FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 10 No. 24, December, 2026, pp 378 – 386
DOI: https://doi.org/10.33003/fjs-2026-1024-6576

Antibiogram and Virulence Gene Profiling of Fried Ready-To-Eat Food Borne Bacterial Isolates

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Received: 16 October 2026
Accepted: 15 November 2026
Published: 19 November 2026

ABSTRACT
Street-vended ready-to-eat (RTE) foods pose notable public health risks due to microbial contamination and antimicrobial resistance. This follow study evaluated the phenotypic virulence attributes, antimicrobial susceptibility profiles, and virulence gene markers of bacterial strains isolated from 288 RTE foods collected across Edo and Delta States in Southern Nigeria. Standard cultural techniques, phenotypic assays (hemolysis, protease activity, biofilm formation), and PCR analyses were performed. Results revealed that all tested isolates (*Escherichia coli* strains 1 and 2, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Bacillus cereus*, and *Staphylococcus aureus* strains 1 and 2) were biofilm producers, with widespread protease and hemolytic activities. Multiple antibiotic resistance (MAR) indices ranged from 0.2 to 0.7, with high resistance observed against amoxycillin, clindamycin, cefuroxime, erythromycin, and tetracycline. Molecular screening confirmed the presence of several resistance and virulence determinants, including *bla*$_{CMY-2}$, *tetA*, *mecC*, *aac(3)-II*, *Hb1A*, and *hlyA*. These findings underscore the severe public health hazard associated with the anthropogenic consumption of contaminated street-vended RTE foods acting as reservoirs for multi-drug resistant pathogens harboring critical virulence genes.

Keywords: Antibiogram Profile, Biofilm Formation, Multiple Antibiotic Resistance (MAR), Ready-To-Eat (RTE) Foods, Virulence Genes

INTRODUCTION

The public health burden associated with the occurrence of foodborne infections/diseases has remained high in various regions across the world especially the middle- and low-income countries (Grace, 2023). In several cases, it is difficult to connect illness or death to contaminated food sources. Across Nigeria, several important foodborne pathogens such as; *Escherichia coli* O157:H7, *Salmonella, Vibrio, Shigella. Staphylococcus aureus* and *Clostridium* species have been documented (Ifeadike *et al*., 2012). Strains of these pathogens have been detected in ready-to-eat foods, vegetables and other street vended foods commonly sold in cafeterias, markets, schools, fast-food outlets and urban centres (Beshiru *et al*., 2020; Igbinosa *et al*., 2020; Igbinosa *et al.,* 2021; Okanlawon *et al.*, 2026). Among these pathogens, diarrheagenic *E. coli* (DEC) has been and is widely linked with gastrointestinal infections associated with contaminated ready-to-eat foods. DEC strains are grouped into six pathotypes, including enterotoxigenic *E. coli* (ETEC), Shiga toxin-producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and enteroinvasive *E. coli* (EIEC) (Lima *et al*., 2017). Each group possesses distinct virulence genes which are responsible for development of diseases in humans (Lima *et al*., 2017).

Pathogenic *E. coli* pathotypes (STEC, ETEC, and EPEC) are known to utilize specific virulence genes (*stx*, *lt*, *st*, *bfpA*, and *eae*) to cause serious gastrointestinal infections, especially among children (Canizalez-Roman *et al.*, 2013). Human exposure mainly occurs *via* foodborne routes, where post-processing contamination frequently arises from environmental persistence despite prior thermal treatment (Beshiru *et al*., 2022).

Weak regulatory enforcement and poor vendor hygiene practices in Nigeria frequently expose consumers to ready-to-eat (RTE) foodborne infections characterized by headache, vomiting, diarrhea, and stomach pain (Beshiru *et al*., 2022; Adeosun *et al*., 2023). Furthermore, RTE foods are vulnerable to contamination by drug-resistant strains originating from raw agricultural materials (Holvoet *et al.,* 2013). Processing facilities are known to act as microbial reservoirs supported by protective surface biofilms (Gomes-Neves *et al.,* 2012), while handling, packaging and distribution promote cross-contamination (Guerini *et al.,* 2017), ultimately culminating in gastrointestinal tract colonization and infection in susceptible consumers (Chen *et al*., 2017).

