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# Antibiotic susceptibility pattern of *Staphylococcus aureus* isolated from fresh lettuce (*Lactuca sativa*) and food safety knowledge of vegetable vendors in Birnin Kebbi, Nigeria

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ABSTRACT

In recent years, the consumption of fresh products has increased due to the multiple contributions of nutrients and functional properties. This study aims to determine the susceptibility pattern of *Staphylococcus aureus* in fresh lettuce and assess the food safety practice of vegetable vendors in the Birnin Kebbi metropolis. A total of 28 fresh lettuce samples were collected from various locations (Tudun Wada, Rafin Atiku, Badariya, and Bayan Kara) in the Birnin Kebbi metropolis, chosen randomly from vegetable vendors. A structured questionnaire was utilized to assess food safety practices. *S. aureus* was isolated and identified using cultural and biochemical characteristics and antibiotic sensitivity was conducted by the Kirby-Baur disk diffusion method. Only 7.14% of the vegetable vendors reported using gloves while handling vegetables. 17.86% reported washing their hands properly after handling waste or garbage. Only 7.14% reported wearing an apron while working. 50% reported properly cleaning the vegetable storage area before storing new products. 14.29% reported washing vegetables after purchasing them from the market, while None of the respondents reported covering their products while selling them. Out of a total of 28 samples tested, 21(75%) were found to be positive for *S. aureus*. Antibiotic susceptibility pattern of *S. aureus* showed resistance in Ampicillin (100%), Amoxicillin (28.57%), Tetracycline (17.86%), Rifampicin (39.29%), Chloramphenicol (14.29%), Neomycin (53.57%), Methicillin (89.29%), Cefpodoxime (35.71%) respectively. Furthermore, none of the *S. aureus* were resistant to Gentamycin. The high occurrence of *S. aureus* found in fresh lettuce suggests contamination could have occurred before or after harvesting. It is recommended that cleaning and sanitation practices be introduced to enhance the safety and quality of this vegetable.

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## 1. Introduction

Fruits and vegetables are considered to be crucial elements of a healthy diet due to their low-fat and low-energy density. They are also relatively high in essential vitamins, minerals, and other beneficial bioactive compounds. Additionally, they are a valuable source of dietary fiber (1). Leafy greens like lettuce, spinach, and kale are commonly eaten raw or lightly cooked, making them susceptible to potential health risks from harmful

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bacteria or pathogens on their leaves. Additionally, being grown close to the ground increases the chances of contamination from soil and other environmental sources, further emphasizing the need for proper handling and washing before consumption (2).

Lettuce is an annual leafy vegetable belonging to the Asteraceae family, commonly consumed raw in the form of salad. Indeed, lettuce is susceptible to contamination due to various factors, including inadequate sanitation practices, poor drainage systems, and unhygienic planting, harvesting, packaging, transportation, and storage methods that are sometimes employed in its production and distribution (3).

Additionally, lettuce can become contaminated with pathogenic microorganisms at various stages of the food production process, from the farm to the market. One common source of contamination is the use of cattle manure as fertilizer or the proximity of vegetable fields or water sources to cattle farms. These sources have been linked to instances of vegetable contamination in the past (4).

Not adequately washing and peeling food items can serve as a breeding ground for different microorganisms, potentially resulting in foodborne illnesses. These illnesses can be caused by different types of pathogens that enter the human body through the oral route, such as by eating contaminated food or drinking contaminated water (5).

While raw fruits and vegetables are known for their numerous health benefits, research has indicated that they rank as the second most common source of foodborne illness outbreaks. These outbreaks not only present a public health risk but also lead to substantial economic losses for the food industry (6). Both in developed and developing regions, foodborne pathogens are a major cause of infectious disease outbreaks, and incidents of foodborne illness related to the consumption of fresh produce have seen a significant increase in recent years (7).

