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Bacteriological assessment of tropical retail fresh-cut, ready-to-eat fruits in south-western Nigeria

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ABSTRACT

Globally, more than 20 kinds of fruits are vended as minimally processed or fresh-cut fruits (FCFs). In Nigeria, pineapple (*Ananas comosus*) and watermelon (*Citrullus lanatus*) are among the commonest FCFs retailed and consumed regularly. However, the consumption of FCFs vended in open markets may constitute health risks owing to microbial contamination. This study assessed the bacteriological safety of FCFs in urban markets across six south-western Nigerian states. One hundred and twenty pineapple and watermelon products, samples of fruit wash water and vendors' hand swabs were randomly sampled in markets and analysed for bacterial contamination by standard bacterial culturing methods, followed by analyses of partial 16S ribosomal RNA (rRNA) gene sequences. Bacterial isolates were further screened for haemolysin and amylase production, as well as subjected to antibiotic sensitivity testing against eight commonly administered antibiotics. *Citrobacter, Enterobacter, Escherichia, Klebsiella, Pantoea* and *Shigella* were the enteric bacterial genera identified in the fruits. Within these genera, 11 species were detected including two potential pathogens, *Enterobacter hormaechei* and *Enterobacter sichuanensis*, that are reported for the first time in vended FCFs in Nigeria. *Klebsiella pneumoniae* was detected across all sampling points and sample types, suggesting its prevalence in the FCFs process chain. About 10% of 102 isolates obtained had haemolytic potentials against erythrocytes, while 9.8% produced amylase. About 76% of the isolates were multidrug-resistant strains. Co-resistance to ampicillin and gentamicin was the most prevalent resistance pattern observed. About 7% of all isolates including those of *Escherichia coli, E. hormaechei, E. sichuanensis* and *Shigella flexneri* were resistant to all the tested antibiotics. Hand swabs from fruit vendors and fruit wash water revealed phylotypes similar to those in the FCFs, sug-

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gesting their involvement as potential sources of contamination of the fruits. Appropriate food safety measures for handlers and consumers of FCFs are thus recommended.

Introduction

Fruits are important sources of essential nutrients (such as vitamins, minerals and easily utilisable sugars) required for human growth and development. These nutrients confer health benefits such as prevention of diet-related deficiencies, reduction in risk of chronic diseases, and detoxification of the human body [3]. Fruits may be vended to consumers as whole fruits or as fresh-cut fruits (FCFs). Globally, more than 20 kinds of fruits are vended as minimally processed or FCFs [34]. In Nigeria, pineapple (*Ananas comosus*) and watermelon (*Citrullus lanatus*) are among the commonest FCFs retailed and consumed.

Tropical FCFs are usually vended after the fruits are washed, peeled, cut and packaged in polyethylene bags. However, the consumption of FCFs processed and vended in open markets may constitute human health risks due to microbial contamination [31]. Microbial contamination of whole fruits may occur at any step of fruit processing, commencing at the farm, through handling and to the consumers [18,31]. Disruption of the protective layers of whole fruits during the process of obtaining FCFs exposes the fruits' mesocarps, thus offering a potential nutrient-rich environment for the proliferation of contaminating spoilage and pathogenic microorganisms [5,26]. Other factors that may contribute to contamination of FCFs include unhygienic handling by human processors, use of contaminated wash water and utensils, or the dirty environment during FCF processing [18,26]. Furthermore, the display of the fruits openly to the atmosphere in marketplaces during handling and processing may encourage frequent visits by flies and other disease vectors, coupled with the direct contact with airborne dust particles [10].

The exact scenario of foodborne diseases caused by contaminated food is 300 to 350 times higher than the reported cases [7,20]. More than 250 types of microbial pathogens have been implicated in different foodborne illnesses [35]. 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 [6,7]. FCFs contaminated by the aforementioned bacteria may cause severe foodborne diseases and possibly death depending on several factors such as the type of bacterial species and the consumers' health and socioeconomic status [26].

Despite the daily consumption of FCFs by fast-paced dwellers in many urban and largely populated cities in Nigeria, there is a paucity of data on the application of 16S rRNA gene-based identification of pathogens in FCFs in south-western Nigeria. The use of 16S rRNA gene offers a more robust and precise identification of bacterial species compared to conventional biochemical-based identification. Furthermore, the available reports on bacterial contamination of fruits in the country [11,15,22,28] are not comprehensive with respect to sampling coverage as the studies were conducted in only single states compared to other studies conducted in Canada [14] and Portugal [19] where multi-sites were covered. Since the food safety and public health threat posed by the consumption of bacterial contaminated FCFs may have dire consequences, routine microbiological surveillance is required. Thus, this study aimed to: (a) determine the diversity of enteric bacteria in the fruits and environmental samples, (b) assess the antibiotic sensitivity patterns in the bacteria isolated from the samples and (c) determine if the potential contamination sources of the fruits were from wash water used during FCFs processing or from the food vendors. The data generated from this study will contribute to establishing food safety policies that target the FCF value chain in order to safeguard public health.

