



## **Bacteria Colonization of Fresh Minimally Processed Fruits and Vegetables from Markets in Nsukka, Southeastern Nigeria**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Consumers' demand for minimally processed fresh minimally fruits and vegetables owing to their reputation of being convenient, fresh, nutritive, healthy, and cheap has been on the increase over the years. Contamination of these commodities during processing has been reported and vendors often ignore proper hygienic processes. However, since fresh produce is often consumed raw, these products could harbor potentially pathogenic bacteria. In this study, 15 randomly selected samples of fresh, minimally processed watermelon, cucumber and garden egg were collected from markets in southeastern Nigeria and evaluated by way of standard plate techniques of serial dilution for bacterial contaminants. Following standard bacteriological practices, dilutions were plated on suitable growth media and incubated for 48 h. Pure cultures of bacterial isolates were investigated for total viable counts and identified both macroscopically and microscopically via Gram staining technique, spore staining technique, motility test and biochemical analysis. Fifty-eight isolates were obtained and the total viable plate count from all samples ranged from  $1.0 \times 10^6$ - $8.0 \times 10^6$  CFU/g with

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watermelon samples recording the highest volume of bacteria loads. The cultural and biochemical characterization revealed the presence of seventeen (17) probable species of bacteria: *Staphylococcus aureus* (40%), *Bacillus* sp.(21%), *Escherichia coli* (18%), *Staphylococcus epidermidis*, *Corynebacterium* sp., and *Citrobacter* sp. (17%), *Lactobacillus* sp., and *Proteus* sp.(12%), *Yersinia* sp., *Serratia marcescens*, *Listeria* sp. and *Pseudomonas* sp.(6%), *Klebsiella* sp., *Streptococcus* sp., *Bacillus cereus* and *Clostridium* sp. (5%), and *Salmonella* sp. (2%). Probable pathogenic bacteria exceeded the standard limit thus requiring urgent public sensitization and education by appropriate regulatory agencies. Therefore, the consumption of these minimally processed fruits and vegetables could lead to foodborne infections.

**Keywords:** *Bacterial contaminants; sanitary measures; vendors; foodborne diseases; natural products.*

## 1. INTRODUCTION

In recent years, there has been an increased demand for fresh produce because they are an essential part of healthy diets globally. Minimally processed fruits and vegetables are fresh produce that are fresh-cut, vended, partially-processed or ready-to-eat food items devoid of any form of additives and require little or no further processing before consumption [1]. They are indispensable food items due to their high nutritive value [2]. They could be processed traditionally or industrially and this minimal processing technique does not influence or alter the nutritional quality and sensory characteristics of such foods [3]. Because these food products are readily available and are usually consumed raw, safety is of great importance [4]. Their surfaces may have a micro-biota of microorganism population. Merits of minimal processing of fruits and vegetables include convenience due to its easy and quick preparation, less severe processing methods, ability to retain quality and freshness of products, and maintenance of products' nutritive and sensory attributes [5]. According to World Health Organization, approximately 1.7 million (2.8%) deaths worldwide are attributable to low fruit and vegetable consumption and it is among the top 10 selected risk factors for global mortality including about 14% of gastrointestinal cancer deaths, 11% of ischaemic heart disease deaths and 9% of stroke deaths (WHO, 2018). Despite the increasing demand for such commodities, they are produced, sold, and consumed with little or no sanitary measures in developing nations [6]. This has led to microbial contamination of such products which may result in foodborne illnesses and a serious public health issue [7]. Several studies in developed and developing countries have reported large microbial contamination of minimally processed fruits and vegetables [6,8,9,10,11,12,13]. The introduction

of microbial populations (bacteria, fungi, viruses, protozoa) via poor hygienic practices, traditional processing methods, and inappropriate holding temperatures leads to product contamination.

Many pathogenic and non-pathogenic microbes have been associated with fresh produce and since they are often consumed raw, they are an important means by which various commensal bacteria are introduced into the GIT [14,15]. The human pathogenic microbes associated with fresh produce mostly comprise of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* sp., *Listeria monocytogenes*, *Klebsiella* sp., some *Bacillus* sp., *Clostridium* sp., and *Serratia* sp., viruses e.t.c. [16].

Foods associated with foodborne illnesses worldwide are categorized into raw foods of animal origin including raw or undercooked meat and poultry, raw eggs, unpasteurized milk, raw or undercooked fish, shellfish, or other seafood; fruits and vegetables. Researchers have reported bacteria as the largest pathogen found on fresh fruits and vegetables thus the interest in bacterial pathogens of concern [17,18,19]. Available reports on bacterial contamination of minimally processed fruits and vegetables in Nigeria [6,20,21,22] are not comprehensive with respect to sampling coverage as the studies were conducted in few states compared to other studies conducted in developed countries [23] where multi-sites were covered in various states of the country. Also, among the 20 globally studied minimally processed fruits and vegetables, the bacterial colonization and biofilm formation of minimally processed watermelon, garden egg and cucumber have not been studied despite their high rate of consumption among dwellers of the study area. In the study area, where minimally processed fruits and vegetables are commonly sold in open markets with no regulation from

appropriate authorities, microbial contamination is a major concern and so routine microbiological surveillance is required. Thus, this study was designed to: (a) determine the diversity of bacteria in the fruits and vegetable samples from various markets (b) to investigate the microbiological quality of fresh minimally processed fruits and vegetables in Nsukka, Southeastern Nigeria. The data generated from this study will contribute to establishing food safety policies that target the minimally processed fruits and vegetable value chain to safeguard public health.

## 2. MATERIALS AND METHODS

### 2.1 Study Area and Site Justification

This study was carried out in Nsukka, Southeastern Nigeria. This study was carried out in Nsukka because similar studies have been conducted in other few states of the country but non of such studies have been conducted in Nsukka. The city is located in a tropical rain forest, with an average annual rainfall of about 2,000 milliliters with heavy rainfall during the rainy season and has a mean daily temperature of 26.7°C.

