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Microbial contamination of some ready-to-eat vended fruits in Sango open-market, Saki, Oyo State, Nigeria

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ABSTRACT

Background: Fruits are widely exposed to microbial contamination through contact with soil, dust, water and handling at harvest, during or postharvest processing. It has been recognized that, they can be a source of food borne illnesses that can majorly result to poor hygiene practices and unsanitary conditions at fruit vending points. The main objective of the study was to assess the hygiene status as well as microbial contamination in fruit-vending-businesses in Saki; a Nigerian City. **Methods:** The microbial contamination of ready-to-eat vended fruits in Sango market was examined using standard microbiological methods. A total of eight (8) fruits samples comprising two from each of fresh apples (*Malus domestica*), cucumber (*Cucumis sativus*), orange (*Citrus sinensis*) and carrot (*Daucus carota*) of vended fruits were screened for total bacterial count. Identifications was made through their colony appearance, microscopic examination on nutrient Agar, selective and differential Agar (EMB, MacConkey, SSA, MRS, Biochemical tests (catalase, sugar,) and Gram staining of the samples. **Results:** Four (4) bacterial species were identified as: *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp* and *Shigella spp* from the vended fruit samples. The total aerobic plate count ranged from 0.8×10^4 - 0.4×10^4 CFU ml⁻¹ in apple with the highest count among carrot samples and the lowest among orange samples. **Conclusion:** The isolated organisms from the vended fruits showed that contamination mainly occurred due to poor hygiene and environmental factors.

Introduction

Fruits are an extraordinary dietary source of nutrients, micronutrients, vitamins and fibre for humans and are also vital for health and well-being. Well balanced diets, rich in fruits have been reported to help to prevent vitamin C and vitamin A deficiencies and to reduce the risk of several

diseases [1]. Fruits are widely exposed to microbial contamination through contact with soil, dust and water and also by mishandling during harvest or post-harvest processing. They therefore harbour a diverse range of microorganisms including pathogens [1]. Vended fruits are fruits that have been cut or sliced open and carried around by street

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vendors or hawkers at local markets or streets and such fruits are eaten without necessarily having to cut, peel or rinse them before consumption because they have already been prepared or packaged by the vendors [2, 3]. They are usually packaged in small polyethylene bags for sale. Over the last few years, there has been a significant increase in the consumption of vended fruits in Nigeria. This is because they are easily accessible, conveniently, and most importantly, they are cheaper than the whole fruits. Other reasons include modern lifestyle, industrialisation, economic downturn, materialism and lack of time to prepare proper meal [4]. The increased consumption coupled with the associated risk of disease to which consumers may be exposed, is a matter of great concern. Most times, it is difficult for one to attest to the hygiene of the processors or the sanitary conditions during preparation. This is worsened by the fact that vended fruits are done without adequate storage conditions, thereby, exposing the fruits to flies, dust and other pathogens [5]. These vended fruits such as watermelon, pineapple, carrots, cucumber, tiger nuts (also known as aki hausa) are sold by unlicensed vendors or local hawkers who have little or no knowledge on food hygiene. This therefore increases the risk of food-borne diseases caused by a wide range of pathogens such as bacteria (*Salmonella spp*, *Staphylococcus aureus*, *Enterobacteriaceae*), fungi, viruses and parasites [6]. These pathogens could invade these fruits during washing, peeling, slicing, trimming, packaging, handling and marketing [7]. The use of dirty utensils encourages rare visits of cockroaches, flies and rats [5]. Poor hygiene practices coupled with low standards of environmental and personal hygiene, improper handling of fruits, improper storage occur with street vended fruits raising health concerns such as food borne illnesses.

Street food vendors are usually unlicensed obstructing vehicle and pedestrian traffic [8]. This raises concern with respect to their potential for serious food poisoning outbreaks and exposure of the sliced fruits to flies, dust and other disease causing agents. Few studies have been done in this context and especially in determining the hygiene and safety of minimally processed ready-to-eat fruits.

The samples were collected from different fruit vendors in Sango Main Market, Saki, Oyo State. Sango Main Market, Saki, is one of the largest market in Oke-Ogun, Oyo State. With different people selling different items like foodstuffs, fruits,

vegetables, wears and other exciting goods. And most of them are sliced or processed because most of their customers may not be able to afford or have time to process the fruits properly.

