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Prevalence and Antibiotic Resistance Profiles of *Escherichia coli* and *Staphylococcus aureus* in Leafy VegeTables from Open Markets in Abakaliki, Nigeria: Implications for Food Safety and Public Health

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ABSTRACT

Leafy vegeTables are vital component of a healthy diet but can harbor pathogenic bacteria, posing significant public health risks when consumed raw or inadequately washed. This study aimed to isolate and evaluate the antimicrobial resistance profiles of *Escherichia coli* and *Staphylococcus aureus* on commonly consumed leafy vegeTables from open markets in Abakaliki, Nigeria. Fifteen vegeTable samples, including Ugu, Green, Curry, Scent, and Bitter leaf, were analyzed using standard microbiological techniques for bacterial isolation and enumeration of colonies. *Escherichia coli* and *Staphylococcus aureus* were identified using the VITEK 2 automated system, while antibiotic susceptibility testing was performed via the Kirby-Bauer disc-diffusion method. The results of colonies enumeration indicated an average counts exceeding 1.3 x 10^1 CFU/ml and 3.6 x 10^1 CFU/ml for *S. aureus* and *E. coli respectively*. Results of contamination levels, revealed that 60% of the samples were positive for *E. coli* and 86.7% for *S. aureus*, including 60% co-contaminated with both pathogens. *Escherichia coli* exhibited high resistance to Tetracycline (66.7%) and Cotrimoxazole (55.5%) while *S. aureus demonstrated* significant resistance to Erythromycin (61.5%), Ampicillin, and Cloxacillin (53.9%). Multiple Antibiotic Resistance Index (MARI) values ranges from 0 to 0.6 among isolates. *Escherichia coli* isolates were susceptible to Gentamicin (100%) while *Staphylococcus aureus* isolates were susceptible to both Chloramphenicol, and Ciprofloxacin (84.6 %) and Gentamicin 92.3%. Our findings emphasize the urgent need for improved hygiene practices in leafy vegeTable handling and stricter regulations to mitigate the prevalence of antibiotic resistance and foodborne disease risks in Abakaliki.

Keywords: E coli, S. aureus, antibiotic resistance, leafy vegeTables

Introduction

Leafy vegeTables are known to possess phytonutrients, vitamins, and minerals that support metabolic functions, and are essential constituent of a healthy diet (WHO, 2020; Udu-Ibiam *et al.,* 2022). Despite their significant nutritional benefits, leafy vegeTables can serve as reservoir for the dissemination of pathogenic bacteria, when inadequately washed or consumed raw (Biswas *et al*., 2020). In Nigeria and other regions of the world, the risk of bacterial contamination is facilitated by exposure to unsanitary conditions during harvesting/production, vending,

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transportation and open display for sale (Losio *et al*., 2015). Among the pathogenic bacteria of public health concern, *Escherichia coli* and *Staphylococcus aureus* have significant been implicated with foodborne illnesses that are difficult to treat due to increasing antibiotic resistance to multiple drug classes (Joseph *et al.,* 2023; Orji *et al.,* 2024; Orji *et al.,* 2025). VegeTables typically have 10^3 to 10^7 microorganisms/cm^3 or microorganisms/g with most common bacteria genera include *Enterococcus*, lactic acid bacteria*, Pseudomonas, Corynebacterium*, *Proteus,* and *Micrococcus* (Sharma *et al.,* 2023).

Escherichia coli, a common indicator of fecal contaminant, is associated with diarrheal diseases, while *S. aureus* a ubiquitous microbiota expresses virulence by producing heat-sTable enterotoxins that cause food poisoning (Kothe *et al*., 2023; Peter *et al.,* 2024). Contamination of leafy vegeTables can occur through numerous routes, including exposure to contaminated irrigation water, soil, animal waste use as manure and unhygienic handling (Bishop and Okwori, 2017).

Furthermore, the frequent misuse of antibiotics in both human medicine and agricultural settings as growth promoter has aggravated the emergence and rapid spread of multidrug-resistant bacteria (MDR), limiting treatment options for MDR infections (Nomeh *et al*., 2023).

