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CTXM and *OXA* Genes in Foodborne Bacteria from Cooked Street Foods Sold in the South Western States of Nigeria

Olufeyikemi A. Adeleke* and Abimbola A. Owoseni

Microbiology Programme, College of Agriculture, Engineering and Science, Bowen University, P.M.B 284 Iwo, Osun State, Nigeria

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ABSTRACT

The emergence of new antibiotic-resistant bacteria implicated in foodborne illnesses has led to increasing drug treatment failures that have become a great public health concern. This work identified the presence of *CTXM* and *OXA* resistance genes located on plasmids in some multi-antibiotic resistant bacteria isolated from cooked street-vended food sold in South Western Nigeria using both phenotypic and molecular techniques. Fifty (50) bacteria were isolated and identified using conventional phenotypic techniques. Ceftazidime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), cefixime (5 µg), amoxicillin (30 µg), nitrofurantoin (300 µg), and augmentin (30 µg) were used to examine the isolates' resistance patterns. For bacteria resistant to four or more antibiotics, plasmid curing and plasmid DNA were extracted using conventional techniques. *CTXM* and *OXA* genes were subsequently amplified using the specific primers (PCR methods) from the extracted bacterial plasmid DNA. High resistance was observed to ceftazidime and augmentin (96%) as well as cefuroxime (86%), whereas susceptibility to ciprofloxacin, ofloxacin, gentamicin and nitrofurantoin was at 80%, 78%, 76% and 60% respectively. Fourteen of the isolates studied showed the presence of *CTXM*, fifteen carried *OXA* genes while five carried both *CTXM* and *OXA* genes on their plasmid. There is, therefore, a need for standard food quality policies guiding the establishment of ready-to-eat food outlets.

***Keywords*:** Bacteria, *CTXM*, *OXA*, Plasmid DNA.

Introduction

Resistance in bacteria has become a major concern as resistant bacteria, which are growing more common in the human environment has frequently resulted in treatment failure that adds to the cost of healthcare. Furthermore, resistant bacteria may spread and cause more severe infection-control issues, not only in healthcare facilities but even within communities.[1] Antibiotics have been used for several decades and in different areas of the world (including Nigeria). This has revealed that many infectious microbes have evolved throughout time, with an alarmingly high rate of antibiotic-resistant species able to evade the antibiotics' inhibitory effects.[2] Resistance has dramatically increased in frequency among bacteria of clinical relevance, presumably because of selective pressure imposed by the extensive use of commercial antibiotics in human and veterinary medicine.[3] These resistance abilities have been widely transferred from one species of bacteria to another through the actions of genetic substances carried by these bacteria. These genes can carry as many as one to ten resistant abilities to the same or different classes of antibiotics. Extended-spectrum β-lactamases (ESBLs) are a predominant cause of β-lactam resistance in Gram-negative bacilli (GNB).[4] Incidences of infections caused by ESBLs producing Gram-negative bacteria are increasing in prevalence worldwide, both in the healthcare as well as community settings, posing significant therapeutic challenges. ESBLs are most often a plasmid-mediated heterogeneous group of *β*-lactamase enzymes that confer resistance to a wide range of commonly used β-lactam antibiotics.

*Corresponding author. E mail: feyikemi.adeleke@bowen.edu.ng
Tel: +2348033509690

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Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

The molecular class D β-lactamases were originally relatively rare and always plasmid-mediated but in the past decade, the *CTX-M* type and *OXA* ESBLs have become the most widely distributed and globally dominant genotypes.[5] This study was aimed at identifying the presence of *CTXM* and *OXA* resistance genes in plasmid-mediated antibiotic-resistant bacteria isolated from cooked street-vended food sold in South Western Nigeria using both phenotypic and molecular techniques.

Materials and Methods

Sample Collection

Food samples for this study were purchased from the point of sale between March-August, 2021 in six different South Western States of Nigeria (Ondo, Ekiti, Osun, Oyo, Ogun and Lagos) for a period of six months. The food samples were kept in clean plastic containers, ice-packed food coolers and taken for processing and analysis. Different media were used in the course of the study, namely nutrient agar, peptone water, xylose lysine deoxycholate (XLD) agar, Mannitol salt agar, and Mueller-Hinton agar media. Serial dilutions of the samples were done and all the media used for the isolation of the bacteria were prepared according to the manufacturer's specifications and the bacteria were isolated and identified using standard morphological and biochemical tests.

