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ORIGINAL ARTICLE

Bacterial Contamination and Multi-drug Resistance in Lettuce Leaves: A Case Study from Abakiliki Metropolis, Nigeria

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ABSTRACT

Lactuca sativa is widespread consumption without adequate heat treatment makes it a potential vehicle for the transmission of pathogenic microorganisms, to the uses. The aim of this study was to assess bacterial contamination and multidrug-resistant bacteria in lettuce leaves sold in Abakaliki metropolis Nigeria. A total of 20 leafy lettuce samples were purchased in Hausa Quarter Market of Abakaliki, and were analyzed using standard microbiology procedures. The bacterial isolates were subjected to antibiotic susceptibility testing using the Kirby-Bauer method. The results revealed that the total aerobic plate counts were within the range of 2.2×10^6 to 8.4×10^6cfu/g, with the average mean of 3.8×10^6cfu/g. Six different bacteria species were identified there included *Escherichia coli, Pseudomonas* species, *Salmonella* species, *Shigella* species, *Staphylococcus aureus And Proteus* species. The Percentage occurrence of isolates highlights that *Escherichia coli* 7(28%) had the highest occurrence S*taphylococcus aureus* 5(20%) *Pseudomonas* species and *Shigella* species 4(16%) each. *Salmonella* species and *Proteus* species recorded 3 (12%) and 2(8%) respectively. The result obtained from antibiotic susceptibility of bacterial isolates revealed that *E. coli* showed 100% resistant to CIP, CTX, AMC, CAZ, W, AMP, CRO and SXT. *Salmonella* spp, *Pseudomonas* spp., *Shigella* spp and *S. aureus* equally showed 100% resistant to CTX, AMC, CAZ, W and AMP. *S. aureus* showed 100% resistant to IPM and CRO. *Proteus* spp. showed 100% resistant to CIP, CTX, AMC, CAZ, W and AMP. The six bacterial isolates had an average MARI value of 0.57. Presence of pathogenic organisms, and significant antibiotic resistance patterns observed in this study indicate poor hygienic practices and a high risk of public health hazards associated with the consumption of lettuce sold in Hausa Quarter markets.

Keywords: *Lactuca sativa*; Bacterial; Antibiotic; Public health; Multidrug-resistant.

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1.0 INTRODUCTION

Lettuce (*Lactuca sativa*) is a widely consumed leafy vegetable valued for its rich nutritional composition, including vitamins, minerals, dietary fiber, and antioxidants that contribute to human health (Okafor-Elenwo& Imade, 2020). It is commonly eaten raw as part of salads and other ready-to-eat (RTE) meals, which makes it particularly susceptible to microbial contamination since it does not undergo heat treatment before consumption (Ibrahim et al., 2019). The increasing consumption of fresh vegetables in urban areas has been associated with rising concerns about food safety, especially in developing countries such as Nigeria where sanitary practices may be inadequate (Bala et al., 2018; Amoah et al., 2020). Post-harvest contamination occur through improper handling, unhygienic storage conditions, and exposure to contaminated surfaces and environments (Paul-Igwe et al., 2025). In many Nigerian markets, lettuce is often displayed in open environments where it is exposed to dust particles, flies, and frequent human contact, thereby increasing the risk of contamination with pathogenic microorganisms (Onifade et al., 2025).Several studies have reported the presence of pathogenic bacteria in ready-to-eat vegetables with common bacterial contaminants include *E.coli*, *Salmonella spp.*, *Shigella spp.*, *S. aureus*, and *Klebsiella spp.*, which are known to cause foodborne illnesses ranging from mild gastrointestinal infections to severe systemic diseases (Ibrahim et al., 2019). *E.coli* for example, is widely recognized as an indicator of faecal contamination and poor hygienic practices, while *Staphylococcus aureus* is commonly associated with contamination from human handling (Okeh et al., 2022). The presence of these pathogens in ready-to-eat lettuce poses a serious public health risk, particularly in areas where regulatory oversight of food safety is limited (Gbolabo et al., 2025).A more critical concern in recent years is the emergence of multidrug-resistant (MDR) bacteria in food including fresh vegetables. Multidrug resistance refers to the ability of bacteria species to resist the action of multiple classes of antibiotics, making infections difficult to treat and increasing the risk of treatment infection(Igbinosa et al., 2023). However, the presence of MDR bacteria in ready-to-eat lettuce is alarming because these organisms can transfer resistance genes to other bacteria within the human gastrointestinal tract, thereby contributing to the global burden of antimicrobial resistance (AMR) (Allende et al., 2017). Studies have demonstrated high prevalence rates of MDR bacteria in ready-to-eat vegetables, with many isolates showing resistance to commonly used antibiotics such as ampicillin, tetracycline, and gentamicin (Onifade et al., 2025).The development and spread of multidrug-resistant

