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RESEARCH ARTICLE

Preponderance of antibiotic-resistant bacteria associated with partially damaged tomato (*Solanum lycopersicum* L.) obtained from local markets in Southwest Nigeria

Omotayo Olumide Ekundayo[1]*, Faniyi Fayokemi Blessing[2], Akinola Omowumi Temitayo[2] & Oyeku Oyeshina Gideon[1]

[1]Pure and Applied Biology Programme, College of Agriculture, Engineering and Science, P.M.B 284, Iwo, Osun State, Nigeria
[2]Microbiology Programme, College of Agriculture, Engineering and Science, P.M.B 284, Iwo, Osun State, Nigeria

*Email: olumide.omotayo@bowen.edu.ng

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Abstract

Ripened tomato fruits tend to rapidly deteriorate after harvest due to the physiological activities of plant hormones and their naturally high-water content, which makes them susceptible to spoilage by microorganisms. This study was therefore carried out to isolate, characterize and identify pathogenic bacteria associated with partially rotten tomato fruits. Partially rotten tomato fruit samples were sourced from local markets in Southwest Nigeria and subjected to bacterial analysis. The level of susceptibility of the bacteria isolates to commonly used β-lactamase antibiotics was evaluated via the disc diffusion method. The bacterial isolates with the highest resistance to all the antibiotics tested were selected for molecular characterization using 16S rRNA gene profiling. Forty-two bacterial isolates were obtained from the tomato samples, namely *Bacillus* spp. (26.19%), *Klebsiella* spp. (16.67%), *Serratia* spp. (14.29%), *Citrobacter* spp. (14.29%), *Staphylococcus* spp. (7.14%), *Pseudomonas* spp. (4.76%), *Micrococcus* spp. (4.76%), *Enterobacter* spp. (4.76%), *Providencia* sp. (2.38%), *Proteus* sp. (2.38%) and *Salmonella* sp. (2.38%). The highest level of antibiotic resistance of the bacterial isolates was against Ciprofloxacin (10 µg) at 100%, followed by Zinnacef (20 µg) at 72.77%, while the highest susceptibility was against Streptomycin (30 µg) at 94.74%. Using phylogenetic analysis of their 16S rRNA gene sequence, three bacteria that were resistant to every single drug tested were identified as *Klebsiella pneumoniae*, *Enterobacter asburiae* and *Enterobacter cloacae*. This study shows that antibiotic-resistant bacteria are present in partially damaged tomatoes; thus, consumption of improperly cooked tomatoes could pose a significant public health risk.

Keywords

antibiotic resistance; bacteria; spoilage microorganisms; 16S rRNA; tomato

Introduction

Food security remains an emerging challenge in the 21st century, particularly in the developing economies of Africa (1, 2). Several factors, including climate change, insecurity, poor energy economics and inadequate/unavailable infrastructural availability for food preservation, especially in rural areas, all contribute to the dwindling food resources available to the teeming masses on the African continent (3). The tomato fruit accounts for about 18% of average daily consumption in Nigeria, which makes it a very

important food crop for the average Nigerian (4). Tomato is often grown in the southwestern part of the country in small farm holdings under natural rain-fed conditions, while in the northern parts, it is cultivated under irrigation systems (5).

Tomato spoilage is the term used to describe unfavorable alterations in tomato quality caused mostly by biological and physical reasons. These could be modifications to the fruits' flavor, aroma, look or texture (6). Bacterial contamination of tomatoes has been identified as a considerable risk to consumers by causing food-borne illnesses and food poisoning (7). Tomato fruits are frequently arranged in baskets and on benches in open markets in many underdeveloped nations on the African continent, such as Nigeria. This exposes potential buyers to opportunistic microbial illnesses, including mycotoxins (8). The researchers in a meta-analysis review of common fruits and vegetables sold and purchased in Nigeria also implicated the dishonest attitudes of vendors in mixing the sale of contaminated fruits with good fruits, which leads to further bacterial infections (9).

Antibiotics are antimicrobial substances that can inhibit the growth of microorganisms or kill them and are widely used for the treatment of bacterial infections in humans and animals, as well as in non-medical applications (10). Beta-lactam antibiotics are common first-line antibiotics usually consulted for combating bacterial-based infections for a wide range of diseases in sub-Saharan Africa. They include penicillin, cephalosporins, monobactams and carbapenems, which are among the most widely used antibiotics today (11). Their applications in managing bacterial-borne diseases have also been reported by some workers on the African continent (12, 13).

Partially rotten tomato fruits (also referred to locally as èsà) are often readily purchased and consumed by the local populace in a local town setting such as Iwo, Nigeria, due to the cheaper prices for these products compared with the fresh products. This study was thus carried out to isolate and identify microorganisms associated with partially damaged tomatoes obtained from local markets commonly patronized by the local community. The specific objectives of the study were to identify and characterize the bacterial population on partially rotten tomato fruits using both classical and modern molecular sequencing methods. The study also assessed the impact of different conventional antibiotics as effective antimicrobials on the growth of the identified spoilage microorganisms of the tomato fruits.