Fresh vegetables can become contaminated by microbes at many points along the farm-to-fork supply chain. Fresh produce cultivation, harvest, preparation, washing, transportation to stores, distribution chains, and even the final stage in the consumer's kitchen can all result in contamination. Both contaminated soil and water might result in contamination during agriculture. Food contamination may also be influenced by the environment in which these foods are handled, such as food contact surfaces or processing equipment (8).

*Staphylococcus aureus* is a common bacterium found in 30% of healthy individuals and can cause infections ranging from mild to severe (9). It has various virulent genes that encode toxins and enzymes. Humans are the primary reservoir, and food contamination can occur through direct or indirect contact. In recent years, there has been a notable surge in bacterial resistance to antibiotics (10). This rise in antimicrobial drug resistance among foodborne pathogens is primarily linked to the overuse of antibiotics in livestock, aquaculture, and agricultural practices for both preventive and therapeutic reasons (11). It is crucial to note that antibiotic-resistant bacteria and antimicrobial resistance genes can transfer between animal and human populations through various routes, including direct contact with animals or their environment and indirect contact through contaminated food sources (12). Staphylococcal enterotoxins, which are the cause

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of staphylococcal food poisoning, can develop and be produced by *S. aureus*. In outbreaks of foodborne illness, pathogenic Staphylococcus strains' enterotoxins are frequently involved. Food contamination with an enterotoxigenic strain of *Staphylococcus* spp. is the primary precondition for staphylococcal food poisoning (13). Temperature, pH, and water activity requirements must be met for enterotoxins to be created. upon being produced by the strains, staphylococcal enterotoxins are resistant to a variety of environmental conditions, such as freezing, drying, heat treatment, and low pH conditions, which easily destroy the enterotoxin-producing strains themselves (14).

*S. aureus* is a bacterial species known for its ability to develop resistance to antibiotics. The β-lactam antibiotics, which include penicillin, methicillin, flucloxacillin, dicloxacillin, nafcillin, oxacillin, and cloxacillin, are typically effective in treating infections caused by *S. aureus*. However, some strains of *S. aureus* have acquired the mecA gene, leading to methicillin-resistant *S. aureus* (MRSA), which is resistant to all penicillins, cephalosporins, and carbapenems. The widespread distribution of MRSA worldwide and its ability to acquire resistance to multiple antibiotics have raised concerns about treatment options (15).

Nigeria is a significant producer of fruits and vegetables, contributing to the livelihoods of both rural and urban communities. Leafy vegetables are commonly used for cooking to improve the taste of the soup and for nutritional purposes, medicinal properties and are used to manage ailments such as diarrhea, stomach issues, coughing, and malaria (16). Typically, these vegetables are cooked in soups or stews and served with a carbohydrate-rich staple food (17). Regrettably, in Nigeria, fruits and vegetables are often unclean and not properly sanitized before being sold in the market, leading to unsafe and substandard produce. There has not been a comprehensive investigation on the issue of antibiotic resistance in fresh lettuce in Birnin Kebbi. Additionally, there is a lack of data regarding comprehensive the prevalence of antibiotic-resistant *Staphylococcus aureus* and food safety knowledge level among street vendors that sell fresh produce in the Birnin Kebbi metropolis. Understanding the prevalence and antibiotic resistance patterns of *Staphylococcus aureus* in fresh lettuce and the level of knowledge among street traders is essential to ensure the safety of the food supply and to prevent the spread of infections. Thus, this research aims to establish the susceptibility of *Staphylococcus aureus* in fresh lettuce to commonly available antibiotics and evaluate the food safety knowledge of vegetable vendors in the Birnin Kebbi metropolis.

## 2. Materials and Methods

2.1. Collection of fresh lettuce

A total of 28 fresh lettuce samples were collected from various locations (Tudun Wada, Rafin Atiku, Badariya, and Bayan Kara) in the Birnin Kebbi metropolis, chosen at random from vendors who display their vegetables openly for customers to see and purchase. Lettuce samples were obtained from each vendor using the same approach as a regular customer, and then individually placed in sterile plastic bags. These samples were subsequently transported in a cool box at low temperatures to the Microbiology Laboratory of

Kebbi State University of Science and Technology Aliero for further analysis.