Standardization of Inoculum and Antimicrobial Susceptibility Testing

The agar disk diffusion method was used to assess antibiotic resistance and susceptibility profiles. The disk diffusion procedure was meticulously standardized and carried out following the Clinical and Laboratory Standards Institute's regulations.[6] From an overnight growth on agar, four to five colonies were selected, inoculated into tryptone broth, and incubated at 37°C for 18 hours. By modifying the inoculum with sterile saline, the inoculum was standardized to 0.5 McFarland standard. The density of the McFarland standard was tested by using a Jenway 6305 spectrophotometer at a wavelength of 625 nm and an adjusted inoculum suspension count of roughly 10^8 CFU/ml to measure the absorbance (between 0.08-0.13).[7]

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The antibiotics ceftazidime (30 µg), cefuroxime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), cefixime (5 µg), amoxicillin (30 µg), nitrofurantoin (300 µg), and augmentin (30 µg) were used in antimicrobial susceptibility tests on Mueller-Hinton agar plates. After applying the antibiotic disks to the infected plates and incubating them at 37°C for 24 hours, the diameter of the zones of full inhibition was measured to the nearest whole millimeter. Multiple-antibiotic-resistant isolates were defined as those that were resistant to three or more antibiotics.[7]

Plasmid curing, Extraction of Plasmid DNA, PCR Assay, Amplification of Resistance Genes and Agarose Gel Electrophoresis

Antibiotic-resistant bacterial isolates were sub-cultured onto fresh agar plates and grown overnight; plasmid curing of the resistant isolates was performed by adding 50 mL of acridine orange 0.10 mg/ml to Luria-Bertani (LB) broth in test tubes, fresh colonies of isolates found to be resistant were then inoculated into the sterilized LB broth containing acridine orange and incubated for 24 hours at 37°C. After incubation, the cultures were swabbed onto fresh Mueller-Hinton agar plates and incubated at 37°C for 24 hours and a new antibiotic sensitivity test was performed. The antibiotic sensitivity test result was recorded. Plasmid DNA was extracted from isolates that were shown to be mediating antibiotic resistance via their plasmids.

The amplification of the DNA was done and was separated on a 2.0 percent agarose gel and electrophoresis was performed using a Vari-gel horizontal gel electrophoresis device at 150V, 250 Ma, 50 W for one hour. Following electrophoresis, DNA bands were detected using ethidium bromide staining and a high-powered UV, using a 100 base pair (100bp) (Solis Biodyne) DNA ladder as a molecular weight marker. Primer sequence for the detection of *CTXM* and *OXA* resistance genes for PCR amplification is presented on Table 1.

Results and Discussion

Fifty (50) bacteria were identified from cooked street foods from which plasmid-mediated antibiotic resistance abilities were studied. Figure 1 shows the cumulative sensitivity percentage profiles of the isolates to the antibiotics. According to the findings, resistance to augmentin and ceftazidime was 96% each, and cefuroxime recorded 86% resistance. However, ciprofloxacin (80%), nitrofurantoin (60%), ofloxacin (78%), and gentamicin (76%) recorded good susceptibility levels. Table 2 shows the occurrence of plasmid-mediated antibiotic-resistant bacteria from the vended foods sampled while the multiple-antibiotic resistance pattern of the plasmid-mediated antibiotic-resistant bacteria from the cooked vended food is presented in Table 3.

Table 1: Primer sequence for the detection of *CTXM and OXA* resistance genes used for PCR amplification

Name	Sequence (5' – 3')	Amplicon size	Annealing Temperature (°C)	Reference
CTX-M - F	CGCTGTTGTTAGGAAGTGTG	569	52	8
CTX-M - R	GGCTGGGTGAAGTAAGTGAC			
OXA – F	CGCTGTTGTTAGGAAGTGTG	701	52	9
OXA – R	GGCTGGGTGAAGTAAGTGAC			

Table 2: Occurrence of Plasmid-mediated antibiotic-resistant bacteria from cooked vended food in South-Western Nigeria

