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Occurrence of *Escherichia Coli* and *Staphylococcus Aureus* in Sliced Pineapple and Water Melon Fruits Sold in Akpan Andem Market, Uyo, Akwa Ibom State, Nigeria

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Abstract- The aim of this study was to carry microbiological assessment of the prevalence of *Staphylococcus aureus* and *Escherichia coli* associated sliced ready-to-eat water melon and pineapple fruits. A total of 10 sliced pineapple and water melon fruits were purchased from different vendors at Akpan Andem Market, Uyo. The fruit samples were aseptically transported to the laboratory and processed using standard techniques. Enumeration of total heterotrophic bacteria and fungi, and isolation, characterization and identification of *Staphylococcus aureus* and *Escherichia coli* were all done using standard microbiological techniques. Antibiotic sensitivity test was carried out using the Kirby Bauer disk diffusion test. The results of the aerobic bacterial and fungal counts indicated that the highest bacterial count was 1.85 x 10^4 for water melon and 1.80 x 10^4 cfu/g for pineapple while those of the fungi were 1.3 and 1.4 x 10^3 cfu/g, respectively. For *E. coli*, the least and highest zones of inhibitions were 6.00 ± 1.05 and 23.37 ± 1.20 mm for isolates WS5 and PA3, respectively against CEP and CPX which were *Ciprofloxacin* and *Ceporex.* A total of 4 out of 10 isolates showed multi-drug resistance (resistance to at least 2 antibiotics) and these were isolates PA4, PA1, WS4, and WS5.

Keywords: water melon and pineapple fruits, bacteria, prevalence of staphylococcus aureus, escherichia coli, heterotrophic bacteria, and fungi.

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Keywords: water melon and pineapple fruits, bacteria, prevalence of staphylococcus aureus, escherichia coli, heterotrophic bacteria, and fungi.

I. Introduction

a) *Background of study*

In developing countries like Nigeria, millions of people on a daily basis consume ready-to-eat fruits and vegetables that are sold by vendors on the streets of major cities and towns (Mahale *et al.,* 2008; Okechukwu *et al.,* 2016). These fruits include oranges, pineapples, water melons, cucumber, and apples to mention but a few. The fruits are often desired and consumed fresh by the populace. Studies have shown that fruits are an excellent dietary source of nutrients, micronutrients and vitamins, water, and fibre which are needed for healthy and proper living (Mahale *et al.,* 2008; Okechukwu *et al.,* 2016). Fruits have been shown to help balance vitamin A and C deficiencies and also lower the risk of some diseases (Kalia and Gupta, 2006).

Due to increased knowledge of the health benefits of these fruits, their consumption is on the increase across the length and breadth of Nigeria. This is due to the accessibility, modern lifestyle of consumers, availability and affordability (Nielsen, 2006; Okechukwu *et al.,* 2016). Furthermore, due to the high costs of the whole fruits as seen with water melons and pineapple, some of these fruits are sold in small sliced pieces so as to make the fruits affordable to many Nigerians. These are usually sliced with stainless knives and then packaged in transparent nylons or polythene bags. Because they are preprocessed by the vendors, when purchased by consumers, they are consumed without rinsing or further processing (cutting and peeling) (Okechukwu *et al.,* 2016).

The processing of these fruits by vendors comes with potential risks of contamination and transmission of pathogens from the fruits and vendors to consumers. In an earlier study, it was shown that enteric pathogens are readily transmitted from ready-to-eat fruits (Mensah *et al.,* 1999). Bacteria and fungi are the common contaminants of fruits and they could be easily transferred from the vendors to the processed fruits through mishandling. The consumption of ready-to-eat fruits directly from street vendors or hawkers potentially increases the risk of food-borne diseases and poisoning caused by a wide variety of pathogens. It is also difficult to attest to the hygiene of these vendors or to the sanitary conditions at points of processing as well as those of the packaging materials. Frequently isolated bacteria from ready-to-eat fruits include *Escherichia coli*, *Salmonella sp*, *Pseudomonas sp*, *Staphylococcus aureus*, *Shigella sp*, *Mucor sp,* viruses and even parasites have been associated with the transmission of enteric pathogens (Mensah *et al.,* 1999).

Amongst these microbial contaminants, the presence of *Staphylococcus aureus* and *Escherichia coli*

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stand out from a public health point of view. Their presence in foods and fruits indicates recent fecal contamination and overall poor hygiene in the handling and processing of such foods. *E. coli*, a normal flora of the small intestine of healthy humans and animals has been linked to several cases of food poisoning and diarrheal illnesses (Prescott *et al.,* 1999). One strain, *E. coli* O157: H7 has been linked to several food-borne illnesses around the world (Yang *et al.,* 2017).

On the other hand, *Staphylococcus aureus* is a normal flora of the skin of human (Prescott *et al.,* 1998). Thus, their presence in food indicates poor hygiene in the processing steps. Unlike *E. coli*, the genus *Staphylococcus* is very diverse and contains over fourty known species (Brooks *et al.,* 2010). Among these species, *Staphylococcus aureus* is the most commonly isolated. It is also frequently associated with food poisoning cases and the bacterium has outstanding ability to secrete enterotoxin that causes food poisoning (Hennekinne *et al.,* 2012; Brooks *et al.,* 2010).

Furthermore, these sliced fruits are sold by unlicensed vendors whose hygiene and general health status are questionable and unascertained by health authorities. In Nigeria as a whole, there is shortage of data on the incidence of *E. coli* and *Staphylococcus aureus* in street vended sliced fruits.

II. Literature Review

a) Classification of water melon

Watermelon (*Citrullus lanatus*) botanically considered as the fruit is belonging to the family Cucurbitaceae (Edwards *et al.,* 2003). Cucurbitaceae family ranks among the highest of plant families for number and percentage of species used as human food (see Figure 1). The common name of watermelon is Tarbooz (Hindi and Urdu), Tarbuj (Manipuri), Kaduvrindavana (Marathi), Eriputccha (Telegu), Kallangadiballi (Kannada), Tormuj (Bengali), Indrak (Gujarati). Watermelon is originated from Kalahari Desert of Africa but nowadays cultivated abundantly in tropical regions of the world. It has great economic importance with 29.6 million tones estimated production worldwide (Reetu and Tomar, 2017).

b) Physical Characteristics

It is a large, sprawling annual plant with coarse, hairy pinnately-lobed leaves and yellow flowers. It is grown 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 seeds embedded in the middle third of the flesh (Wehner *et al.,* 2001). 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. India grows approximately 25 commercial varieties, a few of which have delightfully interesting names: New Hampshire Midget, Madhuri 64, Black Magic, Sugar Baby, Asahi Yamato, Arka Jyoti, Arka Manik, Improved Shipper, Durgapura Meetha and Durgapura Kesar to name a few. Watermelon varieties fall into three broad classes based on how the seed was developed: open-pollinated, F1-hybrid and triploid or seedless (Reetu and Tomar, 2017).