Materials and methods

Sample collection

Freshly cut pineapples and watermelons were conveniently sampled from a total of 12 purposely selected urban markets situated in the six states of Ekiti, Lagos, Ogun, Ondo, Osun and Oyo in south-western Nigeria. Ten samples each of pineapple and watermelon were collected per state. Two vendors per fruit type were purposely selected to minimise confounding variables at a particular market; however, when a vendor sold both pineapples and watermelons, fruit samples were purchased at one spot only. In a lot of whole fruits sold by a particular vendor, each FCF sample was randomly collected after an interval of five whole fruits that were processed into FCFs. Also, 20 mL of fruit wash water and samples of hand swabs (using sterile swab sticks) were aseptically collected from five consenting vendors. The fruit samples were wrapped in sterile polyethylene bags while wash water was collected into sterile plastic bottles. Samples were immediately placed on ice and transported to the laboratory for analyses. This study received approval from the Babcock University Health Research Ethics Committee under the reference number: BUHREC 611/18.

Abbreviations

CLSI	clinical and laboratory standards institute
DNA	deoxyribonucleic acid
FCFs	fresh-cut fruits
rRNA	ribosomal ribonucleic acid
OTU	operational taxonomic unit
PCR	polymerase chain reaction

Bacteriological analyses of samples

Ten-fold serial dilutions of fruit wash water, vendors' hand swabs and homogenised fruit mesocarps were spread-plated onto MacConkey agar (Hi Media, India), thiosulfate citrate-bile salts-sucrose agar (TCBS; Oxoid Basingstoke, UK) and Salmonella-Shigella agar (SSA; Hi Media, India). Swab sticks were inserted into test tubes containing 9 mL sterile peptone water and mechanically agitated prior to serial dilution. The inoculated plates were incubated at 37°C for 24 h. All bacteriological analyses were performed in duplicate.

An average of five isolates was picked from each sample. Selection of isolates was based on cultural characteristics such as colour, elevation and edges. Distinct colonies were streaked on MacConkey agar, TCBS and SSA, as appropriate, until pure cultures were obtained. Cell morphologies of the bacteria were determined by microscopy after Gram staining.

Presumptive lactose fermenting colonies on MacConkey agar were transferred onto freshly prepared eosin methylene blue (EMB) agar and incubated at 37°C for 24 h. Colonies with a green metallic sheen characteristic of *E. coli* were identified accordingly.

16S rRNA gene analysis of isolates

Genomic DNA extraction and PCR

Genomic DNA was extracted from non-*E. coli* isolates. Precisely 1 mL of overnight suspension cultures of isolates in nutrient broth (at 37°C with shaking) were used for DNA extraction. Briefly, cells were recovered from suspension by centrifugation at 10,000 rpm for 2 min. Thereafter, DNA was extracted from the recovered cells (pellets) by using the ZR Fungal/Bacterial Soil Microbe DNA MiniPrep kit (Zymo Research, California, USA) according to the manufacturer's instruction.

Amplification of the 16S rRNA gene of the isolates was performed using universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TGACTGACTGAGGCTACCTTGCGA-3′). PCR reaction volume of 25 μL contained 12 μL 2 x DreamTaq PCR Master Mix (Thermo Fischer Scientific, Waltham, Massachusetts, USA), 1 μL DNA template, 10 μL nuclease-free water and 1 μL each of forward and reverse primers (10 mM). The thermo-cycling (T100™ Thermal Cycler, Bio-Rad Laboratories Pte Ltd, Singapore) conditions were an initial denaturation step at 95°C for 5 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 61°C for 30 s and extension at 72°C for 5 min, and an additional final extension step at 72°C for 7 min. Amplicon sizes (bp) were verified by electrophoresis on 1% agarose gel.

DNA sequencing and phylogenetic analyses

Sequencing of purified amplicons was conducted at Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa. Briefly, sequencing PCR reactions were performed using the PRISM™ Ready Reaction Dye Terminator Cycle Sequencing Kit (Sanger sequencing) with reverse primer 907R (5′-CCGTCAATTCCTTTGAGTTT-3′) and electrophoresed with a model ABI PRISM® 3500XL DNA Sequencer (Applied Biosystems, Foster City, CA, USA). Sequence electropherograms were manually inspected and edited for ambiguous nucleotide positions and poor quality initial sequences at the 5′ and 3′ ends using Chromas Lite software version 2.1 (Technelysium Pty Ltd, South Brisbane, Australia). High-quality partial 16S rRNA gene sequences from all isolates were then clustered into operational taxonomic units (OTUs) at 99% similarity using the Mothur software [33]. Furthermore, OTU representative sequences were assigned to taxonomic groups by aligning against 16S rRNA gene sequences in the EzBiocloud database (http://www.ezbiocloud.net/; [37]). Phylogenetic reconstruction of OTU representative sequences with closely related database sequences was performed in MEGA X [24] as previously described by Adedeji *et al.* [1]. Sequences obtained in this study are available in the NCBI GenBank under the accession numbers MK918511–MK918572.

Determination of haemolysin and amylase production in the isolates

We performed haemolysis assay and amylase production test on all isolates to obtain insights into their pathogenic and fruit spoilage (based on starch metabolism) potentials, respectively. Importantly, these two tests are preliminary (i.e. not definitive) but relatively easy to perform of all other pathogenicity and spoilage tests. Haemolysis assay was performed on freshly prepared blood agar (5% antibiotic-free human blood in 1000 mL nutrient agar; v/v; while amylase test was conducted using starch agar (w/v: 0.5% NaCl, 0.5% peptone, 0.3% yeast extract, 1.5 % Bacto agar, 0.2% soluble starch).