Materials and Methods

Collection of samples

A total of fifteen samples of four (4) openly vended carrots, cucumber, apple and orange were purchased. Sliced orange were bought from four different fruit vendors while cucumber, apples and carrot were also purchased from four different fruit vendors. They were all collected and tagged into different white sterile polyethylene bags to differentiate them based on the vendors they were bought from.

Sterilization of used materials

Glassware such as test tubes, pipettes, conical flask, beaker, glass, slides, were sterilized and washed them thoroughly with detergent. Aluminium foil was used to cover the openings of glass wares and placed in the hot air oven at 160⁰c for 3 hours.

Media and stains used in isolation

The media used were Sabroud Dextrose Agar (SDA) (Lot no, 0000378929, Manufacturer ; Himedia laboratory Pvt, LTD), nutrient agar (Lot no.00337097, , Manufacturer ; Himedia laboratory Pvt, LTD), eosin methylene blue agar (EMB) (0000331508, Manufacturer ; Himedia laboratory Pvt, LTD) and MacConkey Agar (246245770 Manufacturer ;Himedia laboratory Pvt, LTD) while the stains include: crystal violet solution, safranin solution and Gram's iodine. Solvent used was ethanol solution and distilled water.

Media preparation and identification of isolates

The different media which included nutrient agar, SDA, MacConkey agar, Salmonella-Shigella Agar; and peptone water were prepared according to the manufacturer's instruction. Isolation of micro-organisms from the vended fruit samples: About 10g of each of the fruit samples were weighed and homogenised in 90ml of sterile distilled water using an electric blender. Then, ten-fold dilutions of the homogenates were made with sterilized peptone water; after that 1ml of the 10⁻⁴ dilutions of the homogenates were dispensed into the Petri-dishes that were labelled based on the agar used by pour plate method and allowed to solidify. The Petri-dishes that contained Mannitol Salt Agar, Nutrient Agar, MacConkey Agar and Salmonella-shigella Agar were incubated at 37°C for 24hours while the

Petri-dishes that contained SDA were incubated at 25°C for 3 days.

The nutrient agar, MacConkey agar, Mannitol salt agar and Salmonella-Shigella agar were used to check for total bacterial count, presence of *Staphylococcus aureus*, *Salmonella* and *Shigella spp* respectively.

At the end of the incubation period, the plates were brought out of the incubators and the colonies were counted using a colony counter device and each count was expressed in colony forming unit per ml (CFU ml⁻¹).

The distinct colonies on nutrient agar and SDA were carefully examined using microscope for their morphological characteristics like colour. Then these colonies were sub cultured on nutrient agar using streaking method and were incubated at 37°C for 24 hours.

Gram staining and other biochemical tests were carried out based on the method of Cheesbrough [9]. The biochemical tests performed here included catalase test and oxidase test.

Biochemical tests

Catalase test: The discrete colonies of each of the isolates were collected with a wooden stick and emulsified in a drop of hydrogen peroxide (H₂O₂). Bubbles of gas indicated a positive result according to [9].

Oxidase test: A piece of filter paper was placed in a clean Petri dish and 2-3 drops of freshly prepared oxidase reagent was added. With the aid of a wooden stick, discrete colonies of the isolates were collected separately and smeared on the filter paper. A positive result gave a purple-blue colouration after 10 seconds while a negative result gave no such colour after 10 seconds according to [9].

Gram staining: A thin smear of the isolates were made on different slides with the aid of a wire loop

and left to dry and after they were heat fixed and allowed to cool. Then the different smears were covered with crystal violet stain for 30-60 seconds and rapidly washed off with clean water. Then the smears were covered with Lugol's iodine for 30-60 seconds and rapidly washed off with clean water. The smears were decolourised rapidly with alcohol and washed out immediately with clean water. Then the smears were covered with safranin for 30-60 seconds and washed immediately with clean water. The stained smears were then allowed to air-dry. After drying, a few drops of oil immersion were dropped on the stained smears and viewed with the aid of a microscope (×100 oil objective lens) to check for the microscopic properties of the organisms like the Gram reaction, morphology [9].

