is beneficial in enhancing micronutrients deficiency among the population in a country (Balali *et al*., 2020). However, fruits are also susceptible to microbial contamination from various sources which includes the soil, dust, water, and improper handling during harvesting and post-harvest processing (Kalia and Gupta, 2016). This contamination can accommodate a lot of microorganisms, including pathogens, on the fruit (Ogofure *et al*., 2017). The nature of fruits makes them vulnerable to microbial contamination and poor handling practices further contribute to the spoilage of fruits (Mailafia *et al*., 2013). According to (Uzor and Dick 2022), street vendors with limited education often handle and package sliced fruits like Pineapple, Watermelon, Apple, Orange, and Pawpaw, which are commonly sold in markets. However, these vendors typically lack sufficient knowledge and training in food safety and adequate hygiene practices (Muinde and Kuria, 2005). Generally, fruits are known to carry a natural non-pathogenic microflora, and they possess an outer skin layer of cells that resist the penetration of microorganisms (Ogofure *et al*., 2017). This can lead to cross-contamination of fruits due to unhygienic processing and packaging methods. Common foodborne pathogens associated with fresh produce include *Escherichia coli, Salmonella* spp., *Staphylococcus aureus, Listeria monocytogenes*, and *Shigella* spp. (Amoah *et al*., 2017; Oranusi *et al*., 2012). These pathogens gain entrance to these fruits during washing, peeling, cutting, trimming, packaging and handling (Barro *et al*., 2007; Khali and Mazhar 1994). The use of dirty utensils such as knives and trays, as well as the display of these fruits in a sordid environment attracts insects such as cockroaches and flies (Bryan *et al*., 1992). Furthermore, the use of untreated waste water and organic manure for fruit cultivation in some regions contribute to contamination, World Health Organisation (WHO, 2015). Increased in the consumption of contaminated ready-to-eat fruits in Nigeria has led to outbreaks of foodborne diseases promoting the need for proper assessment and stricter measures on food safety (Pradhan *et al*., 2019). However, the safety of fruits, particularly those sold in open markets in Benin city, is a growing concern due to the risk of microbial contamination. In Benin city, where open markets are a common platform for fruit sales, there is a need to assess the microbiological quality of ready-to-eat fruits to ensure consumer safety.

1.2 Aim:

The aim of this study was to assess the microbiological quality and safety of selected ready-to-eat fruits

1.3 Objectives of the study:

1. To isolate and characterize micro-organisms(Bacteria, Fungi) associated with ready-to- eat fruits.
2. To determine the microbial load content in ready-to-eat fruit.
3. To determine the antibiotics sensitivity pattern of bacterial isolates.
4. To determine the multi-drug resistance index of bacteria isolates.

CHAPTER TWO

2.0 LITERATURE REVIEW

Fruits are widely acknowledged for their vitamin and mineral content, along with their high nutritional value (Chumber *et al*., 2007). People use fresh fruits for making various consumables like juices, jams, beverages, wines, or eat them directly because of their nutritional value (Little and Metchell, 2004). In many tropical countries, fruits are a common food for people and are often sold in public places and roadside shops. There have been reports of foodborne illnesses linked in consuming fruits, which mainly affect the gastrointestinal tract and can be transmitted through eating contaminated fruits (Ambekar *et al*., 2008). A fruit is the mature ovary of a flowering plant that is made up of seeds and serves to spread for fertilization or reproduction.(Ellie M *et at.,* 2020). Fruits are also a part of the flowering plants that are gotten from a particular tissue of the flower, containing more than one ovary, and some accessories tissues. Humans and animals are dependent on fruits as a source of food (Lewis R.A *et al*., 2002)

2.2 *Citrullus lanatus* (Watermelon)

2.2.1 Scientific Classification of Watermelon

Kingdom: Plantae

Order: Cucurbitales

Family: Cucurbitaceae

Genus: *Citrullus*

Species: *lanatus*

2.2.2 *Citrullus lanatus* (Watermelon): The watermelon (*Citrullus lanatus*), also known as *Citrullus vulgaris*, is a succulent fruit and a vinelike plant belonging to the gourd family (Cucurbitaceae). Native to tropical Africa and now cultivated worldwide. The fruit is a good source of vitamin A and contains some vitamin C, and

is typically eaten raw. The rind can also be preserved and made into a pickle. Watermelons have a long history, with evidence of their cultivation going back more than 4,000 years. An ancient Sanskrit word for watermelon exists, and the fruit is depicted in early Egyptian art. Over time, domestication and selective breeding have led to the development of intensely sweet, large fruits with tender flesh and fewer seeds. Some modern “seedless” varieties have almost no viable seeds. The fruit has a smooth hard rind usually green with dark green stripes or yellow spots and a juicy, sweet interior flesh usually deep red, orange, yellow or white with numerous seeds.

2.2.3. History of Watermelon

Watermelon is native to Southern Africa, where it originally grew wild. In the 1800s, botanist Alphonse de Candolle identified it as indigenous to tropical Africa. Archaeological evidence shows it was cultivated in the Nile Valley, and seeds have been found in Twelfth Dynasty sites and in the tomb of Tutankhamun. Cultivation spread to India by the 7th century, and by the 10th century it had reached China. Today, China produces more watermelon than any other country in the world.

2.2.4. Cultivation and Harvesting of watermelon

Watermelons are tropical plants that can survive under temperatures greater than 25°C. Seeds are firstly sown in pots under cover and transplanted into well drained sandy loam with a pH between (5.5-7.0) and medium nitrogen levels. If humidity is high, the plant is prone to insects and plant diseases like powdery mildew and mosaic virus (Brickell *et al*.,1992). Watermelons have a longer period of growth than other melons and can often take 85days or more from the time of transplanting for the fruit to mature. Watermelons are considered optimum for eating when their flesh matures to produce a sweet flavour, crisp texture and deep red colour. Some newer cultivars, however, range in colour from light red to yellow. Determining the maturity of melons without tasting each is difficult. External rind appearance does not always predict good internal flesh quality and full maturity of the fruit. Harvesting and handling costs are much higher than growing costs. Therefore, melons must be harvested at the proper stage of maturity and handled carefully to avoid damage and to ensure market quality (Brickell *et al*.,1992). Watermelons are cut from the vine rather than pulled, twisted or broken off. Pulling stems out creates an entrance for micro-organisms(Bacteria and Fungi) to invade. It can cause souring and also deteriorates the internal flesh. As melons are cut from their vines, the bottoms, which are subject to sunscald, are turned down. Direct contact from the sun can affect the quality of watermelon after harvest. Sunburn develops very fast on melons that are exposed whether on the ground or loaded in a truck. Temperatures above 90° F cause internal flesh breakdown and increase decay. These effects may need several days to become apparent. Shading is a necessary protection against direct sunlight and heat (Brickell *et al*., 1992).

2.2.5. Diseases and Pests

Diseases are vital in determining the success or failure of the production of watermelon. Certain diseases have destroyed the entire watermelon crops in some areas while weather conditions favoured their development. If disease control practices are not followed, some loss can be expected every year from foliage and stem diseases. All watermelon foliage diseases spread in a similar manner. Some diseases can easily be brought into an area in or on the seed. Seed grown in dry, arid regions have the best chance of being free of seed-borne diseases. Locally grown seeds are more likely to be infested with diseases (Larkin and Fravel, 1998). Disease-

causing fungi can live from year to year on undecayed vines of watermelon and such fungi produce millions of spores on susceptible plants. The spores are sticky when wet, any time humans, animals or machines move through wet vines, these diseases can be spread. Splashing rain or runoff water can also spread these fungi from one area to another. The amount of disease pressure in any given year is directly related to environmental conditions. Rain is the most important factor in the spread of foliage diseases. Under ideal weather conditions, some diseases can destroy entire fields within a few days. Watermelons suffer from similar diseases and pests like the cucumber like gummy stem blight, anthracnose, fusarium wilt, downy mildew, aphids, thrips, root maggots, wireworms etc. Damping-off is caused by a seedling disease complex that usually involves *Phythium* spp, *Rhizoctonia* spp or *Fusarium* spp. The amount of damping-off is usually directly related to litter from the previous crop and to environmental conditions. In some years, seedling diseases reduce stands by 50 percent; in other years, seedling diseases are rare. Good cultural practices and seed treatment are essential in preventing damping-off of young watermelon seedlings. Root-knot nematodes are small eel-like worms that inhabit the soil and feed on the roots of plants. Root-knot nematodes cause severe damage to watermelons when planted to infested fields. All species of root-knot nematodes are capable of causing serious damage to watermelons. The lack of a good, easy-to-use nematicide for watermelons makes the worms a major problem. Although chemicals like fumigants are the best for reducing the population of nematodes, the waiting period required after application can cause delays in planting (Gordon and Martyn, 1997). Watermelon mosaic virus I and II are now known as papaya ringspot virus-watermelon type (PRSV-W) and watermelon mosaic virus (WMV), respectively. These are two common viruses that are present in watermelons. Several other viruses affect watermelon; all have similar symptoms. The most common symptom is mottling of the leaf. However, mottling may be hard to see under certain weather conditions. Some plants are stunted with abnormal leaf shapes, shortened internodes and bushy erect growth habits of some runner tips. Bumpy and mottled appearance of the surface of the fruits shows the first symptom on the fruits. This disease symptom is strongly expressed in periods of extended high temperatures, which occur just before watermelon harvests. These viruses are spread by aphids, which can spread through an entire planting during the growing season.

