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Beta-Lactamase production and antibiotic susceptibility screening of *Staphylococus aureus* isolated from ready to eat fruits sold in some parts of Offa Metropolis, Nigeria

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ABSTRACT

The global menace of community-acquired antibiotic resistance of Beta Lactamase-producing *Staphylococcus aureus* has been traced to the increased consumption of Ready-to-eat Foods/Fruits. Samples each of ready-to-eat whole and sliced fruits (sliced pawpaw, apple, sliced watermelon, garden egg, cucumber, pear, guava, sliced coconut, berry and date fruit) were collected randomly from vendors in Offa, Kwara State, in Nigeria. Isolation and characterization of *Staphylococcus aureus* from the samples were done. The isolates were screened for Beta-Lactamase production and susceptibility to some antibiotics using standard microbiological techniques. A total of twenty-two (Twenty coagulase-positive and two coagulase-negative) *Staphylococcus aureus* was isolated. The total Staphylococal count was highest in sliced pawpaw ($23.30 \pm 2.75 \times 10^5$ cfu/g) while the least was recorded in apple ($3.0 \pm 0.01 \times 10^5$ cfu /g). Twenty (20) isolates were recorded to be Beta Lactamase producers. All the Beta Lactamase producers were 100 % resistant to Aztreoname, 80 % to Amoxicillin Clavulate, 45 and 35 % to Ceftazidine and Ceftriaxone. Thirty percent of the isolates were found to be susceptible to Ceftazidine only while 25 % were susceptible to Ceftriaxone only. The study concluded that increased incidence of Community-Acquired Multidrug-Resistant *Staphylococcus aureus* could be traceable to the consumption of unhygienic processed Ready-to-eat Fruits. The ripple effects could be dangerous to human health.

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1. Introduction

Beta-lactam antibiotic-resistant *Staphylococcus aureus* is one of the leading causes of food poisoning, a form of gastroenteritis with a rapid onset of symptoms (1). *Staphylococcus aureus* is commonly found in the environment (soil, water and air) and is also found in the nose and on the skin of humans (2, 3).

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It is variously associated with many diseases and is foodborne (4). The pathogenesis of *S. aureus* infection depends on the production of surface proteins that mediate bacterial adherence to host tissues, secretion of a series of extracellular toxins, and enzymes that destroy the host cells and tissues, the host immune defense, and growth and spread of bacterial in host cells (5-7). *Staphylococcus aureus* has generated a lot of interest over the last half century due to its ability to rapidly adapt to antibiotic pressure and develop

antibiotic resistance. The health burden attributable to Methicillin-resistant *Staphylococcus aureus* (MRSA) has been summarized as significant (5). MRSA species has been shown to demonstrate higher rates of associated septic shock and discharge to long-term care than methicillin-susceptible species (4).

Antibiotics can cure disease because they can efficiently inhibit cell wall synthesis, protein synthesis, or DNA replication to kill pathogenic bacteria or inhibit their growth (9). The emergence and spread of strains of *S. aureus* that are resistant to first-line antibiotics is of public health importance. Methicillin-resistant *S. aureus* is a special strain of *S. aureus* that is resistant to the antibacterial activity of methicillin-based and other related antibiotics of the penicillin class. MRSA has acquired genes encoding antibiotic resistance to all methicillin-based, including resistance of pathogenic organisms to countenance antibiotics has become a worldwide tragedy with serious consequences on the treatment of infectious diseases and street vended food has been reported as a major vehicle (5, 10). The β-lactams are a large of diverse compounds and due to their excellent safety profile and broad antimicrobial spectrum are considered to be the most widely used therapeutic class of antimicrobial spectrum are considered to be the most widely used therapeutic class of antibacterial prescribed in human and variety clinical practices (2, 11). The persistent exposure of bacterial strains to a multitude of β-lactams has induced dynamic and continuous production and mutation of β-lactamases in these bacteria, expanding their activity even against the newly developed β-lactam antibiotics (1). These enzymes are known as extended-spectrum β-lactamases (7). Emergence and increasing occurrence of resistant is putting a lot of pressure and presents challenge to healthcare experts. Resistance to β-lactam compound is mainly due to the production of beta- lactamases (BLs) that hydrolyze and thereby inactivate beta lactam antibiotics (12).