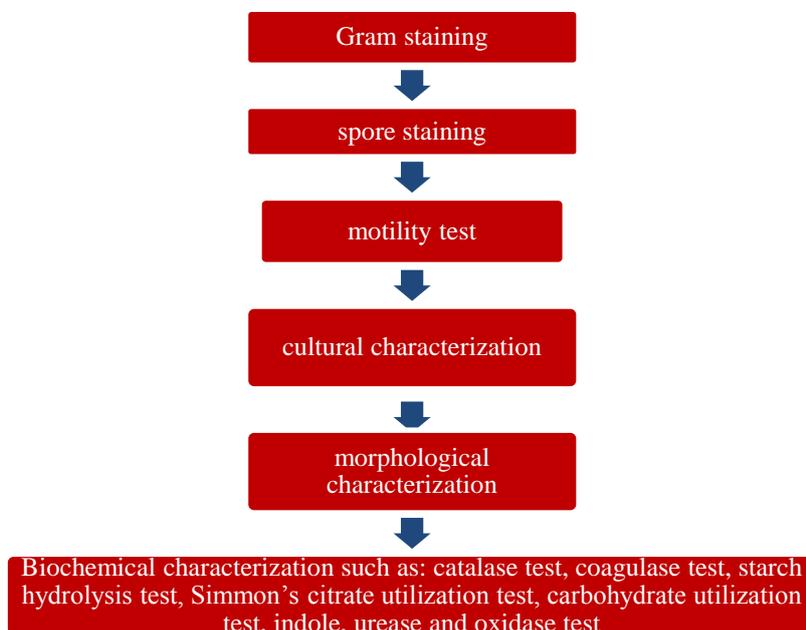
### 2.2 Collection and Transportation of Samples

A total of fifteen (15) samples, three each of fresh minimally processed fruit and vegetable type namely: watermelon (*Citrullus*

*lanatus* Thunb.), garden egg (*Solanum melongena* L.), and cucumber (*Cucumis sativus* L.) were collected in sterile polythene bags from each of the markets sampled (Ogige market, Ikpa market, OrieOrba market, Orielgboeze market, and Eke-Edeoballa market) within Nsukka metropolis. We decided to sample fruits and vegetables from these five markets because these are the largest markets in Nsukka where varieties of foodstuffs, fruits, vegetables, wears and other exciting goods are obtainable. Greater number of traders in these markets is fruits and vegetable sellers and most of these fruits and vegetables are minimally processed because most of their customers may not be able to afford the products as whole or have time to process the fruits properly. Each sample was transported in cold chain to the pharmaceutical microbiology and biotechnology laboratory for processing. The samples not worked on were kept in the refrigerator at 4°C for a maximum of 24 hrs.

### 2.3 Preparation of Culture Media and Sterile Normal Saline

The culture media for cultivation and isolation were prepared following standard microbiological practice in batches according to the manufacturer's specifications for a total of fifteen (15) samples [24]. Sterile normal saline was appropriately prepared aseptically according to standard specifications for serial dilution.



## 2.4 Cultivation and Isolation of Microorganisms from Minimally Processed Fruits and Vegetable

The modified method of Dashwood et al. [25] and Balali et al. [26] were adopted using the pour plate method. Surfaces of each sample were sliced using a sterile blade, chopped in small sections, then 1 g of each sample was rinsed with 10 ml of distilled water to form an enrichment stock. Ten- fold serial dilution was carried out by distributing 9 ml of sterile normal saline into thirty (30) sterile test tubes arranged in rows of ten (10) and columns of three (3) and labeled appropriately.

## 2.5 Isolation of Microbes from the Diluted Solutions

Using pipette, 1 ml of each diluted solution from tubes 1-10 were transferred into 19 ml of already prepared, sterilized, and cooled molten nutrient agar, MacConkey agar, eosin methylene blue agar, and mannitol salt agar respectively in Mac Cartney bottles, mixed thoroughly and aseptically poured into different sterile petri dishes. The content of the petri dishes was mixed thoroughly by rotating the plate gently three times in a circular movement, anti-clockwise, another three times clock-wise and then three times vertically and horizontally to ensure homogenous distribution of cells. All plates for nutrient agar, MacConkey agar, eosin methylene blue agar and mannitol salt agar were allowed to solidify before they were incubated in an inverted position after labeling each plate and their dilutions for 24 - 48 h at 37 °C. After incubation, all thirty (30) plates each for nutrient agar, MacConkey agar, eosin methylene blue agar and mannitol salt agar were examined carefully for the presence of characteristic colonies of microbial growth. The procedure above was carried out in other samples of minimally processed fruits and vegetables until all fifteen (15) samples were exhausted. This was carried out in triplicate for each sample. The colonies were counted using the digital colony counter and the viable count/ml of each plate was calculated using the different dilution factors.

## 2.6 Purification and Storing of Isolate

Discrete colonies were picked by streaking 0.1ml of each inoculum carefully and evenly with the aid of a sterile wire loop in an aseptic condition over the entire surfaces of freshly prepared

media and were incubated at 37 °C for 24 h for bacteria isolation. The plates were sub-cultured and maintained on nutrient agar slants. Each bacteria isolate was prepared as suspension by aseptically inoculating single colonies into 2 ml of sterile nutrient broth to form a bacteria suspension prior to their identification on the basis of morphological, cultural and biochemical tests.

## 2.7 Identification of Isolates and Biochemical Characterization

Bergey's Manual for Determinative Bacteriology was used to confirm bacteria identities. Data generated were presented using simple descriptive and inferential methods such as frequencies and percentages, pie chart figures and bar charts figures.

