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Research Article

PREVALENCE OF *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI* ON LEAFY VEGETABLES SOLD AT A COMMUNITY MARKET

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ABSTRACT

Objective: The aim of this study is to evaluate the presence of *Staphylococcus aureus* and *Escherichia coli* in leafy vegetables sold at a community market as well as determine their antibiotic susceptibility patterns on some commonly used antibiotics.

Methods: A total of thirty (30) different leafy vegetables of different species were bought from different vendors at Eke-Agbani market in Nkanu West L.G.A. The bacteria were isolated and identified using standard microbiological methods. Antibiotic susceptibility patterns were determined using the disk diffusion method.

Results: The mean bacterial load of *S. aureus* and *E. coli* from these samples ranged from 4.2×10^6 to 8.3×10^6 cfu/g and 4.7×10^6 to 7.6×10^6 cfu/g, respectively. The number of positive samples and negative samples are 18 (60%) and 12 (40%), respectively. The percentage distribution of both *S. aureus* and *E. coli* was 50%, respectively. The susceptibility of the isolates to antibiotics showed that all *E. coli* isolates had 100% sensitivity to ciprofloxacin, tarivid, reflacin, ceporex, and augmentin. All the *S. aureus* isolates had 100% sensitivity to gentamycin, amoxicillin, and ciprofloxacin. All the *S. aureus* isolates had 100% resistant to chloramphenicol.

Conclusions: The results revealed the presence of *S. aureus* and *E. coli* in the vegetables screened. The presence of these bacteria may pose a serious threat to human health as some of these vegetables are consumed without proper cooking. Therefore, adequate measures should be taken by consumers to ensure proper washing before consuming as well as advising the public on antibiotic use.

Keywords: Leafy vegetable, *Staphylococcus aureus, Escherichia coli,* bacterial contamination, antibiotic resistance

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INTRODUCTION

Leafy vegetables provide important food nutrients in our diet. The consumption of leafy vegetables, often called fresh produce has increased in recent years due to its multiple contributions of nutrients and functional properties [1]. A diet rich in fruits and vegetables has been shown to protect against various types of cancer and chronic illnesses, including coronary heart disease [2], at the same time, they are associated with a growing number of food-borne outbreaks due to microbial contamination [3]. Leafy vegetables are contaminated by the presence of pathogenic bacteria, viruses, and protozoa [4]. The contamination can occur during pre- or post-harvest and may originate from manure, soil, sewage, irrigation surface water, or wildlife sources [5]. It may also occur during washing, slicing, soaking, packing, and food preparation [6]. Microbial cross-contamination may also occur if particular water used to wash one type of vegetable is used to wash another. A number of microbial infections have been associated with the consumption of fresh vegetables. These infections are caused by bacteria and viruses [7]. Among the bacteria associated with foodborne illnesses from consumption of leafy vegetables are *Listeria monocytogenes, E. coli, Shigella soney, Salmonella,* and *Staphylococcus aureus* [8]. It is important to emphasize that leafy vegetables are prone to contamination at every step including cultivation, harvesting, transporting, packing, storage, and selling to the final consumers. Some sources of pre-harvest contamination include fecal matter, soil, irrigation water, improperly composted manure, air, and wild and domestic animals contact, while post-harvest sources include harvesting equipment, wash and rinse water, transport vehicles, improper storage, improper packaging, cross-contamination, and improper human handling [9,10]. Vegetables can be eaten either raw or cooked. They play an important role in human nutrition, being mostly low in fat and carbohydrates, but high in vitamins, minerals, and dietary fiber. Many nutritionists encourage people to consume plenty of fruit and vegetables, five or more portions a day often being recommended [11]. In developing countries, such as Nigeria, the use of untreated wastewater for irrigation, domestic animal wastes, and inadequately compost manure as fertilizer for the production of vegetables is a major contributing factor to bacterial contamination [12].

The consumption of fresh fruits and vegetables is expected to increase between 8 and 9% over the next 5 years [13]. As consumption increases, the number of recorded foodborne diseases involving fresh produce may also become more common in many countries, including Nigeria [14]. There are some factors that determine the survival and growth of bacteria on leafy vegetables. They are: fruit ripeness, environmental conditions, plant development, bacterial resistance to the plant metabolic processes, and pre- and post-harvest processes [15].