In Abakaliki, Southeastern Nigeria, open market remains the primary source of open display of fresh leafy vegeTables. There is paucity of data on the bacterial quality and antibiotic resistance pattern of associated bacterial pathogens.
Earlier published studies on fresh produce, in other regions have reported high prevalence of antimicrobial resistance and microbial contamination, revealing the need for local assessments of fresh leafy vegeTables (Udu-Ibiam *et al.,* 2022; Yafetto *et al*., 2019; Obeng *et al*., 2018). This study therefore aims to: (i) Isolate *E. coli* and *S. aureus* in leafy vegeTables e. g., curry, *ugu*-pumpkin, green, bitter leaves and scent) from Abakaliki markets and (ii) also determine their antibiotic resistance profile to assess resistance trends and provide evidence-based recommendations to improve food safety and public health practices. By addressing the outline objectives, this study contributes to the growing body of evidence-based research on food-borne pathogenic bacteria and antibiotic resistance surveillance in Nigeria, while informing policy and consumer awareness initiatives to mitigate health risks associated with contaminated vegeTables.

Materials and Methods

Study area and description

The present study was conducted in Abakaliki, located at Latitude 6° 19’ 60.00” N and Longitude 8° 05’ 60.00” E. Earlier report by United Nations (2024), shows the metropolitan population of Abakaliki in 2024 is estimated at 693,000 (Nwojiji *et al*., 2025a). The residents of Abakaliki primarily engage in petty trading and farming as key economic activities. The samples for this study were collected from two prominent market namely; International Market and Kpiri-Kpiri market. International and Kpiri-Kpiri markets are both located in densely populated areas of Abakaliki. In Abakaliki, the International Market, is an ultramodern central market that serves not only peoples in Abakaliki, but also surrounding suburbs and Ebonyi State.

Collection of leafy vegeTable Sample

A total of fifteen fresh leafy vegeTable samples, comprising five different types bitter leaf, *Ugu* (pumpkin), curry leaf, green vegeTable (African spinach), and scent leaf, were purchased in duplicates from selected vendors at the Kpiri-Kpiri and International markets in Abakaliki. Each leafy vegeTable sample were placed separately in a sterile polythene bag (manufactured by Aqua Rapha Investment Nigeria Limited, Enugu, Nigeria) and immediately transported to the Department of Microbiology for further analysis. All samples were analyses within 1–2 hours of collection.

Processing of VegeTable Samples

The vegeTable samples were processed using the methodology described by Yafetto *et al.* (2019). Briefly, 10 g of each of the following bitter leaf, *Ugu* (pumpkin), curry leaf, green vegeTable (African spinach), and scent leaf, were separately weighed, washed with sterile water, and transferred into a 250 mL conical flask. Subsequently, 90 mL of sterile 0.85% normal saline (diluent) was added. The mixture was gently agitated for 5 minutes to dislodge surface-adhered microorganisms. The resulting suspension was decanted and used as the stock solution for serial dilution. Serial dilutions were prepared by aseptically transferring 1 mL of the stock solution into a test tube containing 9 mL of sterile distilled water (10^{-1} dilution). Using a sterile micropipette, this process was repeated sequentially to obtain dilutions of 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} (Joseph *et al*., 2023). The same procedure was replicated for the remaining four vegeTable types to ensure consistency in sample preparation for microbiological analysis.

