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Microbial Profile and Safety Assessment of Vended Fruits in Port Harcourt, Nigeria.

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ABSTRACT

A study on the microorganisms associated with fruits (pineapple, apple, orange, mango and strawberry) obtained from Spar Shopping Mall, Port-Harcourt, was carried out using standard cultural techniques. Nutrient agar and dextrose agar were used as growth media for the isolation of heterotrophic bacteria and fungi. The bacteria were identified as Escherichia coli, Serratia species, Micrococcus species, Salmonella species, Staphylococcus aureus, Lactobacillus species, Acetobacter species, Bacillus cereus, Leuconostoc species, Phythium species, Streptococcus pyogenes, and Agrobacterium tumefaciens while the fungi were identified, on the basis of their colonial and microscopic characteristics, were Penicillium species, Aspergillus niger, Cladosporium species, Rhizopus, Mucor species. Escherichia coli and Aspergillus niger were the predominant isolates amongst the bacteria and fungi respectively, with mean colony counts of 1.84 x 102 and 33.67 x 105 respectively. The presence of the organisms is a public health risk because of the diseases associated with these organisms. It is therefore imperative that adequate hygienic practices must be put in place during storage and handling of fruits. Antimicrobial susceptibility test using common antibiotics as treatments on the isolates revealed that 80% of the isolates were sensitive to Gentamycin, amoxycillin, ceftraxine, norflaxacin, levofloxacin, erythromycillin and chloramphenicol, whereas 20% were sensitive to rifamipicin, ampliclox, and ciprofloxacin. It is established in this study that several microorganisms are associated with the selected fruits, many of which are pathogenic and can pose serious health hazards if consumed without proper pre-treatment, and when consumed as spoilt fruits. It is therefore recommended that consumers of fruits should be properly informed of the impending dangers involved in eating fruits of which may taste palatable or nutritious.

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INTRODUCTION

Fruits constitute commercially and nutritionally important and indispensable food commodity. They play a vital role in human nutrition by supplying the necessary growth factors such as vitamins and essential minerals in human daily diet and that can help to keep a good and normal health. Fruits are widely distributed in nature. One of the limiting factors that influence the fruits economic value is the relatively short shelf-life period caused by pathogens attack. The high concentration of various sugars, minerals, vitamins and amino acids also provide a good platform for the successful growth and survival of various parasitic and saprophytic forms of micro-organisms (Fatima *et al.,* 2010).

Several micro-organisms such as acid tolerant bacteria and fungi (moulds, yeast) use them as a substrate for their growth. Yeasts form the main flora of fruits before processing because of acidic pH. On the basis of the nutrient, they are capable of supporting bacterial growth; of both the Gram negative and Gram-positive forms. Fruits are highly perishable and maintain an active metabolism during the storage phase. During postharvest period diseases can affect the quality of fruits. Postharvest deterioration of fruits may take place in any stages viz: storage, transit or trans-shipment, during handling processes required to move the crop from the grower to the whole sale dealer and to the retailer and finally to consumers. It is estimated that about 20-25% of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries (Sharma et al., 2009). In developing countries like Nigeria, post-harvest losses are often more severe due to inadequate storage and transportation facilities. In Port-Harcourt, southern part of Nigeria, there is always a high demand for fresh fruit product (juice or fruit salad). Fruits are nutritious commonly taken as Part of a diet or used as refreshment; they have great taste and have profound health benefits. It's commonly taken in as fruit juice or as fruit salad. They are however, not free of microbial loads consisting of bacteria and fungi. Several incidences of illnesses due to foodborne diseases associated with the consumption of fruits have been reported in several places (Suaad & Hamed, 2008). Fruits are spoiled not only by the degradation by micro-organisms but also through contamination of toxin-producing bacteria and fungi.

Botanically, fruit is the structure resulting from the growth of a flowering plant. The fleshy component which is normally the portion eaten, serves to protect and eventually nourish the seed(s) as part of the natural development of the original plant's progeny. The morphology of fruits varies substantially as does the plant tissue consumed. Fruit types dictate the mechanics and ease of juice extraction (Arthey & Ashurst, 1996). There are about nine categories of fruit/vegetable types (Kader, 1992).

