



BACTERIOLOGICAL QUALITY OF FRESH-CUT-FRUITS: A CASE STUDY OF IBADAN, NIGERIA



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Received: February 11, 2023 Accepted: April 15, 2023

Abstract

The consumption of fresh-cut-fruits constitutes health risks owing to the effects of microbial contamination. This study assessed the bacteriological safety of fresh-cut-pineapples and -watermelons vended in Ibadan, Nigeria. Sixteen samples were randomly obtained from clusters of retailers in Challenge and Odo-ona markets, Ibadan and analyzed for loads of bacterial groups by standard culturing methods. The isolates were subjected to various biochemical tests, and screened for haemolytic activities and antibiotic resistance. The counts of total bacteria, *Escherichia coli*, coliforms, and *Staphylococcus aureus* ranged from 1.00 ± 0.33 to 5.60 ± 0.56 , 1.50 ± 0.50 to 4.50 ± 0.50 , 0.80 ± 0.30 to 5.10 ± 0.40 and 0.93 ± 0.33 to 4.90 ± 0.31 Log₁₀ CFU/g respectively. A total of 36 isolates were obtained and identified as *S. aureus* (58.30%), *E. coli* (19.40%), *Salmonella* sp. (13.90%), and *Pseudomonas* sp. (8.40%). Among the bacterial isolates, 13 were β -haemolytic, 19 and four were α - and γ -haemolytic respectively. Bacterial isolates demonstrated species/strain specific resistance to different antibiotics. Strains of *E. coli*, *Salmonella* sp. and *Pseudomonas* sp. showed 100% resistance to Efuraxime and Cloxacilin, while only strains of *Pseudomonas* sp. were 100 % resistant to Augmentin, Gentamycin and Ceftriaxone. Nineteen resistance patterns were demonstrated by the isolates, with multiple antibiotic indices ranging from 0.46 to 1.00. The results from this study suggest that fresh-cut pineapples and watermelons vended in Ibadan are highly contaminated with multiple antibiotic resistant pathogenic bacteria, could constitute public health risks. Therefore, increased awareness of hygienic practices and training vendors of this category of products on food safety measures are recommended.

Keywords: Fresh-cut-fruits (FcFs), Antibiotics resistance, Food safety, Pineapple, Watermelon.

Introduction

Fruits are important sources of nutrients required for human growth and development. These nutrients confer health benefits such as reduction in risk of chronic diseases, prevention of diet-related deficiencies and detoxification of the human body (Alotzman *et al.*, 2009). Fruits may be vended to consumers as whole fruits or as fresh-cut-fruits (FcFs). Over the years, there has been a significant increase in the consumption of cut-fruits because they are easily accessible, convenient, ready-to-eat and most especially, more affordable than the whole fruits (Raffo and Paoletti, 2022). In Nigeria, pineapple (*Ananas comosus*) and watermelon (*Citrullus lanatus*) are among the most prominent FcFs retailed in open markets and along the streets (Oyedele *et al.*, 2020). FcFs are usually vended after the fruits are washed, peeled, sliced, and packaged in polyethylene bags (Iturralde-Garcia *et al.*, 2022). However, the consumption of FcFs may constitute human health risks due to microbial contamination (Quadri *et al.*, 2015). Disruption of the protective layers of whole fruits to obtain FcFs exposes the fruits' mesocarps, thus offering a potential nutrient-rich environment for the proliferation of contaminating spoilage and pathogenic microorganisms (Beuchat, 2002; Mahfuza *et al.*, 2016). Other factors that may contribute to contamination of FcFs include unhygienic handling by human processors, use of contaminated wash water, utensils and packaging materials, or the dirty environment during processing (Francis *et al.*, 2012; Mahfuza *et al.*, 2016; Ogunremi *et al.*, 2021a). Furthermore, the display of the fruits openly to the atmosphere in marketplaces during processing and display may allow contact of flies and other vectors, coupled with the direct contact with airborne dust particles (Corbo *et al.*, 2010). The FcFs are processed and sold by vendors who are

untrained and uncertified in food hygiene (Barro, *et al.*, 2007). Studies have revealed that they do not observe adequate personal hygiene and ensure acceptable sanitary conditions at points of preparation and retail, where they make use of rudimentary facilities like wheel barrows, trays, mats, tables and make-shift stalls (Ogunremi *et al.*, 2021).

