



ASSESSMENT OF *Staphylococcus aureus* PREVALENCE IN VEGETABLES SOLD AT LOCAL MARKETS IN BENIN CITY: IMPLICATIONS FOR FOOD SAFETY.

^{1,2}ISICHEI-UKAH, B.O., ^{1,3}AKINNIBOSUN, O. and ^{*1,2}IGBINOSA, E.O.

¹Applied Microbial Processes & Environmental Health Research Group, Faculty of Life Sciences, University of Benin, PMB 1154, Benin City 300283, Nigeria.

²Department of Microbiology, Faculty of Life Sciences, University of Benin, PMB 1154, Benin City 300283, Nigeria.

³Department of Microbiology, Faculty of Science, Federal University of Health Sciences Otukpo, PMB 145, Otukpo 927101, Nigeria.

^{*}Corresponding Author's E-mail: eigbinosa@gmail.com; Phone: 08114744531

ABSTRACT

Vegetables are an essential part of people's diet globally but they could aid the transmission of potential pathogens and channel the dissemination of resistance genes. This study aimed at evaluating the prevalence and antibiogram profile of *Staphylococcus aureus* in vegetables obtained from local markets in Benin City, Nigeria. A total of 50 fresh vegetables were collected from five local markets and investigated. These include 15 green leafs (*Amaranthus tricolor*), 15 water leafs (*Talinum fruticosum*), and 20 pumpkin leafs (*Telfairia occidentalis*). The samples were screened for *Staphylococcus aureus* using cultural, biochemical and Analytical Profile Index (API) Staph 32 identification protocols. The phenotypic characterization of antibiotic resistance was evaluated using the Kirby-Bauer disc diffusion technique. The distribution of *Staphylococcus aureus* as observed was green leafs [3/14 (21.4%)], water leafs [6/14 (42.9%)] and pumpkin leafs [5/14 (35.7%)]. The distribution of *Staphylococcus aureus* based on market location was showed the highest occurrence at the Oba market (40%) and New Benin market (40%) while the least occurrence was observed at New-market (10%). Overall, the total *Staphylococcus aureus* positive samples were 14/50 (28.0%) in all markets. The antibiotic-resistance profile of *Staphylococcus aureus* demonstrated by the isolates showed that the highest resistance was recorded for penicillin while no resistance was observed for nitrofurantoin and gentamicin. The multiple antibiotic resistance (MAR) index of *Staphylococcus aureus* in this study ranged from 0.14 – 0.71. Findings from this study and observed retail practices in local markets ascertained the possibility of vegetables getting contaminated via unhygienic agronomic and food handling practices.

Keywords: Antibiotic resistance, Benin City, Food Safety, *Staphylococcus aureus*, Vegetables

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INTRODUCTION

Vegetables form a major part of the most nutritious diet due to their enriched minerals, vitamins, and phytonutrients contents (Losio *et al.*, 2015). These high nutritive values have immensely increased the consumption of vegetables over the years (Degaga *et al.*, 2022). Similarly, vegetables play prominent roles as prophylactic supplements in obesity, diabetes, cancer, heart disease and deficiency in micronutrient therapies in most undeveloped and emerging nations (Septembre-Malaterre *et al.*, 2018). However, despite all the nutritional and economic benefits of cultivating and consuming leafy vegetables, the consumption of raw and underprepared vegetable products could trigger several health risks such as food poisoning resulting from the activities of diverse microorganisms including *Staphylococcus aureus*.

In most developing countries, vegetable farmers employ the use of wastewaters for irrigation purposes and animal wastes in soil fertilization to subdue the limited access to clean water and synthetic fertilizers (Kouassi *et al.*, 2019). These practices make fresh vegetables potential vehicles of foodborne microbial pathogens which can be detrimental to the health of consumers (Mir *et al.*, 2018; Attien *et al.*, 2020). Foodborne pathogens could cause disease manifestations resulting from the activities of enterotoxins (Bennett *et al.*, 2013). Globally, an estimate of about tens of million cases of vegetable-borne diseases often with deaths has been reported, with most cases emerging from Africa (Attien *et al.*, 2020). Several investigations have reported contamination of leafy vegetables by microbes including *S. aureus* (Seo *et al.*, 2010; Hong *et al.*, 2015).

