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RESEARCH ARTICLE OPEN ACCESS

Characterisation of multi-drug resistance and virulence genes from Gram-negative bacterial isolates from street-vended fruits

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Abstract
Objectives: To determine the prevalence and characterise multidrug resistance and virulence genes from Gram-negative bacteria isolates from street-vended fruits and salads.
Method: Street-vended, ready-to-eat (RTE) fruits were sampled from five popular markets and locations (Watt, Marian, Edim-Otop, Highway, and Goldie/Etta Agbor), from which a total of 60 RTE fruit and 30 salad samples were collected. Microbiological analysis was conducted using standard protocols. The multidrug-resistant (MDR) isolates (n = 12) were profiled for the SHV, CTX-M, and IcaC genes.
Results: The identified MDR isolates were *Escherichia coli*, *Klebsiella pneumoniae (n = 2),* Morganella *morganii (n = 6), Enterobacter cloacae, Acinetobacter baumannii,* and *Proteus mirabilis*. A total of five (5) were positive for the IcaC and the SHV genes, while six (6) were positive for the CTX-M genes. Half (n = 3) of the *M. morganii* showed the presence of all three genes.
Conclusion: With bacteria's ability to spread resistance genes among themselves, resulting in worse clinical outcomes of infection they cause, there is an urgent need for studies that holistically address all the lacunae, especially their sources in fruits, and ways to mitigate their spread, given the enormous public health implications of these genes in these pathogens.

Keywords: Beta-lactamase, Virulence genes, Gram-negative, Antibiotic resistance, Fruits

Plain English Summary

The study was to evaluate the prevalence and diversity of bacteria with multidrug resistance and virulence genes isolated from RTE fruits and salads. The results revealed the presence of various Gram-negative bacteria commonly implicated in hospital- and community-acquired infections. Furthermore, all the isolates exhibited multidrug resistance to antibiotics and harboured various virulence genes.

Background

It is estimated that by 2050, Nigeria's population will reach 400 million (1). Out of this number, a policy report states that at least 40 million Nigerians are 'middle class" (2). This middle-class population is fully aware of the nutritional

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benefits of consuming fresh fruits (3, 4). The perceived benefits of consuming fresh fruits have led to an increase in the number of people consuming ready-to-eat (RTE) fruits and vegetable salads across major cities in Nigeria over the last decade (5, 6). Sadly, the consumption of RTE fruits and salads, though beneficial to humans (7, 8), is also a common route for food-borne illnesses globally (9). A report by the World Health Organisation (WHO) (10) proposed that an estimated 600 million people fall ill, 420,000 lives are lost each year, and USD 110 billion is lost annually in terms of productivity to illness caused by food-borne pathogens. Several studies have revealed the frequency of Gram-negative food-borne microorganisms in Nigeria (6, 11, 12, 13). In an earlier study, Oyedele *et al.* (6) used molecular characterisation to identify 11 species within six genera: Citrobacter, Enterobacter, Escherichia, Klebsiella, Pantoea, and Shigella. These species were found in RTE fruits sold in Lagos State, Nigeria. One important source of these potential pathogens in fruits is the handlers or sellers of the fruits and salads (14, 15). In addition to the food-borne diseases caused by these pathogens, they possess an alarmingly high rate of multidrug-resistant genes, especially genes that confer resistance to beta-lactam antibiotics (6, 16, 17).

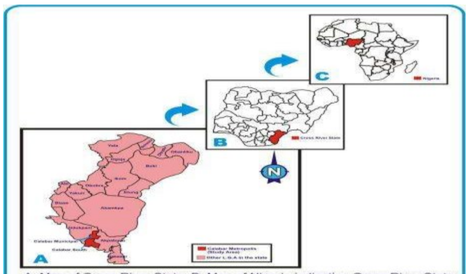
The beta-lactam antibiotics, exemplified by the penicillins, carbapenems, cephalosporins, and monobactams, are the most prescribed antibacterial agents, with the cephalosporins taking the lead (18). These antibiotics are widely prescribed and used because they are well-tolerated and effective. However, their efficacy is increasingly threatened by the emergence and spread of beta-lactamase-producing bacteria, which confer resistance to the beta-lactam antibiotics by beta-lactamase enzymes, including but not limited to SHV and CTX-M, among others. Once limited to clinical isolates, beta-lactam-resistant (multidrug-resistant [MDR]) bacteria have now spread to other environments, including fruits sold by street vendors in major cities worldwide (6, 18, 19, 20, 21). The transfer of resistance from environmental to clinical settings (22, 23) carries significant public health implications, potentially worsening clinical outcomes for patients (24, 25).