Figure 2.1: Water melon fruit

c) Cultivation of water melon

Watermelon is grown in sandy loam soil rich in organic matter with good drainage and pH range for 6.5-7.5 (Kumar *et al.,* 2013).In North Indian plains, watermelons are sown in February-March whereas in Northeastern and Western India best time of sowing is from November to January. In South and Central India, these can be grown almost round the year. Watermelon is a warm season crop grown mainly in sub-tropical and hot-arid regions. Temperature range of 24-27°C is considered as optimum for the growth of the vines. Cool nights and warm days are ideal for accumulation of sugars in the fruits. The seed germinates best when temperatures are higher than 20°C. High humidity at the time of vegetative growth renders the crop susceptible to various fungal diseases.

i. *Planting and transplanting*

Watermelon can be direct seeded in the field or grown as transplants seedling in pots and then transplanted to the field. Before sowing seeds are soaked in warm water for 12 hours. Normally 3.5 kg of seed of watermelon is required for planting one ha area. The hills are usually spaced 1 to 1.5 meters apart in the rows also 2 to 2.5 meters apart. A variation of spacing hills 4meters apart in the rows 1.5 meters apart are also commonly used in the tropics. Apply FYM 20 t/ha, P 55 kg and K 55 kg as basal and N 55 kg/ha 30 days after sowing.

ii. *Weeds and insect control*

Depending upon the season about 2-3 weeding operations is required. The first weeding should be done 20-25 days after sowing while subsequent weeding is done at an interval of one month. The biggest watermelon pest is the leaf-eating beetles, they damage the flowers. The other main problem with growing watermelons is mildew, a fungus that makes the leaves look as if they were coated with white powder.

iii. *Yield and yield components*

The total yield of watermelon is a function of marketable yield, fruit count, percent cull, percent early fruit and fruit size (Dia *et al.*, 2012a; Dia *et al.,* 2012b; Dia *et al.,* 2012c). Marketable yield ranges from a high of 80.44 to a low of 27.43 Mg/ha. Total fruit count ranges from 1.61 to 6.31 thousand fruits/ha. Similarly, percent cull fruit, percent early fruit and fruit size range from 23.42-20.55%, 49.9-17.4%, and 01.72-14.56 kg/fruit, respectively (Dia *et al.,* 2016). Among quality traits, lycopene and sugar range from 8.76 to 52.15 mg/kg and 8.47 to 14.02 mg/kg, respectively (Dia *et al.,* 2016). Variation in watermelon yield and quality is governed by fluctuation in the external environment (Dia *et al.,* 2016c).

iv. *Harvesting and storage*

The crop is ready for harvest in about 75-100 days after sowing. For local market, harvesting should be done at full maturity while for transporting to distant markets, it is done slightly earlier. Watermelons can be stored for 14 days at 15°C. Watermelons should not be stored with apples and bananas as the ethylene produced during storage from these fruits hastens softening and development of off flavour to watermelons (Retu and Tomar, 2017).

d) Nutritional value of fresh watermelon

Watermelon is one of the commonly consumed fruits in many countries. Watermelon contains more than 91% water and up to 7% of carbohydrates. It is a rich source of lycopene and citrulline. Watermelon rind contains more amounts of citrulline then flesh. Additionally, watermelon has a number of essential micronutrients and vitamins (Retu and Tomar, 2017). See more nutritional details in the table below.

e) Health Benefits of Watermelon

i. *Heart health*

Watermelon contains high levels of lycopene that is very effective in protecting cells from damage and lowers the risk of heart disease. Watermelon extracts help to reduce hypertension and lower blood pressure in obese adults. 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 stroke and coronary heart diseases (Le *et al.,* 2005).

ii. *Anti-inflammatory and antioxidant support*

Anti-inflammatory foods can help with overall immunity and general health. The 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 (Edwards et al., 2003). It also contains a good amount of vitamin-B6 (pyridoxine), vitamin C and manganese. Consumption of food rich in vitamin C helps the body develop 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 immunity.

Table 2.1: Nutritive value per 100g of flesh

Components	Nutritive value	% Recommended daily allowance
Energy	30 Kcal	1.5%
Carbohydrates	7.6 g	6 %
Protein	0.6 g	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%
Electrolytes		
Potassium	112 mg	2.5%
Iron	0.24 mg	3 %
Manganese	0.038 mg	1.5 %
Zinc	0.10 mg	1%
Phytonutrients		
Carotene-alpha	303 µg	-
Lycopene	4532 µg	-

Adapted from USDA National Nutrient Database cited by Reetu and Tomar (2017)

iii. *Hydration and digestion*

Watermelons are the perfect example of a food that can help you stay hydrated. Watermelons are nature gift to beat summer thirst due to rich in electrolytes and water content. The watermelon contains fibre, which encourages a healthy digestive tract and helps keep you regular (Retu and Tomar, 2017).

iv. *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.

v. *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 India, Lycopene help in reducing prostate cancer cell proliferation. Consumption of natural fruits rich in vitamin-A is known to protect from lung and oral cavity cancers (Le *et al.,* 2005).

vi. *Edible seeds and rind*

Most people throw away the watermelon rind and seeds. Rind not only contains plenty of health-promoting and blood-building chlorophyll, but the rind actually contains important amino acid citrulline than the flesh. Citrulline is a non- protein amino acid and was first identified from watermelon. Citrulline is used in the nitric oxide system in humans and has antioxidant and vasodilatation roles (Rimando *et al.,* 2005). Citrulline improves circulation by reducing muscle soreness and heart rate. Many people prefer seedless watermelon varieties, but black watermelon seeds are quite healthy and edible. They contain iron, zinc, protein, and fibre (Kumar *et al.,* 2013).

f) Watermelon seed antioxidants

Antioxidants can be defined as substances whose presence in relatively low concentrations but significantly inhibits the role of oxidation of the targets (Rakesh *et al.,* 2010). Antioxidants are considered important nutriceuticals with many health benefits (Sharma and Bhat, 2009). Plants are known to be rich in biologically active substances such as the flavonoids, phenolic acids, anthocyanins, ethereal oils, and tannins, many of which exhibit antioxidant activity. Some of the antioxidants found in plants, such as tocopherols (vitamin E), ascorbic acid (vitamin C), and carotenoids are substances of major significance in human physiology. Most of these antioxidants are phenolics. Based on carbon structure, phenolics can either be classified as flavonoid compounds (flavones, isoflavones, flavanones, flavonols, and anthocyanidins) or non- flavonoid compounds (benzoic acid, stilbenes, and hydroxycinmamic acids). Watermelon varieties are said to contain high amounts of antioxidants, including citruline and lycopene. Watermelon seeds contain an antioxidant known as cucurbocitrin, which is extracted and used in lowering blood pressure and improvement of kidney function (Oseni and Okoye, 2013).

g) Pineapple

Pineapple [*Ananas comosus* (L.) Merr. Family: Bromeliaceae] is one of the most important commercial fruit crops in the world. It is known as the queen of fruits due to its excellent flavour and taste. Pineapple is the third most important tropical fruit in the world after Banana and Citrus .Pineapples are consumed or served fresh, cooked, juiced and can be preserved. This fruit is highly perishable and seasonal (Baruwa, 2013; Bartholomew *et al.,* 2003). Thailand, Philippines, Brazil and China are the main pineapple producers in the world supplying nearly 50 % of the total output. Other important producers include India, Nigeria, Kenya, Indonesia, Mexico, Costa Rica and these countries provide most of the remaining fruit (FAO, 2004; FAO, 2005).

