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Microbial Quality of Ready-To-Eat Pineapple (*Ananas comosus*) and Watermelon (*Citrullus lanatus*)

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ABSTRACT

Spoilage of fruits from contamination by microorganisms through irrigation water, harvesting/processing equipment, transporting, personal handling, soil, dust and even manure is a source of concern to human existence. This study assesses the microbial quality and antibiotics sensitivity of bacteria of ready-to-eat Pineapple (*Ananas comosus*) and Watermelon (*Citrullus lanatus*) in five markets in Port Harcourt using standard microbiological techniques. Total heterotrophic bacterial count for pineapple and watermelon ranged between 1.1×10^5 - 1.9×10^6CFU/g and 1.0×10^4 - 8.7×10^6CFU/g respectively. Fungal count ranged between 1.3×10^3 to 7.2×10^4CFU/g and 1.0×10^3 - 4.6×10^4CFU/g respectively. Isolated bacteria and fungi and frequency of occurrences (%) from pineapple were *Staphylococcus* sp(25%), *Citrobacter* sp(6.3%), *Proteus* sp(12.5%), *Salmonella* sp(6.3%), *Bacillus* sp(18.8%), *Klebsiella* sp(12.5%), *Escherichia coli*(18.7%), and *Candida* sp(21.4%), *Aspergillus* sp(28.5%), *Penicillium* sp., (21.4%) and *Fusarium* sp(7.1%). Isolated bacteria and fungi and frequency of occurrences (%) from watermelon were *Staphylococcus* sp(27.2%), *Proteus* sp(18.2%), *Bacillus* sp(18.2%), *Klebsiella* sp(18.2%), *Escherichia coli*(18.2%), *Candida* sp(15.4%), *Aspergillus* sp(15.4%), *Penicillium* sp(38.4%), *Fusarium* sp(7.7%), and *Mucor*(23.1%). All *Staphylococcus* and *Bacillus* isolates were 100% resistant to Ampiclox, Norfloxacin, and Gentamycin. While 57.14% and 80% of *Staphylococcus* and *Bacillus* respectively was susceptible to Leofloxacin. 75% of *Klebiella* was susceptible to Augmentin while 75% was resistant to Amoxacillin, and Ofloxacin. 75% of *Proteus* was susceptible to streptomycin while all the isolates were 100% resistant to Sparfloxacin. *Salmonella* was 100% susceptible to Septrin, Sparfloxacin, Ciprofloxacin, Ofloxacin and 100% resistant to Augmentin, Gentamycin Nalidixic acid, and Streptomycin. Multiple antibiotics resistance (MAR) index was greater than 0.2 for all isolates. Statistical analysis showed that there were no significant differences ($P>0.05$) in the microbial counts between Pineapple and Watermelon. Isolated microorganisms are responsible for microbial contamination of fruits sold in the markets. This therefore calls for serious attention in creating awareness in the control of human infectious diseases associated with consumption of contaminated fruits.

Keywords: Pineapple (*Ananas comosus*), Watermelon (*Citrullus lanatus*), microbial quality, antibiotic susceptibility, food safety

Introduction

Food safety in a society with growing urbanization, sustainable city-region and resilient food systems are essential in ensuring food and health security. Food crops such as pineapple and watermelon contribute to food and nutrition security**.** Pineapple (*Ananas comosus*) is a tropical plant with an edible fruit. Pineapple is a cylindrical false fruit (pseudo fruit) of the family Bromeliaceae and consists of a thickened, fleshly, very juicy axis core and inedible, scaly, warty skin, resembling a pine core.

Only the polygonal, flattened outsides of the individual fruits are visible at the surface of the multiple fruit (syncarp). The fruit is topped by a crown of prickly leaves. It is a vegetative propagated fruit crop and one of the few crops in which cultivars are derived from spontaneous mutations and natural evolution without controlled breeding (Oset-Kofi *et al.*, 1997).

Raw pineapple pulp is 86% water, 13% carbohydrate, 0.5% protein and contains negligible fat. Pineapple fruits and peels container diverse phytochemicals among which are polyphenols including Gallic acid, syringic acid, vanillin, ferulic acid, sinapic acid etc.

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Pineapple give one third value of vitamin C which aids in tissue growth and repair, also help fight cancer, heart disease. It contains manganese which helps in bone formation, immune response and metabolism; and it gives healthy dose of several B vitamins like niacin, thiamin, B6 and folate. They help in forming new red blood cells. Pineapple contains bromelain which aids digestion by breaking down protein. Pineapple and watermelon can be taken regularly to keep the body cool and safe from sunstroke.

Watermelon (*Citrullus lanatus*) is known to be a popular staple summer fruit found in the world and it is mostly consumed as fruit salad, drinks (Alim-un-Nisa *et al*., 2012, Perkins-Veazie *et al*., 2013) or as a dessert (Blohm *et al*., 2020; Paris, 2020). Watermelon is a flowering plant species of the cucurbitaceae family. The sweet, juicy flesh is usually deep red to pink and many black seed (Renner *et al.*, 2021). Water melon has a natural source of antioxidants, Vitamin C and lycopene (Naz *et al*., 2014). Watermelon helps improve human health as a result of the presence of lycopene. It is known to control chronic diseases such as diabetes, cardiovascular events, and some forms of cancer (Figueroa *et al*., 2011). Similarly, Perkins-Veazie *et al*., 2001 reported that lycopene, a carotenoid, has antioxidant properties that may reduce the incidence of certain cancers. Water melon has the ability to control hypertension, diabetes, cancer, and some coronary heart diseases (Maoto *et al*., 2019). Fresh-cut fruits can easily be contaminated and degraded due to the application of various preparation steps such as washing, peeling, cutting, and slicing (Yousuf *et al.*, 2019). Contamination and degradation of fresh-cut watermelon occur due to its low acidity and growing conditions (Wang *et al*., 2018*;* Wanwimolruk *et al.*, 2015).Water melon is regarded as a potentially hazardous food.

The microbial flora of a fruit consists of the microorganisms associated with the raw fruit, and those acquired during harvesting and preparation.