Inoculation and Isolation of Bacteria

Serially diluted sample of dilution factor 10^{-3} and 10^{-4} was used for bacterial isolation. A 0.5ml serially diluted sample from each dilution factor 10^{-3} and 10^{-4} were pipetted onto two separate sterile petri dish before 20ml of sterile Mannitol salt agar (Thermo Scientific™, U. S.A) and Eosin methylene blue Agar (EMB) (Thermo Scientific™, U. S.A) was added onto the plates and mixed prperly. After overnight incubation, colonies were counted and reported as described by Joseph *et al.* (2023). Colonies with golden-yellow and greenish metallic sheen on Mannitol salt agar (Thermo Scientific™, U. S.A) and Eosin methylene blue Agar (EMB) (Thermo Scientific™, U. S.A) respectively were sub-cultured through successive streaking and incubated at 37°C for 24 hours to obtain pure cultures. *E. coli* and *S. aureus* were characterized using standard microbiological protocol, including motility testing, methyl red test, Gram staining, Voges-Proskauer test, oxidase test, indole test, citrate utilization, triple sugar iron (TSI) test, methyl red test, and carbohydrate fermentation tests (lactose, sucrose, mannitol, glucose) (Iroha *et al.,* 2019; Nwojiji *et al.,* 2025a; Nwojiji *et al.,* 2025b. The *E. coli* and *S, aureus* were further identification, using the VITEK 2 automated system (bioMérieux, France) according to the manufacturer's instructions (Nwojiji *et al.,* 2025b).

Antimicrobial Resistance Testing

Antibiotic susceptibility testing of *E. coli* and *S. aureus* was determined using the Kirby-Bauer disk diffusion method (CLSI, 2022; Nwojiji *et al.,* 2025b). Bacteria inoculum equivalent to 0.5 McFarland standard of the isolate was streaked on entire solidified Mueller-Hinton plates. The following antibiotic; Cotrimoxazole (25 µg), Erythromycin (15 µg), Ampicillin (30 µg), Tetracycline (30 µg), Streptomycin (25 µg), Ciprofloxacin (5 µg),, Ofloxacin (5 µg), Piperacillin (30 µg), ceftriaxone (30 µg), Roxithromycin (15 µg), Levofloxacin (5 µg), Cloxacillin 30 µg), Chloramphenicol (5 µg), Gentamicin (10 µg) were placed aseptically on the inoculated plate and incubated at 37°C. After overnight incubation, zone of inhibition was measured and interpreted as Resistant **(R)** and Susceptible **(S)** to each of the tested antibiotic as per recommended standard (CLSI, 2022; Nwojiji *et al.,* 2025b).

Determination of Multiple Antibiotic Resistance Index

The multiple antibiotic resistant indexes (MARI) for each isolates were equally derived using the mathematical expression of Edemekong *et al.* (2022) and John-Onwe *et al.* (2023), which is given in equation 1:

$$\textbf{MAR index} = \frac{a}{b} \qquad \textbf{(1)}$$

Where:

a represent the number of antibiotics to which the isolates was resistant

b is the total number of antibiotics against which an individual isolate was tested.

Results

Mean Bacterial Counts of Leafy VegeTables Obtained From International Market, Abakaliki, Ebonyi State, Nigeria.

The bacterial contamination levels of *S. aureus* and *E. coli* in different leafy vegeTables purchased from the International Market are shown in Table 1. Spinach samples from Lot 1 showed the highest bacterial loads, with mean *S. aureus* and *E. coli* counts of 4.4×10^4 CFU/g and 8.3×10^2 CFU/g, respectively. Across all samples, *Staphylococcus aureus* counts ranged from 1.3×10^1 CFU/g to 4.4×10^4 CFU/g, while coliform counts varied between 1.5×10^1 CFU/g and 8.3×10^2 CFU/g.

Occurrences of *E. coli* and *S. aureus* based on type of VegeTables from different Location

The occurrence of *E. coli* and *S. aureus* across different vegeTable types and sampling locations is shown in Tables 3. Among the 15 samples comprising five leafy vegeTable varieties: *E. coli* was found in 9 samples accounting for 60.0% while *S. aureus* showed high contamination in 13 samples recording 80.0%. Co-contamination with both pathogens occurred in 9 samples. *Ugu* (pumpkin leaves) and scent leaf exhibited the highest contamination levels. Green vegeTables from three locations showed

Table 1: Mean Bacterial Counts of Leafy VegeTables Obtained from International Market, Abakaliki, Ebonyi State, Nigeria