Figure 2.2: Pineapple fruit

h) Aculty of natural and applied sciences: Nutritional value

Pineapple is a wonderful tropical fruit having exceptional juiciness, vibrant tropical flavor and immense health benefits. Pineapple contains considerable amount of calcium, potassium, vitamin C, carbohydrates, crude fibre, water and different minerals that is good for the digestive system and helps in maintaining ideal weight and balanced nutrition. Pineapple is a common fruit in Bangladesh and it has minimal fat and sodium. It contains 10-25 mg of vitamin. Pineapple composition has been investigated mainly in the edible portion. Pineapple contains 81.2 to 86.2% moisture, and 13-19% total solids, of which sucrose, glucose and fructose are the main components. Carbohydrates represent up to 85% of total solids whereas fibre makes up for 2-3%. Of the organic acids, citric acid is the most abundant in it. The pulp has very low ash content, nitrogenous compounds and lipids (0.1%) (Sabahelkhier *et al.,* 2010; Debnath *et al.,* 2012).

i) Uses as Food

Pineapple fruits exhibit high moisture, high sugars, soluble solid content ascorbic acid and low crude fibre. Thus pineapple can be used as supplementary nutritional fruit for good personal health. The pineapple fruits are normally consumed fresh or as fresh pineapple juice. Field ripe fruits are best for eating fresh, and it is only necessary to remove the crown, rind, eyes and core. Pineapple may be consumed fresh, canned, juiced, and are found in a wide array of food stuffs - dessert, fruit salad, jam, yogurt, ice cream, candy, and as a complement to meat dishes. In Panama, very small pineapples are cut from the plant with a few inches of stem to serve as a handle. The flesh of larger fruits is cut up in various ways and eaten fresh, as dessert, in salads, compotes and otherwise, or cooked in pies, cakes, puddings, or as a garnish on ham, or made into sauces or preserves. Malayans utilize the pineapple in curries and various meat dishes. In the Philippines, the fermented pulp is made into a popular sweetmeat. The pineapple does not lend itself well to freezing, as it tends to develop off flavours. Canned pineapple is consumed throughout the world. In Africa, young, tender shoots are eaten in salads. The terminal bud or "cabbage" and the inflorescences are eaten raw or cooked. Young shoots, called "*hijos de pina*" are sold on vegetable markets in Guatemala (Debnath *et al.,* 2012; Kader *et al.,* 2010).

j) Medicinal values of pineapple

Pineapple can be used as supplementary nutritional fruit for good personal health. Pineapple fruits are an excellent source of vitamins and minerals. One healthy ripe pineapple fruit can supply about 16.2% of daily requirement for vitamin C. Vitamin C is the body's primary water soluble antioxidant, against free radicals that attack and damage normal cells. A powerful antioxidant, vitamin C supports the formation of collagen in bones, blood vessels, cartilage and muscle, as well as the absorption of iron. Vitamin C also retards the development of urinary tract infections during pregnancy and reduces the risk of certain cancers, including colon, esophagus and stomach. Malic acid makes up 13 percent of pineapple juice's acidic content. Malic acid is also beneficial for health. It boosts immunity; promotes smooth, firm skin; helps maintain oral health; and reduces the risk of toxic metal poisoning. Pineapple is also a good source of vitamin B1, vitamin B6, copper and dietary fibre. Pineapple is a digestive aid and a natural anti-inflammatory fruit. Fresh pineapples are rich in bromelain used for tenderizing meat. Pineapple contains a proteolytic enzyme bromelain, which digests food by breaking down protein (Rahmatullah *et al.,* 2009).

Pineapple creates low blood pressure, cure inflammation disease, used for weight loss, control the death rate and prevent diabetes & radical damage. It cures the damaged teeth and makes them strong and healthy. Also help to cure sinusitis and throat problem. Cure different diseases like asthma, obesity, swelling in the body, problems of digestion and heart problem. Pineapples are rich source of manganese which creates strong bones and muscular body. Atherosclerosis and immune disease can be also cured due to high antioxidant content of pineapple. It prevent cancer, heart attack, nausea and gives the long natural hairs. It is use to solve acne, wrinkles, age problem and create strong nails, soft lips and thick hair (Rahmatullah *et al.,* 2009; Debnath *et al.,* 2012).

k) Penetration of micro organisms into fruits

Fruits have an epidermal layer of cells which provides a barrier for penetration of microorganisms. The internal tissues are nutrient rich and many, especially vegetables, have a PH near neutrality. Their structure is comprised mainly of the polysaccharide's cellulose, hemicelluloses, and pectin. The principal storage polymer is starch; microorganisms exploit the host using extracellular lytic enzymes that degrades these polymers. Microorganisms can also enter the fruits during fruits development, either through the calyx cuticle. Fruits possess an outer protective epidermis, typically covered by a natural waxy cuticle layer containing. In addition microorganisms can also penetrate fruits during processing via a number of ways. They can enter via the vendors poor hygiene, water used in washing, packaging materials, and other materials used in processing the fruits such as containers, boards, and even cutleries. Furthermore, the use of dirty utensil, as well as the open display of food encourages sporadic visits by flies, cockroaches, rodents and dusts (Agbo *et al.,* 2017).

l) Staphylococcus food poisoning

Staphylococcal food poisoning (SFP) is one of the most common food-borne diseases in the world following the ingestion of staphylococcal enterotoxins (SEs) that are produced by enterotoxigenic strains of coagulase-positive staphylococci (CPS), mainly Staphylococcus aureus and very occasionally by other staphylococci species such as *Staphylococcus intermedius* When outbreaks occurred during large social events, chaotic situations resulted requiring the rapid implementation of medical care for a high number of cases (Bonnetain *et al.,* 2003; Do Carmo *et al.,* 2004).

The first description of food-borne disease involving staphylococci was investigated in Michigan (USA) in 1884 by Vaughan and Sternberg. This food poisoning event was because of consumption of a cheese contaminated by staphylococci. The authors stated: 'It seems not improbable that the poisonous principle is a ptomaine developed in the cheese as a result of the vital activity of the above-mentioned Micrococcus or some other microorganisms which had preceded it, and had perhaps been killed by its own poisonous products. Ten years later, Denys (1894) concluded that the illness of a family who had consumed meat from a cow that had died of vitullary fever was owing to the presence of pyogenic staphylococci.

