



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

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Authors' contributions

This work was carried out in collaboration with all authors. Author MOE designed the experiment, including the methodology and carried out statistical analysis. Author ICU edit and proof read literature review and give scientific suggestions. Author THE provided some laboratory equipment for laboratory analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SAJRM/2019/v4i330106

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Complete Peer review History: <http://www.sdiarticle3.com/review-history/50184>

Original Research Article

Received 09 May 2019
Accepted 20 July 2019
Published 19 August 2019

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 2.44x10⁸ CfU/mL to 1.00 x10⁸ CfU/mL. In Calabar road market, the highest cell counts were observed in leeks with 2.44 x10⁸ CfU/mL followed by cabbage with 2.13 x10⁸ CfU/mL both for *Bacillus* species. The least count, 1.08 x10⁸ CfU/mL was obtained from green peas for *Listeria* and *Staphylococcus* species. In Marian market, the highest counts were 2.13 x10⁸ CfU/mL for *Bacillus* species obtained from cabbage while the lowest count was 1.00 x10⁸ CfU/mL for *Staphylococcus*

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species from green peas and cabbage. The most prevalent bacterial 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 the 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 foodborne 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.

Keywords: Fresh salad vegetables; Marian market; Calabar market; *Streptococcus*; *Bacillus* species; bacteriological quality.

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. [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 the 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 preservation. According to the 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, and onions. 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 the 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 to provide 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. The identity of each sample was affirmed by a botanist Professor S. Udoh CRUTECH. A 5 g 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} .

2.2 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® 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°C was added

and the Petri dish gently swirled until the contents mix thoroughly. The agar was allowed to set before incubation at 37°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 [10]. The identity of Gram-negative rods was confirmed using the Analytical Profile Index 20E (Biomérieux SA, France) kit.

2.3 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 2.44×10^8 Cfu/mL for *Bacillus* in leeks to 1.4×10^8 Cfu/mL for *Staphylococcus* species in cabbage. The least contaminated vegetable was lettuce with just 1 isolate with a prevalence of 5% in Calabar road (Table 1). In Marian market, the mean cell count ranged from 2.13×10^8 Cfu/mL of *Bacillus* in cabbage to 1.00×10^8 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).

3.2 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. Fig. 1 shows a comparison of the 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 an 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 (Fig. 1).

3.3 Distribution of Bacterial Isolates According to Vegetable Types in Both Markets

In both markets put together, *Staphylococcus* occurred 10 times among a total of 37 bacterial isolates with an occurrence frequency of 27% in all vegetables except for 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 (Fig. 2).

3.4 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 (Fig. 3).

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 2.44×10^8 Cfu/mL and 2.13×10^8 Cfu/mL obtained from the screened vegetables in both markets shows a high level of contamination which implies