Materials and Methods

Sample collection

The materials used for this study included partially rotten tomatoes collected from 3 community markets in different locations at Iwo, Osun State, Nigeria (Odori market, Oja Oluwo and Oja Ale). They were placed aseptically inside clean nylon pouches and conveyed to the Microbiology Laboratory of the Department of Biological Sciences, Bowen University (BU), for further analysis to be carried out.

Media preparation

The culture media (Nutrient Agar, MacConkey Agar, Mannitol Salt Agar and Potato Dextrose Agar) were prepared by the manufacturer's specifications. For the slant samples, sanitized McCartney bottles were used and the sterilized Petri dishes were filled aseptically with media after being allowed to cool on a sterile workbench.

Isolation of microorganisms

The ten-fold serial dilution method was used to isolate spoilage microorganisms from the partially rotten tomato samples. The streaking method was used to subculture distinct bacterial colonies. The plates were then incubated at 37°C for 24 h in an inverted position. Pure isolates obtained after incubation were aseptically stored on nutrient agar slants in McCartney bottles and labelled appropriately. The bottles were kept in the refrigerator for further identification and study.

Biochemical characterization of bacterial isolates

The bacterial isolates obtained in pure culture were characterized based on their colonial morphology, reaction to gram staining and standard biochemical tests such as the indole test, methyl-red, citrate utilization, catalase, Voges-Proskauer test, sugar fermentation, starch fermentation and motility test.

Antibiotics sensitivity test

The isolates were tested for antimicrobial susceptibility using the Kirby-Bauer agar disc diffusion method (14). A loopful of 24 h pure bacterial culture was suspended into 1 mL sterile water to a turbidity equivalent to 0.5 McFarland standard. The suspensions obtained were then streaked on a Mueller-Hinton agar plate using sterile swab sticks. The discs used include Pefloxacin (PEF) 10 µg, Gentamycin (CN) 10 µg, Ampiclox (APX) 30 µg, Zinnacef (Z) 20 µg, Amoxicillin (AM) 30 µg, Rocephin (R) 25 µg, Ciprofloxacin (CPX) 10 µg, Streptomycin (S) 30 µg, Septrin (SXT) 30 µg and Erythromycin (E) 10 µg for gram-positive bacterial isolates. The discs used for gram-negative isolates included Septrin (SXT) 30 µg, Chloramphenicol (CH) 30 µg, Sparfloxacin (SP) 10 µg, Ciprofloxacin (CPX) 10 µg, Amoxicillin (AM) 30 µg, Augmentin (AU) 30 µg, Gentamycin (AU) 10 µg, Tarivid (OFX) 10 µg and Streptomycin (S) 30 µg. The antibiotic discs were gently but firmly placed on the inoculated plates before the plates were incubated at 37°C for 24 h. After incubation, zones of inhibition were measured in millimeters and interpreted according to the Clinical and Laboratory Standard Institute Standard (15). The experiment was carried out in duplicate to ensure the reproducibility of the results.

Molecular characterization and phylogenetic study of the antibiotic-resistant isolates

Four bacterial isolates that showed resistance to all the β-lactam antibiotics were selected for molecular identification and phylogenetic analysis.

DNA extraction

Chromosomal DNA of the isolates was extracted by boiling lysate method as described earlier (16). Briefly, 100 µL of

broth culture for each specimen was added into individual micro-centrifuge tubes and centrifuged at 10000 rpm for 5 min. 500 µL of lysis buffer were then added into each tube, and the mixture was vortexed and incubated at 56°C for 10 min. Centrifugation was done at 10000 rpm for a minute. After spinning, 200 µL of absolute ethanol was added to each tube. The mixture in each tube was subsequently transferred into a separate spin column before centrifugation was carried out at 10000 rpm for 30 sec. The flow-through was discarded and the collection tube was blotted on a tissue paper. 500 µL of wash buffer 1 were added to each spin column and then centrifugation was performed at 10000 rpm for 30 sec. The flow-through was discarded and the collection tubes were blotted on a tissue paper. The DNA was then extracted using centrifugation, which was run for 1 min at 10000 rpm. At -20 °C, the eluted DNA was kept.

PCR amplification of 16S rRNA gene

The polymerase chain reaction was carried out to amplify the 16S rRNA gene of the bacteria using the primer pairs 27F- 5′- AGAGTTTGATCCTGGCT CAG -3’ and 1492R 5’- GGTTACCTTGTTACGACTT -3’ (17). The PCR reaction was carried out using the Solis Biodyne 5X HOT FIREPol Blend Master mix. PCR was performed in 25 µL of a reaction mixture and the reaction concentration was brought down from 5x concentration to 1x concentration containing 1x blend master mix buffer (Solis Biodyne), 1.5 mM $MgCl_2$, 200 µM of each deoxynucleoside triphosphates (dNTP) (Solis Biodyne), 25 pMol of each primer (BIOMERS, Germany), 2 unit of Hot FIREPol DNA polymerase (Solis Biodyne), proofreading enzyme, 5 µL of the extracted DNA and sterile distilled water was used. The electrophoresis procedure and viewing of DNA bands was carried out according to the previously described protocol (18).