Table 2.2: Incidence of *Staphylococcus aureus* food poisoning

Year	Location	Incriminated food	Number of cases
1968	School children, Texas	Salad	1300
1971	UK army	Sausages rools, sandwiches ha	100
1975	Flight from Japan to Denmark	Ham	197
1976	Rio to NYC	Chocolate eclairs	80
1980	Canada	Cheese curd	62
1982	North Carolina and Pennsylvania	Ham and cheese sandwich; stuffed chicken	121
1983	Caribbean cruise ship	Dessert cream pastry	215
1984	Scotland	Sheep's milk cheese	27
1985	France, UK, Italy, Luxembourg	Dried lasagna	50
1985	School children, Kentucky	2% chocolate milk	> 1000
1986	Country Club, New Mexico Turkey,	Poultry, gravy	67
1969	Various US states	Canned mushrooms	102
1990	Thailand	Eclairs	485
1992	Elementary school, Texas	Chicken salad	1364
1997	Retirement party, Florida	Precooked ham	18
1998	Minas Gerais, Brazil	Chicken, roasted beef, rice and beans	4000

2000	Osaka	Low-fat milk	420
2006	Ile de France area, France	Coco nut pearls (Chinese dessert)	17
2007	Scouts' camp	Belgium Hamburger	15
2007	Elementary school, Austria	Milk, cacao milk, vanilla milk	166
2008	French district	Pasta salad	100
2009	Nagoya university festival,	Japan Crepes	75

Source: Hennekkinne et al (2011)

m) Escherichia coli

Escherichia coli, one of the 30 members of the bacterial family of *Enterobacteriaceae*, is a coliform bacterium and is one of the 6 types of *Escherichia* species (*E. adecaroxylate, E. blattae, E. fergusonii, E. hermannii* and *E. vulneris*). It is a gram-negative, non-spore-forming, facultative, anaerobic, rod shaped, mesophilic bacterium that grows in 7–45°C. The group of coliform bacteria consists of *Citrobacter, Enterobacter, Klebsiella* and *Escherichia*. While there are bacteria of fecal origin among coliform bacteria, there are also bacteria of plant origin such as *Enterobacter aerogenes, Citrobacter freundii*, and *Klebsiella pneumoniae*. Presence of coliform group in food is indicative of fecal contamination, poor hygienic conditions or existence of enteric pathogens. For instance, the presence of coliform bacteria in raw milk is an indication of poor hygiene in milking or storage conditions.

The presence of coliform bacteria in raw or frozen fruits and vegetables is not important as *Enterobacter, Citrobacter* and *Klebsiella* are naturally present in the microbiota of plants. However, *E. coli* presence in fruits and vegetables is very important in terms of inadequate hygiene. *E. coli* is an important pathogen as it is an indicator of fecal contamination in foods and drinking water. Due to this characteristic, it is considered as an indicator bacterium in food safety and hygiene. Being the prominent bacterium in the facultative anaerobic microbiota of the intestines, *E. coli* is widespread in stool and the environment. Some of its pathogenic strains both cause intoxication by creating toxins and cause gastroenteritis, pathologic kidney and brain damage by causing infection-type food poisoning through cellular increase. Some enterotoxin producing *E. coli* strains are divided into two groups as heat-stable and heat-labile. The heat-stable toxin is known as stable toxin (ST) and the heat-labile toxin is known as labile toxin (LT).

n) Risk groups for Escherichia coli

E. coli is a type of bacteria that normally live in the intestines of people and animals. However, some types of *E. coli*, particularly *E. coli* O157:H7 can cause intestinal infection. *E. coli* O157:H7 and other strains that cause intestinal sickness are called Shiga toxin–producing *E. coli* (STEC) after the toxin that they produce. People with weakened immune systems, pregnant women, young children, and older adults are at increased risk for developing these complications. Most intestinal infections are caused by contaminated food or water. Proper food preparation and good hygiene can greatly decrease your chances of developing an intestinal infection. Most cases of intestinal *E. coli* infection can be treated at home. Symptoms generally resolve within a few days to a week (Prescott *et al.,* 1999).

o) Strains of E. coli

There are six strain of *E. coli* implicated in diarrhea illness in humans. These are briefly explained below.

i. *Enterotoxigenic E. coli (ETEC)*

People living in developing countries have often been reported to have this pathotype in their feces and shown to have developed immunity against this microorganism. Being a cause of mortality in children under 5, the most frequently observed microorganism in childhood diarrhea is ETEC and it is also responsible for 30–60% of travelers' diarrhea. Infection is characterized by watery diarrhea and, depending on the person; its course may range from a normal course to cholera-like defecation with the addition of symptoms such as vomiting and high fever (Prescott *et al.,* 1999; Ekici and Dumen, 2019). Among these potential pathogens, the most common cause of diarrhea in children under five is the ETEC (heat-stable – ST and/or heat-labile – LT type toxin) producing *E. coli* strains. Through the production of fimbrial or non-fimbrial adhesins, ETEC strains cause hypersecretion of fluids by producing enterotoxins that disrupts fluid and electrolyte homeostasis in the epithelial cells of small intestines, leading to watery diarrhea. Without rehydration, moderate or severe diarrhea could lead to dehydration and acute mortality (Prescott *et al.,* 1999; Ekici and Dumen, 2019).

ii. *Enteropathogenic E. coli (EPEC)*

It is known to be the oldest *E. coli* serotype causing diarrhea and its most important characteristic is adherence. In EPEC infections, vomiting and low body temperatures are observed in addition to watery diarrhea. It is known to cause diarrhea in infants and outbreaks can occur in neonatal care units. Humans, pigs and bovines may be infected with this microorganism. EPEC is transmitted from person to person, however; rarely, it is also known to spread through contaminated food and water. The ability to produce attaching and effacing (A/E) lesions is a distinctive phenotype for EPEC. Bacteria cause extensive deterioration on microvilli by strongly adhering to the host cell membrane. As a distinctive factor, all EPEC strains lack the Shiga toxin (*stx*) producing genes. Among single-pathogen infections, EPEC has the second highest severity score after rotavirus, followed by ETEC (Prescott *et al*., 1999; Ekici and Dumen, 2019).

iii. *Enteroaggregative E. coli (EAEC)*

This pathotype is a food-borne enteropathogen observed in acute and persistent diarrhea cases in children, patients with suppressed immune systems in developing countries and people traveling to endemic regions. Growth disorders and cognitive disorders in children living in developing countries, stem from EAEC infections. In the pathogenesis of EAEC, the first step is the strong adherence to the intestinal mucosa. The second step is leading to the development of enterotoxins and cytotoxins and the third step is known to be characterized with the ability to induce mucosal inflammation (Prescott *et al.,* 1999; Ekici and Dumen, 2019).