In Nigeria, a large amount of most fruit crops harvested is still done by hands and farming implements. There are some simple but essential rules to be considered during harvesting. They include; fruit picked by hand should be carefully placed in harvesting baskets. Further handling has to be done carefully to avoid microbial contamination and mechanical damages.

The harvesting basket and hands of the harvester should be clean. The fruit should be picked when it is ready to be processed, to reduce the chances of contamination. Once the harvesting is done, the nutritional value of the fruits deteriorates in varying degrees. Spoilage is any damage or deterioration of the original value, texture and flavored of the fruits. The fruit becomes harmful to people and unsuitable for human consumption due to the activities of microorganisms. Spoilage can also be defined as any sensitive change (tactile, visual, olfactory or flavor), which the consumer considers to be unacceptable (Tsige *et a*l., 2008). Some of the primary culprits of spoilage of fruits are air, moisture, light, temperature, and microbial growth. Most fruits spoil easily because of damage caused by microorganisms. Microorganisms such as bacteria, yeast and molds need water and nutrients for growth, energy and reproduction. With an average water content of 90 percent or more, fruits grow on the outside of food or within the holes or cracks and spoil quickly.

Microorganisms still find their way into fruit due to poor sanitary practices of the harvesters and unclean utensils used in cutting these fruits (Tsige *et al.*, 2008). Microbes are responsible for the contamination of watermelon and pineapple resulting in bad flavor, deterioration of the nutritional value etc. rendering it unacceptable for human uses. Based on the nutritional content of fruit, they are able to support the growth of bacteria of both gram positive and gram negative forms (Tsige *et al*., 2008).

Ripening pineapple fruit is susceptible to infection by a variety of disease-causing microorganisms including fungi. These disease tend to develop and damage the fruit during fruit nutrition, starting from twenty days before the fruits are harvested until they reach the consumer as fresh fruits or are processed in cannery as canned pineapple, thus the internal quality of the fresh fruit is reduced significantly due to attack by a complex of microorganisms such *as Penicillium, Fusarium* and Yeasts which are believed to cause black spots of pineapple fruits (Tsige *et al*., 2008)..

The type of microorganisms growing in a fruit juice depends on the kind predominant in the raw fruit as well as on the temperature of storage. He asserted that at a temperature below 15.6°C wild yeast may grow, but that the lower the temperature, the more likely is the growth of bacteria and mould rather than yeast.

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Apart from storage temperature, water activity (aw), presence and amount of oxygen and other gases, the relative humidity in addition to the acidity of product are all contributing factors that determine the type of microorganisms associated with fruit juices.

In developing countries such as Nigeria, post-harvest losses are often more severe due to inadequate storage and transportation facilities (Droby, 2006). Pineapple and watermelon infection may occur during the growing season, harvesting, handling, transport, post-harvest storage and marketing conditions or after purchasing by the consumer. Another major source of contamination is the washing water. The process of infection follows the development of fungal penetrating structure called aspersorium. The colonization process by fungi involves their ability to establish themselves within the host. This is initiated when the fungi following adhesion and release of enzymes depolymerize certain cell wall polymers such as prospection, the cementing substance of the produce. The primary cell wall of pineapples is composed of approximately 10% proteins and 90% polysaccharides which can be divided into three groups: cellulose, hemicelluloses and pectin (Nathalie, 2006). Numerous cell wall degrading enzymes can be secreted by fungi to breach and use the plant cell walls as nutrient sources. These fungi produce an abundance of extracellular pectinases and hemicellulases that are important factor in their spoilage of pineapples (Nathalie, 2006) leading to reduced post-harvest life and the development of undesirable quality and soft rot (Miedes and Lorences, 2004). In addition, many fungal species are capable of producing mycotoxins, which are secondary metabolites that are highly toxic to humans and animals.

The flesh and juice of the pineapple and watermelon are prepared and sold on road sides in Nigeria as a snack and their consumption has been on the increase. This is so because they are easily accessible, nutritious and relatively cheap (Nwachukwu *et al.*, 2008). The increase in consumption has been linked with a parallel increase in food borne illnesses (Mensah *et al.*, 2002).

In Nigeria, cut Pineapple and Watermelon fruits are processed and sold by unlicensed street vendors with poor education and lack of training in food hygiene. Also, almost half of the fruit served in cafes and restaurants are contaminated with dangerous bacteria such as *Salmonella* (Tsige *et a*l., 2008).

The problem is due to dirty kitchen utensils used in cutting a different fruit at one time and leaving it in stainless steel jugs where it heats up, allowing bacterial to thrive.

This study is designed to enumerate bacterial and fungal population in ready-to-eat pineapple and watermelon, and to determine the antibiotic susceptibility of the isolates. This is to ascertain the microbiological quality of ready-to-eat pineapple and watermelon and to know the antibiotics that can be used to treat the infections caused by these organisms.

Materials and Methods

Sampling Technique

Already cut or sliced water melon fruits and pineapple fruits were randomly purchased from two vendors at each of the following locations with their Map coordinate indicated; Rivers state university (N – 4.805433° E – 6.986187°), Mile I (N – 4.79369° E – 6.99601°), Kampala (N – 4°47’44.1” E – 6°59’40.5”), Illoabuchi (N – 4.7947° E – 6.9858°) and Agip (N – 4811115° E – 6.974747°). All samples were packaged in zip-lock bags and placed on ice packed cool box and immediately transported to the laboratory. Whole pineapple and watermelon fruit samples were processed under hygiene conditions in the laboratory and regarded as control samples for the study fruits. Microbial analyses were performed using commercially available dehydrated media, and the manufacturer’s instructions were followed.

Sample Preparation

The samples were analyzed in the microbial laboratory at Halden laboratory Trans-Amadi, Port Harcourt, Nigeria. The rinsed watermelon and pineapple samples were serially diluted and aseptically plated on Petri-dishes containing the solidified medium then a sterilized glass spreader was used to make an even spread. All samples were incubated in an aerobic incubator at a temperature of 35±1°C for 24-48 hours. Following incubation, colonies developed were enumerated and transformed into colony forming units per millimeter (CFU/g) of the samples. Sabouraud Dextrose Agar (SDA) was used for the enumeration of fungi at a temperature of 28 ±2.0°C for 3-5 days. Multiple tube testing was used for the enumeration of coliforms at a temperature of 37°C for total coliform and 44.5°C for feacal coliform for 24 hours.