From the foregoing, several issues such as prevalence of foodborne pathogens in different food types consumed in Nigeria, contamination routes and environmental vectors coupled with hygiene and food processing facilities serving as microbial reservoirs supported by protective surface biofilms have been investigated by several researchers (Gomes-Neves *et al.,* 2012; Holvoet *et al*., 2013; Guerini *et al*., 2017; Beshiru *et al.,* 2020; Igbinosa *et al.,* 2020; Adeosun *et al*., 2023). Despite the extensive documentation of foodborne pathogens and general contamination pathways, there still exists, a critical dearth of targeted research explicitly assaying commonly prepared and commercially available Nigerian RTE foods for pathogenic bacteria harboring specific, high-risk virulence genes. There is also a corresponding gap in generating empirical data to elevate consumer awareness with respect to the specific food safety risks directly associated to the consumption these commercial RTE items. To address these gaps, this study was aimed at

ascertaining selected virulence gene profiles, phenotypic virulence factors and antibiogram profiles as well as Multiple Antibiotic Resistance (MAR) indices harbored by previously characterized cultured RTE-borne bacterial strains.

MATERIALS AND METHODS

Description of Study Area

The study was conducted within major commercial hubs and transit corridors across Benin City, Edo State and parts of Delta State, Nigeria—regions characterized by high commuter traffic where hawked and vended ready-to-eat (RTE) foods serve as vital nourishment for travelers and a source of income for local vendors. Three sampling locations were visited and about 288 RTE food samples were purchased from hawkers at these locations. The locations were; Adesuwa junction situated on the Benin -Sapele expressway, Edo State ($6°19'15"N, 5°37'30"E$), Isihor axis situated on the Benin-Shagamu expressway, Edo State($6°24'10"N, 520'35"E$) and Sapele Roundabout (Amukpe Section) situated on the Benin-Sapele expressway, Delta State ($5°53'40"N, 5°41'10"E$). A map indicating the respective sampled locations is shown in Figure 1.