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bacteria are largely attributed to the indiscriminate use and misuse of antibiotics in human medicine, agriculture, and animal husbandry (Ashefo& Oboh, 2025). In many developing countries, antibiotics are often used without proper prescription, leading to the selection of resistant bacterial strains in the environment. These resistant organisms can contaminate water sources, soil, and agricultural produce, thereby entering the food chain (Ochei et al., 2025). Once present on vegetables such as lettuce, these bacteria can persist and proliferate under favorable environmental conditions, posing a continuous risk to consumers (Ibrahim et al., 2019).

In Nigeria, several studies have highlighted the increasing prevalence of multidrug-resistant bacteria in fresh produce sold in local markets. For example, Onifade et al. (2025) reported a high level of contamination and multidrug resistance among bacterial isolates from ready-to-eat fruits in Kano metropolis. Similarly, Igwe et al. (2024) observed significant antibiotic resistance patterns among *Escherichia coli* and *Staphylococcus aureus* isolated from leafy vegetables and water in Abakaliki. These findings suggest that fresh vegetables, particularly those consumed raw, may serve as reservoirs and transmission routes for multidrug-resistant bacteria. Hausa Quarter in Abakaliki metropolis represents a densely populated urban area characterized by active street vending and informal food distribution systems. The hygienic conditions under which ready-to-eat lettuce is handled and sold in this area may not meet standard food safety requirements, thereby increasing the likelihood of contamination. Despite the widespread consumption of lettuce in this locality, there is limited information on the microbiological quality and antibiotic resistance patterns of bacteria associated with these products. This lack of data creates a gap in understanding the potential public health risks associated with the consumption of contaminated lettuce in the area. Therefore, this study is aimed at evaluating the level of bacterial contamination and multidrug-resistant bacteria in ready-to-eat lettuce leaves sold in Hausa Quarter, Abakaliki metropolis, Ebonyi State, Nigeria. The findings of this study will provide valuable insights into the microbial safety of lettuce consumed in the area and contribute to efforts aimed at improving food hygiene practices and controlling the spread of antimicrobial resistance. Furthermore, the study will serve as a baseline for future research and public health interventions targeted at ensuring the safety of ready-to-eat vegetables.

2.0 MATERIALS AND METHODS

Study area and description: The study was carried out in Abakaliki Local Government Area of Ebonyi State, Nigeria. Ebonyi State is located in the southern part of Nigeria, approximately between longitudes 7°30[1] and 7°E and, latitude 5°40[1] and 6°45[1] N. Ebonyi State is bounded to the east by Enugu State to the west by Cross River State and to the north by Benue State, and south by Abia State respectively as shown in Figure1.