The consumption of ready-to-eat fruits has become a global trend and has been reported to be associated with occurrence of resistance in *S. aureus* (4, 10). This is due to their accessibility, convenience, and relatively cheaper prices than the whole fruits (13). Thus, they have gradually become staples due to the recent modernization, industrialization, economic downturn, materialism, and unavailability of ample time to prepare a proper meal in some Food Quality and Safety. The surge in the rate of consumption of street vended food/fruits especially in the developing world has been reported to signal a great risk to consumer health (4, 10) because it is difficult to ascertain the hygienic processes the fruits are subjected to after harvesting, during processing, and before packaging (14). Food contamination during processing and distribution is directly documented as being responsible for staphylococcal Foodborne poisonings (1).

2. Materials and Methods

2.1. Sample collection and preparation

Samples of ready-to-eat sliced fruits (Apple, pear, guava, sliced watermelon, sliced coconut, sliced pineapple, cucumber, berry, sliced pawpaw and date) were collected randomly from the street vendors at Offa metropolis in Kwara State. The fruits were collected in a sterile polythene bag at about 9:00 -10:00 am and transferred to the laboratory for immediate processing.

2.2. Isolation of microorganisms

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A Ten-fold serial dilution of the sample was performed and 1 ml of the appropriate dilution was inoculated unto a sterile Mannitol salt agar using Pour Plate Technique. Isolates that were able to ferment Mannitol salt were taken as *Staphylococcus*. Pure cultures of isolates were maintained on agar slant for further analysis (3).

2.3. Identification of isolates

The routine methods of (15) and others were used to characterize isolates based on their Gram's reaction, catalase and coagulase tests.

2.4. Gram reaction of isolates

A smear of bacteria was made on a clean grease free slide subjected to Gram reaction, catalase and coagulase test (16).

2.5. Catalase test

A drop of 3 % H_2O_2 was introduced unto a grease free slide and mixed with a loop of bacteria. Formation of bubbles confirmed positive test (17).

2.6. Coagulase test

A drop of physiological saline was placed on two separate grease free slide, a loop of bacterial isolate was emulsified on the two slides. A drop of human plasma was added to one of the slide and mixed gently. Clumping was confirmed for Positive test (17).

2.7. Screening of isolates for beta lactamase production

The screening of each isolate for the presence of beta lactamase was done by preparation of Penicillin starch test strip and beta lactam assay was conducted.

2.8. Preparation of penicillin starch paper strips

Benzyl Penicillin containing 1,000,000 IU of antibiotic was dissolved in 10 mL of phosphate buffered saline at pH 7.3 such that each of the solution will contain 100,000 IU of benzyl penicillin. Two percent (2 %) of starch powder was added to 98 mL of phosphate buffered saline; the mixture was warmed until the starch dissolved completely. A Whatman No. 3 filter paper was cut into strips of size 7 cm by 4 cm to fit the bottom of the Petri dish. Ten ml of benzyl penicillin solution was added to 90 mL of 2 % starch solution and mixed properly. The paper strips was soaked in the mixture for 5 min, removed and spread on a hood to dry. The dried strips were stored in the refrigerator at 40°C before use (12)

2.9. Beta lactamase assay

A strip of the Penicillin starch paper strip was spread smoothly in a sterile petri dish. Two millimeter sterile bacteriological loop was used to collect bacterial culture from the surface of the culture medium and transferred to the test paper in a Petri dish; this was spread smoothly over an area of 2-3 mm, inoculums was placed at least 1.5 cm apart. The paper strip containing inoculums was incubated at 37 °C for 30 min after which the paper was flooded with iodine solution and drained immediately. The result was observed for de-colourization to show positive test while a blue-black colour was recorded as negative test (17).

2.10. Determination of antibiotic susceptibility profile of isolates

The broth suspension of the organisms to be tested was prepared (18) and standardized by adjusting to the turbidity of ≥ 0.5 McFarland standard (one mL of inoculum was equivalent to approximately 1.5 x 10^8 cfu/ mL). Antibiotic susceptibility test was carried out as described and used by (19). One ml of inoculum was swabbed on Mueller Hinton agar surface. Five different antibiotic discs based on guidelines set by the National Committee for Clinical Laboratory Standards (20);

(Amoxycillin/clavulate, aztreonam, ceftriaxone, cefoxitin and ceftazidime) were aseptically placed on the inoculated surface and gently pressed down within 15 min of inoculation. The plates were inverted and incubated for 24 h at 37°C. The diameter of the zone of inhibition was measured in millimeters. The result was interpreted as sensitive, intermediate or resistant according to guidelines set by the National Committee for Clinical Laboratory Stadards (20). All the antibiotics used were manufactured by Rapid Labs Ltd., Unit 2 & 2A Hall Farm Business Center, Bently, UK.

