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Carriage of carbapenemase genes among multidrug resistant *Escherichia coli* isolates from contact surfaces in food vending outlets within Nasarawa State, Nigeria

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ABSTRACT

Introduction: Contamination of food contact surfaces by *Escherichia coli* increases the risk of food-borne diseases through cross-contamination which becomes more complicated with the development of multidrug resistance by the pathogen. This study was aimed at investigating multidrug resistance and carriage of carbapenemase genes among *Escherichia coli* isolates from food contact surfaces in Nasarawa State, Nigeria.

Materials and Methods: A total of 924 swab samples (522 from Lafia and 402 from Nasarawa Eggon) were collected from various food contact surfaces in food vending outlets and screened for multidrug resistance and carbapenemase genes in *E. coli* isolates using standard culture, modified Kirby-Bauer disk diffusion, PCR amplification and agarose gel electrophoresis techniques. Data obtained were statistically analyzed and p-value set at 0.05 confidence level.

Results: Highest overall *Escherichia coli* contamination prevalence of 43.33 % was obtained from hawkers' outlets while table top surfaces had 35.43% with highest risk (odd ratio) of 1.94. Eateries and Hotels had no *E. coli* contamination. The prevalence values were significantly different ($p<0.05$) among the food contact surfaces, vending outlets and the two communities. Isolates from street vendors obtained highest resistance to OFX^f, REF^f, STR^a, CEP^c, NAL^f, SEP^s, AMP^p antibiotics group with MDRI of 0.7. The $bla_{-OXA-48}$, bla_{-VIM} and bla_{-KPC} carbapenemase genes were harbored by representative isolates.

Conclusion: The presence of multidrug resistant *E. coli* with carbapenemase genes from food contact surfaces in vending outlets serves as a public health challenge and the need for personal hygiene and strict adherence to antibiotics protocols by food vendors is highly encouraged.

1. Introduction

The availability and comparatively low prices of prepared foods from vendors relative to the processed and household preparations have increased their reliability to customers. This has also contributed to the increase in their popularity and preference by low- and medium-income earners in developing countries.[1,2] The rapid increase in human population especially in developing countries has augmented the activities of food vending services among youths, students and itinerary workers which has resulted in many patronizing foods vending outlets. The rapid development of the food industry has led to the establishment of various food vending options geared

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towards increasing the consumers' preferences to dine outside rather than cooking their meals at home.[3] The responsibilities of good hygienic practices at home have therefore been shifted to food vendors even though some hardly observe such practices.[4,5] In most developing countries where unemployment rates are high, food vending outlets serve as a reliable source of food security to low-income earners and also act as a major form of generating income and employment.[6,7] Despite the numerous advantages associated with the sales of cooked food, contaminated food has been reported to present serious safety and health concerns to consumers and food handlers basically due to their diversity, inadequate food safety knowledge and practices, insufficient basic hygiene, and lack of public awareness.[3,6]

Escherichia coli has been reported as one of the most common commensals in the gastrointestinal tract of humans and has therefore been used as an indicator of faecal contamination. However, it has also been implicated as a major cause of death especially among children in developing countries. Extra-intestinal *E. coli* has also been implicated in infections such as urinary tract infections, pneumonia, septicemia and nosocomial infections.[8] It has been established that the presence of bacterial contaminants on food contact surfaces increases the danger of foodborne illnesses through cross-contamination as a result of poor handling by food vendors.[9–13]

The consumption of street food increases the potential risk of foodborne illnesses such as diarrhea or traveler's diarrhea as is the case with *E. coli*.[14,15]The severity of these diseases depends specifically on the preparation process and exposure during sales, storage processes and or mode of handling of the foods. Moreover, illness resulting from the consumption of contaminated foods has become one of the most widespread public health challenges in contemporary society.[16,17]

There are indications that foods could be contaminated to unsafe levels at the points of consumption with air flora and other microorganisms from handlers, equipment, utensils and raw food materials, though epidemiological evidences on outbreak of foodborne diseases are scarce.[18]

E. coli has been shown to survive for extended periods on wooden, stainless steel and plastic surfaces.[19,20]Food contaminated with antibiotic-resistant pathogenic bacteria is an important threat to public health.[21] Aside from infecting humans, they act as potential reservoirs of antimicrobial resistance and the pathogens easily convey antibiotic-resistant elements to unrelated and related bacterial species.[22] Globally, there is an increase in the occurrence of antimicrobial resistance among foodborne bacterial pathogens in recent years.[23,24] The emergence and spread of carbapenemase-producing bacteria further serves as a serious threat to public health.[25] It has been discovered that carbapenemase-producing *E. coli* are not only present in clinical settings such as hospitals and clinics, but also can be transmitted by food vendors, meat vendors, and domestic animals.[26]

