

Chapter One

Introduction

1.1 Background of Study

Fruits are products of agriculture, possess tissues and are subjected to living thing features which includes respiration, excretion and metabolism etc¹. Fruit can be eaten wholly, sliced or blended to smoothie for drink². They are served in retail stalls, during events and ceremonies³. They are used in food recipes for improving quality of soup and African dishes for healthy living⁴. Fruits dietary values are water, fiber, vitamins, sugar, protein and phytochemical properties. Fruits provides vital sources of nutrient to human being. They supply the body with necessary vitamins, minerals, and oil for human growth and well-being development⁵. International bodies and organization including the World Health Organization (WHO), the Food and Agricultural Organization (FAO), and Centers for Disease Control and Prevention (CDC) have encouraged people to eat more fresh fruits⁶. Regular intake of fruit and vegetables (F&V) has been related epidemiologically with reduced risk of many non-communicable diseases. In addition, fiber found in fruits have been proved to reduce intestinal passage rates by forming a mass, leading to a more gradual nutrient absorption hence preventing constipation⁷. Fruits can be fermented in the colon of human, increasing the concentration of short chain fatty acids, having anticarcinogenic properties and maintaining gut health ⁸. Several studies have shown fruits play a role in suppressing risk of cardiovascular disease (CVD) risk when its intake in human is high. The intake are strongly associated with lower cardiovascular risk factors such as lower blood pressure (BP), cholesterol therefore preventing premature cardiovascular disorder⁹. Recently, Habauzit et al reported that fruits containing high amount of anthoxanins, flavonoids, and procyanidins, such as grapes, berries and pomegranate are

responsible for decreasing cardiovascular risk while citrus fruits and apple have apparent effect on blood pressure and blood lipid level¹⁰. Consumption of carotenoid rich fruits maintains the cholesterol level in blood stream of human¹¹. Fruits have also been highlighted to prevent osteoporosis in adults mainly for their rich sources of calcium and other vitamins which are vital in bone health¹². Furthermore, phytochemicals present fruits such as gooseberry, curcumin, and soya is flavones have shown to be protective against lens damage which occurs due to hyperglycemia. Certain flavonoids in fruits such as quercetin can prevent oxidative stress in the pathogenesis of glaucoma¹³. Interestingly, some phytonutrients has being found to acts as an anti-obesity agent and weight loss¹⁴. Higher fruits consumption is found to have lower risk of cognitive decline hence proved beneficial for mental health¹⁵.

Nigeria is blessed with favorable climate condition for growth of varieties of fruits such as paw paw, almond, apple, water melon, mango, cucumber to mention a few¹⁶. Paw-paw, pineapple, watermelon, salad vegetables, cucumbers, carrots and pears are sliced Nigerian food recipes eaten in Nigeria. Such pathogens may become internalized, survive and grow within the fruit and consequently become health hazard to consumers¹⁷. Salmonella spp has been reported to survive and grow rapidly on water melon held at room temperature and the level of contamination did not change when the melon was stored at refrigeration temperature¹⁷. Outbreaks of listeriosis and salmonellosis have also been associated with the consumption of ready-to-eat fruit salad¹⁷. Microbial Cross contamination from the use of animal manure for fertilization, and poor hygiene of workers are not exceptions¹⁸. Research highlighted by Moses and co-workers on the prevalence of *Escherichia coli* O157 in fruits, vegetables and animal faecal waste used as manure in farms of some communities of Akwa Ibom State, Nigeria identified cow and goat dung as potential sources of *E. coli* O157 contamination of farm

productions, when used as manure¹⁹. On this basis, contaminated irrigation or water channel, poor sanitation and improper maintenance of equipment, the use of animal manure for fertilization, and poor hygiene of workers are contributing factors¹⁸

1.2 Statement of the Problem

In Nigeria, there is increased consumption of fruits in recent years especially since a whole piece of fruit can be divided into fractions to make it affordable and vended for sale. More microorganism therefore has access to the fruits sold in fraction since the innate properties of the fruit has been compromised. This allows both spoilage and pathogenic microorganism access to such fruit

Pathogenic organisms evolve from time to time, acquiring resistance genes especially when exposed to challenging environments, and these fruits are constantly being exposed to challenging environment, and theses fruits are constantly being exposed to different chemical preservative to prevent their spoilage. There is urgent need to carry out antibiotics susceptibility test periodically to ascertain emerging antimicrobial resistance gene.

1.3 Justification of the Study

Investigation into the diversity of microorganisms, environment and conditions that lend itself to the acquisition of antibiotics resistance by microorganism will help researcher's, the government as well as the health care organization acquire an in depth knowledge of the extent to which fruit play a role in antibiotics resistance , and also aid the development of more holistic solution to these problem

1.4 Aims and Objectives of the Study

The main objectives of the research work is to identify bacteria with multidrug resistance obtained from some selected fruit vended in some parts of Ibadan metropolis using molecular methods. The selected fruits include Water Melon-*Citrullus lanatus*, Paw Paw - Papaya papaya, Pineapple-*Ananas comosus*.

Specific Objectives of the study are to:

- i. determine the mesophilic bacteria plate count of selected frit samples in Ibadan.
- ii. determine antibiotics susceptibility of the pathogenic bacteria isolate present in the sample.
- iii. characterize and identify pathogenic bacteria with multidrug resistance using molecular methods.

1.5. Research Questions

1. Are there emerging antibiotics resistance bacteria associated with selected fruits
2. Highest number of bacterial contamination's present in selected fruits
3. What is the precise pathogen bacteria present in selected fruit
4. Recommend antibiotics to be administered to patient with food-borne illness

1.6 Significance of the Study

The complete profiling of pathogenic bacteria associated with selected fruits among fruits vendor will identify prevalence of emerging strains of pathogenic bacteria responsible for food borne illness. It is noted that pathogenic bacteria in fruits can cause life threatening infection in

human after ingestion of fruits especially within children at the age of 0 to 5 years and immune compromised people.

Antibiotics are used in treatment of microbial infection and infectious disease for years thereby alleviating morbidity and mortality. In recent years, formation of antibiotics resistance bacteria was formed over the misuse of antibiotics drugs and prolonged use of antibiotics drug. Antibiotics resistance bacteria has resulted in complicating patient management and increased patient morbidity and mortality. The study will reveal overhaul list of antibiotics drug that pathogenic bacteria are resistant to as well as those they are susceptible to among antibiotics to.

1.7 Scope of the Study

The research will focus on identifying pathogenic organisms in selected fruits in Oje and Bodija market, Ibadan, Oyo state.

1.8 Limitation of the Study

This study is limited to only the mesophilic bacteria isolates present in the selected fruit samples used.

1.9 Operational Definition of Terms

- **Anticarcinogenic:** This is a substance that is responsible for inhibition of effects of carcinogen growth in the body or cancer.
- **Cardiovascular Disease:** are disease that affects the circulatory system including heart and blood vessels.
- **Phytochemicals Properties:** These are composition of bioactive nutrient plant chemicals in fruits, vegetables and grain. They provide desirable health benefits aside nutritional value.

- **Flavonoids:** They are group of plant metabolites thought to provide health benefits through cell signaling pathways and antioxidant effects.
- **Glaucoma:** A condition of increased pressure within the eyeball causing gradual loss of sight.
- **Non-Communicable Diseases:** These are disease or infection that cannot be passed from one person to another.
- **Hyperglycemia:** This is a condition where there is excess glucose in the bloodstream.
- **Gastroenteritis:** is an inflammation of the lining of the intestines caused by virus, bacteria or parasites.
- **Food Poisoning:** are illness caused by bacteria or their toxins in food. This is characterized by vomiting, nauseating or diarrhea in human.
- **Antimicrobial Resistance:** it's a state that's occur when bacteria, viruses, fungi and parasites change its form and no longer responds to drug mediated for the treatment of an infection in a host.
- **Immunocompromised:** is a condition hereby a human immune system's defenses are low. This state can either be permanent or temporary.

Endnotes

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Chapter Two

Literature Review

Fruit plays an important activity in maintaining human health because they contain essentials nutrients required for human livelihood. It's contains water, sugars, pectin, starches and organic acids¹. Carbohydrates remains the most abundant macro-nutrients found in fruits while fats and protein are also present at minimal proportion. Fruits are the major source of vitamins and some contains insoluble fibers that said digestion, healthy bowel function and stimulates anaerobic bacteria fermentation in the colon of human². Fruits are recommended in dietary guidance because of their high concentrations of dietary fiber, vitamins, minerals, especially electrolytes; and more recently phytochemicals, since they possess antioxidants activities³

2.1 Health Benefits Attached to Fruit Consumption

Fruits are vital sources of nutrient to human beings. They give the body the necessary vitamins, fats, minerals and oil in the right proportion for human growth and development⁴. Fruits plays a very important role in the nutrition and health especially as they contain substances which regulate or stimulate digestion, act as laxatives or diuretics, pectins and phenoic compounds which play a part in regulating the pH of the intestines⁵

2.1.1 Mental Wellness

Fruits contain a variety of micronutrients vital for physical and mental function⁶. Antioxidants such as vitamin C and carotenoids are said to play a critical role in protecting the body against oxidative stress, which is responsible for the causation and progression of neurodegenerative diseases, and chronic inflammatory. Furthermore, water-soluble vitamins such as vitamin C, and B vitamins, and certain minerals including calcium, magnesium, and zinc, are important for optimal cognitive and emotional functioning⁶.

Recently there are emerging strong evidence that the more the ingestion of fruits, the better mental state. Research has established that people who eat more fruits have a lower cases of mental disorders, including low rates of depression, perceived stress, and negative mood⁷. People who consume more fruits also have a higher likelihood of optimal mental states, such as greater happiness, positive mood, life contentment, and socio-emotional flourishing, which captures feelings of meaning, purpose, and fulfillment in life⁸.

2.1.2 Digestive System

Fruits play a vital role in digestion, they possess insoluble fiber such as cellulose, lignin to mention a few which regulate or stimulate digestion. They act as laxatives or diuretics, pectins and phenolic compounds which play a vital role in regulating the pH of the intestines and relieve constipation⁵

2.1.3 Maternal Health

Female maternal health relies on having achieved food security during girlhood as well as a diet rich in micro-nutrients during conception, pregnancy and the first few months after childbirth⁵. The well-being of a woman before conception have an impacts on their health during pregnancy and child birth. Majority of pregnant women in the developing countries suffers from anemia and other malnutrition related deficiencies. This leads to complications for the fetus during and after childbirth. Fruits have the micro-nutrients diets that's support women during pregnancy and after childbirth⁵

2.1.4 Combat HIV/AIDS, Malaria and other Chronic Disease

The human body's immune system relies heavily on a balanced diet, rich in micro-nutrients, to be function efficiently. Diets rich in fruits increase the body's immune system, helping it to resist HIV/AIDS, malaria, diarrhea, tuberculosis and many other infectious diseases⁹. A balanced diet, rich in micro-nutrients, is likely to reduce the number of chronic infections, prevent some infections and help the body to tackle the severe infectious diseases which prevailed in developing countries and causative for adult and childhood mortality. Fruits are also considered by FAO and WHO as the primary nutritional value to prevent non-communicable and micro-nutrient deficiency related diseases¹⁰. Diabetes type 2, obesity and certain cardiovascular diseases and cancers can be significantly reduced through increased consumption of fruits and vegetables.

Table2.1: Shows Catalogue of Medicinal Fruits and their Functions among Nigerian People⁸⁴

Fruit Name	Botanical Name	Family	Benefits
Cashew	<i>Anacardium occidentale</i>	Anacardium	Treats ringworm infection. Acts as diuretic
Paw Paw	<i>Carica papaya</i>	Caricaceae	Antimalarial management. Diuretic agent
Mango	<i>Mangifera Indica</i>	Anarcadiaceae	Treats typhoid
Guava	<i>Psidium guajava</i>	Myrtaceae	Cures diarrhea and dysentery
African Pear	<i>Dacryoides edulis</i>	Burseraceae	Used in treatment of skin parasite and remedy for heat condition

2.2 Other Socio-economic Benefits of Fruit Trade

Major fruits produced in Nigeria include mango, pineapple, plantain/banana, citrus, guava, pawpaw, water melon⁵. Fresh-cut fruits on the market today includes water-melon, pawpaw, oranges, grape-fruit, pineapple, and salad vegetables, cucumber, carrots, cabbage and pears¹¹

2.2.1 Source of Income

Fruits sales and production contributes to livelihood of Nigerian citizen. It serves as source for income for both rural and urban dwellers since it's create job. On average, its doubles the amount of employment per hectare of fruits production compared to cereal crop production⁵. Collectively, they play a role in gross domestic profits of the nation as some fruits are exported to another country for foreign exchange. The shift from cereal production towards high value fruit production is an important contributing factor to employment opportunities in developing countries⁵

2.2.2 Eradicates Extreme Poverty

The value of fruits and vegetables per unit area is significantly higher than the value of the cereal crops. Although the costs of cultivation such as labor can be costly, the profits are higher and the income thus generated can be used for many different purposes in terms of eradicating hunger and affording access to education and preliminary health care. Over two billion people suffers from micronutrient deficiencies through poor diets⁵. Fruits are the most appealing and affordable sources of these micronutrients. Diet improvement increases a person's productivity, reduces health care illness and disease which in-turn reduce healthcare costs.⁵

2.2.3 Promotes Gender Equality

The horticultural sector generates off-farm labor, for women. The case functions during export and value-added processing agro-industries which are important sectors of the Nigerian economy. Fruits production empowers women to more economic opportunities. Women are the leading producers of most horticultural crops in developing countries. And are predominantly engaged in the value-added activities from production and marketing of fruits production.⁵

2.3 Phytochemical in Fruits

Fruits consumption are excellent source of phytochemicals for human diet either eaten fresh or processed. About 200,000 phytochemicals are identified so far and 20,000 of them have been highlighted as originating from fruits, vegetables and grains¹². Phytochemicals has many health-related benefits as they are used as antioxidants against many diseases or antibacterial, antifungal, antiviral, cholesterol lowering, antithrombotic, or anti-inflammatory conditions¹². Phytochemicals are applicable for various purposes such as pharmaceuticals, agrochemicals, flavors, fragrances, coloring agents, biopesticides and food additives¹²

Fruits phytochemicals properties are composed of chemical structures such as phenolics, alkaloids, saponins and terpenoid. These compounds are identified as secondary metabolites having various recognized structures, although a benzene ring with one or more hydroxyl group is a common feature¹³. They are popularly classified as flavonoids (anthocyanins, flavan-3-ols, flavanols, pro-anthocyanidins or flavones, non-hydrolysable tannins, isoflavones and flavanones) and non-flavonoids (hydroxycinnamic, hydroxybenzoic acid, hydrolysable tannins, benzoic acids and stilbenes).¹³ Fruits phytochemicals are originated from sugars, acids and polysaccharides coupled with the plant secondary metabolites also known as antioxidant activity and other properties¹⁴. Recently, there have been research on plant antimicrobial,

antiviral, or antibacterial effects¹². Lastly, phytochemicals are among significant natural preservative structures to reduce and inhibit pathogenic microorganism growth and preserve the overall quality of food products¹³

The antioxidant activities of fruits phytochemicals are considered to be beneficial for health. Many studies have as recommended that's a high intake of polyphenol-rich foods may have cardiovascular benefits and provide some level of cancer chemo-preventive activities and beneficial effects against mild or devastating illnesses such as urinary bladder dysfunctions and Alzheimer's disease¹³. Furthermore, nutritionist and food scientists suggest that phytochemicals offer many health benefits when consumed as part.

2.4 Effect of Heavy Metals in Fruits

The last three decades has seen an increase in global concern over health disease¹. Most of these diseases are however not easily detected and may be acquired during childhood and manifest in adulthood². Although modern technology advancement has improved the living condition of many people, one cannot totally overlook its adverse impact on the environment. Industrialization and urbanization has increased the production of hazardous waste affluent that are dangerous to human health³. Agricultural revolution that lead to commercial farming involving the use of chemical fertilizer, pesticides, herbicides and other synthetic chemicals for farming. These heavy chemicals long run affects human health and overall ecosystem directly⁴. One of the issues is the presence of heavy metals. Heavy Metal toxicity human diet has shown to be one of the major threats to health with several health risk associated with it ⁵. Heavy metals are naturally occurring metallic elements that have high atomic weight and a density at least five times greater than that of water. They are metals with metallic properties and atomic number > 20 ⁷. They are elements that have a relatively high density that is toxic or poisonous

at low concentration. They are released in the environment by both natural and anthropogenic sources such as industrial discharge, automobile exhaust and mining. Generally heavy metals are not biodegradable; they have long half-life with the potential for accumulation in different body organs leading to unwanted side effects ^{8,9}. They include Pb, Cd, Zn, Cu, Co, Ni, Ar, Hg and Cr ¹⁰. Fruits and vegetables are edible plant products that are good for health which are widely used for culinary purpose¹¹. They are widely used to increase the quality of soups (leafy vegetables) also for their dietary purposes ¹². They are good source of fiber, selected minerals, vitamins and antioxidants. They are made up of chiefly cellulose, hemi- cellulose and pectin substance that gives their texture and firmness ¹². They contain water, calcium, iron, sulphur and potash which make them to be of importance in our diets ¹². Due to the presence of these vitamin and mineral in fruits and vegetable, they play a unique role in healthy living. However, plants are known to contain both essential and toxic metals which they uptake, translate and accumulate through various transportation mechanism⁷. Although fruits and vegetables are of great health benefit, the accumulation of their contaminants in the bodies of consumer over a long period of time is of major concern as it can result to serious health conditions ¹³. In addition to the soil, other sources of heavy metals for plants are rainfalls in atmospheric polluted area, traffic density, use of oil or fossil fuels for heating, atmospheric dusts, plant protection agents, irrigation water and fertilizer which could be absorbed through aerial parts such as the fruits and leaves ^{10,14,15}. They can leach into living systems from natural ore deposits and other sources such as waste disposal of heavy metal containing waste which accounts for higher percentage of most heavy metal in the environment¹⁶. Some heavy metals like iron, copper, manganese and zinc are nutritionally essential for a healthy life when present in food in small quantity. Few also are of great negative impact to human health. Heavy metals like lead

and cadmium are among the most abundant heavy metals and are particularly toxic ¹³. Excessive amount of these metals in food is associated with etiology of a number of diseases especially with cardiovascular, kidney, nervous as well as bone disease ^{14,17}. Cadmium last for a long time in the body and can cause renal damages, abnormal urinary excretion of protein and decrease in bone calcium. Although zinc is an essential mineral due to its biological and public health significance, it can be toxic when consumed in excess ¹⁸. Arsenic is the 20th most abundant element on earth and 3rd on the periodic table. Human are exposed to arsenic via air, water and food ⁵. Long exposure to arsenic can cause blood vessel destruction, gastro intestinal tissue, heart and brain dysfunction, skin pigmentation, cancer of skin, bladder, lungs, liver colon and kidney ^{19,20}. Mercury can be harmful at a very low concentration because it's high toxicity and ability to bio accumulate. It is one of the most toxic elements among the studied trace metals and exposure to high level could permanently damage the brain, kidney, liver, immune system, pituitary gland, developing fetus ^{11,21,22}. Copper and zinc are essential minerals due to their biological and public health significance; however zinc can be toxic when consumed in excess¹⁸. Excess zinc can cause copper deficiency, autism, nausea, vomiting, stomach pain and diarrhea²³. Excess copper intake can cause Liver kidney and Heart Parenchymatous injury. Despite the variations in the heavy metal contents of different fruits and vegetable, there is a permissible guideline of heavy metals in fruits and vegetables set by the Food Nutrition Board of World Health Organization. Since heavy metals is one of the most important considerations in food quality assurance, there is a need for constant test and analysis of most consumed leafy vegetable and fruits to ensure that their heavy metal content.