3. Results

3.1. Total bacterial count of ready-to-eat fruits

Microbial load varied with the fruit samples ranging from 3.00 ± 0.01 × 10^5 to 23.30 ± 2.75 × 10^5 cfu/mL for all samples except the date that had no growth. The highest microbial load was recorded on Pawpaw and Pineapple with 23.30 ± 2.75 × 10^5 cfu/mL and 20.30 ± 3.35 × 10^5 cfu/mL respectively. The lowest growth was recorded on Apple and Berry with a microbial load of 3.0 × 10^5 cfu/mL (Table 1).

3.2. Colonial morphology and gram reaction of bacterial isolates

The colonial morphology and Gram's reaction to the isolates are presented in Table 2. The colonies were yellowish, pinkish and oily. The isolates were all Gram-positive cocci in clusters.

3.3. Biochemical characteristics of the bacterial isolates

All Twenty-two (22) isolates were catalase positive; Twenty (20) of the isolates were coagulase positive while two (2) isolates were negative (Table 3).

Table 1. Staphylococcal Count (cfu/mL)

Sample	(x10^5 cfu/ml)
Apple	3.00 ± 0.01
Pear	4.30 ± 1.65
Guava	6.30 ± 0.50
Watermelon	20.60 ± 3.77
Coconut	12.00 ± 2.59
Pineapple	20.30 ± 3.35
Cucumber	7.60 ± 3.10
Berry	3.00 ± 0.50
Pawpaw	23.30 ± 2.75
Date	0.00

*All data are means of triplicate readings and standard deviation

3.3. Beta-lactamase production by bacterial isolates

This study was confirmed that twenty (20) of the isolated *Staphylococcus aureus* that were coagulase positive were also beta lactam producing bacteria. The two (2) isolates that were coagulase negative were non beta lactam producers.

3.4. Antibiotic susceptibility pattern of *Staphylococcus aureus*

*A*ll the beta lactam producing *Staphylococcus aureus* isolated were found to be 100 % resistance to Aztreonam and Cefoxitin (Table 5). Eighty percent (80 %) were resistant to Amoxycillin clavulanate while 20 % were susceptible to the antibiotics. Thirty five percent (35%) of *Staphylococcus aureus were* resistant to Ceftriaxone and 45 % were resistant to Ceftazidime.

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Table 2. Colonial morphology and Grams reaction of isolates

Isolates	Colour	Shape of cell	Gram's Reaction
BN 1	Yellow	Cocci	+ve
Bn 2	Yellow	Cocci	+ve
Bn 3	Yellow	Cocci	+ve
Bn 4	Yellow	Cocci	+ve
Bn 5	Yellow	Cocci	+ve
Bn 6	Yellow	Cocci	+ve
Bn 7	Yellow	Cocci	+ve
Bn 8	Pink and oily	Cocci	+ve
Bn 9	Yellow	Cocci	+ve
Bn 10	Yellow	Cocci	+ve
Bn 11	Yellow	Cocci	+ve
Bn 12	Yellow	Cocci	+ve
Bn 13	Yellow	Cocci	+ve
Bn 14	Yellow	Cocci	+ve
Bn 15	Yellow	Cocci	+ve
Bn 16	Yellow	Cocci	+ve
Bn 17	Pink and oily	Cocci	+ve
Bn 18	Yellow	Cocci	+ve
Bn 19	Yellow	Cocci	+ve
Bn 20	Yellow	Cocci	+ve
Bn 21	Yellow	Cocci	+ve
Bn 22	Yellow	Cocci	+ve