			Mean MSA strains and EMS strains Bacterial Counts (Cfu/g)	
S/N	Vegetable samples	Sample Code	Yellow Colonies on MSA	Deep pink Colonies on E.M.B
1.	Ugu (pumpkin) from (Lot1)	UG1	6.9×10^3	4.8×10^2
2.	Ugu (pumpkin) from (Lot 2)	UG2	2.4×10^2	1.5×10^1
3.	Ugu (pumpkin) from (Lot 3)	UG1	7.6×10^2	6.4×10^1
4.	Bitter leaf (Lot 1)	BL1	2.3×10^2	1.6×10^2
5.	Bitter leaf (Lot 2)	BL2	3.2×10^2	4.3×10^2
6.	Bitter leaf (Lot 3)	BL3	2.4×10^2	2.9×10^2
7.	Scent leaf, (Lot 1)	SL1	8.7×10^2	5.4×10^2
8.	Scent leaf, (Lot 2)	SL2	4.2×10^2	6.3×10^1
9.	Scent leaf, (Lot 3)	SL3	2.8×10^2	1.8×10^1
10	Green vegetable (A. spinach) (Lot 1)	GR1	4.4×10^4	8.3×10^2
11.	Green vegetable (A. spinach) (Lot 2)	GR2	2.8×10^2	3.1×10^2
12.	Green vegetable (A. spinach) (Lot 3)	GR3	1.30×10^2	1.8×10^2
13	Curry leaf (Lot 1)	CL1	1.3×10^1	1.9×10^1
14./	Curry leaf (Lot 2)	CL2	4.2×10^2	3.6×10^1
15.	Curry leaf (Lot 3)	CL3	6.7×10^2	4.2×10^1

KEY: E.M.B- Eosin methylene Blue Agar, MSA- Mannitol Salt Agar significant pathogen presence. While samples from Abakaliki International Market recorded the highest *Staphylococcus* counts (Table 1), this did not consistently correlate with prevalence patterns across all sites (Table 2).

Antibiotics Resistance profile of *Escherichia coli* and *Staphylococcus aureus* Isolates From Leafy VegeTable Sold Within International Market, Abakaliki

The antibiotic resistance patterns of the bacterial isolates are presented in Table 4. Among nine *E. coli* isolates tested against a panel of 12 antibiotics, the following susceptibility profile was observed: Most effective antibiotics Gentamicin demonstrated 100% efficacy against all isolates. High susceptibility was observed for: Chloramphenicol: 88.9% (8/9 isolates); Ciprofloxacin 88.9% (8/9 isolates), Levofloxacin: 77.8% (7/9 isolates). Moderately effective antibiotics (66.7% susceptibility): Ceftriaxone, Roxithromycin

Table 2: Ocurrence of *E. coli* and *S. aureus* and their co-contaminations on leafy VegeTable sold in International Market, Abakaliki, Nigeria

Vegetable samples	Sample Code	*Escherichia coli*	*Staphylococcus aureus*	Co-contaminations
Ugu (pumpkin) from (Lot 1)	UG1	+	+	+
Ugu (pumpkin) from (Lot 2)	UG2	-	+	+
Ugu (pumpkin) from (Lot 3)	UG3	+	+	+
Bitter leaf (Lot 1)	BL1	-	+	-
Bitter leaf (Lot 2)	BL2	+	-	-
Bitter leaf (Lot 3)	BL3	-	+	-
Scent leaf, (Lot 1)	SL1	+	+	+
Scent leaf, (Lot 2)	SL2	-	+	-
Scent leaf, (Lot 3)	SL3	+	+	+
Green vegetable (Africa spinach) (Lot 1)	GR1	+	+	+
Green vegetable (Africa spinach) (Lot 2)	GR2	+	+	+
Green vegetable (Africa spinach) (Lot 3)	GR3	+	+	+
Curry leaf (Lot 1)	CL1	+	-	-
Curry leaf (Lot 2)	CL2	-	+	-
Curry leaf (Lot 3)	CL3	-	+	+
Number (%) Positive Sample		**9 (60.0)**	**13 (86.7)**	**8 (60)**