2.2.6. Nutritional value of watermelon:

Every part of the watermelon, from the rind to the seeds, has nutritional value. People most often eat the pinkish-red flesh raw, just as it grows. Other common preparations include pickled watermelon rind, deep-fried watermelon, watermelon cake, and watermelon lemonade. (Tlili *et al*., 2011).

In southern Africa, this plant has been grown alongside crops like maize and sorghum since pre-colonial times. Its young leaves and immature fruits are cooked as greens, and the flesh can be prepared as a porridge with maize meal. It also serves as important livestock feed, particularly during droughts. The hollowed-out fruit is sometimes used to store berries, and the pulp and seeds are consumed after being prepared in various ways. The flat brown seeds have a much higher food value than the flesh and have a nice nutty taste. Significant amounts of vitamin C, minerals, fat, starch and riboflavin have been obtained from them. They can be dried, roasted and eaten as such or ground into flour to produce bread. The flour is made of saponin

and is also used as a detergent. The seed consists of a high percentage of oil which is similar to pumpkin seed oil and can be used in cooking (Tlili *et al*., 2011). In West Africa, the seeds are made into pulp and added as thickener to soups. They are also fermented to produce a sweetener locally called 'ogiri' or they are pounded, roasted, or wrapped in leaves and then boiled to produce another type of sweetener called 'igbãlo'. The residue from oil extraction is made into balls that are fried to produce a local snack called 'robo' in Nigeria, or is used as cattle feed (Loiy Elssir *et al*., 2011). Immuno-histochemical analysis showed watermelon effectively protected pancreatic cells from death, which suggests that watermelon has a beneficial effect on diabetes (Jiyun *et al.*, 2011). Watermelon has also shown to have a significant gastroprotective effect in Indomethacin-induced gastric ulceration (Francis *et al*., 2013). Watermelon acts as a good antioxidant, anti-inflammatory and analgesic potential and may be used as a future food medicine (Gill *et al*., 2010). The results of the extraction of aqueous fruit pulp from *Citrullus lanatus* (watermelon) showed a significant laxative activity of watermelon (Swapnil *et al*., 2011).

2.3. *Ananas comosus* (Pineapple)

2.3.1 Scientific Classification of Pineapple.

Kingdom: Plantae

Order: Poales

Family: Bromliaceae

Subfamily: Bromeliodeae

Genus: *Ananas*

Species: *comosus*

2.3.2. History of Pineapple:

The pineapple is a herbaceous plant that can be biennial or perennial, native to tropical regions. It produces a multiple fruit made of fused berries and is the most economically important species in the Bromeliaceae family. Valued worldwide for its sweetness and distinctive flavor, pineapple is considered one of the most desirable fruits. It provides vitamins A and B, is a fair source of vitamin C, and contains minerals such as calcium, magnesium, potassium, and iron. Pineapple is also known for bromelain, a digestive enzyme. The fruit is eaten fresh, cooked, juiced, or preserved. Christopher Columbus and his crew encountered pineapple in 1493 on an island in the Lesser Antilles. In 1557, a Brazilian clergyman first recorded the name "Ananas," derived from the Tupi word Nana. The English name "pineapple" arose because the fruit's scaly exterior resembles a pine cone. It has various names across languages, piña in Spanish, abacaxi in Portuguese, ananas in Dutch, German, French, and in former French and Dutch colonies, and nanas in parts of South and Southeast Asia. (McKenzie et al., 2010).

2.3.3. Cultivation and harvesting of Pineapple:

Pineapples can be grown in a variety of soil types but prefer mildly acid soils within the pH of (5.5 - 6.5). However, there are specific requirements for successful production of pineapple, which include: Soil preparation,, Trees removal, stumps and stones, subsoil (rip) to a depth of

800 to 900mm under dry conditions, ploughing and tilling of the soil a number of times, to achieve a fine tilt, for effective plant rooting ridge the soil for better drainage, temperature and to improve aeration and have soil samples analysed at least 6 months before planting to determine fertilization and fumigation requirements. Unlike many other crops grown from seed, pineapples are grown by planting different parts of the plant according to the cultivar, where it is going to be produced, and the cultivation methods practised in the area. Although crowns are mostly used as planting material for the Cayenne cultivar, they are considered uneconomical for the Queen cultivar because of the long period of time they take to develop. Suckers are planted in the case of Queen Pineapple production. Pineapples are grown between July and December and they are grown on clayey, loamy or sandy soils (Sanewski and Scott, 2000). Pineapples contribute to over 20% of the world production of tropical fruits and as a crop are second only to bananas as the most important harvested fruit.

2.3.4 Diseases and Pests of Pineapple: The pineapple plant is most productive under a dry environment where low rainfall is supplemented by irrigation in well-drained soils. Once the root system (adventitious roots) is damaged or destroyed, it does not regenerate significantly. Several characteristics of the pineapple plant and commercial pineapple production systems have contributed to the severity of several pest and disease problems (Rohrbach *et al.,* 2003). Common pests infesting vegetative propagates (crowns, slips, suckers) are mealy bugs, scale and pineapple red mites. In addition to these pests, the diseases termed heart rot, root rot, fruit rot and butt rot may be major problems when handling, storing or planting fresh pineapples (Pegg *et al.,*1993). The bacterial heart rot and fruit collapse is caused by a bacterium called *Erwinia chrysanthemi.* It causes water-soaked lesions on the white basal sections of leaves in the central whorl giving it an olive green colouration with bloated appearance. This disease progresses from the juice of the infected fruits and gain entrance to the leaves through the wounds made by ants. The butt rot, black rot and white leaf rot are caused by the fungus called *Chalara paradoxa* which causes a soft rot that begins from the area where the seed detaches from the mother plant; the whole seed may become rotten; black rot of fruit causes a soft watery rot which darkens with time. The fungus survives in soil and pineapple residue. Marbling is another disease caused by a bacterium, *Acetobacter* spp, *Erwinia herbicola*, which causes a yellow to red or very brown discoloration of fruit flesh. The infected tissues develop a granular texture with woody consistency and speckled colour and the emergence of the disease is favoured by warm, wet weather (Rohrbach *et al*., 2003). These diseases can be prevented and controlled by removing or destroying the infected fruits, use of insecticides to control ants; seed material should be properly stored and the removal of debris.

2.3.5. Nutritional value of Pineapple The nutritional content of pineapple can vary significantly between varieties as illustrated by the Vitamin C content of the pineapple varieties. In addition to the fruit the pineapple plant also provides fibre (used in clothing and industry) and the enzyme bromelain (Arshad *et al.,* 2014). Bromelain's medicinal uses include relief for arthritis sufferers, as a digestive aid, in the reduction of blood clotting, as an anti-inflammatory agent, and for skin debridement of burns (Chaurasiya *et al*., 2015). Bromelain also has industrial uses including meat tenderisation, clarification of beer, production of vegetable oils and the dehydration of eggs and soya milk. Bromelain content is

known to vary by up to 50% between varieties. **2.4. *Malus domestica* (Apple) 2.4.1 Scientific classification of Apple**.