*Bn = Isolate; +ve = Positive; -ve = Negative

Table 3. Biochemical characteristics of bacterial isolates

Isolates	Catalase	Coagulase	Isolated Bacteria
Bn 1	+Ve	+ve	*Stapylococcusaureus*1
Bn 2	+ve	+ ve	*Stapylococcusaureus*2
Bn 3	+ve	+ve	*Staphylococcus aureus*3
Bn 4	+Ve	+Ve	*Staphylococcus aureus*4
Bn 5	+Ve	+Ve	*Staphylococcus aureus*5
Bn 6	+Ve	+Ve	*Staphylococcus aureus*6
Bn 7	+Ve	-Ve	*Staphylococcus aureus*7
Bn 8	+Ve	+Ve	*Staphylococcus aureus*8
Bn 9	+Ve	+Ve	*Staphylococcus aureus*9
Bn 10	+Ve	+Ve	*Staphylococcus aureus*10
Bn 11	+Ve	+Ve	*Staphylococcus aureus*11
Bn 12	+Ve	+Ve	*Staphylococcus aureus*12
Bn 13	+Ve	+Ve	*Staphylococcus aureus*13
Bn 14	+Ve	+Ve	*Staphylococcus aureus*14
Bn 15	+Ve	+Ve	*Staphylococcus aureus*15
Bn 16	+Ve	-Ve	*Staphylococcus aureus*16
Bn 17	+Ve	+Ve	*Staphylococcus aureus*17
Bn 18	+Ve	+Ve	*Staphylococcus aureus*18
Bn 19	+Ve	+Ve	*Staphylococcus aureus*19
Bn 20	+Ve	+Ve	*Staphylococcus aureus*20
Bn 21	+Ve	+Ve	*Staphylococcus aureus*21
Bn 22	+ve	+ve	*Staphylococcus aureus*22

* Bn = Isolate; +ve = Positive; -ve = Negative

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Table 4. Beta Lactamase production by isolates

Isolates	Beta Lactamase Production
Bn 1	+ve
Bn 2	+ve
Bn 3	+ve
Bn 4	+ve
Bn 5	+ve
Bn 6	+ve
Bn 7	-ve
Bn 8	+ve
Bn 9	+ve
Bn 10	+ve
Bn 11	+ve
Bn 12	+ve
Bn 13	+ve
Bn 14	+ve
Bn 15	+ve
Bn 16	-ve
Bn 17	+ve
Bn 18	+ve
Bn 19	+ve
Bn 20	+ve
Bn 21	+ve
Bn 22	+ve

* +ve = Positive; -ve = Negative

Table 5. Susceptibility pattern of *Staphylococcus aureus* to selected Beta Lactam antibiotics as indicated by zones of inhibition

Isolates	Amoxy + cluvanate (30 µg)	Cefoxitin (30 µg)	Aztreoname (30 µg)	Ceftriaxone (30 µg)	Ceftazidime (30 µg)
	Zones of Inhibition (mm)				
S. *aureus*1	12.00	10.50	-	20.00	18.00
S. *aureus*2	15.00	10.00	-	20.00	14.50
S. *aureus*3	32.00	10.50	-	19.00	19.50
S. *aureus*4	16.00	11.00	-	12.00	9.00
S. *aureus*5	15.50	10.50	-	18.50	8.00
S. *aureus*6	16.00	10.50	-	20.00	13.00
S. *aureus*8	34.00	12.00	-	24.50	19.00
S. *aureus*9	15.00	10.50	-	13.00	9.00
S. *aureus*10	15.00	10.00	-	23.50	14.50
S. *aureus*11	16.00	10.00	-	24.00	18.00
S. *aureus*12	16.00	10.50	-	13.00	9.50
S. *aureus*13	15.50	10.50	-	19.50	14.00
S. *aureus*14	16.00	11.00	-	12.50	8.00
S. *aureus*15	30.00	10.00	-	24.00	19.00
S. *aureus*17	15.00	10.00	-	13.00	14.50
S. *aureus*18	32.00	12.00	-	24.00	19.00
S. *aureus*19	16.00	10.50	-	13.00	9.00
S. *aureus*20	16.00	10.00	-	19.00	8.50
S. *aureus*21	16.00	10.00	-	12.50	8.00
S. *aureus*22	16.00	11.00	-	19.00	8.50

Table 6. Antibiotic susceptibility pattern of isolated strains of *Staphylococcus aureus*