Table 1
Phylogenetic similarities of 16S rRNA gene operational taxonomic units (OTU) of isolates from fresh cut fruits and environmental samples.

OTU cluster number	Lab ID of OTU representative	Number of isolates per OTU cluster	EzBiCloud Database		Accession number of representative sequences
			Closest Match	Similarity (%)	
OTU1	FF-09	14	*Klebsiella pneumoniae* subsp. *ozaenae*	99.83	MK918565
OTU2	FF-22	11	*Enterobacter hormaechei*	100	MK918522
OTU3	FF-1	11	*Shigella flexneri*/*Escherichia fergusonii*	99.49	MK918511
OTU4	FF-58	9	*Enterobacter sichuanensis*	100	MK918541
OTU5	FF-47	9	*Klebsiella pneumoniae* subsp. *rhinoscleromatis*	99.83	MK918567
OTU6	FF-58B	3	*Klebsiella quasivariicola*	99.66	MK918560
OTU7	FF-39-2	2	*Citrobacter freundii*	100	MK918554
OTU8	FF-21	1	*Pantoea dispersa*	100	MK918521
OTU9	FF39B	1	*Citrobacter europaeus*	99.49	MK918555
OTU10	FF-34	1	*Enterobacter asburiae*	100	MK918528
OTU11	FF2	1	*Enterobacter roggenkampii*	100	MK918512

[a] OTU: Operational Taxonomic Unit.

Antibiotic sensitivity profiling of isolates

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 [9]. Isolates were suspended in 10 mL normal saline and adjusted to 0.5 McFarland's standard before testing. 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 against the isolates were: ampicillin (AMP, 10 µg), ampicillin/sulbactam (SAM, 10/10 µg), azetreonam (ATM, 30 µg), ceftazidime (CAZ, 30 µg), gentamicin (CN, 10 µg), imipenem (IPM, 10 µg), levofloxacin (LEV, 5 µg) and tetracycline (TET, 30 µg). Seeded plates were incubated at 37°C for 24 h and examined for zones of inhibition (measured in millimetres).

In order to determine multidrug-resistant isolates, the multiple antibiotic resistance (MAR) indices were calculated according to Annapurna and Rashmi [4]. The formula: MAR = a/b; was applied (where: a, is the number of antibiotics to which an isolate is resistant; b, is the total number of antibiotics tested).

Results

Occurrence of Enterobacteriaceae in fruits and environmental samples

A total of 172 bacterial isolates (FCFs = 137; fruit wash water = 28; vendors' hand swabs = 7) were recovered in this study. Thirty-nine (22.7%) isolates were considered as *E. coli* based on green metallic sheen colonies on EMB agar. Of the remaining 133 isolates, 63 were selected for 16S rRNA gene sequencing-based on distinct colonial morphology, resulting in a total of 102 isolates being considered for data interpretation in this study. Based on the partial 16S rRNA gene sequences similarity at $\geq$ 99%, the 63 isolates clustered into 11 operational taxonomic units (OTUs) (Table 1), which are related as depicted in the phylogenetic tree in Fig. 1. Taxonomically, all the bacterial isolates were predominantly enteric species and were widely distributed into seven genera; *Escherichia/Shigella* (49%), *Klebsiella* (25.5%), *Enterobacter* (21.6%), *Citrobacter* (2.9%) and *Pantoea* (1.0%) (Table 1). The distributions of the 11 OTUs/species across the analysed samples are presented in Fig. 2. *Escherichia coli*, which was the highest occurring enteric species, was present in all sample types apart from hand swabs. Hand swabs had the least diversity of bacterial isolates. On the other hand, *Klebsiella pneumoniae* subsp. *ozaenae* was the only OTU that was detected across all sample types (Fig. 2).

Species belonging to *Enterobacter, Escherichia, Shigella* and *Klebsiella*, and specifically those clustered in OTUs 1–4, were widely distributed across the states. These species occurred in samples from at least three of the states sampled. Among the prevalent species found, *K. pneumoniae* subsp. *ozaenae* (representing species in OTU 1) was detected across the six studied states. Comparing OTUs found in fruits and environmental samples, bacterial OTU/species identified as *K. pneumoniae* subsp. *ozaenae* was detected at all sampling points, including the FCFs, fruit wash water and hand swabs of vendors (Fig. 2).

Occurrence of haemolytic and starch-degrading bacteria in samples

The pathogenicity potential of the isolates was tested by screening for hemolysin production on blood agar, which resulted in the classification of the 102 bacterial isolates as either α-haemolytic (n =5, 4.9%), β-haemolytic (n =5,4.9%) or γ-haemolytic (n = 92, 90.2%) (Fig. 3). *Klebsiella pneumoniae* subsp. *ozaenae, Enterobacter hormaechei, Shigella flexneri*/*Escherichia fergusonii* and *Klebsiella pneumoniae* subsp. *rhinoscleromatis* constituted the α-haemolytic cluster, while *Enterobacter asburiae, E. hormaechei, E. sichuanensis* and *Klebsiella pneumoniae* subsp. *rhinoscleromatis* haboured the β-haemolytic strains (Fig. 3).