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Rosale

Family: Rosaceae

Genus: *Malus*

Species: *domestica*

2.4.2 History of Apple

Apple trees (Malus domestica) are perennial plants.They produce fruits for many years. Apples are a temperate crop, they need a required number of chill hours to produce fruit and do not thrive in tropical lowlands. Apple belongs to the Rosaceae family. Apples are a source of vitamin C and contain some vitamin K. They are also rich in fiber and antioxidants. Apples (*Malus domestica*) have traces back to Central Asia, specifically the mountains of Kazakhstan. Wild apples (Malus sieversii) are considered the ancestors of today's domesticated apple spread. Apples were spread through trade routes like the Silk Road to Europe, Asia, and beyond cultivation. It have been cultivated for thousands of years. Apples were brought to North America by the European colonizers and have been a staple fruit in many cultures, used in food, drinks, and traditions. They are typically harvested in late summer to autumn depending on the variety and location. Timing varies by climate and apple type (like early season, mid-season, or late-season varieties). Apples are picked by hand or with mechanical aids and are picked when they are ripe for eating fresh or for processing. Apples can be stored for months in controlled conditions to extend availability. Apple trees need proper care like pruning and pest management. Trees usually start bearing fruit between (2-5) years after planting.

2.4.3. Diseases and pest of Apple Apple trees (*Malus domestica*) are susceptible to various diseases which are Apple scab (*Venturia inaequalis*), Fungal disease causing lesions on leaves and fruit *Powdery mildew*, *Podosphaera leucotricha* affects leaves and shoots. Fire blight (*Erwinia amylovora*), bacterial disease damaging blossoms, leaves, branches.Codling moth (*Cydia pomonella*) major pest causing damage to fruit Aphids sap-sucking insects affecting tree mites can cause damage to leaves of plants health.

2.4.4. Nutritional value of Apple Apple are low in calories about 52 calories per 100g. They are a good source of dietary fiber about (2.4g per 100g) and contain vitamin C. Antioxidants rich in antioxidants like quercetin, catechin. Fiber in Apple aids in digestion. Antioxidant effects may help reduce risk of chronic diseases.

Fiber and antioxidants may support heart health. Nutrients vary by apple variety, ripeness, and growing conditions. Apples (Malus domestica) are a nutritious fruit with various health benefits.

2.5 MICRO ORGANISMS ASSOCIATED WITH CONTAMINATION OF FRUITS. Many of these organisms are normally non-pathogenic for humans. The numbers of bacteria present will vary depending on seasonal and climatic variation and may range from 10^2 to 10^4 per gram. The inner tissues of fruits and vegetables are usually regarded as sterile (Lund *et al*., 1992). However, bacteria can be present in low numbers as a result of the uptake of water through certain irrigation or washing procedures. If these waters are contaminated with human pathogens these may also be introduced. About two thirds of the spoilage of fruits and vegetables is caused by moulds. Members of the genera *Penicillium, Aspergillus, Sclerotinia, Botrytis* and *Rhizopus* are commonly involved in this process. The primary pathogens involved in the consumption of contaminated fruits include *Escherichia coli, Salmonella enterica, shigella* spp*, Listeria monocytogenes*. (Oliveira M, *et al*., 2015).

2.5.1. *Escherichia coli:*

The *Escherichia coli* O157:H7 strain is a major food safety concern, especially in meat and poultry, because it has been linked to numerous outbreaks and sporadic illnesses. Most *Escherichia coli* strains live naturally in the guts of humans and other mammals. People usually get infected with pathogenic strains by eating contaminated food such as undercooked meat or tainted produce or through direct contact with animals that carry the bacteria (Yoo BK, *et al.,* 2015). The infection can also spread directly between people when hygiene is poor. Food, drinking water, irrigation water, and recreational water can all become contaminated if they come into contact with fecal material.(Berger CN *et al*., 2010) Since *Escherichia coli* O157:H7 is found in livestock feces, it is essentially unavoidable in animal manure and slurry (Kudva *et al*., 1998). Beyond farm animals, *Escherichia coli* O157:H7 has also been found in the feces of wild birds like starlings (Moller Nielsen *et al.,* 2004) and gulls (Wallace *et al.,* 1997). Leafy greens and fresh fruit are the foods most often tied to *Escherichia coli* infections. Apple juice is an exception worth noting its acidity usually inhibits bacterial growth. *Escherichia coli* O157:H7 is commonly present in ruminant feces, so when livestock graze in orchards, fallen apples can become contaminated (Stopforth *et al.,* 2004) The bacteria can also multiply in damaged apple tissue, which creates a risk of contamination in unpasteurized juices and ciders (Janisiewicz *et al.,*1999). Fruit flies have been shown to act as a vector for *Escherichia coli* O157:H7 as well, spreading it to apples both before harvest and in packing facilities..

2.5.2. *Salmonella* spp: *Salmonella* species are an important cause of gastrointestinal illness in humans. *Salmonella enteritidis* and *Salmonella typhimurium* are the most frequently reported non-typhoidal serotypes in many countries and outbreaks have been associated with a diverse range of food vehicles. Salmonellosis is characterised by diarrhoea, fever, abdominal cramps and vomiting usually lasting 4-7 days (Anon *et al*., 2001). Most *Salmonella* infections resolve on their own, but in a small number of cases they can progress to bacteraemia. In industrialized countries, the case-fatality rate remains below 1% (Anon *et al.,* 2001).

2.5.3. *Shigella* spp: *Shigella sonnei* is primarily spread from person-to-person route although food and waterborne

transmission can occur. *Shigellosis* can be endemic in institutional settings such as prisons, markets, hospitals and nursing homes, where population densities are high and poor hygiene conditions may be present. *Shigella* infection usually presents as abdominal cramps, fever and diarrhoea, which may contain blood and mucus. The duration of illness ranges from (4-7) days (Anon *et al*., 2001).

2.5.4 *Vibrio cholerae*:

Cholera epidemics are caused by *Vibrio cholerae* serogroups O1 and O139. It is mainly spread through contaminated water, and a large dose of bacteria is usually required to cause infection. Even so, several outbreaks linked to fruits and vegetables have been documented. (Wachsmuth *et al.* 1994; Faruque *et al*. 1998; Anon 2001). The hallmark profuse watery diarrhea in cholera results from a heat-labile enterotoxin produced by the bacteria in the intestine. Symptoms appear quickly, and if untreated, the disease can cause severe dehydration and death within hours. Untreated illness typically lasts 3–7 days. (Anon *et al*., 2001).

2.5.5 *Pseudomonas* species:

Pseudomonas species grow readily in moist settings like soil and water, and are often present in high numbers on fresh produce. They can contaminate fruits and vegetables when sewage-polluted water is used for washing or rinsing. Species such as *Pseudomonas syringae*, along with related types including *Pseudomonas avellanae*, *Pseudomonas avii*, and *Pseudomonas savastanoi* cause significant diseases in many plants, particularly fruit trees. The resulting damage lowers crop yields through blossom blast and fruit lesions, and can also destroy scaffold branches and create cankers that may eventually kill the trees (Megan *et al*., 2007).

2.5.6 *Staphylococcus aureus*:

Staphylococcus aureus is a leading cause of food poisoning. Illness results from consuming enterotoxins that form in food left at room temperature. (Walls and Scott, 1997). Poor storage of fruits and vegetables can therefore allow this bacterium to multiply. There are five classical staphylococcal enterotoxins SEA, SEB, SEC, SED, and SEE including newer enterotoxins and enterotoxin-like superantigens ranging from SEG to SEU (Chiang *et al*., 2008). Outbreaks are often linked to produce that is handled extensively during preparation and then stored without refrigeration. *Staphylococcus aureus* can grow between 7–48 °C, with optimal growth at 35–37 °C, which is common in warm climates. (Bazaar *et al.,* 2007).

Foodborne viruses originate in human feces. Many of these infections are mild and short-lived, and most cases go undetected because routine testing doesn't screen for all viruses known to cause infectious intestinal disease. Spread occurs mainly through person-to-person contact, inhalation of airborne droplets, and fecal contamination(Hedberg and Osterholm). Numerous outbreaks tied to fresh fruits and vegetables have been reported. Using (Kaplan *et al.,*1982) epidemiological criteria, an estimated 32–42% of foodborne enteric infections in the U.S. are viral, particularly those affecting the digestive tract.