2.5 Selected Fruit Sample for Research

Fruits were carefully selected basically on high demand, nutritional values, cost effective and conveniences.

2.5.1 Water Melon

Watermelon (*Citrullus lanatus*) a fruit crop, botanically herbaceous creeping plant belonging to the family Cucurbitaceae. It is mainly propagated by seeds and thrives best in warm environment¹⁵. It is cultivated in tropical climate and they require a lot of sunshine and high temperature of over 25°C for optimum growth. Watermelon thrives best in a drained fertile soil of fairly acidic nature. Watermelon does well in both forest and savannah ecologies. It is planted in the North and it equally does well in the south, timing of planting however differ¹⁶

2.5.1.1 Scientific Classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Cucurbitales

Family: Cucurbitaceae

Genus: *Citrullus*

Species: *Citrullus lanatus*

2.5.1.2 Morphology Characteristics

Water melon is a big, sprawling annual plant with coarse, hairy pinnately-lobed leaves and yellow flowers. It is known for its edible fruit, which is a special kind of berry botanically called a pepo. The watermelon fruit has deep green smooth thick exterior rind with grey or light green vertical stripes. Inside the fruit is red in colour with small black or dark brown seeds embedded in the middle third of the flesh¹⁷

Watermelons range in shape from round to oblong. Rind colours can be light to dark green, with or without stripes. Flesh colours can be dark red, red or yellow. Watermelon varieties fall into three broad classes based on how the seed was developed: open-pollinated, F1-hybrid and triploid or seedless¹⁷



Figure 1: Water Melon (*Citrullus lanatus*)¹⁸

2.5.1.3 Nutritional Value of Fresh Watermelon

Watermelon is one of the most commonly consumed fruits in many countries. Watermelon contains more than 91% of water and up to 7% of carbohydrates. It is a rich source of lycopene and citrulline. Watermelon rind contains more amounts of citrulline than flesh. Another, watermelon has a number of essential micronutrients and vitamins¹⁷

Table 2.2: Nutritive Value Per 100g of Flesh¹⁷

Component	Nutrient Value	Percentage of Recommended Daily Allowance
Energy	30 Kcal	1.5%
Carbohydrates	7.6 g	6 %
Protein	0.6%	1%
Total Fat	0.15 g	0.5%
Dietary Fiber	0.4 g	1%
Vitamins		
Niacin	0.178 mg	1%
Pantothenic Acid	0.221 mg	4.5%
Vitamin A	569 mg	19%
Vitamin C	8.1 mg	13.5%
Electrolyte		
Potassium	112 mg	2.5%
Iron	0.24 mg	3%
Manganese	0.038 mg	1.5%
Zinc	0.10 mg	1%
Phyto-nutrients		
Carotene-alpha	303 µg	-
Lycopene	4532 µg	-

2.5.1.4 Health Benefits of Watermelon

It is known to be low in calories but highly nutritious and thirst quenching. Watermelon can be used as fresh salad, dessert, snack, and for decorations. Smoothies can also be made from the watermelon pulp. In Namibia, the juice can be collected and ferments into a refreshing, light alcoholic drink¹⁹. In some parts of Africa, the rind is sliced, dried, cooked and eaten. Pickled watermelon rind is widely eaten in some parts of USA. It is also high in fibre and citrulline; an amino acid the body uses to make arginine²⁰. Watermelon seeds are known to be highly nutritional; they are rich sources of protein, vitamins B, minerals (such as magnesium, potassium, phosphorous, sodium, iron, zinc, manganese and copper) and fat among others as well as phytochemicals²⁰. The fruit is known to be a good source of lycopene and carotenoid. It helps quench the free radicals that contribute to conditions like asthma, atherosclerosis, diabetes, colon cancer and arthritis.¹⁵

2.5.1.5 Heart Health

Watermelon contains high levels of lycopene that is very efficient in protecting the human cells from damage and lower the risk of heart disease. Watermelon extracts help to reduce hypertension and lower blood pressure in obese adults. Furthermore, Watermelon fruit is also a good source of potassium. Potassium is an important component of cell and body fluids that helps controlling heart rate and blood pressure. Thus, it prevents against stroke and coronary heart diseases¹⁷

2.5.1.6 Anti-Inflammatory and Antioxidant Support

Anti-inflammatory foods can help with overall immunity and general health. The presence of lycopene in watermelon makes it an anti-inflammatory fruit. Lycopene is an inhibitor for

various inflammatory processes and also works as an antioxidant to neutralize free radicals. It also contains a good amount of vitamin-B6 (pyridoxine), vitamin C and manganese.¹⁷

Consumption of food rich in vitamin C is effective in developing the body resistance against infectious agents and scavenge harmful oxygen-free radicals. Manganese is used by the body as a co-factor for the antioxidant enzyme, superoxide dismutase. Watermelon is an excellent source of Vitamin A, which is a powerful natural antioxidant. It is one of the essential vitamins for vision and immunity¹⁷

2.5.1.7 Hydration and Digestion

Watermelons are the source of food that helps keep the human body hydrated. Watermelons are rich in electrolytes and water content which are responsible for thirst quenching. The watermelon contains fibre, which supports a healthy digestive tract and helps keep you regularly fit¹⁷

2.5.1.8 Skin and Hair Benefits

Vitamin A helps keep skin and hair moisturized and it also encourages healthy growth of new collagen and elastin cells. Vitamin C is also beneficial in this regard, as it promotes healthy collagen growth.¹⁷

2.5.1.9 Cancer Prevention

Like other fruits and vegetables, watermelons may be helpful in reducing the risk of cancer through their antioxidant properties. According to the National Cancer Institute, Lycopene help in reducing prostate cancer cell proliferation. Ingestion of natural fruits rich in vitamin-A is known to protect from lung and oral cavity cancers.¹⁷²

2.6 Pineapple

Pineapple (*Ananas cosmos*) is a commercial fruit crop in the tropics and parts of the subtropics of the world.²¹ It is one of the tropical fruits of great demand on the international market, with world production in 2004 accounting for 16.1 million metritonnes (mt). Of this total, Asia produces 51% (8.2 million mt), with Thailand (12%) and the Philippines (11%) the two most productive countries. America and Africa contribute 32% and 16% of world production, respectively, with Brazil (9%) and Nigeria (6%) also being major producers.²²

Pineapple is a perennial monocotyledonous herb, with a short basal stem, producing adventitious roots below and a crown of spirally arranged leaves. It produces a single syncarpous fruit on a terminal inflorescence.²¹ The fruit is juicy with sweet flavor to taste. Pineapple is now considered to be the third most important fruit crop in world production after banana and citrus.²¹ The tropical plant, grows optimal and produces better quality fruit at temperatures ranging from 22 to 32°C and with a daily amplitude of 8 to 14°C. In temperatures above 32°C the plant grows less optimal and if exposed to high solar radiation can burn the fruit during the maturation phase.²¹

2.6.1 Scientific Classification

Kingdom: Plantae

Division: Tracheophytes

Class: Magnoliopsida

Order: Poales

Family: Bromeliaceae

Genus: *Ananas*

Species: *Ananas comosus*

2.6.2 Morphology Characteristics

The pineapple (*Ananas comosus* L., Merrill) is a tropical plant, a monocotyledon and a herbaceous perennial, of the family Bromeliaceae, with approximately 50 genera and 2,000 known species. In addition to the fruit as a food, many species are grown for their leaf fibre from which bagging material is produced, and other species are grown as ornamentals²¹

The plant has a short, thick stem around which grow narrow, rigid trough shaped leaves, and from which auxiliary roots develop. The root system is superficial and fibrous and generally grows no deeper than 30 cm and is rarely more than 60 cm from the soil surface. Adult plants for the commercial purposes measure 0.80 m to 1.20 m in height and 1.00 to 1.50 m in diameter²¹

Inserted into the stem is the peduncle which supports the flowers and, later on, the sorosis type fruit. Plantlets develop from auxiliary buds located in the stem (suckers) and the peduncle (slips), which are most used as planting material²¹



Figure 2: Pineapple (*Ananas cosmos*)²³

2.6.3 Nutritional Value and Composition

Pineapple contains sugars, organic acids, essential minerals, vitamins and fiber suitable for human nutrition. The fruits are also rich in antioxidants such as ascorbic acid, flavonoids and carotenoid compounds required to boost human immune system. The chemical composition of pineapple (sugars, organic acids, minerals, fiber, aromatic compounds, vitamins, amino acids, flavonoids, carotenoids, etc.) depends greatly on the it's fruits varieties.²¹

Fresh fruit has small but little amounts of vitamin A, and flavonoid pigments such as β - carotene, xanthin, lutein and β -cryptoxanthin. Collectively, these compounds play vital roles in antioxidant and vision functions. Vitamin A is also required for sustaining integrity of mucosa and skin. Consumption of natural fruits rich in vitamin A and carotenes has been found to protect from lung and oral cavity cancers. One healthy ripe pineapple fruit can supply about 16.2% of daily requirement for vitamin C. It is one of the rare fruits that are rich in B-complex group of vitamins. It contains very good amounts of vitamin B6 (pyridoxine), niacin, riboflavin and folic acid. Moreover, pineapple fruit is rich in minerals with high biological activity²¹.

The principle amino acids in pineapple are tyrosine and tryptophan. Other amino acids include asparagine, proline, aspartic acid, serine, glutamic acid, α -alanine, aminobutyric acid, tyrosine, valine and isoleucine. Amino acids and sugar content play a vital role in the development of maillard reaction in the products (non-enzymatic browning) mainly during storage and processing. The organic acid content of pineapple also adds to the taste and flavour of pineapple products. Volatile organic acids found in pineapple fruit are citric, malic, quinic acids, etc. but citric acid remains as the chief organic acid. The increase in acidity is directly related to the increased concentration of citric acid in pineapple²¹

Pineapple contains a proteolytic enzyme, bromelain which helps in the digestion process by breaking down proteins. Bromelain has anti-inflammatory, anti-clotting and anti-cancer properties. Furthermore, it can also interact with other medications. Care should be taken when consuming fresh pineapple with medications (antibiotics, anticoagulants, blood thinners, anticonvulsants, barbiturates, benzodiazepines, insomnia drugs and tricyclic antidepressants) due to the possibility of certain side effects associated with it²⁴

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Table2.3: Composition of Pineapple Fruit (Per 100 g of Fresh weight)²⁵

Nutrients	Quantity	Unit
Water	86.45	G
Energy	48	Kcal
Protein	0.54	G
Total lipid	0.12	G
Carbohydrate (by difference)	12.66	G
Sugar (total)	9.35	G
Sucrose	5.53	G
Glucose	1.73	G
Fructose	2.05	G
Fiber (total dietary)	1.4	G
Minerals		
Calcium, Ca	13	Mg
Iron, Fe	0.265	Mg
Magnesium, Mg	16.5	Mg
Phosphorus, P	11	Mg
Potassium, K	151.5	Mg
Sodium, Na	1.5	Mg
Zinc, Zn	0.14	Mg
Copper, Cu	0.097	Mg
Manganese, Mn	1.205	Mg
Selenium, Se	0.05	Mg
Vitamins		
Vitamin C	36.65	Mg
Thiamin	0.079	Mg
Riboflavin	0.031	Mg
Niacin	0.306	Mg
Vitamin B6	0.11	Mg
Folate, DFE	9.5	Mg
Vitamin A, RAE	3	Mg
Vitamin A, IU	55	IU
Vitamin E (α -tocopherol)	0.01	Mg
Vitamin D (D2 + D3)	0.0	Mg
Vitamin D	0.0	UI
Vitamin K (phyloquinone)	0.7	Mg

2.7 Potential Anti-Inflammatory and Digestive Benefits

Bromelain is a complex mixture of substances that can be extracted from the stem and core fruit of the pineapple. Among dozens of components known to exist in this crude extract, the best studied components are a group of protein-digesting enzymes (called cysteine proteinases). Originally, researchers believed that these enzymes provided the key health benefits found in bromelain, a popular dietary supplement containing these pineapple extracts. In addition, researchers believed that these benefits were primarily limited to help with digestion in the intestinal tract. However, further studies have shown that bromelain has a wide variety of health benefits, and that many of these benefits may not be related to the different enzymes found in this extract. Excessive inflammation, excessive coagulation of the blood, and certain types of tumor growth may all be reduced by therapeutic doses of bromelain when taken as a dietary supplement.²⁶

Most of the laboratory research on bromelain has been conducted using stem-based extracts. Healthcare practitioners have reported improved digestion in their patients with an increase in pineapple as their "fruit of choice" within a meal plan, even though there are no published studies that document specific changes in digestion following consumption of the fruit (versus supplementation with the purified extract. However, it is suspected that the fruit core will eventually turn out to show some unique health-supportive properties, including possible digestion-related and anti-inflammatory benefits²⁶.

2.7.1 Antioxidant Protection and Immune Support

Vitamin C is the body's primary water-soluble antioxidant, defending all aqueous areas of the body against free radicals that attack and damage normal cells. Free radicals have been shown

to promote the artery plaque build-up of atherosclerosis and diabetic heart disease, cause the airway spasm that leads to asthma attacks, damage the cells of the colon so they become colon cancer cells, and contribute to the joint pain and disability seen in osteoarthritis and rheumatoid arthritis. This would explain why diets rich in vitamin C have been shown to be useful for preventing or reducing the severity of all of these conditions. In addition, vitamin C is vital for the proper function of the immune system, making it a nutrient to turn to for the prevention of recurrent ear infections, colds, and flu²⁶.

2.7.2 Manganese and Thiamin for Energy Production and Antioxidant Defenses

Pineapple is an excellent source of the trace mineral manganese, which is an essential cofactor in a number of enzymes important in energy production and antioxidant defenses. For example, the key oxidative enzyme superoxide dismutase, which disarms free radicals produced within the mitochondria (the energy production factories within our cells), requires manganese. Just one cup of fresh pineapple supplies 128.0% of the DV for this very important trace mineral. In addition to manganese, pineapple is a good source of thiamin (Vitamin B1) that acts as a cofactor in enzymatic reactions central to energy production²⁶.

2.8 Pawpaw (*Carica papaya*)

Pawpaw is a fast-growing herbaceous, soft-wooded perennial tree-like plant in the Caricaceae family that have a live span of about 5-10 years. Pawpaw plant is grown across all tropical countries such as Nigeria and many sub-tropical regions of the world. Pawpaw trees are grown throughout the year in Nigeria, its fresh and ripe fruits are available all year round with its peak period towards the end of the raining season.²⁷ Pawpaw fruits are one of the most nutritious and cheapest fruits found and consumed in Nigeria and its Vernacular names include Hausa-

Gwandar gida, IgboMgbimngbi, Yoruba- Ibepe and Tiv-Mbuawe. However, the production of this crop remains low as compared to the country's population²⁷.

2.8.1 Scientific Classification

Kingdom: Plantae

Division: Tracheobionta

Class: Magnolipsida

Order: Brassicales

Family: Caricaceae

Genus: *Carica*

Species: *Carica papaya*

2.8.2 Morphology

There are two distinct types of *C. papaya* plants known these are dioecious papayas that have male and female flowers on separate trees and gynodioecious papayas that bare female flowers on some trees and bisexual (hermaphrodite) flowers on others²⁸. Pawpaw normally grows as single-stemmed tree growing from 5 to 10 m which is about 16 to 33 ft tall with a crown of large palmate leaves with seven lobes and about 20-28 inches emerging spherically from the top of the trunk however, the tree may become multistemmed if the stem re-grows after a damaged²⁸.

Its fruit is a fleshy berry about 7-30 cm long, weighing up to 9kg, ovoid-oblong to nearly spherical in shape if there are from the pistillate flowers; pyriform, cylindrical or grooved if

there are from hermaphrodite flowers with its central Cavity full of seeds which are white when unripe and turn deep grey to black when ripe. The fruits also have a thin skin which is smooth to feel, green in colour when unripe, turning yellowish or orange when it's ripe, with a mild and pleasant flavour.²⁸ The typical percentage composition of an average typical pawpaw fruit contains seed (8.5 %), skin (12 %) and pulp (79.5%).²⁸



Figure 3: Pawpaw (*Carica papaya*)¹⁸

2.9 Phytochemical and Nutritional Composition

It reported that 100 g edible portion of fresh and ripe pawpaw fruits contains approximately: 88% - water, 10% - sugar, 0.5% - protein, 0.1% - fat, 0.1% - acids, 0.6% - ash and 0.7% - fibre.²⁷ Pawpaw is a vital source of vitamin A, E, pantothenic acid, folate as well as vitamin C, it also contains magnesium, potassium, calcium and dietary fibre²⁸. The latex contains the enzymes papain that effectively treats trauma, allergies and sports injuries and chymopapain as well²⁹. Collectively, papain and chymopapain contains protein digesting and milk clotting properties. The vitamin A and C content of one medium papaya fruit (approx 350 g edible

portion) exceed the dietary reference minimum intake established for adults by the US Food and Nutrition Board and much higher than tomatoes or oranges²⁸. Papaya fruit is marked as a power house of many minerals and vitamins necessary for wellbeing²⁸. The extracts from an unripe C. papaya fruit may contain substantial amount of terpenoids, alkaloids, flavonoids, glycosides, saponins and steroids³⁰.