The samples used were pineapple, orange, strawberry, mango and orange. Pineapple is a tropical plant with edible multiple fruit consisting of coalesced berries. It is classified under the Kingdom: Plantae, Order: *Poales*, Family: *Bromeliaceae*, Subfamily: *Bromelioideae*, Genus: *Ananas*, Species: *Ananas comos*.

commerce. Orange is the fruit is classified under the Kingdom: Plantae, Order: *Sapindales*, Family: *Rutaceae*, Genus: *Citrus*, Species: *Citrus sinensis*. Apples are scientifically classified under the Kingdom: Plantae, Order: *Rosales*, Family: *Rosaceae*, Genus: *Malus*, Species: *Malus pumila.* **M**ango is a juicy stone fruit and it is classified under the Kingdom: Plantae, Order: *Sapindales*, Family: *Anacardiaceae*, Genus: *Mangifera*, Species: *Mangifera indica* and it is native to southern Asia, especially Burma and eastern India. Strawberry fruit is not botanically a berry but an aggregate accessory fruit (meaning that the fleshy part is derived not from the plants ovaries but from the receptacle that holds the ovaries) and it is classified under the Kingdom: Plantae, Order: *Rosales*, Family: *Rosaceae*, Subfamily: *Rosoideae*, Genus: *Fragaria*, Species: *Fragaria xananassa*

Fruits are wonderful gifts of nature and are of tremendous benefit to mankind. They are valuable in diverse respects as they contain several nutritional elements and components such as minerals, vitamins, anti-oxidants etc. They are also having several health benefits because of their richness in minerals, antioxidants, vitamins and micronutrients (Kamala, & Rajagopal, 2018).

This study is justified by the several health hazards associated with fruits due to the microbial loads and as most consumers eat them without prior knowledge of the health implications which may be a serious threat to life and personal economy. The study intends to isolate, identify and carry out antimicrobial susceptibility tests on isolated microorganisms from the fruits.

MATERIALS AND METHODOLOGY

Five fruit samples (pineapple, orange, mango, apple, and strawberry) were purchased from Spar Shopping Mall, Port-Harcourt. The samples were transported to the laboratory that same day in sterilised polythene within one hour. The external part of the fruits were swabbed using sterile swab sticks and the internal part of the fruits were processed using electrical blender and poured in sterile container.

Microbiological Analysis was carried out using Sabouraud dextrose agar for the isolation of yeast and mould, while Nutrient agar was used as a multi-purposed medium for the examination of water and dairy products. Both culture media were prepared using manufacturers' instructions. Diluents were also prepared accordingly. All media, diluents and glassware were sterilized by autoclaving at 121^0C for 15minutes.

Preparation of samples for microbiological analysis and isolation of micro-organisms was carried out by swabbing the external parts of the fruits using sterile swab sticks. Then the sterile fruit juice containers were passed through flame to avoid contamination.

Prepared fruit juices were serially diluted by placing 1ml of the sample with 9ml of sterile saline in a test-tube. This was vortexed to form solution of 10^{-1} concentration. The stock was subjected to decimal dilution by using sterile pipettes to form 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. Then an aliquot of 0.1ml from 10^{-2}, and 10^{-3} dilutions were transferred using a 1ml sterile pipette onto the surface of a dried nutrient agar plate and 0.3ml aliquot from 10^{-2} and 10^{-3} dilution were transferred also using another sterile pipette onto the surface of a dried Sabouraud dextrose agar plates. The inoculum was spread evenly glass spreader sterilised by flaming. The plates were then inverted and incubated at 37^0C for 24hours for the Nutrient agar plates and 72- 96 hours for Sabouraud dextrose agar. After incubation, number of colonies for each plate was counted.