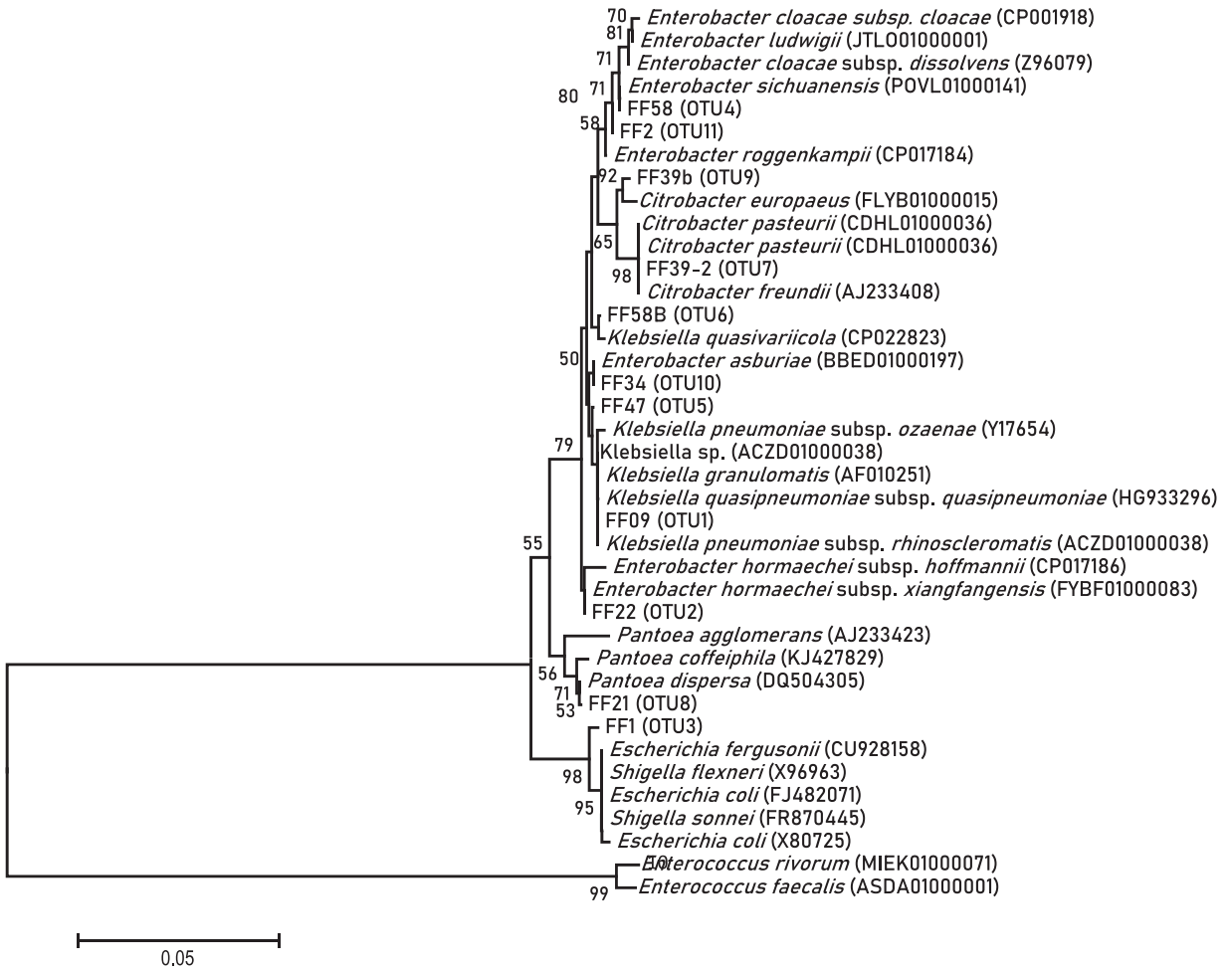

Fig. 1. Neighbour-Joining phylogenetic tree generated by multiple sequence alignment of the almost complete 16S rRNA gene sequences of bacterial isolates obtained from fresh cut fruits (pineapple and watermelon) and environmental samples (fruit wash water and vendors' hand swabs), and DNA sequences downloaded from the EzBiCloud database. The operational taxonomic units' clusters of the sequences are indicated and Bootstrap values of 1000 replications are indicated on the tree nodes. Accession numbers of GenBank sequences are in parenthesis.

On the other hand, the potential of the bacterial isolates to cause fruit spoilage by carbohydrate metabolism was preliminarily investigated by amylase production based on starch hydrolysis. Only 9.8% (n =10) of the screened isolates (n = 102) were positive for amylase production. Among amylase producers were *K. pneumoniae, E. hormaechei, E. sichuanensis* and *S. flexneri/Escherichia fergusonii* (Fig. 3).