Table 2.4: Ripe Pawpaw Fruit Nutritional Values (Per 100 g edible portion only).³¹

Nutrient	USDA Nutrient Database
Water	88.06 g
Energy	43 Kcal
Protein	0.47g
Fats	0.26 g
Carbohydrate (total)	10.82 g
Carbohydrate (Sugar)	7.82 g
Dietary fibre	1.7g
Cholesterol	Nil
Sodium	3 mg
Potassium	182 mg
Calcium	20 mg
Magnesium	21 mg
Iron	0.25 mg
Beta-carotene	47 µg
Thiamin	0.023 mg
Riboflavin	0.027 mg
Niacin	0.357 mg
Vitamin c	60.9 mg
Vitamin a	47 µg
Vitamin K	2.647 µg
Vitamin E	0.3 mg

2.10 Health Benefits

Papaya fruits does not only have luscious taste and bright yellow color of the tropics, they're also rich sources of antioxidant nutrients such as carotenes, vitamin C and flavonoids; the B vitamins, folate and pantothenic acid; and the minerals, potassium and magnesium; and fiber. Together, Together, these nutrients promote the health of the cardiovascular system and also

provide protection against colon cancer. In addition, papaya contains the digestive enzyme, papain, which is used like bromelain, a similar enzyme found in pineapple, to treat sports injuries, other causes of trauma, and allergies³²

2.10. 1 Protection against Heart Disease

Papaya may be very efficient for the prevention of atherosclerosis and diabetic heart disease. Papayas are an excellent source of vitamin C as well as a good source of vitamin E and vitamin A (through their concentration of pro-vitamin A carotenoid phyto nutrients), three very powerful antioxidants. These nutrients help prevent the oxidation of cholesterol. Only when cholesterol becomes oxidized it is able to stick to and build up in blood vessel walls, forming dangerous plaques that can eventually cause heart attacks or strokes. One way in which dietary vitamin E and vitamin C may exert this effect is through their suggested association with a compound called Paraoxonase, an enzyme that inhibits LDL cholesterol and HDL cholesterol oxidation³².

Papayas are also a good source of fiber, which has been shown to lower high cholesterol levels. The folic acid present in papayas is important for the conversion of a substance called homocysteine into benign amino acids such as cysteine or methionine. If unconverted, homocysteine can directly damage blood vessel walls and, if levels get too high, is considered a significant risk factor for a heart attack or stroke³².

2.10.2 Promotes Digestive Health

The nutrients in papaya have also been shown to be helpful in the prevention of colon cancer. Papaya's fiber is able to bind to cancer-causing toxins in the colon and keep them away from the healthy colon cells. In addition, papaya's folate, vitamin C, beta-carotene, and vitamin E have each been associated with a reduced risk of colon cancer. These nutrients provide

synergistic protection for colon cells from free radical damage to their DNA. Increasing your intake of these nutrients by enjoying papaya is an especially good idea for individuals at risk of colon cancer³².

2.10.3 Anti-Inflammatory Effects

Papaya contains several unique protein-digesting enzymes including papain and chymopapain. These enzymes have been shown to help lower inflammation and to improve healing from burns. In addition, the antioxidant nutrients found in papaya, including vitamin C, vitamin E, and beta-carotene, is also very good at reducing inflammation. This may explain why people with diseases that are worsened by inflammation, such as asthma, osteoarthritis, and rheumatoid arthritis, that the severity of their condition is reduced when they get more of these nutrients³²

2.10.4 Immune Support

Vitamin C and vitamin A, which is made in the body from the beta-carotene in papaya, are both needed for the proper function of a healthy immune system. Papaya may therefore be a healthy fruit choice for preventing such illnesses as recurrent ear infections, colds and flu³²

2.10.5 Protection against Macular Degeneration

Mother's especially mentions to children that taking carrots would keep your eyes bright as a child, but as an adult, it looks like fruit is even more important for keeping your sight. Data reported in a study published in the Archives of Ophthalmology indicates that eating 3 or more servings of fruit per day may lower your risk of age-related macular degeneration (ARMD), the primary cause of vision loss in older adults, by 36%, compared to persons who consume less than 1.5 servings of fruit daily. Its reported already which involved over 110,000 women and

men, researchers evaluated the effect of study participants consumption of fruits; vegetables; the antioxidant vitamins A, C, and E; and carotenoids on the development of early ARMD or neovascular ARMD, a more severe form of the illness associated with vision loss. While, surprisingly, intakes of vegetables, antioxidant vitamins and carotenoids were not strongly related to incidence of either form of ARMD, fruit intake was definitely protective against the severe form of this vision-destroying disease. Three servings of fruit may sound like a lot to eat each day, but papaya can help you reach this goal. Add slices of fresh papaya to your morning cereal, lunch time yogurt or green salads. Cut a papaya in half and fill with cottage cheese, crab, and shrimp or tuna salad. For an elegant meal, place slices of fresh papaya over any broiled fish³².

2.10.6 Protection against Rheumatoid Arthritis

Studies suggested that high doses of supplemental vitamin C makes osteoarthritis, a type of degenerative arthritis that occurs with aging, worse in laboratory animals, another indicates that vitamin C-rich foods, such as papaya, provide humans with protection against inflammatory polyarthritis, a form of rheumatoid arthritis involving two or more joints. The findings, presented in the *Annals of the Rheumatic Diseases* were drawn from a study of more than 20,000 subjects and focused on subjects who developed inflammatory polyarthritis and similar subjects who remained arthritis-free during the follow-up period. Subjects who consumed the lowest amounts of vitamin C-rich foods were more than three times more likely to develop arthritis than those who consumed the highest amounts³².

2.10.7 Promote Lung Health

If you or someone you love is a smoker, or if you are frequently exposed to secondhand smoke, then making vitamin A-rich foods, such as papaya, part of your healthy way of eating may save your life, suggests research conducted at Kansas State University. While studying the relationship between vitamin A, lung inflammation, and emphysema, Richard Baybutt, associate professor of nutrition at Kansas State, made a surprising discovery: a common carcinogen in cigarette smoke, benzo (a) pyrene, induces vitamin A deficiency. Baybutt's earlier research had shown that laboratory animals fed a vitamin A deficient diet developed emphysema. His latest animal studies indicate that not only does the benzo (a) pyrene in cigarette smoke cause vitamin A deficiency, but that a diet rich in vitamin A can help counter this effect, thus greatly reducing emphysema³².

2.10.8 Skin Infection Treatment

Some constituents of *Carica papaya* exhibit an alkaline pH thus when combined with borax or potassium carbonate and they have showed good results in treatment of warts, corns, sinuses, eczema, coetaneous tuberculosis and other hardness of the skin³⁰. Some constituents of *Carica papaya* exhibit an alkaline pH thus when combined with borax or potassium carbonate and they have showed good results in treatment of warts, corns, sinuses, eczema, coetaneous tuberculosis and other hardness of the skin³⁰.

2.10.9 Cure Erection Dysfunction

Green fruits of papaya are used to stimulate reproductive organs there by boosting male fertility. It contains an enzyme called arginine which is known in the medically community to boost blood flow around the man-hood where it boosts nitric acid in the body to relax the muscles

surrounding the blood vessels that supply the man-hood with blood. These blood vessels then dilate and increase blood flow, a more concentrated form of arginine is used to treat erectile dysfunction³⁰

2.10.10 Fights Cancer and Regulates Blood Circulation

Papaya has an abundance of cancer fighting lycopene which is a key intermediate in the biosynthesis of many important carotenoids, such as beta-carotene and xanthophylls and thus another useful compound not readily found in the plant kingdom but found in pawpaw is Fibrin. Fibrin reduces the risk of blood clots and improves the quality of blood cells, optimizing the ability of blood to flow through the circulatory system and also important in stroke prevention³²

2.11 Microorganism Associated with Fruits

Microorganisms, bacteria, fungi and viruses are ubiquitous and could be found resident on fresh fruits for their survival³³. Therefore, they are recognized as suitable vehicles for the transmission of human pathogens. Outbreak of food borne pathogens due to contamination of fruits beyond acceptable limits over the past decades cannot be overemphasized³⁴. In both developed and developing countries, there is increase in the incidence of food poisoning and gastroenteritis due to consumption of raw foods, especially ready-to-eat sliced fruit used in preparing home-made smoothies³⁵

Bacteria are the most common cause of food borne diseases and exist in a variety of shapes, types and properties. Some pathogenic bacteria are capable of spore formation and thus, highly heat-resistant (e.g. *Clostridium botulinum*, *C. perfringens*, *Bacillus subtilis*, *Bacillus cereus*).³⁶ Some are capable of producing heat-resistant toxins (e.g. *Staphylococcus aureus*, *Clostridium botulinum*). Most pathogens are mesophilic with optimal growth temperature range from 20 °C

to 45 °C. However, certain food borne pathogens (i.e. psychrotrophs), such as *Listeria monocytogenes*, and *Yersinia enterocolitica* are capable of growth under refrigerated conditions or temperatures less than 10 °C³⁶

2.11.1 *Escherichia coli*

Escherichia coli otherwise known as *E. coli* is a Gram-negative versatile bacterium, easily found and adapts to natural and random genetic alteration.³⁷ *E. coli* are short rod bacilli of the family Enterobacteriaceae. They are facultative anaerobes and non-sporulating. *E. coli* strains with the K1 capsular polysaccharide antigen cause approximately 40% of cases of septicemia and 80% of cases of meningitis³⁸

There is a large collection of sequenced *E. coli* genomes which exhibit different sizes and genomic diversity among commensal and pathogens, indicating a great assortment within the same bacterial species. They comprise of non-pathogenic bacteria that may act as commensals and belong to the normal intestinal microbiota of humans and many animals. There are also pathogenic variants, divided as diarrheagenic and extraintestinal pathogens, with different pathotypes and various natural hybrid strains. These variants can be facultative or obligate pathogens. The facultative bacteria are part of the intestinal tract and may act as opportunistic pathogens when outside of their natural habitat, causing various types of extraintestinal infections. On the other hand, intestinal obligate pathogenic variants cause infections in distinct conditions, from moderate diarrhea to more threatening cases, as lethal outcome³⁸

Based on virulence factors in *E. coli* genomes and phenotypic traits, the human pathotypes of diarrheagenic *E. coli* (DEC) are differentiated from non-pathogenic *E. coli* and extraintestinal pathogenic *E. coli* (ExPEC). The ExPEC are classified as uropathogenic *E. coli* (UPEC),

sepsis-causing *E. coli* (SEPEC) and neonatal meningitis-associated *E. coli* (NMEC)³⁷. Recent pathogenomics and phenotypic classification have revisited the DEC group as nine distinct pathotypes, proposed by their differential features and the essential virulence genes defining each subgroup, such as Shiga toxin-producing *E. coli* (STEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely-adhering *E. coli*(DAEC), adherent-invasive *E. coli*(AIEC), and cell-detaching *E. coli* (CDEC)³⁹

Table 4.5: *E. coli* Pathotypes Main Features: Extraintestinal (ExPEC) and Diarrheogenic (DEC)³⁷

E. coli Pathotype (DEC and ExPEC)	Main Virulence Traits	Clinical Manifestation	References
Shiga toxin producing (STEC)	Shiga-toxin	Not associated with human diseases	40
Enterohemorrhagic (EHEC)	EscF, EscC, EspA, EspB, EspD, Intimin, Tir, and Shiga-toxin	Foodborne Bloody diarrhea and HUS	40
Enteropathogenic (EPEC)	EscF, EscC, EspA, EspB, EspD, Intimin, Tir, EAF plasmid (tEPEC) and Bfp (tEPEC)	Watery diarrhea	41
Enteroadhesive (EAEC)	pAA plasmid, aggregated fimbriae adhesion (AAF), AggR regulator and dispersin	Acute and chronic diarrhea	42
Enteroinvasive (EIEC)	Plasmid pINV and invasins	Bacillary Dysentery	39
Enterotoxigenic (ETEC)	Thermostable (ST) and thermolabile (LT) enterotoxins	Watery diarrhea, known as traveler's diarrhea	39
Diffusely-adhering (DAEC)	Afa/Dr adhesins	Acute diarrhea to asymptomatic cases	42
Adherent-invasive (AIEC)	type VI secretion system, type I pili, long polar fimbriae	Chronic gut inflammation and Crohn's disease	(Barrios-Villa et al., 2018)
Cell-detaching (CDEC)	K-hemolysin, pyelonephritis-associated pili and cytotoxic necrotizing factor 1 (CNF1)	Diarrhea in infants, cell detaching, and linked to Crohn's disease cases	44
Uropathogenic (UPEC)	P fimbriae, certain other mannose-resistant adhesins, and type 1 fimbriae, K capsule, Hemolysin, Aerobactin	Urinary and Bloodstream infections	45

Sepsis-causing (SEPEC)	Type 1, P, and S fimbriae, K capsule K1/K5, hemolysin, aerobactin, yersiniabactin, salmochelin, CNF1, secreted autotransporter toxin, serum resistance, and colicin V	Bacteremia sepsis	and ⁴³
Neonatal meningitis-associated (NMEC)	ompTp, hlyF, cvaC, etsA, cvaA, etsB, cvaB, iss, iutA, and tsh	Meningitis, bacteremia neonates	and ⁴⁵ in

2.11.2 *Salmonella typhi*

Salmonellae are aerobic, non-spore-forming, flagellated bacilli. *Salmonella typhi*, the causative agent for typhoid fever, is a member of the salmonella genus which belongs to the Enterobacteriaceae family of Gram-negative bacteria. Members of this genus have a variety of pathogenic effect. *Salmonella typhi* cells are rod shaped 2-3 µm long and 0.4-0.6 µm diameter³⁶. *Salmonella* is one of the common pathogens causing sporadic cases or outbreaks of gastroenteritis⁴⁶. According to estimates by the World Health Organization, there were 153 million cases of worldwide non-typhoid *Salmonella* enteric infections in 2010, of which 56,969 were fatal, and about 50% of them were foodborne⁴⁷

In contrast to infection with non-typhoidal *Salmonella*, infection with typhoidal strains (primarily serovars Typhi and Paratyphi) presents as a systemic, often serious, disease. After invading the epithelial cell through intestinal mucosa, typhoidal strains disseminate through a transient primary bacteremia that may occur without diarrhea. Following hematogenous dissemination, some individuals will develop typhoid fever, which involves high temperature

(>39° C), vomiting, and headache, sometimes with complications that include neurologic involvement, intestinal perforation and death⁵⁰

In recent years, the antibiotic resistance rate of *S. Typhimurium* has been increasing, making it a global issue of increasing concern that might result in more severe health outcomes⁴⁸. Some studies have shown that the proportion of multidrug resistant bacteria in *S. Typhimurium* is very high and the most frequently observed antibiotic resistance patterns are the ASSuT (ampicillin, streptomycin, sulfonamides, and tetracycline) and ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline) tetra- and penta-resistant patterns, respectively⁴⁹. Multidrug resistant *S. Typhimurium* has brought difficulties to clinical treatment resulting in increased morbidity and mortality. Outbreaks of *S. Typhimurium* have occurred throughout the world, and most of them were caused by multidrug resistant *S. Typhimurium*⁵¹

2.11.3 *Bacillus cereus*

Bacillus cereus are members of the family Bacillaceae; they are Gram-positive, motile rods, and they have the ability to form spores.³⁶ Most *Bacillus* spp. are found throughout the environment, including soils, fresh and marine water environments. Spores produced by *B. cereus* possess appendages and/or pili and are more hydrophobic than any other *Bacillus* spores. These properties enable the spores to adhere to many different types of surfaces and to resist removal during cleaning and sanitation.³⁶

Vegetative cells of *B. cereus* grow at temperatures ranging from 4–15 to 35–55 °C but prefer 30–40 °C, depending on the strain. The organism grows at pH 4.9–9.3, but the inhibitory effect of pH is reduced in foods as evidenced by limited growth on meat at pH 4.35. The minimum

aw, for growth has been established at 0.93, but it has been suggested to use 0.912 as the minimum required for growth, because fried rice tends to have aw values ranging from 0.912 to 0.961 and readily supports *B. cereus* growth.³⁶

B. cereus produces two types of toxins, the emetic (vomiting) and the diarrhoeal one, causing two types of illness. The emetic syndrome is caused by emetic toxin produced by the bacteria during the growth phase in the food. The diarrhoeal syndrome is caused by diarrhoeal toxins produced during growth of the bacteria in the small intestine. The rapid onset of the emetic type is characterized by nausea and vomiting while the late onset of the diarrheal type is characterized by diarrhea and abdominal pain. Both syndromes (i.e., diarrheal and emetic) are a result of *B. cereus* endospores surviving the cooking process, after which germination and subsequent proliferation of vegetative cells occurs at some point during storage. Foods that are frequently implicated in *B. cereus* diarrheic food poisoning include meat products, soups, vegetables, puddings, sauces, milk and milk products³⁶. Symptoms are characterized by abdominal pain, nausea, and diarrhea after an incubation period of approximately 8.16 h. Diarrheal syndrome symptoms generally persist no longer than 12.24 h. After a 1h incubation period, emetic syndrome symptoms include primarily nausea and vomiting and persist for 6–24 h³⁶

A broad range of foods have been implicated as vehicles of *B. cereus*. These include boiled or fried rice, cooked vegetables and meats, pasta, vanilla sauce, custards, casseroles, pastries, salads, soups, ice cream, and herbs and spices⁵². The emetic form of illness (vomiting) is most frequently associated with improperly refrigerated starch dishes, such as fried rice; whereas, the diarrheal form of illness is associated with foods containing meat and vegetables⁵³.