S. aureus is an important cause of food intoxication throughout the world. This bacterium can contaminate and produce several types of enterotoxins on various foods, including minimally processed ready-to-eat vegetables and processed meat products and produce several types of enterotoxins [16,17].

Escherichia coli, as an enteric pathogen, is becoming increasingly important from the viewpoint of public health. Pathogenic strains of *E. coli* particularly the O157:H7 strain can multiply on minimally processed vegetables and meat products even at 4–12°C causing hemorrhagic colitis following consumption [18]. The spread of enteric pathogens from livestock to food crops occurs through the application of manure, irrigation with contaminated water, dispersal by air, and dispersal through biological vectors [19]. In many cities in Nigeria,

ready-to-eat leafy vegetables are not subjected to further processing before consumption and are not monitored by any food protection agency. Although some are used as ingredients in cooked dishes, but many are consumed raw without cooking or given any treatment that would have destroyed pathogenic microorganisms present on them. As such, there is a high risk of consuming fresh produce contaminated with infectious agents, such as *E. coli* and *S. aureus*.

Foodborne diseases resulting from contamination of leafy vegetables has become a serious public health concern in both developed and developing countries [20]. Thus, there is a need to continually determine the microbiological safety of these fresh leafy vegetables. The aim of this study is to evaluate the presence of *S. aureus* and *E. coli* in leafy vegetables sold at a community market in Nkanu West Local Government Area of Enugu State, Nigeria. Some studies have reported the contamination of leafy vegetables by bacteria [21], but not much has been done in the area of *S. aureus* contamination on leafy vegetables in Nkanu West L.G.A.

METHODS

Sample collection

A total of thirty leafy vegetables were bought from different vendors at Eke-Agbani Market in Nkanu-West LGA, Enugu State, Nigeria. The leafy vegetables include: *Murraya koenigii* (curry leaf); *Ocimum gratissimum* (scent leaf), *Brassica oleraca var. capitate* (cabbage leaf), and *Teifairu occidentalis* (fluted pumpkin leaf). The samples were packed in a sterile poly bag and taken to the Microbiology Laboratory of Enugu State University of Science and Technology for bacteriological analysis.

Bacteriological analysis

The samples were analyzed within 1 h of sample collection following the method of [20]. Briefly, one gram of each leafy vegetable was rinsed with 10 ml of sterile distilled water, this was known as stock. A total of 1 ml was taken from the stock and added to 9 ml of sterile distilled water in a test tube. This was then swirled evenly to make the mixture homogenized. Then, ten-fold dilutions of the homogenates were made with sterile distilled water up to a dilution of 10^{-6}. After that, 0.1 ml of the 10^{-3} and 10^{-4} dilutions were dispensed into sterile mannitol salt agar and MacConkey agar petri dishes, respectively. Golden yellow, clusters, a small colony with a smooth surface on mannitol salt agar is presumptive of *S. aureus* while bright pink, raised, shiny, and smooth colonies on MacConkey agar represent *E. coli*. The plates were incubated at 37°C for 24 h. After incubation, the representative colonies on the plates were counted and recorded. Selected presumptive colonies showing distinct morphological characteristics were picked and subcultured to get pure isolates. Pure isolates were then transferred onto nutrient agar slant and stored for further identification.

Identification of bacterial isolates

Bacterial isolates were further identified by Gram reaction and some biochemical tests.

Gram staining

A bacteria isolate smear was prepared on a microscopic slide, air-dried, and heat-fixed. The slides were flooded with crystal violet for 60 s and washed off with water, then each slide was flooded again with iodine solution for 1 min and was washed off with water. Thereafter, the slide was decolorized with acetone until the solvent draining from the slide appeared colorless and was immediately washed with water. It was counterstained with safranin for 30 s and washed off with water. The slides were blotted and air dried and observed under an oil immersion objectives lens (×100).

