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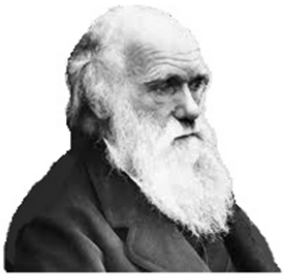

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Occurrence and Antibiotic Resistance Profile of Bacteria Isolated from Freshly Sliced Ready to Eat Fruits Sold Across Major Retail Outlets within Owerri Metropolis

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ABSTRACT

In recent years, there has been a substantial rise in the consumption of sliced ready-to-eat fruits sold across numerous retail outlets in Nigeria. This is because of their high accessibility, convenience, and, most significantly, their lower cost than whole fruits. Nevertheless, these food items have continually acted as carriers for human diseases on a global scale. This study evaluated the occurrence and antibiogram of bacteria from ready-to-eat sliced fruits sold across major retail outlets in the Owerri metropolis. One hundred and fifty samples comprising oranges, watermelons, pineapples, and paw-paws were analysed using standard microbiological techniques. Bacteria isolates were identified using cultural, biochemical, and molecular methods. Antibiotic sensitivity was performed using the Kirby-Bauer diffusion technique. Results showed that orange samples had the highest total viable count (2.50 log cfu/g), total coliform count (2.70 log cfu/g), and faecal coliform count (2.05 log cfu/g), respectively. *Staphylococcus aureus* predominated in the fruit samples with a percentage occurrence of 29.4%. Isolates exhibited different resistance levels, with *Staphylococcus aureus* and *Bacillus* spp showing a high resistance against Gentamycin and ofloxacin. These findings demand further investigation due to the possible health risks of coliform bacteria, faecal coliform and antibiotic resistance. Therefore, monitoring and implementing food safety standards is necessary to protect consumers.

***Keywords*:** Antibiotics, bacteria, slice fruits, resistance, contamination, pathogens, *Staphylococcus*

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1. INTRODUCTION

The escalating prevalence of antimicrobial resistance (AMR) has become a substantial public health issue. It has been identified as an apparent danger to public health in the twenty-first century by the World Health Organization (WHO) [1 and 2]. The threat that antimicrobial resistance (AMR) poses to global sustainability and the progress of endeavours like the United Nations Sustainable Development Goals (SDGs) has been widely recognised [3]. Particularly worrisome in terms of antibiotic resistance is the distribution of sliced fruits, which poses an exceptionally high risk of infection and subsequent resistance development [4].

Sliced fruits are susceptible to contamination at multiple phases, including washing, peeling, slicing, trimming, packaging, handling, and marketing. These processes create potential entry points for pathogens to infiltrate the surfaces of the fruits [4].

Sliced fruits, specifically those packaged in polyethene, frequently fail to satisfy the prescribed quality standards due to elevated counts of total bacteria and coliforms.

Furthermore, it should be noted that while polyethene bags are chemically resistant and impermeable, they may not consistently inhibit bacterial contamination, which could result in substandard poor quality in the packaged fruits [5, 6]. Research has indicated that slicing fruits may facilitate the transmission of bacteria from the skin to the edible flesh, thereby augmenting the potential for contamination. Additionally, the significance of guaranteeing the microbiological safety of sliced fruits is underscored by the exponential proliferation of bacterial pathogens on such fruits, particularly when stored at ambient temperature [7].

Identifying antimicrobial resistant bacteria in fruits and vegetables can facilitate the formulation of suitable approaches to guarantee food safety across the entire fresh fruit and vegetable value chain, from cultivation to consumption. Additionally, it can provide insights into the potential hazards of foodborne illness exposure for the nearby populace. Despite the numerous reports examining the antimicrobial resistance profile of bacteria isolated from sliced fruits in different regions of Nigeria [8-12], there is a paucity of Information regarding the antimicrobial resistance profile of bacteria isolated from sliced fruits consumed in Owerri, Consequently, this research endeavour aimed to assess the prevalence of antimicrobial resistant microorganisms in sliced fruits sold across major retail outlets in Owerri metropolis.

2. MATERIAL AND METHODS

2. 1. Study Design and Collection

A cross-sectional study was conducted across major retail outlets in Owerri. Owerri, the capital of Imo state, Nigeria, lies between latitude 5.483°N to 6.40°N and longitude 7.033°W. According to the Koppen-Geiger climate classification, it is characterized by a tropical wet or monsoon climate. It has the wet season when rains fall for about six months between April-September with a break in August, and a dry season from October to March with a drier dusty Harmattan from December to February [13]. As the heart of southeastern Nigeria, Owerri has an array of markets ranging from large to small street markets.

2. 2. Sample collection

Samples of freshly sliced fruits comprising pineapples, paw-paw, oranges, and watermelon were collected from five major retail outlets in Owerri, namely Akwakuma

Junction, Amakohia Market, Orji Market, Ama Wire Junction, and Relief Market. In the context of this research, freshly sliced fruits are defined as fruits that have been cut and remain in a fresh condition. A total of 150 samples consisting of 30 samples, each of four different fruits, were collected. Eight samples of each fruit included in the research were collected from each location. Sliced pineapple and pawpaw samples were displayed in wrapped translucent polythene bags, while watermelons were presented on silver platters. Samples were collected in sterile containers, tagged, and delivered on ice to the Department of Microbiology for microbiological examination within 8 hours after collection.

