

# **Bacteriological Quality of Fresh Salad Vegetables Sold in Calabar Road and Marian Markets, Calabar, Cross River State, Nigeria.**

## **Abstract.**

The consumption of fresh vegetables is on the increase because of the recent awareness of the numerous health benefits. Most people prefer them raw in form of salads to retain the natural taste and heat labile nutrients. Thus, evaluating the bacteriological quality of these vegetables for public safety is of primary importance. The bacteriological quality of fresh salad vegetables sold in Calabar markets was evaluated using standard techniques. The mean bacterial cell counts ranged from  $2.44 \times 10^8$  Cfu/mL to  $1.00 \times 10^8$  Cfu/mL. In Calabar road market, the highest cell counts was observed in leeks with  $2.44 \times 10^8$  Cfu/mL followed by cabbage with  $2.13 \times 10^8$  Cfu/mL both for *Bacillus* species. The least count,  $1.08 \times 10^8$  Cfu/mL was obtained from green peas for *Listeria* and *Staphylococcus* species. In Marian market, the highest counts was  $2.13 \times 10^8$  Cfu/mL for *Bacillus* species obtained from cabbage while the lowest count was  $1.00 \times 10^8$  Cfu/mL for *Staphylococcus* species from green peas and cabbage. The most prevalent bacteria isolate was *Staphylococcus* species with percentage occurrence of 30% in Marian market and 24% in Calabar road. The most contaminated of the vegetables was carrots with percentage occurrence of 18% (7 out of 37). The order of contamination in analysed vegetables was; carrots > leeks = cucumber > lettuce = green beans = green peas > cabbage = onion = green bell pepper in both markets. The significance of the findings is that vegetables used as salad recipe could be sources of food borne infection, particularly during the beginning of the rains when new vegetables are harvested from the ground level. This calls for thorough washing of salad vegetables before consumption.

**Key words:** *Fresh salad vegetables; Marian market; Calabar market; Streptococcus and Bacillus species.*

## **1. INTRODUCTION**

Vegetables constitute an important human diet in every culture worldwide and they are variously valued for their high concentrations of dietary fibre, vitamins, minerals, electrolytes and phytochemicals, especially antioxidants [1]. In recent times renewed promotions of their health benefits has resulted in increased consumption [2]. It has even been observed that low intake of these vegetables results in poor health and increased risk of chronic diseases. According to Lian *et al.* (2012) [3], vegetables reduce meal energy intake when taken as a first course, thus, contributing to weight loss and reduction in risk of obesity [4, 5]. On account of the numerous health benefits, the Food and Agricultural Organization (FAO), in 2003, launched a global initiative to promote consumption of fruits and vegetables [6, 7].

A lot of vegetables are consumed raw as salads to retain the natural taste and preserve the heat labile nutrients; while some are heat-treated under controlled pH in hermetically sealed containers for the purpose of preservation. According to Oxford Dictionary, salad is any dish that contains a mixture of small pieces of different fresh vegetables served at room temperature or chilled. Fresh salad items include; cabbage, carrot, cucumber, green peas, green beans, lettuce, tomato, onions etc. The canned types come in different varieties such as Hot dog, sweet corn, green peas, red kidney, baked beans, corn beef, tomato pastes etc.

The safety of vegetables that are eaten raw is of great concern, as they have been shown severally to be contaminated with pathogenic bacteria and parasites [8]. The contributing

factors to this are; Poor hygienic practices during production, inadequate washing before consumption and contamination during post-harvest [9]. These serve as sources or vehicles for possible transmission of pathogenic organisms responsible for outbreaks of some human infectious diseases. High level of salad consumption has aroused interest of evaluating and monitoring the bacteriological safety of these items based on locality for the safety of the public. Thus, this research sets out to identify pathogenic bacteria associated with different types of fresh salad vegetables sold in Calabar Markets in Cross River State with the aim of providing data that could guide policy to improve food safety and safeguard public health in the state.

## 2. Materials and Methods

### 2.1. Sample Collection and Preparation

Twenty samples each of Cabbage, carrot, cucumber, green peas, green bell pepper, green beans, leeks, lettuce, onions and tomato were randomly purchased in the two experimental sites (Calabar road and Marian market). Each sample was placed in a sterile polythene bag and transported to Microbiology laboratory, Cross River University of Technology. A 5g weight of each item was cut into tiny pieces and homogenized in a sterile blender (LANDERS-Y-CIA.S.A.) for 2 minutes. Approximately 1g of the homogenate was suspended in 4 mL of sterile normal saline. Taking 1mL of the suspension, a ten-fold serial dilution was done in sterile normal saline down to  $10^{-10}$ .