More than 250 types of microbial pathogens have been implicated in different foodborne illnesses (Tambekar *et al.*, 2008). The most common foodborne pathogens include *Bacillus cereus*, *Escherichia coli* 0157:H7, *Salmonella typhi*, species of *Shigella* and *Vibrio cholerae*, which causes diarrhoea, typhoid fever, dysentery, and cholera (Buck *et al.*, 2003; CDC, 2019). Fresh cut fruits contaminated by the bacteria may cause severe foodborne diseases, ranging from mild, self-limiting to life-threatening conditions, depending on the virulence of the pathogen and consumers' health and socioeconomic status (Mahfuza *et al.*, 2016).

The spread of antimicrobial resistance in bacteria isolated from FcFs has increased overtime (Vicas and Singh, 2010). This has become a concern to consumers, care givers and public health administrators. Elevated levels of antibiotic resistance have been reported among food-borne pathogens such as *Salmonella* and *Shigella* (Mache, 2002). Although, it is difficult to prove a direct role of drug resistance in bacteria contaminating food items with increased clinical cases of resistant infections, the presence of such bacteria in food items could play a role in the spread of antimicrobial resistance amongst food-borne pathogens (Farzana *et al.*, 2009).

Thus, this study aimed to access the bacteriological quality of selected FcFs retailed in Ibadan, Nigeria and the safety of the bacterial isolates by determining their haemolytic properties and antibiotic resistance patterns. The data generated from this study will contribute to establishing food safety policies that target the fresh-cut fruits value chain in order to safeguard public health.

Materials and Methods

Collection of fresh-cut pineapple and watermelon samples

A total of 16 fresh-cut fruits (FcFs) samples of pineapple (*Ananas comosus*) and watermelon (*Citrullus lanatus*) were randomly obtained from different clusters of retailers from two different markets (Challenge and Odo-ona) in Ibadan. The samples were aseptically collected in a sterile and labelled ziplock bags and transported to Microbiology Laboratory, Department of Microbiology and Biotechnology, First Technical University, Ibadan, Oyo state for microbiological analysis.

Enumeration and isolation of bacteria from fresh-cut pineapple and watermelon samples

Ten-fold serial dilutions of homogenized fruit mesocarps in sterile peptone water, were pour-plated in nutrient agar, Mannitol Salt agar and MacConkey agar for the enumeration of total bacteria, *S. aureus* and *E. coli* and coliforms respectively. The plates were incubated at 37 °C for 18-24 h and distinct colonies were randomly selected and streaked on nutrient agar, until pure cultures were obtained (Cheesbrough, 2005). Pure cultures were preserved on nutrient agar slants and renewed at two-weekly intervals.

Characterization and identification of bacterial isolates

Morphological examinations and biochemical tests were carried to determine the characteristics of pure bacterial cultures. They include; colony and cell morphology, Gram reaction, catalase, oxidase, indole, nitrate reduction, urease, methyl red, Voges-Proskauer, Kligler's Iron Agar (KIA) and sugar fermentation tests (Cheesbrough, 2005). The isolates were identified by comparing their morphological and biochemical characteristics with the characteristics of reference organisms as described in Bergey's Manual for Determinative Bacteriology.

Determination of haemolytic activity of the isolates

Fresh bacterial cultures were streaked in triplicate on blood agar plates (5 % w/v defibrinated sheep blood) and incubated at 37 °C for 24 h. The plates were examined for β-haemolysis (clear zones around colonies), α-haemolysis (green zone around colonies), and γ-haemolysis (no zones around colonies) (Banwo, *et al.*, 2012).

Antibiotic susceptibility profile

The sensitivity of all the isolates to selected antibiotics was investigated using the disc diffusion method as recommended by the Clinical and Laboratory Standards Institute guidelines (CLSI, 2016). Isolates were suspended in 10 mL normal saline and adjusted to 0.5 McFarland's standard, equivalents to 1.5 X 10⁸ CFU/mL. The suspension

of each test isolate was aseptically spread- plated onto freshly prepared Mueller-Hinton agar plates and allowed to stand for an hour prior to the placement of antibiotic-impregnated discs on the seeded agar. The antibiotics (Oxoid, Basingstoke, UK) used include: Ceftazidime (CAZ) (30g), Efuroxime (CRX) (30g), Gentamicin (GEN) (10g), Cefixime (CXM) (5g), Ofloxacin (OFL) (5g), Augmentin (AUG) (30g), Nitrofurantin (NIT) (30g), and Ciprofloxacin (CPR) (5g). Seeded plates were incubated at 37°C for 24 h and examined for zones of inhibition. The resultant diameter of visible zones of inhibition were measured in millimeters (mm) and classified as resistant (R), intermediate (I) or sensitive (S) in accordance to the guidelines of the Clinical and Laboratory Standard Institute (CLSI, 2018).