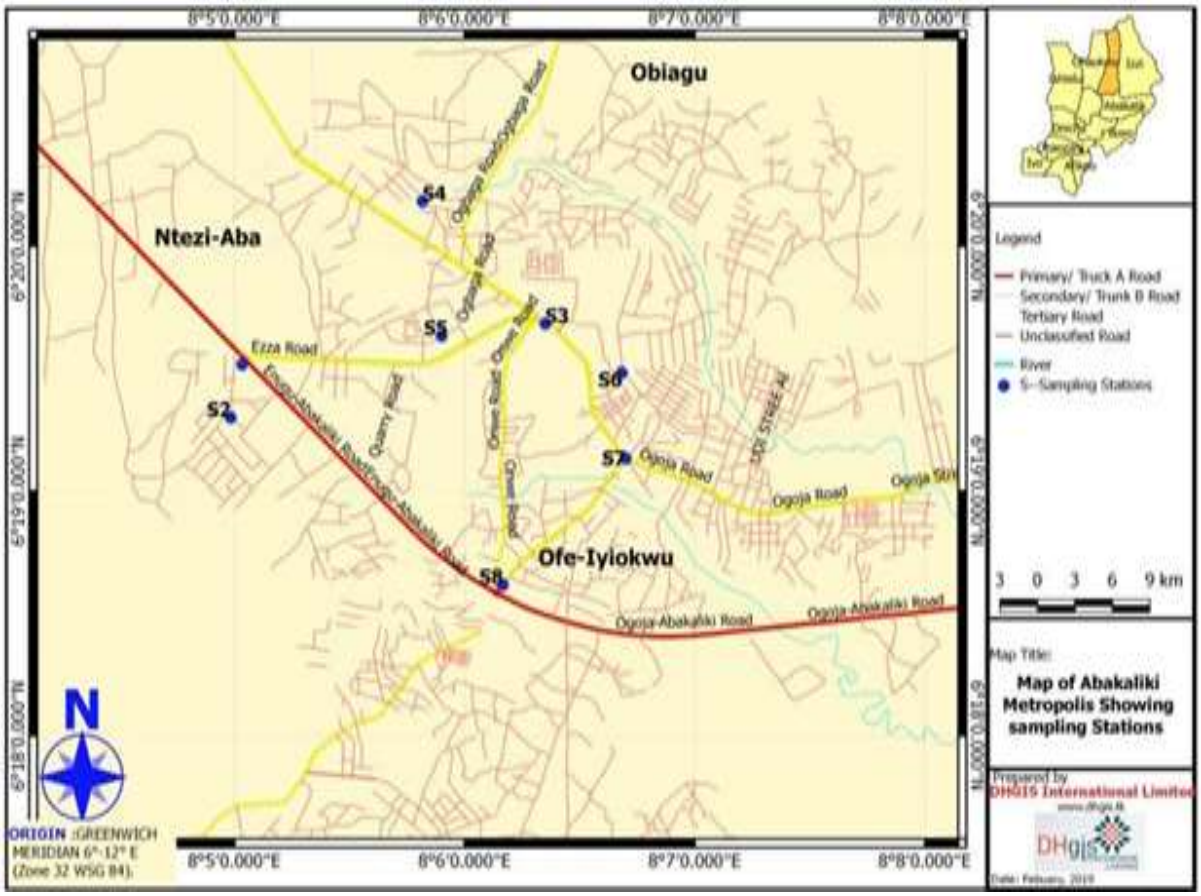


Figure 1: Map of Abakaliki Local Government Area

2.1 Collection of samples

A total of 20 leafy lettuce samples were purchased in Hausa Quarter Market of Abakaliki capital city. The lettuce samples were taken to Applied Microbiology Laboratory Unit in polythene bags for bacteriological analysis within 5 h of purchase.

2.2 Bacteriological analyses

The samples were processed by weighed 25g and then placed it in a sterile electric blender with 100mL of 0.1% peptone water for homogenization. The resulting wash was serially diluted to 10^{-5} in peptone water and 0.5 mL of 5th dilution was placed on Plate count agar and Nutrient agar. The plates were packed and incubated at 37^{0}C for 24 h. The plates were removed and discrete colonies counted and multiplied by the dilution factor (CLSI, 2020). Distinct colonies were severally sub-cultured on fresh nutrient agar plates to obtain pure cultures.

2.3 Identification and characterization of bacterial isolates

The bacterial isolates were identified on the basis of colonial morphology followed by microscopic examination after gram-staining. Biochemical tests including catalase, oxidase, motility, indole test, coagulase test and methyl red test were carried out to characterize the isolates as described by (Cheesbrough, 2006).

2.4 Standardization of test bacteria

All the test bacteria isolated were standardized before use by inoculating 5mL normal saline in sterile test tubes with loopful of a 24h culture of the test bacteria from a nutrient agar slant. The dilutions using loopful of the test bacteria and sterile water was carried out in order to obtain microbial population of 10^5colony forming unit per mL (cfu/mL) by comparing it with 0.5 Mcfarland turbidity standard (CLSI, 2020).