16S rRNA sequencing

All PCR products were purified with Exo sap and sent to Epoch Life Science (USA) for Sanger sequencing. The corresponding sequences were identified using the online blast search at http://blast.ncbi.nlm.nih.gov/Blast.cgi.

Molecular phylogenetic analysis

The sequences that were obtained in this study (i.e., OR343125 - *Klebsiella pneumoniae;* OR343126 - *Enterobacter* sp.; OR343127 - *E. cloacae*) were aligned with existing sequences of *Klebsiella*, *Enterobacter, Serratia, Escherichia* and *Pseudomonas* in the GenBank database. A sequence of *Bacillus cereus* from the same database was used as an out -group. Sequence alignment was performed using the MUSCLE algorithm in Geneious® v.2023.2 software for Windows(19). Twenty-four sequences in total were used for the 16S rRNA gene sequence phylogenetic analysis. The phylogenetic relationship between the sequences was examined using the Bayesian posterior probability (BI) analysis. This was carried out on the Geneious® software for Windows using MrBayes 3.2.6 (20). The Hasegawa-Kishono-Yano model with Gamma distribution was used for the BI analysis. Two million (2.0×10^6) generations were used in the phylogenetic analysis(21). A sampling frequency of 1000 generations and a burn-in value of 200 were also selected according to the previous protocol (22).

PCR amplification of ESBL resistance genes

The bacterial isolates exhibiting phenotypic resistance to greater than three beta-lactam antibiotics used in this study were screened for the presence of genes encoding ESBLs (*bla*CTX-M, *bla*TEM, *bla*SHV) using multiplex primers according to the procedure described earlier (23). The primer sequences and corresponding temperature used in multiplex PCR reactions are listed in Table 1.

Table 1. Primer sequences and corresponding temperature used in multiplex PCR reactions

Primer name	Sequence	Annealing temperature	Base pair (bp)	References
Bla SHV-F	GATGAACGCTTTCCCATGATG	58	214	
Bla SHV-R	CGCTGTTATCGCTCATGGTAA			
Bla TEM-F	AGTGCTGCCATAACCATGAGTG	58	431	(23)
Bla TEM-R	CTGACTCCCCGTCGTGTAGATA			
Bla CTX-MF	GACAAAGAGAGTGCAACGGATG	58	501	
Bla CTX-MR	TCAGTGCGATCCAGACGAAA			

Results

The bacterial isolates were morphologically identified as they showed varied types of growth, arrangements, elevation and density according to their different types. The bacterial groups were also characterized based on standard gram-staining and reference biochemical tests as shown in Table 2. Forty-two bacterial isolates were obtained from the partially rotten tomato samples and identified based on ABIS and Microrao online bacterial identification tools. The distribution of the isolated bacteria genera isolated and identified across the respective markets sampled in this study included *Bacillus* sp. (26.19%), *Klebsiella* spp. (16.67%), *Serratia* spp. (14.29%), *Citrobacter* spp. (14.29%), *Staphylococcus* spp. (7.14%), *Pseudomonas* spp. (4.76%), *Micrococcus* spp. (4.76%), *Enterobacter* spp. (4.76%), *Providencia* sp. (2.38%), *Proteus* sp. (2.38%) and *Salmonella* sp. (2.38%). For the Odori market, the highest percentage of isolated bacterial genus was *Bacillus,* with *Enterobacter, Providencia* and *Proteus* showing similarly lower occurrence values (Fig. 1). At the Oja-Oluwo market, *Klebsiella* was the highest observed bacterial genus, while the lowest was *Staphylococcus* (Fig. 2). In the Oja-Ale market, the highest occurrence of isolated bacterial genus was also *Klebsiella*, while the lowest occurring bacterial genera were *Citrobacter, Serratia* and *Salmonella* (Fig. 3).

Healthy tomato samples and partially spoilt tomato samples used in the study from which the bacteria were isolated are shown in Fig.4, respectively. The gram-negative bacteria isolated included those in the genera *Klebsiella*, *Enterobacter*, *Providencia* and *Citrobacter,* amongst others (Table 2). *Klebsiella pneumoniae* of the genus *Klebsiella* was observed as rod-shaped, coliform bacteria with a dark pinkish to mucoid pink colony appearance.