Determination of multiple antibiotics resistance (MAR) indices of isolates

Multiple antibiotic resistance (MAR) index of each strain was calculated using the formular of Oyedele *et al.* (2020) below:

$$\text{MAR} = \frac{\text{Number of antibiotics to which strain is resistant}}{\text{Total number of antibiotics tested}}$$

Statistical analysis

Data was collected in duplicate and presented in mean ± standard deviation of replicate values. Counts were expressed in logarithmic units of microorganisms per gram (log₁₀ CFU/g).

Results and Discussion

Enumeration and identification of bacteria in fresh-cut pineapple and watermelon samples

The average microbial loads of the vended FcFs samples are shown in Table 1. The total bacteria, *E. coli*, coliform and *Staphylococcus aureus* counts from ranged from 1.00±0.33 to 5.60±0.56, 1.50±0.50 to 4.50±0.50, 0.80±0.30 to 5.10±0.40 and 0.93±0.33 to 4.90±0.31 cfu/g respectively. The highest bacterial and *E. coli* counts were recorded in a pineapple sample (POA₁). Based on morphological and biochemical characterization, the 36 bacterial isolates were identified as *Staphylococcus aureus* (58.30%), *E. coli* (19.40%), *Salmonella* sp. (13.90%) and *Pseudomonas* sp. (8.40%) (Figure 1). The presence of these groups of bacteria was previously reported in fruits, including pawpaw (*Carica papaya*), watermelon (*Citrullus lanatus*) and pineapple (*Ananas comosus*) (Nwachukwu *et al.*, 2014; Afolabi *et al.*, 2015; Allamin, *et al.*, 2015). The contamination of cut fruits has been linked, to processing and rinsing with faecally polluted water, and the exposure of these products to low temperature (Edeghor *et al.*, 2019). The presence of these microorganisms in counts beyond the safe limits may be due to the enriched nutrients and favourable water activity in the fruit. It is been reported that presence of *E. coli* in food samples was an indication of faecal contamination and improper hygienic practices by food vendors (Edeghor *et al.*, 2019). The presence of *S. aureus*, *Pseudomonas* sp., *Salmonella* sp. and *E. coli* was reported from pedegre-cut fruits sold in Ilorin (Odebisi *et al.*, 2015). In agreement with this study, *Salmonella* sp. were earlier reported in ready-to-eat fresh produce retailed in some Nigerian open markets

Bacteriological quality of fresh-cut-fruits in Ibadan

(Ogunremi *et al.*, 2021b). Pathogenic bacterial are the common contaminants of fruits, they could be easily transferred from the vendors to the processed fruits through unhygienic and improper food handling. Ogunremi *et al.* (2021a) reported high contamination of food retail surfaces in an open market with multiple antibiotic resistance bacterial strains of *Bacillus* sp., *E. coli*, *Proteus* sp., *Pseudomonas* sp., *Salmonella* sp., and *Staphylococcus aureus*. The

consumption of ready-to-eat sliced fruits directly from street vendors or hawkers could constitute a likely health risk and potentially increases the risk of food-borne diseases caused by a wide variety of pathogens, because it is difficult to monitor the hygiene of these vendors or the sanitary conditions at points of processing as well as the packaging (Owabumoye *et al.*, 2015).