S. aureus is one of several pathogens associated with cross-contamination of food materials (Kadariya *et al.*, 2014). Despite the fact that *S. aureus* is a ubiquitous microbiota, they could opportunistically trigger the development of food intoxication and other related diseases (Wu *et al.*, 2018). *S. aureus* produces several virulence factors which include leukocidin, haemolysin, staphylococcal enterotoxins (SEs), and toxic shock syndrome 1 (TSST-1) which influences their pathogenicity potential (Wu *et al.*, 2018). Globally, the activities of enterotoxigenic *S. aureus* are a foremost cause of foodborne diseases of staphylococcal origin (Hennekinne *et al.*, 2012). *S. aureus*'s capability of acquiring determinants of antibiotic resistance to diverse antimicrobial agents has also been reported (Argudin *et al.*, 2012; Jamali *et al.*, 2015; Attien *et al.*, 2020). The alarming surge of antimicrobial-resistance in *S. aureus* has been detected in several developed and emergent nations over the years (Wang *et al.*, 2017; Kashani *et al.*, 2018). *S. aureus* strains isolated from vegetables including pumpkin leaves, water leaves, green leaves, bitter leaves and lettuce have notably demonstrated multi-drug resistance which could induce considerable menace to man's health (Kemajou *et al.*, 2017; Wu *et al.*, 2018). This connotes the potential of food materials as a probable route of transmitting antibiotic resistance to humans (Gutiérrez *et al.*, 2012). It is therefore very necessary to routinely assess microorganisms associated with food sources using monitoring tools such as phenotypic characterization of antibiotic resistance. This study evaluated the prevalence and antibiogram profile of *S. aureus* associated with vegetables obtained from local markets in Benin City, Edo State.

MATERIALS AND METHODS

Study Area

This study was carried out in Benin City, Edo State. It lies between latitude 6°20'00 N and longitude 5°37'20 E in South Southern region of Nigeria. The mean temperature is about 27°C and over 2000mm annual rainfall.

Samples Collection

Fifty (50) vegetable samples were collected from five local markets using and assessed in the study. The samples include 15 green leafs (*Amaranthus tricolor*), 15 water leafs (*Talinum fruticosum*) and 20 pumpkin leafs (*Telfairia occidentalis*). The markets were Uselu Market, Ekiosa market, New Benin Market, New Market, and Oba Market. Uselu market is located at Uselu, Ugbowo; Ekiosa market is located between Second/Third East Circular Road and Sakponba Road; Oba market is located at Ring Road; New Benin market is located along New Lagos Road and Upper Mission Road while New market is located along Second East Circular Road, Benin City. The samples were collected using sterile polystyrene containers and conveyed immediately for analysis in the laboratory within 4 h after collection.

Isolation and Identification of *Staphylococcus aureus*

Staphylococcus aureus isolation and identification were carried out as described previously (Wu *et al.*, 2018). Twenty-five grams (25g) of each sample were mixed in a sterile plastic bag containing 225mL normal saline solution and homogenized in a stomacher for about 2 minutes. An aliquot of 100 μ L from each of the homogenized samples was aseptically pipetted into 5 mL tryptone soy broth supplemented with 10% NaCl (Merck, Darmstadt, Germany) and subsequently incubated for 18-24 h at 37°C. After incubation, loopful bacteria culture from tryptone soy broth was inoculated using the streak technique on mannitol salt agar plates (Lab M, Lancashire, UK) and was incubated at 37°C for 18-24 h. Distinct golden yellow colonies were classified as presumptive *Staphylococcus aureus*. Presumptive *Staphylococcus aureus* was sub-cultured on Nutrient agar (Lab M, Lancashire, UK) and further characterized via Gram reaction, KOH test, coagulase test, oxidase test and catalase test. Presumptive *Staphylococcus aureus* was Gram-positive cocci, KOH negative, coagulase-positive, oxidase negative and positive to catalase reaction. The isolates were subsequently screened and confirmed via Analytical Profile Index (API) Staph 32 (BioMerieux, France) identification techniques according to the manufacturer's instruction.

Antibiotic Susceptibility Test

Staphylococcus aureus was screened for antibiotic susceptibility using Kirby-Bauer disc diffusion technique (Bauer *et al.*, 1966). Bacterial suspension was standardized using 0.5 McFarland's relative turbidity and was smeared on mueller-hinton agar (Lab M, Lancashire, UK). The mueller-hinton agar plates were impregnated with antibiotic discs via aseptic techniques and incubated for 18 h at 37°C. Antibiotics tested include clindamycin (2 μ g), tetracycline (30 μ g), nitrofurantoin (300 μ g), cefepime (30 μ g), erythromycin (15 μ g), penicillin G (10 units) and gentamicin (10 μ g) (Oxoid, Hampshire, UK). The results were interpreted using the diameter of inhibition zones as recommended by CLSI, (2018).