Antibiotic-resistant bacteria profiles in samples

The antibiotic sensitivity profiles of enteric bacteria contaminating the fruit and environmental (fruit wash water and vendors' hand swab) samples were determined. Only 3% of 102 isolates were susceptible to all antibiotics tested. MAR indices of 0.9 and 0.6 were more frequent than other MAR indices, occurring at 23.8% and 19.8% respectively (Fig. 4). Approximately 76% of the isolates also had MAR > 0.4 and were classified as multidrug-resistant (MDR) strains. At least 48 antibiotic-resistant patterns were detected among the bacterial isolates tested; 38 (79.2%) patterns consisting of at least four of the same antibiotics to which the isolates were resistant (Table 2). The highest MDR patterns ($n = 22$) occurred among *E. coli* strains, while *K. pneumoniae* subsp. *ozaenae, S. flexneri, E. hormaechei* and *E. sichuanensis* displayed $\geq$ 7 MDR patterns. Other species displayed one or two MDR patterns. Co-resistance to ampicillin and gentamicin was the most common resistance pattern among the bacterial isolates.

Approximately 7% of all the isolates, particularly those obtained from the fruits, were resistant to all eight test-antibiotics. These resistant strains belonged to *E. coli* ($n = 3$), *S. flexneri* ($n = 2$), *E. sichuanensis* ($n = 1$) and *E. hormaechei* ($n = 1$) phylotypes.

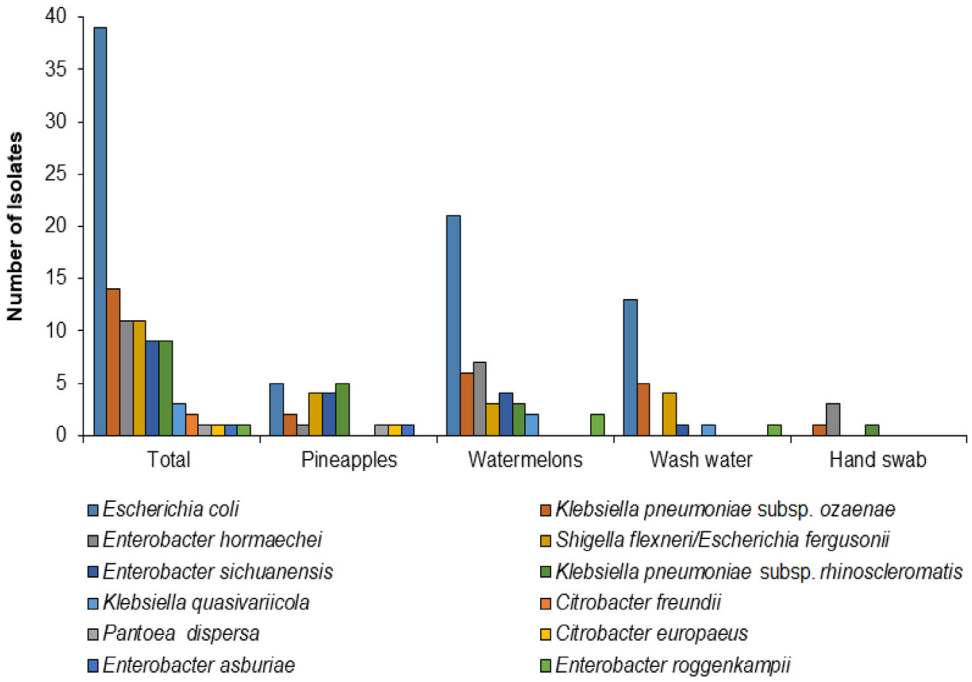

Fig. 2. Distribution of 102 bacterial isolates in fresh cut fruits (pineapple and watermelon) and environmental samples (fruit wash water and vendors' hand swabs).

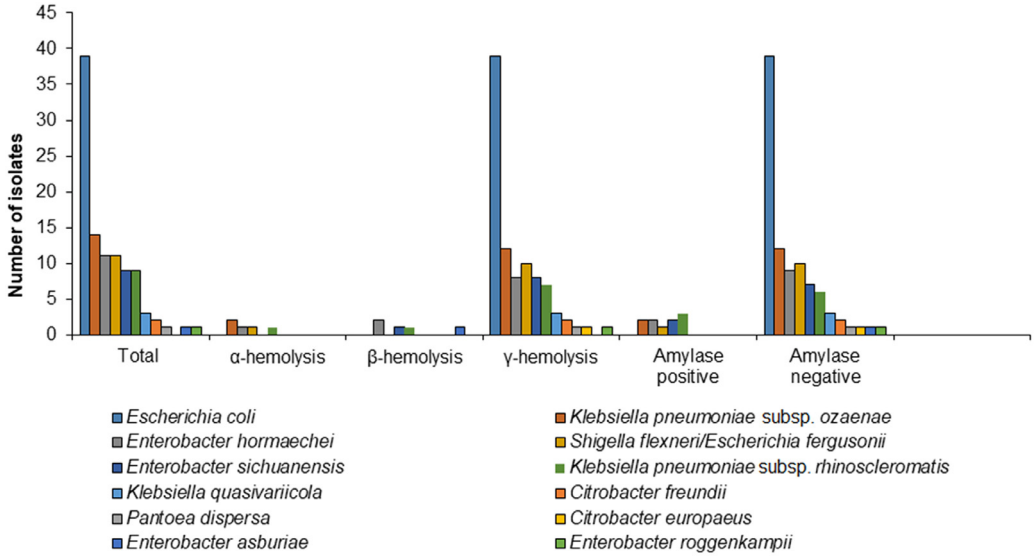

Fig. 3. Haemolysin and amylase production in 102 bacterial isolates obtained from fresh cut fruits (pineapple and watermelon) and environmental samples (fruit wash water and vendors' hand swabs).