The consumption of fruits contaminated with potential pathogens possessing multidrug resistance (MDR) genes represents a significant public health concern (11, 13, 17, 20). Even more concerning is the poor handling of these fruits and the lack of hygiene practices among vendors, which remain unregulated at both the state and national levels in Nigeria (1, 3, 5). On virtually every street corner in various Nigerian cities, there are fruit vendors, which is probably due to the low capital required to establish such businesses (5, 6, 13, 15). It is, therefore, imperative to profile the various beta-lactamase genes that the potential pathogens harbour to plan any future intervention to control their spread. Several studies have profiled bacterial isolates in fruits using cultural techniques (6, 21, 26). However, there is a dearth of studies utilising molecular techniques to characterise resistance genes to beta-lactam antibiotics among the bacterial isolates from fruits (6). Similar studies have been conducted in some parts of Nigeria, but not in Cross River State, Nigeria. Thus, this study aimed to evaluate the virulence genes and beta-lactamase genes elaborated by Gram-negative bacilli from street-vended fruits and salads.

Materials and Methods

Study area

The study was conducted in Calabar, Cross River State, Nigeria. It is in the southern part of Cross River State and lies at latitudes 4˚50'N and 5˚10'N and longitudes 8˚17'E and 8˚20'E (Figure 1). It is bordered to the north and east by Odukpani and Akpabuyo Local Government Areas of the State (27). Its annual rainfall is estimated to be 2750 mm, with a total land mass of 274.593 km^2. It possesses abundant fertile soil that promotes the growth of fruits and luxuriant forests. The city is also home to numerous tourist attractions (28). The capital has experienced a significant population increase, rising from 10,000 during the colonial era to 371,022 as of the 2006 national census (29). The city has several densely populated areas whose markets were sampled in this study.



A: Map of Cross River State; B: Map of Nigeria indicating Cross River State; C: Map of Africa indicating Nigeria

Figure 1: Map showing the study site (30).

Study design

For this study, a cross-sectional study design was used. Cross-sectional studies are essential for assessing the prevalence of diseases or any health outcome of interest (31). Thus, we evaluated fruit samples and their corresponding salads from some locations/markets in the state capital. The sample size was determined based on previous reports (32, 33).

Sample collection

Ninety (90) fruits and their salad samples were collected from five purposively selected locations/markets (Watt, Marian, Goldie/Etta Agbor, Edim-Otop, and Highway (Mobil) in Calabar due to the high number of vendors. From these locations, 15 vendors were randomly selected for the collection of the samples. The samples were aseptically collected using sterile plastic containers. The collected fruits were pineapple (n = 15), pawpaw (n = 15), cucumber (n = 15), banana (n = 15), and watermelon (n = 15), in addition to the vegetable salads (which included cabbage, carrot, cucumber, and lettuce) (n = 15). The collected samples were transported on ice within 1 hour of collection to the laboratory for further analysis.

Sample preparation

The collected samples were first allowed to attain room temperature before being processed further (34). The same kinds of fruits from the same market were first grouped. Thereafter, they were mechanically macerated using a sterile pestle and mortar to obtain a uniform mixture for each type of fruit and vegetable salad for each location/market. From these, composite samples were obtained for each fruit type and location/market.