S/N	Bacterial Genera	n (%)						
		EKITI	ONDO	OSUN	OYO	OGUN	LAGOS	TOTAL
1.	*Bacillus*	-	-	1(2)	-	2(4)	2(4)	5(10)
2.	*Staphylococcus*	2(4)	1(2)	2(4)	-	-	-	5(10)
3.	*Providencia*	1(2)	-	-	-	-	1(2)	2(4)
4.	*Escherichia*	-	1(2)	1(2)	-	-	2(4)	4(8)
5.	*Vibrio*	-	2(4)	-	-	-	-	2(4)
6.	*Clostridium*	1(2)	2(4)	-	-	1(2)	-	4(8)
7.	*Shigella*	-	1(2)	2(4)	-	-	-	3(6)
8.	*Proteus*	-	1(2)	1(2)	-	-	-	2(4)
9.	*Serratia*	-	-	-	-	-	1(2)	1(2)
10.	*Salmonella*	1(2)	-	1(2)	-	1(2)	-	3(6)
11.	*Klebsiella*	-	2(4)	1(2)	1(2)	-	-	4(8)
12.	*Streptococcus*	-	1(2)	1(2)	-	-	1(2)	3(6)
13.	*Acinetobacter*	-	1(2)	-	-	-		1(2)
14.	*Enterobacter*	-	-	-	1(2)	-	1(2)	2(4)
15.	*Hafnia*	-	-	1(2)	-	-	-	1(2)
16.	*Bifidobacterium*	-	-	1(2)	-	-	-	1(2)
17.	*Lactobacillus*	-	-	-	-	-	1(2)	1(2)
18.	*Pseudomonas*	-	1(2)	1(2)	-	-	-	2(4)
19.	*Citrobacter*	-	-	1(2)	-	-	-	1(2)
20.	*Neisseria*	-	1(2)	-	-	2(4)	-	3(6)
	TOTAL	5(10)	14(28)	14(28)	2(4)	6(12)	9(18)	50(100)

Key: n= number of bacteria; % = frequency of occurrence in percentage

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Table 3: Multiple-antibiotic resistance pattern of Plasmid-mediated antibiotic-resistant bacteria from cooked vended food in South-Western Nigeria

S/N	Bacteria Genera	Distribution patterns of MAR	Number of MAR
1.	*Bacillus*	CRX, CPR, AUG, AMP, CAZ	5
		CRX,GEN, AUG, NIT, AMP, CAZ	6
2.	*Staphylococcus*	CRX, AUG, AMP, CAZ	4
		AUG, AMP, CAZ	3
3.	*Providencia*	CRX, AUG,AMP,CAZ	4
		CRX, CPR, AUG, AMP, CAZ	5
4.	*Escherichia*	CRX, AUG, AMP, CAZ	4
		CRX, AUG, NIT, AMP, CAZ	5
5.	*Vibrio*	CRX, AUG, AMP, CAZ	4
6.	*Clostridium*	CRX, CPR, AUG, AMP, CAZ	5
		CRX, AUG, AMP, CAZ	4
		CRX, GEN, AUG, AMP, CAZ	5
7.	*Shigella*	CRX, AUG, AMP, CAZ	4
8.	*Proteus*	CRX, AUG, AMP,CAZ	4
		AMP, CAZ	2
9.	*Serratia*	CRX, CPR, AUG, AMP, CAZ	4
10.	*Salmonella*	CRX, AUG, AMP, CAZ	4
		CRX, CPR, AUG, AMP, CAZ	5
11.	*Klebsiella*	CRX, AUG, AMP, CAZ	4
12.	*Streptococcus*	CRX, GEN, CPR, OFL, AUG, NIT, AMP, CAZ	8
		CRX, CPR, AUG, AMP, CAZ	5
13.	*Acinetobacter*	CRX, GEN, AMP, CAZ	4
14.	*Enterobacter*	CRX, AUG, NIT,AMP,CAZ	5
		CRX, GEN, AUG, AMP, CAZ	5
15.	*Hafnia*	CRX, AUG, AMP, CAZ	4
16.	*Bifidobacterium*	CRX, AUG, AMP, CAZ	4
17.	*Lactobacillus*	CRX, AUG, AMP, CAZ	4
18.	*Pseudomonas*	CRX, AUG, AMP, CAZ	4
19.	*Citrobacter*	CRX, AUG, AMP, CAZ	4
20.	*Neisseria*	CPR, AUG, AMP, CAZ	4

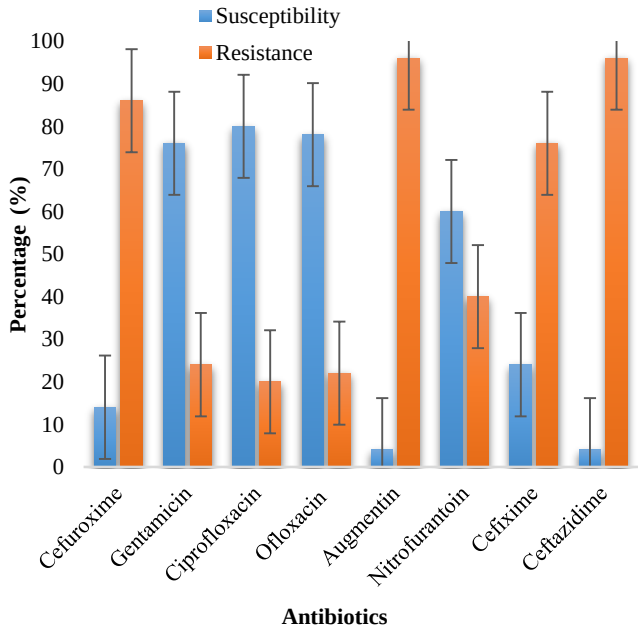

Figure 1: Cumulative percentage sensitivity pattern of the isolates to the antibiotics