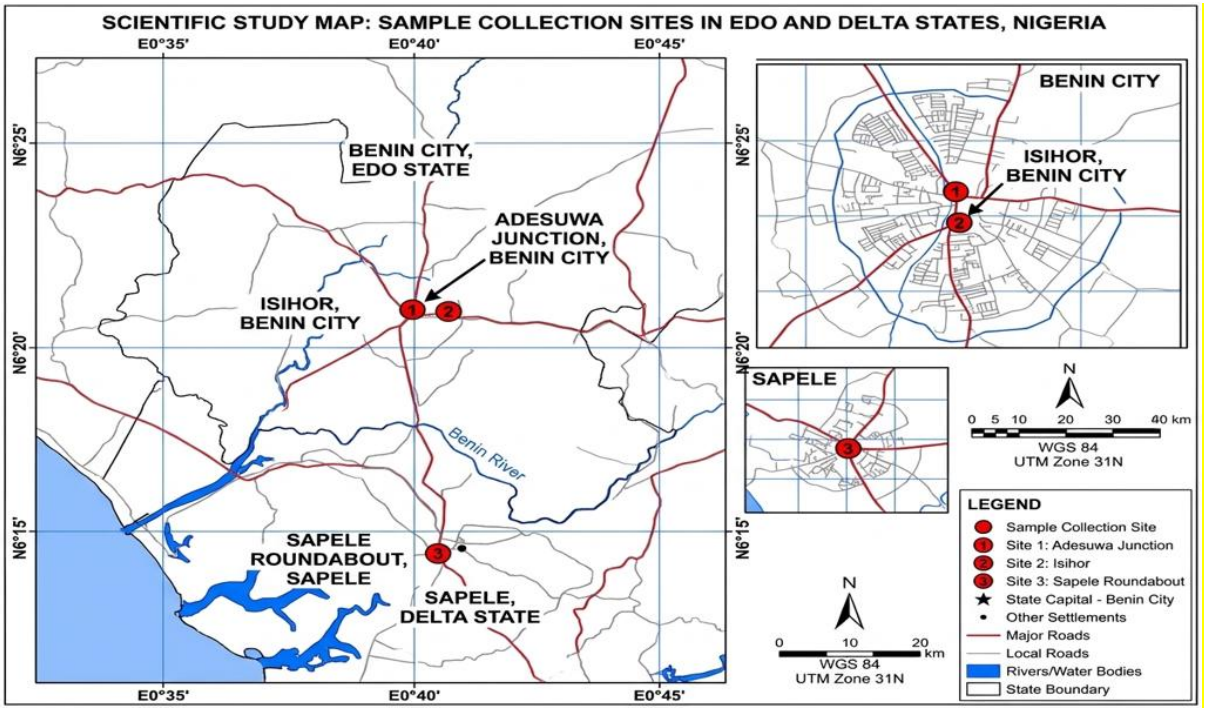


Figure 1: Map revealing the respective sampling locations

Collection and Handling of RTE Food Samples

Samples of ready-to-eat (RTE) foods were randomly collected over a six-month period from February to July 2024. Food Samples were collected in triplicates and duplicates from different street vendors and sellers across the sampling locations. The three types of RTE food samples collected from the respective sellers included; Fried edible palm weevil larvae (*Rhynchophorus phoenicis*, locally called orhu or edible worm), fried giant land snail specimens (*Achatina achatina*) and cooked/fried pork meat (*Sus domesticus*). Immediately upon purchase from the vendors at each mapping point, the respective food samples were carefully transferred into clean, sterile labeled polyethylene bags under aseptic conditions to prevent environmental cross-contamination. The bagged samples were securely stored, kept in coolers which contained ice packs and promptly transported under ambient field-to-laboratory transit conditions to the Post Graduate Microbiology Laboratory in the Department of Microbiology, Faculty of Life Sciences, University of Benin, for immediate bacteriological analysis.

Bacteriological analysis of the RTE food samples revealed the presence of eight bacterial isolates; *E. coli* strain 1, *E. coli* strain 2, *Bacillus subtilis Klebsiella pneumoniae*, *Streptococcus pneumoniae, Bacillus cereus*, *Staphylococcus aureus* strain 1 and *Staphylococcus aureus* strain 2 (Abina *et al*., 2026). The isolation frequencies of each food borne isolates revealed that 23 individual strains of *S. pneumoniae*, *E. coli* strain 1, *E. coli* strain 2 and 24 individual strains of *K. pneumoniae, B. subtilis, B. cereus, S. aureus* strain 1 and *S. aureus* strain 2 were respectively cultured (Abina *et al*., 2026).

Determination of Phenotypic Virulence Factors of the Bacterial Isolates

The potential hemolytic attributes of the foodborne bacterial strains were ascertained by culturing the respective bacterial cultures on blood agar using streaking procedure as described by Sharma (2009). The blood agar utilized was brain heart infusion agar supplemented with 5% defibrinated rabbit blood and the streaked blood agar incubated at 37 °C for 24 hrs. The presence of a partial halo or total hemolysis was documented as a hemolysin-positive result. The ability of each isolate to produce biofilm was evaluated by culturing the isolate on Brain heart infusion broth supplemented with 5% sucrose and Congo red stain as described by Nuryastuti (2014). The agar plates were incubated at 37 °C for 24 hrs. After expiration of the incubation period, the agar plates were observed and the visualized reactions reflecting matrix production and cellular aggregation were documented. The food borne isolates were also screened for protease activity by streaking purified cultures on skim milk agar as described by Pereira and Vanetti (2015). The agar plates were incubated

at 37 ºC for 24 hrs and upon the expiration of the incubation period, the agar plates plates were inspected for clear zones (proteolysis/casein degradation) surrounding or along the streak lines, and visualized reactions were documented.

PCR Analysis of Selected Bacterial Virulence Genes

PCR sequencing mix contained GoTaq buffer, $MgCl_2$, dNTPs, primers [27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5′-AAGGAGGTGATCCAGCC-3′] and Taq DNA polymerase (Promega, USA), water and DNA template. Amplification was carried out using a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with initial denaturation at 94°C for 5 min; 30 cycles of denaturation, annealing at 50°C ad extension at 72°C followed by a final extension at 72°C. Product was held at 4ºC before downstream analysis.

Determination of Amplified Gene Integrity

The PCR product integrity was confirmed using agarose gel electrophoresis. Agarose gel was prepared in 1× TAE buffer, stained, cast and allowed to solidify. PCR samples mixed with loading dye were then loaded alongside a 100 bp DNA ladder run at 120 V for 45 mis. Bands were visualized under UV light and fragment sizes estimated by comparison with the ladder.

Purification and Sequencing of Amplified Product

PCR products were purified using ethanol, washed, dried and resuspended in sterile water before been stored in a freezer at -20ºC. Purified fragments were then confirmed on 1.5% agarose gel and quantified using NanoDrop 2000. Sequencing was carried out using an Applied Biosystems Genetic Analyzer 3120 ×l using the BigDye Terminator v3.1 kit, while BioEdit ad MEGA 6 were used for genetic analysis.

Molecular Analysis of Selected Virulence Genes

Virulence genes were detected by PCR using region specific primers (see Table 1 below) on extracted DNA. Each reaction contained SYBR green buffer, $MgCl_2$, dNTPs, primers, Taq polymerase, sterile water and DNA template, while buffer control was included to avoid false amplification. PCR amplification was then performed using Applied Biosystem GeneAmp 9700 Thermal Cycler using primer-specific conditions (Table 1).