Discussion

Tropical FCFs widely vended and consumed in the urban areas of Nigeria serve as the main source of the recommended daily fruit intake allowance for the busy and fast-paced urban dwellers. In the present study, we applied a culture-dependent method to elucidate the bacterial contaminants of food safety and public health importance in two commonly retailed FCFs across the six states in south-western Nigeria.

Findings of the present study showed that the bacterial contaminants in the FCFs samples belonged to the Enterobacteriaceae family represented by 11 operational taxonomic units. Although similar bacteriological surveys have previously highlighted the occurrence of some of these enteric bacteria in ready-to-eat pineapples and watermelons vended in markets

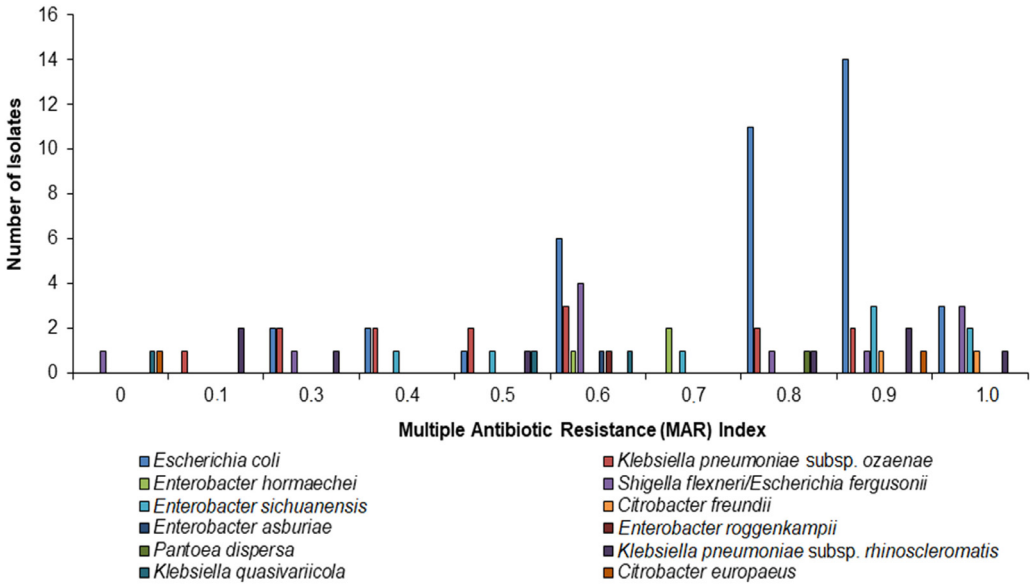

Fig. 4. Multiple antibiotic resistance indices among bacterial isolates ($n = 102$) obtained from fresh cut fruits (pineapple and watermelon) and environmental samples (fruit wash water and vendors' hand swabs).

Table 2
Antibiotic-resistant patterns observed among bacterial isolates obtained from fresh-cut fruits (pineapple and watermelon) and environmental samples (fruit wash water and vendors' hand swabs).