KEY: E.M.B- Eosin methylene Blue Agar, MSA- Mannitol Salt Agar

Table 3: Ocurrence of *E. coli* and *S. aureus* based on type of VegeTables from different Location

Vegetable samples	Number of Samples Examined	Number (%) Containing *E.coli*	Number (%) containing *S. aureus*
Ugu (pumpkin)	3	2 (66.7)	3 (100)
Bitter leaf	3	1 (33.3)	2 (66.7)
Scent leaf	3	2 (66.7)	3 (33.3)
Green vegetable	3	2 (66.7)	2 (66.7)
Curry leaf	3	1 (33.3)	2 (66.7)
Total sample Positive Sample (%)	**15**	**9 (60.0)**	**13 (86.7)**

and Ampicillin. The highest resistance patterns was against Tetracycline: 66.7% resistance (6/9 isolates) and Cotrimoxazole 55.6% resistance (5/9 isolates). Analysis of 13 *S. aureus* isolates against 12 antibiotics revealed: highest susceptibility to Gentamicin at 92.3% (12/13 isolates). Moderate susceptibility to Chloramphenicol: 84.6% (11/13) and Ciprofloxacin 76.9% (10/13). A significant resistance to Erythromycin accounted: 61.5% (8/13) and Ampicillin and Cloxacillin 53.9% (7/13 each).

Antibiotics Resistant Pattern, Multiple Antibiotic resistant Index (MARI) of *Escherichia coli* Isolates from VegeTables Sold within International Market, Abakaliki

The Multiple Antibiotic Resistance Index (MARI) analysis demonstrated: resistance spectrum with CL1-E showed highest resistance (MARI 0.6; resistant to 6/12 antibiotics) and SL1-E exhibited minimal resistance (MARI 0.1; resistant to 1/12 antibiotics) as shown in Table 5

The distribution profile revealed that 40% of isolates showed moderate resistance (MARI 0.3-0.5) while 20% displayed high resistance (MARI ≥0.5)

Clinical Implications: The observed resistance patterns, particularly: the 50% resistance rate in CL1-E and elevated MARI values (>0.2) in 60% of isolates.

Antibiotics Resistant Pattern and Multiple Antibiotic resistant Index (MARI) of *Staphylococcus aureus Isolates* Isolates from VegeTable Sold within International Market, Abakaliki.

The overall antibiotic resistance patterns of *S. aureus* isolates against a panel of 12 antibiotics are presented in Table 6. The analysis reveals resistance profiles among the *S. aureus*, as characterized by their Multiple Antibiotic Resistance Index (MARI) values ranging from 0-0.5.

The proportion of highly resistant isolates with MARI of 0.5 were as follows: BL1-S, BL3-S, SL1-S, and SL3-S exhibit resistance to 6-7 antibiotics. These isolates represent significant multidrug-resistant (MDR) strains

The proportion of moderately resistant isolates with MARI of 0.2 were as follows: UG3-S and SL2-S showed low resistance patterns. This suggests that the bacteria originated from environments with

Table 3: Antibiotics Resistance profile of Escherichia coli and Staphylococcus aureus Isolates From Leafy VegeTable Sold Within International Market, Abakaliki