Antibiotic	Potency (*µg*)	Number *S. aureus* (%)		
		Sensitive	Intermediate	Resistant
AmoxycillinClavulanate	20/10	20	0	80
Cefoxitin	30	0	0	100
Aztreonam	30	0	0	100
Ceftriaxone	30	25	40	35
Ceftazidime	30	30	25	45

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3.5. Antibiotic zone of inhibition on bacterial isolate

The zone of inhibition varies based on type of antibiotics, sensitivity to Amoxycillin Cluvalate as indicated by the zones of inhibition ranges from 12 to 34 mm, Cefoxitin ranges from 10 to 12 mm and Ceftriazone ranged from 12 to 24 mm while Ceftazidime ranged from 8 to 19.50 mm.

4. Discussion

Staphylococcus aureus is reported to be responsible for many food poisoning of bacterial origin. The close association of this bacterium with humans has been incriminated (2-4). The problem of multi-drug-resistant community-acquired species calls for serious attention and swift action (5). Ready-to-eat food and food vendors have been incriminated as the vector (4). The Staphylococcal count recorded on the ready-to-eat fruit samples examined in this study was high. The observation could be traceable to the processing and packaging methods used by vendors. It was recorded that sliced fruits have a higher microbial load than whole fruits. This result is similar to the findings of (21) in a review of the consumption of ready-to-eat fruits and vegetables. All the *Staphylococcus aureus* isolated were coagulase positive except two (isolates 7 and 16). They were coagulase-negative *Staphylococcus aureus* and were also negative for beta-lactam production. Coagulase-negative staphylococci were reportedly identified from food, and food handlers along processing and distribution lines (6, 22).

Date palm fruit was found to have zero staphylococcal count in this study. The absence of *Staphylococcus aureus* on date could probably be due to low moisture content and of the fruits as well as the high sugar content. Similar result was reported by (23). *Staphylococcus aureus* been a commensal of human can be found in close connection with processed foods essentially when processing does not involve heating. The equipment handler's hands; packaging and surfaces where the fruits are displayed are usually common sources of *Staphylococcus* and other food borne pathogens (24). The nutritional status and moisture content of the fruits allow for rapid multiplication of the bacterial contaminants. The result is a very high bacterial count in the fruits (1, 25).

The slicing process further exposes the fruits to more contamination by increasing the surface area prone to bacterial attack. The natural barriers in terms of back, skin, shell, rind among others, have been removed making the fruits more vulnerable to pathogen (16). The production of Beta lactamase by this isolates is an indication confirms their resistance to Beta lactam antibiotics (26). Bacteria with ability to produce Beta lactamase usually are resistant to methicillin based antibiotics (27). Furthermore the high percentage of Beta lactamase producer is a pointer to increase in community acquired resistant *Styaphylococcus aureus* strains, thus infection from these strains becomes difficult to treat leading to high morbidity and mortality rate (28).

Antibiotic susceptibility pattern of the isolated *Staphylococcus aureus* revealed 100% resistant to Aztreonam and Cefoxitin which are among notable and commonly recommended beta lactam antibiotics (Table 5). The high percentage of resistance could pose serious health challenge to public health as ailment of staphylococcal origin will not be treatable by these antibiotics. These agrees with the findings of (1, 25) who recorded similar trends in resistance of

Staphylococcus aureus in their studies. Bacteria often develop resistance to beta-lactam antibiotics by synthesizing a beta-lactamase, an enzyme that attacks the beta-lactam ring. To overcome this resistance, beta-lactam antibiotics are often given with beta-lactamase inhibitors such as Clavulanic acid (12). The isolated *Staphylococcus aureus* was also resistant to amoxicillin clavunate though a small percentage (20%) of the isolate were susceptible, they were however moderately susceptible to Ceftriaxone and Ceftazidine (35 and 45 %) respectively. This trend of resistance indicates a high tendency to increase in virulence of *Staphylococus aureus* and poor sensitivity to second and third generations of beta-lactam antibiotics.

Microbial resistance to third-generation cephalosporin drugs has been increasing significantly as the findings of the present study indicated. Moreover, previous studies have it that those strains that developed resistance to third-generation antibiotics were also resistant to multiple drugs which could make treatment of infectious diseases, triggered by these microbial strains challenging (6,21,29). Therefore, the right medications should be selected based on susceptibility data of causative agents towards the drugs for the treatment of the right disease agents.