Catalase test

A drop of hydrogen peroxide was made on one side of the clean microscopic slide and at the other end, a drop of water to serve as the control. A colony was then collected with a sterile applicator stick and smeared on the end containing hydrogen peroxide and the same was done for the control, side containing water. The presence of bubbles on the hydrogen peroxide within 10 s of application indicated positive. Results were noted and recorded.

Oxidase test

A drop of prepared oxidase reagent (tetramethyl p-phenylenediamine dihydrochloride) was made on Whatman filter paper. A colony of each isolate was collected with a sterile applicator stick and smeared on the soaked filter paper. The presence of a purple color which indicated a positive result was observed and recorded.

Indole test (kovac's method)

Peptone water (5 ml) was dispensed into test tubes and sterilized at 121°C/15p.s.i, and allowed to cool. Using a wire loop, each isolate was independently added into each test tube and incubated for 3 days. After incubation, Kovac's reagent (3 drops) was placed into the test tubes. The presence of a red ring, which indicated a positive result was observed and result recorded.

Vogues-Proskauer Test (Omeara's method)

A colony of the isolates was inoculated into already prepared sterile glucose phosphate peptone water (4 ml) medium and incubated at 37°C for 5 days. A spatula edge of creatinine was added to the cultured tubes. Sodium hydroxide (4 ml) was also added. Observation for a color change to deep blue or purple within 10–30 s was recorded as a positive result.

Coagulase test

A drop of normal saline was made on a clean slide; a colony was then collected with a sterile applicator stick and smeared on the slide. Test suspensions were treated with a drop of plasma and mixed well with the applicator. Observations for clumping were made and results recorded.

Sugar fermentation

Each of the isolates was tested for its ability to ferment a given sugar with the production of acid and gas or acid only. Since most bacteria especially gram-negative bacteria utilize different sugars as sources of carbon and energy with the production of both acid and gas or acid only, the test is used as an aid in their differentiation. The growth medium used was peptone water.

Briefly, 100 ml of peptone water were prepared in five different conical flasks, for the four sugars, 0.5 ml was discarded from each flask (which serves as a control for the test) and 0.5 ml of Andrade indicator was added in each conical flask; then 1g of each sugar was added into the flask and was shaken properly for the sugars to dissolve. 3 ml from each flask and 5 ml from glucose was measured out into 5 different test tubes. Durham's tube was added into the tubes containing the sugars for gas collection, the tubes with their content were then sterilized by autoclaving at 121°C for 15 min. The tubes were then inoculated with 24-h-old culture of the isolates and incubated at 37°C for 24 h. Acid production was indicated by a color change from orange to pink color while gas production was indicated by the presence of gas in Durham's tube; the control tube was not inoculated.

Antimicrobial sensitivity test

An antimicrobial sensitivity test was performed using the disc diffusion method.

The 0.5 Macfarland turbidity standards were used to adjust the turbidity of the inocula for the antimicrobial susceptibility testing. The 0.5 MacFarland turbidity standard was prepared by adding 0.5 ml of a 1.175% (wt/vol) Barium chloride dehydrate ($BaCL_2\,2H_2O$) solution to 99.5 ml of 1% (vol/vol) sulphuric acid (H_2SO_4). The turbidity standard was then aliquoted into screw-capped test tubes identical to those used to prepare the inoculum suspensions; the test tubes were then sealed with wax to avoid evaporation.

An inoculating needle was used to select the isolated colonies and these were transferred into test tubes containing sterile saline. The mixture

was vortexed thoroughly. The test tube containing the turbidity standard was also vortexed so that the white precipitate of Barium sulfate could be mixed well. The bacterial suspensions were then compared to the 0.5 MacFarland turbidity standards. During comparison, those bacterial suspensions that did not appear to be the same density as the 0.5 MacFarland turbidity standards were either reduced by adding sterile saline or increased by adding more bacterial cells.