2.6. MICROBIAL ASSESSMENT OF SOME VENDED FRUITS.

Microbial risk assessment is a systematic method for understanding intricate food systems. It changes the potential for pathogens in food preparation, processing, and production environments into reports detailing the likelihood and extent of a food safety risk, defined by negative public health outcomes (Havelaar *et al*., 2010). Due to the

significant health risks associated with ready-to-eat fruits, various experiments have been conducted to determine their safety for consumption. **Table 1** shows the microbial assessment of vended fruits carried out by (Orji *et al.,*2016). He evaluated the microbial contamination of ready-to-eat (RTE) fruits in Abakpa main market Abakaliki southern part of Nigeria. It was done using standard microbiological methods. About 17 fruit samples (Watermelon, Pineapple, Tigernut, Carrot, Cucumber) were used and screened to determine the total bacteria count and Fungi counts. The analysis shows that Total aerobic plate count range from 3.5×10^5 to 1.03×10^6 cfu/ml with the tiger-nut having the greatest count while cucumber has the least count. The total fungal count ranged from 1.1×10^5 to 1.42×10^6 cfu/ml with carrot having the greatest count while sliced pineapple have the least count, whereas tiger-nut had no growth at all. The isolated organisms in all studies including their infections are shown in **Table 2.** The organisms isolated from the fruits were *Escherichia coli, Salmonella* species, *Staphylococcus, Pseudomonas*, and *Mucor* which is the only fungal isolate present in the ready-to-eat fruit. Another study was carried out by (Ugwu and Edeh, 2019). Forty (40) ready-to-eat fruit samples (Pineapple, Watermelon, Cucumber, Pawpaw) were purchased from a fruit vendor in the market and were screened to ascertain the microbial count. The results showed that the microbial count. The result shows that the mean bacteria load of the samples ranged from 6.0×10^5 to 8.2×10^5 cfu/g with pineapple and watermelon showing the greatest count while the mean fungi load ranged from 1.3×10^5 to 1.7×10^5 cfu/g with pawpaw showing the greatest count. The total of five bacterial (*Escherichia coli, Klebsiella*, *Shigella, Salmonella, Staphylococcus*) species and two Fungal (*Aspergillus and candida*) species were isolated. *Staphylococcus aureus* (70%) had the greatest occurrence, *Escherichia coli* (62.5%), *Salmonella* (50%), *Klebsiella* (40%), *Shigella* (37.5%), *Candida*(37.5%) and *Aspergillus* (17.5%) which had the smallest count. (Ugwu and Edeh, 2019) declared that the detection of these organisms in the screened fruits shows poor hygiene practices during processing of vended fruits. In another study carried out by (Oranusi and Olorunfemi, 2011) on the safety evaluation of street sliced RTE fruits that were sold. The total of sixty (60) samples of ready-to-eat fruits which includes (sliced watermelon, sliced pineapple, apple, sliced pawpaw and packaged fruits salad) were purchased from vendors from the local market and university cafeteria. Thereafter, they were subjected to microbial count. The mean total aerobic plate count ranges from 2.0×10^6 to 8.2×10^8 cfu/g on pineapple and watermelon purchased from the local market and from 6.0×10^4 to 2.7×10^7cfu/g on apple and fruit salad from the cafeteria of the university. The samples were all contaminated with coliform and fungi count ranging from 2.2×10^5 to 4.2×10^6cfu/g and 2.0×10^1 to 1.0×10^3cfu/g in the samples from the cafeteria and 2.0×10^5 to 3.5×10^6cfu/g and 2.0×10^2 to 1.1×10^3cfu/g in the samples from local market. Organisms found were (*Escherichia coli*, *Salmonella* species, *Staphylococcus aureus, Pseudomonas* species, *Proteus*, *Enterobacter, Klebsiella, Bacillus*, *Penicillium* species, *Mucor* species, *Aspergillus niger, Pseudomonas aeruginosa, micrococcus*, *Lactobacillus* species). He concluded that the coliform found with a count of atleast 10^6 in some samples shows the sanitary quality of the processing of the produce. Therefore the consumption of RTE fruit was claimed to be unsafe. Apart from the case study in Nigeria, different experiments on the quality of vended fruits have been carried out in some other countries such as (India, Bangladesh, Ghana, U.S.A). **Table 1**: Shows microbial assessment of ready-to-eat fruits and organisms isolated from it.

Vended fruits tasted	**Microbial assessment**	**Organisms found**	**References**
Sliced Pineapple, Watermelon, Cucumber, Carrot, Tiger-nut	Total bacterial count and fungal count	*Escherichia coli*, *salmonella* spp, Shigella spp, *staphylococcus aureus, Pseudomonas aeruginosa,mucor*.	(Orji *et al.,* 2016)
Watermelon, Pineapple, Pawpaw, Cucumber	Bacterial and Fungal load	*Staphylococcus aureus, Klebsiella pneumoniae*, *Escherichia coli, salmonella and shigella* spp*, Candida, Aspergillus niger*	(Ugwu and Edeh, 2019)
Apple, sliced Pineapple, sliced Watermelon, Pawpaw, Packaged fruits salad	Total aerobic plate	*Enterobacter, Staphylococcus aureus, Escherichia coli, Bacillus spp, Proteus, micrococcus, Klebsiella pneumoniae, Lactobacillus, Penicillium, Aspergillus niger.*	(Oranusi and Olorunfemi, 2011)

Table 2: Identified organisms, diseases and infections of ready-to-eat fruits.

Probable organisms isolated in ready-to-eat fruits	**Diseases and infections**	**References**
Escherichia coli	Pneumonia, diarrhoea, intestinal lining damage	**(** Wendolf *et al* ., 2015)
Klebsiella pneumoniae	Urinary tract infections, pneumonia, sepsis and meningitis.	(Turton *et al*., 2004)
Salmonella spp	Paratyphoid fever	(Vieira-pinto *et al* ., 2011)
Shigella spp	Dysentery and indigestion	(Rooney *et al* ., 2004)

Pseudomonas aeruginosa	Necrotising inflammation	(Ouzari *et al .,* 2008*)*
Staphylococcus aureus	Food poisoning and skin infections	(Halablab *et al*., 2010)
Micrococcus	Skin infections	(Jayaprakash *et al., 2005*)
Proteus	Urinary tract infections, wound infections, sepsis and meningitis	(Cao *et al*., 2014)
Lactobacillus	Urinary tract infections	(Matsumoto *et al.,* 2005)
Aspergillus spp	Asthma, cystic fibrosis, pulmonary infections	(Panackel *et al*., 2003))
Mucor	Sinus or nasal congestion	(Thiyam and Sharma *et al*.,2013)
Candida	Destruction of the immune system	(Hirota *et al*., 2017)

2.7. MICROBIAL CONTAMINATION OF VENDED FRUITS FUNGAL PATHOGENS ASSOCIATED WITH FRUIT CONTAMINATION INCLUDE:

Mucor, Aspergillus, Penicillium, Rhizopus species are the isolated organisms in fruits. These organisms are soil inhabitants and could contaminate the fruits during post-harvesting. Moisture content appeared to be one of the major factors that support fungal growth in dates (Hill and Waller, 1999) as both the semi dry and soft types had all the six species identified from them. Storage facilities such as sacks, polythene bags and natural fibre, which are air-tight, should be used for storage of all the varieties of fruits and vegetables that might have encouraged the growth of such species.

2.8. SOURCES OF MICROBIAL CONTAMINATION IN FRUITS.

Bacteria can be introduced to crops during growth from multiple contamination sources such as soil, seeds, irrigation water, and animal waste. Once on the plants, these bacteria may persist and multiply if environmental conditions are favorable (Park *et al*., 2012).

2.8.1. PRE-HARVEST SOURCES: **(i) Soil**: Soil is a major source of contamination for fresh produce, particularly when the land was formerly used for livestock farming, waste disposal, or treated with manure as fertilizer. (Uyttendaele *et al*.,2015). Although many foodborne pathogens originate from animals, Listeria monocytogenes is widely present in the environment and can be isolated from soil even when there's no clear link to animal sources.(Vivant *et al*., 2013; Smith *et al.,* 2018).

Root vegetables like carrots, which grow underground, are at a higher risk of contamination because they are in direct contact with the soil. If these vegetables are eaten raw without being thoroughly washed or peeled, they can pose a greater risk. Extreme weather events, such as flooding, can also lead to outbreaks of foodborne illnesses (Castro-Ibáñez *et al*., 2015; Bergholz *et al*., 2016). Additionally, with the rise in global warming, dust events are becoming another

source of contamination. For example, a dust storm in Australia in 2018 was thought to have contaminated rock melons with Listeria monocytogenes (NSW Department of Primary Industries, 2018). Similarly, a dust storm in the UK in 2022 during a very dry period was linked to the contamination of salad crops with STEC from a nearby animal farm. Contamination from soil can occur when leaves come into direct contact with the ground or through splash events, where pathogens are transferred from the soil onto the plant's leaves (Cevallos-Cevallos *et al*., 2012; Girardin *et al.,* 2005; Lee *et al*., 2019). The risk is heightened by the ability of these pathogens to persist for extended periods in soil. *Salmonella* Typhimurium can survive for up to 231 days (Islam *et al*., 2004b), *Escherichia coli O157:H7* for up to 217 days (Islam *et al.,* 2004), and *Listeria monocytogenes* for up to 360 days (Piveteau *et al*., 2011).