#### 2.1.1. Bacterial Cell Count, isolation and characterisation of Bacterial species

Bacterial cell count was done by the pour plate method using nutrient and MacConkey agar (Liofilchem<sup>®</sup> s.r.l., Italy), respectively. A 1mL amount of  $10^{-9}$  and  $10^{-10}$  dilution of the vegetable homogenate suspension was placed in a clean sterile Petri dish; then, 20 mL of molten Nutrient or MacConkey agar at about  $44^{\circ}\text{C}$  was added and the Petri dish gently swirled until the contents mix thoroughly. The agar was allowed to set before incubation at  $37^{\circ}\text{C}$  for 24 hours in a humidified incubator [6].

After 24 hours of incubation, the plates were examined for growth and the emergent colonies counted and recorded. Thereafter, discrete colonies were isolated, purified by three successive sub-culturing and re-isolations on Nutrient agar and characterized by standard bacteriological techniques as described by Cheesbrough (2006) [10]. The identity of Gram negative rods were confirmed using the Analytical Profile Index 20E (Biomérieux SA, France) kit.

#### 2.1.2. Data Analysis

The statistical analysis was done using SPSS version 20 for descriptive statistics. The student Unpaired T-test compared the means of fresh vegetables that were significant at  $P = .05$ .

## 3. RESULTS

- 3.1. Bacterial Cell Count:** The bacterial cell count obtained from vegetables purchased from Calabar road ranged from  $2.44 \times 10^8$  CfU/mL for *Bacillus* in leeks to  $1.4 \times 10^8$  CfU/mL for *Staphylococcus* species in cabbage. The least contaminated vegetable was lettuce with just 1 isolate with prevalence of 5% in Calabar road (Table 1). In Marian market, the mean cell count ranged from  $2.13 \times 10^8$  CfU/mL of *Bacillus* in cabbage to  $1.00 \times 10^8$  CfU/mL of *Staphylococcus* species in green beans. Out of 10 vegetables analysed, 7 (70%) were contaminated with 2 bacterial strains each. The least contaminated were green peas and green bell pepper each yielding 1 bacterial isolate (Table 2).

**Table 1: Mean Bacterial Cell Counts in Colony Forming Unit(Cfu/mL) from various Vegetables from Calabar Road**

| <b>Vegetables</b> | <b><i>Staphylococcus</i></b> | <b><i>Streptococcus</i></b> | <b><i>Bacillus</i></b> | <b><i>Salmonella.</i></b> | <b><i>Micrococcus</i></b> | <b><i>Listeria</i></b> | <b><i>Shigella</i></b> | <b><i>E .coli</i></b> | <b><i>Klebsiella</i></b> |
|-------------------|------------------------------|-----------------------------|------------------------|---------------------------|---------------------------|------------------------|------------------------|-----------------------|--------------------------|
| Green beans       | 0                            | 0                           | 1.92 x 10 <sup>8</sup> | 0                         | 0                         | 0                      | 0                      | 1.2 x 10 <sup>8</sup> | 0                        |
| Lettuce           | 2.24 x 10 <sup>8</sup>       | 0                           | 0                      | 0                         | 0                         | 0                      | 0                      | 0                     | 0                        |
| Leeks             | 1.36 x 10 <sup>8</sup>       | 0                           | 2.44 x 10 <sup>8</sup> | 0                         | 0                         | 0                      | 0                      | 0                     | 0                        |
| Cabbage           | 1.41 x 10 <sup>7</sup>       | 0                           | 1.68 x 10 <sup>8</sup> | 0                         | 0                         | 0                      | 0                      | 0                     | 0                        |
| Carrots           | 1.76 x 10 <sup>8</sup>       | 1.10 x 10 <sup>8</sup>      | 0                      | 0                         | 0                         | 0                      | 0                      | 0                     | 0                        |
| Onions            | 1.16 x 10 <sup>8</sup>       | 1.88 x 10 <sup>8</sup>      | 0                      | 0                         | 0                         | 0                      | 0                      | 0                     | 0                        |
| Green pea         | 1.08 x 10 <sup>8</sup>       | 0                           | 0                      | 0                         | 0                         | 1.08 x 10 <sup>8</sup> | 1.17 x 10 <sup>8</sup> | 0                     | 0                        |
| Tomatoes          | 0                            | 0                           | 0                      | 0                         | 0                         | 1.96 x 10 <sup>8</sup> | 0                      | 0                     | 1.24 x 10 <sup>8</sup>   |
| Cucumber          | 0                            | 0                           | 1.28 x 10 <sup>8</sup> | 1.21x10 <sup>8</sup>      | 0                         | 0                      | 0                      | 0                     | 0                        |