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Preservation of Bacteria Isolates

Pure cultures of the bacterial isolates were preserved in bijou bottles containing 10% prepared glycerol. Prior to storage, 5mL glycerol suspension were transferred into bijou bottles and were sterilized by autoclaving at 121ºC for 15 psi. The pure isolates were transferred into labeled bijou bottles containing the sterile glycerol suspensions. After which, the bottles were kept frozen in the refrigerator. This was used for subsequent identification tests.

Grams Staining Technique

Slide with warm fixed smear was flooded with crystal violet (essential stain) and permitted to represent 60seconds. Following 60 seconds the stain was delicately flushed under faucet water. Furthermore, the smear was delicately overflowed with gram's iodine (a mordant) and left to stand for 60 seconds after which it was washed under faucet water. Decolourization was finished with 95% acetone for 5 seconds and was promptly flushed under faucet water after which Counter stain (safranin) was overflowed and permitted to stand for 60seconds. It was again flushed under faucet water. The gram stained smear was viewed with light-microscope under oil-immersion (x100) and the Grams' reaction was recorded.

Motility Test

Some bacteria have the ability to move about with the aid of a structure called flagella (a few move about with axial filaments). Motility test help to detect such motile bacteria and it was done as shown below. On a sterile semi-solid medium (in test tubes), a 24 hours isolate was stabbed along the center deep down with the aid of an already heat flamed straight wire loop making a single stab. The tubes were incubated at 37ºC for 24 hours. Non-motile bacteria only grow on the stab-line while motile-bacteria diffuse and spread along the medium having a hazy growth. Some bacteria were left to up to seven (7) days of incubation before result was taken.

Other biochemical and physiological tests carried out using the isolated bacteria were; Catalase Test, Oxidase test, Indole Test, Simmon's Citrate Agar Test, Voges Proskauer Test, and Sugar Fermentation test. The sugar fermentation test is used to determine their ability of the microorganisms to ferment sugars.

Four (4) sugars used in this work were lactose, sucrose, mannitol and fructose. The test isolates were identified by comparison of their tat results with those of already established taxa of Bergey's manual of determinative bacteriology (Holt *et al*., 1994).

Isolation of Fungi

A sterile pipette was used to transfer the sample into the Petri dishes containing the already prepared SDA before spreading. The spread sample was then incubated at room temperature (27-37^{0}C) for 7 days before identification.

Identification of Fungi

The isolated fungi were identified by their colonial and microscopic characteristics by carrying out a wet mount technique for the fungi isolated. A drop of lactophenol in cotton blue was aseptically dropped on a grease free clean slide. A piece of fungal hyphae under test was teased into it using two sterile needles. The teasing was done carefully and slowly so as to make good spread of the fungal hyphae. Each prepared slide was gently covered with a cover slip to avoid air bubble. The slides were observed under low and high power objective, and observation recorded as the cultural characteristics, sporangia, conidia, arthrospores, and vegetative mycelium, septate and non-septate hyphae according to Barnett and Hunter (1998).

Most Probable Number (MPN) Technique for Estimation of Coliform

The multiple tube fermentation technique is a three stage procedure (presumptive test, confirmed test and Completed test). This technique is called the most probable number (MPN) technique. The MPN test was done using fifteen (15) test tubes for each aliquot (10ml, 1ml and 0.1ml) sample of juice. Ten (10) ml of juice sample was inoculated into 10ml of the first set of five test tubes of sterile double strength of Mac-Conkey broth with Durham tubes inserted in them.

One (1.0) ml of juice sample was inoculated into 10ml of the second set of five test tubes sterile single strength of Mac-Conkey broth with Durham tubes inserted in them. The third set of five test tubes had 0.1ml aliquot of juice inoculated into 10ml of sterile of single strength of Mac-Conkey broth with Durham tubes inserted in them. All the test tubes were incubated at 37^{0}C for 48 hours.

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The changing of Mac-Conkey broth colour to deep yellow and the presence of gas in Durham tube shows positive presumptive test while no gas after 48 hours of incubation constitute a negative test that is absence of coliform in juice sample. The most probable number of the organism was recorded and the results were compared with the MPN index and 95% confidence limits for various comparisons of positive results when five tubes are used for dilutions (10ml, 1ml, 0.1ml) (most probably number Table) and results are expressed in Most Probable Number per 100ml (MPN/100ml) (Verma *et al*., 1999).

Mueller-Hinton Agar Preparation

The Mueller-Hinton agar preparation was done according to the manufacturer specifications and sterilized in an autoclave at 120^{O}C for 15minutes at 15 pounds per square inch. The pH of the medium was confirmed to be 7.2 and poured to the appropriate depth in the Petri dish to avoid false reading of the zones of inhibitions.

Preparation of 0.5M Mcfarland Turbidity Standard

About 1% v/v solution of Sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of water and properly mixed. About 0.5g of dehydrated Barium Chloride ($Bacl_2.2H2_o$) was dissolved in 50ml of distilled water to prepare 1% w/v of Barium Chloride Solution (CLSI, 2017). About 0.6ml of the Barium Chloride solution was added to 99.4ml of the sulphuric acid solution and properly mixed. A prepared turbid solution was transferred to a capped tube and kept in well-sealed container in the dark at room temperature (25-28ºC).

Antibiotics Resistance Profiling of the Isolates by Kirby-Bauer Disk Diffusion Method

A sterile swab stick was dipped into the tube containing the bacteria suspension which it's turbidity is equivalent to 0.5m McFarland Turbidity Standard and the swab was used to swab the surface of the Petri dish evenly which contain already prepared Mueller Hinton agar in three dimension and rotating the plates to about 60º to ensure even distribution of the organism. The agar was allowed to dry for about 3-5minutes.