2.2. Food safety practice data collection

To assess the demographic characteristics and food safety practices of vegetable vendors, a structured questionnaire was utilized. Trained research assistants and the researchers themselves conducted interviews with the participants, using the structured questionnaire which included questions related to the demographic information of the respondents adapted from previous works (18,19). Moreover, questions regarding food safety practices were adapted from previously published studies (20) and were presented on a five-point scale, with a score of 1 representing strong disagreement and a score of 5 representing strong agreement.

2.3. Samples preparation

To prepare each fresh lettuce sample for analysis, the initial step involved slicing or cutting it into small pieces using a sterilized tray and knife while wearing latex gloves for sanitary purposes. Subsequently, 25 g of each sample were weighed and combined with 225 mL of sterile normal saline solution. The mixture was homogenized using a stomacher device and then incubated at 37°C for a duration of 24 h.

2.4. Isolation and identification of *S. aureus*

Following homogenization and incubation, one milliliter of each sample was extracted and inoculated onto a sterile Mannitol Salt Agar (MSA) plate, which was subsequently streaked and incubated at 37°C for 24 h. The typical colonies obtained from each MSA agar plate were further sub-cultured on nutrient agar to facilitate identification.

From the total of 28 samples, one presumptive culture of *Staphylococcus aureus* was randomly chosen and identified using culture characteristics on Mannitol Salt Agar, gram-staining, and biochemical reactions following the guidelines outlined in Bergey's Manual of Systematic Bacteriology (21).

2.5. Antibiotic sensitivity testing

The confirmed *S. aureus* isolates underwent antibiotic susceptibility testing using the Kirby Bauer disk diffusion technique, following the guidelines provided by the Clinical and Laboratory Standard Institute (CLSI). Eight different antibiotics were utilized in this process, namely ampicillin (10 µg), amoxicillin (30 µg), tetracycline (30 µg), gentamycin (10 µg), rifampicin (5 µg), chloramphenicol (30 µg), neomycin (10 µg), methicillin (5 ug), and cefpodoxime (10 µg). The inhibition zone diameters were measured and compared to predetermined breakpoints outlined in the CLSI protocol (22). Based on the results, the antibiotic susceptibility of the isolates was interpreted as sensitive, intermediate, or resistant.

## 3. Results

3.1. Prevalence of *S. aureus* in fresh lettuce samples

This study collected fresh lettuce samples (n=28) from street vegetable vendors in Birnin kebbi. Table 1 presents the number of positive samples for *S. aureus* along with their prevalence percentage. Out of a total of 28 samples tested, 21(75%) were found to be positive for *S. aureus*, while the remaining 7(25%) were negative.

**Table 1.** Prevalence of *S. aureus* (n=28) in fresh lettuce samples

| Organism | Number (%) positive | Number (%) negative |
|---|---|---|
| *Staphylococcus aureus* | 21(75) | 7(25) |

**Table 2.** Demographic characteristics of vegetable vendors

| Parameter | Frequency | Percentage |
|---|---|---|
| **Gender** | | |
| Male | 28 | 100 |
| Female | 0 | 0 |
| **Age** | | |
| <18 | 4 | 14.23 |
| 19-40 | 20 | 71.43 |
| 41-60 | 4 | 14.23 |
| >60 | 0 | `0 |
| **Education** | | |
| Primary | 18 | 64.29 |
| Secondary | 5 | 17.86 |
| Higher | 0 | 0 |
| No formal education | 5 | 17.86 |
| **Number of years in business** | | |
| 0-5 | 5 | 17.86 |
| 6-10 | 15 | 53.57 |
| >10 | 8 | 28.57 |
| **Marital status** | | |
| Single | 20 | 71.43 |
| Married | 8 | 28.57 |
| Divorced | 0 | 0 |
| **Food safety training** | | |
| Yes | 0 | 0 |
| No | 28 | 100 |