|                   |   |                    |   |   |                    |   |   |   |   |
|-------------------|---|--------------------|---|---|--------------------|---|---|---|---|
| Green bell pepper | 0 | $1.81 \times 10^8$ | 0 | 0 | $1.48 \times 10^8$ | 0 | 0 | 0 | 0 |
|-------------------|---|--------------------|---|---|--------------------|---|---|---|---|



**Table 2: Mean Bacterial Cell Counts in Colony Forming Unit(Cfu/mL) from various Vegetables from Marian Market**

| <b>Vegetables</b> | <b><i>Staphylococcus</i></b> | <b><i>Streptococcus</i></b> | <b><i>Bacillus</i></b> | <b><i>Salmonella.</i></b> | <b><i>Listeria</i></b> | <b><i>E.coli</i></b>   | <b><i>Klebsiella</i></b> | <b><i>Shigella</i></b> | <b><i>Enterococcus</i></b> |
|-------------------|------------------------------|-----------------------------|------------------------|---------------------------|------------------------|------------------------|--------------------------|------------------------|----------------------------|
| Cabbage           | 0                            | 1.07 x 10 <sup>8</sup>      | 2.13 x 10 <sup>8</sup> | 0                         | 0                      | 0                      | 0                        | 0                      | 0                          |
| Carrots           | 0                            | 0                           | 1.45 x 10 <sup>8</sup> | 0                         | 0                      | 0                      | 0                        | 1.31x10 <sup>8</sup>   | 0                          |
| Cucumber          | 0                            | 0                           | 0                      | 1.21 x 10 <sup>8</sup>    | 1.48 x 10 <sup>8</sup> | 0                      | 0                        | 0                      | 0                          |
| Green peas        | 0                            | 1.50 x 10 <sup>8</sup>      | 0                      | 0                         | 0                      | 0                      | 0                        | 0                      | 0                          |
| Green bell pepper | 1.78 x 10 <sup>8</sup>       | 0                           | 0                      | 0                         | 0                      | 0                      | 0                        | 0                      | 0                          |
| Green beans       | 1.00 x 10 <sup>8</sup>       | 0                           | 0                      | 0                         | 0                      | 1.19 x 10 <sup>8</sup> | 0                        | 0                      | 0                          |
| Lettuce           | 0                            | 0                           | 0                      | 0                         | 0                      | 1.15x10 <sup>8</sup>   | 1.52x10 <sup>8</sup>     | 0                      | 0                          |
| Leeks             | 1.10 x 10                    | 0                           | 0                      | 0                         | 0                      | 0                      | 0                        | 0                      | 1.15 x 10                  |
| Onions            | 1.84 x 10                    | 0                           | 0                      | 0                         | 0                      | 0                      | 0                        | 0                      | 0                          |