With Sterile forceps, the impregnated antimicrobial discswas placed evenly on the surface of the inoculated plate and the disc was placed 15mm away from the edge of the plate. The head of the forceps was used to Press down each antibiotics disc slightly to make contact with the agar. After applying the discs, the plates were incubated in an inverted position aerobically at 35ºC for 16-18h. After incubation, the test plates were examined to ensure confluence growth or near confluence. The diameter of each zone of inhibition was measured in mm using a ruler on the underside of the plate and recorded for reference purpose (CLSI, 2017).

Determination of Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotics resistance is the resistance of isolates of *Staphylococcus aureus* to three or more antibiotics. Multiple antibiotic resistance (MAR) index was ascertained for each isolate by using the formula MAR = a/b, where a stands for the number of antibiotics to which the test isolate depicted resistance and b stands for the total number of antibiotics to which the test isolate has been evaluated for susceptibility.

Results

Table 1 shows the microbial count for the different microbial groups in the cut pineapple and watermelon and their control samples. Total heterotrophic bacteria count for pineapple, RSU had the highest count with the total of 4.5×10^6 and for watermelon; Illoabuchi had the highest count with the total of 7.1×10^6. Statistical analysis using ANOVA for pineapple shows that there is no significant difference between the counts of the five locations; $P > 0.05$ while for watermelon there is a significant difference between the counts of the five locations; $P < 0.05$. The Staphylococcal count for pineapple and watermelon, Kampala had the highest count with the total of 7.1×10^6 and 1.8×10^4 respectively. For pineapple, Agip had the highest fungal count with the total of 8.6×10^4CFU/g and for watermelon, RSU had the highest count with the total of 4.1×10^4 Statistical analysis for pineapple shows that there is a significant difference between the counts of the five locations; $P < 0.05$ while for watermelon there is no significant difference between the counts of the five locations; $P > 0.05$. Control counts were far lower.

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Table 1: Microbial Counts (CFU/g) of cut Pineapple and Watermelon fruit samples

Locations	Total heterotrophic bacteria (CFU/g)		Total Staphylococcal count (CFU/g)		Total heterotrophic fungi (CFU/g)	
	Pineapple	Watermelon	Pineapple	Watermelon	Pineapple	Watermelon
Control	7×10^{2}	4×10^{2}	ND	3×10^{1}	5×10^{1}	10×10^{1}
Agip	4.4×10^{6}	1.4×10^{6}	0	7.2×10^{3}	8.6×10^{4}	1.4×10^{4}
Mile I	4.5×10^{6}	1.1×10^{5}	9.7×10^{2}	5.1×10^{2}	1.24×10^{3}	1.2×10^{4}
RSU	4.9×10^{6}	1.12×10^{5}	2.6×10^{2}	7.0×10^{2}	9.9×10^{3}	4.1×10^{4}
Illoabuchi	5.1×10^{5}	7.1×10^{6}	1.1×10^{3}	5.7×10^{2}	3.8×10^{4}	1.2×10^{4}
Kampala	3.0×10^{5}	6.6×10^{5}	3.5×10^{3}	1.8×10^{4}	4.1×10^{4}	1.4×10^{4}

The microscopic and macroscopic characteristics of fungal isolates from the different locations are shown in Table 2.

Figure 1 shows the percentage occurrence of the funngi isolated from pineapple and watermelon.

The most occurring fungi in pineapple was *Aspergillus* sp. with 36.36% occurrence, while for watermelon it is *Penicillium* sp with the percentage of 33.33%.

Table 3 shows the cultural and biochemical characteristics of the different bacteria isolates which matches the tentative organisms.

Table 2: Macroscopic and Microscopic Characteristics of Fungi Isolated from Isolated Pineapple and Watermelon

Isolates	Macroscopy	Microscopy	Probable Identity
A	Cream large round	Oval budding blastoconidia	*Candida* sp
B	White, cottony, flat	Septate hyphae and half moon-shaped microconidia	*Fusarium* sp
C	Green powdery surface surrounded by white lawn, brown reverse	Septate hyphae with septate conidiophores bearing conidia	*Penicillium* sp
D	Grayish brown, white reverse	Non-septate mycelium	*Mucor*
E	Light green lawn surrounded by white lawn-like growth	Septate hyphae with aseptate conidiosphore bearing conidia	*Aspergillus* sp

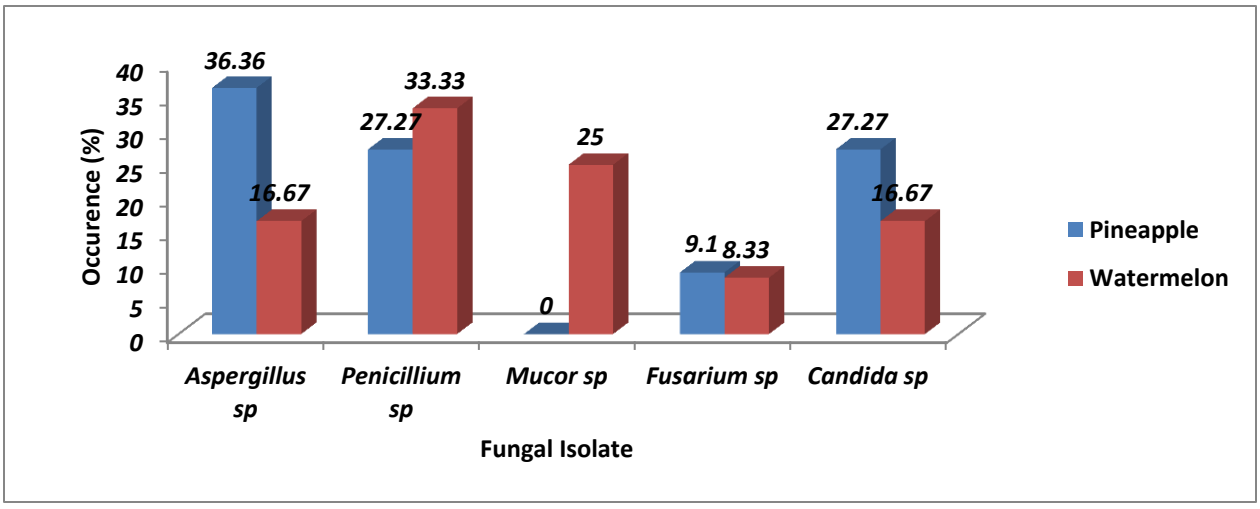

Fig. 1: Percentage Occurrence of Fungal Isolated from Pineapple and Watermelon

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Table 3: Cultural and Biochemical Characteristics and Tentative Identity of Bacteria Isolated from Pineapple and Watermelon Samples

