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# Evaluation of Bacterial Contamination of Fruits and Vegetables Obtained in Markets in Abia State, Nigeria

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**Abstract**
The study explored bacterial contamination of fruits and vegetables obtained from markets in Abia State, Nigeria. It was a cross sectional descriptive survey. A sample size of 360 fruits and vegetables were collected from different markets in Abia State, Nigeria. It used laboratory systematic manipulation of a few variables at a time while others that may affect the outcome of the result were held constant. Data was analyzed using descriptive statistics of frequency counts, normative, percentage, barchart and inferential statistics of chi-square (×2). The results showed high bacterial contamination of fruits and vegetables. Five bacterial isolates exhibited varying levels of occurrence, Escherichia coli occurring highest in the three zones of Abia State; Abia South (42%), Abia central (34.5%) and Abia North (23.5%), followed by Staphylococcus aureus, Salmonella sp, Klebsiella pneumoniae and Shigella sp, were detected in (26,%), (12%), (18%), and (10%) respectively in (Abia South). Salmonella sp, was highest in Abia Central followed by Abia South and Abia North. Prevalence rate of salmonella paratyphi and typhi were highest in Abia central (211 & 83.07), followed by Abia South (208 & 68.42) and Abia North (154 & 75.12). In conclusion, this investigation recorded a high load of bacteria isolates in fruits and vegetables sold in six markets across the three senatorial zones of Abia state, Nigeria. Microorganisms identified are both of health importance and spoilage value. Poor sanitation in markets, fruits and vegetables displayed on dirty table surfaces/grounds in Abia state can be identified as a major sources of microbial contamination of fresh produce.

**Keywords:** Salmonella Spp, Staphylococcus Aureus, Klebsiella Pneumonia, Raw Vegetable, Raw Fruits, Escherichia Coli

## Introduction

Fruits and vegetables are vital sources of essential nutrients (such as vitamins, minerals and simply utilisable sugars) needed for human growth and development (Ezenwaka & Amuzie, 2021; Rabiu, Jamilu, Adamu & Muhammad, 2021) [8, 13]. These nutrients confer health edges like hindrance of diet-related deficiencies, reduction in risk of chronic diseases, and detoxification of the body system (Daphey, Ito & Onybiguwa, 2023) [5]. Fruits and vegetables contribute to healthy living globally. Fruits and vegetables embody a various cluster of plant foods that modify greatly in content of energy and nutrients (Vincent, Nkanga, Okita & Sunday, 2022) [16]. Also, fruits and vegetables provide fibre, and its intake is coupled to lower prevalence of obesity and cardiovascular disorders. Fruits and vegetables conjointly provide vitamins and minerals to the diet and square measure sources of phytochemicals that perform as antioxidants and anti inflammatory agents and alternatively aid in protective mechanisms (Shola *et al.*, 2022) [14]. Fruits and vegetables have typically control an area in dietary steering as a result of their concentrations of vitamins, particularly vitamins C and A; minerals, particularly electrolytes; and additional phytochemicals, particularly antioxidants (Bashir, Babaginda, Abdullahi & Haladu, 2020) [3]. Nigeria is blessed with favourable climate and seasons that permits the expansion of different types of fruits and vegetables. They're low-cost, accessible and available readily to customers. Tropical fruits and vegetables, relatively are sold as whole foods or as prepackaged goods in synthetic resin baggage.