	Escherichia coli (n =9)			*Staphylococcus aureus (n =13)*		
Antibiotics Disc (µg)	**S (%)**	**I (%)**	**R (%)**	**S (%)**	**I (%)**	**R (%)**
Cotrimoxazole (25)	2 (22.2)	2 (22.2)	5 (55.51)	8(61.5)	1 (7.7)	4(30.8)
Erthromycin (15)	NA	NA	NA	4(30.8)	1 (7.7)	8(61.5)
Ampicillin (10)	6 (66.7)	2 (22.2)	1 (11.1)	3 (23.1)	3 (23.1)	7(53.9)
Tetracycline (30)	1 (11.1)	2 (22.2)	6 (66.7)	5 (38.5)	3 (23.1)	5 (38.5)
Streptomycin (30)	NA	NA	NA	7(53.9)	2(15.4)	4(30.8)
Cefotaxime (10)	5 (55.51)	1 (11.1)	3 (33.3)	**NA**	**NA**	**NA**
Ciprofloxacin (10)	8 (88.9)	1 (11.1)	0(0.0)	11(84.6)	1 (7.7)	1 (7.7)
Ofloxacin (10)	3 (33.3)	3 (33.3)	3 (33.3)	10(76.9)	2(15.4)	1 (7.7)
Roxithromycin (15)	6 (66.7)	0(0.0)	3 (33.3)	6(46.15)	4(30.8)	3 (23.1)
Levofloxacin (10)	7 (77.7)	0(0.0)	2 (22.2)	10(76.9)	0(0.0)	3 (23.1)
Ceftriaxone (10)	6 (66.7)	1 (18.75)	2 (22.2)	NA	NA	NA
Cloxacillin (5)	NA	NA	NA	5 (38.5)	1 (7.7)	7(53.9)
Piperacillin (10)	4 (44.4)	3 (33.3)	2 (22.2)	NA	NA	NA
Chloramphenicol (30)	8 (88.9)	1 (6.25)	0 (5.0)	11(84.6)	2(15.4)	0(0.0)
Gentamicin (10)	9 (100.0)	0 (12.5)	0(6.25)	12(92.3)	1 (7.7)	0(0.0)

Key: **S**- Susceptible, **I-** Intermediate**, R-**Resistance**, NA-**Not Applicable**, %- Percentage, µg-** microgram, **n**-number of isolate.

Table 5: Antibiotics Resistant Pattern, Multiple Antibiotic resistant Index (MARI) of *Escherichia coli* Isolates from VegeTables Sold within International Market, Abakaliki

		Escherichia coli (N =9)		
Escherichia coli	**No. of Antibiotics**	**Resistant Pattern**	**MARI**	**(%) Resistant to 12 Antibiotic**
UG1-E	9	BA-TE	0.2	16.7
UG1-E	9	BA- AS- TE-CF	0.3	33.3
BL2-E	9	TE- OF-LE- PT	0.3	33.3
SL1-E	9	LE	0.1	8.3
SL3-E	9	BA-AS- OF- RO- CR	0.4	41.7
GR1-E	9	AS-TE-RO- PT	0.3	33.3
GR2-E	9	BA- CF	0.2	16.7
GR3-E	9	AS- TE	0.2	16.7
CL1-E	9	BA-AS- CF- OF- RO-LE	0.6	50.0

Key: MARI- Multiple Antibiotic resistant Index, Antibiotics Used Cotrimoxazole (BA) 25 µg, Erythromycin (E) 15 µg, Ampicillin (AS) 10 µg, Tetracycline (TE) 30 µg, Streptomycin (S) 10 µg, Ciprofloxacin (CP) 5 µg, Ofloxacin (OF) 5 µg, Roxithromycin (RO) 15 µg, Levofloxacin (LE) 5 µg, Cloxacillin (CX) 1 µg, Chloramphenicol (C) 30 µg, Gentamicin (GN) 10 µg.

Table 6: Antibiotics Resistant Pattern and Multiple Antibiotic resistant Index (MARI) of *Staphylococcus aureus* Isolates from VegeTable Sold Within International Market, Abakaliki

Staphylococcus aureus isolates Code	Antibiotics Tested	Resistant Pattern	MARI	(%) Resistant to 12 Antibiotic
UG1-S	12	BA- E- TE-CF	0.3	30.8
UG2-S	12	TE- OF-LE- RO -PT- S- CX	0.3	53.8
UG3-S	12	E-CX	0.2	15.4
BL1-S	12	BA-E-AS-OF-CR-S-CP	0.5	53.8
BL3-S	12	AS-E-TE-RO- PT- CX	0.5	50
SL1-S	12	BA-E-S-RO-CF-CX	0.5	50
SL2-S	12	AS- TE	0.2	15.4
SL3-S	12	BA-E-AS-CF-OF-LE	0.5	50.0
GR1-S	12	TE- S- RO –CX	0.3	30.8
GR2-S	12	E- AS- TE- S	0.3	30.8
GR3-S	12	E- AS-LE- CX	0.3	30.8
CL2-S	12	0	0	0
CL3-S	12	E- AS- CX	0.2	23.1