Result

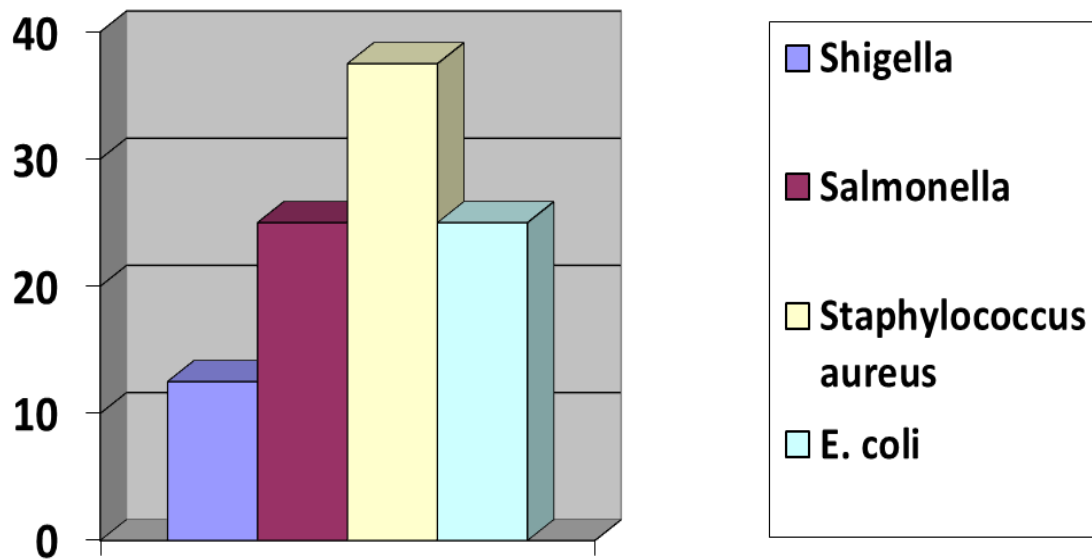
The results of the microbial contamination of the processed vended fruit samples collected from different fruit vendors in Sango Main market, Saki are presented in the samples in colony forming unit per ml (CFU ml⁻¹). It reveals that carrot and Apple has the highest average total aerobic plate count of 0.8 × 10⁴, 0.8 × 10⁴, while orange has the lowest, 0.4 × 10⁴ shows the result of the morphological and biochemical characteristics of the microbial isolates from the ready-to-eat vended fruit samples. It reveals that a total of six (4) micro-organisms were isolated. Out of these isolates, five were bacterial isolates namely: *Salmonella spp*, *Escherichia coli*, *Shigella spp* and *Staphylococcus aureus*. All the bacterial isolates are rod shaped and Gram negative except for *Staphylococcus aureus* that is cocci in shape and Gram positive. Moreover, all bacterial isolates are catalase positive.

Table 1. Average microbial load of ready-to-eat vended fruit samples (CFU ml⁻¹).

| Vended fruit samples | Vendors | Total Aerobic plate count (TAPC)(CFUml ⁻¹) |
|----------------------|----------------|--|
| Carrot | A ¹ | 0.8 x 10 ⁴ |
| | A ² | 0.6 x 10 ⁴ |
| Apple | B ¹ | 0.7 x 10 ⁴ |
| | B ² | 0.8 x 10 ⁴ |
| Orange | C ¹ | 0.4 x 10 ⁴ |
| | C ² | 0.6 x 10 ⁴ |
| Cucumber | D ¹ | 0.5 x 10 ⁴ |
| | D ² | 0.7 x 10 ⁴ |

Table 2. Morphological and biochemical characteristics of the microbial isolates from the ready- to-eat vended fruit samples.

| Vended fruit samples | Biochemical CA OX | Gram reaction +ve/_ve shape arrangement | Morphological characteristics | Suspected organisms |
|----------------------|----------------------|--|-------------------------------|-------------------------|
| Carrot | + + | -ve Rod Single | Pale-white/black | <i>Salmonella spp</i> |
| | + + | -ve Rod Single | Pink | <i>E. coli</i> |
| Orange | + + | -ve Rod Single | Yellow | <i>S. aureus</i> |
| | + + | -ve Rod Single | white/black | <i>Salmonella Spp</i> |
| Apple | + - | -ve Rod Single | Pink | <i>E. coli</i> |
| | + + | -ve Rod Single | Pale White | <i>Shigella species</i> |
| Cucumber | + + | +ve Cocci Single | Yellow | <i>S. aureus</i> |
| | + + | +ve Cocci Single | Yellow | <i>S. aureus</i> |

Figure 1. Frequency distribution (%) of the microbial isolates on the ready-to-eat vended fruit samples.