Table 2. Probable identification of the bacterial isolates from partially rotten tomatoes in South-west Nigeria using morphological and biochemical characteristics

S/N	Isolate	GR	Cat	Mot	SH	IMVC				Sugar fermentation				Probable organism
						Ind	MR	VP	CU	Glu AG	Lac AG	Man AG	Suc. AG	
1.	A1	- rod	+	+	-	-	-	+	+	AG	AG	AG	AR	*Enterobacter sp*
2	B2	- rod	+	+	-	-	-	+	-	AG	AG	A	A	*Serratia marcesens*
3	B3	- rod	+	+	-	-	-	+	-	AG	AG	AG	AG	*Enterobacter aerogens*
4	B4	- rod	+	+	-	-	-	+	-	AG	AG	A	A	*S. marcesens*
5	C2	- rod	+	+	-	-	+	+	+	AG	AG	AG	AG	*Citrobacter freudnii*
6	C1	- rod	+	-	-	-	-	+	+	AG	AG	AG	AG	*Klebsiella pnuemoniae*
7	C4	- rod	+	+	-	-	+	+	+	AG	AG	AG	AG	*C. freudnii*
8	C3	- rod	+	-	-	-	-	+	+	AG	AG	AG	AG	*K. pnuemoniae*
9	D1	-rod	+	-	-	-	-	+	+	AG	AG	AG	AG	*K. pnuemoniae*
10	D3	- rod	+	-	-	-	-	+	+	AG	AG	AG	AG	*K. pnuemoniae*
11	D4	- rod	+	+	-	-	+	-	+	AG	-	-	AG	*Salmonella spp*
12	D2	- rod	+	-	-	-	-	+	+	AG	AG	AG	AG	*K. pnuemoniae*
13	A1	+ rod	+	-		-	+	+	+	AG	-	-	-	*Bacillus anthracis*
14	A2	- rod	+	-	+	-	+	-	+	A	-	-	AG	*Providentia sturartii*
15	A3	+ rod	+	-		-	+	+	+	AG	-	-	-	*B. anthracis*
16	A4	- rod	+	+	-	-	+	+	+	AG	-	-	-	*Proteus mirabilis*
17	B1	- rod	+	+	-	-	+	+	+	AG	AG	AG	AG	*C. freudnii*
18	B2	+ rod	+	-	-	-	+	-	+	AG	AG	AG	AG	*Bacillus endophyticus*
19	B3	- rod	+	+	-	-	-	+	-	AG	AG	A	A	*S. marcesens*
20	B4	- rod			-	-	+	+	+	AG	AG	AG	AG	*C. freudnii*
21	C1	+ rod	+	-		-	+	-	+	AG	AG	AG	AG	*Bacillus megaterium*
22	C2	+ cocci	+	-	-	+	-	-	-	AG	AG	AG	AG	*Staphylococcus simulans*
23	C3	- rod	+	+	-	-	-	+	-	AG	AG	A	A	*S. marcesens*
24	C4	- rod	+	+	-	-	+	+	+	AG	AG	AG	AG	*C. freudnii*
25	D1	+ rod	+	-	-	-	+	-	+	AG	AG	AG	AG	*B.endophyticus*
26	D2	- rod	+	+	-	-	+	+	+	AG	AG	AG	AG	*C.freudnii*
27	D3	+ rod	+	-	-	-	+	-	+	AG	AG	AG	AG	*B. endophyticus*
28	D4	+ cocci	+		-	+	-	-	-	AG	AG	AG	AG	*Staphylococcus intermedus*
29	A1	+ rod	+	-		-	+	+	+	AG	-	-	-	*B.anthracis*
30	A2	+ rod	+	-		-	+	+	+	AG	-	-	-	*B. anthracis*
31	BI	+ cocci	+	-	-	-	-	-	+	AG	AG	-	AG	*Micrococcus halobios*
32	B2	+ co	+	-	-	-	-	-	+	AG	AG	-	AG	*M. halobios*
33	B3	+ rod	+	-	-	-	+	-	+	AG	AG	AG	AG	*B. endophyticus*
34	C2	+ rod	+	-	-	-	+	-	+	AG	AG	AG	AG	*B. endophyticus*
35	D1	+ cocci	+	-	-	-	-	-	+	AG	A	AG	A	*Staphylococcus aureus*
36	D2	+ rod	+	-	-	-	+	-	+	AG	AG	AG	AG	*B. endophyticus*
37	B1	- rod	+	+	-	-	-	-	+	AG	-	AG	AG	*Pseudomonas aeruginosa*
38	B2	- rod	+	+	-	-	-	-	+	AG	-	AG	AG	*P. aeruginosa*
39	CI	- rod	+	-	-	-	-	+	+	AG	AG	AG	AG	*K. pnuemoniae*
40	C2	- rod	+	+	-	-	-	+	-	AG	AG	A	A	*S. marcesens*
41	D1	- rod	+	-	-	-	-	+	+	AG	AG	AG	AG	*K. pnuemoniae*
42	D2	- rod	+	+	-	-	-	+	-	AG	AG	A	A	*S. marcesens*

GR= Gram reaction, **NA**= Nutrient agar, **Cat**= Catalase, **Mot**= Motility, **Ind**= Indole, **MR**= Methyl red, **VP**= Voges-Proskauer, **CU**= Citrate utilization, **SH**= Starch hydrolysis, **Glu**= Glucose, **Lac**= Lactose, **Mal**= Mannitol, **Su**= Sucrose, **A**= Acid production, **G**= Gas production.