The consumption of raw or processed fruits and vegetables sold in markets could represent human health risks in relation to microbic contamination. Microbic flora in fruits and vegetables are determined by factors like introduced non-resident microorganisms through manure, waste material and human handling (Bhunia *et al.*, 2018; Vincent *et al.*, 2022) [4, 16]. Enteric diseases have been associated with consumption of fruits and vegetables sold in open markets (Adekanle *et al*., 2015) [2]. Enteric pathogens like E. coli and enterobacteria have been associated with greater disease prevelence during food related outbreaks (Bhunia *et al*., 2018) [4]. In Nigeria, for an example, market sanitation, fruits/vegetables sellers' personal hygiene as well as proper handling of goods sometimes are not considered hence, exposing fruits and vegetables to contaminants. Proper handling of fruits and vegetables that are void of contamination are vital to maximise the nutritional values provided by these produce. Adequate washing of fruits and vegetables is crucial for contamination void. Water supplemented with variable concentrations of organic acids, like carboxylic acid, acid and sorbic acids, has been seen to reduce the impact of microbes in fruits (Negebenebor *et al*., 2019) [11]. Fruits harbour natural non-pathogenic microorganisms from their surrounding and deterioration. However, they may be contaminated via handling and packaging processes (Orpin & Usmain-Sani, 2020) [18]. This is as a result of poor processing of fruits/vegetables and lack of internal control. Microbes like enterobacteria spp., enteric bacteria spp., Camphylobacter spp. and E. coli will contaminate sliced fruits and vegetables through contact with waste material, contaminated water used in washing and processing.

In Nigeria and other developing countries, ready-to-eat sliced fruit and vegetables have become rampant because of high patronage, astonishingly; a number of these fruits and vegetables are not washed before being consumed. Pathogens, spongers and chemicals are agents of contamination of fruits and vegetable which may defile produce at different points of handling from production, harvesting, transportation, storage, preparation and consumption processes. The natural hazards that mostly defile fruits and vegetables usually come from either animal or human activities and can be grouped into spore forming bacteria, non-spore forming bacteria, contagions, spongers and prions (Oluwakemi *et al*., 2020) [17]. Resident flora (e.g *E.coli & Staphylococcus aureus* etc) seen in humans are also found in fruits and vegetable. They could also be contamination from soil, terrain, and inoculation via water irrigation. According to WHO, position of microbial contamination in product systems can do because of multitudinous variables including- crop practices, original terrain, workers' health and hygienic conditions. Although, microbial contamination of vended fruits and vegetables can change at every stage of the food chain, from civilization to processing and point of consumption, poor aseptic conditions and environmental pollution during planting could also increase the trouble of contamination. The high moisture content of fruits and vegetables provide favorable environment for the growth and multiplication of microorganisms which contaminate them. Cutting or peeling of these produce can rupture their tissue thereby favoring unwanted microorganism growth which in turns pollute them. Most sellers are not conscious of personal hygiene; hence they expose their products to polluted air, unkept environment, use not potable water to wash or wet their produce and use dirty packaging bags in some occasions (Ezenwaka *et al.*, 2021) [8].

## Methods

### Research Design

The study employed a cross-sectional descriptive survey that involved direct observation, laboratory study and analysis of fruits and vegetables borne microorganisms sold in the markets of Abia State.

### Collection of Samples

A total of 180 fruits and 180 vegetable samples (5 different types of fruits and vegetables from each market) were collected in sterile bags from 6 open markets (2 markets from each senatorial zone). The markets are; Eke-Amiyi and Eke Elu (Abia North), Ubani Central market and Orie Ntigha (Abia Central), Ahiaohuru and Ariaria (Abia North). The fruit samples were Guava (*Psidium guajava*), Orange (*Citrus sinensis*), Lemon (*Citrus limon*), Apple (*Malus domestica*) and Mango (*Mangifera indica*) while the vegetable samples were Waterleaf (*Talinium triangulare*), Fluted pumpkin (*Telfairia occidentalis*), Carrot (*Daucus carota*), Cabbage (*Brassica oleracea*) and Eggplant (*Solanum melongena*). The samples were immediately taken to the microbiology laboratory for analysis.