Discussion

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 increase the risk of food-borne diseases caused by a wide variety of pathogens, because it is difficult to attest to the hygiene of these vendors or to the sanitary conditions at points of processing as well as the packaging materials. These micro-organisms isolated were *Escherichia coli* (25.0%),

Salmonella spp (25.0%), *Staphylococcus aureus* (37.5%), and *Shigella spp* (12.5%). All the microbial isolates in this study apart from *Shigella spp* was reported in the work of [10] in Ilorin, Kwara State. Also, isolated *Staphylococcus aureus*, *Salmonella sp* and *Escherichia coli* from sliced pineapple and paw-paw. This is further supported by the work of [11] that isolated *Staphylococcus aureus*, *Pseudomonas spp*, *Salmonella spp* and *Escherichia coli* from ready-to-eat fruits sold in Otta, Ogun State. Also isolated *Staphylococcus aureus*, *Salmonella spp* and *Escherichia coli* from

street vended fruits juices in Amravati, India. Moreover, the result of this study is in consonance with the report of [12] from air contaminated vended foods sold in Lokoja, Kogi State, Nigeria. Most of the isolates in this study may have been introduced into these fruits through fecally polluted water used in washing utensils like knives, trays and polyethene bags used for the packaging of the fruits after slicing or cutting and also exposure of these fruits to low temperatures which encourage the microbial growth of these pathogens [8]. The presence of *Staphylococcus aureus*, *Salmonella spp* and *Escherichia coli* was in total agreement with the work of [10] from pre-cut fruits sold. *Staphylococcus aureus*, *Salmonella spp*, *Shigella spp*, and *Escherichia coli* are environmental isolates and they have been isolated from plants, human skin, animal and dairy products. Their presence in these ready-to-eat fruits may have been through contaminated hands of the vendors, contact with sewage and contaminated water [13]. This implies that, the fruit samples could serve as a vehicle in the transmission of these pathogens to the consumers of these contaminated fruits. The presence of *Staphylococcus aureus* may have been introduced into the ready-to-eat fruits through body contact of vendors with the fruits because the organism is a normal flora of the nasal passage, hands and skins of healthy individuals [10] reported *Staphylococcus aureus* to have the highest occurrence in fruits and foods respectively. It was recorded to be the second highest occurring isolate with the frequency of occurrence of 4(66.7%). It was reported [13] that the incidence of *Staphylococcus aureus* in food is an indication of environmental and human contamination. This high incidence may have occurred due to the use of polyethene bags for the packaging of these fruits after slicing or cutting them. Such high occurrence may have occurred as a result of the exposure of these ready-to-eat fruits to dusty or muddy areas. Most of these fruit vendors stay near stagnant water of gutters which may serve as an entry for fruit contamination [14] reported that the microbial load on leafy vegetables and fruits increase with time during storage. When these fruits are stored at in appropriate temperatures, they tend to attain temperatures that are suitable for the microbial growth of these pathogens to cause diseases when ingested. The results show that *Escherichia coli* had the highest frequency of occurrence of 5(83.3%) and it conforms to the report by [8, 15] in sliced fresh fruits sold in Minna & Bida

metropolis respectively. *Escherichia coli* is regarded as primary indicator for microbiological quality of food and water and this shows that these fruits are not safe for human consumption. According to [16], the main transmission of *Escherichia coli* was through faecal contaminated food or water. The high occurrence may have occurred in the contact of contaminated water with the fruits during washing of the fruits and also the inadequate washing of hands by the fruit vendors. Some of these fruit vendors get their water from unclean sources like dirty streams and also they could use very little quantity of water to wash or rinse all the fruits. The low occurrence of *Shigella spp* was also reported by [12] in the assessment of air contaminated vended foods sold in Lokoja, Kogi state. These ready-to-eat fruits may get contaminated from knives used for cutting or slicing, improper human handling and processing, tables or trays used during peeling and cutting, rinsed water, washing buckets and packaging materials as these fruits are cut, washed, wrapped with transparent polyethene bags and sold to the consumers. The presence of these possible pathogens in the analysed fruit samples should be of great importance to the vendors, consumers and concerned arms of government.

Although the amount of samples studied was little and sample size varied among vendors thanks to inconvenience of some turn out, we have a tendency to believe this study provides a general summary of the microbiological quality of fresh fruits and vegetables sold in Niger-Congo market, Saki, Oyo State, Nigeria.

Recommendation

Most of the fruits get contaminated from poor handling by the vendors; more awareness should be created to educate both the vendors and the consumers on personal hygiene. Regular hand washing doctrine should be enforced by sanitary officers on food vendors.

Conflict of interest: None.

Financial disclosure: None.

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