## 3. RESULTS AND DISCUSSION

In a descending order of numbering (Fig. 1), water melon sample from Eke Ede-Oballa market (WE) had the highest number of isolated bacteria, followed by water melon sample from Orie-Orba (WOR) and Orie Igbo-eze (WIG) both having the same number of isolates (5). Samples W1, WO, GI, GO, GOR, GIG, and GE had same number of isolates (4) while COR, CIG, and CE had same number of isolates (3). The least number of isolated bacteria were from CI, and CO each having 2 bacteria isolate. Generally, water melon samples collected from all markets had greatest number of isolates (WI, WO, WOR, WIG, and WE) and cucumber samples from all markets had the least number of isolates. However, this implies that water melon samples collected from different markets showed highest level of contaminants, while cucumber samples had the lowest level of contaminants (Fig. 1). The results of total viable plate count from all samples in Table 1 ranged from  $1.0 \times 10^6$  -  $8.0 \times 10^6$  CFU/g with water melon still having the highest viable bacterial count ( $8.0 \times 10^6$  CFU/g) from all markets and cucumber having the lowest count ( $1.0 \times 10^6$  CFU/g). The total viable plate count is an indication of the microbiological quality of any food product. According to WHO and Food and Agricultural Organization, standard values for microbial limits should not exceed  $10^5$  cells/ml for total aerobic bacteria,  $10^3$  cells/ml for enteric bacteria and salmonella and *E.coli* should totally be absent, but unfortunately, the total viable count of bacteria from all samples of all markets exceeded the standard limit and also harboured

salmonella and *E.coli* [27,28]. The presence of these bacteria in numbers exceeding the recommended microbiological standard is an indication of product unwholesomeness reported to be associated with foodborne illnesses.

Watermelon samples had the highest bacteria count. A study by Olamide et al. [29] on microbiological analysis of street - vended packaged sliced fresh fruits (watermelon and pineapple) in Apata, Ibadan, Oyo State, Nigeria revealed a higher total viable bacterial count for watermelon samples. Another study by Okechukwuet al. [30] on microbial contamination of ready-to-eat vended fruits in Abakpa main market, Abakaliki, Ebonyi State, Nigeria, cucumber was observed to have the lowest viable bacterial count compared with all other vended fruit sampled. From these studies, cucumber had the lowest number of bacterial colonies showing that it is least contaminated of all fruit and vegetable sample, while water melon samples were the most contaminated sample as with other related studies. Samples from Orié Orba market had the highest bacterial count whereas those from Ogige market had the lowest count. This could be attributed to the fact that Orié Orba market is a rural market where the level of literacy as to how these commodities are handled is very low compared to Ogige market which is an urban market. It implies that minimally processed watermelon is more exposed to microbial contamination. This can also be validated from the bar chart in Fig. 1 showing water melon as the highest contaminated sample. One probable reason for watermelon having the highest bacterial count is handling by vendors as they are sliced, high water content of watermelon which encourages bacterial proliferation and hence the observed higher microbial levels. This clinically implies that consumers of minimally processed water melon are more exposed to bacterial infection compared to consumers of garden egg and cucumber.

Figs. 2 and 3 show the microscopic views of bacteria isolates from some fruit and vegetable samples. Similar other studies have also observed that Gram-positive bacteria when viewed properly under the microscope are purple while Gram-negative bacteria are red (Figs. 2 and 3) [31]. Percentage distribution of the organisms according to their Gram reaction and cell morphology (Fig. 4) shows that 45 % of all isolates were Gram-positive cocci (highest number of isolates), followed by Gram-positive

rods, with 29 % and Gram-negative short rods, with 26 %. From the study, Gram-positive genera were most abundant microbes from all samples. A study conducted by Ankita et al. [32] on microbial contamination of raw fruits and vegetables in India revealed Gram-positive genera to be dominantly present on outer surfaces of minimally processed fruits and vegetables studied. This present study also identified Gram-positive genera as the dominating genera on surfaces of minimally processed fruits and vegetables. Table 2, the spore staining test of Grampositive rods revealed a total of twelve (12) isolates as spore formers and six isolates as non-spore formers. The positive spore staining bacteria suggests *Bacillus* sp. or *Clostridium* sp while non-spore formers are suggestive of *Corynebacterium* spp., *Listeria* spp or *Lactobacillus* spp. The terminal shaped singly dispersed spores are suggestive of *Clostridium* spp. They were further grown on thioglycolate broth to confirm their anaerobic nature. Table 3, motility test conducted to differentiate non-spore formers revealed that all non-spore formers with the exception of cucumber sample from Orié Igbo-eze (CIG<sub>2</sub>) gave a negative motility test. This further confirms the identity of each non-spore forming bacteria.

Cultural and biochemical characterization of isolated bacteria from all minimally processed fruits and vegetables revealed the presence of seventeen (17) species of bacteria (Table 4) namely: *Yersinia* sp.(n=1), *Serratia marcescens* (n=1), *Listeria* (n=1), *Salmonella* sp. (n=1), *Pseudomonas* sp.(n=1), *Klebsiella* sp. (n=2), *Bacillus cereus* (n=2), *Clostridium* sp.(n=2), *Streptococcus* sp. (n=2), *Lactobacillus* (n=2), *Proteus* sp. (n=2), *Citrobacter* (n=3), *E. coli* (n=3), *Corynebacterium* sp. (n=3), Other *Staphylococcus* sp. such as *Staphylococcus epidermidis* (n=7), *Bacillus subtilis* (n=8), and *Staphylococcus aureus* (n=17). Their percentage occurrence as shown in Figs. 5 and 6 revealed *Staphylococcus aureus* (40%) with the highest occurrence followed by *Bacillus* sp. (21%), *Escherichia coli* (18%), *Staphylococcus epidermidis*, *Corynebacterium* sp., and *Citrobacter* sp. (17%), *Lactobacillus* sp. and *Proteus* sp. (12%), *Yersinia* sp., *Serratia marcescens*, *Listeria* sp., and *Pseudomonas* sp.(6%), *Klebsiella* sp., *Streptococcus* sp., *Bacillus cereus* and *Clostridium* sp.,(5%), and *Salmonella* sp.(2%) was the least in occurrence.