### Isolation and Identification of Bacteria

Ten (10) grams of the selected raw vegetables and fruits each were measured and finely chopped aseptically. The weighed samples were washed in 100ml of sterile distilled water. 1ml of the wash water was then subjected to ten-fold serial dilution series. 1ml from $10^4$ dilutions were spread plated in duplicate onto Nutrient Agar plates and MacConkey Agar plates and then incubated at 37$^o$C for 24 hours (Benjamin *et al*, 2018). Identification of bacteria was done using colony characteristics and biochemical properties of the isolates. Pure cultures were sub-cultured onto nutrient agar slants and stored at 4$^o$C in a refrigerator.

### Gram's staining

This was used to distinguish Gram negative and Gram positive organisms. In this form of staining, a thin smear was made on a clean grease- free glass slide. The smear was air dried and heat fixed by rapidly passing the smear over the flame of Bunsen burner three times. The fixed smear was flooded with crystal violet for 60 second. After 60 seconds, the stain was rapidly washed off with distilled water and then flooded with Lugol's iodine solution for one minute. The iodine was washed off with distilled water and the smear was decolorized with acetone for 15 seconds. The slide was washed immediately with distilled water and counterstained with safranin for 60 seconds. After that, the stain was washed off with distilled water and air dried. The smear was then observed under the microscope using oil immersion with X100 objective lens.

### Catalase Test

In this test, 2ml of hydrogen peroxide solution was poured into a test tube and a glass rod was used to remove some colonies of the test organism and immersed in the hydrogen peroxide solution. Observation for Bubbles of oxygen gas 5-10 seconds after was done immediately.

### Oxidase Test

To test for the presence of the enzyme cytochrome oxidase in the test organism, a piece of filter paper was placed in a clean Petri dish and three drops of freshly prepared Kovac's oxidase reagent was added. A glass rod was used to remove

a colony of the test organism and smeared on the filter paper and then allowed for 10 seconds. Development of purple colour would indicate a positive result while no purple colour formation would indicate a negative result.

**Sugar Fermentation**

This test was carried out to determine the ability of the test organism to metabolize sugar with the production of acid or gas. The following sugars were prepared and used for the test-glucose, maltose, lactose, arabinose, fructose, sucrose, mannose and mannitol. In the test, 0.2g of each of the sugars was dissolved in 20 ml of peptone water solution. Phenol red was added as indicator and 5 ml aliquots were dispensed into test tubes containing Durham tubes and autoclaved at 121ºC for 15 minutes (121ºC for 3 minutes for lactose, sucrose, arabinose and maltose as they may break down if sterilized further). It was then allowed to cool and then inoculated under aseptic conditions with the test organism using sterile wire loop and afterwards incubated at 37ºC for 24 hours. A change in colour from purple to yellow indicates a positive result while gas production was shown by the presence of bubbles in the Durham tubes.

**Methyl Red Test**:

The medium for this test is glucose phosphate broth also called Methyl Red-Voges-Proskauer broth. It was prepared by mixing 5g of peptone and 5g of dihydrogen phosphate in one litre of distilled water. The mixture was steamed until the solid dissolved after which it was filtered. Five grams of glucose was added and mixed well. It was then distributed in 5 ml volume into test tubes and sterilized at 121ºC for 15 minutes. During sterilization, the tubes were placed on a solid bottom container to protect them from contact with steam as this may make the medium become straw yellow in colour. After sterilization, the medium was allowed to cool and the test organism was inoculated into the broth and incubated for four to seven days at 37ºC. After incubation, 5 drops of the indicator methyl red were added to the broth. It was then observed for the formation of red colour after few minutes to indicate a positive result while yellow colour was taken as negative result.

**Urease Test**

An overnight culture of the test organism was streaked on the surface of urea agar slant under aseptic conditions. The cap of the test tube was closed loosely and incubated at 37°C for 48 hours for up to 7 days. It then examined for the development of a pink colour after 4 to 7 days of incubation.

**Coagulase Test**

In this test, a drop of water was placed on each end of a slide. A colony of the test organism was emulsified in each of the drops to make thick suspension. A loopful of plasma was added to the suspension and mixed gently. The clumping of the organism was checked within 10 seconds.