Tomato

0

0

0

0

0

0

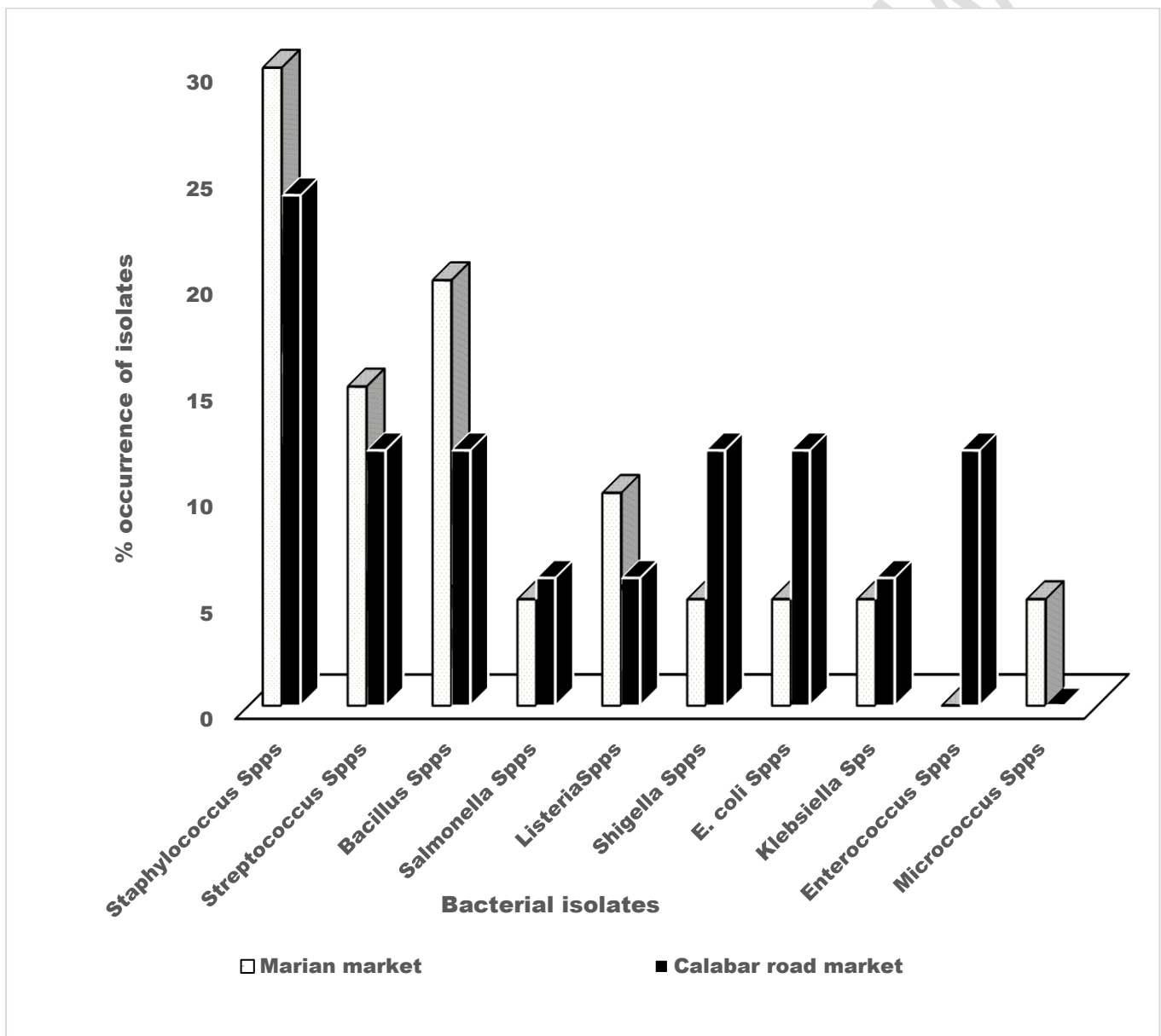
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$1.06 \times 10$

$1.10 \times 10$

UNDER PEER REVIEW

**3.1.2. Distribution of Bacterial Isolates According to Markets:** In Marian market, the most frequently isolated organisms were *Staphylococcus*, *Bacillus*, *Streptococcus* and *Listeria* species occurring at respective frequencies of 35%, 24%, 18%, and 12%. *Salmonella*, *Shigella*, *E. coli*, *Klebsiella*, and *Micrococcus* each occurred just once (5%) in various vegetables obtained from Calabar road. Figure 1 shows a comparison of frequency occurrence of the bacterial contaminants in the vegetables from the two market sources. *Staphylococcus* appeared to be the most frequently isolated in both the Marian market (30%) and Calabar Road market ( $\approx 25\%$ ). *Bacillus spp* occurred at a higher frequency (20%) in Marian market vegetable samples than in samples from Calabar Road market. However, both strains occurred at approximately equal rate in vegetable samples from Calabar Road market ( $\approx 12\%$ ). *Enterococcus* was isolated only from Calabar Road vegetable samples; similarly *Micrococcus* appeared only in Marian market vegetable samples (Figure 1).