Table 1: Selected Virulence Gene Primers

Virulence gene		Primer sequence 5'-3'	PCR conditions
blaCMY-2	*blaCMY-2*-F	TGGCCAGAACTGACAGGCAAA	An initial denaturing 5min at 94ºC, then 35 cycles of 94ºC for 30s, 53ºC for 30s, 72ºC for 30 seconds and terminate at 72ºC for 10min
	blaCMY-2-R	TTTCTCCTGAACGTGGCTGGC	
TetA	*TetA*-F	GGTTCACTCGAACGACGTCA	An initial denaturing 5 min at 94ºC, then 35 cycles of 94ºC for 30 s , 46ºC for 30 seconds, 72 ºC for 35 s and terminate at 72ºC for 10min
	TetA-R	CTGTCCGACAAGTTGCATGA	
SHV(sulf hydryl variable)	*mecC* -F	GCTCCTAATGCTAATGCA	An initial denaturing 5min at 94ºC , then 35 cycles of 94ºC for 30s 48ºC for 30s and 72ºC for 30s and terminate at 72ºC for 10min
	mecC -R	TAAGCAATAATGACTACC	
Aac(3)-II	*Aac(3)-II* -F	GCTCGGTTGGATGACAAAGC	An initial denaturing 5min at 94ºC, then 35 cycles of 94ºC for 60s, 55ºC for 30s and 72ºC for 30s. and terminate at 72ºC for 10mins
	Aac(3)-II -R	AGGCGACTTCACCGTTTCTT	
entA	*entA*-F	AAATATTATGGAAATGGAGTGTAT	An initial denaturing 5min at 94ºC, then 35 cycles of 94ºC for 60s, 55ºC for 30s and 72ºC for 30s. and terminate at 72ºC for 10 mins
	entA-R	GCACTTCCCTGGAATTGCTCACAT	
HblA1	*HblA1*-F	GCTAATGTAGTTTCACCTGTAGCAAC	An initial denaturing 5 min at 94ºC, then 35 cycles of 94ºC for 60s, 55ºC for 30s and 72ºC for 30s. and terminate at 72ºC for 10 mins
	HblA1-R	AATCATGCCACTGCGTGGACATATAA	
hlyA1	*hlyA*-F	GTCTGCAAAGCAATCCGCTGCAAATAAA	An initial denaturing 5 min at 94ºC, then 35 cycles of 94ºC for 60s, 55ºC for 30s and 72ºC for 30s. and terminate at 72ºC for 10mins
	hlyA-R	TGTCCACGAGTTGGTTGATTAGCTGC	

Determination of the Antibiogram Profiles of the Bacterial Cultures

Bacterial antimicrobial susceptibility was assessed using the modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar inoculated via the spread plate technique (Cappuccino & Welsh, 2020; Vandepitte *et al*., 2003). Inocula were standardized to a 0.5 McFarland turbidity standard using a 0.36 Normal barium sulphate opacity standard (Vandepitte *et al*., 2003). Growth inhibition zones for the selected antimicrobial agents were measured and interpreted against standard breakpoints (CLSI, 2020).

The antibiotic sensitivity/resistance profile of the respective bacterial isolates was evaluated using the modified Kirby-Bauer disc diffusion method as described by Vandepitte *et al.* (2003) and the resultant growth inhibitory zones were interpreted using standard antibiotic breakpoints as documented by the Clinical and Laboratory Standards Institute (CLSI, 2020).

Commercially available antibiotic discs utilized were; Clindamycin (30 µg CAZ), cefuroxime (30 µg CRX), gentamicin (10 µg CN), erythromycin (30 µg E), amoxycillin (30 µg AMX), ciprofloxacin (10 µg CPX), ofloxacin (5 µg OFX), tetracycline (30 µg TET) and augmentin (30 µg AUG). The resultant visible zones of inhibition were measured across the disc using a ruler. The multiple antibiotic index value for each exposed isolate strain cluster was determined using a formula described by Mir *et al.* (2021). The formula is given below:

MAR= $A \div B$

Where; A: Number of antibiotic drugs, isolate was resistant to, B: Total number of antibiotic drugs isolate was exposed to

RESULTS AND DISCUSSION

The expressed phenotypic virulence profiles of the respective RTE food borne bacterial isolates are presented in Table 2. All the test food borne strains were biofilm producers and with the exception of S. *pneumoniae* exhibited varying levels of protease activity when exposed to milk protein (Table 2). The test isolates exhibited different types of hemolytic activity with only two isolates; *S. pneumoniae* and *B. cereus* displaying alpha hemolytic activities (Table 2). Only *E. coli* strain 2, *B. subtilis* and all the S. *aureus* strains exhibited beta hemolytic activities. For gamma hemolysis, only *E. coli* strain 2 and *K. pneumoniae* amongst the test isolates displayed this type of hemolytic activity (Table 2).