2.5 Antimicrobial susceptibility testing

Antibiotic susceptibility of the isolates was done using the disc diffusion method and interpreted according to the guidelines of Clinical Laboratory Standards Institute (CLSI, 2020). Mueller-Hinton agar was prepared following the manufacturer's instructions. The medium was cooled to 50°C and poured into plates. The inoculum was aseptically inoculated on the surface of Mueller-Hinton (MH) agar plate(s) using sterile swab sticks. After this, the inoculated Mueller-Hinton agar plate was dried for a 5 min at 25 ± 2^0C. After the agar surface has dried for few min, antibiotic impregnated discs (Oxoid, UK) of known concentrations, was aseptically impregnated on the inoculated Mueller-Hinton agar plates using sterile forceps. The plates was incubated at 37°C for 24 h. After incubation, the inhibition zone diameters (IZDs) produced by the antibiotic disks was measured with a ruler to the nearest millimeter and recorded. CLSI breakpoints were used to interpret the results and recorded as resistant, intermediate and susceptible (CLSI, 2020)

2.6 Determination of multiple antibiotic resistant index (MARI)

Multiple antibiotic resistant index (MARI) was determined to know the resistant level of the isolates according to the method described by (CLSI, 2020).

$$MARI = \frac{a}{b} \quad (1)$$

Where;

a**:** Number of antibiotics to which the test isolate showed resistance.

b:Total number of antibiotics to which the test isolate has been evaluated for sensitivity.

2.7 Statistical analysis

The raw data obtained in the course of the study was presented as mean in tables and bar charts while relevant data was interpreted using simple descriptive statistics such as minimum, maximum, and one way analysis of variance (ANOVA) with the aid of IBM Statistical Package for Social Sciences (SPSS) version22 and Microsoft Excel 2013 software.

3.0 RESULTS

3.1 Morphological and biochemical characteristics of isolated bacteria

The results revealed that the total aerobic plate counts were within the range of 2.2 × 10^{-6} to 8.4 × 10^{-6}cfu/g, with the average mean of 3.8 x10^{-6}cfu/g Table 1. The morphological and biochemical characteristics of the probable organisms isolated from samples retailed for human consumption Hausa Quarter Markets within Abakaliki. It revealed that a total of six bacteria were identified. The bacteria include *E. coli*, *Salmonella* Spp, *Shigella* Spp, *Pseudomonas* Spp, *Proteus* Spp and *S. aureus*. Out of the six bacteria, five are gram negative except *Staphylococcus aureus*. also, five of the bacteria are oxidase negative, while four are indole negative (Table 2).

Table **1**. Bacterial load of lettuce leaf samples

S/N	Total aerobic plate count (cfu/g)
1	2.2 x 10^6
2	3.8 x 10^6
3	4.1 x 10^6
4	3.8 x 10^6
5	3.6 x 10^6
6	2.2 x 10^6
7	5.0 x 10^6
8	5.4 x 10^{-6}
9	2.2 x 10^6
10	2.2 x 10^6
11	8.4 x 10^6
12	5.1 x 10^6
13	2.4 x 10^6
14	5.8 x 10^6
15	3.8 x 10^6
16	3.9 x 10^6
17	3.0 x 10^6
18	3.1 x 10^6
19	3.2 x 10^6
20	3.3 x 10^6

Sample Source: Hausa Quarter.

Table 2: Morphological and biochemical characteristics of isolated bacteria

SN	Source	Biochemical				Motility	MR	Gram RXN	Shape	Arrangement	Morphological CHX	Probable organism
		CA	CO	OX	IND							
1	Hausa quarters	-	-	-	+	+	+	-	Rod	Single	Pink	*Escherichia coli*
		+	-	-	-	+	-	-	Rod	Single	Pale white with black edges	*Salmonella* spp.
		-	-	-	-	-	-	-	Rod	Single	Pale white	*Shigella* spp.
		+	-	+	-	+	-	-	Rod	Single	Yellow-green	*Pseudomonas* spp.
		+	+	-	+	-	+	+	Cocci	Group	Yellow	*Staphylococcus aureus*
				-	-	+		-	Rod		Large, circular, gray smooth colonies	*Proteus*spp

Key: CA = Catalase test, OX = Oxidase test, IND = Indole test, -= Negative, += Positive, MR=methyl red

3.2 Percentage occurrence of pathogenic bacteria

Tables 3 show percentage occurrence of pathogenic Bacteria Isolated from lettuce samples retailed from Hausa Quarters. The result revealed that *Escherichia coli* 7(28%) had highest occurrence isolates among others. S*taphylococcus aureus* 5(20%) *Pseudomonas* species and *Shigella* species 4(16%) each. While *Salmonella* species and *Proteus* species recorded 3 (12%) and 2(8%) respectively. Table 4 presents the results of multiple antibiotic resistance index (MARI) of pathogenic bacteria isolated from lettuce samples purchased from Hausa Quarters, revealed an average MARI value of 0.57.