2.11.4 *Yersinia enterocolitica*

The genus *Yersinia* belongs to the family Enterobacteriaceae and includes 10 established species, although only 3 are considered pathogenic to either humans or animals. *Yersinia pestis* is the causative agent of plague, *Yersinia pseudotuberculosis* is primarily an animal pathogen but may infect humans after the ingestion of contaminated food or water, and *Yersinia enterocolitica* has surfaced as a cause of foodborne gastroenteritis in humans.³⁶ *Yersinia* spp. are Gram-negative or gram-variable, non-spore-forming rods that grow under both aerobic and anaerobic conditions but are considered facultative anaerobes. With the exception of *Y. pestis*, all *Yersinia* spp. possess peritrichous flagella and are motile at 22–30 °C but not at 37 °C³⁶

Y. enterocolitica are widely distributed throughout the environment and have been isolated from raw milk, sewage-contaminated water, soil, seafood, humans, and many warm-blooded animals such as poultry and, most importantly, pigs³⁶. As a psychrotroph, *Y. enterocolitica* may pose a health hazard in contaminated refrigerated foods, although under refrigeration temperatures the pathogen is usually outgrown by other competing psychrotrophs³⁶.

Y. enterocolitica grow at temperatures between 0 and 45 °C but prefer an optimum temperature between 25 and 30 °C. This psychrotroph can survive alkaline conditions as well as any other gram-negative bacterium but does not survive well in acidic environments, because growth occurs between pH 4.0 and 10.0, with pH 7.6 being optimum. Additionally, *Y. enterocolitica* can grow in the presence of sodium chloride at concentrations as high as 5%³⁶

Not all serotypes of *Y. enterocolitica* are enteropathogenic, and the specific serotypes of *Y. enterocolitica* involved in human yersiniosis are prevalent primarily in swine. Ingestion of contaminated water or food, more specifically raw or undercooked pork, is a source of

foodborne infection in humans, resulting in symptoms appearing after an incubation period of a few days to a week. Intestinal yersiniosis may persist for 1–2 weeks in adults and as long as 4 weeks in children and may include symptoms such as watery, sometimes bloody, stools or bloody diarrhea in conjunction with fever, vomiting, and abdominal pain³⁶.

Immunocompromised individuals and children under the age of 15 are most commonly infected, and extraintestinal infections associated with yersiniosis include septicemia, meningitis, Reiter syndrome, myocarditis, glomerulonephritis, thyroiditis, and erythema nodosum. *Y. enterocolitica* toxin is heat stable, resists enzymatic degradation, remains stable during prolonged storage, and is of similar pH stability as the thermostable enterotoxin produced by ETEC³⁶

2.11.5. *Listeria monocytogenes*

Listeria monocytogenes is a Gram-positive facultatively anaerobic rod and is responsible for almost all *Listeria* infections in humans; although rare cases of infection caused by *L. ivanovii* have been reported. *Listeria monocytogenes* is one of the leading causes of death from food-borne pathogens especially in pregnant women, newborns, the elderly, and immunocompromised individuals⁵⁵. Infections in pregnant women can be devastating to the fetus, resulting in miscarriages, stillbirths, and birth defects.⁵⁵

It is found in environments such as decaying vegetable matter, sewage, water, and soil, and it can survive extremes of both temperatures (1–45 °C) and salt concentration marking it as an extremely dangerous food-borne pathogen, especially on food that is not reheated and is carried asymptotically by numerous animal species³⁶ The bacterium has been found in a variety of

raw foods, such as uncooked meats and vegetables, as well as in foods that become contaminated after cooking or processing³⁶

It can spread from the site of infection in the intestines to the central nervous system and the fetal-placental unit. It can cause meningitis (inflammation of the membrane surrounding spinal cord and brain), gastroenteritis (inflammation of mucous membranes of stomach and intestine), and septicemia (systemic spread of bacteria and toxins in the blood) can result from infection⁵⁵. It has 13 serotypes, including 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7; among them, serotypes 1/2a, 1/2b, and 4b have been associated with the vast majority of foodborne infections⁵³

Listeriosis is a serious infection usually caused by eating food contaminated with *L. monocytogenes*. Although it is a relatively rare disease with a high mortality rate (20–30 %) that makes it one of the deadliest food-borne threats³⁶. Unlike many other foodborne pathogens, *Listeria* multiplies in cold environments such as refrigerators. It can quickly spread in damp buildings, dripping off pipes or ceilings onto food. Once *Listeria* bacteria get into a food-processing factory, they can live there for years, sometimes contaminating food products³⁶

2.11.6 *Campylobacter jejuni*

Campylobacter spp. are small (0.2–0.9 µm wide and 0.2–5.0 µm long), spiral formed, Gram-negative bacteria with 18 species, six sub-species and two biovars³⁶. *Campylobacter* genomes are relatively unstable; several mechanisms that may lead to this genetic instability have been proposed, including bacteriophage activity, DNA recombination and transformation⁵³. They are very different from other pathogens associated with foodborne disease in that they are essentially microaerophilic, growing best in an atmosphere containing approximately 10% CO₂

and approximately 5% O₂. The species pathogenic for man also have a rather narrow temperature range for growth with a maximum temperature of ~46 °C and a minimum of 30 °C. These are classified as thermophilic campylobacters³⁶.

Campylobacter spp. are members of the family Campylobacteriaceae and *Campylobacter jejuni* is one of the most common causes of diarrheal illness. *C. jejuni* is responsible for approximately 850,000 illnesses, 8,500 hospitalizations, and 76 deaths in the US each year. The World Health Organization (WHO) estimates that ~1% of the population of Western Europe will be infected with campylobacters each year³⁶. Extensively found throughout nature, *C. jejuni* can colonize the intestines of both mammals and birds, and transmission to humans occurs via contaminated food products. This organism can invade the epithelial layer by first attaching to epithelial cells, then penetrating through them. Diarrhea results from damage to the epithelial cells. Systemic infections can also occur causing more severe illnesses⁵⁶

Campylobacter spp. are part of the normal intestinal flora of a wide variety of healthy domestic and wild animals, including cattle, sheep, goats, pigs, chickens, ducks, geese, wild birds, dogs, cats, rodents, and marine mammals, and are often found associated with bodies of water such as water troughs and streams. Most cases of campylobacteriosis are associated with eating raw or undercooked poultry meat, unpasteurized milk, contaminated water, or from cross-contamination of other foods by these items. All animals used for food can be campylobacter-positive as can many companion species (domestic pets). Samples from the natural environment, such as groundwater, will also frequently contain these pathogens.³⁶ Ready-to-eat fresh produce contaminated with enteric pathogens presents a risk to consumers. However, its importance as a source of campylobacters is unclear. The number of documented foodborne outbreaks associated with raw fruits, vegetables and unpasteurised fruit juices has increased.

Such foods can present a campylobacteriosis risk to public health as a consequence of using contaminated irrigation or washing water³⁶.

2.11.7 *Vibrio cholerae*

Vibrio cholerae (*V. cholerae*) belongs to the family of Vibrionaceae, a facultative anaerobe with a flagellum used for mobility⁵⁷. It is Gram-negative, bean-rod shaped, and oxidase positive, but it does not form spores⁵⁸. Fresh isolates are prototrophic, and in suitable media they breed very fast within a maximum growth rate of 30 min. They exhibit maximum growth in an aerobic condition, though they are facultative organisms. *V. cholerae* fare well in an alkaline medium but are destroyed in any condition below pH 6 and can be found mainly in aquatic habitats (freshwater, saltwater or brackish water) or in the intestine, vomit and stool of a human host⁵⁹

The bacterium is distinguished serologically on the O antigen of its lipopolysaccharide into cholera vibrio (pathogenic) and non-cholera vibrio (non-pathogenic) variants. Cholera toxin-producing strains, O1 and O139 serogroups, cause cholera disease (acute enteric human diarrhoea), while the non-toxigenic O1/O139 group causes non-epidemic periodic diarrhoea, wound infection, gastroenteritis, septicemia and skin infections. *V. cholerae* O1 is further classified into Ogawa, Inaba and Hikojima biotypes that may either be classical or El Tor serotypes⁵⁹

V. cholerae infections in humans start by ingesting polluted water and/or food. Once the organisms have successfully escaped from the acerbic hurdle of the abdominal cavity, the pathogenicity of *V. cholerae* is exhibited in their ability to invade human hosts and their ability to secrete cholera toxin from their TCP production⁶⁰

Antagonism of bacteria to antimicrobials has become an essential civic health issue that is mainly connected to disease management and control⁶¹. *Vibrio spp.* was earlier reported to be highly sensitive to most clinically used antibiotics⁶². However, emergence of antibiotic resistance strains has occurred and is escalating as the years go due to the abuse of antibiotics intake and assortment stress triggered by the use and misuse of antimicrobials in aquaculture, food production and animal farms⁶³. Treating animals, especially those eaten by man, with antibiotics has become a public health concern because it may result in the resistance of human pathogens to such antibiotics, which may be the only vital human drug available to treat a particular human infection⁶⁴.

2.11.8 *Staphylococcus aureus*

Staphylococcus aureus is one of the most important microorganisms causing foodborne outbreaks⁶⁵. These bacteria are non-motile, gram-positive cocci that appear singly or in pairs, tetrads, short chains, or characteristic “grapelike” clusters. Staphylococci are facultative anaerobes that, with the exception of *Staphylococcus saccharolyticus* and *Staph. aureus* subsp. anaerobius, grow more rapidly under aerobic conditions³⁶. In fact, *S. aureus* is an opportunistic pathogen that can induce food poisoning by producing enterotoxins⁶⁶. They are non-spore forming and can be easily different from streptococcus spp via oxidase test. *S. aureus* is oxidase positive while streptococcus is oxidase negative.⁷⁰

Staphylococcal enterotoxins (SEs) are members of a family of more than 20 different staphylococcal and streptococcal exotoxins.⁶⁷ These toxins share a common phylogenetic relationship, structure, function, and sequence homology⁶⁸. *Staphylococcal enterotoxins* A and B (SEA and SEB) are the most significant enterotoxins produced by *S. aureus*⁶⁹. SEA is often associated to foodborne outbreaks by *S. aureus* and SEB causes food poisoning.⁶⁹ Many foods

can be contaminated with SEs, particularly foods that contain carbohydrates and proteins (such as meats, eggs and dairy products). Most of the SE food poisoning cases are due to direct contact of hands with foods⁶⁹.

S. aureus grow, depending on the strain, at temperatures ranging from 7 to 47.8 °C and produce enterotoxins between 10 and 46 °C but prefer an optimum temperature between 40 and 45 °C. The bacterium grows between pH 4.5 and 9.3, with an optimum between 7.0 and 7.5, and is very tolerant to high levels of salt (>10% sodium chloride); enterotoxin production requires a minimum aw of 0.86, whereas growth has been demonstrated at an aw of 0.83⁵³.

S. aureus typically causes infections involving the skin, such as boils, cellulitis, impetigo, and postoperative wound infections, but can also be associated with more serious infections like bacteremia, pneumonia, osteomyelitis, cerebritis, meningitis, and abscesses of muscle, urogenital tract, central nervous system, and various abdominal organs³⁶. Toxic shock syndrome, a condition resembling septic shock and resulting from the production of toxic shock syndrome toxin 1, has been attributed to *Staph. aureus* infection³⁶. Further, the virulence of *S. aureus* has risen with existence of antibiotics resistance strains such as Methicillin resistant *S. aureus* (MRSA) and Vancomycin resistance *S. aureus* (VRSA)⁷¹. The resistance strains increased the challenge in treating the infections caused by them⁷⁰

2.11.9 *Shigella* spp.

The cells of the *Shigella* species are Gram-negative bacilli, non-motile, non-spore-forming, facultative anaerobic rod⁷². *Shigella* is differentiated from the closely related *Escherichia coli* on the basis of pathogenicity, physiology (failure to ferment lactose or decarboxylation lysine) and serology. They are generally catalase positive and oxidase and lactose negative. They

ferment sugars, usually without forming gas. The strains are able to at temperatures ranging from 200^oC and 460^oC, with an optimum at 370^oC and at a pH range of 5.0 to 7.5. The common selective or differential agar media used for the culturing of *Shigella* are MacConkey (MAC), Xylose Lysine Deoxycholate (XLD), Hektoen (HEK), Salmonella-Shigella (SS), Deoxycholate Citrate Agar (DCA). The genus is divided into four serogroups with over 2,500 identified multiple serotypes⁷².

The genus *Shigella* was divided into four species viz., *Shigella dysenteriae* (serogroup A), *Shigella flexneri* (serogroup B), *Shigella boydii* (serogroup C) and *Shigella sonnei* (serogroup D). Based on the variations in their O- polysaccharide portion of their LPS, the species were further classified into several serotypes, as *S. dysenteriae* known to have 15 serotypes, *S. flexneri* have 14 serotypes and sub serotypes, *S. boydii* 20 serotypes and *S. sonnei* with a single serotype.

Shigella spp. is the causative agent of the disease shigellosis. This severe intestinal infection is also called as bacillary dysentery. Globally, this disease is known as a major burden in public health care⁷². It is first and foremost a disease of humans. The disease is characterized by injure of the colonic epithelium followed by intracellular and intercellular spread, infection in the nearby or near to cells and the hosts acute inflammatory reactions leads to colitis of the mucosa. It results in the leakage of blood and mucous in the intestinal lumen⁷²

Shigella infections are very transmissible; transmission of the disease is mainly by person-to-person contact through contaminated hands. Outbreaks in children consistently occur under situation associating close physical contact, such as those come across in day-care centres, nursing homes, custodial institutions, cruise ships, aboriginal reservations and packed refugee

camps, with poor hygiene practices and contaminated food or water serving as the vehicle for infection⁷²

Shigella infections also may be acquired from consumption of contaminated food. In the developed and developing countries, incidence of foodborne illness is cited through foodNet, a reporting system used by public health agencies that occupies foodborne illness in over 13% of the population of the ten pathogens; Salmonella, Campylobacter and Shigella are responsible for most cases of foodborne illness. An estimated 20% of the total number of cases of shigellosis involved food as the vehicle of transmission⁷²

Antimicrobial resistance in pathogenic bacteria is developing and increasing risk to human health. Physicians are progressively attentive that antimicrobial resistance is increasing in bacterial pathogens and that, as a result, patients who are recommended antibiotics are at increased risk for promoting antimicrobial-resistant various infections⁷².

2.12 Antibiotics

Antibiotics are widely used most effective medication since the twentieth century against bacterial infections. In 1928, Alexander Fleming discovered Penicillin as the first natural antibiotic. He observed that Penicillium molds produced a diffusible extract that had antibacterial activity against Staphylococci⁷³. S.A. Waksman defined an antibiotic as a chemical substance, produced by micro-organisms, which can inhibit the growth of and even to destroy bacteria and other micro-organisms. Antibiotics can be prepared by either naturally or synthetically. Antibiotics inhibit bacterial growth by targeting essential cellular processes such as the synthesis of the bacterial cell wall, DNA/RNA, and proteins and retard growth and cause cell death⁷⁴

Resistance comes as a curse with antibiotics that occurs when bacteria change in some way that reduces or eliminates the effectiveness of drugs, chemicals or other agents designed to cure or prevent infections. Most of the antibiotics on the market were discovered in the mid to late 20th century. Thus, there is a limited arsenal of drugs to high resistance bacteria and bacteria can be resistant to multiple drugs at a time⁷⁶. Bacteria evolve rapidly so the mechanism of developing resistance grows fast. Antibiotic consumption continues to rise among the low- and middle income countries in recent years⁷⁸. World Health Organization has named antibiotic resistance as one of the three most important public health threats of the 21st century⁷⁹. It has been reported by the Centers for Disease Control and Prevention (CDC) estimated more than 2.8 million antibiotic resistance infections occurred in the U.S each year, and more than 35,000 people die as a result⁷⁷. Thus, the number is increasing alarmingly⁷⁵. Antibiotic resistance spread over due to overuse of antibiotics. So, if however, bacteria are exposed to drugs below the dose required to kill all bacteria in a population (the minimum bacterial concentration or MBC), they can mutate and resist antibiotic treatment via natural selection for resistance-conferring mutations⁷⁶

2.12.1 Classification OF Antibiotics

The most common classification of antibiotics is based on their molecular structures, mode of action, route of administration (injectable, oral, and topical), and spectrum of activity⁸⁰. Antibiotics are shown similar effectiveness, toxicity and allergic activity having the same chemical structure. The most common classes of antibiotics are Beta-lactams, Macrolides, Tetracyclines, Quinolones, Aminoglycosides based on chemical or molecular structures⁸⁰

2.12.1.1 Beta-Lactams

The beta-lactam groups are mostly prescribed groups of antibiotics. The most prominent members of the beta-lactam class include Penicillins, Cephalosporins, Monobactams and Carbapenems⁸⁰. All members belong to beta-lactam groups are containing beta-lactam ring. The chemical structure of beta-lactam, penicillin, cephalosporin, monobactams, and carbapenems are shown in Figure 4

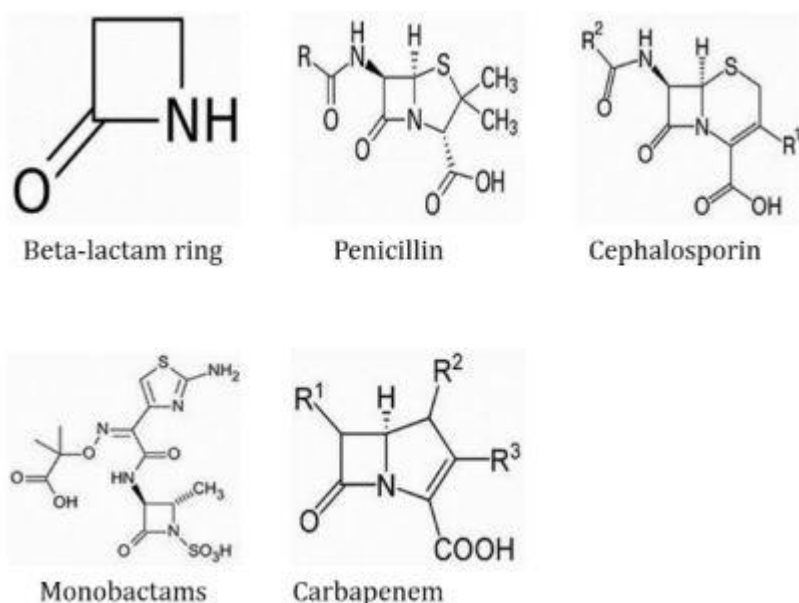


Figure 4: The Chemical Structures of the Member of Beta-lactam Antibiotic Groups³².