### 3.2. Demographic characteristics of vegetable vendors

The socio-demographic profile of respondents is presented in Table 2. Out of 28 respondents, all 100% were males. The majority of the participants are aged between 19-40 (71.43%), followed by those aged below 18 (14.23%) and those aged 41-60 (14.23%). In terms of education, 64.29% of the participants have completed primary education, while 17.86% have had no formal education, and only 17.86% have completed secondary education. Regarding the number of years in business, 53.57% of the participants have been in business for 6-10 years, while 28.57% have been in business for more than 10 years, and 17.86% have been in business for 0-5 years. In terms of marital status, 71.43% of the participants are single, while 28.57% are married. Finally, none of the participants received food safety training.

### 3.3. Food safety practices of vegetable vendors

Table 3 represents the responses (in percentages) to various food safety questions. All 28 respondents (100%) reported eating or drinking at their workplace. Only 7.14% reported using gloves while handling vegetables, while 71.23% reported not using gloves. 17.86% reported washing their hands properly after handling waste or garbage, while 82.12% reported not doing so. All 28 respondents (100%) reported washing their hands after using the toilet. Only 7.14% reported wearing an apron while working, while 92.86% reported not wearing one. 50% reported properly cleaning the vegetable storage area before storing new products, while the other 50% did not. All 28 respondents (100%) reported using clean polyethylene bags for packaging. 14.29% reported washing vegetables after purchasing them from the market, while 85.71% reported not doing so. None of the respondents reported covering their products while selling them.

### 3.4. Results of antibiotic susceptibility pattern for *S. aureus* isolates from fresh lettuce

The antibiotic-resistant pattern of *S. aureus* from lettuce samples is shown in Table 4. All 28 isolates (100%) were resistant to ampicillin. The majority of isolates (28.57%) were resistant to amoxicillin. 17.86% of the isolates were resistant to tetracycline. None of the isolates were resistant to gentamycin. A significant proportion of isolates (39.29%) were resistant to rifampicin. 14.29% of the isolates were resistant to chloramphenicol. More than half of the isolates (53.57%) were resistant to neomycin. The vast majority of isolates (89.29%) were resistant to methicillin. 35.71% of the isolates were resistant to cefpodoxime. Overall, the resistance pattern of the *S. aureus* isolates indicates that there is significant resistance to several antibiotics, particularly ampicillin, methicillin, and neomycin.

**Table 3.** Food safety practices of vegetable vendors

| Questions | Response (n)% | |
|---|---|---|
| | YES | NO |
| Do you eat or drink at the workplace? | 28(100) | 0(0) |
| Do you use gloves while handling vegetables? | 2(7.14) | 20(71.23) |
| Do you wash your hands properly after handling waste/garbage? | 5(17.86) | 23(82.12) |
| Do you wash your hand after using the toilets? | 28(100) | 0(0) |
| Do you wear an apron the whole working? | 2(7.14) | 26(92.86) |
| Do you properly clean the vegetable storage area before storing new products? | 14(50) | 14(50) |
| Do you use clean polyethylene bags for packaging? | 28(100) | 0(0) |
| Do you wash the vegetables after purchase from the market? | 4(14.29) | 24(85.71) |
| Do you cover your product while selling? | 0(0) | 28(100) |