Table **3**. Percentage occurrence of pathogenic bacteria isolated from lettuce samples purchased from Hausa Quarters

S/N	Isolates	Isolates (n)	Prevalence (%)
1	*Escherichia coli*	7	28
2	*Pseudomonas*spp	4	16
3	*Salmonella*spp	3	12
4	*Shigella*spp	4	16
5	*Staphylococcus*spp	5	20
6	*Proteus* spp	2	8

Antibiotics percentage resistance of bacteria isolated from lettuce samples purchased from Hausa Quarters. It revealed that *Escherichia coli* showed 100% resistant to CIP, CTX, AMC, CAZ, W, AMP, CRO and SXT. *Salmonella*spp, *Pseudomonas* spp., *Shigella*spp and *Staphylococcus*

aureus equally showed 100% resistant to CTX, AMC, CAZ, W and AMP. Also, *Staphylococcus aureus* showed 100% resistant to IPM and CRO. *Proteus* spp. showed 100% resistant to CIP, CTX, AMC, CAZ, W and AMP (Figure 1).

Table **4**: Multiple antibiotics resistance index (MARI) of bacteria isolated from lettuce sample purchased from Hausa Quarters Market

S/N	Organism	MARI value
1	*Escherichia coli*	0.4
2	*Escherichia coli*	0.6
3	*Escherichia coli*	0.7
4	*Escherichia coli*	0.5
5	*Escherichia coli*	0.5
6	*Escherichia coli*	0.5
7	*Escherichia coli*	0.4
8	*Salmonella* species	0.6
9	*Salmonella* species	0.7
10	*Salmonella* species	0.7
11	*Pseudomonas* species	0.5
12	*Pseudomonas* species	0.6
13	*Pseudomonas* species	0.7
14	*Pseudomonas* species	0.6
15	*Shigella* species	0.5
16	*Shigella* species	0.6
17	*Shigella* species	0.6
18	*Shigella* species	0.5
19	*Staphylococcus aureus*	0.6
20	*Staphylococcus aureus*	0.7
21	*Staphylococcus aureus*	0.5
22	*Staphylococcus aureus*	0.6
23	*Staphylococcus aureus*	0.6
24	*Proteus* species	0.5
25	*Proteus* species	0.6
	Total	**14.3Average= 0.572**