In Nigeria, the sales of ready-to-eat food in markets and streets as well as laterally within the road for travelers is a norm. Unfortunately, none of these food vendors is monitored or licensed by appropriate agencies or organizations to certify the safety of foods. As a result of the conditions and manner these food vendors display their items, there are possibilities of contamination with bacteria pathogens. The role of food contact surfaces in food vending outlets as major routes for the transmission of foodborne pathogens such as *E. coli* can therefore not be underestimated.[27–32] The safety of served meals can therefore be evaluated through the assessment of the level of contamination of food contact surfaces by notorious food pathogens such as *Escherichia coli* in food vending establishments.[7,33]

2. Materials and Methods

2.1. Sample collection

A total of 924 swab samples (402 from Nasarawa Eggon Community and 522 from Lafia Metropolis) were collected aseptically from food contact surfaces (table tops, spoons, plates and cups) in various food vending outlets using swab sticks and immersed into sterile packs containing normal saline. Samples were immediately transported to the Microbiology Laboratory, Federal University of Lafia, for further analyses within 6 hours.

2.2. Isolation and identification of Escherichia coli

Each diluted sample was spread on MacConkey agar (Difco) using a sterile bent glass rod and incubated for 24 hours at 45 °C. Suspected E. coli colonies with pink coloration were subcultured on Eosin Methylene Blue (EMB) agar plates and incubated for 24 hours at 37 °C under aerobic conditions. Colonies with greenish metallic sheens were isolated and placed on nutrient agar slants and refrigerated at 4^{o}C for biochemical analysis.

Identification of the *E. coli* isolates was carried out using cultural and morphological (colonial) appearance on nutrient agar plates. Characteristics such as shapes, size, consistency, color, and elevation of colonies. Gram staining was also performed and characteristic biochemical tests for *E. coli* such as urease, motility, indole, Methyl Red/Voges-Proskauer (MR-VP), citrate, and sugar fermentation on triple sugar iron agar (TSI) were carried out to confirm its identification.[34]

2.3. Antibacterial susceptibility testing

2.3.1. Inoculum preparation

The inoculum was prepared using a 24-hours old resuscitated culture of the test organism suspended in sterile peptone water (Difco) and incubated for 2 hours to obtain the log phase of growth. The culture suspension was then diluted to match the turbidity standard (McFarland 0.5) containing approximately 1.5 x 10^8 CFU/mL.

2.3.2. Antibiotics susceptibility analysis

Each of the isolates (*E. coli*) was subjected to antibiotic susceptibility testing using the Kirby–Bauer disc diffusion method as standardized and evaluated by the methods of the National Committee for Clinical Laboratory Standards.[35] Isolates resuscitated overnight on Nutrient Agar were suspended in sterile normal saline (0.9% w/v NaCl).The following antibiotics discs were in the panel: Tarivid (10 μg), Reflacine (10 μg), Ciprofloxacin (10 μg), Augmentin (30 μg), Gentamycin (10 μg), Streptomycin (30 μg), Ceporex (10 μg), Nalidixic acid (30 μg), Cotrimoxazole (30 μg), and Ampicilin (30 μg). A sterile swab was dipped into the standardized bacterial cell suspension and used to evenly inoculate the entire surface of sterile Mueller-Hinton agar plate and allowed for about 5 minutes for the agar surface to dry. The appropriate antibiotic discs were aseptically placed using sterile forceps, and all plates were incubated (Gallenkamp England, model IH-150) at 37°C for 24 hours. The diameters of the zones of inhibition were measured to the nearest millimeters as described by Cheesbrough.[34]

2.4. PCR screening for carbapenemase genes

2.4.1. DNA extraction (Boiling method)

The DNAs of *Escherichia coli* were identified by molecular methods with minor modifications.[36] Six (6) *E. coli* colonies (one each from the six food vending outlets that produced positive colonies) were randomly collected and plated on Luria Bertani Agar. The culture was mixed with 200 μl of double-distilled water in 1.5-ml micro-centrifuge tubes and boiled for 10 min in a water bath followed by snap chilling in ice for 5 min. The heat-treated bacterial suspensions were centrifuged at 10000 rpm for 5 min to pellet down the cell debris, and the supernatants were used as DNA templates in the PCR. The DNA was purified and quantified using Nano drop 1000 Spectrophotometer (Scale Tech., South Africa).

2.4.2. Amplification for Carbapenemase Genes

Primers (Table 1) for the carbapenemase genes were amplified by PCR method. Reaction mixtures in final volume of 25 μl was prepared with 10 μmol of each primer, 200 mM of dNTP, 1 unit of Taq polymerase, 2.5 μl of 10X reaction buffer, 1.5 mM $MgCl_2$ in final concentration (Inqaba Biotec, South Africa) and 100 ng DNA template. Amplification reactions was carried out in a thermocycler (Eppendorf master cycler, MA) under the following conditions: 94 ^{o}C for 5min, followed by 30 cycles of s94^{o}C for 25 sec, 52^{o}C for 40 sec, 72 ^{o}C for 50 sec, and 72 ^{o}C for 6 min for the final elongation step.