iv. *Diffusely-adherent E. coli (DAEC)*

Hep-2 or HeLa cell cultures are called DAEC due to their diffuse adherence characteristics. DAEC serotypes are known to cause chronic diarrhea in children between the ages of 1 and 5. They cause degradation in the intestinal epithelium by binding to proteins that accelerate degradation. Mild diarrhea void of fecal leukocytes is the indication of infection. In France, DAEC strains were found out to be widespread in diarrhea cases observed in inpatients from a hospital with no other enteropathogen. This situation indicates that DAEC strains may be an important diarrheagenic pathogen in developed countries. Recent studies show that some DAEC strains contain virulence factors present in uropathogenic *E. coli* (UPEC) strains (Prescott *et al.,* 1999; Ekici and Dumen, 2019).

v. *Enteroinvasive E. coli (EIEC)*

EIEC strains causing inflammatory damage in intestinal mucosa and submucosa are very similar to those produced by *Shigella*. These microorganisms have the same spreading and reproducing abilities inside epithelial cells. However, clinically, EIEC-related watery diarrhea is much more commonly observed than dysentery caused by *Shigella*. O antigens of EIEC can cross-react with O antigens of *Shigella*. The disease starts with severe abdominal cramping, weakness, watery stool, difficulty urinating and fever. It could rarely aggravate and turn into watery stool containing blood or mucus. The fecal leukocytes observed in shigellosis may also be observed in the mucus smear of a person infected with EIEC. EIEC infections are endemic to less developed countries and are reported to be rarely observed infection in developed countries. The incubation period is observed as 10–18 hours. There is evidence showing that EIEC is transmitted through contaminated foods. Just like in shigellosis, cases of diarrhea with entero-invasive strains can be treated by using antimicrobials effective against *Shigella* isolates (Prescott *et al.,* 1999; Ekici and Dumen, 2019).

vi. *Enterohemorrhagic E. coli (EHEC)*

EHEC are also named Shiga toxin producing *E. coli* (STEC) and also verotoxin producing *E. coli* (VTEC). All strains of EHEC produce Shiga toxins that destroy Vero cells similarly to Shiga toxins produced by Shigella. *E. coli* O157: H7, first defined after the outbreak associated with the consumption of rare cooked minced meat in 1982, is the primary cause of EHEC infection in industrialized countries including the USA, Canada and England. O26, O103, O111 and O145 can be listed among the other EHEC serogroups responsible for food-borne diseases. Even though the O157 strains are the ones that draw the most attention, the strains of other EHEC serogroups, especially O111, are gradually getting reported more and more around the world. Based on the severity of the disease, EHEC is regarded as the most serious *E. coli* strain among foodborne pathogens. *E. coli* O157:H7, differ from the other *E. coli* serotypes because of some of its characteristics, which are: not being able to grow in or above 42°C, not being able to ferment sorbitol, not having β-glucuronidase enzymes and producing enterohemolysins (Prescott *et al.,* 1998; Ekici and Dumen, 2019).

III. Materials and Methods

a) Materials

The materials used in this study were Binocular microscope, Autoclave, Hot air oven, Incubator, Weighing balance (electric), Bunsen Burner, Refrigerator, Wire loop, Hand gloves, Spatulas, Qubec colony counting chamber, Conical flasks, Petri dishes (disposable), Measuring cylinder, Slides, Cover slips, Durhams tubes, Beaker, test tubes, test tube racks, aluminum foil paper, cotton wool, forcep, masking tapes, and permanent marker. Other include consumables such Eosin Methylene Blue Agar, Nutrient Agar, Saboudraud Dextrose Agar, Mannitol Salt Agar, Muller Hinton Agar, Peptone water, Distilled water, Ethanol (70%), peptone water, nutrient broth, Gram staining reagents, Citrate Agar, Kovac reagent, Oxidase test strip, 3% hydrogen peroxide and MR-VP reagents.

b) Collection of fruit samples

Pineapple and watermelon fruits used in this study were purchased fresh from Akpan Andem Market in Uyo Metropolis, Akwa Ibom State, Nigeria. Five samples each of both fruits were collected from five different fruit vendors. The fruits were purchased as sliced by vendors and packaged in transparent polythene bags. The purchased fruits were then further wrapped with separate aluminum foil papers, placed in sterile polythene bags, and transported within one hour of purchase to the microbiology laboratory for microbiological analyses.

c) Processing of samples

This was carried out using a method previously described by Agbo *et al.* (2016) and Abubakari *et al.* (2015) but with a little modification. Each of the samples was placed in a separate sterile beaker and then gently rinsing them with sterile distilled water (50ml). After rinsing the fruits, the edible parts were sliced gently. For each of the fruits, the sliced outer most parts of the fruits together with the water that was used in rinsing them were then homogenized using a sterile electric blender. Following blending, 1ml of the homogenized samples for each of the fruit was then subjected to a ten-fold serial dilution.

d) Enumeration of total aerobic bacteria and fungi counts

Following serial dilutions, 10^{-3} dilutions were used for enumeration of total aerobic bacteria and 10^{-2} for fungi. This was done using the pour plate method previously described (Prescott *et al.,* 1999). From each of the selected serial dilution, 1ml was taken and poured into a sterile petri-dish. Then freshly prepared nutrient agar and Saboudraud dextrose agar which were first allowed to reach 45°C were then poured into the plates and allowed to solidify. The plates were then incubated inverted at room temperature for 24 hours and 48 hours, respectively for nutrient agar and Saboudraud dextrose agar. After incubation, the colonies which developed were counted and expressed as colony forming units per ml (cfu/g).

e) Isolation of Staphylococcus aureus and E. coli

The *S. aureus* isolates were isolated by pour plating the 10^{-3} dilutions onto freshly prepared mannitol salt agar plates and incubating at room temperature inverted for 24 hours. The discrete colonies (total of 11) were selected and sub-cultured twice in order to purify them. They were stored in freshly prepared nutrient agar slants for biochemical characterization and sensitivity analysis. For *E. coli*, the 10^{-3} dilution were plated onto freshly prepared Eosin Methylene Blue agar plates and incubated overnight at room temperature. Similarly, the selected discrete colonies were sub- cultured twice in order to purify them. After pure colonies were obtained, they were stored in freshly prepared nutrient agar slants for biochemical characterization and sensitivity analysis.

f) Gram staining

A bacterial smear was made on a slide, the slides was placed on a staining tray and the smear was covered and allowed to stain for 60seconds, the slide was tilted and gently rinsed with distilled water until the stain was removed. The smear was covered with Gram's Iodine and allowed for 60 seconds, the slide was gently rinsed with distilled water, the slide was tilted and 2 or 3 drops of ethanol run over the slide. It was rinsed with distilled water, the smear was covered with Safranin and stained for 60 seconds and rinsed with distilled water. The slides were allowed to dry and observed using oil immersion. Those that were Gram positive appeared as purple while those that were Gram negative showed red\pink colour.