However, the duration of their survival is influenced by several factors; **(a) Soil Characteristics**:Pathogens tend to persist longer in loamy soils compared to sandy soils.Jechalke *et al.,* 2019). **(b) Microbial Diversity**:When soil prokaryote diversity declines, *Salmonella* survives for a shorter time. (Schierstaedt *et al.,* 2020).

(c) Plant-Microbe Interactions: Some Rhizobacteria can either enhance or inhibit the colonization of plant roots by *Listeria monocytogenes* (Schoenborn *et al*., 2021). Additionally, plant root exudates may act as attractants for human enteric pathogens, causing them to migrate toward the roots of plants like Arabidopsis and lettuce (Karmakar *et al*., 2019; Klerks *et al*., 2007). Once contaminated, pathogens can spread from the seed to the roots, stems, and cotyledons of the growing plant, as demonstrated in laboratory studies with a variety of vegetable seeds (Cui *et al*., 2017; Liu *et al*., 2018; Milillo *et al*., 2008; Shenoy et al., 2017). The sprouting process itself can facilitate the proliferation of bacteria, leading to a significant enrichment of pathogens Indeed, in the laboratory, both *Salmonella* and *Escherichia coli* can attach directly to seeds, although greater populations of *Salmonella* were generally supported (Cui *et al*., 2017; Liu *et al., 2018*). **(ii) Irrigation water:**

Irrigation water can carry enteric pathogens when it picks up runoff from sewage, soil, or animal waste. (United Nations, 2018). Urbanization is making this problem worse. The UN projects that 68% of people will live in cities by 2050, up from 30% in 1950. (United Nations, 2018). With more people and aging sewage systems that aren’t properly managed, greater amounts of waste and pathogens are entering the environment.(United Nations, 2018).

Contamination from irrigation water is a problem in high-income countries (Abraham, 2011). For instance, a 2005 S. Newport outbreak in the U.S. was linked to a pond used for irrigating tomatoes (Greene *et al*., 2008), while *Escherichia coli O157:H7* outbreaks from romaine lettuce in the U.S. and watercress in the U.K. were traced to irrigation water near cattle farms (Bottichio *et al*., 2020; Jenkins *et al.,* 2015). As a result, the Leafy Green Food Safety Task Force suggested increasing buffer zones between farms and concentrated animal feeding operations (Bottichio *et al*., 2020). The method of irrigation also affects contamination levels. Laboratory studies showed that overhead sprinklers, which apply water directly to foliage, led to greater recovery of *Escherichia coli* on lettuce and spinach compared to drip and furrow systems (Fonseca *et al*., 2011; van der Linden *et al*., 2013; Mitra *et al*., 2009). However, this difference was not observed for *Salmonella* (Van der Linden *et al.,* 2013). To mitigate these risks, several strategies can be used to reduce microbial load. These include treating water with filtration, chlorination, UV treatments, and other methods (Banach and Van Der Fels-Klerx, 2020). Simple measures, such as using exclusion fences to restrict livestock access to streams, have also

been shown to decrease *Escherichia coli* populations in the water (Bragina *et al*., 2017). While effective, many of these solutions are too costly for low-income countries to implement (Banach and Van Der Fels-Klerx, 2020). Contamination from irrigation water is also a problem in high-income countries (Abraham, 2011). For instance, a 2005 S. Newport outbreak in the U.S. was linked to a pond used for irrigating tomatoes (Greene *et al*., 2008), while *Escherichia coli* O157:H7 outbreaks from romaine lettuce in the U.S. and watercress in the U.K. were traced to irrigation water near cattle farms (Bottichio *et al*., 2020; Jenkins *et al.,* 2015). As a result, the Leafy Green Food Safety Task Force suggested increasing buffer zones between farms and concentrated animal feeding operations (Bottichio *et al*., 2020). The method of irrigation also affects contamination levels. Laboratory studies showed that overhead sprinklers, which apply water directly to foliage, led to greater recovery of *Escherichia coli* on lettuce and spinach compared to drip and furrow systems (Fonseca *et al*., 2011; van der Linden *et al.,* 2013; Mitra *et al*., 2009). However, this difference was not observed for *Salmonella* (Van der Linden *et al*., 2013). To mitigate these risks, several strategies can be used to reduce microbial load. These include treating water with filtration, chlorination, UV treatments, and other methods (Banach and Van Der Fels-Klerx, 2020). Simple measures, such as using exclusion fences to restrict livestock access to streams, have also been shown to decrease *Escherichia coli* populations in the water (Bragina *et al*., 2017). While effective, many of these solutions are too costly for low-income countries to implement (Banach and Van Der Fels-Klerx, 2020).

(iii) Domestic and wild Animals: Animals are a significant source of pre-harvest contamination of fresh produce due to their role as both a reservoir and a vector for enteric pathogens. These pathogens, including *Escherichia coli O157:H7*, *Salmonella*, and *Listeria monocytogenes*, are commonly found in the intestines and feces of healthy livestock and wild animals (Gopinath *et al*., 2012). Animals are the primary host for *Escherichia coli O157:H7* in healthy cattle, which can shed the bacteria through their feces (Wells *et al*., 1991). This contamination can be spread to crops through soil, water runoff, or direct deposit onto foliage. Wild animals also pose a significant risk, as demonstrated by the isolation of Salmonella enterica from the faeces of deer, mice, coyotes, and stray dogs in California's Salinas Valley, an area responsible for producing a large portion of the state's salads (Kilonzo *et al*., 2013; Jay-Russell *et al*., 2014). Outbreaks linked to this pathway include an *Escherichia coli O157:H7* outbreak in Oregon tied to deer faeces on strawberries (Laidler *et al*., 2013) and an outbreak in Canadian spinach from feral swine feces (Jay *et al*., 2007). Birds can act as long-distance vectors, transporting pathogens across large areas, and have been shown to carry *Escherichia coli*, *Salmonella*, and *Listeria monocytogenes* (Navarro-Gonzalez *et al.*, 2020; Smith *et al*., 2022).

(iv) Manure and Slurry: Using uncomposted animal manure and slurry as fertilizer can introduce pathogens into crops. This practice has been directly tied to outbreaks, including *Escherichia coli* infections linked to lettuce and spinach. (CDC, 2018; Grant *et al*., 2008). Quantitative microbial risk assessments show that applying untreated manure or slurry to cropland exposes people to *Listeria monocytogenes* and pathogenic *Escherichia coli*. (Nag *et al*., 2022). The risk may grow as organic farming expands and relies more on natural fertilizers. Using untreated animal manure or human biosolids, which often contain high levels of harmful pathogens, substantially raises the likelihood of contamination. (Gutierrez-Rodrigues E, *et al.,* 2017).

Animal manure in the soil can create conditions that allow enteric pathogens, such as *Listeria monocytogenes, Salmonella* spp, *Escherichia coli O157:H7*, and *Campylobacter* spp., to survive for long periods (Iwu and Okoh, 2019). These pathogens have been isolated from improperly treated manure and can contaminate fresh produce grown in that soil (Alegbeleye *et al*., 2018; Balali *et al*., 2020).

2.8.2. POST HARVEST CONTAMINATION:

Postharvest processing and handling practices can influence the safety of ready-to-eat fresh produce. Key factors include storage, transport, and retail temperatures; the quality of water used for rinsing and disinfection; sanitation of facilities, surfaces, and machinery; worker hygiene; and packaging methods. (Castro-Ibáñez *et al.,* 2017). Horticultural products typically undergo several processing steps like cutting, shredding, and dewatering before reaching consumers, and these stages can introduce contamination risks. (Buchholz *et al*., 2012). Temperature management after harvest is especially important, as it has a major impact on both the quality and safety of fresh produce.(Brasil and Siddiqui, 2018).
Improper temperature control can create conditions for pathogens to multiply, leading to foodborne illness and outbreaks. (Zeng *et al.,* 2014). For instance, holding ready-to-eat foods at a slightly elevated range of 7–10°C can allow *Listeria monocytogenes* to grow.(Kotzekidou, 2013). Therefore, temperature management, which includes maintaining low temperatures during transportation, processing, storage and retail sales, is critical in combating losses due to microbial invasion, changes in quality attributes and ageing (Nunes, 2008). The hygienic design and status of different equipment surfaces used during fresh produce processing is an important consideration, as they can harbour pathogen populations and greatly increase the risk of contamination (Castro-Ibáñez *et al*., 2017). Poorly cleaned and unhygienic equipment surfaces (handling surfaces, shredders and conveyor belts) have been identified as hotspots for the growth of pathogens, including *Listeria* monocytogenes, *Escherichia coli* and *Salmonella* spp. (Buchholz *et al*., 2012). Wash water of poor sanitary quality and contaminated with enteric pathogens is another potential source of microbial contamination of RTE crops (Castro-Ibáñez *et al.,* 2017, Holvoet *et al*., 2012).