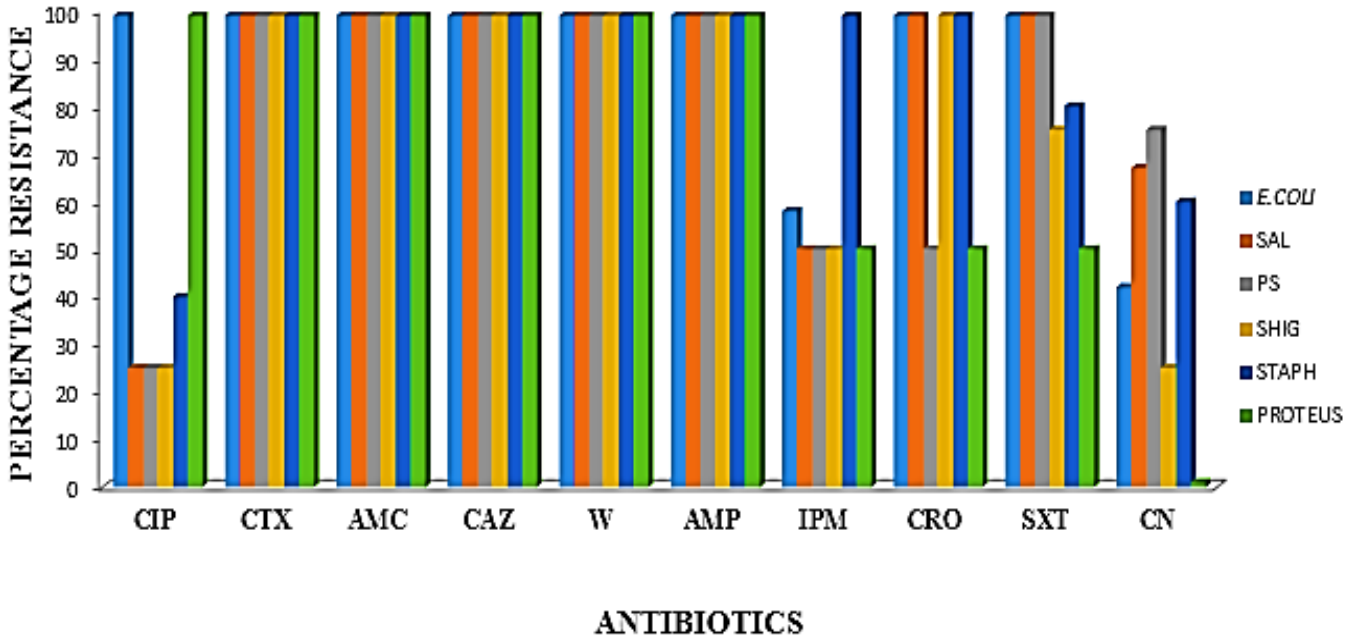


*Figure **1**. Antibiotics percentage resistance of organisms isolated from Lettuce purchased from Hausa Quarters market in Abakaliki. Ps = Pseudomonas aeruginosa, Sal = Salmonella spp, SHIG = Shigella spp, STAPH = Staphylococcus spp, Proteus =Proteus spp, CIP = ciprofloxacin, AMC = Amoxicillin/clavulanic Acid, W = Trimethoprim, AMP = Ampicillin, IPM = Imipenem, CTX = Cefotaxime , CRO = Ceftriaxone , SXT = Sulfamethoxazole /Trimethoprim, CN = Gentamicin, CAZ=cefltazidime***.**

4.0 DISCUSSION

The increasing prevalence of food borne pathogens on green leafy vegetables has been attributed to the bacteria ability to be internalized from contaminated manure or water into the leafy plant tissue. These food borne infections often cause gastrointestinal illnesses especially people leaving in rural region like Ebonyi state in south-east Nigeria Igwe at al. (2026).The results revealed that the total aerobic plate counts were within the range of 2.2×10^6 to 8.4×10^6cfu/g, with the average mean of 3.8 x10^6cfu/g Table 1. These values are considerably higher than the acceptable microbiological limits for fresh vegetables, which are generally recommended to be $\leq 10^5$ cfu/g for ready-to-eat produce (ICMSF, 2018). The high bacterial load observed suggests poor hygienic handling, contamination from irrigation water, soil, manure, transportation, and market display conditions. Similar high microbial loads in leafy vegetables have been reported by studies conducted in different parts of Nigeria and other developing regions, where vegetables are often irrigated with untreated wastewater and handled under unhygienic conditions Allende et al. (2017) and Oluwapelumi et al. (2019). The high counts recorded in this study are consistent with the findings of Nwuzo et al. (2024) reported bacterial loads ranging from 10^5 to 10^7 cfu/g in vegetables sold in open markets, indicating widespread contamination. The isolation of pathogenic bacteria such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Pseudomonas* spp., *Proteus* spp., and *Staphylococcus aureus* further highlights the microbiological risks associated with the consumption of raw lettuce Table 2 and 3. The predominance of *Escherichia coli* (28%) in this study is particularly concerning, as it is widely recognized as an indicator of fecal contamination and poor sanitary conditions during production and handling (WHO, 2022). Similar findings have been reported by (Dineo et al., 2025), where *E. coli* was the most prevalent isolate in fresh vegetables sold in Nigerian markets. The presence of *Salmonella* and *Shigella* species is also of serious public health concern, as these organisms are well-known etiological agents of foodborne gastroenteritis and dysentery (CDC, 2023).The occurrence of *Staphylococcus aureus* (20%) in this study suggests contamination from human handling, as this organism is commonly found on the skin, nose, and hands of food handlers. This observation aligns with previous studies which identified poor personal hygiene among vendors as a major source of contamination in ready-to-eat foods (Tsado et al., 2013). Additionally, the isolation of *Pseudomonas* and *Proteus* species indicates environmental contamination, as these organisms are commonly associated with soil and water (Okeh et al., 2022).The study also in agreement with the report of Ivie et al. (2022) in Benin city Edo State, Nigeria. The antibiotic resistance patterns observed in this study (Figure1) are alarming. The 100% resistance of *E. coli* to commonly used antibiotics such as ciprofloxacin, ampicillin, cefotaxime, ceftriaxone, and sulfamethoxazole/trimethoprim suggests widespread misuse and overuse of antibiotics in both human medicine and agriculture. Similar high resistance patterns have been reported globally, particularly in low- and