Identified bacteria were tested for the presence of *CTXM* and *OXA* resistance genes and the PCR amplification process revealed the existence of these genes present on the bacterial plasmid (Table 4).

Plasmids carry genes that confer extra-chromosomal abilities advantageous to bacteria just as observed in this study. From this study, street cooked food were observed to be carriers of antibiotic resistant bacteria as 50 bacterial isolates were obtained from cooked street foods sold within the South Western States of Nigeria. These findings are consistent with observations made in Ethiopia.[10] 50% of the isolates were positive for ESBL genes. Of the positive isolates 95.8% (68/71) carried *blaCTX-M* genes all the *blaCTX-M* positive Enterobacteriaceae showed a multidrug resistant (MDR) phenotype with remarkable co-resistances (non-susceptibility rates) to aminoglycosides (92.2%), fluoroquinolones (78.1%) and trimethoprim/sulfamethoxazol (92.2%).

Figure 1 shows the cumulative sensitivity percentage profiles of the isolates to the antibiotics. According to the findings, augmentin (96%), ceftazidime (96%), and cefuroxime (86%) have significant levels of antibiotic resistance, but ciprofloxacin (80%), nitrofurantoin (60%), ofloxacin (78%), and gentamicin (76%) have good susceptibility levels. Fourteen (28%) of the isolates studied showed the presence of *CTXM* while fifteen (30%) carried *OXA* genes on the bacterial plasmid but five (10%) carried both genes on their plasmids. This supports a study on Enterobacteria isolates from ready-to-eat foods where numerous antibiotic resistance patterns were identified. All the isolates obtained showed high resistance levels to ceftazidime and augmentin.[11] A study was conducted in Brazil on isolates from domestic food-related environments, at least one antibiotic resistance was found in 125 isolates tested, while 6.4% of the isolates carried multiple antibiotic resistance abilities.[12] Also as reported in a study carried out on effluent receiving surface water, some antibiotic-resistant bacteria possess resistant genes borne on plasmids that help to confer resistance to sulfamethoxazole *(sul)* and trimethoprim *(dfr).*[13]

Table 4: Identified bacteria with Plasmid-mediated *CTXM* and *OXA* genes from Southwest Nigeria

S/N	Identified Bacteria	Resistance genes		Sampled Southwestern State
		CTXM	*OXA*	
1.	*Providencia* sp	+	+	Lagos
2.	*E. coli*	+	+	Ondo
3.	*Vibrio* sp	-	+	Osun
4.	*Shigella* sp	+	+	Ondo
5.	*Staphylococcus* sp	+	-	Ondo
6.	*Proteus* sp	+	-	Osun
7.	*Klebsiella* sp	+	-	Ondo
8.	*E. coli*	+	-	Osun
9.	*E. coli*	+	+	Oyo
10.	*Salmonella* sp	+	-	Oyo
11.	*Staphylococcus* sp	+	+	Ekiti
12.	*Klebsiella* sp	-	+	Ogun
13.	*Bacillus* sp	-	+	Osun
14.	*Pseudomonas* sp	-	-	Lagos
15.	*Acinetobacter* sp	-	+	Ekiti
16.	*Shigella* sp	-	+	Osun
17.	*Streptococcus* sp	-	+	Ondo
18.	*Clostridium* sp	-	+	Oyo
19.	*Bifidobacterium* sp	-	+	Ogun
20.	*Clostridium* sp	-	+	Ogun
21.	*Bacillus* sp	+	-	Lagos
22.	*Clostridium* sp	-	+	Osun
23.	*Citrobacter* sp	+	-	Ogun
24.	*Shigella* sp	+	-	Ogun
25.	*Lactobacillus* sp	+	-	Lagos