Microbiological analyses

From each macerated composite sample, a 100-fold serial dilution was carried out, as previously reported (34). From the second dilution, inoculation was carried out via pour plating onto freshly prepared nutrient agar and *Salmonella-Shigella* agar plates in triplicate for each sample type. The plates were then incubated overnight in an inverted position at 37 °C. After overnight incubation, discrete colonies were sub-cultured on nutrient agar twice to purify them. The purified isolates were then stored in sterile agar slants in Bijou bottles until required (35). Initially, the bacterial isolates were identified based on their colony morphology and pigmentation; subsequently, Gram staining was performed. The Gram-negative isolates were further screened using a battery of biochemical tests, including catalase, oxidase, citrate, methyl red, indole, motility, fermentation/gas production, and urease (36, 37, 38). The tentatively identified isolates were subjected to an antimicrobial susceptibility test using the Clinical Laboratory Standard Institute's (CLSI) disc diffusion protocol (39, 40). The culture was first reconstituted by growing them on freshly prepared nutrient agar plates overnight at 37 °C. The isolates were inoculated in normal saline and then mixed gently to obtain a homogenous suspension. The concentration was adjusted to the McFarland standard (0.5) using a turbidometer. A total of ten different (10) antibiotic discs (Oxoid) were used for the susceptibility test. These were four (4) quinolone antibiotics (ofloxacin (5µg), pefloxacin (5µg), ciprofloxacin (5µg), and nalidixic acid (30µg), three (3) beta-lactam antibiotics (amoxicillin/clavulanate (30µg), cephalexin (10µg), and ampicillin (30µg). The rest were two (2) aminoglycosides (streptomycin (30µg) and gentamicin (10µg) and one (1) folate pathway antagonist (sulphamethoxazole/trimethoprim (30µg)). Using a sterile bent-glass spreader, 0.1 ml of each inoculum from the McFarland standard stock was evenly distributed onto the surface of freshly prepared Mueller-Hinton Agar (MHA) and allowed to stand for 15 minutes before placing the antibiotic discs. The plates were then incubated overnight at 37 °C. The observed zones of inhibition were recorded and interpreted following the CLSI guidelines (39) as sensitive (S), intermediate (I), and resistant (R).

Determination of antibiotic resistance indices

Following the antimicrobial susceptibility test, antibiotic resistance indices, including multiple antimicrobial resistance (MAR) and multidrug resistance (MDR), were computed. These are defined below and were done as previously reported (41). The MAR was calculated by dividing the number of antimicrobial agents to which the isolates showed resistance by the number of antibiotics used in the susceptibility test against that particular isolate. MDR was interpreted as an isolate that is not susceptible to ≥1 agent in ≥3 antimicrobial categories.

Extraction of DNA from the MDR isolates

The MDR isolates were further identified using molecular techniques. The purified isolates were first reconstituted by subculturing them onto freshly prepared nutrient agar and incubated overnight at 37 °C. The resulting pure isolates had their DNA extracted using the Zymo Fungal/Bacterial DNA Extraction Kit (Zymo, USA). The extraction was done according to the manufacturer's recommended protocol. The extracted DNA was quantified using the Nanodrop 1000 spectrophotometer. The extracted DNA was stored at 4^{o}C until further use.

Amplification of the 16S rRNA

The extracted DNA from each of the MDR isolates had their 16s RNA amplified using the 27F: 5'-AGAGTTTGATCTGGCTCAG-3 and 1492R: 5'-CGGTTACCTTGTTACGACTT-3 primer pair on an Applied Biosystems ABI 9700 thermal cycler with a final volume of 40 microliters and 35 cycles (42). The X2 Dream Taq Master mix, which consisted of a Taq polymerase, DNTPs and $MgCl_2$, was used in the amplification together with the primer pair at a concentration of 0.5 microlitre, and the extracted DNA as template. The polymerase chain reaction (PCR) conditions for 35 cycles were set as follows: an initial denaturation (95°C for 5 minutes), followed by subsequent denaturation at 95°C for 30 seconds, annealing at 52°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 5 minutes. The result was visualised on a blue light transilluminator after being resolved on a 1% agarose gel at 130V for 30 minutes (43). The amplified DNA was stored for further use.

Sequencing and identification of isolates

The amplicons were sequenced using a 3510 ABI sequencer and the BigDye Terminator kit. The BigDye® terminator v1.1/v3.1, 0.25 µL of the 10 µM PCR primer, 2.25 µL of the 5x BigDye sequencing buffer, and 2-10ng of the PCR template per 100bp were used in the sequencing, which was done at a final volume of 10 µL. There were 32 cycles of 96°C for 10 seconds, 55°C for 5 seconds, and 60°C for 4 minutes in the sequencing conditions. The sequence of the MDR isolates obtained from Sanger sequences was first edited using the bioinformatics tool TraceEdit and the Plasmid Editor (APE) tools to remove non-nucleotide sequences. The cleaned sequences, one after the other, were then loaded onto the Basic Local Alignment Search Tool (BLAST) hosted at the National Centre for Biotechnology Information (NCBI) and identified by comparing them with the closest sequence in terms of similarity (> 99%). In addition, the sequences of similar isolates previously deposited on NCBI and assigned accession numbers were retrieved in FASTA format, as previously described (44), and used for phylogenetic tree analysis. The deposited sequences were assigned accession numbers (PQ357274.1 to PQ357280.1).