Isolate Code	Texture	Elevation	Colour	Gram Stain	Oxidase	Indole	Methyl red	Voges –Proskauer	Citrate	Motility	Catalase	Starch hydrolysis	*Lactose*		*Maltose*		*Sucrose*		*Mannitol*		Tentative Identity
													Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	
P_1	Smooth	flat	clear	-rod	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	*E. coli*
W_1	Smooth	flat	clear	-rod	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	*E. coli*
P_{13}	Smooth	flat	clear	-rod	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	*E. coli*
W_2	Smooth	flat	clear	-rod	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	*E. coli*
P_{14}	Smooth	flat	clear	-rod	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	*E. coli*
P_2	Smooth	flat	Cream	+ cocci	-	-	-	+	+	-	+	+	+	-	+	-	+	-	+	-	*S. aureus*
P_{15}	Smooth	flat	yellow	+ cocci	-	-	-	+	+	-	+	+	+	-	+	-	+	-	+	-	*S. aureus*
P_3	Smooth	flat	Cream	+ cocci	-	-	-	+	+	-	+	+	+	-	+	-	+	-	+	-	*S. aureus*
W_3	Smooth	flat	yellow	+ cocci	-	-	-	+	+	-	+	+	+	-	+	-	+	-	+	-	*S. aureus*
P_{16}	Smooth	flat	yellow	+ cocci	-	-	-	+	+	-	+	+	+	-	+	-	+	-	+	-	*S. aureus*
W_4	Smooth	flat	Yellow	+ cocci	-	-	-	+	+	-	+	+	+	-	+	-	+	-	+	-	*S. aureus*
W_5	Smooth	flat	Cream	+ cocci	-	-	-	+	+	-	+	+	+	-	+	-	+	-	+	-	*S.aureus*
P_4	Smooth	flat	Creamy	-rod	-	-	+	-	+	+	+	-	+	+	+	+	-	+	+	+	*Citrobacter* sp
P_6	mucoid	raised	white	-rod	-	-	-	+	-	+	+	-	+	-	+	-	+	-	+	+	*Klebsiella* sp.
P_7	mucoid	raised	white	-rod	-	-	-	+	-	+	+	-	+	-	+	-	+	-	+	+	*Klebsiella* sp.
W_6	mucoid	raised	white	-rod	-	-	-	+	-	+	+	-	+	-	+	-	+	-	+	+	*Klebsiella* sp.
W_7	mucoid	raised	white	-rod	-	-	-	+	-	+	+	-	+	-	+	-	+	-	+	+	*Klebsiella* sp.
P_8	smooth	flat	white	-rod	-	-	+	-	-	+	+	-	-	-	+	-	-	-	+	+	*Salmonella* sp.
W_8	smooth	flat	Creamy	-rod	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	*Proteus* sp
W_9	smooth	flat	Creamy	-rod	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	*Proteus* sp
P_9	smooth	flat	Creamy	-rod	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	*Proteus* sp
P_{10}	smooth	flat	Creamy	-rod	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	*Proteus* sp
W_{10}	rough	flat	Cream	+rod	-	-	-	+	+	+	+	+	-	-	+	-	+	-	+	-	*Bacillus* sp
P_{11}	rough	flat	Cream	+rod	-	-	-	+	+	+	+	+	-	-	+	-	+	-	+	-	*Bacillus* sp
W_{11}	rough	flat	Cream	+rod	-	-	-	+	+	+	+	+	-	-	+	-	+	-	+	-	*Bacillus* sp
P_{12}	rough	flat	Cream	+rod	-	-	-	+	+	+	+	+	-	-	+	-	+	-	+	-	*Bacillus* sp
P_{13}	rough	Flat	Cream	+rod	-	-	-	+	+	+	+	+	-	-	+	-	+	-	+	-	*Bacillus* sp

Key: P = Pineapple; W = Watermelon; A=Acid; + = Positive; - = Negative

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Figure 2 shows the percentage occurrence of the bacteria isolated from pineapple and watermelon. *Staphylococcus aureus* had the highest occurrence in both pineapple and watermelon. There was no occurrence of *Citrobacter* sp. and *Salmonella* in the watermelon samples.

The result for the estimation of coliform in the fruit juice is shown in Table 4.

Fruits from Agip market had the highest count for total coliform; all the tubes were positive while fruits from Kampala market had the highest count 5(10ml), 5(1ml), and 5(0.1ml) for feacal coliform. These results were compared with the MPN index and 95% confidence limits for various comparisons of positive results when five tubes are used for dilutions (10ml, 1ml, 0.1ml).