The highly reactive beta-lactam ring contains 3-carbon and 1-nitrogen in the ring, and this ring disrupts protein synthesis of bacterial cell wall and thus leading cell death or inhibits their growth. However, this ring also interferes with the synthesis of peptidoglycan in the cell wall of bacteria by blocking a penicillin-binding protein (PBP), resulting in the cell lysis and death⁸¹

2.12.1.2 Macrolides

Erythromycin A was the first macrolides discovered and isolated in 1952 by J. M. McGuire from soil-inhabiting fungus *Saccharopolysporaerythraea*.⁸⁰ It is a broad-spectrum antibiotic and primarily used for respiratory, skin and soft tissue infections. Demonstrated as broad-spectrum antimicrobial activity and was used primarily for respiratory and skin and soft tissue infections. The members of Macrolides are erythromycin, azithromycin, and clarithromycin⁸². Macrolides can reversibly bind to the 23S rRNA of bacteria and thus, inhibit protein synthesis by blocking elongation.⁷⁵

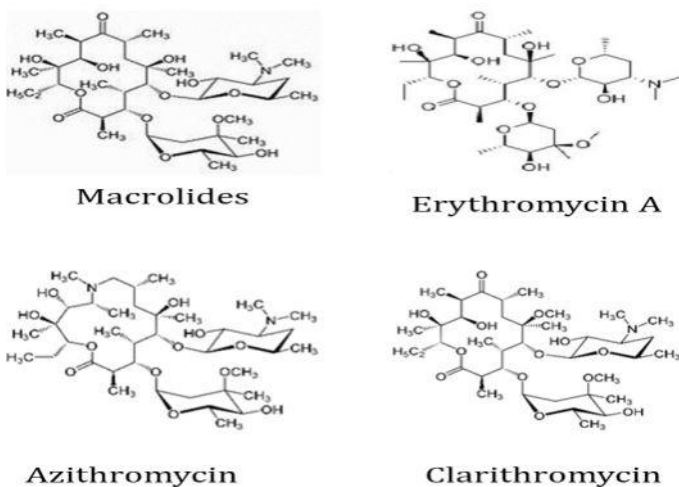


Figure 5: The Chemical Structure of the Members of the Macrolides Group of Antibiotics.⁷⁵

2.12.1.3 Tetracycline

Tetracycline was discovered by Benjamin Duggar in 1945 from a bacterium *Streptomyces*⁷⁵. The chlorotetracycline was the first member of this class. The tetracyclines can be sub-divided into different generations (1st to 3rd) based on the synthesis method. The members of first generation tetracyclines are tetracycline, chlortetracycline, oxytetracycline and demeclocycline derived from biosynthesis⁷⁵

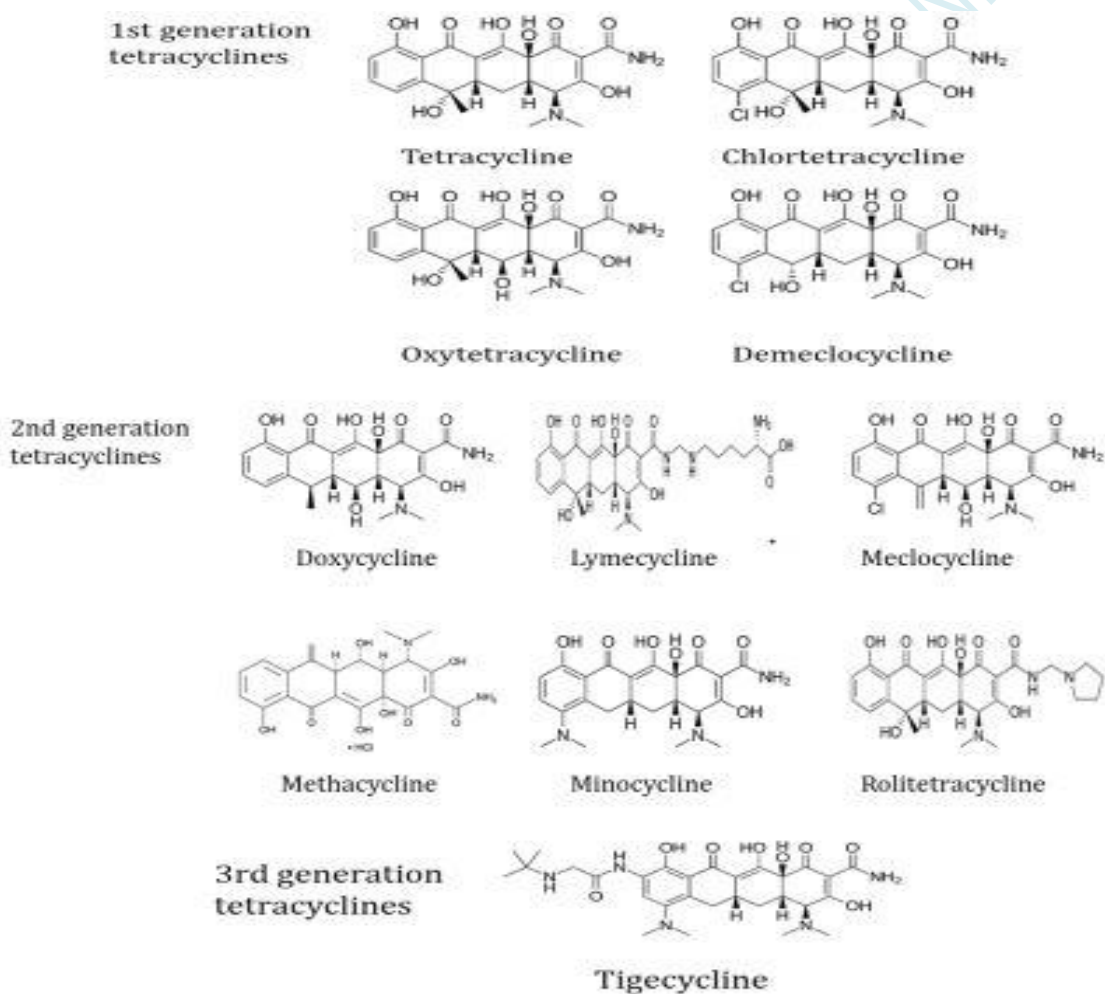


Figure 6: The Chemical Structure of the Members of the Tetracyclines Group of Antibiotics.⁷⁵

The second-generation tetracyclines are doxycycline, lymecycline, meclocycline, methacycline, minocycline, and rolitetracycline those are semi-synthetic. The 3rd generation tetracyclines are total synthetic (e.g., tigecycline)⁷⁵. These classes of antibiotics target to attack bacterial ribosome by disrupting amino acids to polypeptide chains during protein synthesis. Tetracyclines are used in treating malaria, elephantiasis, amoebic parasites and rickettsia⁷⁵

2.12.1.4 Quinolones

Quinolones class of antibiotics was discovered by chemists when Leshner accidentally discovered nalidixic acid as a by-product of the synthesis of the anti-malarial compound chloroquine in 1962⁷⁵. The chemical structure of quinolone is shown in Figure 4. They consist of two rings, though recent generations of quinolones have more rings in the parent structure to increase their spectrum of antimicrobial activity.

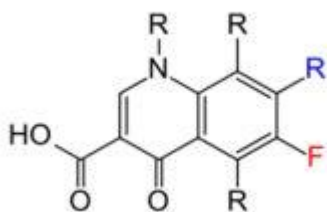


Figure 7: The Chemical Structure of Quinolones⁷⁵

The members of quinolones are commonly cinoxacin, norfloxacin, ofloxacin, ciprofloxacin, temafloxacin, sparfloxacin, nalidixic acid, enoxacin⁷⁵. The quinolones class of antibiotics can interfere with DNA replication and transcription in bacteria. These classes of antibiotics are used in the treatment of urinary, systemic and respiratory tract infections⁸⁰.

2.12.1.5 Aminoglycosides

Streptomycin was the 1st drug of aminoglycosides class of antibiotics which was isolated from soil Actinomycetes in 1943. Drug to be discovered among members of this class of antibiotics was streptomycin, first isolated in 1943⁷⁵. The chemical structure of the members of aminoglycosides classes are shown in Figure 5

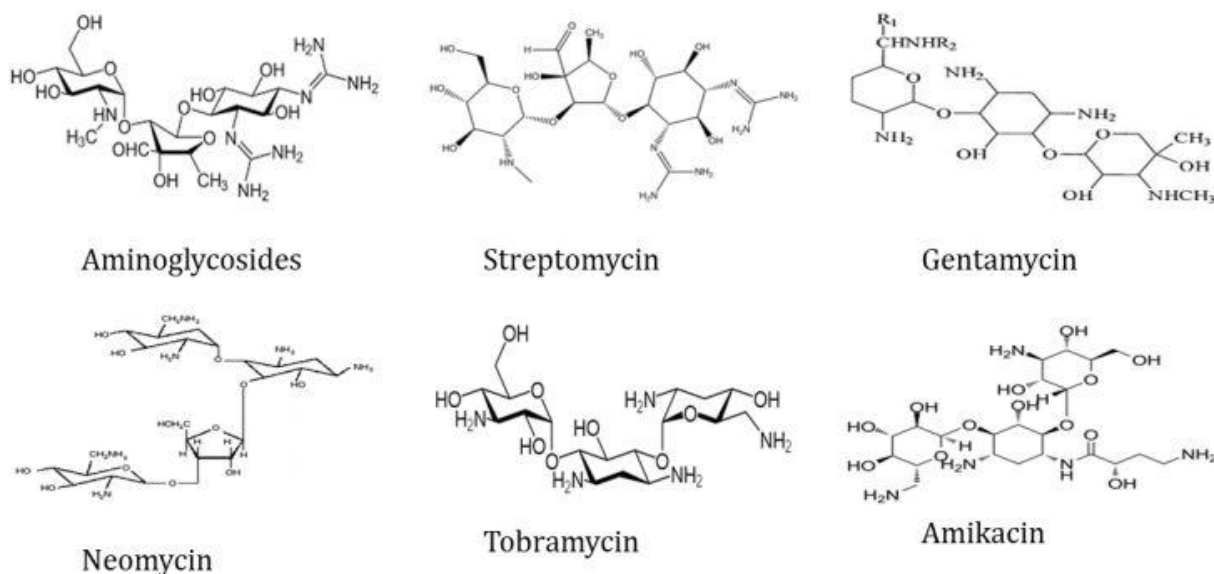


Figure 8: The Chemical Structure of the Members of Aminoglycosides Class Antibiotics⁷⁵

Other members of the aminoglycosides class of antibiotics are gentamicin, neomycin, tobramycin, and amikacin. The aminoglycosides class of antibiotics possess broad-spectrum antimicrobial activity. These classes of antibiotics can bind ribosomal subunits and thus, inhibit the protein synthesis in bacteria⁷⁵. They are widely used in treating bubonic plague, tularemia, and tuberculosis⁸²

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Chapter Three

Methodology

3.1 Materials

The materials used for this study area; fruit sample (water melon, pineapple and pawpaw), distilled water, peptone water, slant bottles petri-dishes(disposable), measuring cylinder, cotton wool, hand gloves, face mask, paper tape, foil paper, permanent marker, inoculating loop, beakers(500mls, 250mls, 100mls), conical flask(1000mls, 500mls), needle and syringe, (5mls, 2mls), spirit lamp, matches, pipette and tips, tubes, vials and racks.

3.2 Source of the Fruits

The samples were purchased from two different market in Ibadan, Oyo state (Oje market and Bodija market)

3.3 Equipments

Incubator(U-clear DNP- 9052), water bath(Uniscope SM 101), Auto clave (U- Clear), weighing balance(Mettler Toledo), Centrifuge, Thermocycler, Thermomixer, Refrigerator, Electrophoresis System, Voltex Mixer, Water bath and Dry bath.

3.4 Reagents and Consumables

The Reagents/ chemicals used for this study are; 70% ethanol, distilled water, Sterile peptone water, distilled water, Nutrient agar, Blood agar, Mannitol salt agar, MacConkey agar, Iodine solution, methylene blue, safranin, methylene red broth, Voges- Proskauer brotg

3.5 Sample Collection

Fruits samples were gotten from Oje market and Bodija market(sample K and sample Z). 20g each from the fruit was blended together using a sterile blender and sample was taken to Microbiology Laboratory for analysis same day.

3.6 Sterilization

All glass wears used were thoroughly washed using liquid wash and kept in the oven at 160°C FOR 2-3hours. Also, all surfaces use were swabbed at interval using cotton wool and ethanol.

3.7 Sample Preparation

1ml from sample K(Oje Market) and Z(Bodija Market) was removed and dispensed into 10mls sterile peptone water. It was incubated aerobically and aerobically at 36°C for 24hours.

3.8 Isolation of Organisms

Selected fruits sample were collected from two market metropolis; Oje market labeled sample K and Bodija market labeled as sample Z and brought into the laboratory. The fruits sample were blended into pulps with sterile water and 1ml of each fruit sample was inoculated into 9ml of peptone water aseptically and incubated at 37°C aerobically and anaerobically for 24hours immediately the samples were collected and labelled appropriately.

The labeled samples were removed from the incubator and 1ml of each fruits sample was dispensed into 9ml of sterile distilled water in a test-tube to make a serial dilution 10^{-1} and was serially diluted to 10^{-5} . Each test-tube were labelled accordingly for sample K and Z.

Nutrient agar, MaConkey agar and Mannitol salt agar were prepared according to manufacturer specification. 0.1ml of serial dilution 10^{-1} , 10^{-3} , 10^{-5} of sample K and Z was inoculated into

Nutrient agar, MacConkey agar and Mannitol Salt agar using pour plate method. During pour plate process, 0.1m of each fruit sample was dispensed into molten media and rocked anticlockwise before the media solidified in a petri-dish. The media plates were incubated at 37°C for 24hrs aerobically and anaerobically in an inverted position.

3.9 Preparation of Agars

3.9.1 Nutrient Agar

The preparation of nutrient agar (NA) was done by dissolving 28g of NS powder into 1000mls distilled water according the manufacturers instruction. It was mixed and boiled in water bath for 3 minutes until to homogenize, it was then sterilized in an autoclave at 121°C for 15minutes. Nutrient agar support the growth of no fastidious organisms.

3.9.2 MacConkey Agar (MA)

The preparation of nutrient agar was done by dissolving of powder into 1000ml distilled water according to the manufacturers instruction. It was mixed and boiled in water bath for 3 minutes to dissolve completely, it was then sterilized in an autoclave at 121°C for 15minutes. Nutrient agar support the growth of non fastidious organisms.

3.9.3 Mannitol Salt Agar(MSA)

The preparation of mannitol salt agar was done by dissolving 48.5g into 1000mls distilled water, it was mixed and boiled in water bath for 1- 3minutes according to the manufacturer instruction. It was then autoclave at 121°C for 15minutes. Mannitol salt agar is a selective and differential agar used for the isolation of microorganisms.

3.10 Gram Staining

Staining techniques was done using Danish Method 1884, this test differentiated the bacteria's into gram positive and gram negative bacteria which helped in classification of the organism.

A tiny portion of pure culture of selected isolate was picked with sterile inoculating loop to make a smear by homogenizing it in a drop of distilled water on a clean sterilized microscopic slide, and then spread in a circular motion with the inoculating loop and heat fix. The smear was gently flooded with methylene blue (primary stain) for a minute and gently rinsed with distilled water, few drops of Lugol's was added and allowed to stay for a minute then rinsed with distilled water. The smear was then discoloured with 95% ethanol (discolourer) which was added to the slide in drops until it covered the smear and the rinsed immediately and counter stained with 0.5% safranin (counterstain) solution for about 60 seconds and then rinsed with distilled water. It was air dried and drops of oil immersion was added and then examined under microscope.

3.11 Hemolysis Test

Hemolysis test was performed on freshly prepared Blood agar (5mls) in Nutrient agar. Blood agar is an enriched medium often used to grow fastidious organisms.

The Nutrient agar was autoclaved and allowed to cool (45- 50°C), 5% blood was added and mixed gently after which, was poured onto sterile petri dishes, allowed to solidify, inoculated and incubated at 35°C for 24 hours

3.12 Biochemical Tests

3.12.1 Indole Test.

Peptone water was dispensed into 5mls test tube and sterilized by autoclaving 15minutes at 121°C.the test tube was inoculated aseptically by taking growth of 24 hours cultured organism then added 0.5ml Kovac's reagent to the broth culture. Observed the presence or absence of ring.

3.12.2. Methyl Red Test

Methyl red broth (glucose phosphate broth) was prepared and cooled to room temperature, the broth was dispensed into sterile test tubes and inoculated with pure culture of microorganisms after which it was incubated at 35°C for 48hours. 5 drops of the methyl indicator was added to the culture

3.12.4 Simmon Citrate Test

Simmon Citrate agar was prepared and slightly inoculated on a Sterile petri- dish from a 24 hours cultured organism, it was then incubated at 35°C for 24hours . Colour changes was observed for denoting alkalization

3.12.5. Catalase Test

1-2mls of hydrogen peroxide solution was dispensed into a sterile test tube and inoculated with a 24 old cultured organism. Immediate bubbling was then observed.

3.13 DNA Isolation

Fruits were harvested and frozen immediately in liquid nitrogen. The use of lyophilized tissues offers several advantages. Dry tissue can be efficiently disrupted while the DNA is unhydrated and can be stored for several years with little loss of DNA quality. A 0.3 g of fruit sample was

ground in liquid nitrogen using a mortar and pestle. The pulverized fruits were quickly transferred to liquid nitrogen. 2% of CTAB buffer (1 ml) containing 1% (v/v) mercaptoethanol and 1% PVP was quickly added to the micro centrifuge tube (2 ml) and stirred with a glass to mix. The tube was incubated at 60°C for 30 min with frequent swirling. An equal volume of chloroform:Isoamylalcohol (24:1) was added and centrifuged at 10 000 rpm and 4°C for 15 min to separate the phases. The supernatant was carefully decanted and transferred to a new tube. The above steps, beginning with the addition of chloroform: isoamylalcohol (24:1) and ending with decanting of supernatant, were repeated twice. The supernatant was precipitated with $\frac{2}{3}$ volume of ethanol. The precipitated nucleic acids were collected and washed twice with the buffer (75% ethanol, 3 M sodium acetate, TE). The pellets were air dried and re-suspended in TE. The dissolved nucleic acids were brought to 1.4 M NaCl and re-precipitated using 2 volumes of 75% ethanol. The pellets were washed twice using 100% ethanol⁴, dried and re-suspended in 100 μ l of the buffer. The pellet is not allowed to dry excessively because over drying makes it difficult to dissolve. The tube was incubated at 37°C for 30 min to dissolve genomic DNA, was then added.