**Table 4.** Antibiotic susceptibility pattern for *S. aureus* (n=28) isolates from fresh lettuce

| **Antibiotics** | **Concentration (ug)** | **Zones of inhibition (mm)** | | |
|---|---|---|---|---|
| | | Number (%) of resistant | Number (%) of intermediate | Number (%) of sensitive |
| Ampicillin | (10 µg), | 28(100) | 0(0) | 0(0) |
| Amoxicillin | (30 µg), | 8(28.57) | 6(21.43) | 14(50) |
| Tetracycline | (30 µg), | 5(17.86) | 2(7.14) | 21(75) |
| Gentamycin | (10 µg), | 0(0) | 5(17.86) | 23(82.14) |
| Rifampicin | (5 µg), | 11(39.29) | 1(3.57) | 16(57.14) |
| Chloramphenicol | (30 µg) | 4(14.29) | 3(10.71) | 21(75) |
| Neomycin | (10 µg), | 15(53.57) | 0(0) | 13(46.43) |
| Methicillin | (5 ug) | 25(89.29) | 2(7.14) | 1(3.57) |
| Cefpodoxime | (10 µg). | 10(35.71) | 4(14.29) | 14(50) |

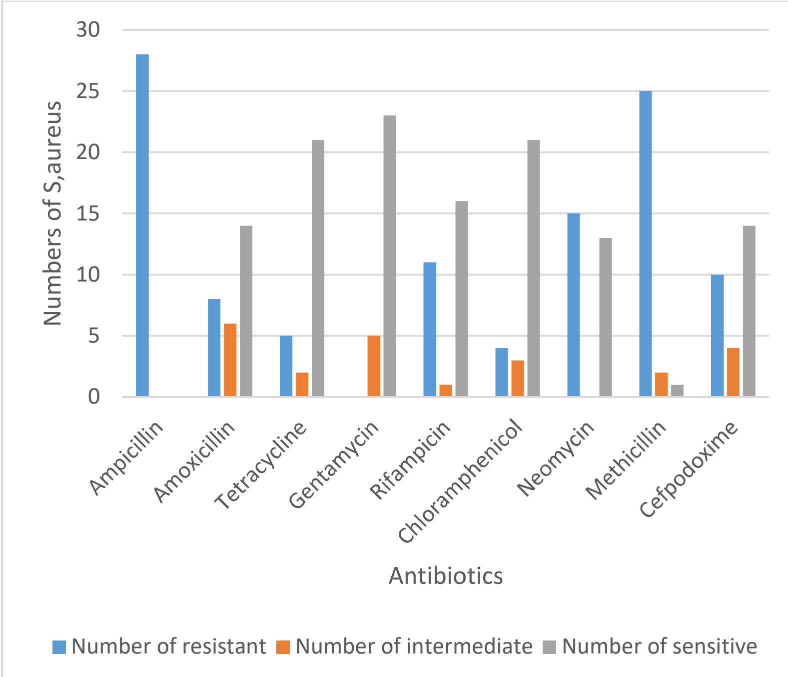


**Figure 1**. Antibiotic susceptibility test for *S. aureus* (n=28) isolates from fresh lettuce

## 4. Discussion

This study presents novel findings regarding the occurrence of antibiotic-resistant *Staphylococcus aureus* in lettuce, as well as the food safety measures implemented by vegetable vendors in the city of Birnin Kebbi, Nigeria. Furthermore, it establishes a foundation of knowledge that can serve as a benchmark for future studies in this area. Fresh produce can become contaminated at different points along the entire production chain, from its cultivation on the farm to its consumption at the table (23). Various factors, including human contact, harvesting equipment, transportation containers, insects, dust, rinse water, ice, vehicles for transport, and processing equipment, can all play a role in postharvest contamination (24).

In this study, only 50% reported properly cleaning the vegetable storage area before storing new products, 14.29% reported washing vegetables after purchasing them from the market and none of the respondents reported covering their products while selling them. This could be contributing factor to the contamination of the vegetable. According to previous research, an inadequate understanding of food safety can result in food handlers adopting unsatisfactory hygiene practices (25). Additional research has indicated that simply providing food safety and hygiene knowledge does not guarantee the adoption of safe food behavior or practices (26).