Multiple Antibiotic Resistance (MAR) Index

MAR index was determined as previously expressed (Chitanand *et al.*, 2010). MAR index equals a/b. "a" represents the number of antibiotics to which the test isolates demonstrated resistance while "b" represents total number of antibiotics tested.

RESULTS

The distribution of *Staphylococcus aureus* as observed were green leafs [3/14 (21.4%)], water leafs [6/14 (42.9%)] and pumpkin leafs [5/14 (35.7%)]. The highest prevalence was detected in water leaf samples while the least was observed in green leafs samples as shown in Table 1. The distribution of *Staphylococcus aureus* based on market location was shown in Table 2. These include Ekiosa market [3/10 (30%)], Uselu market [2/10 (20%)], Oba market [4/10 (40%)], New Benin market [4/10 (40%)] and New Market [(1/10) (10%)]. The highest occurrence was observed at Oba market and New Benin market (40%) while the least occurrence was observed at New market (10%). Overall, the total *Staphylococcus aureus* positive samples were 14/50 (28.0%).

Table 1: Occurrence of *Staphylococcus aureus* from vegetables

Sample Type	Samples assessed	<i>S. aureus</i> positive samples
Green leaf	15	3
Water leaf	15	6
Pumpkin leaf	20	5
Total	50	14/50(28.0%)

Table 2: Distribution of *Staphylococcus aureus* from vegetables based on market location

Market	Samples assessed	<i>S. aureus</i> positive samples
Ekiosa Market	10	3
Uselu Market	10	2
Oba Market	10	4
New Benin Market	10	4
New Market	10	1
Total	50	14(28.0%)

Table 3: Antibiotics susceptibility profile of *Staphylococcus aureus*

Antibiotics class	Antibiotics	Susceptibility profile of <i>Staphylococcus aureus</i> (n=14)		
		Sensitive (%)	Intermediate (%)	Resistance (%)
Lincosamides	Clindamycin (2 µg)	5(35.7)	2(14.3)	7(50.0)
Tetracyclines	Tetracycline (30 µg)	6(42.9)	4(28.6)	4(26.8)
Nitrofurans	Nitrofurantoin (300µg)	11(78.6)	3(21.4)	0(0)
Cephalosporins	Cefepime (30 µg)	6(42.9)	-	8(57.1)
Macrolides	Erythromycin (15 µg)	7(50.0)	2(14.3)	5(35.7)
Penicillins	Penicillin G (10 units)	4(28.6)	-	10(71.4)
Aminoglycosides	Gentamicin (10 µg)	14(100)	0(0)	0(0)

NOTE: Values in parenthesis () represents percentage.

The resistance demonstrated by the isolates were clindamycin [7/14 (50.0%)], tetracycline [4/14 (26.8%)], nitrofurantoin [0/14 (0%)], cefepime [8/14 (57.1%)], erythromycin [5/14 (35.7%)], penicillin G [10/14 (71.4%)] and gentamicin [0/14 (0%)]. The highest resistance was demonstrated towards penicillin with a resistance rate of 71.4% while there was no resistance observed towards nitrofurantoin and gentamicin. However, three isolates demonstrated an intermediate resistance towards nitrofurantoin. The multiple antibiotic resistance index (MAR Index) of *Staphylococcus aureus* was shown in Table 4. It was observed that two isolates were resistant to at least five antibiotics and two isolates demonstrated resistance to four antibiotics.

Table 4: Multiple antibiotics resistance index of *Staphylococcus aureus*

Isolate code	Number of antibiotics	Resistance phenotype	MAR Index
G ₆ , W ₁	5	CLI/TET/FEP/ERY/PEN	0.71
W ₄ , W ₁₃	4	CLI/TET/ERY/PEN	0.57
W ₅ , P ₁ , P ₁₄	2	CLI/FEP	0.29
G ₁	2	FEP/ERY	0.29
W ₁₀ , P ₆	2	FEP/PEN	0.29
G ₇ , W ₇ , W ₁₁ , P ₂₀	1	PEN	0.14

Key: CLI: clindamycin, TET: tetracycline, FEP: cefepime, ERY: erythromycin, PEN: penicillin G, G: Green leaf, W: Water leaf, P: Pumpkin leaf

However, all the isolates except G₇, W₇, W₁₁ and P₂₀ were resistant to at least two antibiotics. The multiple antibiotic resistance index of *Staphylococcus aureus* in this study ranged from 0.14 – 0.71. All isolates except the four mentioned above demonstrated an MAR index ≥ 0.29 .