g) Biochemical test

i. *Catalase test*

A small amount of the culture was picked from the agar slopes, using a clean sterile platinum wire loop. This was inserted in drops of 10% H_20_2 on a clean microscopic slide. Production of gas bubbles indicated a positive reaction.

ii. *Oxidase test*

A fresh and sterile filter paper was soaked in 1% oxidase reagent solution, a few colony of the test organism was rubbed on the filter paper, oxidase reagent was poured over the test organism in a culture, positive results gave a deep purple colour after 10 seconds and no colour change if the organism cannot produce oxidase.

iii. *Methylred*

The test organism was inoculated in a glucose phosphate broth and was incubate at 37c for 3 days, then five drops of 0.04% of methyl red were added and was mixed thoroughly. A red colour indicated a positive result while an orange colour indicated a negative result.

iv. *Voges Proskauer test*

A colony of bacteria isolate was transferred in a test tube containing MR-VP medium/broth using a sterile hours. Following incubation, 2 drops of ¤-naphthol was added and 1 drop of potassium hydroxide (KOH) was added into the medium. The medium was shaken at intervals to ensure maximum reaction for 3-5 minutes. Thereafter the medium was observed for colour change from pink to mahogany red within 15-20 minutes which indicates a positive test.

v. *Citrate utilization*

Simmon's citrate agar was dispensed into 2 tubes and allowed to solidify in that position, the tubes were inoculated by stabbing and incubated at 35-37°C for 24hours with a loosely fitted cap. The presence of growth and blue coloration was for a positive test, no colour change indicated inability to utilize citrate.

vi. *Indole test*

Bacterial colonies was transferred into test tube containing MIO medium broth motility indole ornithine medium using a sterile inoculating loop the tube was incubated at 37°cfor 48 hours. Thereafter, 3 to 5 drops of Kovac's reagent was added and shaken to mix properly. On observation the appearance of red ring on the surface of the tube was taken as positive test while orange colour indicated negative.

vii. *Urease test*

Bacterial colonies were inoculated into urea medium. This was incubated at 37°C for 48 hours.

Urease production was shown by a change of colour in the medium from yellow to pink.

viii. *Motility test*

Motility ornithine indole (MOI) medium was prepared. The medium was stabbed, inoculated and incubated with the surface corked. The medium was viewed for bacterial growth at both the edge of the medium as well as the stab line.

h) Antibiotics sensitivity test

Antimicrobial sensitivity test was carried out on the various *E. coli* and *S. aureus* isolates using specific Gram negative and positive antibiotics discs. The antibiotics used for E. coli were CPX, SXT, S, PN, CEP, OFX, NA, PEF, CN, and AU which represent Ciprofloxacin (CPX), sulfamethoxazole+trimethoprim (SXT), Streptomycin (S), Perfloxacilin (PN), Ceporex (CEP), Ofloxacin (OFX), Nalidixic acid (NA), Reflacine (PEF), Gentamycin (CN), and amoxicillin clavulanic acid (AU), respectively. While the antibiotics for Gram negative ERY – Erythromycin, AMX – Amoxil, CHL – Chloramphenicol, LEV – Levofloxacin, CXP – Ciprofloxacin, STR – Streptomycin, APX – Ampliclox, NFX – Norfloxacin, RIF – Rifampicin, GEN – Gentamycin. The test was done as previously described by Kirby Bauer disk diffusion test (CLSI, 2006). Briefly, each of the purified isolates were first inoculated onto freshly prepared peptone water and incubated overnight. After incubation, the turbidity was adjusted to Mac Farland standard before 0.1 ml of the inoculum were picked and streaked onto freshly prepared Muller Hinton Agar and in duplicates for each isolate. The plates were then incubated for 24 hours and the zones of inhibition measured using a meter rule in millimetre.

i) Statistical analysis

Replicate data generated in this study for microbial counts and antimicrobial sensitivity tests were managed and analyzed using Microsoft Excel 2010. The data were analyzed using analysis of variance (ANOVA) with level of significance set at 95%. The data were then presented as mean ± standard deviation and regarded as significant with p-values less than 0.05.

IV. Results

a) Total aerobic bacteria and fungi counts

The results of study are presented in the Tables 4.1 to 4.8. Table 4.1 and 4.2 show the total aerobic bacteria for the various water melon and pineapple samples used in the study, respectively. From the results (Table 4.1), the that the highest count and least counts were 1.60 and 1.31 x 10^5 cfu/g. Table 4.2 shows the mean aerobic counts for the pineapple samples used in this study. The lowest count was 1.23 x 10^5 from pineapple sample 3 while the highest count was 1.56 x 10^5 cfu/g. Tables 4.3 and 4.4 show the total aerobic fungi counts in the water melon and pineapple samples used in this study. Comparatively, the counts for the aerobic fungi were lower than those of bacteria. From the results in Table 4.3, the water melon sample 5 (WM5) had the highest count of 1.3 x 10^3 CFU/g while WM1 had the least count of 5 x 10^2 CFU/g. For the pineapple samples, the total aerobic fungi counts were 6, 8, 11, 13 and 14 x 10^2 CFU/g for samples 1, 2, 3, 4 and 5, respectively. As can be seen, sample s5 and 1 had the highest and least counts of 6 and 14 x 10^2 CFU/g (Table 4.4).

Table 4.1: Total aerobic bacteria counts in WM Samples (CFU/g)

Samples	10^5
WM1	1.30±0.03
WM2	1.60±0.11
WM3	1.53±0.08
WM4	1.31±0.07
WM5	1.51±0.04

Key: WM = Water melon, CFU = colony forming units

Table 4.2: Total aerobic bacteria counts in PA samples (cfu/g)

Samples	10^5
PA1	1.33±0.01
PA 2	1.56±0.10
PA 3	1.23±0.03
PA 4	1.36±0.11
PA 5	1.50±0.12

Key: PA = Pineapple, CFU = colony forming units

Table 4.3: Total aerobic fungi counts in WM samples (cfu/g)

Samples	10^2
WM1	5
WM2	8
WM3	10
WM4	11
WM5	13

Key: WM = Water melon, CFU = colony forming units

Table 4.4: Total aerobic fungi counts in WM samples (cfu/g)

Samples	10^2
PA1	6
PA 2	8
PA 3	11
PA 4	13
PA 5	14

Key: PA = Pineapple, CFU = colony forming units

b) Biochemical and morphological characterisation of the isolates

Although selective media were used to isolate the *Escherichia coli* and *Staphylococcus aureus*, routine morphological and biochemical characterization were carried out and the results are presented in tables 4.5 and 4.6. The results in Table 4.5 indicates that the all the

10 isolates selected for characterization were gram negative, rod shaped isolates, positive reactions for catalase, indole, and gas production. Furthermore, the isolates in Table 4.5 were negative for Oxidase, methyl red, voges proskauer, citrate, urease and H_2S. All the results provided a tentative identification for *E. coli* in addition to the sheen green metallic sheen colour that was obtained on Eosin Methylene Blue Agar.