## Results

**Total bacterial counts from fruit samples**

The total count of bacteria isolated from fruit and vegetables samples in all the six markets were recorded.

Fig 1 displays the total bacterial count from fruit samples. Highest bacterial counts were observed in isolates from mango samples purchased at Ahiaohuru market with a count of 5.2 X $10^4$. High counts were also observed in mango samples from Ubani market with a count of 4.8 X $10^4$ and also in orange samples at Ekeamiyi with a count of 4.9 x $10^4$. There were also high counts from apple samples purchased at Ahiaohuru and Eke Elu markets (4.7 X $10^4$ and 5.0 X $10^4$ respectively). Lowest counts were observed in isolates from orange purchased at Ahiaohuru market (1.7 X $10^4$).

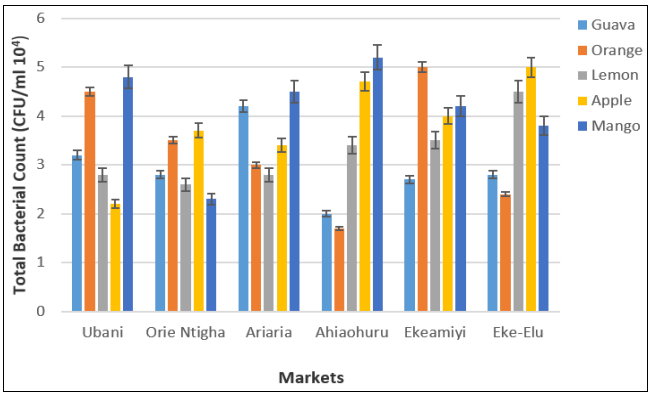


**Fig 1:** Total Bacterial (viable plate count) of Fruit Samples (CFU/ml X $10^4$)

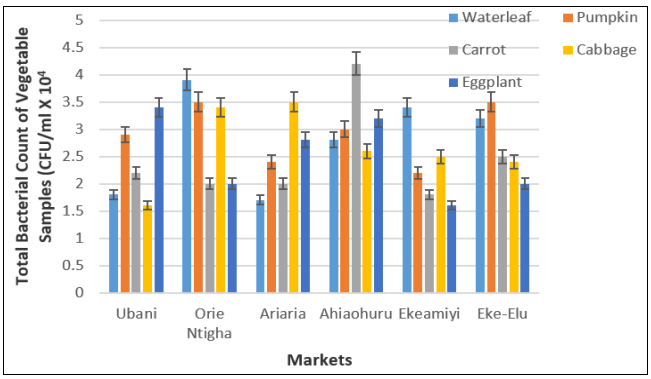


**Fig 2:** Total Bacterial (viable plate) of Vegetable Samples (CFU/ml X $10^4$)

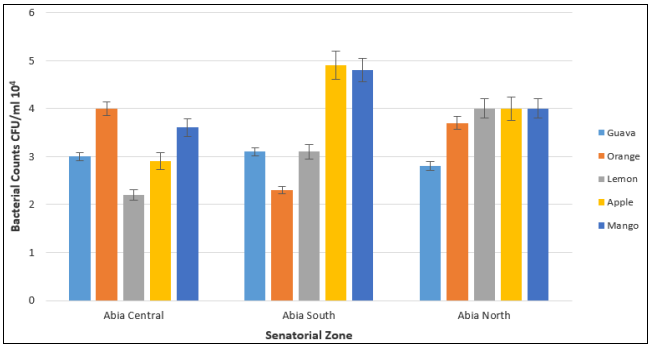


**Fig 3:** Average Bacterial Counts in Fruits in the 3 Senatorial Zones (CFU/ml X $10^4$)

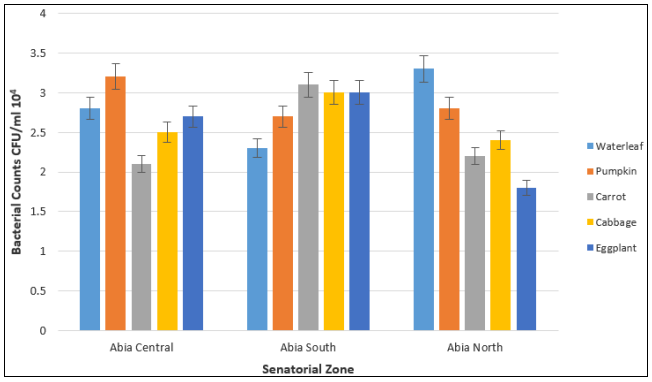


**Fig 4:** Average Bacterial (viable plate) in Vegetables in the 3 Senatorial Zones

Fig 2 displays bacterial counts from vegetable samples in the six senatorial zones. Optimal bacterial counts from vegetable samples were observed in carrot purchased from Ahiaohuru market (4.2 X $10^4$). Counts in Orie Ntigha market were also high with isolates from waterleaf samples having a count of 3.9 X $10^4$. However waterleaf purchased from Ubani and Ariaria markets had lower counts (1.8 X $10^4$ and 1.7 X $10^4$ respectively). Lowest counts were observed in eggplant from Ekeamiyi and cabbage from Ubani markets (1.6 X $10^4$).

In Fig 3, bacterial counts from fruits in the three zones were recorded. Highest counts were observed in mango samples from Abia south senatorial zone (4.8). Least counts were observed in lemon samples from Abia central zone (2.2).