DISCUSSION

Vegetables are agricultural products that are usually in close proximity to the soil. This greatly exposes vegetable crops to the risk of getting cross-contaminated with soil-borne bacteria that are soil microbiota, soil decomposers in organic manure, or microbial-infested irrigation water (Hölzel *et al.*, 2018). In the course of this study, *Staphylococcus aureus* was detected in 14/50 (28.0%) of the vegetable samples assessed in all markets. Detection of *S. aureus* in vegetable has been emphasized in several studies from Nigeria and other countries (Orji *et al.*, 2016; Kibitok and Nduko, 2016; Wu *et al.*, 2018). The presence of *S. aureus* in vegetable samples could be resultant of poor hygiene policies in the chain of food processing and thus signals risks to the health of potential consumers. Additionally, crops such as vegetables which are cultivated in close proximity to the soil are at high risk of contamination with soil-borne bacteria either via natural micro-biota or organic fertilization (Hölzel *et al.*, 2018). This agrees with previous study which reported that microbial contamination of vegetables could have occurred on farms during cultivation, harvesting, or postharvest handling by farmers (Olaimat *et al.*, 2016). Similarly, it could be due to unhygienic handling practices and storage conditions at retail points (Mritunjay *et al.*, 2015). Furthermore, Iyoha and Agoreyo (2015) reported that the unhygienic practices of potential buyers discriminately have contact with vegetables in open stalls during product selection. This agrees with other studies that have also implicated improper handling of food products

to immensely alleviate the risks associated with cross-contamination microbes including *S. aureus* (Elfaki and Elhakim, 2011; Gutiérrez *et al.*, 2012). The varying level of *S. aureus* occurrence among the various local markets could be due to different levels of hygienic practices by the handlers. Farmers and vegetable marketers believed that rinsing vegetables with clean water should eliminate microbial contamination. However, previous research has emphasized that rinsing procedures could not significantly reduce the level of microbial contamination in vegetables to safe limits (De Silva *et al.*, 2013).

The antibiotic-resistant profile of *S. aureus* showed that the isolates were significantly resistant to penicillin antibiotics (71.4%). This is lower than the 83.1% and the 96.3% previously reported in comparable studies by Salmanov *et al.* (2021) and Hong *et al.* (2015) respectively on lettuce, cucumbers, sprouts, tomatoes and other vegetables. High levels of staphylococcal resistance to penicillins have also been demonstrated in other studies (Seo *et al.*, 2010; Jamali *et al.*, 2015). The use of animal wastes as manure and the use of contaminated irrigation water in crops cultivation have been implicated as possible entry source of antibiotic-resistant bacteria (Cerqueira *et al.*, 2019; Hölzel *et al.*, 2018; Amato *et al.*, 2021). Contamination of irrigation water with antibiotic resistant bacteria can be attributed to discharge of untreated wastewater into receiving rivers and other water bodies meant for irrigation purposes (Sidrach-Cardona *et al.*, 2014; Rodríguez-Mozaz *et al.*, 2015). This makes vegetables irrigated with contaminated water a potential vehicle for the dissemination of antibiotic resistance along the food chain (Araújo *et al.*, 2017; Hölzel *et al.*, 2018). Resistance to other antibiotic agents which include tetracycline, erythromycin, and clindamycin is comparably similar to the level demonstrated in other studies (Yang *et al.*, 2016; Wang *et al.*, 2017). The potential of antibiotic-resistant bacteria to spread via vegetables and other food sources is a cause for public health concern. Nevertheless, isolates under study demonstrated no resistance observed towards gentamicin and nitrofurantoin. The multiple antibiotic resistance index of *S. aureus* ranged from 0.14 – 0.71. Most of the isolates demonstrated an MAR index ≥ 0.29 . Previous investigation reported that MAR index that exceeds 0.20 indicates that the bacteria being assessed originated from sources associated with indiscriminate antibiotic use (Gufe *et al.*, 2019). The extensive use of penicillin, tetracycline and β -lactam in veterinary medicine have been widely reported due to their low cost and broad spectrum of activity (Harnisz *et al.*, 2011; Igbinosa and Beshiru, 2019). Hence, regulated use and prescription of antibiotics is important in curtailing the spread of drug-resistant bacteria (Wu *et al.*, 2018).

CONCLUSION

This study affirms the necessity for better health surveillance of consumables to curtail food-borne disease arising from microbial contamination. It is recommended that farmers and food handlers should be enlightened on proper hygiene and crop cultivation practices. Therefore, food materials including vegetable products should be guided from contamination by animals, humans and irrigation water to negate the threat to consumers' health. The consumption of raw vegetables without adequate processing and the use of unsafe water during processing stages should also be discouraged.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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