Within 15 min after adjusting the turbidity of the inoculum suspension, sterile cotton swabs were dipped into the test tubes containing bacteria suspensions. The sterile cotton swab was pressed firmly against the inside wall of the test tubes just above the fluid level. The swabs were rotated to remove excess fluid. Each of the swabs was then streaked over the entire surface of a sterile (Muller-Hinton agar) plate. The plates were rotated at 60°C after each application to ensure even distribution of the inoculum. After the inoculation, the recommended antibiotics for gram-negative bacteria were placed individually with sterile forceps equidistant from each other on the inoculated petri dish. The discs were gently pressed down onto the agar and the agar plates were inverted and incubated at 37°C for 24 h. Clear zones of inhibition produced by the organisms were observed and measured. For each isolate, the test was carried out in triplicate.

Statistical analysis
All statistical analyses were done using the SPSS software package. The bacterial numbers were converted to base-10 logarithms.

RESULTS

Mean bacterial load of the leafy vegetables
A total of 30 samples of leafy vegetables from different vendors at Eke-Agbani Market in Nkanu-West LGA, Enugu State, Nigeria was examined for the presence of *E. coli* and *S. aureus* on MacConkey agar and Mannitol salt agar, respectively. The mean bacterial load (cfu/g) of the leafy vegetables was evaluated and the result is shown in Table 1.

Percentage distribution of positive and negative samples
The leafy vegetables were analyzed, some showed positive growth while some showed negative growth. This is shown in Table 2.

Percentage distribution of *S. aureus* and *E. coli* from the leafy vegetables

The percentage distribution of *S. aureus* and *E. coli* were determined, this is shown in Table 3.

Cultural and morphological characteristics of *S. aureus* and *E. coli* isolates from Leafy vegetables on media

The cultural and morphological characteristics of the two isolates were determined. This is shown in Table 4.

Antimicrobial susceptibility pattern of *S. aureus* isolates
All the *S. aureus* isolates had 100% sensitivity to gentamicin, ciprofloxacin, and amoxicillin. All the *S. aureus* isolates had 100% resistant to chloramphenicol (Fig. 1).

Antimicrobial susceptibility pattern of *E. coli* isolates
The isolates were subjected to different antibiotics. All the *E. coli* isolates had 100% sensitivity to ciprofloxacin, tarivid, reflacin, ceporex, and augumentin (Fig. 2).

DISCUSSION

Recent outbreaks of infectious diseases associated with vegetables have prompted increased interest in all aspects of the microbiological safety of vegetables, particularly those that are eaten raw or subjected to minimal processing before their consumption. Fresh vegetables are vulnerable to microbial contamination from the point of planting to the point of consumption. Fresh vegetables have natural effective immunity against most pathogenic microorganisms and plant spoilage. However, this protection or immunity however could be hindered due to the contamination of these vegetables. There are several factors that promote the contamination and post-harvest deterioration of vegetables by bacteria; these include contamination during field cultivation, harvesting, post-harvest handling, and distribution [22].

The mean colony count of the leafy vegetables ranged from 4.2×10^6 to 8.3×10^6 cfu/g for *S. aureus* and 4.7×10^6 to 7.6×10^6 for *E. coli* (Table 1).

Table 1: Mean bacterial load (cfu/g) of different leafy vegetables bought from Eke-Agbani Market

S. No.	Sample type	Mean of bacterial load (cfu/g)	
		Staphylococcus aureus	*Escherichia coli*
1.	*Murraya koenigii* (curry leaf)	5.1×10^6	6.6×10^6
2.	*Ocimum gratissimum* (scent leaf)	4.2×10^6	5.4×10^6
3.	*Brassica oleraca var. capitate* (cabbage leaf)	8.3×10^6	7.6×10^6
4.	*Telfairia occidentalis* (fluted pumpkin)	7.8×10^6	4.7×10^6

Table 2: Percentage distribution of positive and negative samples on Leafy vegetable Samples

S. No.	Type of samples	Number of samples	Number of Positive samples (%)	Number of Negative samples (%)
1.	*Murraya koenigii* (curry leaf)	7	6 (85.7)	1 (14.3)
2.	*Ocimum gratissimum* (scent leaf)	7	5 (71.4)	2 (28.6)
3.	*Brassica oleraca var*. capitate (cabbage leaf)	8	3 (37.5)	5 (62.5)
4.	*Telfairia occidentalis* (fluted pumpkin)	8	4 (50)	4 (50)
	Total	30	18 (60)	12 (40)