Fig 4 displays mean bacteria counts from vegetables in the three senatorial zones. Highest mean counts were observed in Abia North (3.3) in waterleaf samples. Lowest counts were observed in eggplant samples also in Abia north (1.8). This was in contrast to higher counts observed in eggplant samples in Abia South which had an average count of 3.0.

**Table 1:** Identification of Bacterial Isolates: Morphological and physiological characteristics of the bacterial isolates were investigated according to the method described by Bergey *et al*, 2000.

| | | | Biochemical Test | | | | | | | | | Sugar Fermentation | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **S. No** | **Cultural Morphology** | **Microscopic Features** | **Catalase** | **Coagulase** | **Indole** | **Methyl -red** | **VP** | **Urease** | **$H_2S$** | **Citrate** | **Oxidase** | **Lactose** | **Glucose** | **Motility** | **Gram Reaction** | **Isolates** |
| 1 | Pink colonies on Macconkey Agar | Rod Shape | +<br>+ | - | +<br>- | +<br>- | -<br>+ | -<br>+ | - | -<br>+ | -<br>- | +<br>+ | +<br>+ | +<br>- | - | *Escherichia coli* |
| 2 | Mucoid Pink colonies on Macconkey agar | Rod Shape | + | - | - | - | + | + | - | + | - | + | + | - | - | *Klebsiella Pneumoniae* |
| 3 | Creamy colonies with rough edges on Nutrient agar | Cocci in clusters | + | + | - | + | + | + | - | - | + | + | + | - | + | *Staphylococcus aureus* |
| 4 | Colourless colonies with black centres on SSA | Rod Shape | + | - | - | + | - | - | + | - | - | - | + | + | - | *Salmonella* sp |
| 5 | Colourless colonies on SSA | Rod Shape | + | - | + | + | - | - | - | - | - | - | + | - | - | *Shigella* sp |

Taxanomic identification using biochemical tests confirmed the isolates to be *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* sp, *Klebsiella pneumoniae* and *Shigella* sp.