Table 4.6 the morphological and biochemical testes for the *Staphylococcus aureus* isolates. They were all Gram positive and cocci in shape. Furthermore, they tested positive to catalase, methyl red, voges proskauer, citrate and urease. However, they isolates were negative for motility, oxidase, indole, H_2S and gas production tests. These results provided tentative identification for *Staphylococcus aureus.*

Table 4.5: Biochemical characterization of the *E. coli* isolates

Isolates	GR	Shape	Motility	Catalase	Oxidase	Methyl Red	Voges Proskauer	Indole	Citrate	Urease	H_2S/Gas
Isolate 1	-	Rods	+	+	-	+	-	+	-	-	-/+
Isolate 2	-	Rods	+	+	-	+	-	+	-	-	-/+
Isolate 3	-	Rods	+	+	-	+	-	+	-	-	-/+
Isolate 4	-	Rods	+	+	-	+	-	+	-	-	-/+
Isolate 5	-	Rods	+	+	-	+	-	+	-	-	-/+
Isolate 6	-	Rods	+	+	-	+	-	+	-	-	-/+
Isolate 7	-	Rods	+	+	-	+	-	+	-	-	-/+
Isolate 8	-	Rods	+	+	-	+	-	+	-	-	-/+
Isolate 9	-	Rods	+	+	-	+	-	+	-	-	-/+
Isolate 10	-	Rods	+	+	-	+	-	+	-	-	-/+

Keys: + = Positive, - = Negative.

Table 4.6: Biochemical characterization of the *Staphylococcus aureus*

Isolates	GR	Shape	Motility	Catalase	Oxidase	Methyl Red	Voges Proskauer	Indole	Citrate	Urease	H_2S/Gas	Coagulase
Isolate 1	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 2	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 3	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 4	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 5	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 6	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 7	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 8	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 9	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 10	+	Cocci	-	+	-	+	+	-	+	+	-/-	+
Isolate 11	+	Cocci	-	+	-	+	+	-	+	+	-/-	+

Keys: + = Positive, - = Negative.

The *E. coli* and *Staphylococcus aureus* isolates were subjected to sensitivity testing and the results are presented in Tables 7 and 8, respectively. A total of 4 isolates showed multi-antibiotics resistance (resistance to atleast 2 antibiotics) and these were isolates PA4, PA1, WS4, and WS5, respectively. Apart from PA5 that was resistant to only one antibiotic Gentamycin (CN), the rest of the isolates were sensitive to the test antibiotics. The highest and the least zones of inhibitions were 6.00±1.05 and 23.37±1.20 mm for isolates WS5 and PA3, respectively for CEP and CPX which were *Ciprofloxacin* and *Ceporex.*

On the other hand, the highest and least zones of inhibitions recorded by the 11 *S. aureus* isolates were 24.10 and 6.00 for norfloxacin and ciprofloxacin, respectively against isolates WM10 and WM 11. As also observed amongst the *E. coli* isolates, a total of 5 isolates showed multi-drug resistance and these were isolates PA1, PA3, PA5, WM77 and WM11.

Table 4.7: Sensitivity of *Escherichia coli* isolates

Antibiotics zones of inhibitions (mm)										
Isolates	CEP	OFX	NA	PEF	CN	AU	CPX	SXT	S	PN
WS1	22.07±1.01	19.10±0.85	12.13±0.81	12.17±0.76	NG	13.07±0.90	22.10±1.01	16.13±0.81	16.13±0.91	13.50±1.71
WS2	18.07±1.90	21.10±1.85	21.47±1.36	22.17±0.76	19.07±0.90	20.17±0.76	21.10±0.85	22.07±1.01	21.53±1.28	20.57±1.25
WS3	18.20±2.05	18.67±0.58	20.23±1.05	21.10±1.15	19.13±1.11	17.83±1.01	17.13±1.11	16.30±1.11	18.43±1.30	17.33±1.35
WS4	NZ	18.10±0.90	19.07±1.01	18.13±1.12	18.57±1.01	15.10±0.90	16.23±1.01	NZ	10.87±1.24	15.17±1.12
WS5	NZ	NZ	NZ	8.00±1.00	6.00±1.00	NZ	NZ	NZ	NZ	NZ
PA1	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
PA2	10.37±1.05	14.23±1.27	15.23±1.05	12.33±1.05	12.10±0.90	18.17±1.06	19.80±1.22	20.80±1.30	22.17±1.11	11.57±1.29
PA3	18.00±1.00	18.00±1.91	19.23±0.90	20.10±2.1	21.43±0.81	22.27±0.86	23.37±1.20	20.13±1.03	17.23±1.05	16.20±2.05
PA4	NZ	14.83±0.76	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
PA5	20.00±2.00	21.00±1.00	20.00±1.00	22.00±2.00	NZ	21.00±2.00	23.00±1.00	20.00±1.00	19.00±1.00	18.00±1.00

Key: NZ = No zone of inhibition, CPX, SXT, S, PN, CEP, OFX, NA, PEF, CN, and AU which stood for *Ciprofloxacin, sulfamethoxazole+trimethoprim, Streptomycin, PN, Ceporex, Ofloxacin, Nalidixic acid, Reflacine, Gentamycin, and amoxicillin+clavulanic acid, respectively.*

Table 4.8: Sensitivity of Staphylococcus *aureus* isolates (mm)

Isolates	CXP	STR	NFX	APX	GEN	ERY	AMX	CHL	LEV	RIF
PA1	NZ	NZ	NZ	14.11±1.05	15.03±0.57	18.00±1.13	10.33±0.11	15.23±2.05	18..13±1.12	20.57±1.23
PA2	18.57±1.01	19.00±1.00	NZ	17.00±0.58	16.00±0.90	18.25±0.58	20.00±2.05	21.00±0.90	22.00±1.57	22.00±1.00
PA3	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
PA4	14.01±0.58	17.57±0.41	18.00±1.05	18.57±0.58	19.15±1.01	21.00±1.01	23.00±1.00	24.00±2.00	20.00±1.05	18.33±1.33
PA5	17.00±1.01	16.00±0.47	20.00±1.30	NZ	NZ	18.00±0.57	19.00±0.57	23.00±0.57	19.33±0.57	18.33±0.21
WM6	17.04±2.04	16.57±0.58	19.10±1.02	20.12±1.21	21.00±1.25	13.33±1.05	15.10±0.90	19.17±1.08	19.08±1.02	20.08±1.03
WM7	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
WM8	17.00±1.01	16.80±1.02	16.40±0.90	19.00±1.25	20.12±1.25	13.00±0.80	22.50±1.21	18.13±0.71	16.05±0.72	15.50±1.71
WM9	17.00±0.90	22.30±1.21	16.33±0.90	16.13±1.91	18.20±0.57	20.00±1.00	23.20±2.25	18.50±0.31	20.50±1.21	17.80±0.41
WM10	21.00±1.01	23.00±1.00	24.10±2.00	20.00±1.05	18.33±1.33	19.00±1.05	15.01±1.01	18.00±1.00	20.00±1.00	17.00±0.33
WM11	6.00±0.57	7.15±0.90	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ