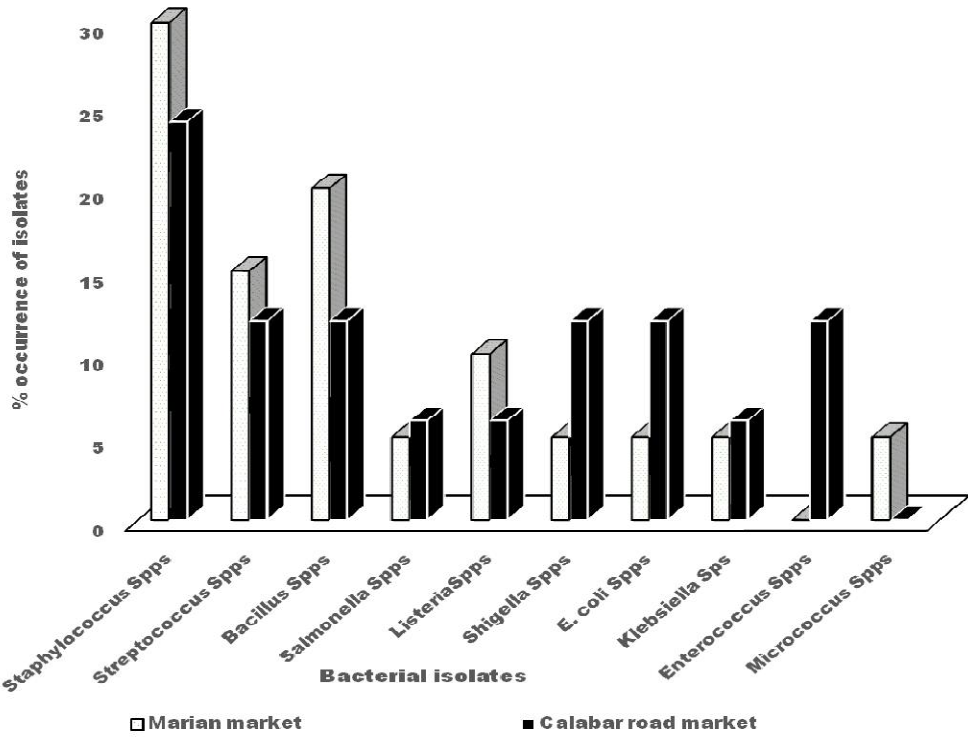


Fig. 1. Distribution of bacterial isolates according to markets

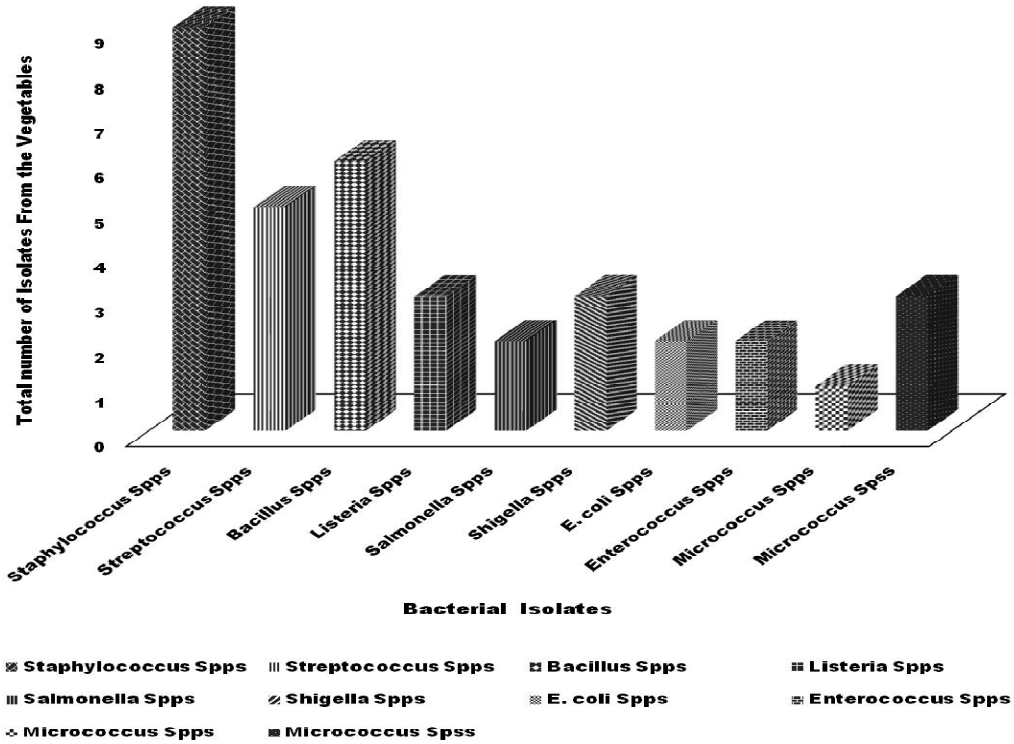


Fig. 2. Total number of bacterial isolates from both markets

potential health risk to consumers. Halablab et al. [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 is 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, and gastrointestinal tract infection [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, [10], there is potency in any pathogen recover in 10^{-5} of any diluent.