Phylogenetic Analysis

Identical sequences were labelled after retrieval from the NCBI database and used together with our MDR sequences for the construction of a neighbour-joining phylogenetic tree. Multiple alignment was performed using the MAFFT (Multiple Alignment using Fast Fourier Transform) tool version 7. Following multiple alignment, the sequences were trimmed at both ends to have an even set of sequences in terms of length. The evolutionary history was inferred using the Neighbour-Joining method in Molecular Evolutionary Genetics Analysis (MEGA) version 6.0 (45). The genus of the organisms under study was assumed to be represented by the bootstrap consensus tree generated from 500 replicates (46). The Jukes-Cantor technique was employed to assess phylogenetic differences (47).

Extended-spectrum beta-lactamases detection

From the MDR isolates, the CTX-M, IcaC, and SHV genes were amplified. The CTX-M genes from the isolates were amplified using the CTX-M **F**: 5'-CGCTTTGCGATGTGCAG-3' and CTX-M **R**: 5'-ACCGCGATATCGTTGGT-3' primers. While for the IcaC and SHV genes, the primer set was F: 5'-ATGAGTATTCAACATTTCCGTG-3' and IcaC R: 5'-TTACCAATGCTTAATCAGTGAG-3', and SHV gene F: 5'-CGCCTGTGTATTATCTCCCT-3' and SHV R: 5'-CGAGTAGTCCACCAGATCCT-3 3', respectively. The primer sets were amplified using an Applied Biosystems thermal cycler at a final volume of 40 microliters for 35 cycles. For all the genes, the X2 Dream Taq Master mix, which comprised Taq polymerase, dNTPs, and $MgCl_2$, along with primers at a concentration of 0.4 microlitres and 50 ng of the extracted DNA, was used. The polymerase chain reaction (PCR) conditions for 35 cycles were set as follows: an initial denaturation (95°C for 5 minutes), followed by subsequent denaturation at 95°C for 30 seconds, annealing at 52°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 5 minutes. This was done as previously reported, but with minor modifications (48). The result was visualised on a UV transilluminator after being resolved on a 1% agarose gel at 120V for 25 minutes.

Statistical analysis

The data collected were collated and analysed using simple descriptive statistics (percentages, charts, and mean) on Microsoft Excel version 2013.

Results

Microbial isolates identified from the study

As summarised in Table 1, a total of 40 microbial isolates were isolated from all samples. The isolates were *Acinetobacter baumanii, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterobacter cloacae,* and *Morganella morganii.* The percentage occurrence of the isolates indicates that *M. morganii* was the most common, accounting for 55.0% of all isolates (22/40). *K. pneumoniae* followed this with 17.5%

(7/40) of all isolates. The least abundant isolate was *E. cloacae,* with a prevalence of 2.5% (1/40).

Table 1: Percentage occurrence of the isolates and samples

Isolates	Fruits (%)	Salad (%)
Acinetobacter baumanii	2(6.25)	1 (12.50)
Escherichia coli	1 (3.13)	2 (25.00)
Klebsiella pneumoniae	6 (18.75)	1 (12.50)
Proteus mirabilis	3 (9.38)	1 (12.50)
Enterobacter cloacae	0 (0.00)	1 (12.50)
Morganella morganii	20 (62.5)	2 (25.00)
Total	**32 (100)**	**8(100)**

Antibiotic resistance of the isolates

From Table 2, it was observed that the isolates exhibited varying degrees of resistance to the different antibiotics used. *Acinetobacter baumanii* and *P. mirabilis* showed 100% resistance to ciprofloxacin, nalidixic acid, and sulphamethoxazole/trimethoprim. This was followed by *P. mirabilis,* which showed 75% resistance to ofloxacin, pefloxacin, ciprofloxacin, cephalexin, and ampicillin. *K. pneumoniae* was 71.4% resistant to pefloxacine. *E. coli showed* 66.7% resistance to ciprofloxacin, nalidixic acid, and ampicillin. *M. morganii* showed 63.6% resistance to pefloxacine and nalidixic acid. The lowest percentage was observed in *E. cloacae,* which showed no resistance to all the antibiotics used (0%).