2.9. FACTORS AFFECTING READY-TO-EAT FRESH PRODUCE

The safety of ready-to-eat fresh produce (RTEFP) is significantly impacted by several post-harvest factors, including temperature control, sanitation, and hygiene during processing and handling. These steps are crucial because they can introduce or encourage the growth of harmful pathogens.

(i) Temperature Management: Maintaining proper temperatures is critical for ensuring the quality and safety of fresh produce (Brasil and Siddiqui, 2018). Temperature abuse during processing, transportation, storage, and retail can lead to the proliferation of pathogens and increase the risk of foodborne illnesses (Zeng *et al*., 2014). For instance, storing RTE foods at slightly elevated temperatures (7–10°C) can promote the growth of dangerous bacteria like Listeria monocytogenes (Kotzekidou, 2013). Therefore, keeping produce at low temperatures throughout the supply chain helps prevent microbial growth and spoilage (Nunes, 2008).

(ii) Sanitation and Hygiene The hygienic condition of processing facilities, equipment, and water is a major concern. Processing steps like cutting and shredding can be potential contamination points (Buchholz *et al*., 2012). Poorly cleaned equipment surfaces,

such as conveyor belts and shredders, can become breeding grounds for pathogens like *Listeria monocytogenes*, *Escherichia* coli, and *Salmonella* spp., increasing the risk of contamination (Buchholz *et al*., 2012). Similarly, using contaminated wash water is another potential source of microbial contamination for RTE crops (Holvoet *et al.,* 2012; Castro-Ibáñez *et al*., 2017). An example is the contamination of tomatoes with *Salmonella javania* and of parsley with *Shigella sonnei*, which caused large outbreaks in the US (CDC, 2005). Mangoes exported to the US from Brazil were found to be infecting consumers with Salmonella (Sivapalasingam *et al*., 2000). However, there was no evidence that consumers in Europe were being infected. The infection was traced to contaminated hot water used to kill fruit-flies in the mangoes. Washing helps to reduce the microbial load of raw fruits and vegetables. Ensuring proper sanitation of processing facilities and equipment is vital to minimize this risk (Castro-Ibáñez *et al*., 2017). **(iii) Worker Hygiene and Packaging**

Maintaining worker hygiene and using clean packaging materials are key to avoiding cross-contamination. If handling and packaging are unsanitary, both the safety and shelf life of the product can be affected. (Castro-Ibáñez *et al*., 2017). Keeping a clean, hygienic environment during the packaging of ready-to-eat fresh produce after drying, along with sterile packaging materials, helps reduce the risk of cross-contamination and extends shelf life (FAO, 2010, Jacxsens *et al*., 2003).

CHAPTER THREE

3.0 MATERIAL AND METHOD

3.1. Sample collection:

Fruits of three varieties namely; *Citrulus lanatus* (Watermelon), *Malus domestica* (Apple) and *Ananas comosus* (Pineapple) were purchased from Uselu market in Benin city and were packaged in a clean polythene bag and was transported immediately for processing in the Department of Microbiology Laboratory.

3.2. Preparation of culture media:

All media Nutrients agar (N.A), Potato dextrose agar (P.D.A), Muellar-Hinton agar, Citrate agar, used for culturing were prepared according to the standard specification by the manufacturer's instructions and were placed in an autoclave for sterilisation at 121°C for 15minutes.

3.3. Sample preparation:

The three samples were washed properly under running water. Ten (10g) of each sample was measured using a weighing balance. It was cut into tiny pieces and transferred into a sterile conical flask. A measuring cylinder was used to obtain 90ml of distilled water and was transferred into the conical flasks containing the samples and kept in a few hours to form a stock.

3.4. Serial dilution:

Serial dilution of samples aims to
enumerate the microorganisms present in the ready-to-eat fruit samples, a series of ten-fold dilutions were prepared for each sample. This procedure was carried out under aseptic conditions to prevent external contamination. Ten grams(10g) of homogenized fruit sample was aseptically weighed and transferred into a conical flask containing 90 mL of distilled water. This mixture was homogenized for 2 minutes to create the primary dilution, which was a 10^{-1} dilution. From the primary dilution (10^{-1}), 1 mL of the suspension was aseptically transferred into a sterile test tube containing 9 mL of sterile peptone water, using a new sterile pipette for each transfer. This created the second dilution, a 10^{-2} dilution.
This process was repeated sequentially to create a series of dilutions up to 10^{-9} by transferring 1 mL of the suspension from the previous tube into a new test tube with 9 mL of distilled water. The resulting dilutions (10^{1}, 10^{-2}, 10^{-3}) were then used for the inoculation of various culture media for the enumeration of different microbial groups.

3.5. Microbial Enumeration and Isolation:

Petri-dishes consisting of Nutrient agar were incubated at 37°C for 24hours to allow the growth of bacteria. Petri-dishes with potato dextrose agar were kept separately at room temperature at 25°C for 3-5 days to support the growth of Fungi. The plates were examined to detect the growth of microorganisms, the colonies were counted carefully and the quantification was expressed as Colony forming units (c.f.u/g).

3.6. Isolation of Bacteria pure culture:

A single colony was selected from a primary plate and transferred to a newly prepared nutrient agar medium. The culture was then incubated at a temperature of 37°C for 24hrs to obtain an uncontaminated and isolated sample. The pure isolates were then transferred to a new nutrients agar(slant and stored in a refrigerator at 4°C until needed. The isolates were identified based on the colonial morphology, biochemical test (Gram staining, citrate, catalase, indole, oxidase and triple sugar iron test).

3.7. Cultural Characteristics Morphological Tests:

3.7.1 Gram staining:

Gram staining is carried out to determine if the bacteria is a Gram negative or a Gram positive. Using a wire loop a thin smear of the bacteria isolates were made on a slide and were allowed to heat-fix and then cool for a few seconds. A drop of crystal violet (Primary stain) was placed on the slide containing the smear for one minute and was washed immediately with clean water, a drop of iodine (Lugol's iodine) was placed on the slide for one minute and washed off immediately with clean water. The smears were discolored by the application of alcohol and were washed off instantly using clean water. A drop of safranin(Secondary stain) was placed on the smear for one minute and was washed with clean water. The stained smear was allowed to air-dry. After drying, a few drops of oil immersion were applied on the stained smear and were viewed under the microscope (×100) objective lens. The immersion oil aids in viewing the organism clearly. Phenotypic and morphological characteristics were seen (shape, size, margin, colour, elevation, opaque, transparency).

3.7.2 Citrate test:

Citrate test is carried out to determine if bacteria utilizes citrate as its sole source of carbon or energy. Citrate agar (Dark green) is required and is placed in a petri-dish and then allowed to solidify. A small loop of bacteria was streaked on the pretri-dishes containing the agar and was incubated at 37°C for 24hrs. Changes from green to blue colouration, shows citrate is positive, but when it still remains green (no colour change) it shows citrate is negative.

3.7.3 Catalase test:

The Catalase test is used to detect the presence of the enzyme catalase which helps in breaking down hydrogen peroxide (H202) into water and oxygen. Three(3%) of hydrogen peroxide (H2O2) was introduced into a clean slide. A smear of bacteria was made. Bubble formation was observed for each bacterial isolate (Mahon 2011). The formation of bubbles shows catalase positive while no bubble formation shows catalase negative. The Catalase test is used for distinguishing between Gram-positive bacteria like *staphylococcus* from Gram-negative bacteria like the *streptococcus.*

3.7.4 Indole test:

Peptone broth was prepared by weighing (15)ml of peptones broth in test-tubes and was sterilized in the autoclave at 121°C for 15 minutes. A full loop of the bacteria culture was inoculated in broth and incubated 24-48hrs at 37°C. Two (2) drops of kovac reagent were dispensed into test-tubes and are mixed together after sterilisation. The indole test determines the ability of bacteria to produce indole from an amino acid called tryptophan. The test is used for identifying members of the Enterobacteriaceae family (*Escherichia coli*).