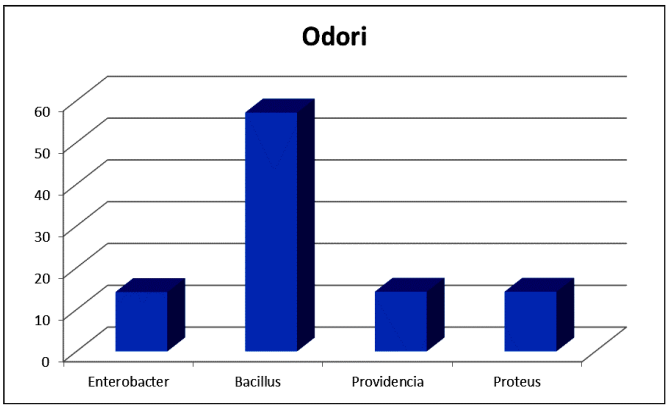

Fig. 1. Percentage distribution of bacterial isolates from Odori market.

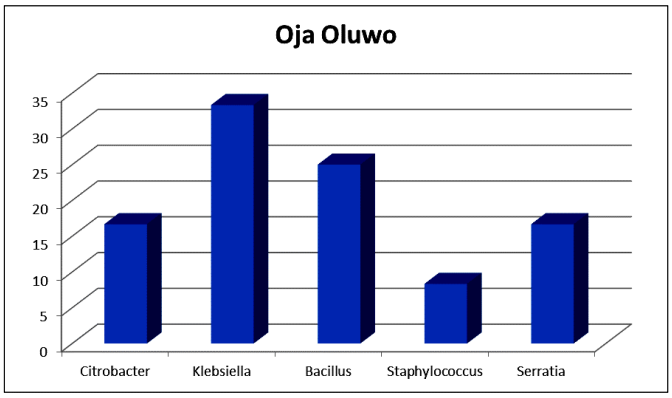

Fig. 2. Percentage distribution of bacterial isolates from Oja Ale market.

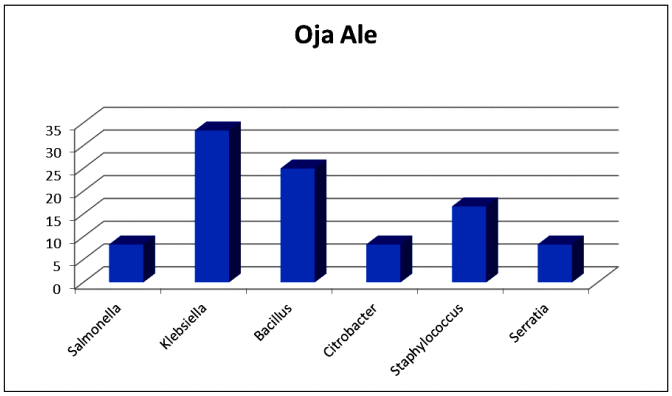

Fig. 3. Percentage distribution of bacterial isolates from Oja Ale market.

Citrobacter fruendii was observed as a rod-shaped bacillus with creamy-coloured colony appearance of the genus *Citrobacter*. Similarly, *Serratia marcescens* was observed as a rod-shaped bacillus with creamy-coloured colony appearance of the genus *Serratia*.

Antibiotic susceptibility tests were carried out on all the bacterial isolates (Fig. 5). The isolates were tested against 10 conventional antibiotics. The results are shown in Fig. 6 and 7. All the antibiotics used exhibited various effects on the bacterial isolates. The most effective antibiotics against the gram-positive bacteria were Erythromycin (E), with 94.44% susceptibility and 100% resistance to Ciprofloxacin (CPX). Conversely, the most effective antibiotic against gram-negative bacteria was Tarivid with 100% susceptibility, Streptomycin with 94.74% susceptibility and 100% resistance to Ciprofloxacin. Fig. 8 illustrates the occurrence of beta-lactam resistance genes in the four selected bacterial isolates. For the ESBL genes examined in this study, no positive amplifications were found in the corresponding bacterial samples. Therefore, the bacterial isolates may be considered non-ESBL producers.

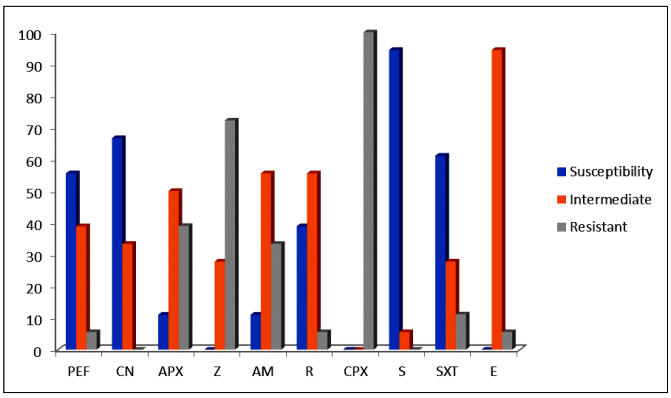

Fig. 4. Percentage susceptibility of gram-positive bacterial isolates to conventional antibiotics. **PEF** = pefloxacin; **CN** = Gentamycin; **APX** =Ampiclox; **Z** =Zinnacef; **AM** =Amoxacillin; **R** = Rocephin; **CPX** = Ciprofloxacin; **S** = Streptomycin; *SXT* = Septrin; **E** = Erythromycin.