Mbata et al. [33] conducted a study on bacteriological status of water melon sold in mile

III market Port-Harcourt, the study revealed that among all bacteria strains isolated from the sliced water melon, *Staphylococcus aureus* had the highest percentage (41.4-45.8%). Aguoru et al. [24] conducted a study in Nigeria on bacteria contaminants on surfaces of some edible fruits sold in makurdi metropolis, Benue State. Several bacteria genus were isolated from samples of vegetables and fruits including *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus* sp., *Salmonella* sp., *Lactobacillus* sp., *Proteus* sp., *Klebsiella* sp., and *Pseudomonas aeruginosa*. Again, the percentage occurrence of *Staphylococcus aureus* was the highest (45%) in the above study. Therefore, various studies carried out on minimally processed fruits and vegetables indicated *Staphylococcus aureus* as the most abundant bacterial strain, followed by *Bacillus* sp. and *Salmonella* sp. having the least abundance from all samples. The result of this study is in tandem with studies on minimally processed fruits and vegetables where *Staphylococcus aureus* is usually the most abundant bacterial strain, followed by *Bacillus* sp. *Salmonella* sp. had the least abundance from all samples.

According to Brackett [34], all types of food and food products have the potential to harbour pathogens including *Shigella* sp., *Salmonella* sp., enterotoxigenic and enterohemorrhagic *Escherichia coli*, *Campylobacter* sp., *Listeria monocytogenes*, *Yersinia enterocolitica*, *Bacillus cereus*, *Clostridium botulinum*, viruses and parasites such as *Giardia lamblia*, *Cyclospora cayatanensis*, and *Cryptosporidium parvum*, all of which are of global health concern. Ankita et al. also isolated various bacteria genera from raw fruits and vegetables in India which included *Bacillus* (14), *Lactobacillus* (8), *Corynebacterium* (35), *Streptococcus* (25), *Staphylococcus* (25), *Micrococcus* (6), *Pseudomonas* (5), and *Enterobacteriaceae* (7) [32]. According to Oliveira et al., organisms such as *Pseudomonas* sp., *Bacillus* sp., *Lactobacillus*, *Corynebacterium* sp., *Streptococcus* sp., *Staphylococcus* sp., *Micrococcus* sp., *Listeria* sp., *E. coli*, *Salmonella* sp., *Yersinia* sp., and *Citrobacter* sp., are bacteria colonies that can be isolated from fresh cut fruits and vegetables [35]. Another study conducted in India that examined different vegetables such as carrots, radishes, tomatoes, lettuce, cabbage, cucumbers, and coriander reported presence of *Staphylococcus aureus*, *E. coli*, *Enterobacter* sp., *Klebsiella* sp., *S. typhi*, *Serratia* sp., *Providencia* sp. and *P. aeruginosa* [36].

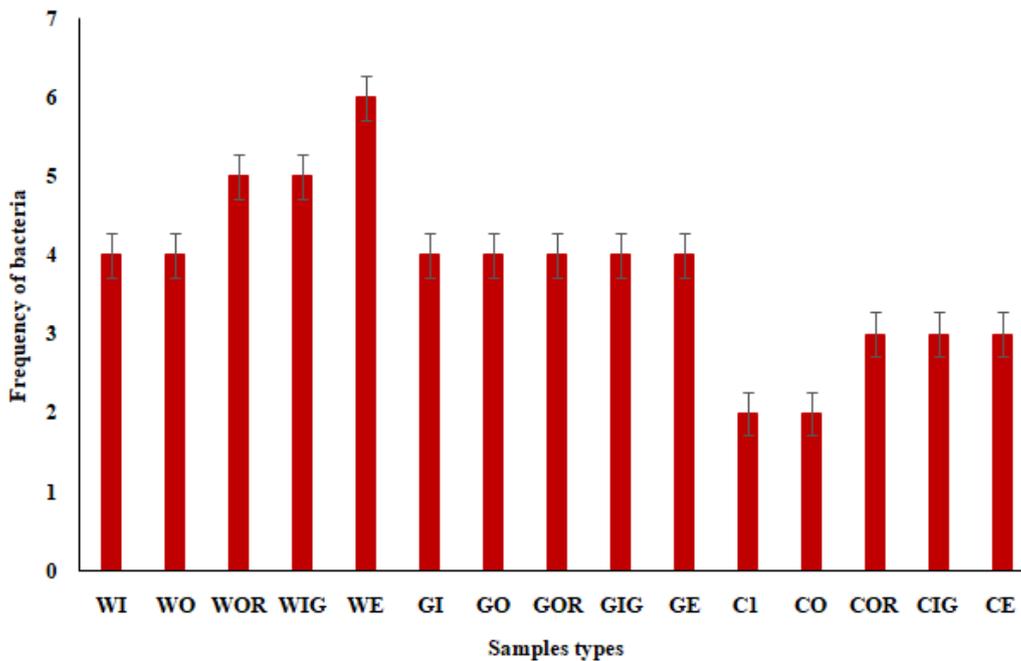
Ehimemen et al. carried out a study on the prevalence of bacterial loads on some fruits and vegetables sold in Kaduna central market, northwestern Nigeria. The result revealed a total of six bacterial species including *Staphylococcus aureus*, *Streptococcus* sp., *Enterobacter* sp., *Escherichia coli*, *Citrobacter* sp., and *Klebsiella* sp. *Staphylococcus aureus* was the most abundant bacterial strain while *Streptococcus* sp. were the least from the isolates [37]. Another study carried out in different markets of Enugu metropolis, Southeastern Nigeria on the microbial quality of ready-to-eat fruit samples (pineapple, watermelon, pawpaw, and cucumber) by Ugwu et al. revealed the presence of bacterial and fungal species including *Shigella* sp., *Staphylococcus aureus*, *Klebsiella* sp., *E. coli*, *Salmonella* sp., *Candida* sp., and *Aspergillus* sp. *Staphylococcus aureus* (70%) had the highest occurrence followed by *E. coli* (62.5%), *Salmonella* sp. (50%), *Klebsiella* sp. (40%), *Shigella* sp. (37.5%), *Candida* sp. (37.5%), and *Aspergillus* sp. (17.5%) [12]. The presence of these bacterial species in fruit and vegetable samples are of public health concern. It has also been revealed that majority of these microbes cause nosocomial infections [37]. The above studies conform to the findings of this present study.