Table 2: Phenotypic Virulence Activity of the Food Borne Bacterial Isolates

Isolate (n)	Hemolytic activity			Protease activity	Biofilm formation activity
	Alpha	Beta	Gamma		
Escherichia coli strain 1(23)	_	_	+++	++	+++
Escherichia coli strain 2(23)	_	+++	_	+++	+++
Bacillus subtilis (24)	_	+++	_	+++	+++
Klebsiella pneumoniae (24)	_	_	+++	+	+++
Streptococcus pneumoniae (24)	+++	_	_	_	+++
Bacillus cereus (24)	+++	_	_	+++	+++
Staphylococcus aureus strain 1 (24)	_	+++	_	+++	+++
Staphylococcus aureus strain 2 (24)	_	+++	+++	+++	+++

KEY; n; number of strains, -; No activity, ++: moderate activity, +++; Strong activity, +; weak activity

The result of the PCR analysis of selected virulence gene markers in the respective isolates is presented in Table 3. For all the test bacterial strain cultures, only *B. cereus*, *B. subtilis*, *E. coli* strain 1 and *E. coli* strain 2 was observed to harbor ESBL- $_{BlaCMY-2}$ gene. Amongst all the test isolates, only *S. aureus* strain 1 harbored *mecC* gene. Four isolates; *S. aureus* strain 2, *B. subtilis*, *E. coli* strain 1 and *E. coli* strain 2 were observed to harbour the *tetA* gene. The presence of *aac3* virulence gene was observed in five bacterial cultures; *S. aureus* strain 1, *S. aureus* strain 2, *E. coli* strain 1, *E. coli* strain 2 and *K. pneumoniae* respectively. Amongst all the examined isolates, only *B. cereus* harbored Hb1A gene. The presence of *HlyA* gene was observed in *S. aureus* strain 2, *E. coli* strain 2 and *K. pneumoniae*. None of the examined food borne bacterial cultures harbored *entA* gene (Table 3).

Table 3: Molecular (PCR) Profiling of Virulence Gene Markers in the Food Borne Respective Isolates

Virulence gene markers	*S. pneumoniae* (23)	*S.aureus* 1(24)	*S.aureus* 2 (24)	*B. cereus* (24)	*B. subtilis* (24)	*E. coli 1* (23)	*E. coli 2* (23)	*K. pneumoniae* (24)
ESBL- $_{BlaCMY-2}$	-	-	-	+	+	+	+	-
MecC	-	+	-	-	-	-	-	-
tetA	-	-	+	-	+	+	+	-
aac3	-	+	+	-	-	+	+	+
Hb1A	-	-	-	+	-	-	-	-
HlyA	-	-	+	-	-	-	+	+
entA	-	-	-	-	-	-	-	-

KEY: -: Absent, +: Present, number in bracket; number of cultured strains

The results of the antibiogram analysis for all the RTE food borne isolates are presented in Table 4. All the cultured strains of the non-cured *E. coli* strain 1 and 2 were resistant to amoxycillin, clindamycin, erythromycin, cefuroxime and tetracycline. All the wild type *E. coli* strains were sensitive towards ciprofloxacin and ofloxacin. The multiple antibiotic resistance (MAR) index of the exposed wild type *E. coli* strains was 0.7. The exposed non-cured *B. subtilis* and *K. pneumoniae* exhibited sensitivity towards ciprofloxacin and ofloxacin. The MAR index of the wild type *B. subtilis a*nd *K. pneumoniae* were 0.3 and 0.7. Both exposed wild type *S. pneumoniae* and *B. cereus* strains were sensitive to ofloxacin. The MAR index of both *S. pneumoniae* and *B. cereus* strains were 0.2 and 0.3 respectively. All cultured non-cured *S. aureus* strains exhibited resistance towards augmentin, cefuroxime, erythromycin, clindamycin, tetracycline and amoxycillin. All the wild type *S. aureus* strains displayed sensitivity towards ciprofloxacin. For the test wild type S. *aureus* strains, a derived MAR value of 0.7 was documented for these culture strains.