Table 2: Antimicrobial resistance profile of the isolates

Antibiotic	***A. buamanii*** **(n=3) (%)**	***E. coli*** **(n=3) (%)**	***K. pneumoniae*** **(n=7) (%)**	***P. mirabilis*** **(n=4) (%)**	***E. cloacae*** **(n=1) (%)**	***M. morganii*** **(n=22) (%)**
OFX	1 (33.3)	0 (0.00)	3 (42.9)	3 (75.0)	0 (0.00)	12 (54.4)
PEF	1 (33.3)	1 (33.3)	5 (71.4)	3 (75.0)	0 (0.00)	14 (63.6)
CPX	3 (100.0)	2 (66.7)	4 (57.1)	3 (75.0)	0 (0.00)	10 (45.6)
NA	2 (66.7)	2 (66.7)	3 (42.9)	4 (100.0)	0 (0.00)	14 (63.6)
AU	1 (33.3)	0 (0.00)	4 (57.1)	2 (50.0)	0 (0.00)	9 (40.9)
CEP	1 (33.3)	0 (0.00)	2 (28.6)	3 (75.0)	0 (0.00)	11 (50.0)
PN	2 (66.7)	2 (66.7)	3 (42.9)	3 (75.0)	0 (0.00)	12 (54.4)
CN	0 (0.00)	1 (33.3)	3 (42.9)	0 (0.00)	0 (0.00)	8 (36.4)
S	1 (33.3)	0 (0.00)	2 (28.6)	1 (25.0)	0 (0.00)	8 (36.4)
SXT	1 (100.0)	0 (0.00)	2 (28.6)	0 (0.00)	0 (0.00)	10 (45.6)

Key: OFX = Ofloxacin; PEF = Perfloxacin; CPX = Ciprofloxacin; NA = Nalidixic acid; AU = Amoxicillin/Clavulanate; PN = Ampicillin; CN = Gentamicin; S = Streptomycin and SXT = Sulphamethoxazole/trimethoprim.

Molecular characterisation of the multi-drug resistant (MDR) isolates and their virulence genes

Following susceptibility testing, a total of 12 multidrug-resistant isolates that showed absolute resistance were selected for molecular characterisation. The amplicons were loaded in lanes 1 to 12, while the ladder (1500bp) lane L displayed the respective band sizes (Figure 2). The resulting sequences were identified using the Megablast tool to find extremely comparable sequences in the non-redundant nucleotide (nr/nt) database of the National Centre for Biotechnology (NCBI) to identify the sequenced amplicons. The isolates' 16S rRNA sequences displayed 100% similarity to those of other species (Figure 3). Furthermore, their virulence genes IcaC, CTX-M, and SHV were also evaluated (Figures 4, 5, and 6). Figure 4 shows the bands (1-5 and 7-12) for the various isolates evaluated for the IcaC gene. The result indicates the presence of IcaC genes at 600 bp in lanes 1, 5, and 7-11, implying that a total of 7 isolates possessed the IcaC gene. The isolates were *E. coli*, *E. cloacae, M. morganii* (n = 3), *P. mirabilis,* and *K. pneumoniae.* Figure 5 shows the result for the SHV gene at 200bp. Only four isolates, *K. pneumoniae, A. baumannii*, *M. morganii,* and *P. mirabilis,* were positive for the SHV gene. The CTX-M evaluation presented in Figure 6 indicates that a total of five isolates were positive for the CTX-M gene, comprising *E. coli*, *K. pneumoniae*, *M. morganii* (n = 2), and *P. mirabilis.*

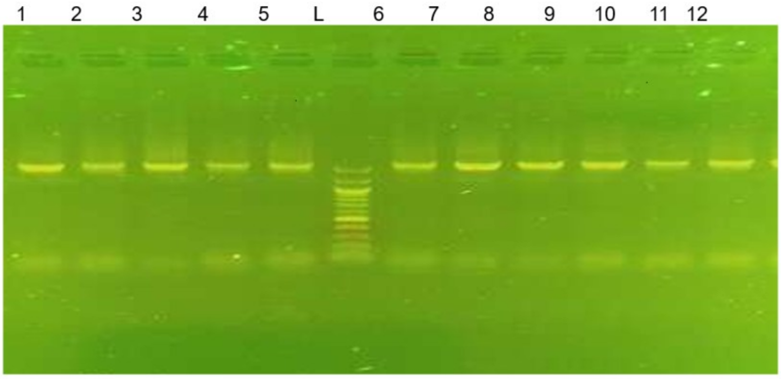


Figure 2: Gel electrophoresis showing the 16S rRNA bands.
Key: 1 = *E. coli*, 2= *K. pneumoniae*, 3, 4, 7, 10, 11 and 12 = *M. morganii*; 5 = *E. cloacae;* 6 = *A. baumanii*; 8 = *P. mirabilis*; 9 = *K. pneumoniae*; L =Molecular ladder marker (1500bp)