Antibiotic[a] resistant pattern	N[b]	Sample type[c] (OTU/Species[d])	Antibiotic[a] resistant pattern	N[b]	Sample type[c] (OTU/Species[d])
AMP	3	M (OTU2, OTU5), H (OTU2)	LEV-TET-AMP-CN-CAZ	1	W (*E. coli*)
CN	1	M (OTU1)	ATM-LEV-TET-AMP-CAZ	1	W (*E. coli*)
TET-AMP	1	W (*E. coli*)	TET-SAM-AMP-CAZ-IPM	2	M (OTU4), H (OTU2)
AMP-CN	3	P (OTU3, OTU5), W (OTU1)	LEV-TET-CN-CAZ-IPM	1	M (OTU2)
AMP-IPM	1	W (*E. coli*)	ATM-TET-SAM-AMP-IPM	1	W (OTU3)
AMP-CAZ	1	W (OTU1)	ATM-SAM-AMP-CN-IPM	1	M (OTU6)
SAM-AMP-CN	1	M (OTU4)	TET-SAM-AMP-CN-CAZ	1	M (OTU2)
TET-AMP-CN	1	W (OTU1)	ATM-LEV-TET-SAM-AMP-CAZ	1	P (*E. coli*)
AMP-CN-IPM	3	W (*E. coli*), P (OTU2), M (OTU1)	ATM-TET-SAM-AMP-CN-CAZ	2	P (OTU3), W (*E. coli*)
TET-AMP-IPM	1	W (*E. coli*)	ATM-TET-SAM-AMP-CAZ-IPM	1	M (*E. coli*)
TET-SAM-AMP-CN	3	P (*E. coli*), P (OTU10), M (OTU1)	ATM-SAM-AMP-CN-CAZ-IPM	3	M (OTU1, *E. coli*), P (OTU5)
TET-AMP-CN-IPM	2	M (OTU6), W (OTU4)	ATM-TET-AMP-CN-CAZ-IPM	2	W (*E. coli*), M (OTU4)
ATM-AMP-CN-IPM	3	M (OTU1), H (OTU1, OTU2)	ATM-LEV-AMP-CN-CAZ-IPM	2	P (OTU8, *E. coli*)
AMP-CN-CAZ-IPM	1	P (OTU1)	TET-SAM-AMP-CN-CAZ-IPM	1	P (*E. coli*)
ATM-SAM-AMP-IPM	1	M (OTU5)	ATM-LEV-TET-CN-CAZ-IPM	1	M (OTU1)
ATM-TET-SAM-AMP-CAZ	2	P (OTU1), M (*E. coli*)	ATM-LEV-TET-SAM-AMP-CN	3	M (*E. colix 2*), W (*E. coli*)
ATM-TET-AMP-CN-CAZ	2	P (OTU3), H (OTU2)	LEV-TET-SAM-AMP-CN-CAZ	1	M (*E. coli*)
ATM-AMP-CN-CAZ-IPM	2	P (OTU11), M (*E. coli*)	ATM-LEV-TET-SAM-AMP-CN-CAZ	3	M (*E. colix 3*)
TET-SAM-AMP-CN-IPM	2	P (*E. coli*), W (*E. coli*)	ATM-TET-SAM-AMP-CN-CAZ-IPM	8	M (OTU7, *E. coli*), W (OTU1, OTU5, *E. colix 2*), P (OTU4, OTU5)
LEV-TET-SAM-AMP-CN	1	W (OTU3)	ATM-LEV-TET-SAM-AMP-CAZ-IPM	4	M (*E. colix 3*), W (*E. coli*)
SAM-AMP-CN-CAZ-IPM	1	P (OTU3)	ATM-LEV-TET-AMP-CN-CAZ-IPM	6	M (*E. colix 3*), W (OTU3), P (OTU4), P (OTU9)
LEV-AMP-CN-CAZ-IPM	1	M (OTU2)	ATM-LEV-SAM-AMP-CN-CAZ-IPM	1	W (OTU1)
TET-AMP-CN-CAZ-IPM	2	W (OTU2), M (OTU2)	LEV-TET-SAM-AMP-CN-CAZ-IPM	4	M (OTU3, OTU4, OTU5, *E. coli*)
ATM-LEV-SAM-AMP-CAZ	1	M (OTU2)	ATM-LEV-TET-SAM-AMP-CN-CAZ-IPM	7	P (OTU4), M (OTU7, OTU3 × 2, *E. colix 3*)
			No Pattern (All susceptible)	3	W (OTU3, OTU6, OTU9)

[a] ATM- Azetreonam; LEV- Levofloxacin; SAM- Ampicillin/ Sulbactam; CN- Gentamicin; TET- Tetracycline; IPM- Imipenem; CAZ- Ceftazidime; AMP- Ampicillin.

[b] Number of isolates.

[c] P: pineapple; M: watermelon; W: fruit wash water. H: vendors' hand swabs.

[d] OTU1, *Klebsiella pneumoniae* subsp. *ozaenae*; OTU2, *Enterobacter hormaechei*; OTU3, *Shigella flexneri/Escherichia fergusonii*; OTU4, *Enterobacter sichuanensis*; OTU5, *K. pneumoniae* subsp. *rhinoscleromatis*; OTU6, *Klebsiella quasivariicola*; OTU7, *Citrobacter freundii.*; OTU8, *Pantoea dispersa*; OTU9, *Citrobacter europaeus*; OTU10, *Enterobacter asburiae;* OTU11, *Enterobacter roggenkampii; xN* represents the number of resistant isolates >1 per sample type.

across Nigeria [15,22,28], the present study is the first molecular-based report on the bacterial isolates from the two FCFs in Nigeria. Enteric bacteria, though mostly opportunistic pathogens, widely serve as indicators of faecal contamination of food and water, thereby indicating potential health hazards for consumers. Cases of dysentery and diarrhoea, which resulted in hospitalisation, have been linked to the consumption of food materials contaminated with foodborne bacterial species, such as *E. coli, K. pneumoniae* and *S. flexneri,* that were detected in fruits analysed in the present study ([13,21]; Brisse and Verhoef, 2011; [23]). According to the Centre for Science in the Public Interest (2014), the major cause of hospitalisations in the United States between 2002 and 2011 was attributed to the consumption of contaminated fresh produce. However, the case is no different in Europe, where a 10-year survey also implicated contaminated fresh produce as the main cause of life-threatening illnesses between the years 2006 and 2016 [27]. These data are pointers to the urgent need for proper monitoring of the processing, storage and distribution stages for fresh-cut fruits in order to minimise microbial contamination and outgrowth, and ultimately to safeguard public health. Factors that influence microbial susceptibility of FCFs include atmospheric exposure of fruit surfaces due to cutting and removal of peels, lack of heat-processing before consumption, the perishable nature of fruit itself, poor hygiene of handlers, dirty processing and packaging materials, abuse of storage time and temperature among others [38]. With respect to the geographical spread of the identified bacterial species, *K. pneumoniae* subsp. *ozaenae* was detected across the six studied states, thereby suggesting that this species is a common bacterial contaminant of FCFs in south-western Nigeria. Worthy of note is the detection of isolates that are phylogenetically related to *E. hormaechei* and *E. sichuanensis* in the vended fruits samples. To the best of our knowledge, this is the first report on the presence of such phylotypes in Nigerian fruit products. *Enterobacter sichuanensis* was recently isolated from the urine of a patient with chronic renal insufficiency in China [36], while strains of *E. hormaechei* have been implicated in foodborne illness outbreak in Mexico [29]. The occurrence of these potentially pathogenic bacterial species in the fruits from most states in fruit wash water, also suggests a regional prevalence of these species.