Table 4: Antibiogram Profiles of the RTE Food Borne Bacterial Isolates

Isolate (n)	AMX (30 µg)	CAZ(30 µg)	CRX(30 µg)	CN(10 µg)	CPX(10 µg)	ERY(30 µg)	AUG(30 µg)	OFX (5 µg)	TET(30 µg)	Multiple Antibiotic Resistance index (MAR)
Escherichia coli strain 1(23)	R(100)*	R(100)	R(100)	I(100)	S(100)	R(100)	R(100)	S(100)	R(100)	0.7
Escherichia coli strain 2(23)	R(100)	R(100)	R(100)	S(100)	S(100)	R(100)	R(100)	S(100)	R (100)	0.7
Bacillus subtilis (24)	R(100)	S(100)	R(100)	S(100)	S(100)	S(100)	I(100)	S(100)	R(100)	0.3
Klebsiella pneumoniae (24)	R(100)	R(100)	R(100)	R(100)	S(100)	R(100)	R(100)	S(100)	R(100)	0.7
Streptococcus pneumoniae (23)	S(100)	I(100)	S(100)	R(100)	I(100)	R(100)	S(100)	S(100)	I(100)	0.2
Bacillus cereus (24)	R(100)	S(100)	R(100)	I(100)	S(100)	S(100)	R(100)	S (100)	S (100)	0.3
Staphylococcus aureus strain 1 (24)	R(100)	R(100)	R(100)	S(100)	S(100)	R(100)	R(100)	S(100)	R(100)	0.7
Staphylococcus aureus strain 2 (24)	R(100)	R(100)	R(100)	I(100)	S(100)	R(100)	R(100)	S(100)	R(100)	0.7

KEY; n; number of strains, Amoxycillin (AMX), Clindamycin (CAZ), Cefuroxime (CRX), Gentamicin (CN), Ciprofloxacin (CPX), Erythromycin (ERY), Augmentin (AUG), Ofloxacin (OFX), Tetracycline (TET), S; Sensitive, I; Intermediate, R; Resistant, *percentage number of exposed strains

Discussion

Both *E. coli* strains 1 and 2 exhibited varying resistance to amoxycillin, clindamycin, erythromycin and cefuroxime. The expressed resistance of the respective *E. coli* could be intrinsic as this antibiotic is generally inactive against Gram negative bacteria (Kakoullis *et al*., 2021; Ma *et al.*, 2024). The expressed drug resistance patterns of both *E. coli* strains are similar to a report by Abass *et al.* (2020) which revealed the resistance of foodborne *E. coli* to amoxicillin-based agents, tetracyclines and macrolides/lincosamides. The PCR profiling of both *E. coli* strains 1 and 2 revealed that *E. coli* strain 2 harboured more virulence gene markers (ESBL-$_{\text{BlaCMY-2}}$ gene, *tetA, aac3* and *HlyA*) in comparison to *E. coli* strain 1 (ESBL-$_{\text{BlaCMY-2}}$ gene, *tetA* and *aac3*). The detection of ESBL-$_{\text{BlaCMY-2}}$ genes in both strains could be the reason for the expressed resistance to cefuroxime as the expression of extended spectrum β-lactamase (ESBL) has been attributed as a reason for cefuroxime resistance by *E. coli* isolates (Li *et al*., 2022). The detection of *tetA* genes in both *E. coli* strains could be the direct reason for the expressed resistance to tetracycline antibiotic by the exposed *E. coli* strains. The presence of ESBL-$_{\text{BlaCMY-2}}$ and *tetA* genes in the respective *E. coli* strains is a worrisome trend from a public health perspective as it can amplify the likely health risk(s) linked with the direct consumption of these foods harbouring these drug-resistant *E. coli* strains (Koga *et al*., 2019; Igbinosa *et al.*, 2023).

B. subtilis and *K. pneumoniae* strains had identical MAR index values with *B. subtilis* displaying resistance to amoxycillin, cefuroxime and tetracycline while *K. pneumoniae* strains were resistant to amoxycillin, clindamycin, cefuroxime, gentamicin, erythromycin, augmentin and tetracycline respectively. The expressed resistance of the RTE food borne *B. subtilis* strains *to* cefuroxime might be intrinsic or chromosomally mediated as the bacterium is known to harbour extra-cytoplasmic function (ECF) sigma factors (specifically σ^M and σ^X) that aids in conferring intrinsic resistance to the cephalosporin group of antibiotics exemplified by cefuroxime (Luo and Helmann, 2012; Helmann, 2016).