3.7.5 Oxidase test:

Two drops of oxidase reagent were placed carefully into the Whatman filter paper and allowed to heat-fix for a few seconds. Using a wire loop, a small quantity of bacteria was collected

carefully and a smear was made on the filter paper, after (5-10) seconds, a blue or purple coloration was seen, indicating a positive result, when there is no colouration, it indicated a negative result. This shows that the micro-organisms were able to produce the enzyme oxidase.

*3.*7.6 Triple sugar iron test:

The T.S.I is a composite test that assesses a bacterium's ability to ferment sugar (Glucose, Lactose, and Sucrose) as well as its ability to produce Hydrogen sulphide (H2S) gas. Pink colouration on the slant shows alkalinity, and it indicates that the organism was able to ferment sugar, (Glucose, Lactose, and Sucrose). while the butt shows red or yellow, indicating that it is acidic. When it still retains the orange colour, it shows that there was no change, so it is negative. A black colouration shows the presence of hydrogen sulphide production. When there is a bubble or cracks it shows that the organism was able to produce gas.

3.8. Determination of Antibiotics susceptibility profile:

The most common method for antibiotics test is the Kirby-Bauer disc diffusion method. 0.5 of the McFarland standard was used to prepare culture of each bacterial isolates. Bacterial isolates (1.5×10^8 cells/ml) was seeded into each sterile Muller Hinton agar and were allowed to stand at room temperature (27°C) for 30minutes to allow inoculated organisms to pre-diffuse in the prepared media. The disc containing antibiotics (Chloramphenicol, Septrin, Ciprofloxacin, Sparfloxacin, Amoxicillin, Augmentin, Gentamicin, Pefloxacin, Streptomycin, Afloxacin) were carefully placed aseptically on Mueller Hinton agar plates. The bacteria can be susceptible, intermediate, or resistant. Bacteria with no zone of inhibition are considered resistants. All plates were placed in an incubator for 24hours at 37°C. Zone of inhibition was measured in millimeters to meet the guideline set by the Clinical Standard Laboratory Institution (CLSI, 2017).

3.9. Identification of Fungi Isolate:

The fungal isolates were identified using cultural morphological features such as the growth of colony, conidial morphology, pigmentation, and pattern. The technique was adopted for the identification of the isolated fungi using Lactophenol cotton blue stain. The identification was archived by introducing a drop of the stain in a sterile glass slide with the aid of a disposable pipette whereby a small portion of the aerial mycelia from the representative fungi culture was removed and is placed in a drop of Lactophenol. The mycelium was spread on the glass slide using the disposable pipette. A cover slip was placed gently with little pressure to remove air bubbles. The glass slide was then mounted and is viewed under the microscope with ×10 and ×40 objective lens for its morphological characteristics and phenotypic features (colour, hyphae, spores).

3.9.1 Statistical analysis:

Bacterial enumeration was done using colony forming unit millimeters(cfu/ml). (ANOVA) was used to compute and arrive at statistical decisions. The Statistical Package for Social Science (SPSS) version was used for Statistical data.

CHAPTER FOUR 4.0 RESULTS

Table 4.1 Shows the heterogeneous bacterial counts of the three samples of ready-to-eat fruits and also the standard deviation.

Table 4.2: Shows the fungal counts of the three samples of ready-to-eat fruits and also the standard deviation.

Table 4.3 : Shows the Cultural, Morphological, and Biochemical characteristics of the bacterial isolates recovered from the fruits samples. The identified organisms are *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter.*

Table 4.4. Shows the frequency and percentage occurrence of fruits bacterial isolates in ready-to-eat fruits.

Table 4.5: Shows the antibiotics susceptibility profile of Gram positive bacteria isolates. *Bacillus* spp and *Staphylococcus aureus* show high sensitivity to Ciprofloxacin, Levofloxacin, Gentamicin, Ampliclox but resistance to Amoxicillin.

Table 4.6: Shows the antibiotics susceptibility profile to Gram negative bacteria isolates. *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella* pneumoniae, and *Enterobacter* were sensitive to Ofloxacin, Gentamicin, Levofloxacin and Ciprofloxacin but showed resistance to other antibiotics.

Table 4.7: Shows the Cultural and Morphological Characteristics of Fungi Isolates.

Figure 4.1: Shows the multiple antibiotics resistance (MAR) index of the Gram positive bacterial isolates.

Figure 4.2: Shows the multiple antibiotics resistance (MAR) index of the Gram negative bacterial isolates.

Table 4.1 showing the total heterogeneous bacterial counts of some selected fruits samples.

Samples.	**THBC ($\times 10^4$ cfu/g)**
Apple	1.80±0.103
Pineapple	5.10±0.48
Watermelon	3.60±0.068

Table 4.2. showing the fungal counts of some selected ready-to-eat fruits.

Samples	**TFC($\times 10^4$cfu/g)**

Apple	6.35±0.033
Pineapple	5.90±0.041
Watermelon	3.85±0.056

Table 4.3. Showing the Cultural, Morphological, and Biochemical Characteristics of Bacteria Isolates.

Characteristics.	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**
Cultural.						
Size	Circular	Irregular	Circular	Circular	Irregular	Irregular
Shape	Small	Large	Small	Medium	Medium	Large
Elevation	Raised	Flat	Raised	Raised	Flat	Raised
Transparency	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Margin.	Entire	Undulate	Entire	Entire	Undulate	Entire
Colour on N.A.	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy
Morphological						
Gram stain	+ve	+ve	-ve	-ve	-ve	-ve
Cell type.	Cocci	Rod	Rod	Rod	Rod	Rod
Arrangement	Cluster	Chain	Single	Pair	Single	Single
Biochemical						
Citrate	+ve	+ve	-ve	+ve	+ve	+ve
Catalase	+ve	+ve	+ve	+ve	+ve	+ve
Indole	-ve	-ve	+ve	-ve	-ve	-ve
Oxidase	-ve	-ve	-ve	-ve	+ve	-ve
Motility	-ve	+ve	+ve	+ve	+ve	+ve
Glucose	+ve	+ve	+ve	+ve	+ve	+ve
Fructose	-ve	+ve	+ve	+ve	+ve	+ve
Lactose	-ve	+ve	+ve	+ve	+ve	+ve
Gas formation	-ve	-ve	+ve	-ve	-ve	+ve
H2S formation	-ve	-ve	-ve	-ve	-ve	-ve
TSI (Slant/ butt)	K/A	K/A	A/G	K/A	K/K	K/A
Organisms Identified	*Staph aereus*	*Bacillus* Spp	*Escherichia coli*	*Klebsiella Pneumoniae*	*Pseudomonas aeruginosa*	*Entero-bacter*

Table 4.4. Showing the percentage occurrence of bacterial isolates from various fruits samples.

Bacterial isolates	Frequency	Percentage (%)
Staphylococcus aureus	1	11.11%
Bacillus spp	3	33.33%
Klebsiella pneumoniae	1	11.11%
Pseudomonas aeruginosa	2	22.22%
Lactobacillus	2	22.22%
Total	9	100%

Table 4.5: Showing the antibiotics susceptibility profile of Gram positive bacteria.

Organisms	ZN	AM	R	CIP	AZ	LEV	E	PEF	GEN	AMP	RI
Bacillus cereus	10(R)	11(I)	10(R)	20(R)	10(R)	18(S)	17(S)	10(R)	19(S)	20(S)	0.40
Staph aereus.	16(S)	9(R)	18(S)	17(S)	18(S	17(S)	18(S)	12(I)	18(S)	10(R)	0.20
Bacillus subtilis	18(S)	20(S)	16(R)	12(I)	19(S)	12(I)	11(I)	10(R)	13(I)	14(I)	0.20

KEYS

Resistance(R) = (0-10 mm)

Intermediate (I) = (11-16 mm)

Susceptible (S) = (≥17 mm)

Stap. aureus= *Staphylococcus aureus*

ZN = Zinnacef (20ug)

AM = Amoxicillin (30ug)

R = Rocephin (23ug)

CIP = Ciprofloxacin (10ug)

AZ= Azithromycin(12ug)

LEV =Levofloxacin(20ug)

E = Erythromycin (10ug)

PEF = Pefloxacin (10ug)

CN = Gentamicin (10ug)

AMP = Ampicillin (30ug)

R.I = Resistance index

Table 4.6. Showing the antibiotics susceptibility profile of Gram negative bacteria.