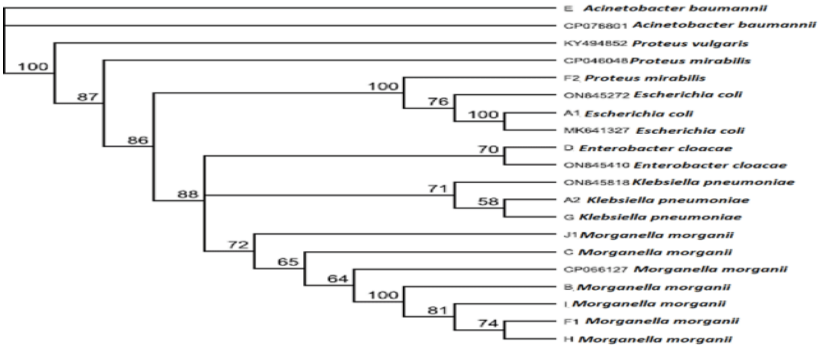


Figure 3: Phylogenetic tree showing the evolutionary distance between the bacterial isolates

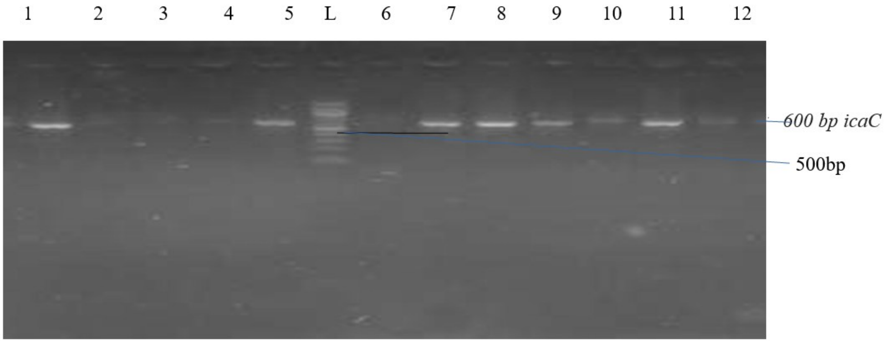


Figure 4: Gel electrophoresis showing the amplified IcaC bands.
Key: Lane 1 = *E. coli;* 5 = *E. cloacae;* 7, 10 and 11 = *M. morganii* 8 = *P. mirabilis* 9 = *K. pneumoniae;* L = IcaC gene marker.

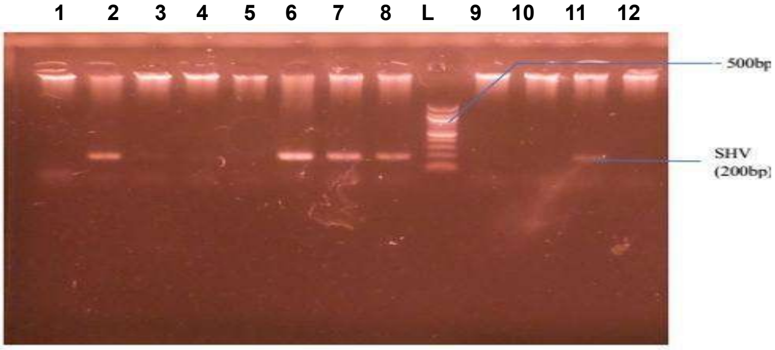


Figure 5: Gel image of the amplified SHV gene.
Key: Lanes 2 (*K. pneumoniae*), 6-8 (*A. baumanii*, *M. morganii,* and *P. mirabilis*) and 11 returned bands at 200bp, while lane L represent the 1000bp ladder.

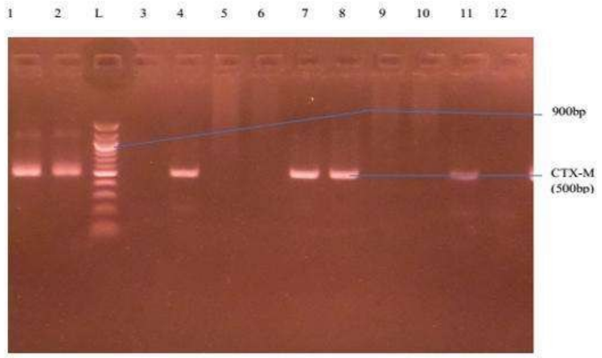


Figure 6: Gel image showing the amplified CTX-M gene.
Key: Band 1 = *E. coli*; Band 2 = *K. pneumoniae*, bands 4 and 7 = *M. morganii*; Band 8 = *P. mirabilis*.