Therefore, the presence of the above probable organisms can be confirmed to be isolates of fresh minimally processed fruits and vegetables as previous studies above have indicated. These microbes have been identified by numerous researches as causes of foodborne diseases [24]. Qadri et al. identified *E. coli* O157:H7, *Salmonella* sp. and *Listeria monocytogenes* as food borne pathogens often present on the surface of fresh produce and may cause public health problem [38]. Sim et al. reported many cases of salmonellosis due to consumption of fresh-cut fruits [39]. It has been reported that some of these identified bacteria have the ability to produce toxins causing a wide variety of infection. An example is the *Staphylococcus aureus* which had the highest percentage occurrence of 40%. This implies a serious health concern because *Staphylococcus aureus* has been clinically implicated in food poisoning, boils, impetigo e.t.c. The United State Food and Drug Administration reported that its presence or its enterotoxins in processed foods or on food processing equipment is generally an indication of poor sanitation and the agency emphasized that *S. aureus* including other species of *Staphylococcus* have been identified as the

causative agent in many cases of food poisoning/infection outbreaks globally [40]. Globally, minimally processed fruits and vegetables are often contaminated with enterotoxigenic strains of this bacterium resulting in public health burden.

The presence of Bacillus and Clostridium species are indications of serious health concerns because they have the ability to form spores. Endospores of their members are able to contaminate fruits and vegetables especially during processing and packaging under conditions for spore germination [41]. Genera of Bacillus have been associated with foodborne disease outbreaks as they are widely found in the soil where these food products are cultivated as well as on the plants (fruits and vegetables). *B. cereus* has been associated with diarrheal-type food poisoning on the consumption of contaminated fruits and vegetables. The Clostridium specie, *C. perfringens* has also been greatly associated with food poisoning [42].

The presence of *Escherichia coli* in the minimally processed samples analyzed is indicative of faecal contamination of such samples. As part of the normal flora of the human intestines, *Escherichia coli* has been linked to urinary tract infection as it has been identified as a urinary tract pathogen [24]. *Klebsiellasp.*, a ubiquitous opportunistic pathogen has been cultured from many sources such as soil, water, raw fruits and vegetables. The presence of *Klebsiellasp.* as an opportunistic pathogen has been identified to inhabit the upper respiratory tract and could cause diseases associated with food. *Salmonella* sp., a non-lactose fermenter has been isolated from raw fruits and vegetables in many countries of the world. Generally, contamination with these organisms could arise from washing fruits with contaminated water or poor handling of fruits by vendors. Clinically, the presence of these organisms poses a public health challenge as majority of them are associated with foodborne diseases.



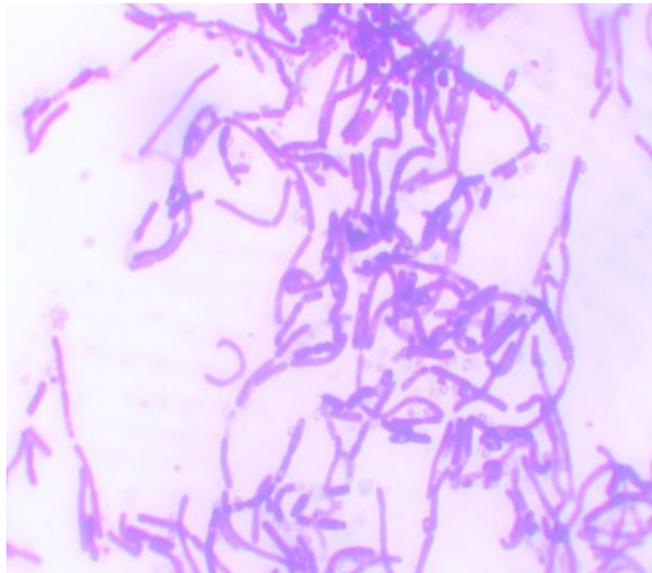
**Fig. 1. Frequency of isolates from each sample type**

Key: WI: Water melon sample from Ikpa market, GI: Garden egg sample from Ikpa market, CI: Cucumber sample from Ikpa market, WO: Water melon sample from Ogige market, GO: Garden egg sample from Ogige market, CO: Cucumber sample from Ogige market, WOR: Water melon sample from Orië-Orba market, GOR: Garden egg sample from Orië-Orba market, COR: Cucumber sample from Orië-Orba market, WIG: Water melon sample from Orië Igbo-eze market, GIG: Garden egg sample from Orië Igbo-eze market, CIG: Cucumber sample from Orië Igbo-eze market, WE: Water melon sample from Eke Ede-Oballa market, GE: Garden egg sample from Eke Ede-Oballa market, CE: Cucumber sample from Eke Ede-Oballa market

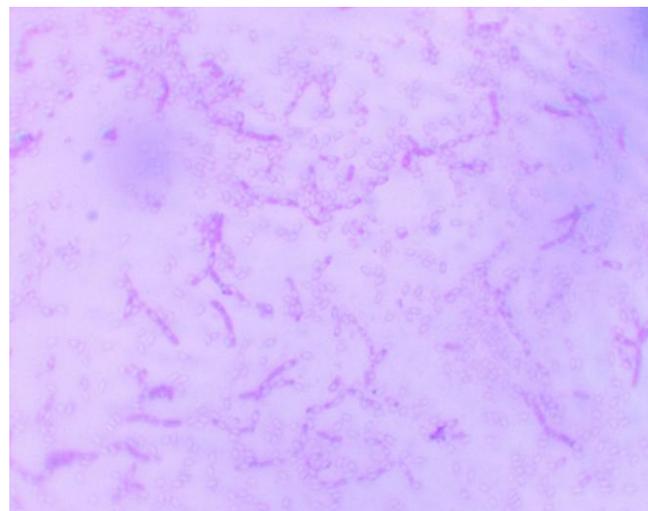
**Table 1. Total viable counts of microbial contaminants in minimally processed fruits and vegetables from various sampling location**