**Figure 1: Distribution of Bacterial Isolates According to Markets.**

### 3.1.3. Distribution of Bacterial Isolates according to Vegetable types in both Markets

In both markets put together, *Staphylococcus* occurred 10 times among a total 37 bacterial isolates with occurrence frequency of 27% in all vegetables with the exception of tomato and cucumber. *Bacillus* occurred 6 times (16% frequency) in cabbage, leeks, cucumber, onion, green peas, green bell pepper and carrots. Two isolates (*Listeria* and *Shigella*) occurred 3 times (8%) in 4 vegetables (tomato, green peas, cucumber and carrots) while *Salmonella*, *E. coli*, *Enterococcus* and *Klebsiella* occurred 2 times in different vegetables and *Micrococcus* occurred once in green bell pepper (Figure 2).

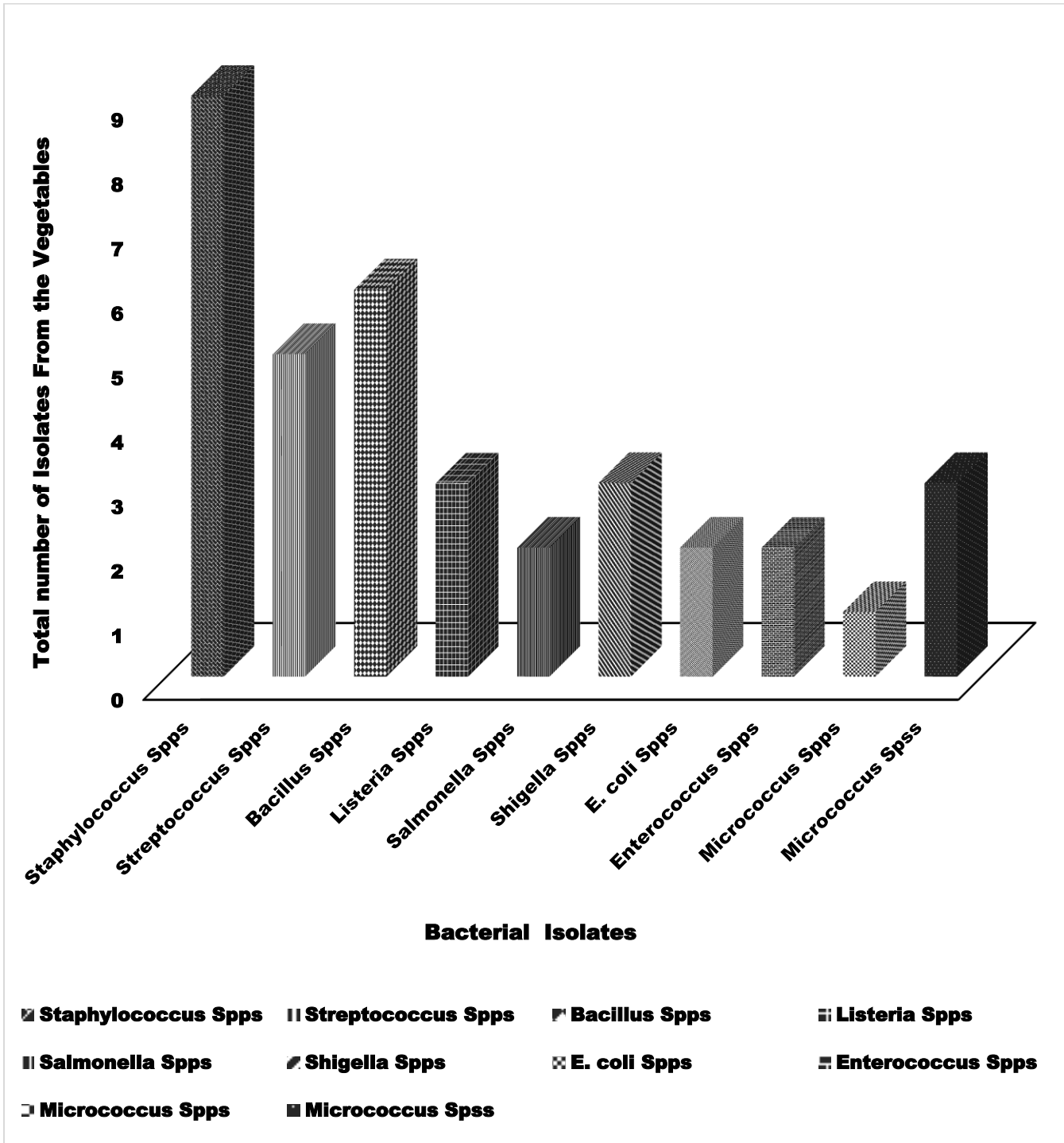
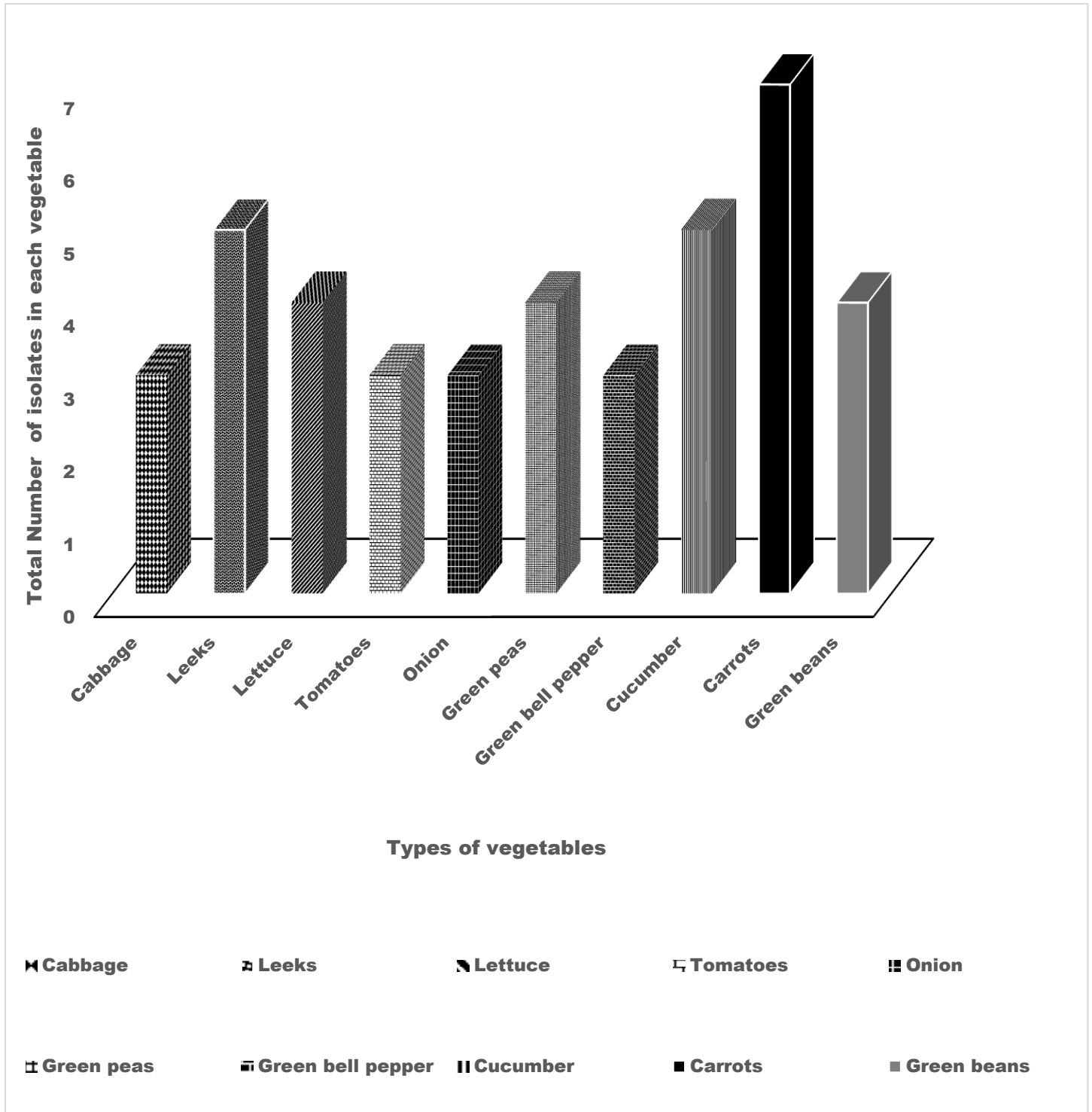


Figure 2: Total Number of Bacterial Isolates from both Markets.



**3.1.3. Total Number of Isolates in Each vegetable in both Markets:** Out of a total of 37 bacterial strains isolated 7 (19%) came from carrot samples, 5 (14%) from leeks and cucumber each, 4 (11%) from lettuce and green pea, each, while the least number 3 (8%) came from cabbage, tomato and onions each (Figure3).