2. 3. Isolation and enumeration of bacteria

Isolation of bacteria from the sliced fruit samples was done following standard microbiological techniques. For each sample, 10 grams were mixed with 90 ml of buffered peptone water, and then a part of the mixture was washed and diluted in buffered peptone water to create dilutions of 10^5. Using the pour plate method, 1ml aliquots were distributed over standard plate count agar (Oxoid, UK) and MacConkey agar (Oxoid, UK). The plates were in an incubator at 37 °C for 24 hours. Afterwards, the colonies on the plates were enumerated using a colony counter. Plate containing colonies ranging from two to ten were selected for sampling. The pure culture of each isolate was maintained on a slanted agar medium.

2. 4. Identification of isolates

Isolates were initially identified using microscopic and biochemical characteristics. Catalase test, oxidase, motility, citrate utilization, vogues Proskauer, methyl red test, indole test, and sugar fermentation were carried out.

2. 5. Identification using 16s rRNA

Total genomic DNA was extracted from the Zymo kit (Zymo Research Corporation, Irvine, CA, USA). PCR amplification was performed with universal primer 27F/ 1492R and sequenced using ChromasPro (Technelysium- AU). Sequence reads were edited and aligned using CLUSTAL (version: 1.2.4) and compared to sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST).

2. 6. Antibiotic sensitivity test

The Kirby-Bauer's disc diffusion method was employed to determine the antibiotic resistant profile of the isolates. A commercial sensitivity disc comprising ofloxacin (10 mcg), erythromycin (10 mcg), ciprofloxacin (10 mcg), augmentin (30 mcg), gentamycin (10 mcg), ceftazidime (30 mcg), nitrofurantoin (10 mcg), cefuroxime (30 mcg), ceftriaxone (30 mcg), cefixime (30 mcg) and cloxacillin (30 mcg) were used for the sensitivity test using a Mueller Hilton agar (Oxoid, UK). The results were obtained by measuring the zone of inhibition and comparing it with the standards set by the Clinical and Laboratory Standards Institute [14].

3. RESULTS

3. 1. Enumeration of microorganisms from sliced ready-to-eat fruits

The mean count of total viable, coliform, and faecal coliform bacteria, as represented in Table I, revealed that counts varied across different fruit samples. Oranges recorded the highest total viable count (2.50 log cfu/g) and faecal coliform (2.05 log cfu/g), while watermelons had the least viable (2.1 log 10 cfu/g), coliform (1.75 log cfu/g) and faecal coliform count (1.05 log 10 cfu/g).

3. 2. Identification of microorganisms from sliced ready-to-eat fruits

A two-phase approach comprising biochemical characteristics and a 16s RNA sequencing method was used to identify isolates. Isolates were identified as *Escherichia coli, Bacillus* sp, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus* spp.

3. 3. Percentage distribution of bacteria isolates from sliced ready-to-eat fruits

The result of the distribution of bacteria from slice fruit samples showed that *Staphylococcus aureus* was the most predominant isolate (29.4%), followed by *Pseudomonas aeruginosa* (23.6%) and *Bacillus* sp (19.4%), while *Enterococcus* spp was the least with 11.8% (Table 2)

Table 1. Total mean of bacterial load of some slice fresh-cut ready-to-eat fruit samples within Owerri Metropolis (log10 cfu/g)

Fruit	Total viable	Total coliform	Faecal coliform
Orange	2.50	2.70	2.05
Watermelon	2.10	1.75	1.05
Pineapple	2.40	2.30	1.65
Pawpaw	2.23	2.15	1.60

Table 2. Percentage distribution of bacteria isolates associated with sliced ready-to-eat fruits

Organism	No of occurrence	Percentage Occurrence
E. coli	8	15.7
Bacillus spp	10	19.6
Pseudomonas aeruginosa	12	23.6
Staphylococcus aureus	15	29.4
Enterococcus spp	6	11.8
Total	51	100

Table 3. 16sr RNA identification of bacteria isolates from sliced ready-to-eat fruits

Organism identity	Percentage similarity	The corresponding organism from NCBI
E. coli	99	*Escherichia coli* CP154880
Bacillus spp	93	*Bacillus* spp KT150211
Pseudomonas aeruginosa	96	*Pseudomonas aeruginosa* WE 41437
Staphylococcus aures	97	*Staphylococcus* aureus CT35692
Enterococcus spp	99	*Enterococcus* spp BT54281

Antibiotic resistant profile of isolates

In this study, Augmentin (21 mm) and Cefuroxime (20 mm) recorded the highest sensitivity against *Pseudomonas aeruginosa*, whereas gentamycin (4 mm) and ciprofloxacin (5 mm) recorded the highest antibiotic resistance against *Escherichia. coli* and *Pseudomonas aeruginosa*, respectively. For gram-positive isolates, *Staphylococcus* was resistant to Ceftazidime (4 mm), gentamycin (5 mm), and ofloxacin (5mm), making it the most resistant isolate from the sliced fruit samples. This was followed by *Enterococcus* sp against Ceftazidime (5 mm) and cloxacillin (4 mm).