S/No	Sampling location	Microbial Level (cfu/g)		
		Cucumber (C)	Water melon (W)	Garden egg (G)
1.	Ikpa (I)	1.4 X 10 <sup>6</sup>	5.5 X 10 <sup>6</sup>	1.6 X 10 <sup>6</sup>
2.	Ogige (O)	1.0 X 10 <sup>6</sup> *	3.4 X 10 <sup>6</sup>	1.7 X 10 <sup>6</sup>
3.	OrieOrba (OR)	3.5 X 10 <sup>6</sup>	8.0 X 10 <sup>6</sup> *	7.6 X 10 <sup>6</sup>
4.	Orie Igbo-Eze (IG)	2.3 X 10 <sup>6</sup>	3.4 X 10 <sup>6</sup>	2.5 X 10 <sup>6</sup>
5.	Ede-oballa (E)	1.5 X 10 <sup>6</sup>	7.7 X 10 <sup>6</sup>	3.0 X 10 <sup>6</sup>

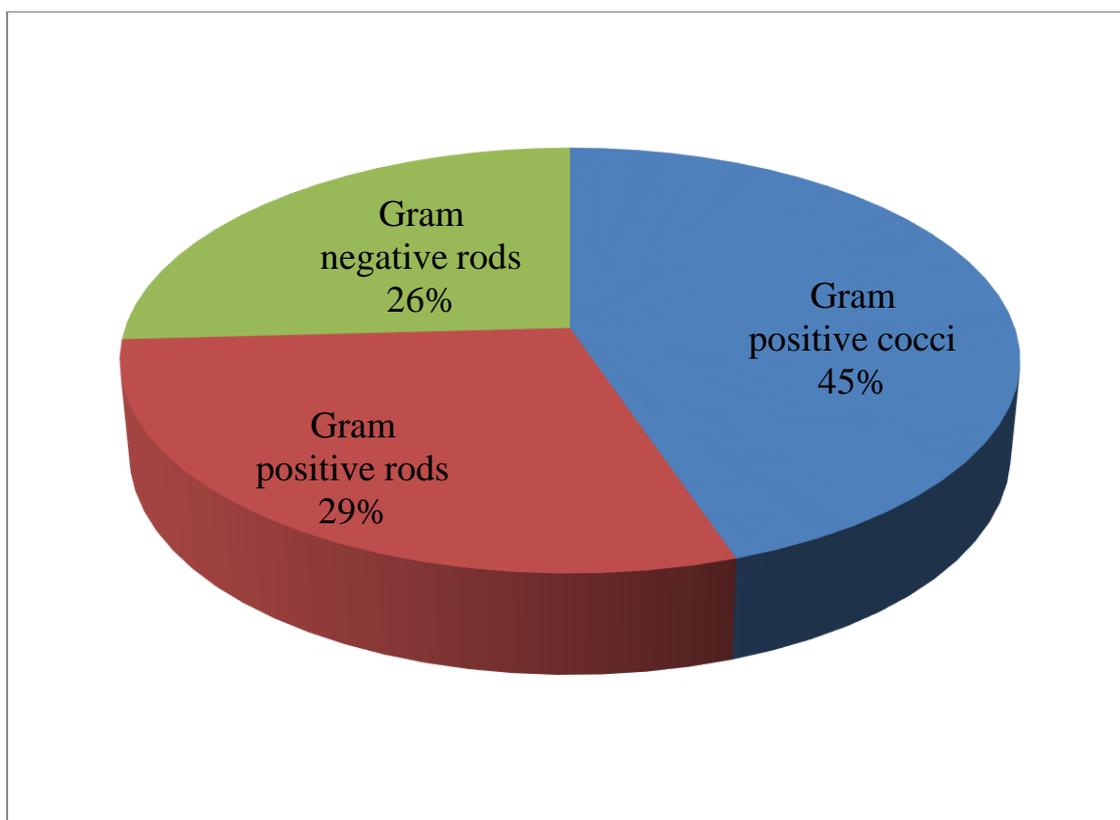
Key: CFU= colony forming unit



**Fig. 2. Microscopic view of W1<sub>3</sub> as Gram positive rod**



**Fig. 3. Microscopic view of W1<sub>4</sub> as Gram positive cocci**



**Fig. 4. Percentage distribution of the bacteria according to their Gram reaction and cell morphology**

**Table 2. Spore formers and non-spore formers with their different arrangements**

S/No	Gram positive bacilli/ rods	Spore forming ability	Arrangements
1.	WI <sub>3</sub>	Spore former	Singly dispersed
2.	CI <sub>2</sub>	Non-spore former	Singly dispersed
3.	WO <sub>2</sub>	Spore former	Singly dispersed
4.	WO <sub>4</sub>	Spore former	Singly dispersed
5.	WOR <sub>1</sub>	Spore former	Singly dispersed
6.	WOR <sub>2</sub>	Spore former	Terminal shaped singly dispersed spores
7.	GOR <sub>1</sub>	Spore former	Singly dispersed
8.	WIG <sub>4</sub>	Non-spore former	Singly dispersed
9.	GIG <sub>1</sub>	spore former	Singly dispersed
10.	GIG <sub>2</sub>	Spore former	Singly dispersed
11.	GIG <sub>4</sub>	Spore former	Singly dispersed
12.	CIG <sub>1</sub>	Spore former	Terminal shaped singly dispersed spores
13.	CIG <sub>2</sub>	Non-spore former	Singly dispersed
14.	WE <sub>4</sub>	Spore former	Singly dispersed
15.	GE <sub>2</sub>	Non-spore former	Singly dispersed
16.	GE <sub>4</sub>	Non-spore former	Singly dispersed
17.	WE <sub>5</sub>	Non-spore former	Singly dispersed
18.	COR <sub>1</sub>	Spore former	Singly dispersed

Key: W=water melon, G=garden egg, C=cucumber, I=Ikpa market, O=Ogige market, OR=Orie-Orba market, IG=Orie Igbo-Eze market, E=Eke Ede-Oballa market.