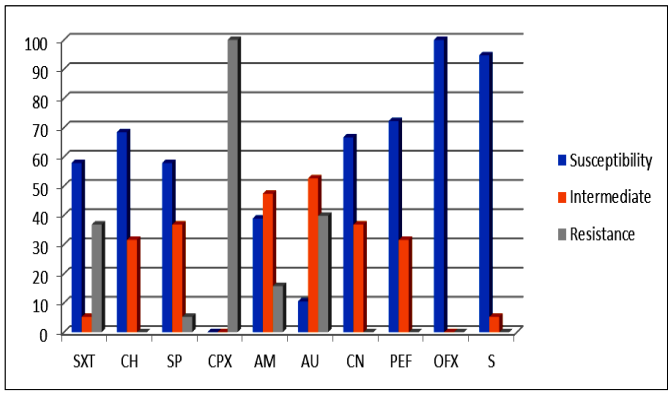

Fig. 5. Percentage susceptibility of gram-negative bacterial isolates to conventional antibiotics. **SXT** = Septrin; **CH** = Chlorophenicol; **SP** = Sparfloxacin; **CPX** = Ciprofloxacin; **AM** = Amoxacillin; **AU** = Augmentin; **CN** = Gentamycin; **OFX** = Tarivid; **PEF** = Perfloxacin.

Fig. 9 depicts the phylogenetic relationship between the sequences of the isolates obtained in this study, i.e., OR343125, OR343126 and OR343127, and those of other isolates of *Klebsiella*, *Enterobacter, Serratia, Escherichia* and *Pseudomonas* obtained from the Genbank database. Nine clades were delineated on the tree. A sequence of *Bacillus cereus* (NR 074540) and *Pseudomonas aeruginosa* (NR 0260780) formed distinct clades (BI value = 0.99 each). The sequence of *K. pneumonia* NGIWBAC5 reported in this study was grouped with those of other isolates (DSM 30104 and AAE) of the same species in a separate clade (BI value = 0.55). One sequence of *K. aerogenes* (NR 102493) constituted another clade with a BI value of 0.76. Singular sequences of strains of *Enterobacter cancerogenus, E. bugandensis* and *Escherichia hermannii* grouped into a separate clade with BI value of 0.62, while those of *Enterobacter amnigena, E. soli* and *Klebsiella oxytoca* formed another clade (BI value = 0.97). The sequence of *S. marcescens* KRED and *E. coli* U 5/41 clustered into a clade (BI value = 0.96) while that of *Enterobacter* sp. NGIWBAC6 obtained in the present study, *E. hormaechei* 0992 -77, *E. mori* YIM Hb-3 and *Enterobacter* sp. Px6-4 is also grouped into another clade (BI value = 0.53). The *E. cloacae* NGIWBAC7 sequence reported in this study clustered separately with those of other strains of the same species (BI value = 1.00), within the *E. cloacae/E. asburiae/ E. ludwigi/E. kobei* clade (BI value = 1.00).

