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Research Article

Antibiotics Resistant Pattern of Bacteria Isolated from Spoiled Avocado Fruit Sold in Sokoto Metropolis

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Abstract

Consuming fruit contaminated with bacteria remains a crucial route of foodborne infection in developing countries and creates a severe public health burden. The research aimed to determine the antibiotic-resistant pattern of bacteria associated with the spoilage of Avocado pear (*Persea americana*) sold in the Sokoto metropolis. Twenty spoilt avocado fruits were obtained from market three in Sokoto Metropolis. The bacteriological analysis was carried out using the pour plate method. The antibiotic resistance pattern was determined using the Kirby-Bauer disk diffusion method. A total of five species of bacteria were isolated and identified in this study. The mean and standard error of total viable bacterial counts of avocado samples across three different locations ranged from 4.20±5.77 to 8.43±33.49 (x 10^4CFU/g). A total of 19 bacterial isolates were identified from avocado samples. *Staphylococcus aureus* had the highest frequency of occurrence, 16 (29.2%), while *Klebsiella* sp. had the lowest frequency of occurrence, 7 (12.7%). Among all antibiotics tested against bacterial species, *Escherichia coli* isolates were found to have 10 (91%) and 9 (82%) resistance against ciprofloxacin and streptomycin, respectively. *Pseudomonas* sp. was found to be 100% resistant to chloramphenicol and Septrin (cotrimoxazole). However, *S. aureus* was found to have 12 (72%) resistance to pefloxacin. Similarly, *Klebsiella* spp. were found to be 7 (100%) resistant to Septrin. Isolation of these bacterial species, especially *E. coli* from avocado samples analyzed, is of public health significance, especially the presence of antibiotic resistance species.

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INTRODUCTION

According to the Centers for Disease Control and Prevention (CDC), fruit and vegetables are one of the sources of food born disease in developing countries and causes serious health problem worldwide. Despite the effort, food safety programs in the African continent remain challenging, leading to poor resource usage, duplication of efforts, and a lack of coordination among the region's countries and stakeholders[1]. As a result, data are scarce on surveillance studies on the microbial safety of food in the African continent. Microbial contamination of fruits can occur from the time of production to consumption[2]. Avocados (*Persea americana*) are fruits widely sold worldwide[3]. According to Ogunwusi and Ibrahim[4], in Nigeria, avocado fruits are grown in the forest, farmlands, and homesteads but can also be found in the Central part of the country. Avocados are available in all the States in Eastern Nigeria and part of the South-South Zone; Imo, Abia, Anambra, Enugu, Ebonyi, Edo, Akwa-Ibom, Delta, and the Cross River States. Avocado fruits can be grown in Plateau and Kaduna States. However, Borno, Bauchi, Gombe, Kano, Katsina, Kebbi, Sokoto, Jigawa, Yobe, Adamawa, and Zamfara are non-avocado-producing states. The fruits are available on sale in these areas in some marketplaces. In Sokoto state, avocado is brought from those producing

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states and distributed to some markets, particularly in some hospital mini-market. More data must be collected on the microbial safety of avocados sold in the Sokoto metropolis.