Organisms	**AM**	**AU**	**CN**	**PEF**	**OFX**	**AZ**	**LEV**	**CF**	**SPF**	**CIP**	**R.I**
E. coli	10(R)	18(S)	12(I)	11(I)	9(R)	13(I)	17(S)	18(S)	11(I)	14(I)	0.20
Klebsiella	10(R)	9(R)	10(R)	12(I)	10(R)	10(R)	15(I)	10(R)	10(R)	18(R)	0.70
Pseudomonas	10(R)	10(R)	17(S)	14(I)	17(S)	10(R)	18(S)	19(S)	9(R)	10(R)	0.40
Enterobacter	14(I)	17(S)	11(I)	11(R)	16(I)	18(S)	10(R)	13(I)	10(R	12(I)	0.30

KEYS

Resistance(R)= (0-10)

Intermediate(I)= (11-16 mm)

Susceptible (S)= (≥17 mm)

E.coli =Escherichia coli

AM= Amoxicillin(30ug)

AU=Augmentin(10ug)

CN=Gentamicin(30ug)

PEF= Pefloxacin (30ug)

OFX=Ofloxacin (10ug)

AZ= Azithromycin (12ug)

LEV= Levofloxacin(20ug)

CF= Cefotaxim(10ug)

SP= Sparfloxacin(10ug)

CPX= Ciprofloxacin (30ug)

R.I= Resistance index

Table 4.7: Showing the Cultural and Morphological Characteristics of Fungi Isolates.

Parameters.	**1.**	**2.**	**3**.	**4**.	**5**.
Colour of mycelium on agar plates	Dark colour growth	Cream front Colour	Green wholly with rapid growth	White	Lemon wholly colour
Colour of reverse plate	Dark	Dark cream	Dark	Darkish	Yellowish
Microscopic Characteristics					
Nature of hyphae	Septate	Septate	Septate	Aseptate	Septate

Types of spores	Conidio-. phores	Ascospores	Conidio-phores	Conidio-phores	Sporangio-phores
Conidia	Present	Present	Present	Present	Present
Rhizoids	Absent	Absent	Absent	Absent	Black
Appearance of Special structure	Dark	Fruiting heading	Dark	Dark	Black
Class of fungi	Ascomycetes	Ascomycetes	Ascomycetes	Ascomycetes	Ascomycetes
Probable Organisms.	*Aspergillus.* niger	Yeast	*Penicillium* spp	*Mucor*	*Aspergillus* spp

Figure 4.1: Showing the multiple antibiotics resistance (MAR) index of the Gram positive bacterial isolates.
Figure 4.2. Shows the multiple antibiotics resistance (MAR) index of the Gram negative bacterial isolates.

CHAPTER FIVE

5.0 DISCUSSION

Micro-organisms, notably Bacteria and Fungi, were identified as the major contaminants in the ready-to-eat fruits sampled, a problem often stemming from vendor mishandling. The microbial contaminants found in the RTE fruits from various vendors in Uselu market confirmed the poor microbiological quality of the products. Within this study, Pineapple exhibited the greatest heterogeneous bacterial count (5.10 cfu/g), while Apple had the least bacterial count (1.80 cfu/g). Conversely, the fungal mean count was observed to be highest in Apple and lowest in Watermelon (3.85×10cfu/g)

The finding that all analyzed fruit samples were contaminated with numerous micro-organisms within the study area is consistent with microbial isolates documented by prior research. This assertion aligns with the findings of (Odebisi *et al.,* 2015), who studied microbial contamination of ready-to-eat fruits in the main market of Abakaliki, Ebonyi state. This is further supported by the research conducted by (Oranusi and Olurunfemi 2011), who successfully identified the

presence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus, Salmonella*, and *Mucor* in commercially available fruits in Otta, Ogun state. Furthermore, (Tambeker *et al.,* 2009) also reported similar findings in their study. The majority of isolates examined in this study were likely introduced into the fruits through the use of contaminated water (potentially with fecal matter) during washing, or via unhygienic utensils such as trays, knives, and polythene bags used for packaging cut fruits. Moreover, the practice of exposing these fruits to low temperatures may inadvertently facilitate the growth of psychrophilic micro-organisms (Jerry *et al*., 2016). Additionally, the introduction of *Staphylococcus aureus* into the ready-to-eat fruits can be attributed to the physical contact between vendors and the fruits. This is due to the fact that *Staphylococcus aureus* is commonly found as part of the normal microbial communities inhabiting the hands, nasal passages, and skin of a healthy individual. (Jerry *et al*., 2016) reported this bacterium to be the most frequently isolated micro-organism, a finding consistent with the current study, and others conducted by (Odebisi *et al*., 2015) and (Ganguli *et al.,*2006). In contrast, (Fowoyo *et al.,*2012) noticed a limited presence of *Pseudomonas aeruginosa* in their research on air-borne contamination in fruits sold in Lokoja, Kogi state.
In antibiotics susceptibility tests, *Staphylococcus aureus* was seen to exhibit the highest zone of inhibition on Zinnacef, Rocephin, Ciprofloxacin, Azithromycin, Levofloxacin, Erythromycin, and Gentamicin, suggesting these antibiotics remain effective against the strains isolated. The contamination by various fungal substances, specifically the high level of *Mucor* species, can be attributed to the ubiquitous nature of these species in the environment. This high contamination level may be a consequence of the exposure of ready-to-eat fruits to dusty or mouldy environments. The frequent operation of fruit vendors in close proximity to stagnant water found in gutters is a significant environmental factor that can further facilitate the introduction of contaminants to fruits. The overall results highlight the urgent necessity for vendors to implement rigorous personal and environmental hygiene practices to mitigate contamination risks associated with ready-to-eat fruit consumption.

5.1 CONCLUSION

The findings of this study conclusively demonstrate that the pineapple and watermelon samples examined exhibited poor microbiological quality. This poor quality is evidenced by high bacterial and fungal loads. Specifically, pineapple sourced from the Uselu market displayed the highest heterogeneous mean bacterial count at (5.10 cfu/g), significantly higher than the lowest recorded count of (1.80 cfu/g) found in the Apple sample. Similarly, pineapple from Uselu market showed the greatest fungal count at (5.90×10^4 cfu/g), while the Watermelon sample recorded the lowest fungal count at (3.85×10^4cfu/g).The probable bacterial isolates identified include: *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa*, *Bacillus subtilis*. The fungal species identified include: *Aspergillus spp, Mucor* spp, *Yeast, Penicillium* spp, *Aspergillus niger*. The presence of these opportunistic and pathogenic microorganisms underscores the critical need for improved personal and environmental hygiene practices among fruit vendors to reduce contamination risks and safeguard public health.

5.2 RECOMMENDATIONS

i. Government agencies should regularly inspect the hygiene standards of fruit vendors in all markets, focusing specifically on the processing and packaging of vended fruits. This oversight must include verifying the source and type of water utilized for washing and processing, as well as the cleanliness and state of utensils employed. The primary objective of these inspections is to significantly mitigate the incidence of fruit contamination across the vending sector.

ii. The government should implement targeted public awareness programs designed to educate fruit vendors on the critical importance of stringent personal and environmental hygiene practices. These programs will serve as a proactive strategy to reduce contamination risks at the point of sale.

iii. Consumers must ensure all fruits are thoroughly washed under clean, potable water prior to consumption to minimize exposure to microbial contaminants.

5.3 CONTRIBUTIONS TO KNOWLEDGE

This study contributes to the existing body of knowledge by providing empirical data on the microbiological quality of selected ready-to-eat fruits sold in open markets (Uselu). Given the limited research on the safety of unprocessed fruits in informal market settings in Nigeria, the findings establish a baseline for understanding the extent of microbial contamination and the potential public health risks associated with their consumption. The identification and quantification of pathogenic and spoilage microorganisms in this study offer evidence that can inform food safety policies, regulatory interventions, and public health education programs. Specifically, the results highlight critical control points in fruit handling and vending practices, thereby providing a scientific basis for targeted hygiene interventions by regulatory agencies such as NAFDAC and state Ministries of Health. Moreover, the study serves as a reference for future research on food safety in open market systems. It demonstrates the practical application of standard microbiological techniques to real-world public health problems and creates a foundation for comparative studies across different locations and time periods.
In essence, this research bridges the gap between laboratory microbiology and community health by translating microbial data into actionable recommendations for vendors, consumers, and policymakers.

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