The resistance of *B. subtilis* strains to amoxycillin can be directly attributed to the production of β-lactamase enzymes that are capable of degrading the antibiotic. This observation was collaborated by the detection of ESBL-$_{\text{BlaCMY-2}}$ gene in the bacterium in the course of ascertaining the virulence gene profiles of the respective food borne bacterial cultures. The resistance of *K. pneumoniae* strains to some of the antibiotics; amoxycillin, cefuroxime and augmentin could be attributed to the production of extended spectrum β-lactamase (ESBL) enzymes by the respective *K. pneumoniae* strains (Li *et al*., 2024). However, this likely phenotypic activity was not collaborated by the results of the PCR profiling of virulence genes which indicated the absence of ESBL-$_{\text{BlaCMY-2}}$ in the bacterium. This observation could suggest that maybe, the expressed β-lactamase (ESBL) enzyme was encoded by a different gene whose primer was not utilized in the PCR analysis. The resistance of *K. pneumoniae* strains to gentamicin can be attributed to likely alterations in cellular permeability of the bacteria against cellular entry of the antibiotic or the likely presence of efflux pump systems (Li *et al*., 2023). Erythromycin resistance by *K. pneumoniae* is likely to be intrinsic as this bacterium has been known to exhibit high levels of resistance to macrolide antibiotics exemplified by erythromycin (Li *et al.*, 2023; Li *et al.*, 2024). The aac(3)-II gene encode for acetyltransferases that inactivate aminoglycosides. Its detection in *K. pneumoniae* was in alignment with literature describing high-level aminoglycoside resistance mechanisms mediated by plasmid- or transposon-borne determinants (Plattner *et al*., 2020; Zhang *et al*., 2021).

B. subtilis and *K. pneumoniae* harboured only two (2) virulence gene markers respectively and with reference to the antibiogram profiles, it can be inferred that the virulence gene markers detected in *B. subtilis* were phenotypically expressed. The presence of *tetA* gene in the foodborne *B. subtilis* isolate alongside its subsequent expression is a troubling observation from a public health perspective as it can increase the risk of these drug-resistant isolates causing severe food borne illness as *B. subtilis* can elaborate toxins that can cause gastrointestinal symptoms in susceptible individuals. It has been reported that foodborne *B. subtilis* strains harboring resistance genes (*tetA*) and expressing diverse hydrolytic or toxigenic profiles represent an under-recognized public health threat (Haque *et al.*, 2022).

The detection of *HlyA* gene in *K. pneumoniae* is noteworthy from a public health perspective as this gene encodes for α-hemolysin which is a very potent toxin that can contribute to the expressed pathogenicity and associated virulence (Bautista-Cerón *et al*., 2022; Paniagua-Contreras *et al.*, 2023). This trend also increases the risk of affected consumers developing severe food borne infection and health complications after consuming foods harbouring virulent live stains of *K. pneumoniae* at infectious doses.

The expressed resistance of *B. cereus* strains to amoxycillin, cefuroxime and augmentin can be attributed to the production of β-lactamase enzymes which can degrade the respective antibiotic medications. The possibility of this bacterium producing β-lactamase enzyme(s) was collaborated by the detection of ESBL-$_{\text{BlaCMY-2}}$ gene in the bacterium. The detection of *Hb1A* gene in the bacterium is worrying from a public health perspective, as this gene is part of the hemolysin BL (HBL) enterotoxin complex and can contribute to the virulence of *B. cereus* when phenotypically expressed (Maćkiw *et al*., 2024). Food borne *B. cereus* strains harbouring *Hb1A* gene are known to be capable of producing toxins that can cause gastrointestinal symptoms which include; vomiting and diarrhoea when ingested (Sánchez-Chica *et al*., 2021). As such, the detection of food borne *B. cereus* harbouring this virulence gene poses a notable health risk to food consumers especially as these RTE foods are usually consumed directly after purchase.

Although, the *aac3* gene which can confer on the host bacterium the ability to tolerate or resist aminoglycoside antibiotic was detected in both *S. aureus* strains, the phenotypic expression of this genetic trait was not observed as the *S. aureus* strains were susceptible to gentamicin. The isolation of multi drug resistant *S. aureus* from the RTE foods in itself is a worrying observation based on the documented pathogenic attributes of food borne *S. aureus* and their ability to cause food borne gastroenteritis and poisoning (Baumgartner *et al*., 2014; Puah *et al.*, 2016; Lin *et al.,* 2019). The likely toxigenic attributes of these food borne *S. aureus* isolates were further collaborated by the detection of *HlyA* gene in both *S. aureus* strains. The presence of this gene in both strains can enhance their likely virulence attributes with the concomitant increase in the risk of these bacteria as a likely cause of severe infections and complications in affected persons. The correlation between toxigenic gene profiling (such as hlyA) and high virulence potential with respect to foodborne *S. aureus* has also been described by several authors (Baumgartner *et al*., 2014; Puah *et al.*, 2016).