In some Global markets, avocado is sold traditionally under poor hygienic conditions, with hawkers with excess flies and specks of dust all over the fruits. These can undoubtedly increase the chance of microbial contamination. Thus, the presence of these microorganisms on these fruits is dangerous for human consumption[4]. Although microflora is dominated by spoilage bacteria, yeasts, and molds, avocados can harbor pathogenic bacteria such as *Salmonella*, *Escherichia coli*, *Bacillus cereus*, *Campylobacter* spp., *Yersinia enterocolitica*, *Listeria monocytogenes*, and *Clostridium botulinum*, as well as some viruses and parasites. In 2019, avocados were recalled in six states of California (Arizona, California, Florida, New Hampshire, North Carolina, and Wisconsin) because of Listeria contamination[5]. García-Frutos *et al*.[3] and Shiferaw and Kibret[6] also reported the presence of *E. coli*, *Salmonella* sp., *Listeria* spp., and *L. monocytogenes* from avocado samples collected from retail markets located in Guadalajara, Mexico. In African continents, the story is not different; avocado fruits are primarily consumed raw, without further processing. Research conducted by Shiferaw and Kibret[6] on the Microbial Quality of avocado and guava fruits used to prepare freshly squeezed juices from juice houses of Bahir Dar Town, Northwest Ethiopia, reported the presence of Salmonella and Shigella species. However, Coetzee *et al*.[7] reported the presence of *E. coli* and *Salmonella* spp. in avocado fruit, although *L. monocytogenes* was not detected in any avocado fruit samples. Research conducted in the Ijebu area of Ogun State, Southwestern Nigeria, showed that some of the avocado fruits and vegetables marketed there were contaminated with different bacterial species, including *E. coli*[8]. A similar study[9] conducted in Benin City, the capital of Edo State, Southern Nigeria, also reported the presence of *E. coli* and other bacterial species. Although many researchers have worked on the microbial analysis of spoiled avocado fruits in Nigeria, there is limited literature on the microbial safety of contaminated avocados sold in Sokoto metropolis as different environmental conditions may spread bacteria with different genetical makeup.

Treatment of infections caused by foodborne pathogens is a global threat to healthcare management due to the development of resistance against commonly prescribed drugs[10]. The resistance or treatment failure varies from one region to another. There needs to be comprehensive data in the African continent to show the burden of antimicrobial treatment failure due to insufficient surveillance studies in the regions[11]. Most researchers focus on isolating and identifying bacteria associated with fruit spoilage in Africa, especially in Nigeria[7,9,12,13]. The empirical treatment of bacterial infections depends on selecting the appropriate antibiotics, determined by the regional susceptibility profile, key indicators in the genomic evolutionary trend, and the efficacy of antibiotics commonly prescribed in a specific locality[14]. For these reasons, studies conducted locally are critical in guiding the selection of the most appropriate antibiotics for empirical therapy, limiting the development of resistance to routinely used medications. Therefore, this study was designed to determine the antibiotic-resistant pattern of bacteria isolated from spoiled Avocado fruit sold in Sokoto Metropolis.

MATERIALS AND METHODS

Materials

A total of nine spoiled avocado fruit samples were collected from three different Markets within the Sokoto metropolis: Shagari Market (three samples), Usmanu Danfodiyo University Teaching Hospital Market (UDUTH) (three samples), and Maryam Abacha Hospital Sokoto Market (three samples). The samples were collected in a sterile polythene bag, labeled accordingly, and transported in an ice-cool box (4°C) to the Microbiology Laboratory at the Department of Microbiology, Kebbi State University of Science and Technology, Aliero, for further analysis.

Methods

Study design

The Experimental laboratory research was used to determine the bacteria associated with the spoilage of avocado and its antibiotics resistance profile. Three different Market places within the Sokoto metropolis were selected for sample collection.

These three locations are significant places where avocados can be found on sale in the Sokoto metropolis, as the fruit is rare in the state. The avocado fruit is mostly brought into the state from the Eastern, South-south zones, Plateau, and Kaduna states. The collected samples were analyzed using standard microbiological methods. The antibiotic resistance profile was determined using modified Kirby-Bauer disk diffusion. All experiments were carried out in the Microbiology laboratory at the Department of Microbiology, Faculty of Life Sciences, Kebbi State University of Science and Technology, Aliero, Nigeria.

Isolation of bacteria[15]

Ten grams of each sample was blended by adding 25 mL of sterile distilled water in a sterile blender. The composite sample was serially diluted up to 10^7. Bacteria were isolated using pour plated method. As much as 1 mL of the serially diluted avocado sample (10^4) was pipetted and poured into a sterile petri-dish. Molten Nutrient agar cooled to 45°C was poured aseptically into the plates and stirred. The plate was allowed to be solidified and incubated at 37°C for 24 hours. Each sample was inoculated in triplicate. After 24 hours of incubation, the plates were observed for growth. Colonies were counted and recorded. The results were expressed as colony-forming units per mL (CFU/mL). Plates with different colonies were subcultured on freshly prepared nutrient agar to have a pure culture.