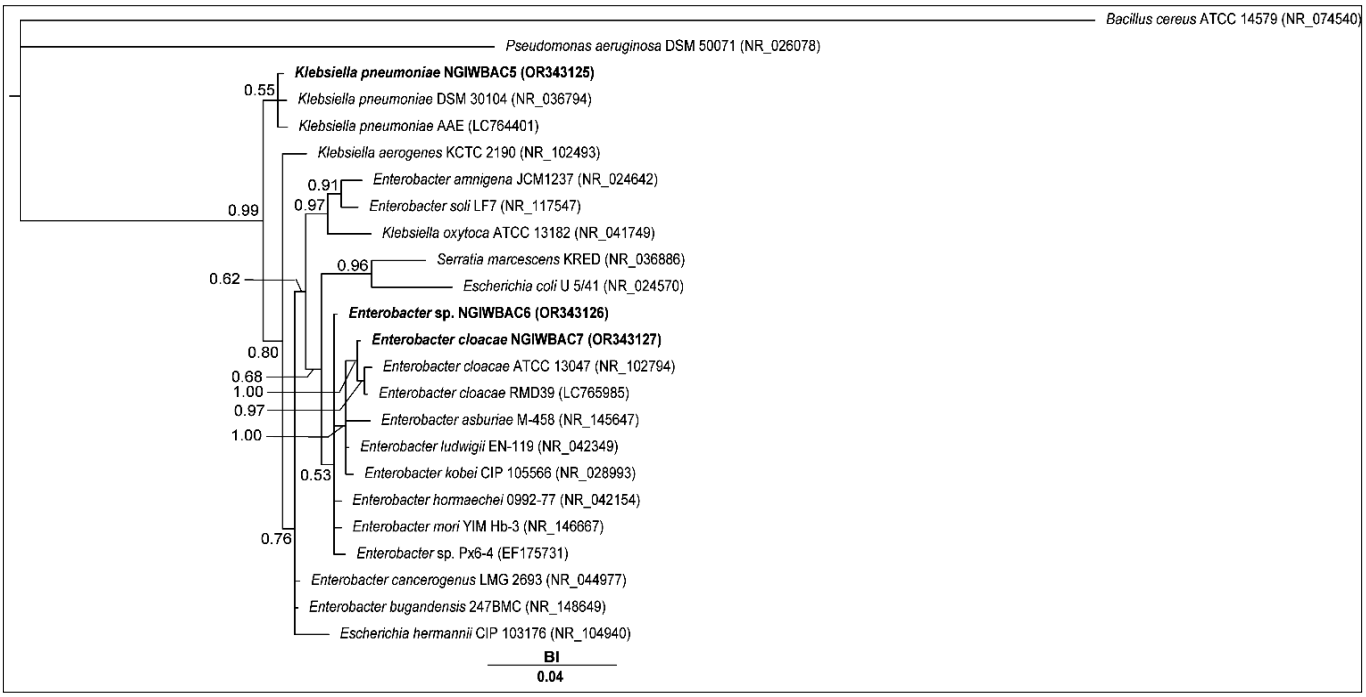

Fig. 6. ITS phylogenetic tree showing the relationship between the bacterial isolates obtained (**in bold**) with similar and other species. Values obtained from the Neighbour-Joining and Bayesian Posterior Probability analysis are presented at the various nodes.

Fig. 7. Healthy tomatoes (**A**) and partially damaged tomatoes (**B**) sampled for the study.

Fig. 8. Zones of inhibition expressed by standard antibiotic discs to spoilage bacteria isolated from partially spoilt tomatoes.

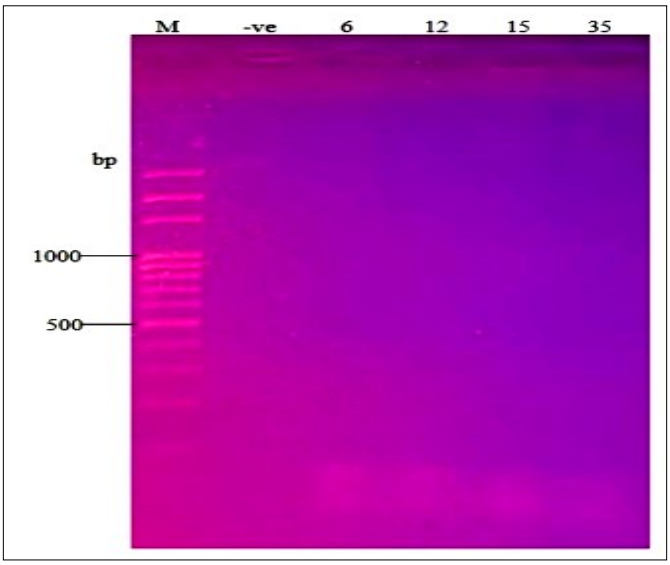

Fig. 9. Amplification of ESBL gene of three bacterial isolates. Isolate 6- *Klebsiella pneumoniae* strain HR16; Isolate 12- *Enterobacter asburiae* strain YT; Isolate 35- *Enterobacter cloacae* strain FC1376.

Discussion

The presence of harmful opportunistic pathogens in commonly consumed foods such as tomatoes is a very real and ever-present public health threat to consumers in local communities of developing nations of the world. The findings from this study showed that the heterotrophic bacteria populations isolated from the assessed partially damaged tomato samples found relatively abundant incidence of *Klebsiella* sp. and *Enterobacter* sp., which is an indication of unhealthy human contact, possibly during transportation of the tomatoes to the markets for sale. Improper handling of tomatoes during market days and unhygienic vehicles being used to convey the products from one market location to another are also implicated in the abundance of pathogenic microbes such as fungi and bacteria on these fruits (24). The bacteria isolated in this study were similar to the one isolated by Oviness et al. (25). This result also agrees with Obeng et al. who isolated *Klebsiella, Enterobacter* and *Citrobacter* as the predominant bacteria associated with spoilt tomatoes (7), . In this study, *Bacillus* sp. had the highest frequency across the 4 locations, which could be probably due to opportunistic contaminations through poor handling processes of tomato fruit. The susceptibility of tomatoes to microbial colonization is due to their differential chemical composition, such as high levels of sugar, low pH (4.9–6.5) and water activity (p>0.99). These characteristics encourage growth of microorganisms in tomatoes, making them a potential source of health risks for humans because they can produce toxins that can cause diseases like gastroenteritis and diarrhoea after consumption (26). Consumption of foods in which large numbers of *Bacillus sp*. have grown can cause severe gastrointestinal-related illnesses.