The prevalence of *Staphylococcus aureus* contamination reported in this study is comparatively higher than rates observed in other regions. For instance, a study conducted in Tehran found that 45 out of 350 (12.85%) vegetable and salad samples tested positive for *S. aureus* (27). In a study conducted by (28), investigating antibiotic-resistant *S. aureus* in leafy greens and clinical sources, it was found that 31 (28.18%) of the retailed vegetables tested positive for *S. aureus*. Among the vegetables, lettuce had the highest prevalence, with 13 out of 15 samples (86.7%) being positive for *S. aureus*. Another study in the Bekaa Valley of Lebanon revealed that lettuce samples had a significantly higher microbial load, including coliforms, *E. coli*, and *S. aureus*, compared to parsley samples (29) Similarly, a study conducted in China to investigate the prevalence and characterization of *S. aureus* in retail vegetables found that 24 (5.73%) samples were positive for *S. aureus*, with lettuce having the highest prevalence at 13 out of 84 samples (15.48%) (30). *Staphylococcus aureus* has the potential to contaminate vegetables when there is contact with unwashed hands during the selection process. Its presence in food raises public health concerns due to its ability to produce enterotoxins, posing a risk of food poisoning (31,32). Recently, there has been a significant alarm in both public and clinical sectors regarding the emergence and spread of antibiotic-resistant strains of *S. aureus*, which has implications for managing staphylococcal infections (33,34). Multiple studies have documented the presence of antibiotic-resistant *S. aureus* strains in various food samples from different countries (28,35,36).

In this particular study, it was found that all of the isolates (100%) demonstrated resistance to ampicillin, while 89.29% exhibited resistance to penicillin, (53.57%) were resistant to neomycin and the vast majority of isolates (89.29%) were resistant to methicillin. MRSA, methicillin-resistant *Staphylococcus aureus*, is notorious for its resistance not only to methicillin and oxacillin but also to various other antibiotics. This bacterium has emerged as a significant cause of infections, posing a major global health threat due to its ability to produce numerous toxins. In recent years, MRSA has been increasingly detected in the community, outside the confines of healthcare facilities. This includes its presence in food and food-producing animals worldwide (37). The presence of MRSA in food raises further awareness about potential routes of transmission and the need for monitoring and controlling antibiotic resistance in both clinical and food environments.

In this particular study, it was found that all of the isolates (100%) demonstrated resistance to ampicillin, while 89.29% exhibited resistance to penicillin. These resistance rates were in agreement with those reported by (38) in their research on leaf vegetables in Korea, where a resistance rate of 96.3% was observed. The resistance patterns to other antimicrobials such as tetracycline, erythromycin, gentamicin, and ciprofloxacin were found to be comparable to the findings of (30). In their study, it was reported that 49.4% of *S. aureus* isolates from food products displayed resistance to tetracycline, 24.1% to erythromycin, and 13.8% to gentamicin. Over time, there have been changes in antibiotics and the resistance patterns exhibited by foodborne pathogens. These patterns can vary among isolates from the same or different sources. Several factors contribute to these differences, including geographical locations, the specific bacterial species involved, the type of animal production systems employed, the extent of antibiotic usage, the techniques used for sampling, and the duration of the sampling period. These diverse factors collectively influence the observed variations and similarities in antibiotic resistance patterns among foodborne pathogens (39,40).

## 5. Conclusion

The findings of this study demonstrate the presence of resistant *Staphylococcus aureus* isolates in lettuce samples, indicating inadequate sanitation and improper handling practices during harvesting and marketing. To mitigate contamination risks, farmers need to adopt the best agricultural and handling practices, while food vendors, processors, and consumers should adhere to good hygiene practices. These results also provide valuable information to clinicians for effective treatment of patients. Therefore, it is crucial for the government to closely monitor both pre-and post-harvest activities of vegetable producers and sellers to minimize disease risks. Additionally, regular surveillance of antibiotic susceptibility is necessary to monitor changes in resistance patterns.

## Conflict of interest

The authors declare that there is no conflict of interest.

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