Table 3: Percentage distribution of *Staphylococcus aureus* and *Escherichia coli* from the leafy vegetables

S. No.	Type of samples	*Staphylococcus aureus*	*Escherichia coli*
1.	*Murraya koenigii* (curry leaf)	2 (40)	3 (60)
2.	*Ocimum gratissimum* (scent leaf)	2 (50)	2 (50)
3.	*Brassica oleraca var.* capitate (cabbage leaf)	2 (100)	-
4.	*Telfairia occidentalis* (fluted pumpkin)	3 (42.9)	4 (57.1)
	Total	9 (50)	9 (50)

Table 4: Cultural and biochemical identification of bacteria isolated from leafy vegetables bought at Eke-Agbani Market

Growth on Nutrient agar	Gram reaction	Catalase test	Oxidase test	Citrate utilization test	Motility test	Indole	Glucose fermentation	Lactose fermentation	Maltose fermentation	Fructose fermentation	Sucrose fermentation	Suspected organism
Circular, raised, shinny, smooth, colorless and non-pigmented colonies	Negative rod	+ve	-ve	-ve	-ve	+ve	A/G	A/G	A	A	A/G	*Escherichia coli*
Golden-yellow, clusters, small colony with smooth surface	Positive cocci	+ve	-ve	-ve	-ve	-ve	A/G	A/G	A/G	A/G	A/G	*Staphylococcus aureus*

Key: –ve=negative, +ve=positive, A=Presence of acid only, A/G=Presence of Acid and gas formation

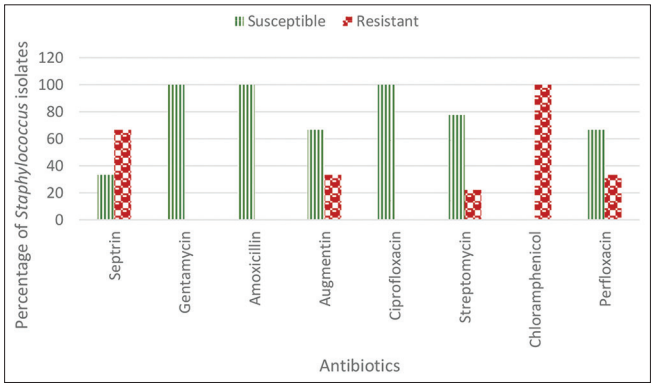

Fig. 1: Antimicrobial susceptibility pattern of *Staphylococcus aureus* isolates

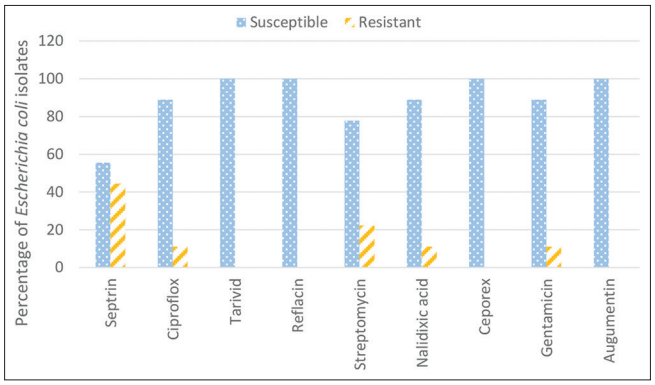

Fig. 2: Antimicrobial susceptibility pattern of *Escherichia coli* isolates

Consistent with previous studies [22-27], the result reports higher bacteria count in commonly consumed vegetables. The high microbial load of the leafy vegetables observed in this study did not meet up with the microbial load safety record of 10^3 cfu/g as recommended by the Food and Drug Agency [28]. This is in line with the report of FAO and WHO which stated that leafy green vegetables currently pose the greatest concern in terms of microbiological hazards. The high bacteria load of the leafy vegetables can be attributed to poor processing methods which may involve washing and spraying with fecal contaminated stream water [29].