Key: MARI- Multiple Antibiotic resistant Index, **Antibiotics Used** Cotrimoxazole (BA) 25 µg, Erythromycin (E) 15 µg, Ampicillin (AS) 10 µg, Tetracycline (TE) 30 µg, Streptomycin (S) 10 µg, Ciprofloxacin (CP) 5 µg, Ofloxacin (OF) 5 µg, Roxithromycin (RO) 15 µg, Levofloxacin (LE) 10 µg, Cloxacillin (CX) 5 µg, Chloramphenicol (C) 30 µg, Gentamicin (GN) 10 µg.

minimal antibiotic exposure.
CL2-S was the only isolate was completely susceptible and exhibited a MARI of 0 to all tested antibiotics

Discussion

Leafy vegeTable offers essential health benefits for human consumption, but if no proper hygienic practices are followed during handling, it may result in public health risk of potential infection and food poisoning. After purchase, most consumers adequately fail to wash vegeTables, increasing the risk of exposure to foodborne pathogen (Baishakhi *et al*., 2020).

Our study shows that fresh leafy vegeTables harbors pathogenic bacteria, acting as potential vehicle for the dissemination of food-borne disease. All screened samples were contaminated with *S. aureus* and *E. coli* strains. From our findings, the highest bacterial counts were observed in green vegeTables (*A. spinach*) from Lot 1, with mean *Staphylococcus* and coliform counts of 4.4×10^4 CFU/g and 8.3×10^2 CFU/g, respectively. The *Staphylococcus* counts ranged from 1.3×10^1 to 4.4×10^4 CFU/g, while coliform counts varied between 1.5×10^1 (lowest) and 8.3×10^2. These high levels of bacterial contamination may indicate unhygienic conditions in open markets that allow for open display of leafy vegeTable.

Among the bacteria isolated, *Staphylococcus aureus* contamination of the leafy vegeTable was more prevalent than *Escherichia coli*. Although *Staphylococcus aureus* is a normal flora human skin, it has the propensity to cause various infections in human. Additionally, human direct contact can facilitates its transfer to leafy vegeTables, making it a significant foodborne pathogen. Several studies has reported *S. aureus* in foodborne outbreaks worldwide (Kothe *et al*., 2021; Al-Kharousi *et* *al*., 2016; Hong *et al*., 2015; Seo *et al*., 2010). For instance, earlier published studies reported 25% *S. aureus* foodborne outbreak in ready-to-eat foods in China (Wang *et al*., 2014) and also associated with over 20% of fruit and vegeTable outbreak of foodborne illness in Brazil (Elias *et al*., 2008).

Pathogenic S. aureus can produces heat-resistant enterotoxins that are lethal to host cell membranes, resulting to foodborne illnesses. The *S. aureus* bacteria population of 5 log CFU/g is sufficient to produce harmful toxin levels (Lindqvist *et al*., 2002), a threshold easily reached through improper handling and storage. *S. aureus* contamination often occurs when buyers excessively handle vegeTables during selection, transferring bacteria from their hands to the vegeTables.

Our study also linked leafy vegeTable consumption with increased risks of virulence enteropathogenic *E. coli* infections. Earlier research by Heaton and Jones (2008) reported the presence of *E. coli* and *S. aureus* in ready-to-eat salads. This also echoes with the findings by Bishop and Okwori (2017), who detected bacterial contamination in carrots and suggested a potential source of contamination as irrigation water, soil, feces, dust, insects, human handling, manure and animal waste.