Identification of bacteria[16]

Bacterial isolates were identified using Gram's staining and confirmed using the biochemical test. Biochemical tests include catalase, oxidase, indole, motility, citrate, mannitol fermentation (sugar), coagulase, lactose fermentation, urease, and methyl red and Vogues-Proskauer Test.

Antibiotic susceptibility testing

The antibiotic resistance pattern of bacteria isolated from Avocado was determined using the disc diffusion method. Fifteen different antibiotics were used, including cefuroxime (CFX, 30 µg) pefloxacin (PEF, 10 and 30 µg), gentamicin (GE, 10 and 30 µg), Ampiclox (APX, 30 µg), Zinacef (Z, 20 µg), amoxicillin (AM, 30 µg), Rocephin (R, 25 µg), ciprofloxacin (CPX, 10 and 30 µg), streptomycin (S, 30 µg), Septrin (SXT, 30 µg), erythromycin (E, 10 µg), chloramphenicol (CH, 30 µg), sparfloxacin (SP, 10 µg), Augmentin (AU, 10 µg), and ofloxacin (OFX, 10 µg). This was done according to the Clinical and Laboratory Standards Institute (CLSI) method[17]. A 24 hours growth of the bacterial isolate was used to prepare the inoculums. The density of the inoculums was adjusted to 0.5 McFarland turbidity standard as described by Katoch *et al*.[18], and plates were inoculated with sterile swab sticks within 15 minutes. Antibiotic discs were seeded aseptically onto the surface of the inoculated agar plates. The plates were incubated at 37°C for 18 hours. The zone of inhibition was measured using a ruler (mm), and the results were interpreted according to CLSI[17] and Awandkar *et al*[19].

Statistical analysis

The results obtained were analyzed with Origin 8 Lab (2007 version). One paired sample t-test and ANOVA were used for assessing the test of significance at a 5% level of probability at df = (n^{-1}).

RESULTS AND DISCUSSION

Foodborne diseases remain a global health problem, especially in developing and underdeveloped countries. Like other Low-Income Countries (LICs), Nigeria is challenged by foodborne infections, which are accompanied by social, economic, and health implications[20]. In 2014, a National Policy on Food Safety and associated implementation strategy were introduced to enhance health via the control of foodborne infections and the reduction and eventual eradication of the risk of diseases connected with poor food cleanliness. However, foods, especially vegetables like avocado, are still handled un-hygienically[21]. Therefore, this research determined the bacterial contamination and antibiotic resistance pattern of bacteria associated with the spoilage of avocados sold in the Sokoto State metropolis.

The mean and standard error of total viable bacterial counts of avocado samples across three different locations ranged from 4.20±5.77 to 8.43±33.49 (x 10^4 CFU/g) (**Table I**). The statistical comparisons between the three samples and three locations using one-way ANOVA showed no significant difference ($p \geq 0.05$). The bacterial counts obtained in this research align with the findings of García-Frutos *et al*.[3], who reported 4.3 to 9.0 CFU/g on Hass avocados sold at retail markets in Guadalajara, Mexico. Similarly, Shiferaw and Kibret[6] reported the mean aerobic mesophilic count of 5.24 log10 CFU/g from avocado peel used to prepare freshly squeezed juices from juice houses of Bahir Dar Town, Northwest Ethiopia. Kechero *et al*.[13] also reported the mean average of total viable counts of the samples of juices and vegetable salads as 5.96 log CFU/g. However, Wogu and Ighile[15] reported bacterial counts of 5.2 to 6.7x10^4 CFU/g from avocado fruit samples from New Benin and Uselu markets in Benin City, Nigeria. This finding was contrary to the finding of Musa *et al*.[22], who reported the mean total bacterial counts of 1.24±0.07x10^5 CFU/mL from avocados sold in selected markets within the Kaduna metropolis. The higher mean of bacterial counts obtained in this study could result from the sample source and time for sample collection. Our samples were collected during the harmattan season with the cool, dry wind that blows from all angles of the market. However, inoculums were prepared using the whole fruit (i.e., avocado peel, exocarp, and mesocarp). These and other market environmental conditions can lead to higher microbial contaminations in fruit sales in most African retail markets[3]. Other researchers also reported that the variance in microbial loads of fruits might be caused by contamination by microorganisms from the soil, irrigation water, the environment during transit, washing/rinsing water, or process of handling, or it could be part of the fruits' natural flora[3,22-24].