All the RTE food borne isolates were phenotypically capable of biofilm production. Biofilm formation is an important aspect of the virulence ability of these isolates. The formed biofilms can aid the bacteria to survive exposure to antibiotic drugs and host immune responses as well as increase the ability of these organisms to adhere to various surface types. This trend in addition to the expressed multi drug resistance profiles of these RTE food borne bacterial strains is a very worrying observation and would reinforce the inference that these foods being sold to unsuspecting members of the public are a major health risk and likely source/cause of food borne illness outbreak.

There are slight variations in the type of biofilm matrix elicited by these food borne bacterial strains. Biofilms produced by *S. aureus* and *B. cereus* are known to comprise of polysaccharides and proteins which are known to facilitate cellular adhesion and aggregation. Biofilms produced by *B. subtilis* are known to comprise of exopolysaccharides and proteins that can facilitate cell to cell adhesion as well as matrix formation. Biofilms produced by *E. coli* comprise of curli fibers and cellulose which contribute to the structural integrity of the biofilm (Archer *et al.*, 2011; DePas *et al.*, 2014; Pandit *et al.*, 2020). Capsular polysaccharides and proteins are associated with biofilms produced by *K. pneumoniae* (Limoli *et al.*, 2014). These biofilm constituents are known to enhance surface adhesion as well as aid the bacterium to evade host immune responses and antibiotic medications. The biofilm-forming capability exhibited by the respective food borne isolates is in alignment with documented microbial ecological findings, which revealed that biofilms protect foodborne pathogens against environmental stressors, sanitizers, host immune responses, and antibiotic penetration (Archer *et al*., 2011; DePas *et al*., 2014).

Varying levels of protease activities was observed for all the food borne bacterial strains with the exception of *S. pneumoniae* strains. The ability of these bacterial strains to elicit caseinolytic activities on skim milk medium at different magnitudes might infer that these isolates can also hydrolyse similar food proteins such as soy proteins. With the exception of both *E. coli* strain 1 and *K. pneumoniae* strains, all the RTE food borne strains exhibited either α or β haemolysis. This trend would suggest that these food borne haemolytic bacterial isolates are capable of causing erythrocyte lysis in a susceptible human host that has ingested amounts of these foods harbouring sufficient infective doses of these suspected pathogens. The expression of protease and hemolytic activities would invariably underscore the capacity of these street-vended RTE isolates to degrade host/food proteins and lyse erythrocytes, significantly increasing the risk of gastrointestinal pathology and systemic complications upon ingestion (Pereira and Vanetti, 2015).

CONCLUSION

It is evident that the examined street-vended ready-to-eat (RTE) foods are heavily contaminated with pathogenic bacterial strains possessing robust phenotypic virulence factors and high multiple antibiotic resistance (MAR) indices ranging from 0.2 to 0.7. All isolated bacterial strains (*E. coli* strain 1 and 2, *Bacillus subtilis, Klebsiella pneumoniae, Streptococcus pneumoniae, Bacillus cereus*, and *S. aureus* strain 1 and 2) demonstrated universal biofilm-forming capabilities alongside diverse hemolytic and proteolytic activities.

Furthermore, molecular profiling confirmed the presence of critical resistance and virulence gene markers—such as bla_{CMY-2}, *tetA*, *mecC*, *aac(3)-II*, *Hb1A* and *hlyA*—which corroborated several of the expressed phenotypic resistance patterns. The widespread dissemination of these multi-drug resistant and virulent pathogens through street-vended RTE foods is representative of a profound public health hazard, increasing the risk of foodborne infections and severe clinical complications among consumers.

RECOMMENDATIONS

i. Regulatory and public health agencies in Nigeria should enforce stricter hygiene standards, mandatory licensing and routine monitoring of street-vended and retail food operations to minimize contamination.
ii. The establishment of comprehensive training programs is recommended for the purpose of educating food vendors on proper food handling, personal hygiene, and cross-contamination prevention during processing, distribution, and preparation.
iii. Continuous epidemiological surveillance systems should be implemented to monitor antimicrobial resistance trends and virulence gene markers in foodborne pathogens circulating within commercial food settings.
iv. Public awareness campaigns should be conducted with the sole purpose of informing likely consumers about the food safety risks linked with consuming commercially available ready-to-eat food items.

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