The subscript numbers stands for different dilutions from which each isolate was obtained

**Table 3. Motility test for non-spore formers**

S/no	Sample code	Motility test
1.	Cl <sub>2</sub>	-
2.	GE <sub>4</sub>	-
3.	WE <sub>5</sub>	-
4.	WIG <sub>4</sub>	-
5.	CIG <sub>2</sub>	+
6.	GE <sub>2</sub>	-

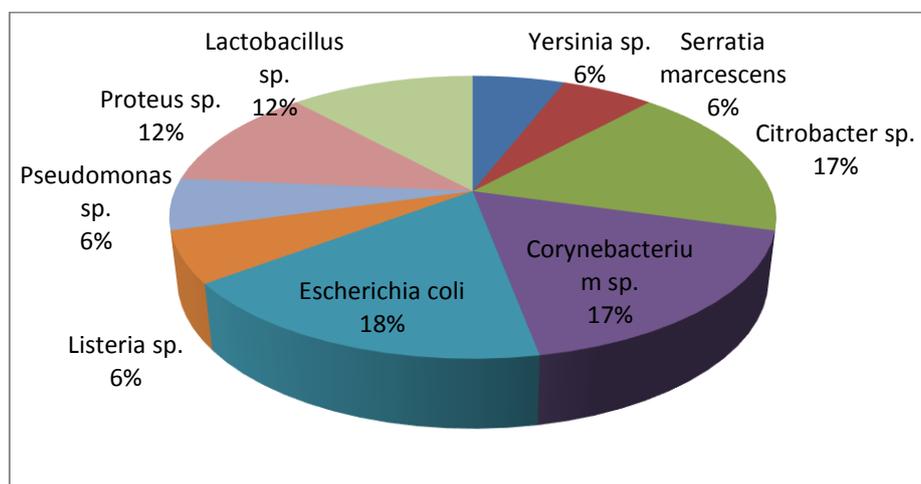
**Key:** + = positive, - = negative, Cl<sub>2</sub>=cucumber sample from Ikpa market, GE<sub>4</sub>=garden egg sample from Eke Ede-Oballa market, WE<sub>5</sub>=watermelon sample from Eke Ede-Oballa market, WIG<sub>4</sub>=watermelon sample from Orié Igbo-Eze market, CIG<sub>2</sub>=cucumber from Orié Igbo-Eze market, GE<sub>2</sub>=garden egg from Eke Ede-Oballa market. The subscript numbers stands for different dilutions from which each isolate was obtained

**Table 4. Sample codes representing various isolated probable bacteria**

S/no	Sample Codes	Probable Bacteria
1.	GIG <sub>3</sub>	<i>Yersinia sp.</i>
2.	WIG <sub>3</sub>	<i>Serratia marcescens</i>
3.	WE <sub>1</sub> , GI <sub>3</sub> , WO <sub>1</sub>	<i>Citrobacter sp.</i>
4.	Cl <sub>2</sub> , GE <sub>4</sub> , CE <sub>5</sub>	<i>Corynebacterium sp.</i>
5.	GO <sub>2</sub> , WI <sub>2</sub> , GOR <sub>3</sub>	<i>Escherichia coli</i>
6.	CIG <sub>2</sub>	<i>Listeria sp.</i>
7.	Cl <sub>1</sub>	<i>Pseudomonas sp.</i>
8.	GOR <sub>4</sub> , WIG <sub>2</sub>	<i>Proteus sp.</i>
9.	WIG <sub>4</sub> , GE <sub>2</sub> ,	<i>Lactobacillus sp.</i>
10.	COR <sub>2</sub>	<i>Salmonella sp.</i>
11.	GOR <sub>2</sub> , GI <sub>4</sub>	<i>Klebsiella sp.</i>
12.	WI <sub>1</sub> , GI <sub>1</sub> , GI <sub>2</sub> , Cl <sub>3</sub> , GO <sub>3</sub> , CO <sub>1</sub> , CO <sub>2</sub> , CO <sub>3</sub> , CO <sub>4</sub> , WOR <sub>3</sub> , WOR <sub>4</sub> , WIG <sub>1</sub> , WE <sub>2</sub> , WE <sub>3</sub> , CE <sub>1</sub> , CE <sub>2</sub> , and CE <sub>3</sub>	<i>Staphylococcus aureus</i>
13.	WO <sub>3</sub> , and CE <sub>4</sub>	<i>Streptococcus sp.</i>
14.	WI <sub>4</sub> , Cl <sub>4</sub> , GO <sub>1</sub> , GE <sub>1</sub> , GE <sub>3</sub> , COR <sub>3</sub> , and CIG <sub>3</sub>	Other <i>Staphylococcus sp.</i> such as <i>Staphylococcus epidermidis</i>
15.	WOR <sub>2</sub> and CIG <sub>1</sub>	<i>Clostridium sp.</i>
16.	WI <sub>3</sub> , WO <sub>2</sub> , WO <sub>4</sub> , GOR <sub>1</sub> , GIG <sub>1</sub> , GIG <sub>2</sub> , GIG <sub>4</sub> and WE <sub>4</sub>	<i>Bacillus sp.</i>
17.	WOR <sub>1</sub> , and COR <sub>1</sub>	<i>Bacillus cereus</i>

**Key:** W=water melon, G=garden egg, C=cucumber, I=Ikpa market, O=Ogige market, OR=Orié-Orba market, IG=Orié Igbo-Eze market, E=Eke Ede-Oballa market.

The subscript numbers stands for different dilutions from which each isolate was obtained.