**Figure 3: Total Number of Isolates in Each vegetable in both Markets.**

#### 4. DISCUSSION

The evaluation of bacteriological quality was conducted on 10 fresh salad vegetables (cabbage, carrot, green peas, green bell pepper, onion, green peas, cucumber, tomato, leeks and lettuce) obtained from Calabar Road and Marian Markets in Cross River State, Nigeria.

The high mean bacterial cell count of  $2.44 \times 10^8$  CfU/mL and  $2.13 \times 10^8$  CfU/mL obtained from the screened vegetables in both markets shows high level of contamination which implies potential health risk to consumers. Halablab *et al.* (2011) [11] reported similar bacterial contamination of fresh vegetables from different regions of Bekaa Valley in Lebanon. *Bacillus spp.*, were predominantly isolated from both market and would be expected given that *Bacillus* is a common soil bacterium that can easily contaminate vegetable which are harvested close to the ground level as those listed above. The spore of the bacterium is ubiquitous, able to resist heat, frizzing, chemicals and able to remain in a state of dormancy for years and re-germinate on exposure to nutrient [12]. This bacterium depending on species can cause two distinct food poisoning syndromes: rapid onset emetic syndrome characterized by nausea and vomiting and a slow onset diarrheal syndrome. Other species such as *B. cereus*, *B. subtilis* and *B. licheniformis* are periodically associated with bacteraemia/septicaemia, wound infection, respiratory tract, gastrointestinal tract infection etc. [13]. In the context of contamination of vegetable salad, it is significant that *Bacillus* species such as *Bacillus cereus* and *B. subtilis* are associated worldwide with food poisoning. The other importance of *Bacillus* as a spore-former is that it is a notable cause of spoilage of cooked foods such as vegetable soups if stored at ambient temperatures.

The high rate of isolation of *Staphylococcus* strains from most vegetables in both markets may suggest poor personal hygiene among the handlers. It is also significant to note that *Staphylococcus* species have been severally implicated in enterotoxin production and toxic shock syndrome [14] and food poisoning occasioning death after chicken salad consumption (CDC, 2012). Other organisms such as *Micrococcus*, *E. coli*, *Klebsiella*, and *Shigella* occurred sparingly. According to Cheesbrough, (2006) [10] there is potency in any pathogen recover in  $10^{-5}$  of any diluent.

The vegetables were contaminated in the order, carrots > leeks = cucumber > green peas = lettuce = green beans = cabbage = tomato = onions = green bell pepper. High contamination of cabbage, obtained from Marian market, with *Bacillus* species strongly indicates environmental factors as inferred by [15] who worked on cabbage and onions sold by vendors in Umuahia main market. Green beans was the least contaminated and may have been protected from contamination to a large extent by being concealed with a pod (the fruit). It is rather surprising that green peas obtained from Calabar Road market were more contaminated by *Bacillus* (mean count =  $1.92 \times 10^9$  CfU/mL) than with *staphylococcus* and *Listeria* species (each with a mean cell count of  $1.08 \times 10^9$  CfU/mL). High bacterial load obtained from this work generally signifies poor hygiene and need for thorough washing/rinsing before using the vegetables to prepare salad which is invariably served raw.

#### CONCLUSION

High mean bacterial cell count of  $2.44 \times 10^8$  CfU/mL and  $2.13 \times 10^8$  CfU/mL were obtained from the screened vegetables in both markets. *Staphylococcus* species appeared to be the most frequently isolated in both Marian (30%) and Calabar Road market ( $\approx 25\%$ ). *Bacillus spp* occurred at a higher frequency (20%) in Marian market vegetable samples than in samples from Calabar Road market. However, both strains occurred at approximately equal rate in vegetable samples from Calabar Road market ( $\approx 12\%$ ). Other organisms such as *Micrococcus*, *E. coli*, *Klebsiella*, and *Shigella* occurred sparingly. According to Cheesbrough, (2006), there is potency in any pathogen recover in  $10^{-5}$  of any diluent.

The vegetables were contaminated in the order, carrots > leeks = cucumber > green peas = lettuce = green beans = cabbage = tomato = onions = green bell pepper. The index research findings signifies poor

hygiene among handlers and need for thorough washing and rinsing of fresh salad vegetables especially when it is to be served raw.

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