The colonial morphology of the different colonies were screened and identified based on the taxonomic schemed and descriptions by Bochanan and Gibbons (1974) for bacteria and Mislavec *et al*., (1992) for yeast and fungi

Isolation and preservation of colonies was done using different colonial morphology observed on each plate and sub-cultured onto dried nutrient agar plate for the bacteria colonies using sterile wire loop, and on the surface of dried sabouraud dextrose agar plate for the fungal colonies using sterile needles. The plates were then incubated at 37ºC for 24hours for bacteria and 48-72hours for fungi o obtain a pure culture of each different colony. Stock cultures were maintained for further studies.

Identification of colonies of fungi and bacteria was carried out. For fungal isolates, a drop of lacto-phenol blue dye was placed on a clean grease free slide with the aid of a sterile wire loop; each fungal isolate was teased in the lacto-phenol blue dye and covered with a cover slip. The preparation was then observed under the low magnifications of light microscope and then increased to higher powers for clear vision. Bacterial identification was carried out using 24-hour-old cultures and observed under microscope by gram staining performing various biochemical tests, and final identification was performed using ABIS online microbial identification system (Stoica & Sorescu, 2012).

Antibiotic susceptibility testing (AST) was carried out to determine which antibiotic will be most successful in treating a bacterial infection *in vivo*.

RESULTS

The results obtained from the microbiological analysis of the five fruits samples showed that bacteria and fungi were associated with them. The total bacterial and fungal isolated from the samples is given. The colonial characteristics of the isolates, their microscopic characteristics and their antimicrobial susceptibility are also given. The samples analysed were both from the external and internal part of the selected fruit purchased from the shopping mall, spar and transported to the laboratory in sterile polythene.

Total Bacterial and Fungal Counts

The total bacterial and fungal load for each product sample for both the external and internal part of the fruit is given below:

Table 4.2.1: total heterotrophic bacterial counts of internal part of the fruits

Replications	Pineapple	Orange	Mango	Strawberry	Apple
1	2.96×10^2	2.11×10^2	1.63×10^2	1.86×10^2	2.01×10^2
2	1.69×10^2	1.74×10^2	1.30×10^2	1.34×10^2	1.75×10^2
3	8.7×10	103×10^2	6.4×10	9.7×10	1.12×10^2
Total	5.52×10^2	4.88×10^2	3.57×10^2	4.17×10^2	4.88×10^2
Mean cfu/ml	1.84×10^2	162.67×10^5	1.19×10^2	1.39×10^2	162.67×10^5
Standard deviation	105.3×10^5	54.8×10^5	50.41×10^5	44.7×10^5	45.8×10^5

Table 4.2.2: Total heterotrophic fungal count of internal part of the fruits

Replication	**Pineapple**	**Orange**	**Mango**	**Strawberry**	**Apple**
1	53	32	41	43	45
2	32	20	29	37	19
3	16	17	23	10	16
Total	1.01×10^2	6.9×10	9.3×10	9.0×10	6.2×10
Mean cfu/ml	33.67×10^5	2.3×10	3.1×10	3.0×10	20.67×10^4
Standard deviation	18.56×10^5	7.94×10^5	9.17×10^5	17.58×10^6	22.34×10^5

Table 4.2.3: total heterotrophic bacterial count of external part of the fruits

Fruit	**value of bacterial count**
Pineapple	2.74×10^2
Orange	2.52×10^2
Strawberry	1.73×10^2
Mango	2.04×10^2
Apple	1.04×10^2

Table 4.2.4: showing total heterotrophic fungal count of external part of the fruits

Fruit	value of fungal count
Pineapple	5.3 x 10
Orange	4.0 x 10
Strawberry	5.5 x 10
Mango	2.7 x 10
Apple	2.5 x 10

IDENTIFICATION OF BACTERIAL ISOLATE

A total of thirteen isolates were finally gotten from both the external and internal part of the fruit samples. The microbiological tests were performed on the bacteria isolates which were obtained from the fruit samples. Based on the results of the gram stain and biochemical tests performed, morphological and biochemical properties of bacteria were explained in the tables below:

Table 4.3.1: morphological characteristics of bacterial isolate in internal part of fruit

Bacterial isolates	Fruits	Colour	Margin	Elevation	shape	Microscopic shape of isolate	Gram reaction
Klebsiella species	Pineapple	White	Undulate	Flat	irregular	Rods	+ve
Salmonella sp.	Pineapple, orange	Creamy	Undulate	Convex	Irregular	Rods	-ve
Bacillus cereus	Pineapple, apple, orange	Off-white	Entire	Convex	circular	Rods in chain	+ve
Staphylococcus aureus	Apple, orange	Yellow	Entire	Convex	circular	Cocci in chains	+ve
Lactobacillus sp.	Apple, strawberry	White	Entire	Convex	circular	Rods	+ve
Acetobacter sp.	Strawberry	Milky	Entire	Flat	circular	Rods	-ve
Escherichia coli	Mango, orange, pineapple, apple, strawberry	Creamy	Entire	Slightly raised	circular	Rods	-ve
Bacillus subtilis	Strawberry	White	Undulate	Flat	circular	Rods in chains	+ve
Agrobacterium tumefaciens	Mango	White	Entire	Convex	circular		-ve
Micrococcus sp.	Orange	Yellow	Entire	Convex	circular	Cocci singly	+ve
Streptococcus pyogenes	Apple, orange	Creamy	Entire	Convex	Circular	Cocci	+ve

Table 4.3.2: showing morphological characteristics of bacterial isolate of external part of the fruits

Bacterial isolate	Fruit	Colour	margin	Elevation	shape	Gram reaction	Microscopic shape of isolate
Staphylococcus aureus	Pineapple, orange, mango	Yellow	Entire	convex	Irregular	+ve	Cocci
Escherichia coli	Pineapple, orange, apple, strawberry	Creamy	Entire	Slightly raised	Cirrcular	-ve	Rods
Bacillus cereus	Pineapple, orange, strawberry	Off-white	Entire	convex	circular	+ve	Rods in chains
Micrococcus sp.	Pineapple, apple	Bright yellow	Entire	convex	circular	+ve	Cocci
Streptococcus pyogenes	Apple, mango	creamy	Entire	convex	circular	+ve	Cocci
Leuconostoc	Apple	Yellow	Entire	convex	circular	+ve	Cocci
Serratia sp.	Orange	creamy	Entire	convex	circular	-ve	Rods
Pseudomonas sp.	Strawberry	Cream	Entire	Flat	circular	-ve	Rods
Lactobacillus sp.	Strawberry, orange	White	Entire	convex	circular	+ve	Rods

4.5 Antibiotic Susceptibility and Zones Sizes of Inhibition

Bacteria	Sensitive	Resistance
Agrobacterium	Ampicillin(8mm) or rifampicin	
Streptoccoccus	Chloramphenicol or streptomycin	Rifampicin, gentamycin
Micrococcus	Gentamycin, chloramphenicol, erythromycin, lefloxacin	Ampiclox, rifampicin, ampicillin
Bacillus cereus	Amoxicillin, erythromycin, zinnat, norflaxacin, gentamycin, streptomycin	Ampiclox, rifamipicin
Staphylococcus aureus	Streptomycin, norfloxacin, gentamycin, erythromycin, ceftraxine	Ciprofloxacin, chloramphenicol, streptomycin, amoxicillin, rifampicin
Streptococcus pyogenes	Gentamicin, lefloxacin, erythromycin, chloramphenicol	Ampiclox, rifampicin, amplicillin

DISCUSSION

In this study 5 fruit samples were examined for microbiological analysis. Factors which determine the colonization of fruits by micro-organisms include $_p$H, redox potential, water activity, nutrients, structures, antimicrobial agents, temperature, relative humidity, and atmosphere

Fruits have high acidic content, nutrient content, redox potential, etc. these factors are an availability of components that are accessible by microbes. Their tentative identification showed the presence of pathogenic bacteria such as *Escherichia coli*, *Salmonella species*, *Bacillus cereus*, *Staphylococcus aureus*, *Micrococcus* and others in both the external and internal part of the fruit samples and they are considered a public health concern.