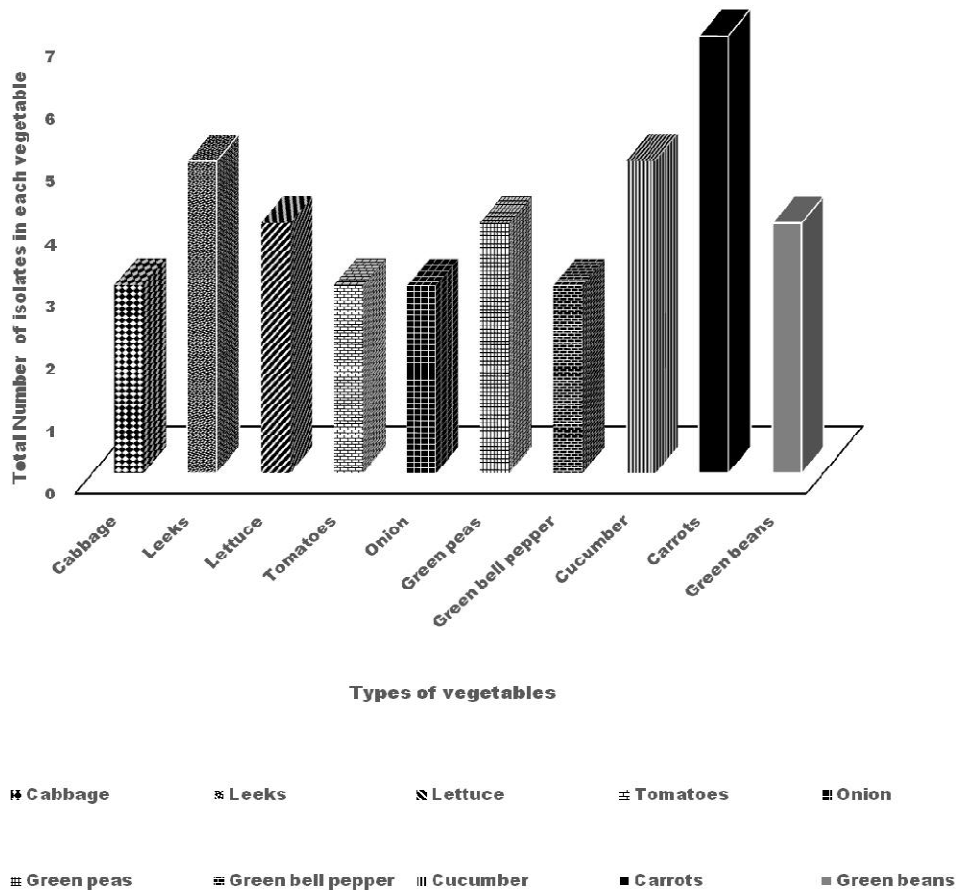


Fig. 3. Total number of isolates in each vegetable in both markets

Table 1. Mean bacterial cell counts in colony forming unit(cfu/ml) from various vegetables from calabar road

Vegetables	<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>Bacillus</i>	<i>Salmonella</i>	<i>Micrococcus</i>	<i>Listeria</i>	<i>Shigella</i>	<i>E . coli</i>	<i>Klebsiella</i>
Green beans	0	0	1.92 x 10 ⁸	0	0	0	0	1.2 x 10 ⁸	0
Lettuce	2.24 x 10 ⁸	0	0	0	0	0	0	0	0
Leeks	1.36 x 10 ⁸	0	2.44 x 10 ⁸	0	0	0	0	0	0
Cabbage	1.41 x 10 ⁷	0	1.68 x 10 ⁸	0	0	0	0	0	0
Carrots	1.76 x 10 ⁸	1.10 x 10 ⁸	0	0	0	0	0	0	0
Onions	1.16 x 10 ⁸	1.88 x 10 ⁸	0	0	0	0	0	0	0
Green pea	1.08 x 10 ⁸	0	0	0	0	1.08 x 10 ⁸	1.17 x 10 ⁸	0	0
Tomatoes	0	0	0	0	0	1.96 x 10 ⁸	0	0	1.24 x 10 ⁸
Cucumber	0	0	1.28 x 10 ⁸	1.21x10 ⁸	0	0	0	0	0
Green bell pepper	0	1.81 x 10 ⁸	0	0	1.48x10 ⁸	0	0	0	0

Table 2. Mean bacterial cell counts in colony forming unit (Cfu/ml) from various vegetables from marian market

Vegetables	<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>Bacillus</i>	<i>Salmonella.</i>	<i>Listeria</i>	<i>E . coli</i>	<i>Klebsiella</i>	<i>Shigella</i>	<i>Enterococcus</i>
Cabbage	0	1.07 x 10 ⁸	2.13 x 10 ⁸	0	0	0	0	0	0
Carrots	0	0	1.45 x 10 ⁸	0	0	0	0	1.31x10 ⁸	0
Cucumber	0	0	0	1.21 x 10 ⁸	1.48 x 10 ⁸	0	0	0	0
Green peas	0	1.50 x 10 ⁸	0	0	0	0	0	0	0
Green bell pepper	1.78 x 10 ⁸	0	0	0	0	0	0	0	0
Green beans	1.00 x 10 ⁸	0	0	0	0	1.19 x 10 ⁸	0	0	0
Lettuce	0	0	0	0	0	1.15x10 ⁸	1.52x10 ⁸	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	0	1.06 x 10	1.10 x 10

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 were 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 = 1.92×10^9 Cfu/mL) than with *staphylococcus* and *Listeria* species (each with a mean cell count of 1.08×10^9 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

The present study showed potential health risk of fresh salad items purchased from two (2) markets in Cross River State, Calabar. High occurrence of *Staphylococcus aureus* in both markets indicated contamination that may be linked to contact with human skin. The presence of *Bacillus* and *E. coli* spp. signified that items might have come in contact with contaminated soil during pre or post-harvest, possibly, the soil might have come in contact with faecal matters. The presence of other isolates (*Streptococcus*, *Salmonella*, *Listeria*, *Shigella*, *Klebsiella*, *Enterococcus* and *Micrococcus* spp) should not be undermined. According to Cheesbrough, (2006), there is potency in any pathogen recover in 10^{-5} of any diluent. The result may be useful in identifying possible bacteria associated with a particular vegetable. Also, it is important that the government set up better surveillance on the activities of fresh vegetable sellers within the local markets to minimize the risk of disease outbreak associated with consumption of contaminated vegetable.

ACKNOWLEDGEMENT

Sincere appreciation goes to my supervisor Prof. C. U. Iroegbu, and Mrs. Tarh, Jacqueline Eboh for their scientific suggestions, careful editing of the work and providing some laboratory

equipment that were needed for successful completion of this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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