Table 1: Microbial count of the fresh-cut pineapple and watermelon samples

Sample	TBC	<i>E. coli</i>	Coliform	<i>S. aureus</i>
Codes	Log ₁₀ CFU/g	Log ₁₀ CFU/g	Log ₁₀ CFU/g	Log ₁₀ CFU/g
WCA ₁	5.31 ± 0.42	4.30 ± 0.56	4.44 ± 0.47	4.33 ± 0.56
WCB ₁	5.30 ± 0.56	4.11 ± 0.5	4.93 ± 0.04	4.53 ± 0.53
WOA ₁	5.30 ± 0.39	3.43 ± 0.50	4.80 ± 0.70	4.60 ± 0.56
WOB ₁	5.50 ± 0.54	3.92 ± 0.32	5.10 ± 0.43	3.89 ± 0.83
WCA ₂	2.12 ± 0.25	1.50 ± 0.50	1.54 ± 0.51	1.73 ± 0.40
WCB ₂	1.24 ± 0.34	1.70 ± 0.42	0.80 ± 0.30	0.93 ± 0.33
WOA ₂	Nil	1.23 ± 0.30	0.80 ± 0.30	1.50 ± 0.30
WOB ₂	1.02 ± 0.50	1.40 ± 0.30	0.95 ± 0.40	1.00 ± 0.43
PCA ₁	5.40 ± 0.40	4.34 ± 0.37	4.90 ± 0.43	4.90 ± 0.31
PCB ₁	5.30 ± 0.40	3.70 ± 0.50	3.99 ± 0.55	4.60 ± 0.49
POA ₁	5.60 ± 0.56	4.50 ± 0.50	4.86 ± 0.55	4.60 ± 0.43
POB ₁	4.80 ± 0.31	3.93 ± 0.44	3.69 ± 0.43	4.00 ± 0.41
PCA ₂	Nil	Nil	Nil	Nil
PCB ₂	1.40 ± 0.50	2.00 ± 0.43	0.80 ± 0.30	Nil
POA ₂	1.00 ± 0.33	1.70 ± 0.35	1.33 ± 0.50	Nil
POB ₂	1.10 ± 0.30	1.90 ± 0.30	1.24 ± 0.33	Nil

Key: TBC-Total Bacterial Count.

Values are mean ± standard deviation of replicate values

Table 2: Antibiotics resistance profile of bacterial isolates from fresh-cut fruits

Antibiotics	Bacterial species											
	<i>Staphylococcus aureus</i> (n = 21)			<i>Escherichia coli</i> (n = 7)			<i>Salmonella</i> sp. (n=5)			<i>Pseudomonas</i> sp. (n =3)		
	R	I	S	R	I	S	R	I	S	R	I	S
AUG	19	2	0	7	0	0	4	1	0	3	0	0
GEN	4	3	14	3	2	2	1	3	1	3	0	0
OFL	1	4	15	4	1	2	0	3	2	1	1	1
CPR	20	1	0	1	1	5	2	0	3	0	0	3
CRX	10	2	9	7	0	0	5	0	0	3	0	0
CTR	2	3	15	6	1	0	3	0	2	3	0	0
NIT	20	1	0	5	2	0	0	2	3	2	1	0
ERY	4	15	2	7	0	0	5	0	0	2	1	0
CXM	20	1	0	7	0	0	5	0	0	2	1	0
CAZ	7	0	14	7	0	0	4	0	1	2	1	0
CXC	19	0	2	7	0	0	5	0	0	3	0	0

Key: Key: AUG – Augmentin, GEN – Gentamycin, OFL – Ofloxacin, CPR – Ciprofloxacin, CRX – Efuraxime, CTR – Ceftriaxone, NIT – Nitrofurantoin, ERY- Erythromycin, CXM – Cefixime, CAZ – Ceftazidime, CXC – Cloxacilin, R- Resistant, I- Intermediate, S- Sensitive, n- Number of isolates

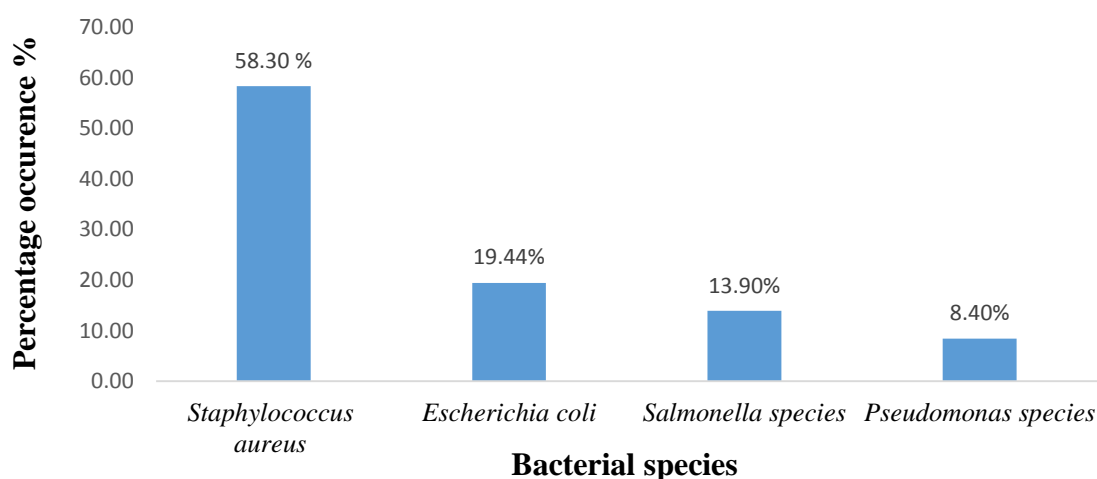


Figure 1: Percentage occurrence of bacterial species isolated from fresh-cut pineapple and watermelon