3. 14 PCR Reactions and Electrophoresis

The primer used was (GACA) 5:5'GACAGACAGACAGACAGACA- 3'. Specific annealing temperature (T_a) determined (GACA) 5 was 62°C. PCR reactions were performed with the Gene Amp PCR System 2400 Perkin Elmer. The PCR conditions were optimised for other thermo-cyclers and annealing temperatures was optimised for each primer set. Each 25 μ l reaction volume contains 2.5 μ l reaction buffer (10x), 2.5 μ l MgCl₂ (25 mM), 2 μ l dNTP mixture (2.5 mM), 4 μ l of primer (10⁻⁴ mol l⁻¹), 0.5 μ l Taq DNA polymerase (Red Gold star™

DNA polymerase, Eurogentec, 5 units/-1) and 1 -1 of DNA (40 ng). PCR consists of one cycle of 94°C, 2 min, which was followed by 27 cycles of 94°C, 1 min; 62°C , 1 min; 72°C, 2 min, and finally one cycle of 72°C, 7 min. The PCR products were analyzed by electrophoresis using a 2% agarose gel in TBE buffer. DNA was stained by soaking the gel in a 0.5 mg/ml ethidium bromidesolution. In the subsequent wash. During ethanol precipitation of nucleic acids from 1.4 M NaCl, polysaccharides remain dissolved in the ethanol³

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Endnotes

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Chapter Four

Discussion of Findings

4.1 Concentration of various Heavy Metals Found in the Fruit Samples

Table 4.1 & figure 1 represents the Heavy Metals Found in the Fruit Samples. The result shows that for the three samples each metals are lesser than permitted limit and within the references range with the exception of Lead that is greater than reference range.

Table 4.2 & figure 2 represents the Heavy Metals Found in the Fruit Samples. The result shows that for the three samples each metals are lesser than permitted limit and within the references range with the exception of Lead that is greater than reference range.

Table 4.3 & figure 3 represents the Heavy Metals Found in the Fruit Sample 3. The result shows that for the three samples each metals are lesser than permitted limit and within the references range with the exception of Lead that is greater than reference range.

The table 4.4 represents the mean concentration of various heavy metals found in the fruit samples. The result shows that mean value **Lead** range from 0.15 to 0.69, mean value of **Chromium** range from 0.16 to 0.76 , mean value of **Cadmium** range from 0.05 to 0.96, mean value of **Chromium** range from 0.16 to 0.76 , mean value of **Copper** range from 0.38 to 0.65, mean value of **Cadmium** range from 0.05 to 0.96, mean value of **Chromium** range from 0.16 to 0.76 , mean value of **Zinc** range from 0.57 to 0.82 and mean value of **Nickel** range from 0.50 to 0.91. this indicated that Nickel and Zinc have the highest mean values among other metals found in the fruit samples. Furthermore, The results shows that mean value of Chromium is significant when compare to Lead, Cadmium, Copper, Zinc and Nickel in the tree samples.

Also, mean value of lead, Chromium, Cadmium were higher when compared to one another while that of Copper, Zinc and Nickel were not higher

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4.1 Concentration of various Heavy Metals Found in the Fruit Sample One

Metals						
	Lead	Chromium	Cadmium	Copper	Zinc	Nickel
Reference	0.02	1.5	0.5	2.0	5.0	1.0
range						
pineapple	0.060	0.120	0.073	0.273	1.173	0.243
Pawpaw	0.117	0.133	0.033	0.343	2.033	0.180
Water	0.031	0.230	0.078	0.510	3.103	0.190
Melon						

Source: Authors Laboratory Research

Table 4.2 Concentration of various Heavy Metals Found in the Fruit Sample Two

Fruit	Lead	Chromium	Cadmium	Copper	Zinc	Nickel
Sample						
Reference	0.3	1.5	0.2	73.3	99.4	67.9
range						
pineapple	0.034	0.30	0.09	0.71	3.13	0.23
Pawpaw	0.035	0.39	0.32	0.30	4.0	0.38
Water	0.043	0.49	0.33	0.42	3.3	0.43
Melon						

Source: Authors Laboratory Research

Table 4.3 Concentration of various Heavy Metals Found in the Fruit Sample Three

Fruit	Lead	Chromium	Cadmium	Copper	Zinc	Nickel
Sample						
Reference	0.02	1.5	0.5	2.0	5.0	1.0
range						
Pineapple	0.03	0.41	0.13	0.65	3.13	0.23
Pawpaw	0.052	0.97	0.21	0.31	4.0	0.38
Water	0.031	0.91	0.32	1.01	3.3	0.43
Melon						

Source: Authors Laboratory Research

Table 4.4 Mean Concentration of various Heavy Metals Found in the Fruit Samples

Values are presented as mean plus or minus standard deviation.

Sample No	Lead	Chromium	Cadmium	Copper	Zinc	Nickel
1	0.69 ± 0.0019 _b	0.16 ± 0.003 _a	0.05 ± 0.001	0.38 ± 0.0143	0.82 ± 0.013	0.91 ± 0.002
2	0.27 ± 0.0024 ^a	0.39 ± 0.009 ^{a,b,c}	0.39 ± 0.009	0.47 ± 0.040	0.71 ± 0.002	0.50 ± 0.004 ^a
3	0.15 ± 0.0012 ^a	0.76 ± 0.001 ^{a,b,c}	0.96 ± 0.09 ^a	0.65 ± 0.1220	0.57 ± 0.021	0.89 ± 0.012

4.2 Enumeration of Fruit Samples

Table 4.8 represents the mean value of enumeration of fruit samples. The result shows that mean value of bacterial count of 10^5 cfu/ml (NA) range from 42.75 ± 2.0 with standard deviation range between 5.12 ± 13.83 , mean value of bacterial count of 10^5 cfu/ml (MAC) range from 23.0 ± 25.5 with standard deviation range between 3.65 to 9.95 and mean value of bacterial count of 10^5 cfu/ml (MSA) range from 2.33 ± 5.33 with standard deviation range between 1.15 ± 2.52 . thus, bacterial count of 10^5 cfu/ml (NA) have the highest mean of all the samples. The p-value of 0.401 ± 0.701 for Aerobic and aneorobic respectively were gather than 0.05 significant level. This indicated that there are no significant differences in the count of both aerobic and anaerobic

Table4.5 Enumeration Result of Fruit Sample 1

Sample Codes	Bacterial Counts 10⁵ cfu/ml (NA)	Bacterial Counts 10⁵ cfu/ml (MAC)	Bacterial Counts 10⁵ cfu/ml (MSA)
K1	34	17	3
Z1	57	32	8
K2	31	25	NG
Z2	49	28	5

Source: Authors Laboratory Research

Table 4.6 Enumeration Result of Fruit Sample 2

Bacterial Counts 10⁵ cfu/ml (NA)	Bacterial Counts 10⁵ cfu/ml (MAC)	Bacterial Counts 10⁵ cfu/ml (MSA)	(MSA)
2K1	41	19	3
2Z1	49	25	1
2K2	52	27	3
2Z2	43	21	NG

Source: Authors Laboratory Research

Table 4.7 Enumeration Result of Fruit Sample 3

Bacterial Counts	Bacterial Counts	Bacterial Counts	(MSA)
10⁵ cfu/ml (NA)	10⁵ cfu/ml (MAC)	10⁵ cfu/ml (MSA)	
3K1	32	14	NG
3Z1	54	29	2
3K2	59	21	3
3Z2	63	37	6

Source: Authors Laboratory Research

Table 4.8 Mean Value of Enumeration Result of Fruit Sample

Sample	Bacterial		Bacterial		P-Value	
	Counts		Counts			
	10 ⁵ cfu/ml (NA)	10 ⁵ (MAC)	cfu/ml	10 ⁵ (MSA)	Aerobic	Anerobic
1	42.75 ± 5.12	25.5 ± 6.35		5.33 ± 2.52		
2	46.25 ± 12.34	23.0 ± 3.65		2.33 ± 1.15	0.401	0.702
3	52.00 ± 13.83	25.25 ± 9.95		2.67 ± 2.08		

Source: Authors Laboratory Research

4.3 Colonial Morphology of Isolates

Table 4.9 and figure 4 represents the Colonial Morphology of Isolates with 14 isolates in total. The results shows that 8 isolates were large and 4 were small in size, 11 isolates were round and 1 isolate was irregular round in shape, 10 isolates were flat, 1 isolates was raised and also 1 isolate was irregular flat in elevation. They were all have smooth surface. Finally, the colour were Yellow mucus, white, irregular Cream, Green-Blue Pigment, Blue Pigment, White Crystalline, Yellow and Cream for all the isolates.

Table 4.9 Colonial Morphology of Isolates

Isolate Code	Size	Shape	Elevation	Surface	Colour
CK1	Small	Round	Raised	Smooth	Yellow mucus
CZ1	Large	Round	Flat	Smooth	White
CK2	Large	irregular	Flat	Smooth	Yellow mucus
CZ2	Large	Round	Flat	Smooth	irregular Cream
2CK1	Small	Round	Irregular Flat	Smooth	Green-Blue Pigment
2CZ1	Large	Round	Flat	Smooth	Blue Pigment
2CK2	Large	Round	Flat	Smooth	White
2CZ2	Large	Round	Flat	Smooth	White
3CK1	Small	Round	Flat	Smooth	White Crystalline
3CZ1	Large	Round	Flat	Smooth	Yellow
3CK2	Large	Round	Flat	Smooth	Cream
3CZ2	Small	Round	Flat	Smooth	Cream

Source: Laboratory Analysis, 2022

4.4 Hemolysis Test

The table 4 and fig represent Hemolysis Test results with the 37 isolates in total. It is shown from the result that 14 (38.9%) tested positive for hemolysis while 22 (61.1%) isolates tested negative. This means that percentage of pathogenic isolates is 38.9% while remaining 61.1% is non pathogenic. The fourteen (14) pathogenic isolates are K1NAi, K1NAiii, Z1NAii, K2NAi, K2NAii, K2NAiii, Z2NAii, K1MACi, K1MACii, Z1MACii, K2MACi, Z2MACi, K1MANi and K2MANi as shown in the table above.

Table 4.10: Hemolysis Test

Isolates	Haemolysis
Codes	
K1NA ⁱ	+
K1NA ⁱⁱ	-
K1NA ⁱⁱⁱ	+
Z1NA ¹	-
Z1NA ⁱⁱ	+
Z1NA ⁱⁱⁱ	-
K2NA ⁱ	+
K2NA ⁱⁱ	+
K2NA ⁱⁱⁱ	+
Z2NA ⁱ	-
Z2NA ⁱⁱ	+
Z2NA ⁱⁱⁱ	-
K1MAC ⁱ	+
K1MAC ⁱⁱ	+
K1MAC ⁱⁱⁱ	-
Z1MAC ¹	-
Z1MAC ⁱⁱ	+
Z1MAC ⁱⁱⁱ	-

K2MAC ⁱ	+
K2MAC ⁱⁱ	-
K2MAC ⁱⁱⁱ	-
Z2MAC ⁱ	+
Z2MAC ⁱⁱ	-
Z2MAC ⁱⁱⁱ	-
K1MAN ⁱ	+
K1MAN ⁱⁱ	-
K1MAN ⁱⁱⁱ	-
Z1MAN ¹	-
Z1MAN ⁱⁱ	-
Z1MAN ⁱⁱⁱ	-
K2MAN ⁱ	+
K2MAN ⁱⁱ	-
K2MAN ⁱⁱⁱ	-
Z2MAN ⁱ	-
Z2MAN ⁱⁱ	-
Z2MAN ⁱⁱⁱ	-

Source: Laboratory Analysis 2022

Source: Authors Laboratory Research

4.5 Biochemical Test

The table represents Biochemical test Identification results with fourteen (14) isolates in total. The result shows that 4(28.5%) isolates that is Z2MACⁱ, Z1MACⁱⁱ, K2MANⁱ and K2NAⁱⁱ yielded *Staphylococcus aureus* portable diseases, 3(21.4%) isolates that is K1MANⁱ, K1MACⁱ and K2NAⁱ yielded *Escherichia coli*, 2(14.28%) isolates that is K2NAⁱⁱ and Z1NAⁱⁱ yielded *Streptococcus sp*, also, 2 (14.28%) of both K1NAⁱ and K1MANⁱ isolates yielded *Bacillus subtilis*, while 1(7.14%) each of K1MACⁱⁱ, K1NAⁱⁱⁱ and Z2NAⁱⁱ yielded *Pseudomonas sp*, *Salmonella typhi* and *Bacillus cereus* respectively.

4.11 Biochemical Test Result

Isolates	Gram staining (colony shape)												Probable Organism
		Catalase	Oxidase	Sucrose	Lactose	Glucose	Simon citrate	Methyl red	Voges proskauer	Indole	Motility	Spore staining	
K1NA ⁱ	+ Purple flagellated Short rods	+	-	+	-	+	+	-	+	-	+	+	<i>Bacillus subtilis</i>
K1NA ⁱⁱⁱ	- pink Short rods	-	+	-	-	+	+	-	-	-	-	-	<i>Pseudomonas sp</i>
K2NA ⁱ	- Pink Short rod	+	-	+	+	+	-	+	-	-	+	-	<i>Escherichia coli</i>
K2NA ⁱⁱ	+ Purple Cocci in chains	-	-	+	+	+	+	+	-	-	ND	ND	<i>Streptococcus sp</i>
K2NA ⁱⁱ	+ Cocci in clusters	+	-	+	+	+	+	+	+	-	-	-	<i>Staphylococcus aureus</i>
K1MA C ⁱ	- Short rods	+	-	+	+	+	-	+	-	-	+	-	<i>Escherichia coli</i>
K1MA C ⁱⁱ	- Short rods	+	-	+	+	+	+	+	+	-	-	+	<i>Salmonella typhi</i>
K2MA C ⁱ	- Short rods	+	-	+	+	+	-	+	-	-	+	-	<i>Escherichia coli</i>
K1MA N ⁱ	+ Short rods	+	-	+	-	+	+	-	+	-	+	+	<i>Bacillus subtilis</i>
K2MA N ⁱ	+ Cocci in clusters	+	-	+	+	+	+	+	+	-	-	-	<i>Staphylococcus aureus</i>
Z1NA ⁱⁱ	+ Purple Short rod in chains	-	-	+	+	+	+	+	-	-	ND	ND	<i>Streptococcus sp</i>
Z2NA ⁱⁱ	+ Short rod	+	-	+	+	+	+	+	-	-	-	+	<i>Bacillus cereus</i>
Z1MAC ii	+ Cocci in cluster	+	-	+	+	+	+	+	+	-	-	-	<i>Staphylococcus aureus</i>
Z2MAC i	+ Cocci in cluster	+	-	+	+	+	+	+	+	-	-	-	<i>Staphylococcus aureus</i>

Source: Laboratory Analysis, 2022

4..12 Antibiotics Susceptibility Test of Isolated Bacterial

The table represents the Antibiotics Susceptibility Test of Isolated Bacterial. The result shows that 8 of the isolates show multi-drug resistance while two of them show extensive drug resistance.