In table 4.2.1, it showed pineapple fruit in its liquid form contains more bacteria than every other fruit sample worked on, its total mean count was 1.8×10^2, higher than the other bacterial counts of the fruits sample. Strawberry fruit has a relatively low count of 1.39×10^2. Also in table 4.2.2, it also shows that pineapple yet again had the highest fungal count of 33.6×10^5 and orange had he lowest of 6.9 x 10. This result indicates that the pineapple used in the study have been infested largely by micro-organisms.

Bacillus cereus is an aerobic spore forming bacteria associated with food poisoning and its presence in almost all the samples may be attributed to its spore forming ability. Its immediate source is usually the plant equipment used, but it may also originate from handling stage.

It was seen that the bacterium *Escherichia coli* was isolated in all the fruit samples. *Escherichia coli* is a foodborne pathogen and it is found in the soil. The presence of this bacterium alongside *Salmonella* and *Staphylococcus aureus* are of primary concern because these pathogens were implicated in a number of outbreaks associated with fruits.

The dominant fungi recorded in fruits belong to *Penicillium specie*, *Cladosporium specie*, *Aspergillus niger*, and *Botrytis specie.* They produce mycelia mats and musty, stale off-flavours in fruits and fruit juices (Lawlor, *et al*., 2009). *Rhizopus* and Mucor are also associated with spoilage of fresh fruits (Renard, *et al*., 2008). Some produce mycotoxins which are of great threat to human health. Major mycotoxins associated with fruits and juices are byssochlamic acid, patulin (produced by *Aspergillus specie)*, citrinin (produced by *Penicillum specie*), and ochratoxin (produced by *Aspergillus specie*).

It is seen that some bacteria found in the external part of the fruit are not found in the internal part of the fruit, the micro-organisms may have been introduced during the course of the work or during the handling stage.

CONCLUSION

This work has established that the fungal and bacteria organisms that were isolated are responsible for seed and seedling rots and causes deterioration of seeds and it was to show the various micro-organisms that cause these diseases and to give an account of where they come from. Micro-organisms are naturally present on al fruits and can be brought in by wind, soil, water, animals, and humans. The presence of pathogenic micro-organisms in fruits is clearly an indication of foodborne outbreaks.

Fruits can become contaminated during their growth, harvesting and transportation, eventually leading to their spoilage. They can encounter bacterial and fungal infestation from the environment such as insect infestation, wound and presence of foreign matter such as sand, dust and debris among others. Some of the identified bacterial and fungal species could have come from any of those sources, constant exposure of these fruits to sales could have aided in deposition of the bacteria of fungal spores on them.

The high prevalence of some fungi demand that appropriate control measures against infection should be employed if farmers expect good performance of their produce. It is therefore necessary that the farmers and marketers should take necessary precaution in preventing the contamination of the produce to reduce their spoilage and the health risk their consumption pose to the consuming public. In

addition, spoilt fruit should be discarded and not consumed by humans.

It is recommended that early intervention measures during fruit harvesting through the use of good agricultural practices (GAP) will provide dramatic reductions in yield loss due to spoilage at all subsequent steps in the food-to-fork continuum and proper hygienic conditions should be maintained to produce good quality fruits and water should be used which will improve the microbiological quality of the fruits and make them acceptable to the quality conscious market world-wide.

Farmers who harvest the fruits and put into bags for transportation, the marketers and consumers should take necessary precaution in preventing contamination and also try to create an environment that will not encourage growth and multiplication of micro-organisms. This will however reduce the risk of mycotoxins associated with fungi contamination which are deleterious to human health.

To prevent food poisoning, contaminated fruits should be sorted and eliminated to avoid reinfection and washing of the fruits with clean water before consumption should be strongly encouraged.

And in case of food poisoning, the sensitive antibiotics can be used or contact a medical practitioner.

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