The occurrence of enteric bacteria in commercial FCFs may be inevitable due to the nature of processing and other factors mentioned earlier. However, in order to assert quality control to minimise microbial contamination, it is important to identify the possible sources of contamination. In this quest, this study identified the co-occurrence of bacterial OTU/species identified as *E. coli* and *K. pneumoniae* subsp. *ozaenae* in all sample types, suggesting a contamination link between the fruits, wash water and vendor's hand. These observations further emphasise the need for quality improvement of fruit vendors' hygiene, wash water, raw fruits and processing utensils, involved in FCF production and marketing. Regular hand washing with soap and clean water, as well as wearing disposable hand gloves are highly recommended for individuals actively involved in fruit processing. Governments' assistance, however, is needed in this regard, especially for subsidising the cost of food-grade sanitisers and disposable hand gloves, as well as in the provision of potable water sources. In addition, it is necessary to sensitise people working with the FCFs about the need to ensure utmost food safety during fruits processing. The impact of fruit contamination by soil microbiota, organic fertilisers and the transport chain, though not investigated in the present study, cannot be overlooked in the overall assessment of FCF contamination. These links have been recognised as possible sources of foodborne pathogens in industrialised countries [30]. Therefore, further expansive studies investigating the complex value chain for fresh-cut fruits in Nigeria are required.

The pathogenic and fruit spoilage potentials of the bacterial isolates obtained in this study were investigated by measuring haemolysin and amylase activities, respectively. Bacterial cells that produce the enzyme haemolysin are capable of degrading or lysing red blood cells; a phenomenon indicative of their pathogenic potential [8]. 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 FCF consumers upon consumption of such poorly processed fruits. The activity of amylase enzyme is critical for food spoilage, particularly of sugar-rich foods such as tropical fruits [12,16]. Approximately 10% of the bacterial isolates screened in the present study were amylase positive, indicating their fruit-spoilage causing potential. It is important to note that food spoilage is not only a function of the presence of spoilage-causing microorganisms but also that of the contaminants' populations reaching certain thresholds where quality depreciation becomes noticeable. However, the focus of the present study was not quantitative but qualitative. From the haemolytic and amylase production data, all amylase producers were haemolytic. Consequently, it could be postulated that these haemolytic amylase-producing species pose an underestimated risk to consumers of the fruits bearing in mind that they could adhere and proliferate in the gastrointestinal tract of FCFs consumers, thereby causing acute gastroenteritis. Further studies are required to verify this postulation

Lastly, the antibiogram of bacterial isolates was determined. Multidrug-resistant pathogens constitute a burden on healthcare management worldwide, by complicating disease treatments, increasing healthcare costs, extending hospital stay, and increasing mortality rates [32]. 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 [17]. *Klebsiella pneumoniae* and *E. coli* showed phenotypic resistance to varying classes of antibiotics such as penicillin, beta-lactam combinations, cephalosporins, monobactams, carbapenems, aminoglycosides, tetracyclines and quinolones in samples from Ibadan (data not shown); a trend observed by Lee *et al.* [25] in clinical isolates responsible for urinary tract infection. However, Al-Kharousi *et al.* [2] reported susceptibility to extended-spectrum beta-lactamases to which isolates from this study were resistant. This trend could be traced to the proximity of FCF vendors to fresh meat vendors within the market as well as the unhygienic fruit processing environments. The fact that higher numbers of antibiotic-resistant bacteria (76% of 102

isolates) were associated with the samples in the present study is worrisome and suggests that FCFs 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 across the six south-western states of Nigeria are contaminated by diverse enteric bacterial species, including two species of *Enterobacter* (*E. hormaechei* and *E. sichuanensis*) reported for the first time in tropical fruits in Nigeria. These bacteria may constitute a threat to FCF consumers. The public health risks may be heightened by the occurrence of several multiple antibiotic-resistant strains in the samples. In addition, we suspect that water used for fruit washing, bacteria on the hands of vendors, compost from animal sources on the farm and knives used for cutting the fruits may be veritable sources of contamination of the fruits analysed. Thus, proper use of potable water for fruit washing and processing equipment, regular hand washing with soap and clean water, and the use of protective disposable (polyethylene) hand gloves are good hygiene practices recommended to fruit vendors. Also, it is imperative to maintain adequate pre- and post- harvest practices of pineapple and watermelon. We also recommend strict regulations and regular monitoring to ensure compliance of fruit vendors to recommended food safety practices. Specifically, the government should set up a compliance task force within food and hygiene regulatory authorities to conduct routine surveillance of vended FCFs and environmental samples; this will facilitate the implementation of the recommendations herein by stakeholders in the FCF value chain. 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 FCF process chain in Nigeria.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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