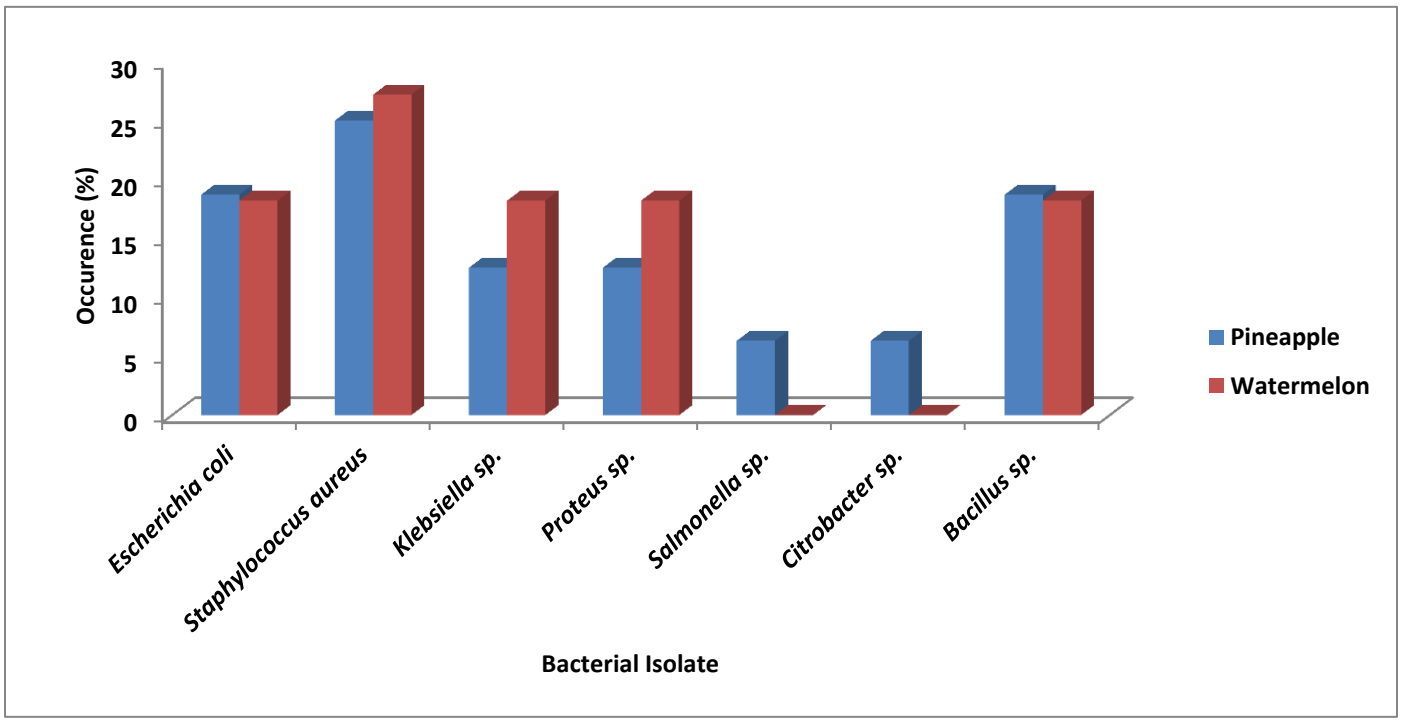

Fig. 2: Percentage Occurrence of Bacteria Isolated from Pineapple and Watermelon

Table 4: Total Coliform and Faecal Coliform Count (MPN/100ml) of Pineapple and Watermelon Juice

Market/Locations		Total Coliform				Faecal Coliform			
		10ml	1ml	0.1ml	MPN/100ml	10ml	1ml	0.1ml	MPN/100ml
Agip	P	5	5	5	≥2400	5	4	4	350
	W	5	5	5	≥2400	5	3	3	180
RSU	P	4	3	1	33	4	2	0	22
	W	4	3	0	34	4	2	0	22
Mile I	P	5	3	2	140	5	3	2	140
	W	5	5	0	240	5	1	2	63
Illoabuchi	P	5	5	2	540	5	5	2	540
	W	4	3	0	27	4	3	0	27
Kampala	P	5	5	4	1600	5	5	4	1600
	W	5	5	5	≥2400	5	5	5	≥2400

Key: P= Pineapple; W = Watermelon

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The results of antibiotics profile of the Gram negative bacterial isolates from pineapple and watermelon samples are presented in Table 5. The isolates were subjected to ten different types of antiobiotics such as Septrin (30µg) Chloramphenicol (30µg), Gentamicin (30µg), Sparfloxacin (10µg), Ofloxacin (10µg), Augmentin (10µg), Ciprofloxacin (30µg), Streptomycin (30µg), Amoxacillin (30µg), and Pefloxacin (30µg) out of which only three namely; Augmentin Ofloxacin and Streptomycin had the highest degree of susceptibility to the gram negative bacterial isolates.

Table 6 presents the antibiotics sensitivity profile of the Gram positive bacterial isolates from pineapple and watermelon samples. The isolates were subjected to ten different types of antiobiotics such as Ciprofloxacin (10µg), Norfloxacin (10µg), Gentamycin (10µg), Amoxil (20µg), Ampiclox (20µg), Erythromycin (30µg), Levfloxacin(20µg), Chloramphenicol (30µg),Streptomycin (30µg), Rifampin(20µg) out of which all the isolates were resistant Gentamycin, Norfloxacin and Ampiclox . Figure 3 and 4 shows the percentage occurrence of MAR indices of Gram + ve and -ve isolates > 0.2.

Table 5: Antibiogram Profile for Gram Negative Isolates

Isolate code	Antibiotics										MAR index
	SXT	CH	SPX	CPX	AML	AUG	CN	PX	OFX	S	
KP8	5(R)	8(R)	15(R)	17(I)	11(R)	19(S)	6(R)	11(R)	3(R)	4(R)	0.8
KP9	8(R)	20(S)	13(R)	7(R)	19(I)	20(S)	17(S)	20(S)	10(R)	19(S)	0.4
PP10	25(S)	20(S)	0(R)	13(R)	19(I)	11(R)	16(S)	8(R)	10(R)	22(S)	0.5
PP11	18(S)	3(R)	7(R)	16(I)	12(I)	19(S)	10(R)	28(S)	19(S)	3(R)	0.4
SP12	22(S)	15(I)	19(S)	22(S)	13(I)	11(R)	13(R)	8(R)	16(S)	4(R)	0.4
CP13	4(R)	10(R)	20(S)	25(S)	14(I)	12(R)	18(S)	17(I)	16(S)	16(S)	0.3
EP14	14(I)	14(I)	8(R)	20(S)	0(R)	15(I)	16(S)	0(R)	18(S)	4(R)	0.4
EP15	8(R)	10(R)	4(R)	25(S)	0(R)	20(S)	12(R)	0(R)	20(S)	8(R)	0.7
EP16	20(S)	18(S)	2(R)	17(I)	0(R)	18(S)	8(R)	3(R)	17(S)	8(R)	0.5
KW6	16(S)	15(I)	16(I)	22(S)	10(R)	30(S)	4(R)	15(I)	8(R)	3(R)	0.4
KW7	11(I)	19(S)	19(S)	25(S)	8(R)	15(I)	22(S)	15(I)	0(R)	0(R)	0.3
PW8	4(R)	16(I)	14(R)	19(I)	9(R)	28(S)	8(R)	14(I)	15(I)	20(S)	0.4
PW9	2(R)	20(S)	11(R)	22(S)	3(R)	15(I)	19(S)	13(R)	17(S)	25(S)	0.4
EW10	10(R)	23(S)	5(R)	12(R)	0(R)	13(R)	14(I)	8(R)	14(I)	5(R)	0.7
EW11	5(R)	20(S)	0(R)	22(S)	0(R)	10(R)	20(S)	10(R)	13(I)	10(R)	0.6