**Table 2:** Distribution of Bacterial Isolates in Samples from the Zones

| **Isolates** | **Distribution of Isolates** | | | | | |
|---|---|---|---|---|---|---|
| | **Abia Central** | | **Abia South** | | **Abia North** | |
| | **Ubani** | **Orie Ntigha** | **Ariaria** | **Ahiaohuru** | **Ekeamiyi** | **EkeElu** |
| *Escherichia coli* | + | + | + | + | + | + |
| *Staphylococcus aureus* | + | + | + | + | + | + |
| *Salmonella* sp | + | - | + | - | + | - |
| *Klebsiella Pneumoniae* | + | + | + | - | + | + |
| *Shigella* sp | - | + | - | + | + | - |

**Key: + = Present, - = Absent.**

## Distribution of Bacteria Isolates in Fruit and Vegetable Samples

The distribution and spread of microbial isolates from fruit and vegetable samples analysed in the six markets are displayed in Table 2. Amongst the bacterial isolates, *Escherichia coli* and *Staphylococcus aureus* were isolated in samples from all six markets. The other bacteria species were isolated from all senatorial zones but not in all markets.

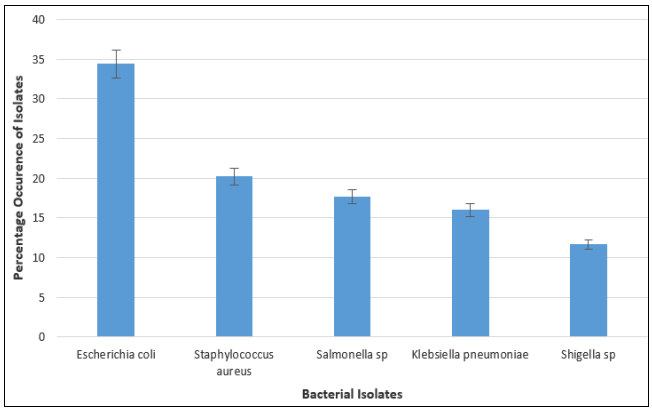


**Fig 5:** Percentage Occurrence of Bacterial Isolates in Fruit and Vegetable Samples in the Abia Central Senatorial Zone

Fig 5 displays the percentage occurrence of bacteria isolates in Abia Central Senatorial zone. *Escherichia coli* was the highest occurring isolate with (34.4%) occurrence. This was followed by *Staphylococcus aureus with* (20.2%) occurrence. There was significant statistical difference in the occurrence rate of bacteria isolates in Abia Central zone.

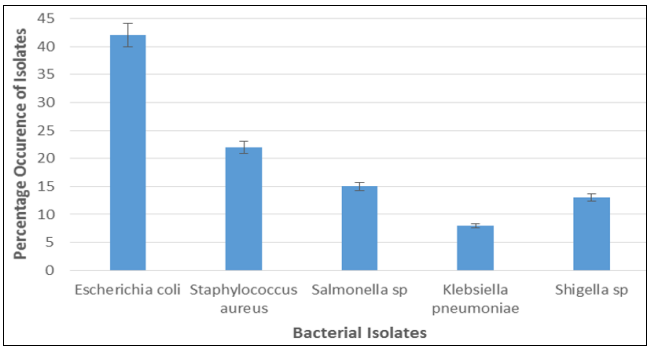


**Fig 6:** Percentage Occurrence of Bacterial Isolates in Fruit and Vegetable Samples in the Abia South Senatorial Zone

Fig 6 displays occurrence rate in percentage of bacteria isolated from fruits and vegetables in Abia South zone. *Escherichia coli* was the highest occurring with (42%) while *Klebsiella pneumoniae* had the least with (8%) occurrence.

There was significant statistical difference in the occurrence rate of bacteria isolates in Abia South.