Discussion

Microbial load in the various samples

A total of 40 species, distributed among six genera, were obtained, and the abundance of the isolates varied in the following order: *M. morganii* > *K. pneumoniae* > *P. mirabilis* > *A. baumannii* > *E. coli* in the fruits. For the salad, the abundance of *E. coli* and *M. morganii* was similar but higher than that of the others. Oyedele *et al.* [6] reported that *K. pneumoniae* was the most prevalent isolate in their study. In another study, Razzaq *et al.* (49) reported the presence of enteric and non-enteric pathogens, including *S. aureus, Pseudomonas aeruginosa, E. coli, Enterobacter* spp., and *Salmonella* sp., in the uncooked vegetable samples examined. Another study reported *S. aureus* as the most frequent isolate (40%), followed by *E. coli* (33.3%) and *Salmonella* spp. (50). Isolates obtained by Jabin *et al.* (51) were similar to ours, except for the detection of *Salmonella* spp. and *Vibrio* spp. in their study. Similarly, Lucky *et al.* (52) reported *Salmonella* spp. and *Shigella* spp. as the least isolated species, while *S. aureus* and *Pseudomonas* spp. were the most isolated species in their study. These differences could be a result of the diversity of organisms colonising the various fruits or samples used in the different studies. The presence of non-enteric pathogens in their study (49, 50) further lends credence to the contamination of fruits and vegetables via soil (*P. aeruginosa*) and personal hygiene (*S. aureus*). The pathogenic potential of these organisms was demonstrated in a study that traced cases of dysentery leading to hospitalisation to the consumption of fruits contaminated with food-borne bacteria, such as *E. coli, K. pneumoniae,* and *S. flexneri* (53). Disease outbreaks have also been reported in various cities, including the United States, between 2002 and 2011, as noted in a report by the Centre for Science in the Public Interest (54) and a 10-year survey (55) in Europe, which attributed these outbreaks to pathogens in food.

Antimicrobial profiles of the isolates

According to Chelaghma *et al.* (56) and the WHO (57), resistance to antimicrobial agents is among the top ten dangers to public health globally. Our results indicate that a total of 20 isolates were MDR, representing 50.0% (n = 40). Several studies have corroborated this prevalence of MDR isolates from fruit samples. Dallal *et al.* (58) indicated the occurrence of eight (8) *E. coli* isolates from 21 that were resistant to ceftazidime and cefotaxime, and another six isolates that were ESBL-producers. Morris and Cerceo (59) identified *E. coli, P. aeruginosa, K. pneumoniae, and A. baumannii* as having the highest rates of resistance to the antibiotics utilised in their study. Oyedele *et al.* (6) reported that 7% of their isolates, which included *E. coli, E. hormaechei, E. sichuanensis,* and *S. flexneri,* showed resistance. Compared to our findings, all our isolates from the fruit samples showed a higher MDR than that of Oyedele *et al.* (6). In another study, varying rates of resistance were observed among their test isolates to various antibiotics (55), as was also observed in our study. El-Sayed isolated resistant *Enterococcus faecalis* and *S* (60). *aureus* from various fruits and fish samples. Although neither isolate was obtained in our study, the isolation of these pathogens elsewhere supports the fact that fruits are a common route of transmission for pathogens to humans (9).

In contrast, a report by Parastan *et al.* (61) highlighted the susceptibility of some isolates in their study to certain antibiotics commonly used in clinical settings. A relatively lower resistance to imipenem and gentamicin was observed in *E. coli, Klebsiella* spp., *Salmonella* spp., *Pseudomonas* spp., and *Vibrio* spp. was also observed by Sarwar *et al.* (62) in street-vended foods from Bangladesh. Despite these reports from Parastan *et al.* (60) and Sarwar *et al.* (62), the prevalence of resistance observed in our study and previous studies (6, 58, 59) is a cause for concern. These differences may be a result of variations in the antimicrobial genes they express, as well as the physiological states of the organisms (63). These AMR observations are a cause for concern as microbes continue to evolve mechanisms to stay resistant to antibiotics, and there is widespread abuse of antibiotics in the population (12, 64).