Table I. Total viable bacterial colony counts in avocado samples obtained from the three locations in the Sokoto metropolis.

Sample code	Total bacterial counts (10^4CFU/mL)	p-value
Shagari Market		
AVO-A1	4.20±5.77	0.5315
AVO-A2	8.43±33.49	
AVO-A3	7.23 ± 43.99	
UDUTH Market		
AVO-B1	5.60 ± 13.85	0.9462
AVO-B2	5.87 ± 16.59	
AVO-B3	6.17 ± 8.01	
Maryam Abacha Hospital Market		
AVO-C1	6.23 ± 15.10	0.8699
AVO-C2	6.17 ±2.73	
AVO-C3	7.43±20.87	

A total of 19 bacterial isolates were identified (**Table II**) from avocado samples collected from three locations in the Sokoto metropolis. *Staphylococcus aureus* had the highest frequency of occurrence with 16 (29.2%), while *Klebsiella* sp. had the lowest frequency of occurrence with 7 (12.7%), in the spoiled avocado samples. However, *E. coli* with 11 (20%) was also observed in some samples. Shiferaw and Kibret[6] also reported the presence of *S. aureus* from avocado peels used to prepare freshly squeezed juices from juice Houses of Bahir Dar Town, Northwest Ethiopia. The higher frequency of occurrence of *S. aureus* recorded in this research could result from the customer's frequent hand touching of the avocado during buying to choose a better one because it is the normal human skin flora of the human skin[25]. Other reasons could be exposed to dust, flies, and contact with damage, which can contribute significantly to cross-contamination. Most of the bacterial species discovered from the studied spoiled avocado are associated with endotoxin characterized by a short incubation period (1-8 hours), violent nausea, vomiting, and diarrhea[26]. The *E. coli* and *Klebsiella* spp. isolated from all the samples are members of the *Enterobacteriaceae* family, which signify fecal contamination from dirty hands, and water used in the processing and handling of the fruits. These bacterial species in avocado fruit samples could lead to foodborne illnesses since the avocado is usually consumed raw[12,22,27]. A study by Coetzee *et al*.[7] reported the presence of *E. coli* from avocado fruit collected from a Food Safety System Certification (FSSC) 22000-certified processing facility in Gauteng, South Africa.

Table II. Distribution of bacterial species isolated avocado sample sold within the Sokoto metropolis.

Bacterial isolates	Frequency of occurrence (%)			Total (%)
	Shagari Market (%)	UDUTH Market (%)	Maryam Abacha Hospital Sokoto Market (%)	
Escherichia coli	3 (5.5)	4 (7.3)	4 (7.3)	11 (20)
Pseudomonas sp.	3 (5.5)	3 (5.5)	2 (3.6)	8 (14.5)
Staphylococcus aureus	6 (10.9)	5 (9.1)	5 (9.1)	16 (29.2)
Staphylococcus spp.	5 (9.1)	4 (7.3)	4 (7.2)	13 (23.6)
Klebsiella sp.	2 (3.6)	2 (3.6)	3 (5.5)	7 (12.7)
Total	**19 (34.6)**	**18 (32.7)**	**18 (32.7)**	**55 (100)**