In the study, plasmids were isolated from 76 *Escherichia coli* from effluent and an effluent-receiving stream carrying sulfamethoxazole-trimethoprim resistance abilities. Inappropriate use of drugs in a way results in elevated levels of the ineffectiveness of such drugs.[14] In this study, high susceptibility levels were observed to ciprofloxacin and ofloxacin. The occurrence of plasmid-mediated antibiotic-resistant bacteria from cooked vended food in South Western Nigeria is presented in Table 2. Of the bacteria genera identified in the course of the study, *Bacillus* had the highest level of occurrence of 10% followed by *Klebsiella* at 8% of the 50 bacterial isolates identified. *Bacillus* sp. and other Gram-negative bacteria, carrying antibiotic resistance genes are known to be borne on plasmids.[15] 19 (36.5%) bacteria isolates from different sources in Iwo were reported to be resistant to more than three antibiotics. Of the multiple antibiotic-resistant isolates five showed the presence of the *CTX-M* gene, a β-lactam resistance gene that confers resistance to the β-lactam group of antibiotics.[7] This finding corroborates the findings of this present study that recorded several antibiotic resistance patterns from bacteria isolated from cooked vended foods sampled.

Conclusion

Discovery of new resistance characteristics in bacteria leads to resistance to available standard drug therapies, a condition that is a key public health challenge. Multiple resistant genes have been discovered in a range of bacteria species from various habitats, making antibiotic resistance in bacteria a major public health concern. The presence of these bacteria in street food is of high concern and there is therefore a need for standard food quality policies guiding the establishment of ready-to-eat food outlets.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Cosgrove SE and Carmeli Y. The impact of antimicrobial resistance on health and economic outcomes. *Clinical Infect Diseases* 2003; 36 (11): 1433–1437.
2. Nikaido, H. Multidrug resistance in bacteria, Annual Review of Bio. 2009; 78:119–146.
3. Baker-Austin C, Wright MS, Stepanauskas R and McArthur J V. Co-selection of antibiotic and metal resistance. Tr. in Mcb. 2006*;* 14:176–182.
4. Ruppé É, Woerther PL, and Barbier F. Mechanisms of antimicrobial resistance in Gram-negative bacilli. Ann Intensive Care 2015; 5(1):1.
5. Adamski CJ, Cardenas AM, Brown NG, Horton LB, Sankaran B and Prasad BV. Molecular basis for the catalytic specificity of the CTX-M extended spectrum β-lactamases. Biochemistry 2014; 54(2):447–57
6. Clinical and laboratory standards (CLSI). Performance Standards for Antimicrobial Susceptibility Testing, 30th ed. CLSI supplement M100. Wayne, PA: Clinical and laboratory Standards Institute 2020.
7. Adeleke OA, and Owoseni AA. Multiple-Antibiotic Resistance and Presence of CTX-M Genes among Enterobacteriaceae Isolates from Different Sources in Iwo, Osun State, Nigeria. *Mcb.* Res J. Int. 2020; 30(9):30-38.
8. Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, Edalatmand A and McArthur AG. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res, 2020; 48(D1):D517-D525.
9. Al-Mijalli SHS. Extended-Spectrum β-Lactamase Enzymes (ESBLs) Produced by *Escherichia coli* Urinary Pathogens at Riyadh, Saudi Arabia. J. of Antimicrob. Agents 2016; 2: 125.
10. Zeynudin A, Michael P, Sören S, Maxim M, Gabriele L, Michael H, Tefara B and Andreas W. Prevalence and antibiotic susceptibility pattern of CTX-M type extended-spectrum β-lactamases among clinical isolates of Gram-negative Bacilli in Jimma, Ethiopia. BMC Infectious Diseases 2018; 18:524.
11. Owoseni AA, and Onilude AA. Antibiotic sensitivity and sequence amplification patterns of genes in multidrug resistant enterobacteria isolates from processed foods in some West African countries. *P.* J. of Mcb. 2011; 60(4):309–316.
12. Azevedo I, Albano H, Silva J, and Teixeira P. Antibiotic resistance of Enterobacteriaceae isolated from the domestic food related environments. J. of Food Quality and Hazards Control 2015; *2*(2):51–55.
13. Suhartono S, Savin M and Gbur E. Genetic redundancy and persistence of plasmid-mediated trimethoprim/sulfamethoxazole resistant effluent and stream water *E. coli*. Water Research 2016; 103:197-204.
14. Adeleke OA and Owoseni AA. Multiple antibiotic resistance pattern of coliform bacteria isolated from different sources in Iwo, Nigeria. Open Sci J Bio. and Bioengineering 2018; 5(3):41-45
15. Christopher M, Paul O, Hamadi B. Association of metal tolerance with multidrug Resistance among environmental bacteria from wetlands of Lake Victoria basin. Agric Biol J.N. Am. 2014; 5:24-32.