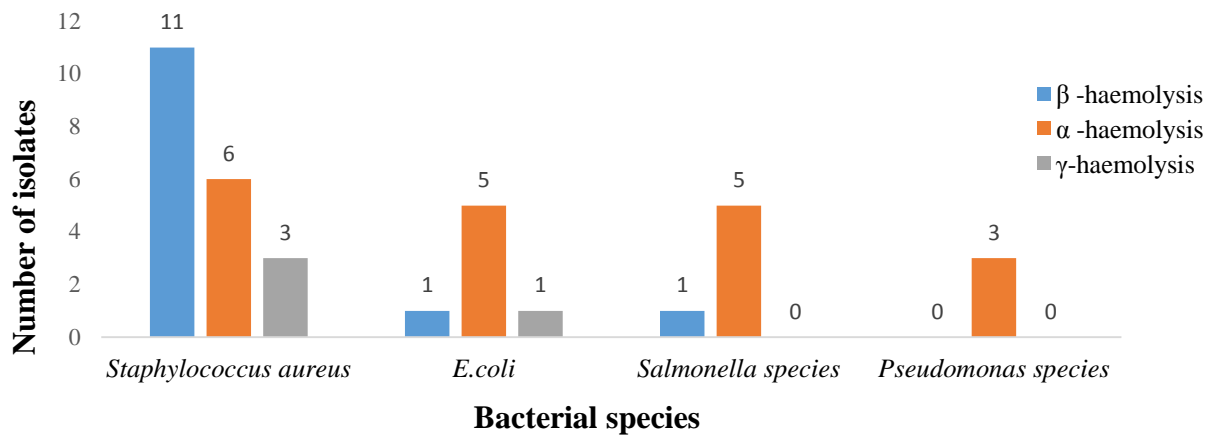


Figure 2: Heamolytic properties of bacterial isolates from fresh-cut pineapple and watermelon

Table 3: Multiple antibiotics resistance pattern of bacterial isolates from fresh-cut fruits

No. of antibiotics	MAR index	Resistance phenotypes	Bacterial species				Total no. of isolates (n =36)
			<i>E. coli</i> (n =7)	<i>Pseudomonas</i> sp. (n =3)	<i>S. aureus</i> (n =21)	<i>Salmonella</i> sp. (n =5)	
5	0.46	AUG, CPR, NIT, CXM, CXC	-	-	2	-	2
6	0.56	AUG, CPR, NIT, ERY, CXM, CXC	-	-	7	-	7
6	0.56	AUG, GEN, CPR, CRX, NIT, CXC	-	1	-	-	1
6	0.56	AUG, CPR, NIT, ERY, CXM, CXM	-	-	1	-	1
6	0.56	CPR, CRX, NIT, ERY, CXM, CXC	-	-	1	-	1
6	0.56	CPR, CRX, NIT, CXM, CAZ, CXC	-	-	-	1	1
7	0.64	AUG, CPR, CRX, NIT, ERY, CXM, CXC	-	-	1	-	1
7	0.64	AUG, CPR, CRX, NIT, ERY, CXM, CXM	-	-	1	-	1
7	0.64	AUG, CPR, CRX, NIT, ERY, CXM, CAZ	-	-	1	-	1
7	0.64	AUG, GEN, CPR, NIT, ERY, CXM, CXC	-	-	3	-	3
7	0.64	AUG, CRX, CTR, ERY, CXM, CAZ, CXC	2	-	-	2	4
8	0.73	AUG, CRX, CTR, NIT, ERY, CXM, CAZ, CXC	1	-	-	1	2
9	0.82	AUG, GEN, CPR, CRX, CTR, NIT, ERY, CXM, CXC	-	-	1	-	1
9	0.82	AUG, CPR, CRX, CTR, NIT, ERY, CXM, CAZ, CXC	-	-	1	-	1
9	0.82	AUG, GEN, CRX, CTR, NIT, ERY, CXM, CAZ, CXC	-	1	-	1	2
9	0.82	AUG, CPR, CRX, CTR, NIT, ERY, CXM, CAZ, CXC	-	-	1	-	1
10	0.91	AUG, GEN, OFL, CRX, CTR, NIT, ERY, CXM, CAZ, CXM	1	-	-	-	1
10	0.91	AUG, GEN, OFL, CRX, CTR, NIT, ERY, CXM, CAZ, CXC	3	1	-	-	4
11	1.00	AUG, GEN, OFL, CPR, CRX, CTR, NIT, ERY, CXM, CAZ, CXC	-	-	1	-	1