The present study showed that positive samples were high in *Murraya koenigii* (curry leaf) with 6 (85.7%) and least in *Brassica oleraca var. capitate* (cabbage leaf) with 3(37.5%) (Table 2). This high number of positive samples could be attributed to the cross-contamination that occurred during the washing of these vegetables where the same water was used to wash almost all the vegetables sold. This is in line with the work of [20] who reported that 10 (16.7%) was the highest positive samples gotten from vegetables at Tirkania.

The result of the isolation and identification of bacteria from leafy vegetables revealed that bacteria were the major organisms responsible for the post-harvest spoilage of leafy vegetables many of which could be pathogenic to humans. A total of 30 leafy vegetables were studied and from these 30 leafy vegetables, 9 (50%) had *E. coli* while 9 (50%) had *S. aureus* (Table 3). This result is in line with the observation of [30] who reported that bacteria were the major sources of contamination and post-harvest spoilage of vegetables. Earlier works such as [22] isolated various strains of bacteria from vegetables and observed *Bacillus cereus* and *E. coli* as the major contaminants and agents of post-harvest deterioration of vegetables. This study is in line with the work of [31], who reported 41.2% of *E. coli* and 33.3% of *S. aureus* from leafy vegetables. *E. coli* was isolated from the vegetable

which shows that this vegetable can be a source of foodborne disease if it is eaten raw or not properly cooked as is the custom with many people in this part of the world. It is believed that half-done vegetables retain their nutrients. With the number of incidences of foodborne diseases resulting from eating raw or not well-cooked vegetables, it will be necessary to decontaminate the vegetables before cooking in a way that will retain their nutrients and free of foodborne pathogens. *E. coli* is regarded as the primary indicator for microbiological quality of food and water and this indicates that the leafy vegetables are not safe for human consumption [32]. The high incidence of *E. coli* may probably be due to handling by buyers and sellers whose hands may have been contaminated with fecal matter or they may have been contaminated from the farm yards when fertilized with human and animal manures.

S. aureus is more likely to have been introduced onto the leaves from the hands of the handlers which include the farmers and the sellers. As ascribed by [33], this could be from the point of harvesting to the packaging, sizing for sale, and also from the utensils and packaging bags used by the sellers. The ingestion of a sufficient load of *S. aureus* is also undesirable due to the ability of *S. aureus* to cause serious food poisoning with fatal consequences.

It has been identified by recent study that some independent variables that demonstrated a significant correlation with bacterial infection are personal hygiene, environment hygiene, consumption of unhygienic vegetables, age, gender, and use of contaminated manure and water to plants [32,33]. It can be said from this study that these vegetables are contaminated with foodborne pathogens and therefore care should be taken when preparing them for consumption.

The antimicrobial susceptibility pattern of the isolates revealed varying levels of resistance to the antibiotics tested. All *E. coli* isolates showed 100%sensitivity to tarivid, reflacin, ceporex, and augumentin. All of the *S. aureus* isolates had 100% sensitivity to gentamycin, amoxicillin, and ciprofloxacin. All of the *S. aureus* isolates had 100% resistant to chloramphenicol. This result was in agreement with that obtained in a similar study carried out in China by [18] who reported high antimicrobial resistance of *S. aureus* from retail vegetables. The reason for this resistance may be due to abuse by people and widespread use of these antimicrobial agents in society for purposes other than therapeutic use by humans [34].

CONCLUSION

The study has revealed that the microbial quality of vegetables sold by different vendors at the community market is of low standard and unfit for human consumption and there is a need for it to be washed very well before consumption. Bacterial contamination was evident by the presence of *E. coli* and *S. aureus* isolated from leafy vegetables. The research showed that bacteria are still a health problem among vegetable consumers in the study area, the presence of the bacteria isolated from leafy vegetables could pose a serious threat to the health of consumers of this vegetable as they are pathogenic and are harmful when consumed. This study has shown that the high levels of drug resistance in humans in this environment are not only due to the abuse or indiscriminate use of antibiotics in humans but also due to the presence of resistant organisms in herbs or foods eaten raw or minimally processed.

AUTHORS CONTRIBUTIONS

Celestina C Ugwu as the lead author and corresponding author drafted the topic, collected the samples, carried out the experiment, and wrote the manuscript.

CONFLICTS OF INTEREST

The author declares that there is no conflict of interest.

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