5. Conclusion

The present study has been able to establish that *Staphylococcus aureus* is predominant on fresh ready to eat fruits since they are common commensal of man and can be easily transmitted during handling or processing of fresh fruits. The widespread use of brood spectrum antibiotics has led to the emergence of antibiotic resistant strains of bacteria. High rates of resistance have been primarily observed in bacteria that cause common health problems. The multidrug resistance pattern in this study has also shown that *Staphylococcus aureus* is capable of rendering the effect of antibiotics useless and as such there is a need for proper hygiene while handling fruits to avoid cross-contamination of infectious diseases in humans. Professionals and other people including farmers and market women involved in the food production industry ranging from production to the market should be made aware of the potential risk associated with various practices and possible chances of contamination. Caution should be taken to make sure one does not use one cycle of water for all vegetables. This might cause further contamination of previous cycles. So, the consumption of whole fresh fruits properly washed is still encouraged by this review but significant measures must be taken to check the safety of these products before consumption.

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Author's contributions

MRA reviewed the methods of research, supervised the lab work and writing of the manuscript, reviewed and edited the manuscript. TOE conceive the project idea, carry out the investigations, collated and analyzed the data, and wrote the manuscript. AEA reviewed and edited the manuscript.

Declaration of competing interest

All authors hereby declared that there was no competing interest

Data availability

Authors hereby state that all data are available at the point of submission

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References

1. Muhammad SM, Aliero AA. Antibiotic susceptibility pattern of *Staphylococcus aureus* isolated from fresh lettuce (*Lactuca sativa*) and food safety knowledge of vegetable vendors in Birnin Kebbi, *Nigeria.* J Food Safe Hyg. 2023; 9(3): 185-96.
2. El-Deeb W, Cave R, Fayez M, Alhumam N, Quadri S, Mkrtchyan HV. Methicillin-Resistant Staphylococci Isolated from Goats and Their Farm Environments in Saudi Arabia Genotypically Linked to Known Human Clinical Isolates: a Pilot Study. Microbiol Spect. 2022; 10.1128/spectrum.00387-22.
3. Izabela S. Identification and study of the behavior of *S. aureus* and *S. epidemis* in fresh and frozen strawberries. Acta Sci Pol Technol Aliment. 2018; 17(1): 27-35.
4. Omoruyi IM, Ibegbulam OS. Ready-to-eat food sold in healthcare facilities could contribute to the increased disease burden of multidrug resistant *Staphylococcus aureus.* J Biol Res Biotech, Bio-Res. 2023; 21(2); 1923-34.
5. Sanabria-Rios DJ, Alequin-Torres D, De Jesus A, Cortes G, Carballeira NM. Vinyl halogenated fatty acids display ntibacterial activity against clinical isolates of methicillin-resistant *Staphylococcus aureus,* Med Res Arch. [online] 2022; 10(7). https://doi.org/10.18103/mra.v10i7.2901
6. Thwala T, Madoroba E, Basson A, Butaye P. Prevalence and characteristics of *Staphylococcus aureus* associated with meat and meat products in African Countries: A Review. Antibiotic 2021; 10, 1108.https://doi.org/10.3390/ antibiotics 10091108
7. Timothy JF. Antibiotic resistance in *Staphylococcus aureus*: Current status and future prospects. FEMS Microbiol Rev. 2017; 007 (41) 430–49.
8. Bhunia AK. *Clostridium botulinum, Clostridium perfringens, Clostridium difficile* Foodborne. Microbial Pathogens. Springer, Berlin, Germany 2018.
9. Wangai FK, Masika MM, Maritim MC. *Staphylococcus aureus* (MRSA) in East Africa: red alert or red herring? BMC Infect Dis. 2019; 19: 596.
10. Esemu SN, Njoh ST, Ndip LM, Keneh NK, Kfusi JA, Njukeng AP. Ready-to-Eat Foods: A Potential vehicle for the spread of coagulase-positive Staphylococci and antimicrobial-resistant *Staphylococcus aureus* in Buea Municipality, South West Cameroon, Hindawi. Canadian J Infect Dis Med Microbiol. 2023; Article ID 9735319, https://doi.org/10.1155/2023/9735319
11. Rahman SH, Aliu T, Aliu I, Khan N, Bohan A, Gaoj J. The growing genetic and functional diversity of extended-spectrum Beta Lactamases. Biomed Res Int. 2018; 1: 1-14.
12. Peterson E, Kaur P. Antibiotic resistance mechanisms in bacteria: relationship between resistance determinants of antibiotic producers, environmental bacteria and clinical pathogens. Frontiers Microbiol. 2018; 9: 2928.
13. Shahbandeh M. Leading Global Producers of Fresh Vegetables 2017; FAO, Rome.
14. Wang S. Spread of *Escherichia coli* O157: H7 during Flume Washing and Drying of Fresh-Cut Romaine Lettuce, Michigan State University, East Lansing, MI, USA 2016.
15. Cheesbrough M. District Laboratory Practice in Tropical countries. Cambridge University Press, Cambridge, 2006; U.K., pp. 62-70.
16. Mir SA, Shah MA, Mir SA Dar BN, Greiner R, Roohinejad S. Microbiological contamination of ready-