middle-income countries, where antibiotic regulation is often inadequate (O'Neill, 2020). The resistance of *Salmonella*, *Shigella*, *Pseudomonas*, and *Staphylococcus aureus* to multiple antibiotics further confirms the emergence of multidrug-resistant strains in the food chain.The result is in agreement with the study of (Okeh et al., 2022). Also in line with the study of (Igwe et al., 2026). showed in their study that the Gram-negative bacteria isolated from several fresh farm produce from various groceries in Ebonyi state were multidrug in nature and were resistant to more than 50 % of the antibiotics tested. In the same study, Nwinyi & Nduchukwuka (2016) also reported that *E. coli* isolated from vegetables was resistant to all the tested antibiotics with exception to amoxicillin. Nwinyi & Nduchukwuka (2016) also reported that *Salmonella* species isolated from vegetables showed intermediate reaction to gentamicin, but susceptible to tetracycline and resistant to clotrimazole, cloxacillin, erythromycin, augmentin, streptomycin and chloramphenicol.Notably, the resistance of *Staphylococcus aureus* to imipenem and ceftriaxone is particularly concerning, as these are considered critical antibiotics for treating severe infections. The Multiple Antibiotic Resistance Index (MARI) values obtained in this study ranged from 0.4 to 0.7, with an average of 0.57 Table 4. A MARI value greater than 0.2 is indicative of high-risk contamination sources where antibiotics are frequently used. Therefore, the high MARI values recorded in this study suggest that the bacterial isolates originated from environments with intense antibiotic exposure, such as hospitals, livestock farms, or areas contaminated with pharmaceutical waste. These findings are consistent with reports by (Dineo et al., 2025), who documented high MARI values among bacterial isolates from vegetables in Nigerian markets. In line with the result of our study,Igwe et al. (2026) also reported in their respective studies carried out in Ebonyi state Nigeria that enteric and non-enteric bacteria isolated from vegetables are multidrug resistant in nature especially to antibiotics in the beta-lactam group, aminoglycosides and fluoroquinolones. The results of our study have shown that some vegetables are heavily contaminated with potent bacterial pathogens that cause infections in humans. Also, the isolated enteric and non-enteric bacteria isolated from the lettuce (*Lactuca sativa*) samples were found to be highly resistant to majority of the antibiotics used especially to those in the beta-lactam family, aminoglycosides and fluoroquinolones.In comparison with other studies, the results obtained in this research are in agreement with findings from similar studies conducted in Africa and beyond. For instance, a study byNwuzo et al. (2024) reported the presence of MDR *E. coli* and *Salmonella* in leafy vegetables sold in open markets, while another study by Krumperman (1983) highlighted high levels of antibiotic resistance among bacteria isolated from fresh produce in Asia. These similarities suggest that the issue of contaminated vegetables and antimicrobial resistance is a global problem requiring coordinated intervention. The public health implications of these findings are significant. The consumption of contaminated lettuce without proper washing or disinfection may lead to foodborne infections, particularly among vulnerable populations such as children, the elderly, and immunocompromised individuals. Moreover, the presence of multidrug-resistant bacteria in food increases the risk of treatment failure, prolonged illness, and increased healthcare costs. The findings of this study support the growing global concern that fresh produce can serve as a reservoir and transmission route for antimicrobial-resistant bacteria.

Conclusion

In conclusion, the high bacterial loads, detection of pathogenic organisms, and significant patterns of antibiotic resistance observed in this study indicate inadequate hygienic practices and pose a considerable public health risk associated with the consumption of lettuce sold in markets within Hausa Quarter. The findings underscore the urgent need for improved food safety practices throughout the production, transportation, marketing, and handling of lettuce. Proper washing and hygienic preparation of vegetables, continuous public health education for food vendors and consumers, routine microbiological monitoring, and stricter regulation of antibiotic use are essential to mitigate the risks identified in this study. These measures will contribute significantly to reducing bacterial contamination, limiting the spread of antimicrobial-resistant organisms, and protecting consumers from foodborne infections.

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Competing Interests

Author has declared that no competing interests exist.

Authors' Contributions

All the authors conceived and designed the study, carried out the sample collection and laboratory investigations, analyzed and interpreted the data, and prepared and approved the final manuscript.

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