Table 6: Antibiogram Profile for Gram Positive Isolates

Isolate Code	Antibiotics										MAR index
	CN	AML	S	RD	E	CH	APX	LEV	NB	CPX	
SP1	12(R)	0(R)	20(S)	3(R)	6(R)	17(I)	0(R)	24(S)	0(R)	2(R)	0.7
SP2	6(R)	11(R)	12(I)	8(R)	3(R)	13(I)	0(R)	16(I)	0(R)	3(R)	0.7
SP3	5(R)	7(R)	9(R)	15(R)	0(R)	25(S)	0(R)	18(S)	0(R)	12(R)	0.8
SP4	0(R)	15(I)	3(R)	12(R)	15(I)	27(S)	0(R)	7(R)	0(R)	16(I)	0.6
BP5	0(R)	22(S)	14(I)	17(I)	0(R)	22(S)	5(R)	19(S)	0(R)	24(S)	0.4
BP6	0(R)	5(R)	19(S)	22(S)	25(S)	27(S)	12(R)	25(S)	0(R)	17(R)	0.5
BP7	0(R)	17(I)	17(S)	20(S)	17(I)	15(I)	11(R)	27(S)	0(R)	20(R)	0.5
SW1	17(S)	23(S)	6(R)	22(S)	7(R)	8(R)	0(R)	17(S)	0(R)	17(I)	0.5
SW2	13(I)	12(I)	24(S)	25(S)	10(R)	4(R)	0(R)	12(R)	0(R)	22(S)	0.5
SW3	0(R)	0(R)	21(S)	20(S)	11(R)	20(S)	0(R)	22(S)	0(R)	25(S)	0.5
BW4	0(R)	17(I)	20(S)	15(R)	3(R)	13(R)	10(R)	17(S)	0(R)	15(R)	0.7
BW5	0(R)	20(S)	22(S)	8(R)	8(R)	27(S)	17(I)	16(I)	3(R)	18(R)	0.5

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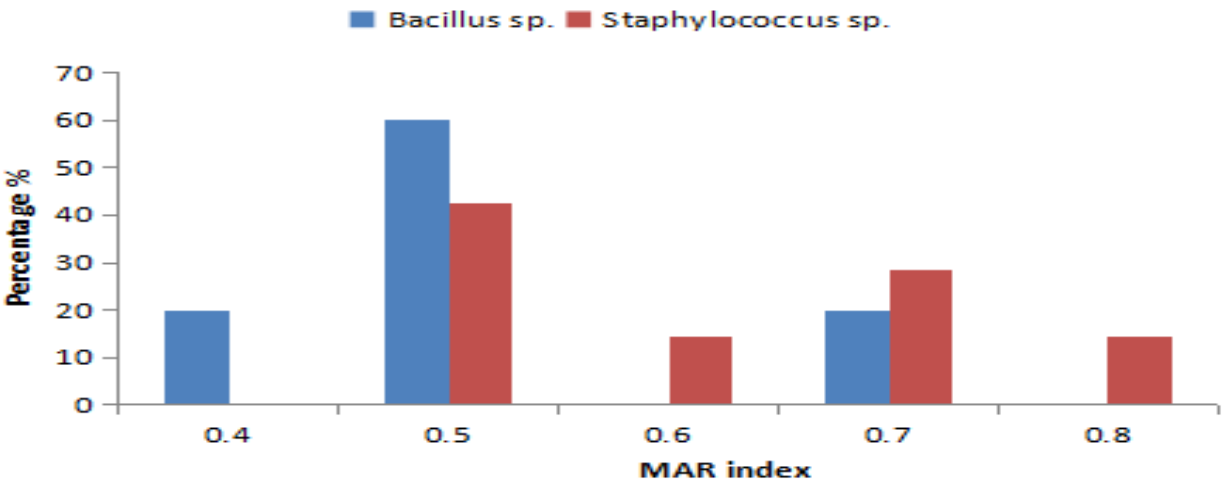