**Fig. 5. A simple pie chart of percentage abundance of nine bacteria isolates**

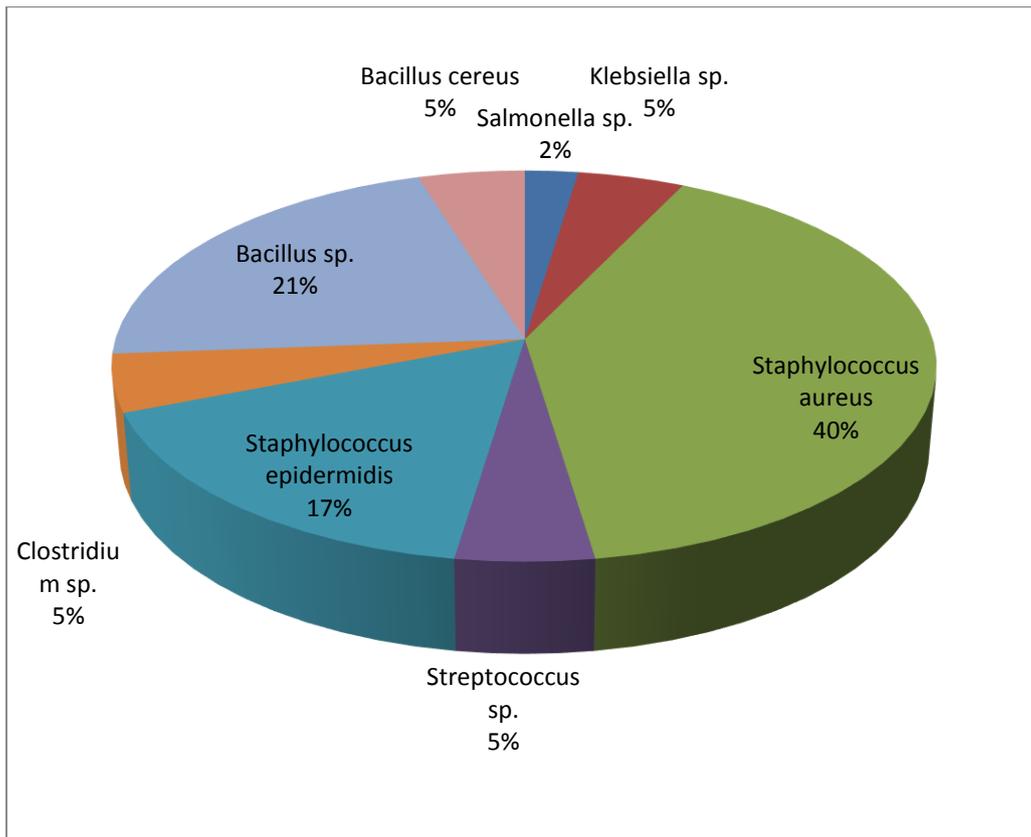


Fig. 6. A simple pie chart of percentage abundance of eight bacteria isolates

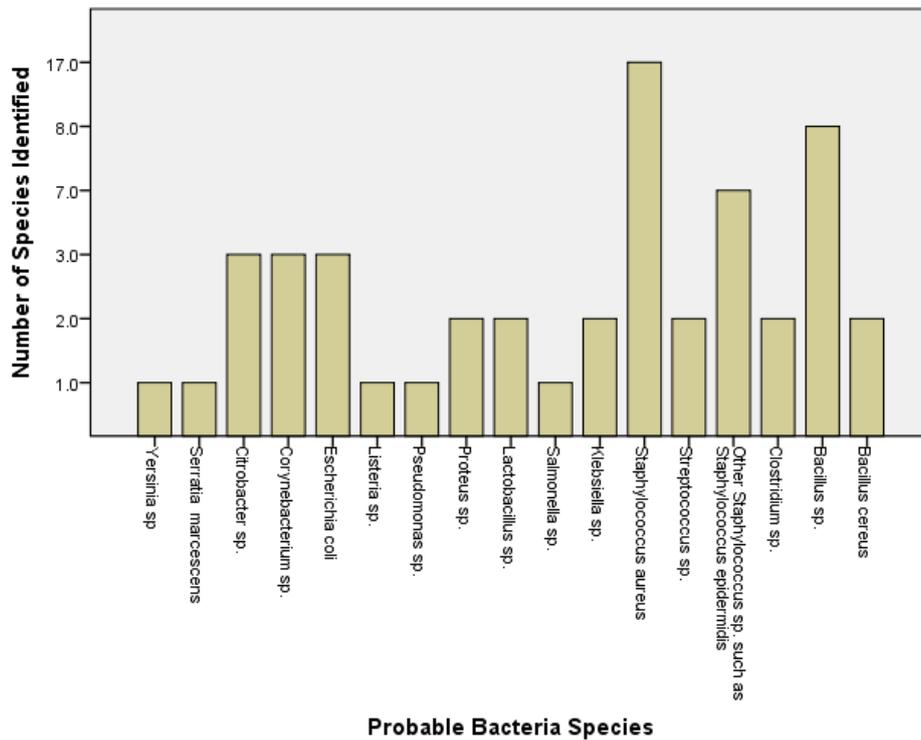


Fig.7. Number of each isolated probable bacteria species

#### 4. CONCLUSION

The findings from this study revealed diverse probable pathogenic bacteria colonizing surfaces of minimally processed fruits and vegetables in numbers exceeding recommended standard limits. Although, surfaces of fruits and vegetables are attractive hosts for some of these bacteria which serve as epiphytic flora for the samples, the high level of bacterial load is a reflection of poor hygienic practices by the vendors. This poses a serious health risk as high level of surface colonization of minimally processed fruits and vegetables cause serious foodborne illness to consumers. Since these food products are usually consumed without further processing, there is need for urgent public sensitization and strict measures to be instituted by appropriate regulatory authorities to oversee the activities of vendors in the study area and other locations where such commodities are sold in Nigeria. It calls for simple hygienic procedures to be employed by vendors because hygienic handling of minimally processed fruits and vegetables is essential for maintenance of food safety. Therefore, consumers are advised to look beyond convenience and always endeavor consumption at home so as to ensure proper washing of such items.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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