Keys: ERY – Erythromycin, AMX – Amoxil, CHL – Chloramphenicol, LEV – Levofloxacin, CXP – Ciprofloxacin, STR – Streptomycin, APX – Ampliclox, NFX – Norfloxacin, RIF – Rifampicin, GEN – Gentamycin, NZ = No zone

V. Discusssion, Conclsuion and Recommendation

a) Discussion

In developing countries like Nigeria, street vended sliced fruits are widely consumed by millions of Nigerians in cities and they are a source of affordable nutrients to many of the inhabitants. However, these fruits and the juices are often linked with diarrhoeal diseases largely due to unhygienic handling and processing of the fruits and its juices that are commonly vended on the street in developing countries (WHO, 2002; Baro, 2006 & Tambekar *et al.,* 2009). The major microbial contaminants are usually enteric and skin associated bacteria and fungi (Mensah *et al.,* 1999). However, to a lesser extent, viruses and even parasites have been associated with the transmission of enteric pathogens in foods (Mensah *et al.,* 1999).

In an earlier study, where the mean bacteria counts for various street vended were examined, they obtained counts for fried yam, fried fish, moi-moi, suya, meat pie and fried plantain were 7.4 x 10^4 cfu/g, 7.4 x 10^4 cfu/g, 1.0 x 10^4 cfu/g, 9.3 x 10^4 cfu/g, meat pie 6.4 x 10^4 cfu/g and fried plantain 7.6 x 10^4 cfu/g respectively (Agbo *et al.,* 2016). In this study, the mean aerobic bacteria counts obtained for water melon and pineapple were 1.85 and 1.867 x 10^4 cfu/g, respectively. In another earlier study, higher counts up to 4.6 x 10^6 cfu/g from juices of pineapple, sweet lime, and vegetables randomly collected from vendors in Nagpur City in India (Titarmare *et al.,* 2009). As expected, the fungal counts were very low compared to their bacterial counterparts. This is in line with earlier studies that showed that fungal abundance always less than those of bacteria in various ecosystems (Edet et *al.,* 2017). In our study, the fungal counts ranged from 0.6 to 1.4 x 10^3 cfu/g for pineapple and 0.5 to 1.3 x 10^3 cfu/g for water melon and these counts were within range of what was obtained in an earlier study where 1.1 x 10^3 – 3.0 x 10^5 and 1.4 x 10^3 – 2.0 x 10^5 cfu/g were obtained for hot and cold food samples, respectively in street vended foods in Abeokuta, Nigeria (Oluwafemi *et al.,* 2013). Furthermore, the fungal species were *Rhizopus* sp; *Aspergillus* spp; *Penicillium* spp. and *Mucor* spp.

Studies abound that have shown several frequently isolated bacteria from ready-to-eat fruits and foods and these include *Escherichia coli*, *Salmonella sp*, *Pseudomonas sp*, *Staphylococcus aureus*, *Shigella sp*, *Mucor sp,* viruses and even parasites have been associated with the transmission of enteric pathogens (Agbo *et al*., 2016; Mensah *et al.,* 1999). In another study, by Titamare (2009) where 38 street vended fruit and vegetables were evaluated, fecal coliforms, *Staphylococcus* and *Salmonella* were all isolated with total coliform and *Staphylococcus* showing no significant difference.

The main aim of our study was to isolate *Staphylococcus aureus* and *Escherichia coli* that stand out from a public health perspective. The presence of both microbes is a direct indication of poor handling and hygiene. Their presence in foods and fruits indicates recent fecal contamination and overall poor hygiene in the handling and processing of such foods (Titamare *et al.,* 2016; Yang *et al.,* 2017). E. coli is a normal flora of human that resides in the small intestine. One of its strain *E. coli* O157:H7 have been linked to several cases of food poisoning and diarrheal illnesses (Prescott *et al.,* 19998; Yang *et al.,* 2017). On the other hand, *Staphylococcus aureus* is a normal flora of the skin of human (Prescott *et al.,* 1999) and their presence in food is an indication of poor hygiene in the processing steps (Brooks *et al.,* 2010). Like *E. coli,* they are frequently associated with food poisoning cases and the bacterium has outstanding ability to secrete enterotoxin that causes food poisoning (Hennekinne *et al.,* 2012; Brooks *et al.,* 2010).

Apart from clinical and environmental samples that were about the only sources of antibiotics resistance, vegetables and fruits have joined the fray. A recent outbreak of enterohemorrhagic *Escherichia coli* (EHCC) in 2011 resulted in a total of 4321 cases with atleast 50 fatalities (Holzel *et al.,* 2018). In an earlier study, *Staphylococcus aureus* isolated from a total of 53 vegetables (lettuce, penilla leaf and sprouts) cultivated in Korea showed multiple drug resistance and as well as single resistance. This is in line with our findings as 5 out of 10 *S. aureus* isolates showed multi-drug resistance to the test antibiotics used in this study. Two of isolates

PA1 and WM7 isolated from pineapple and watermelon showed 100% resistance to all the test antibiotics. In another study, Agbo *et al* (2016) showed that *Staphylococcus aureus* was highly resistant to Norfloxacin (50.0%), Ampiclox (45.0%), Erythromycin (40.0%), Amoxil (35.0%). Furthermore, Kwaku et al (2016) showed that *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Citrobacter* spp. and *Enterobacter isolated from* carrots and lettuce were resistant to all eight (8) antibiotics in their study.

Tadesese *et al* (2018) isolated *E. coli* from fresh cow milk and fruits juice that were highly resistant to ampicillin (70%), sulfamethoxazole-trimethoprim (60%), clindamycin (80%), erythromycin (60%), chloramphenicol (50%), and kanamycin (50%) susceptible to gentamicin (100%), norfloxacin (100%), tetracycline (60%), polymyxin B (90%), and ciprofloxacin (90%). In this study, most isolates showed resistance to all the antibiotics used.

b) Conclusion

This research project has indeed shown that sliced Water melon and Pineapple that are widely consumed in Uyo and other cities in Nigeria have microorganism that are of public health concern. Isolation of *S. aureus* and *E. coli* is an indication of the poor personal hygiene of the various fruit vendors. The multiple antibiotic resistance shown by the *S. aureus* and *E. coli* isolated from the pineapple and water melon samples are even more worrisome.

c) Recommendation

1. Similar studies should be carried out on other fruits types such as Oranges, Apples, Straw berries that are also vended in the study location.
2. Molecular characterization should be carried out such as Sanger sequencing to identify the various strains.
3. Plasmid profiling of the multi-drug resistant isolates should be carried out to ascertain the resistant genes they might be carrying.
4. Fruits vendors should be made to undergo regular health checks and also be exposed to best and acceptable hygienic practice in handling and processing of the fruits they sell to the public.

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