Table III shows the antibiotic resistance pattern of bacterial species isolated from spoiled avocados collected from three markets within the Sokoto metropolis. Among all antibiotics tested against bacterial species, *E. coli* isolates were found to have 10 (91%) and 9 (82%) resistance against ciprofloxacin and streptomycin, respectively. This was contrary to the finding of Sharma *et al*.[28], who reported lower resistance of *E. coli* isolated from Street Fruit Drinks made from avocado in Delhi, India, against ciprofloxacin. However, Kechero *et al*.[13] also reported higher sensitivity of *E. coli* isolated in commonly consumed fruit juices and vegetable salads made from avocado sold in some fruit juice houses in Addis Ababa, Ethiopia, against ciprofloxacin. *Pseudomonas* sp. was found to be 100% resistant to chloramphenicol and Septrin. However, *S. aureus* was found to have higher resistance of 12 (72%) to pefloxacin. Similarly, *Klebsiella* spp. was found to be 100% resistant to Septrin. Resistance of these bacterial species to some of these antibiotics is of public concern, as these are the most commonly prescribed antibiotics in the hospitals in the studied area due to their availability and affordability by common people, especially during the era of COVID-19 which caused the global economic disruption where people from that community were reported to live below the poverty line in the country[29,30].

Table III. Antibiotic resistant pattern of bacteria isolated from avocado samples sold within the Sokoto metropolis.

Bacterial isolates	Antibiotics														
	CFX 30 µg (%)	S 30 µg (%)	SP 10 µg (%)	ERY 10 µg (%)	PEF 10 & 30 µg (%)	GE 10 & 30 µg (%)	APX 30 µg (%)	Z 20 µg (%)	AM 20 µg (%)	R 25 µg (%)	SXT 30 µg (%)	CH 30 µg (%)	CPX 10 & 30 µg (%)	AU 10 µg (%)	OFX 10 µg (%)
E. coli (n = 11)	ND	9 (81.1)	0 (0)	ND	8 (72.7)	0 (0)	ND	ND	6 (54.6)	ND	7 (63.6)	8 (72.7)	10 (90.9)	5 (45.5)	0 (0)
Pseudomonas sp. (n = 8)	ND	8 (100)	4 (50)	ND	8 (100)	5 (62.5)	ND	ND	6 (75)	ND	7 (87.5)	8 (100)	3 (37.5)	5 (62.5)	7 (87.5)
S. aureus (n = 16)	8 (50)	10 (62.5)	0 (0)	0 (0)	12 (75.0)	0 (0)	8 (50)	0 (0)	10 (62.5)	11 (68.8)	ND	ND	ND	ND	ND
Staphylococcus spp. (n = 13)	8 (61.5)	9 (69.2)	0 (0)	0 (0)	10 (76.9)	0 (0)	12 (92.3)	0 (0)	10 (76.9)	11 (84.6)	ND	ND	ND	ND	ND
Klebsiella spp. (n = 7)	ND	7 (100)	4 (57.1)	ND	4 (57.1)	5 (71.4)	ND	ND	6 (85.7)	ND	7 (100)	5 (71.4)	5 (71.4)	5 (71.4)	7 (100)

Note: ND = Not done; % = percentage; cefuroxime (CFX); Pefloxacin (PEF); gentamicin (GE); Ampiclox (APX); Zinacef (Z); amoxicillin (AM); Rocephin (R); ciprofloxacin (CPX); streptomycin (S); Septrin (SXT); erythromycin (E); chloramphenicol (CH); sparfloxacin (SP); Augmentin (AU); ofloxacin (OFX).

CONCLUSION

The overall bacteriological assessment of spoiled avocado fruit samples studied indicated a significant count and contamination with bacterial pathogens. The high counts may pose risks to consumer health, particularly in the current study, where bacterial pathogenic species such as *E. coli*, *Klebsiella* sp., *Pseudomonas* sp., *Staphylococcus* sp., and *S. aureus* were found in spoiled avocado fruit samples. Some bacterial species were found to be 100% resistant to commonly prescribed drugs, such as chloramphenicol and Septrin. Further research should be conducted on the molecular characterization and detection of genes associated with antibiotic resistance in isolated bacterial species.

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AUTHORS' CONTRIBUTION

All authors have an equal contribution to carrying out this study.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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