Key:AUG – Augmentin, GEN – Gentamycin, OFL – Ofloxacin, CPR – Ciprofloxacin, CRX – Efuraxime, CTR – Ceftriaxone, NIT – Nitrofurantoin, ERY- Erythromycin, CXM – Cefixime, CAZ – Ceftazidime, CXC – Cloxacilin; MAR- Multiple Antibiotic Resistance

Haemolytic properties of the bacterial isolates

The haemolytic properties of the bacterial isolates are shown in Figure 2. Thirteen isolates were found to have β - haemolytic, including *S. aureus* (11 isolates), *E. coli* (1) and *Salmonella* sp. (1). Some other isolates of *Staphylococcus aureus* (6 isolates), *Salmonella* (5), *E. coli* (5) and *Pseudomonas* (3) were found to have α -haemolysis activity while *Staphylococcus aureus* (3) and *E. coli* (1) were found to have γ -haemolysis activity. The pathogenicity of the bacterial isolates obtained in this study were investigated by measuring haemolysin activities. Bacterial cells that produce the enzyme haemolysin are capable of degrading or lysing red blood cells; a phenomenon indicative of their pathogenic potential (Cheung *et al.*, 2012). This observation further underscores the health risks that may be associated with the consumption of poorly processed FcFs. However, there is a need to conduct epidemiological-based studies to understand the food safety risks faced by FcFs consumers upon consumption of such unhygienically processed fruits.

Antibiotic resistance pattern of isolates

The 36 bacterial isolates demonstrated species/strain specific resistance to different antibiotics (Table 2). Strains of *E. coli*, *Salmonella* sp. and *Pseudomonas* sp. showed 100% resistance to Efuroxime and Cloxacilin, while only strains of *Pseudomonas* sp. were 100 % resistant to Augmentin, Gentamycin and Ceftriaxone. Some *S. aureus* strains demonstrated resistance to Augmentin, Ciprofloxacin, Nitrofurantoin, Cefixime and Cloxacilin. Absolute susceptibility was only recorded by *Pseudomonas* sp. to ciprofloxacin. Nineteen resistance patterns were demonstrated by the isolates, with multiple antibiotic indices ranging from 0.46 to 1.0. Multidrug-resistant pathogens constitute a burden on health-care management worldwide, by complicating disease treatments, increasing healthcare costs, extending hospital stay, and increasing mortality rates (Raoult, *et al.*, 2019). The recent upsurge in cases of antibiotic resistance among bacterial pathogens emanates from increased evolutionary selective pressures on antibiotic-susceptible strains, as occasioned by the over-application of antibiotics in veterinary and human medicine, thus encouraging the proliferation of antibiotic-resistant strains (FMAEH, 2017). *E. coli*, *Salmonella* sp. and *Pseudomonas* sp. have been reported to have phenotypic multiple resistance to various antibiotics (Lee *et al.*, 2015). This suggests that FSFs may contribute to the transmission of antibiotics resistant bacteria in Nigeria.

Conclusion

The results from this study suggest that fresh-cut pineapples and watermelons vended in Ibadan are heavily contaminated with microorganisms and potentially pathogenic microorganisms. This is an indication of poor handling and processing conditions that the cut-fruits are subjected. A significant proportion of the microorganisms were multiple antibiotic resistant pathogenic bacteria. These could constitute risk to the health of burgeoning consumers of FcFs.

This study recommends strict regulations and trainings to ensure compliance of fruit vendors to food safety practices. Furthermore, comprehensive studies that will explore the culture-independent approaches are required to understand the source, diversity, transmission, and public health risks associated with bacterial endophytes, commensal and pathogens occurring in the FSFs process chain in Nigeria.

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