K1NAi	K1NAii	Z1NAi	K2NA	K2NAi	K2NAii	Z2NAi	K1Mi	KMCii	ZMCi	K2MCi	Z2MC	K1M
	i	i	i	i	i	i					i	i
15	7	11	11	14	11	11	11	13	12	12	12	12
10	11	11	11	10	11	10	12	13	11	12	12	12
10	11	5	12	0	11	6	12	4	5	4	4	10
0	0	0	9	0	0	0	4	0	0	0	0	0
7	0	0	12	0	0	0	0	0	0	0	5	11
15	11	11	11	12	11	11	10	11	11	12	11	12
9	11	11	11	12	11	11	10	11	11	11	12	12
11	11	11	12	5	11	11	11	5	5	4	11	12
11	0	11	12	5	0	10	12	5	5	0	11	12
11	0	10	11	11	10	14	12	9	11	5	12	12

The diameter of susceptibility are measured in (mm)

Source : Laboratory Analysis, 2022

Table 4.13: Characteristics and Properties of Partial 16S Ribosomal RNA Sequences using nBLAST on GenBank

Sample ID	Accession Number	Properties	Identified Species	Details	E-Value
LCU-MCB-22-010	OP114666	1,004 bp	<i>Bacillus cereus</i>	99.20% similarity using BLAST 2.10.0N+	0.0
LCU-MCB-22-011	OP114667	1,140 bp	<i>Bacillus cereus</i>	99.56% similarity using BLAST 2.10.0N+	0.0

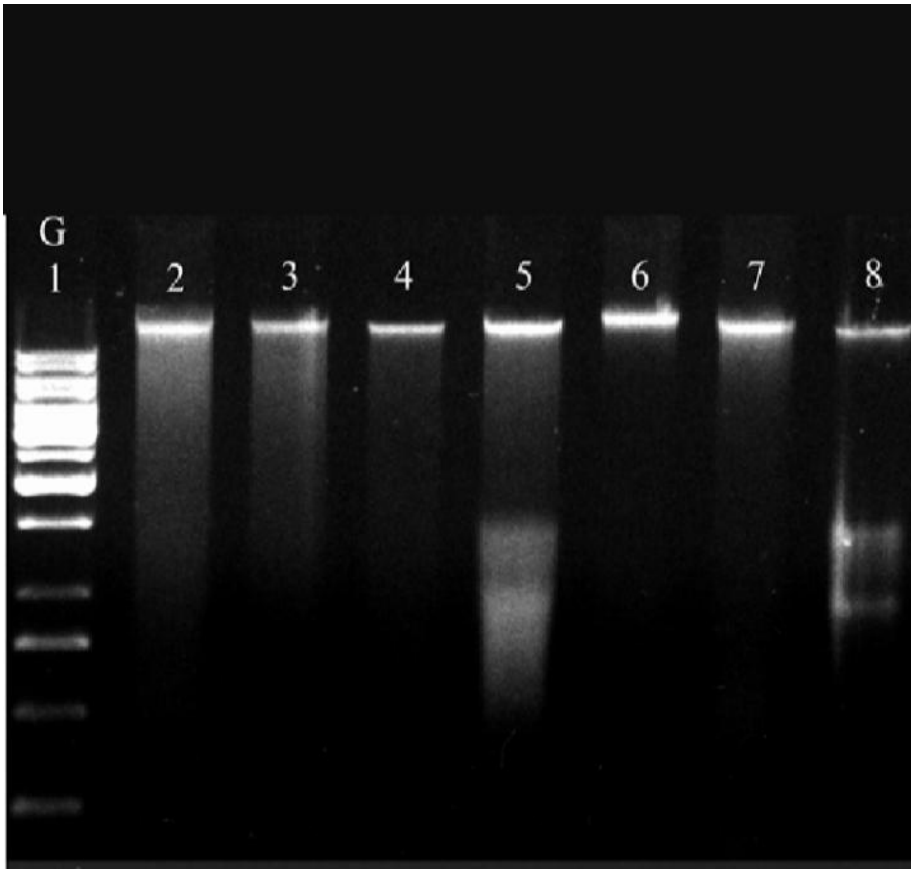
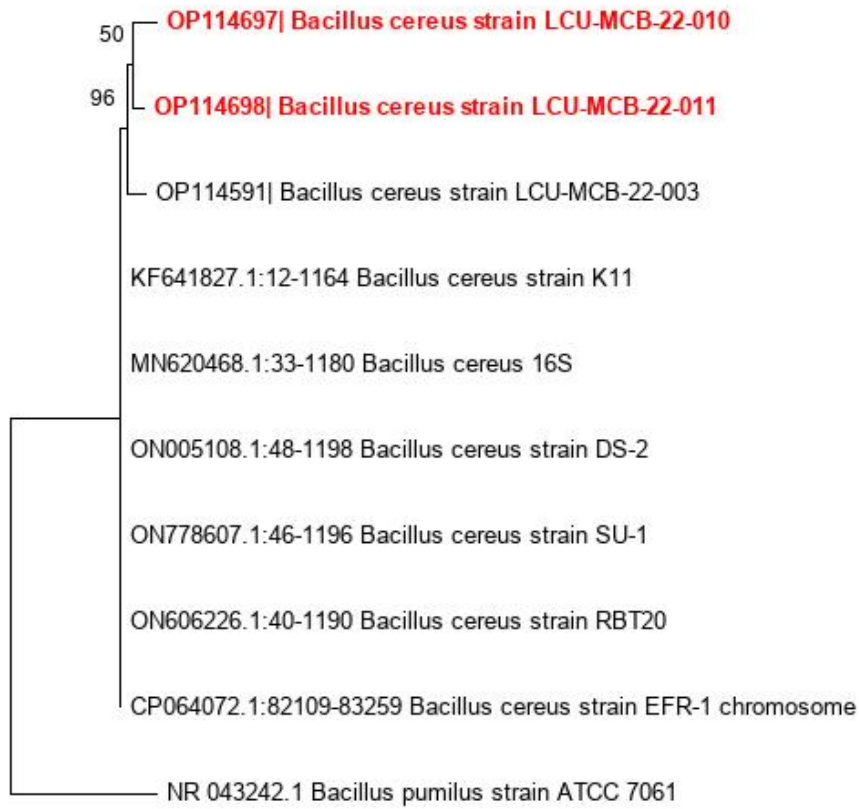


Plate 1: Gel image for PCR of the Eight isolates with molecular marker/ladder. Source: Laboratory Analysis, 2020

Figure 2: Phylogenetic tree showing evolutionary relationship *Bacillus cereus* associated with fruit smoothie in Ibadan, Nigeria (in red) with others strains in the GenBank database.

The evolutionary history was inferred using the Neighbor-Joining method . The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches . The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method [3] and are in the units of the number of base substitutions per site. This analysis involved 10 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1454 positions in the final data set. Evolutionary analyses were conducted in MEGA11 4



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Figure 10: Phylogenetic Tree for *Bacillus cereus*

Source: National Centre for Biotechnology Information, 2022

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4.6 Discussion of Findings

The mean values of Heavy Metals Found in the sampled Fruit were between the permissible ranges which is 0.2 for Cd, 0.3 for Pd, Ni 67.9, Zn 99.4, Cu 73.3, Fe 425.5 but Lead is the only metal having values greater than permissible value according to the finding of this result. This is in agreement with the findings of a study that worked on Cd and Pb contamination of selected species of vegetables and fruits in the form of fresh, frozen, dried, and processed product with the findings that the highest concentrations were recorded in dried products. Several fruit and vegetable samples exceeded the maximum permissible concentrations of Cd and Pb¹. To further support these findings another study, find out that obtained results declared that concentrations of major studied metals were exceeding the recommended maximum acceptable levels proposed by the Joint FAO/WHO Expert Committee on Food Additives including Lead². whereas, this is in contrast with another finding where in fruit samples, Mn and Cd were not present³. Also, another study finds out that concentration of Iron, Lead, Copper, Chromium, and Cadmium heavy metals in vegetables grown near dumpsites of Jimeta and Ngurore areas of Adamawa State, Nigeria were below the limit⁴. This is the same as the case of all metals examined in this study.

T-test result that compare the mean values of aerobic and anaerobic indicated that there is no significant differences between both samples for bacterial counts for 10^5 cfu/ml (NA), 10^5 cfu/ml (MAC) and 10^5 cfu/ml (MSA) respectively. Also, bacterial count of 10^5 cfu/ml (NA) have the highest mean of all the samples. This may be as a results of time lags or duration for both aerobic and anaerobic. Also, the weather condition seems to be favourable for both bacterial when considering the sunshine. This is in line with a study that there was a statistically significant lower bacterial count for both aerobic and anaerobic bacteria in the DL group compared with the Endo group in both S3 samples only after laser application⁵.

Most of the sizes of the isolates were large, also highest shape is round, has regards elevation most isolate were flat and there colour were vary from Cream, Green-Blue Pigment, Blue Pigment, White Crystalline and Yellow. They all have smooth surface. This is the same with the findings that most of isolates were moderate and large in size⁵. For the hemolysis test, the percentage of pathogenic isolates is 14 (38.9%) while remaining 22(61.1%) is nonpathogenic. Also, this is in contrast to a study on several physico-biochemical tests were performed to identify the selected bacterial isolates up to genus level. In the study, 66.67% of the bacterial isolates were Gram positive and 33.33% were Gram negative⁵.

The table represents Biochemical test Identification results with fourteen (14) isolates in total. The result shows that 4(28.5%) isolates that is Z2MACⁱ, Z1MACⁱⁱ, K2MANⁱand K2NAⁱⁱ yielded *Staphylococcus aureus portable diseases*, 3(21.4%) isolates that is K1MANⁱ, K1MACⁱ and K2NAⁱ yielded resemblance of *Escherichia coli*, 2(14.28%) isolates that is K2NAⁱⁱ and Z1NAⁱⁱ yielded *Streptococcus sp*, also, 2 (14.28%) of both K1NAⁱ and K1MANⁱ isolates yielded resemblance of *Bacillus subtilis*, while 1(7.14%) each of K1MACⁱⁱ, K1NAⁱⁱⁱ and Z2NAⁱⁱ yielded resemblance of *Pseudomonas sp*, *Salmonella typhi* and *Bacillus cereus* respectively.

Based on the physico-biochemical characteristics 28.5% of the isolates were resembled *Staphylococcus aureus portable diseases*, follow by *Escherichia coli* with 21,4%, also 14.28% of isolates resembled *Streptococcus sp* and *Bacillus subtilis*.

The most of the isolates show multi-drug resistance while little of them show extensive drug resistance. For K1NAⁱ isolate is resistance to Zinnacef and Amoxicillin, K1NAⁱⁱⁱ is resistant to Pefloxacin, Zinnacef, Amoxicillin, Septrin and Erthromycin, Z1NAⁱⁱ is resistance to Amplicox, Zinnacef and Amoxicillin, K2NAⁱⁱ is Amplicox, Zinnacef, Amoxicillin, Streptomycin and Septrin, K2NAⁱⁱⁱ is resistant to Amoxicillin, Ceftriaxone and Septrin, Z2NAⁱⁱ is resistance to

Amplicox, Zinnacef, and Amoxicillin, K1MCi is resistance to Zinnacef, and Amoxicillin, KMCii is resistance to Amplicox, Zinnacef, Amoxicillin, Streptomycin and Septrin, ZMCii is resistance to Amplicox, Zinnacef, Amoxicillin, Streptomycin and Septrin, K2MCi is resistance to Amplicox, Zinnacef, Amoxicillin, Streptomycin, Septrin and Erthromycin, Z2MCi is resistance to Amplicox, Zinnacef, and Amoxicillin, K1MNI is resistance to Zinnacef and K2MNI is resistance to Amplicox, Zinnacef, Amoxicillin, Ceftriaxone and Ciprofloxacin. Furthermore, K2NAi is susceptible to all the drugs while Gentamycin is susceptible to all the isolates. Also, Amplicox, Zinnacef and Amoxicillin shown extensive resistance to the isolates.

Furthermore, Phylogenetic tree showing evolutionary relationship of *Bacillus cereus* associated with fruit smoothie in Ibadan, Nigeria. Of all the bacterial *Bacillus cereus* were found to be resistant against Z and AM. To support this finding a study evaluated the effect of antibiotics, the bacterial isolates were examined against Ampicillin, Cloxacillin and Gentamicin where, all organisms were found resistant against Ampicillin except *Alcaligenes* sp. (isolate no. A15) and *Achromobacter* sp. (isolate no. A11). Similarly, all of the isolates were resistant to Cloxacillin except for two isolates *Micrococcus* sp. (isolate no. A2) and *Pseudomonas* sp. (isolate no. A4). However, all isolates were found to be sensitive to Gentamicin⁵.

Endnotes

1. N.D. Nanven, J.N. Egila, & Y.N. Lohdip. *Heavy Metal Concentrations in Some Vegetables Grown in a Farm Treated with Urban Solid Waste in KuruJantar, Nigeria* **Br. J. Appl. Sci. Tech.** 2015, 8(2): 139- 147.
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7. R. Singh, *Radiation Resistance of the Micro Flora Associated with Amniotic Membranes.* **World Journal of Microbiology and Biotechnology.** 22:23– 27. Springer 2006
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Chapter Five

Conclusion

5.1 Summary of the Findings

This study emphasizes the importance of constant surveillance of pathogens and multidrug resistance bacteria associated with vended fruits. These vended fruits provide affordable source of nutrient to human; however, this fruit are easily infested with pathogens thereby causing multidrug resistance which poses high risk to human health. Bacteria with multidrug resistance were obtained from fruits in major market in Ibadan and identified as *Bacillus cerus* and *Providencia stuartiia* after subjecting their isolated DNA to 16S ribosomal RNA gene amplification and subsequent sequencing the amplified genes with ascertainment number OP114667 and OP114666 respectively from NCBI database. Causes of antibiotic's resistance are due to many factors which include exposing antibiotics to sub-lethal concentration of antibiotics, exposing them to chemicals used in preserving fruit and many other factors.

5.2 Conclusion

This result shows that they are pathogenic organism in vended fruits and they are also multidrug resistance therefore when consumed, they can cause fruit poisoning and there is tendency that antibiotics that will be administered to the patient may not work especially if the organisms are the drug resistance type. it is also notice that most of the fruit samples has some degree of heavy metals but the content are within the reference range given by WHO. Therefore regulatory bodies should enforce laws on proper fruit handling and hygiene

5.3 Recommendations

1. It is recommended that the bacteria strain obtained in the work be kept for further work
2. Vended fruits should be wrapped in clean film and be put in a show glass to avoid contamination
3. Urgent steps including awareness are needed to enhance personal hygiene and proper fruit handling

5.4 Contribution to Knowledge

From the result obtained from this work, it has shown that the fruit samples that were collected in the major markets in Ibadan are within WHO reference standard for heavy metals. It also shows the presence of pathogenic bacteria.

5.5 Area of Further Research

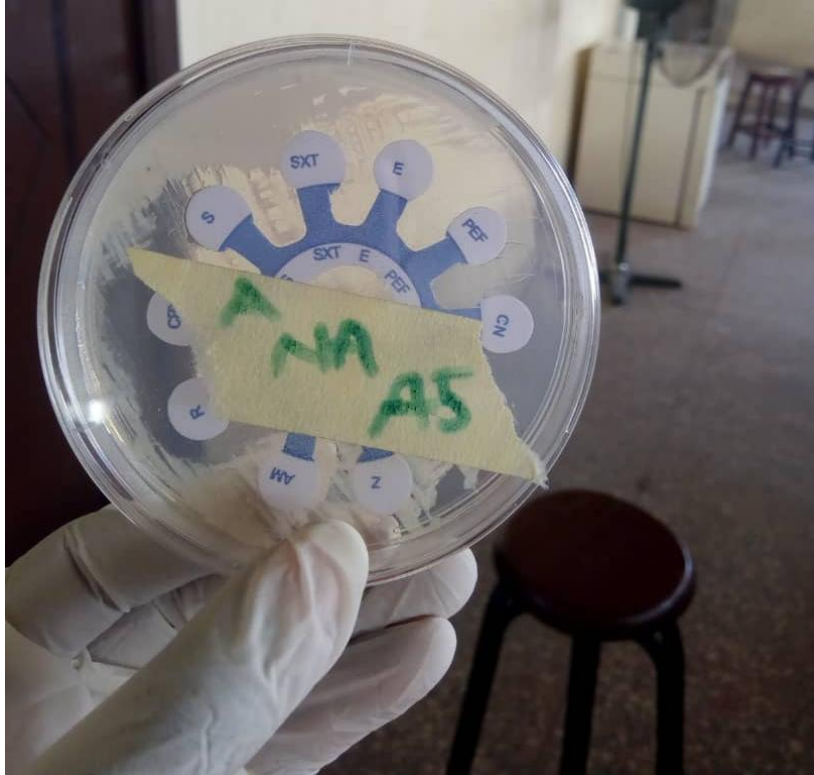
Further research should be done on the gram negative, gram positive and even fungi that are resistance to antimicrobials. Also, more research work should be carried out to determine genotypic studies on the resistance genes of these microorganism