Table 4. Antibiotic resistance profile of Gram negative isolates

Organisms/ZI		CAZ	CRX	GEN	CXM	OFL	AUG	NIT	CPR
E. coli		S (18)	S (12)	R (4)	S (10)	S (17)	S (18)	R (5)	S (11)
Pseudomonas aeruginosa		S (12)	S (20)	S (19)	S (16)	S (16)	S (21)	S(19)	R (5)

KEY: Zone of inhibition (ZI), Ceftazidime (CAZ), Cefuroxime (CRX), Gentamicin (GEN), Cefixime (CXM), Ofloxacin (OFL), Augmentin (AUG), Nitrofurantoin (NIT), Ciprofloxacin (CPR), S = Sensitive, R = Resistant.

Table 5. Antibiotic resistance profile of Gram positive isolates

Organisms/ZI	CAZ	CRX	GEN	CTR	OFL	AUG	CXC	ERY
Bacillus	S (13)	S (21)	R (4)	S (20)	R (4)	S (21)	S (11)	S (20)
Staphylococcus aureus	R (4)	S (15)	R (5)	S (16)	R (5)	S (23)	S (13)	S (13)
Enterococcus sp	R (5)	S (13)	S (16)	S (13)	S (20)	S (18)	R (4)	S (14)

KEY: Zone of inhibition (ZI), Ceftazidime (CAZ), Cefuroxime (CRX), Gentamicin (GEN), Ceftriaxone (CTR), Ofloxacin (OFL), Augmentin (AUG), Cloxacillin (CXC), Erythromycin (ERY), S = Sensitive, R = Resistant

4. DISCUSSION

This study examined the occurrence and antibiogram of sliced ready-to-eat pineapple, watermelon, and orange fruit samples sold across major outlets within the Owerri metropolis and their antibiotic profile against selected antibiotics. The fruit samples showed varied total viable, coliform, and faecal coliform counts. Significantly, the total coliform count was higher than the faecal coliform count, suggesting that soil instead of faecal matter was the primary source of contamination. Numerous other studies have reported similar results [15-20]. Orange samples recorded the highest count of coliform (2.75 log cfu/g) and faecal coliform (2.05 log cfu/g). This result agrees with the reports of [21, 22]. This high count on the peeled oranges may be ascribed to vendors failing to adhere to hygienic practices, as documented instances where vending sites lacked lavatory facilities, which could have facilitated the spread of faecal coliforms. *Escherichia coli* (15.7%), *Bacillus* sp (19.6%), *Pseudomonas aeruginosa* (23.6%), *Staphylococcus aureus* (29.4%), and *Enterococcus* (11.8%) were isolated from the sliced ready to eat fruit samples. *Staphylococcus aureus* was the highest occurring organism, whereas *Enterococcus* was the least isolated. No statistically significant difference was observed among the various bacteria recovered from the fruit samples ($p > 0.05$). These organisms have also been reported in similar works [20-29]

According to [30], healthy persons serve as the main reservoirs of S. aureus in their nasal passages, hands, and skin. While peeling or slicing fruits, *Staphylococcus aureus* may be introduced into the pre-cut fruits when the sellers touch them with their bare hands. *Staphylococcus aureus* is the primary cause of foodborne illnesses, as stated by [30]. [31] and [32] have also reported the *Staphylococcus* in food. The detection of *Escherichia coli* and *Enterococcus* may be attributed to faecal contamination or inadequate hygienic conditions of the vendors [33]. *Enterococcus* species, which are prevalent in the gastrointestinal tract of warm-blooded animals, may lead to many health issues, such as gastroenteritis, diarrhoea, urinary tract infections, endocarditis, and meningitis [34]. The Pseudomonas *aeruginosa* and *Bacillus* spp identified in this research could be indigenous microorganisms found naturally on fruits or originated from soil as pollutants [35].

The antibiogram result showed that the bacterial isolates exhibited different levels of resistance. *Bacillus* spp was susceptible to all the antibiotics tested except gentamycin, ofloxacin, and ceftriaxone; Staphylococcus *aureus* was also resistant to gentamycin and ofloxacin. Other researchers have also reported a similar pattern [5, 30, 31, 36, 37]. The most expansive zone of inhibition was recorded with Augmentin against *Staphylococcus* aureus.

5. CONCLUSION

In conclusion, this current research has shown that the freshly sliced fruits obtained from the different retail outlets are unsuitable for human consumption and pose a health risk to consumers due to poor microbiological quality. The contamination of fruits is mainly caused

by factors such as pollution from farms or producing areas, improper food handling during processing, unhygienic practices during packing, and unfavourable weather conditions. These factors contribute to a significant increase in the presence of microorganisms in the produce. This research highlights the pressing need for a regulatory body to thoroughly evaluate fruit vendors to enhance quality standards and ensure the safety of sliced ready-to-eat fruits at these retail outlets.

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