Our study revealed that of 15 leafy vegeTable screened, *E. coli* and *S. aureus contamination accounted for* 60% and 80 % respectively. Co-contamination by both *E. coli* and *S. aureus* was found in nine samples. *Ugu* and *Scent leaf were found to harbor the* highest contamination levels, followed by green leafy vegeTables across the studied location. Despite the higher bacterial counts in samples from International Market, bacteria prevalence significant varies by sample site.

The significant bacterial presence serve as a public health concern, necessitating immediate intervention to prevent foodborne outbreaks. Similar findings have been published by other researchers (Sahilah *et al*., 2010; Tunung *et al*., 2011).

E. coli isolates were 100 % susceptible to Gentamicin as the most effective antibiotic. Moderate susceptibility was observed against Chloramphenicol and Ciprofloxacin 88.9%. Additionally**,** the isolate was 55.5-77.7 % susceptible to Levofloxacin, Ceftriaxone, Roxithromycin, and Ampicillin while the highest resistance was seen against Tetracycline 66.7%, and Cotrimoxazole 55.5%.

S. aureus isolates showed highest susceptibility to Gentamicin (10 µg) 92.3%, Moderate susceptibility to Chloramphenicol and Ciprofloxacin (10 µg) and demonstrated highest resistance to Erythromycin (61.5%), followed by Ampicillin (53.9%) and Cloxacillin (53.9%). The high resistance to erythromycin and ampicillin likely stems from their widespread use in infection prevention and first-line treatments (Nwode *et al*., 2024). In contrast, low resistance to gentamicin may be due to its controlled parenteral administration. Overuse of low-toxicity antibiotics (e.g., tetracycline, sulfonamides, β-lactams) has contributed to rising resistance (Titilawo, 2015). These findings contrast with Oyedeji *et al*. (2011), who reported no ciprofloxacin resistance in enteric bacteria but noted high ampicillin resistance consistent with other studies (Tula and Osaretin, 2014; Nwode *et al*., 2024).

E. coli recorded a **MARI values** within the ranged of **0.1 to 0.6** while **CL1-E** was the most resistant isolate with MARI value of 0.6, and a significant resistant to **50% of tested antibiotics**. **SL1-E** exhibited the least MARI value of 0.1.

All S. aureus displayed **MARI values** within the ranged of **0 to 0.5** except isolate **CL2-S tha**t was fully susceptible with the MARI value of zero (0). Our isolates with MARI value of 0.1-0.6 indicates that the presence of multidrug-resistant strains originating from environments with heavy antibiotic usage. The complication of such MDR strain is the failure of most antibiotic impacting limited treatment options.

Our study reported resistance patterns underscore widespread antibiotic misuse in clinical and environmental settings (Olukosi *et al*., 2016). The contributing factors facilitating the emergence of MDR include; Over-the-counter antibiotic sales that is widespread in the study area, inappropriate prescribing practices, substandard drugs exerting sub- inhibitory selective pressure and poor regulatory oversight. The antibiotic-resistant bacteria in reported in leafy vegeTables in our study pose a serious public health risk, as they can disseminate antibiotic resistant genes to human gut flora, exacerbating resistance spread as a reservoirs for persistent antibiotic resistant resistance genes (Olukosi *et al*., 2016).

Conclusion

Our study reports a significant bacteria contamination of leafy vegeTables sold in Abakaliki markets, with *Staphylococcus aureus* (86.7%) and *Escherichia coli* (60%) while the co-contamination of the samples accounted for 60% underscoring the potential health risk posed by these pathogens. *Staphylococcus aureus* and *Escherichia coli* were MDR with MARI value ≥0.1 but were highly susceptible to Gentamicin, which could be serve as a better treatment option for food-borne disease associated with the reported strain. These findings underscore the public health risks associated with consuming contaminated vegeTables and call for urgent interventions, including vendor education on hygiene, improved market sanitation, and regulatory measures to curb antibiotic misuse. Addressing these issues is critical to safeguarding food safety and reducing the burden of antibiotic-resistant infections in the region.

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