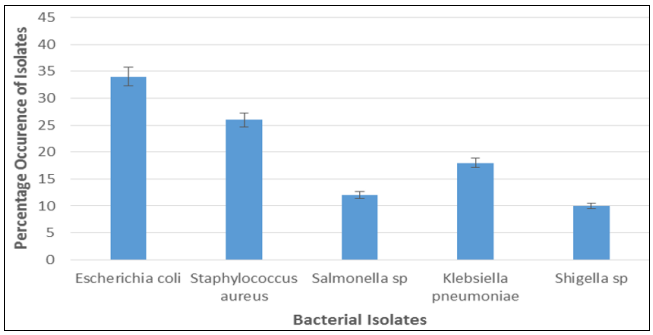


**Fig 7:** Percentage Occurrence of Bacterial Isolates in Fruit and Vegetable Samples in the Abia North Senatorial Zone

Fig 7 exhibits occurrence rate of bacteria isolates in samples from Abia north zone. *Escherichia coli* had the highest occurrence (34%) followed by *Staphylococcus aureus* (26%), *Salmonella* sp (12%), *Klebsiella pneumoniae* (18%) and *Shigella* sp (10%). There was significant statistical difference in the percentage occurrence of bacteria species isolated from samples in Abia North.

## Discussion

Eating unwashed/not properly washed fresh fruits as well as consumption of unhygienic processed vegetables as a habit by majority of the people in our society has posed a serious threat to the health of the people and has greatly contributed to the epidemic of enteric diseases amongst the population. The findings of this study has shown that fruits and vegetables sold in the markets in Abia State were highly contaminated with microorganisms and these microbes cause enteric diseases that are of public health importance in both developed and developing country like Nigeria. However, the endemicity of these enteric diseases have been highly favoured by factors such as illiteracy of the farmers and traders, poor hygiene practices amongst the farmers, traders as well as the consumers. Therefore, this study investigated the presence of bacteria in fruits and vegetables sold in selected markets in Abia State. Bacteria isolated were *Escherichia coli, Staphylococcus aureus, Shigella* sp, *Salmonella* sp and *Klebsiella Pneumoniae*. The presence of enteric bacteria and *Staphylococcus aureus* were similar to the findings by Ogufure *et al*. (2017) [12], Mahfuza *et al*. (2016) [10] in Dhaka, Bangladesh, Ogofure *et al*. (2017) [12] in Benin City Nigeria, Ehimemen *et al*. (2019) [6] in North-West Nigeria, Afolabi *et al*. (2019) in Ogun State, and Ugwu and Edeh (2019) [15] in Enugu State, Nigeria. Highest counts of isolated bacteria were observed in fruit and vegetable samples from Ahiaohuru market, in Aba, Abia South from mango and carrot samples respectively. These high counts can be attributed to the evident indiscriminate disposal of wastes and general poor hygiene in Ahiaohuru market. *Escherichia coli* were the most prevalent bacterial isolate from fruit and vegetable samples in the three senatorial zones. This was similar to reports by Getaneh *et al*. (2018) [9] and Yafetto *et al*. (2019) [19]. This study showed that *E.coli* (36.6%) on average was the most prevalent bacteria while the least occurring bacteria was *Shigella* (13%) and the report is quite lower than the report of Ugwu and Edeh (2019) [15], in Enugu State Nigeria, where the most occurring bacteria contaminant was *E.coli* (62%) and least occurring bacteria was *Shigella* (37.5%) but both studies agreed that *E. coli* and *Shigella* are the most and least bacterial contaminants respectively. The presence of coliforms such as *E.coli*, *Shigella* sp, *Klebsiella pneumoniae* in the samples can be attributed to the use of sewage contaminated water during cultivation of the plants and during processing and packaging of the raw fruits and vegetables (Bhunia, 2018) [4]. *E.coli* and *Staphylococcus aureus* had the widest distribution amongst the isolates as it was identified from samples purchased from the six markets in Abia State. Comparatively, *E.coli* was the most prevalent bacterial isolate from both fruit and vegetable samples, followed by *Staphylococcus aureus* isolates. This was similar to review done by Erhirher *et al* (2020) [7], and was in agreement to their distribution in the six zones. The presence staphylococcus aureus indicates that consumers of these fruits and vegetables are at risk of staphylococcal infections. Consumers are also at risk of contracting shigellosis, typhoid fever, bronchopneumonia and diarrheal infections due to the presence of enteric bacteria.

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