Fig. 3: MAR indices of Gram Positive Bacterial Isolates

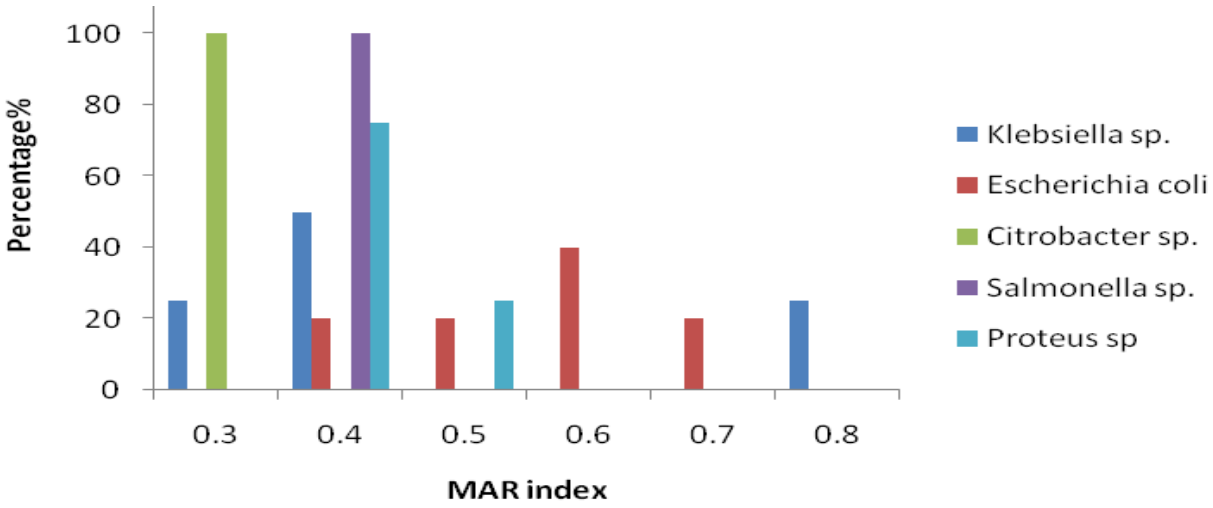

Fig. 4: MAR indices of Gram Negative Bacterial Isolates

Discussion

This study revealed that all the already cut and ready to eat Pineapple and Watermelon fruits obtained from the selected markets were contaminated with microbes. The result of bacterial and fungal counts shows that samples collected from the markets were high, this could be as the result of transfer to produce and cross contamination between the produce during prewashing with the same wash water by the vendor. This could be attributed to unhygienic conditions and practice as well as the extent of exposure to dust under which they are displayed. Some counts for bacteria and fungi in this study are similar to those reported by Asante *et al*. (2019) but most of the counts reported here are higher than those reported by Asante *et al*. (2019) .The microorganisms present in the fruit are as a result of the sanitary quality of the cultivation water, transportation, harvesting, storage and processing of the fruits for consumption (Titamare *et al.*, 2016, Yang *et al.*, 2017).

The high contamination of the sample fruit observed may be as a result of the processing procedure and the long period of storage of the produce before usage. The bacteria isolated during this study were *Citrobacter, Proteus, Escherichia, Kiebsiella, Bacillus, Staphylococcus aureus* and *Salmonella* sp.

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The fungi isolated during this study were *Aspergillus, Mucor, Penicillium, Candida,* and *Fusarium* species. All the bacteria isolated in the study were previously isolated from fruits in other studies elsewhere (Agbo *et al.,* 2016). Tsige *et al*. (2008) reported *Citrobacter, Proteus, Escherichia, Kiebsiella,* and *Bacillus.* They also reported *Aspergillus, Mucor, Penicillium,* and *Candida*. Del Rosario and Beuchat (2015) reported growth of enterohemorrhagic *Escherichia coli* O157:H7 in Cantaloupe and Watermelon. Asante *et al*. (2019) reported *E. coli*, *Salmonella* and yeast from fresh-cut pineapple. Improper storage conditions can encourage growth of pathogen on produce. Most of the organism isolated in the study might have been introduced into these fruits from contaminated water used for washing utensils (e.g. knives, trays, and pans), wrapping materials and the exposure of the product to tropical temperatures. It may also be a result of the failure of food handlers to observe basic sanitary rules.

Bacteria are indicators of some degree of potentially hazardous contamination. Among the genera of bacteria isolated in the study, *Staphylococcus* spp was predominant in watermelon. The contamination could be as a result of discharge into the atmosphere through sneezing or coughing or even to the manner in which the fruits are hawked and sold that continually predisposes them to contamination. *E. coli* were also isolated in this study. *E. coli* count in fruits is widely used and accepted as indicators of fecal contamination (Prescott, 1999). *Staphylococcus* species are readily introduced from handlers. These organisms are known to be associated with food poisoning or food infection. Also outbreaks of food borne diseases are attributed to consumption of contaminated fruits. Other genera isolated from the tested sample include *Bacillus* spp.

The presence of this organism in fruits can be due to ecological and environmental influence since their survival in the atmosphere depends on a number of factor such as nature of microorganism, susceptibility to changes, resistance to new physical environment and their ability to form spores and resistant strains. Dust particles become airborne at intervals during period of human activities in market home and enclosed environments. The environmental factors such as temperature, humidity and harmattan wind favour the spread of spores and whole organisms or fragments from one locality to another.

Wind creates dust from soil which carries microorganisms that inhabits the soil onto food sample during processing. The commonest genera of fungi that were isolated and identified included *Aspergillus* spp, *Mucor, Penicillum* spp, *Fusarium* spp, and *Candida* sp. Poor handling, inadequate transportation system, poor packaging and also congestion of the fruits in containers or bags during transit could be a pore puncture on the fruits thereby making it easier for fungi and other microorganisms to penetrate the biological barriers that is the outer covering.

Fungi especially mould secretes mycotoxin which cause serious intoxication in man and contamination of organism could be attributed to poor hygienic, poor storage condition. Environment to which fruits are normally exposed makes products come in contact with large number of different types of microorganism. Some fungi produce toxins that are carcinogenic. The most thoroughly studied of these carcinogenic toxins are produced by species of *Aspergillus* which are called aflatoxins. Ingestion of aflatoxins in moldy food has been implicated in development of liver cancer.

Microorganisms that cause spoilage in watermelon include *Mucor* sp, *Bacillus, Staphylococcus aureus, Proteus Escherichia coli*. Contamination and degradation of fresh cut watermelon and pineapple is due to the application of various preparation steps like harvesting, peeling, washing, slicing. It also occurs due to low acidity and growing conditions. Spoilage of these fruits is manifested in visible growth, gas production, slime and off odors.

In conclusion, the findings of this study underline the public awareness of the dangers of already cut ready to eat fruits from open markets. These contaminated fruit can cause serious health challenges to the health status of consumers due to unhygienic handling of the product. Proper practices, cleanliness, proper handling and washing of fruits before sale should be followed and highly stressed.

Consumers should thoroughly wash fruits before consumption. Fruit handlers and vendors should be educated on proper sanitary ways of processing the product in order to reduce microbial contamination and poisoning.

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