Molecular identification of their virulence genes

The isolates (n = 12) obtained in this study, identified by 16S rRNA gene sequences, were *E. coli, K. pneumoniae, M. morganii* (n = 6), *E. cloacae, A. baumannii, P. mirabilis,* and *K. pneumoniae.* The isolates were further evaluated for the presence of IcaC, SHV, and CTX-M genes. Our results indicate that 7/12, 5/12 and 6/12 isolates had positive bands for the IcaC, SHV, and CTX-M genes, respectively. This was corroborated by Chelaghma *et al.* (56), who, in their review, reported an alarming rate of extended-spectrum lactamases, cephalosporinases, carbapenemases, and mcr genes in Gram-negative bacteria in vegetables and fruits. Several genes and proteins have been implicated in bacterial pathogenesis, and one of such genes is the gene that occupies the intercellular adhesion (ICA) locus that is responsible for the synthesis of the polysaccharide intercellular adhesion (PIA), a well-known exopolysaccharide in *S. aureus* (61, 65, 66, 67). Others include the icaADBC (biosynthetic) and icaR (regulatory) genes, which are found not only in *S. aureus* but also in all pathogens. The icaADBC genes have all been linked to bacterial pathogenesis as they aid phagocytosis evasion (48). This position implies that the seven isolates positive for the IcaC gene are potential pathogens that can evade the immune system. The study by Mirzaei *et al.* (48) reported that IcaC was the most abundant gene among biofilm-forming *Staphylococcus epidermidis* compared to icaADBC. Parastan *et al.* (61) reported the presence of the icaADBC genes, establishing a causal relationship between these genes and antimicrobial resistance.

The CXT-M and SHV genes have been linked to resistance to the beta-lactamase group of antibiotics via the expression and/or uptake of extended-spectrum beta-lactamase genes (6). Isolates in our study showed the presence of both genes. In their study, Silveira *et al.* (68) reported the presence of blaTEM, blaKPC, and blaCTX-M genes among their study clones from fruit isolates. Montero *et al.* (69) Results indicate that 58% (96) of their isolates were positive for ESBL, with over 50% coming from irrigation water, 11 from vegetables, and 30% from fruits. These findings were further corroborated by Irrgang *et al.* (70), who reported the presence of the blaCTX-M-15 gene in 5.12% (n = 21) of the isolated *E. coli* species in their study. Dallal *et al.* (58) investigated the presence of TEM (*bla*TEM) and SHV beta-lactamase (*bla*SHV) genes in *E. coli* isolated from strawberry samples in Sanandaj, Iran. The differences in the percentages of expressed genes in our study and other studies could be attributed to variations in the abundance of the genes expressed, sources, and concentrations of antibiotics used in the various studies. Our study was limited by the fact that we did not sample all the fruit vendors or all the fruits and salads sold in the locations surveyed. Furthermore, not all markets in the study area of Calabar were included in the study. Despite these limitations, our findings indicate that the MDR isolates obtained from street-vended fruits expressed virulence genes that collectively pose a significant public health challenge, which could worsen clinical outcomes in patients in both community and hospital settings.

Conclusion

This study's findings indicate that ready-to-eat fruit and vegetable salads are often contaminated with pathogenic enteric bacteria. This risk is further heightened by the fact that about half of these isolates were MDR isolates that possessed or elaborated several multiple antibiotic-resistant genes. Specifically, approximately half of the isolates expressed the *IcaC*, *SHV*, and *CTX-M* virulence genes, which have been associated with antimicrobial resistance, biofilm formation, and evasion of the human immune system. Collectively, these genes pose a serious public health concern, as organisms harbouring them have been linked to both community- and hospital-acquired infections. There is a need for routine surveillance of street-vended fruits for MDR organisms, especially those expressing virulence genes.

List of Abbreviations

CLSI:	Clinical Laboratory Standard Institute
MAR:	Multiple Antibiotic Resistance
MDR:	Multi-Drug Resistance
MHA:	Muller Hinton Agar
RTE:	Ready-to-eat
USD:	United States Dollar
WHO:	World Health Organisation

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for Publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution-Non-Commercial 4.0 license.

Availability of Data
All the data generated are within the body of the manuscript.

Competing interests
The authors declare that there are no primary and secondary competing interests.

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Authors' contributions
Conceptualisation: BGP and IEO. Methodology: BGP, UJC, UOE, IEO, UNO, AMO, EAAO, and OOM. Supervision: UOE, BGP and IEO. Manuscript draft writing: BGP and UOE. Review and Approval of Draft: All the authors reviewed, approved the publication of the manuscript.

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