to-eat vegetable salads in developing countries and potential solutions in the supply chain to control microbial pathogens. Food Control. 2018; 85:235–44.

17. Amit KK, Jyoti M. Detection of β-lactamase and antibiotic susceptibility of clinical isolates of *Staphylococcus aureus*. J Bacteriol. 2019; 178: 7248-53.
18. Fawoyo PT, Ogunbanwo ST. Antimicrobial resistance in coagulase-negative Staphylococci from Nigerain Traditional Fermented foods. Annals Clinic Microbiol Antimicrob. 2017; 16:14.
19. Omoruyi IM, Ojubiaja SE. Antibiogram and virulence gene detection in *Escherichia coli* and *Vibrio* species isolated from market dumpsites in Edo South Senatorial District, Nigeria. Sci World J. 2022; 17(1): 17-25.
20. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility Testing; Twenty-fifth informational supplement. CLSI Document M100- S25. Wayme: Clinical and Laboratory Standards Institute 2015.
21. Jia K, Qin X, Bu X, Zhu H, Liu Y, Wang X, et al. Prevalence antibiotic resistance and molecular characterization of *Staphylococcus aureus* in ready to eat fruits and vegetables in Shanghai, China. Sci Direct. 2024; 8, 100669 doi.org/10.1016/j.crfs.
22. Igiehon OO, Ademola EA, Anthony TI. A review on the consumption of vended fruits: microbial assessment, risk, and its control. Food Qual Safe. 2020; 4(2): 77-81.
23. El-Zamkan MA, Mubarak AG, Ali AO. Prevalence and phylogenetic relationship among methicillin-and vancomycin resistant staphylococci isolated from hospital's dairy food, food handlers, and patients. J Adv Vet Animal Res. 2019; 6: 463.
24. Samad MA, Hashim SH, Simarani K, Yaacob JS. Antibacterial properties and effects of fruit chilling and extract storage on antioxidant activity, total phenolic and anthocyanin content of four date palm (*Phoenix dactylifera*) cultivars. Molecules. 2016; 21: 1-14.
25. Kumar V, Mritunjay SK. Fresh farm produce as a source of pathogens: a review. Res J Environ Toxicol. 2015; 9 (2) 59–70.
26. Ogofure A, Bello-Osagie I, Ahonsi C, Ighodaro V, Emoghene A. Bacteriological assessment of ready-to-eat pawpaw (*Carica papaya*) sold in selected locations in Benin City. Afric Sci. 2017; 18: 157–62.
27. McDaniel C, Jadeja R. A review of fresh produce outbreaks, current interventions, food safety concerns and potential benefits of novel antimicrobial sodium acid sulfate. MOJ Food Process Technol. 2019; 7 (3): 59–67.
28. Peacock SJ, Peterson GK. Mechanisms of methicillin resistance in *Staphylococcus aureus*. Annual Rev Biochem. 2015; 84:577–601.
29. Mueller A, Seinige D, Jansen W, Klein G, Erhrich R, Monecke S, Kehrenberg C. Variety of Antimicrobial Resistance and Virulence Factors in *Staphylococcus aureus* Isolates from Meat Products Legally and Illegally Introduced to Germany. PLos ONE. 2016; 11(12): 1-18.