The antibiotic resistance of the bacteria isolated in this study was determined by subjecting them to conventional antibiotics. The highest resistance of the total gram-positive bacteria can be seen in Ciprofloxacin (10 µg) at 100% resistance and the highest susceptibility was seen in Streptomycin 30 µg with 100%. Conversely, the highest resistance to gram-negative bacteria isolated in this study was seen in Ciprofloxacin (10 µg) with 100% resistance, while the highest susceptibility was observed in Tarivid (10 µg) with 100%, followed by Streptomycin (30 µg) with 94.74%. Antibiotic resistance mostly occurs as a result of misapplication of antibiotics in a given environment; thus, if a particular antibiotic is not frequently misused in an environment, it is quite unlikely for resistance patterns to occur in bacteria isolated from such environments. This implies that the use of antibiotics in local tomato production should be completely discouraged.

Four out of the forty-two bacteria that showed the highest resistance to the conventional antibiotics were amplified and classified by PCR using a specific primer for the 16S rRNA genes in these bacteria. The results of the conventional PCR that detected the 16S rRNA region of the four bacterial isolates were equal to the base pair size of 1500 base pairs, according to this study. *Klebsiella pneumoniae, Enterobacter cloacae* and *Enterobacter asburiae* were specifically identified and confirmed through the molecular characterization methods. In a similar study by Abdulsalam et al. , 16S rRNA and ITS gene markers were used to identify specific genera of spoilage bacteria preponderant on harvested tomato crops in South Africa, including *Klebsiella*, *Acinetobacter*, *Pseudomonas*, *Leuconostoc* and other bacterial groups (27). These results underscore the importance of gene sequencing methods as the more reliable standard for proper bacteria identification compared to conventional culture techniques. Most of the bacterial species presented on the phylogenetic tree may be categorized as opportunistic pathogens due to their ubiquitous existence in the native rhizosphere soil of the crop plant, compost used as soil amendments and sometimes within the host plant itself. The sequences of *Enterobacter* sp. and *K. pneumoniae* were observed to cluster together, indicating the genetic similarity of these species. Additional studies on this will help validate the actual taxonomic status of these species. *Enterobacter* sp. were diverse and appeared to form a species complex. The varieties are morphologically and genetically similar; hence, their observation in the same cluster on the phylogenetic tree. The distinct lineage formed by the *Bacillus cereus* isolate reported in this study might be due to geographical genetic variation. This study also indicated that the genus *Enterobacter* was the most diverse bacterial isolate observed on the spoilt tomatoes obtained from Iwo local markets, which implies its preponderance in the local environment where the tomatoes are commonly grown and sent to the market for local consumption.

Antibiotic resistance genes such as extended-spectrum β-lactamases (ESBLs) are frequently reported among the environmental isolates of the bacterial species isolated from fruits and vegetables (28). Thus, they may serve as crucial environmental reservoirs for the emergence of clinically relevant strains. The ESBL genes in question, though, were not discovered in the bacterial chromosomal genome. This could be a result of the existence of antimicrobial resistance mechanisms other than the enzymatic process. The phenotypic resistance can be caused by another mechanism, such as an efflux pump, or it might be plasmid-borne rather than chromosomal (29). According to Adeleke and Owoseni the presence of plasmids in bacteria confers resistant abilities to several drugs and chemicals intended to inhibit the deleterious effects of bacteria (13). Thus, the plasmids identified in the limited size of antibiotic-resistant bacteria identified in this study may be directly responsible for the ability to repel the effect of the tested general antibiotic spectrum applied to them. It is, however, important to note that resistance genes are not always found on the chromosome alone but are also found expressed on plasmids, which was perhaps responsible for the inability of this study to detect resistant genes among the selected bacterial isolates. This is in part because DNA extraction conducted in this study was from chromosomal and not plasmid elements of the targeted bacterial isolates and the absence of resistance genes on chromosomes does not imply that they may not be found on the bacterial plasmid elements. Thus, future

work will be directed at exploring plasmid-related resistant genes of opportunistic bacteria that occur as natural microflora on the plant surfaces of locally produced and consumed vegetable crops in South West Nigeria.

Conclusion

The preponderance of bacterial isolates identified on partially rotten tomatoes (èsà), which are often frequently consumed in many parts of South-West Nigeria, is a noteworthy factor responsible for food-borne infections associated with the consumption of tomatoes and has great implications on the public health status of the human communities in this region of the country. The conventional antibiotic treatments assessed in this study were observed to exhibit notable antibacterial effects on the test isolates. Therefore, it is recommended that simple hygienic practices such as thorough washing of tomatoes to reduce fruit microbial load should be regularly carried out, with proper cooking of tomatoes to eliminate possible pathogenic organisms and, where possible, complete avoidance of the usual practice of consuming partially damaged tomatoes and these measures will surely contribute to reducing foodborne infection related problems in the country.

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Authors' contributions

OEO conceived the study, participated in its design and coordination and drafted the manuscript. OBF carried out the microbiological studies, participated in the sequence alignment and drafted the original manuscript. OTA participated in the design of the study, participated in the sequence alignment and performed microbiological studies. OGO carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

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

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