Appendices

Appendix 1



Haemolysis Test Plate



Antibiotics Susceptibility Test Plate



Citrate Test Plate



Citrate Test Plate

Appendix 2

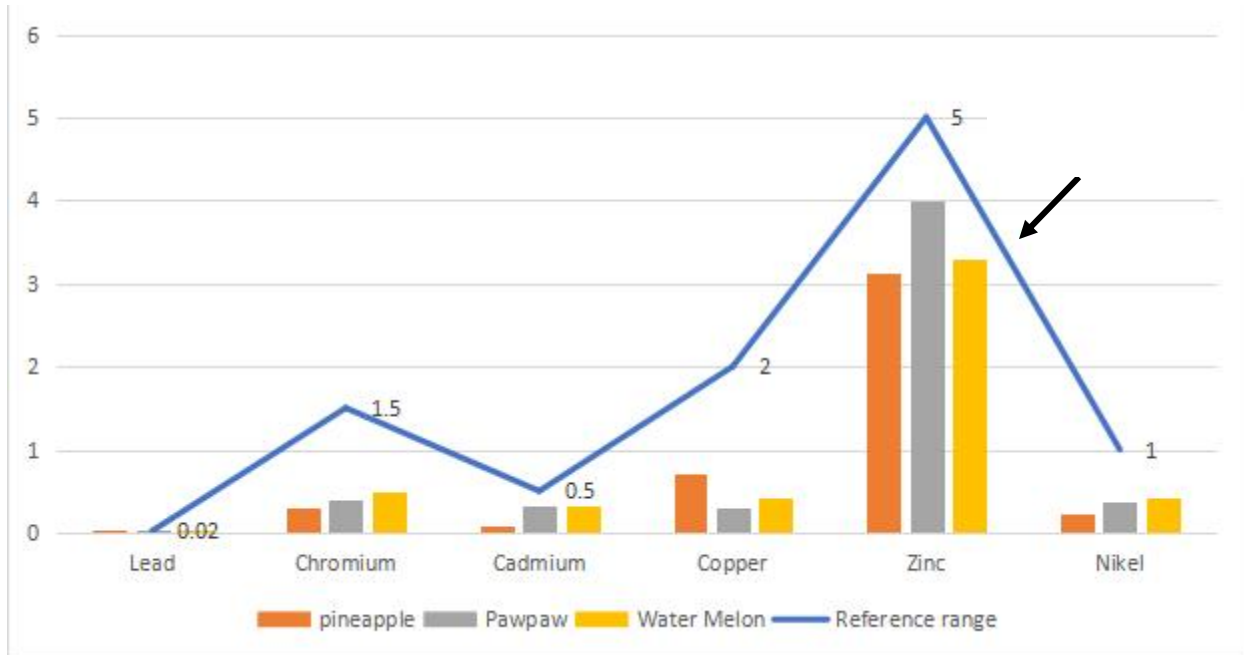


Figure 1: Concentration of various Heavy Metals Found in the Fruit Sample One

Source: Authors Laboratory research

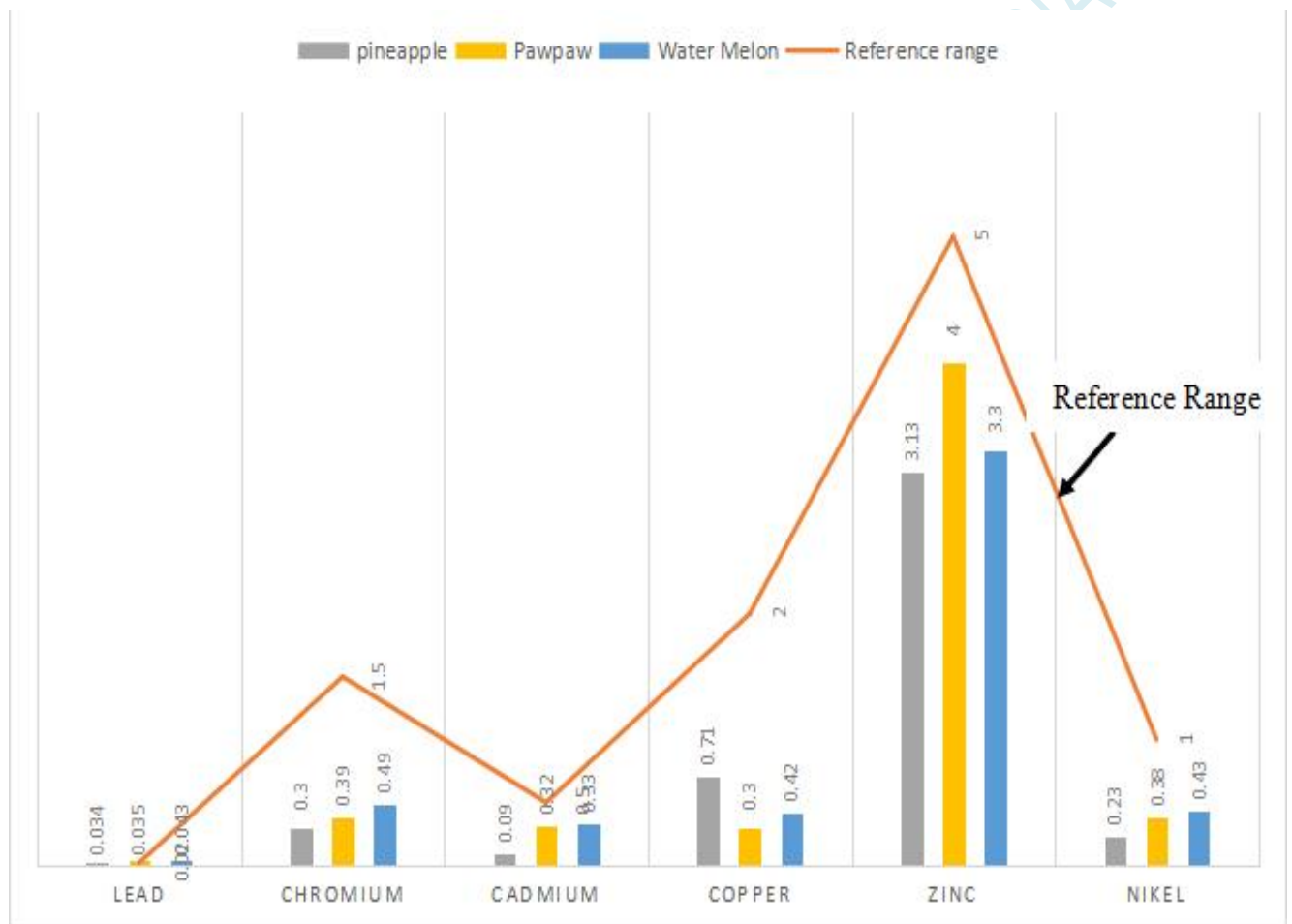


Figure 2: Concentration of various Heavy Metals Found in the Fruit Sample Two

Source: Authors Laboratory research

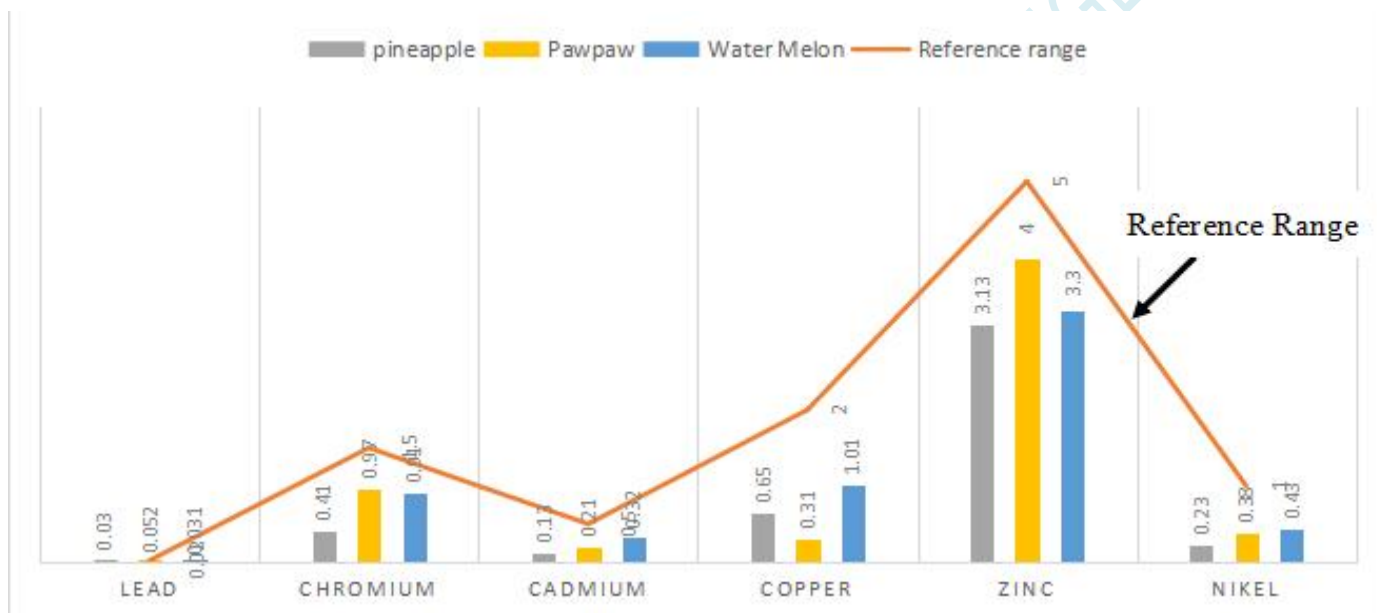


Figure 3: Concentration of various Heavy Metals Found in the Fruit Sample Three

Source: Authors Laboratory research

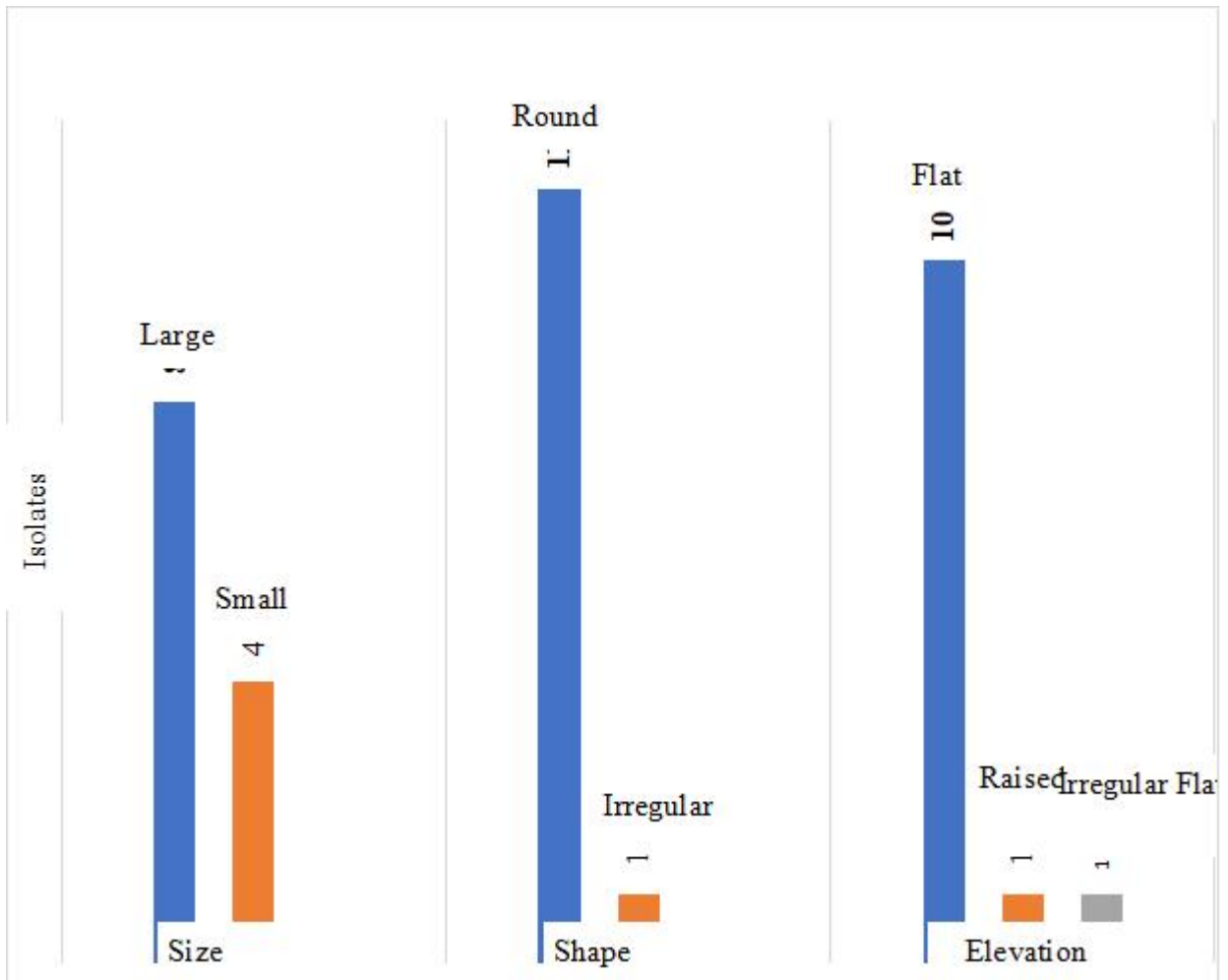


Figure 4: Colonial Morphology of Isolates

Source: Authors Laboratory Research

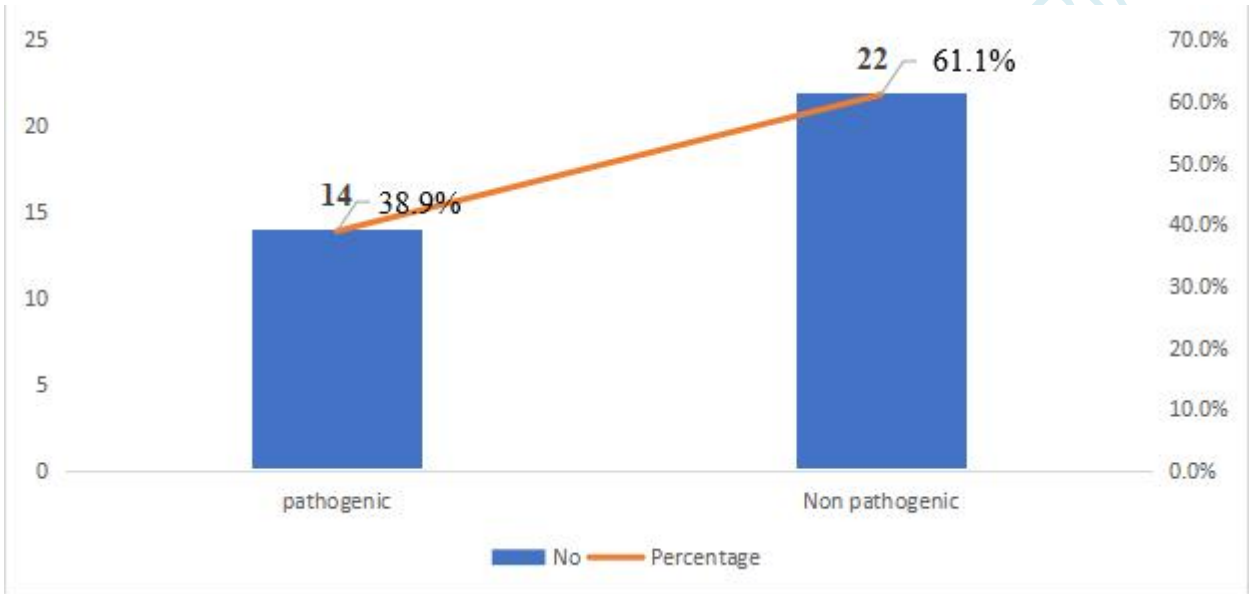


Figure 5: Hemolysis Test Chart

Source: Authors Laboratory Research

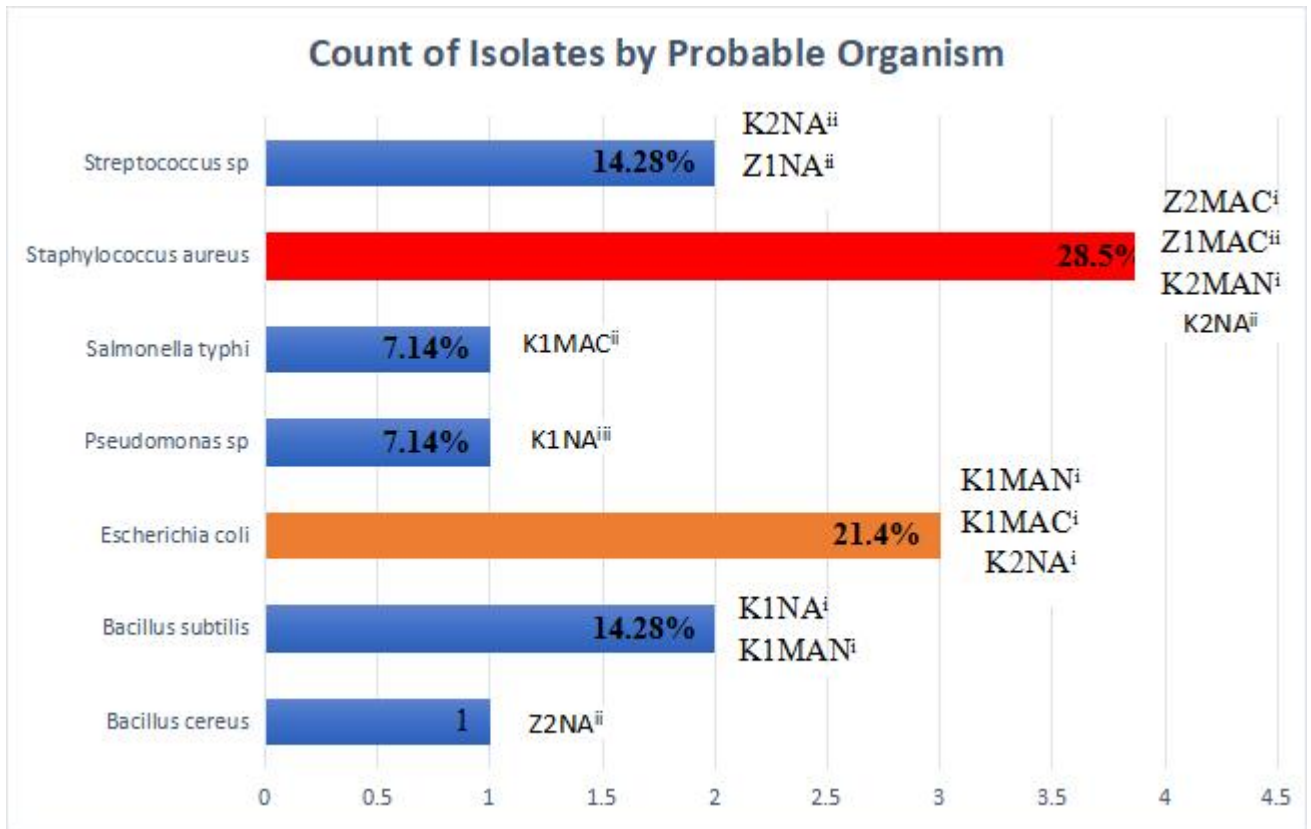


Figure 6: Biochemical Test Result chart

Source: Authors Laboratory Research

Appendix 3

>OP114697| Bacillus cereus train LCU-MCB-22-010 16S ribosomal RNA gene, partial sequence

CCTAATACTTGCAAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGG
CGGACGGGTGAGTAACACGTGGGTAACCTGCCATAAGAGTGGGATAACTCCGGGA
AACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCG
GCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAAC
GGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGG
ACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATG
GACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAA
ACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTAC
CTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACTTAGGTGG
CAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTG
ATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGT
GCAGAATAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTATAGATATGGAG
GAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTA ACTGACACTGAGGCGCGAAAG
CGTGGGGAGCAAACAGGATTAGATAACCCTGGTAGTCCCACGCCGTAAACGATGAGT
GCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAATTAACGCATTAAGCACTCC
GCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCAC

AAGCGGTGGAGCATGTGGTTTASTTCGAAGCAACGCGAAGAACCTTACCAGGTCTTG
ACATCCTCTGAAAACCCTAGAGTTAGGGCTTCTCCTTCGGGAGCAG

>OP114698| *Bacillus cereus* strain LCU-MCB-22-011 16S ribosomal RNA gene, partial
sequence

CCTAATAGATGCAAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGG
CGGACGGGTGAGTAACACGTGGGTAACCTGCCATAAGACTGGGATAACTCCGGGA
AACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCG
GCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAAC
GGCTACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGG
ACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATG
GACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTTCGTAAA
ACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTAC
CTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGG
CAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTG
ATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGT
GCAGAAGAGGAAAGTGGAATTCATGTGTAGCGGTGAAATGCGTAGAGATATGGAG
GAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAAGTACTGACTGAGGCGCGAAAG
CGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGATGAGTG
CTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCG
CCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGGCCCGCAC
AAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTT
GACATCCTCTGAAAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGG

TGGTGCATGGTTGTCGTCAGCTCGTGTCGGGAGATGTTGGGTAAAGTCCCGCAACGA
GCGCAACCTTGGATCTTAGTTGCCATCATTAAAGTTGGGCACTCTTAGGTGACTGCCG
GTGACAAACCGGA

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This is to certify that the thesis of **Adeniji**, Oluwatosin with registration number **LCU/PG/001748**, at the Department of Biological Sciences, Faculty of Natural and Applied Sciences, Lead City University, Ibadan, Nigeria is in full compliance with the University approved form and style.

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