2.5. Agarose gel Electrophoresis

The amplified products were electrophoresed on agarose gels as described by Abimiku et al.[36] An aliquot of 8μl of PCR products stained with ethidium bromide was loaded into 1.0% (wt/vol) agarose gel (Inqaba Biotec, South Africa) wells with a molecular marker and run concurrently at 120 V for 30 min. The DNA bands were visualized and photographed under UV light 595nm.

2.6. Data analysis

SPSS version 20 (Statistical Package for Social Sciences) was used for statistical analysis of the data. One way and a two-way ANOVA (analysis of variance) were used to determine differences in the group means. Statistical importance was set at 0.05 confidence level. Odd ratio was also used to determine the risk factors evaluation for the isolation of *E. coli* from vending outlets.

3. Results

3.1. Occurrence of E coli isolates from various food vending outlets in various locations

Table 1 showed a total of 924 swap samples collected from Nasarawa Eggon and Lafia Local Government Areas of Nasarawa State. Among the samples collected from Nasarawa Eggon, 52/90 (57.78%), 46/120 (38.33%) and 24/120 (20.00%) from Hawkers, Street vendors and Restaurants respectively were positive for *E. coli* while samples from Hotels and Eateries had no *E. coli* contamination. For samples collected in Lafia, a total of 26/90 (28.89%), 29/120 (24.17%) and 23/120 (19.17%) from Hawkers, Street vendors, and Restaurants respectively were positive for *E. coli* while those obtained from Hotels and Eateries had no *E. coli* contamination. The overall *E. coli* positive samples were 200/924 (21.65 %) out of which 122/402 (30.35 %) were from Nasarawa Eggon Community and 78/522 (14.94 %) from Lafia Metropolis. Chi square test indicates that the distributions of *E. coli* were significantly different among the two locations and the vending outlets ($\chi^2 = 15.21$, $p < 0.001$).

3.2. Occurrence of E coli isolates from various contact surfaces

Results of the frequency of isolation of *E. coli* from the various contact surfaces are shown in Table 2. In Nasarawa

Table 1: Primers for carbapenem resistance genes used in this study

S/N	Target genes	Gene sequence	Amplicon size (bp)	Reference
1	bla-OXA-48	F 5´- GCTTGATCGCCCTCGATT -3´ R5´-GATTTGCTCCGTGGCCGAAA-3´	238	36
2	bla-VIM	F 5´- GATGGTGTTTGGTCGCATA-3´ R 5´-CGAATGCGCAGCACCAG-3´	398	36
3	bla-KPC	F5´-CATTCAAGGGCTTTCTTGCTGC-3´ R 5´-ACGACGGCATAGTCATTTGC-3´	498	36

F = forward primer; R = reverse primer

Eggon, highest frequency value of 38/78(48.72%) was obtained from table top swabs while the least value of 20/108(18.52%) was obtained from spoon swabs. Similar results pattern of 28/108(25.93%) and 6/138(4.35%) were obtained from table top and spoon swabs from Lafia Metropolis respectively. Highest overall frequency value of 66/186 (35.46%) was obtained from table top swabs while those from cups, plates and spoons had 57/246(23.17%), 51/246(20.73%) and 26/246(10.57%) respectively. The difference in the frequency values of the individual contact surface and those of the overall values in both locations were significant (x^2= 39.26; p < 0.01).

3.3. Potentials of vending outlets as risk factors for the isolation of E. coli from contact surfaces.

Table 3 showed the risk (odd ratios) associated with the isolation of *E. coli* from food vending outlets as reported in this study. In both Nasarawa Eggon and Lafia, Hawkers swabs had the highest odd ratio of 4.73 and 4.11 respectively with an overall value of 1.94 while overall values for Street vendors and Restaurants were 1.44 and 0.59 respectively.

3.4. Antibiotic resistivity test of E. coli isolates in contact surface swabs from various food outlets.

Table 4 showed the results of antibiotics resistivity test of *E. coli* isolates obtained from restaurants with the tested antibiotics. In a total of 47 *E. coli* isolates tested, 37 (78.72 %) isolates exhibited highest resistance to cotrimoxazole while 8 (17.02%) had intermediate resistance and 2 (4.26 %) were susceptible. All isolates were not resistant to ciprofloxacin, augmentin and gentamycin. Resistant potentials of the isolates to the various antibiotic was significantly different (x^2 = 395.88; p< 0.001).

Table 4 shows the results of antibiotics resistivity test of *E. coli* isolates obtained from street vendors with tested antibiotics. Among a total of 75 *E. coli* isolates tested, more than half of the isolates were resistant to cotrimoxazole (77.33%), ampicillin (73.33%) and streptomycin (53.33%) while none was resistant to ciprofloxacin, augmentin and gentamycin. Resistant potentials of the isolates with the various antibiotic was significantly different (x^2 = 598.77; p< 0.001).

The resistivity potentials of the *E. coli* isolates in contact surface swabs from food hawkers against the various antibiotics is shown in Table 5. In a total of 78 *E. coli* isolates tested, 55/78 (70.50%) were resistant to Ampicillin with intermediate value of 23/78 (29.49%) while none was resistant to Ciprofloxacin, Augmentin and Gentamycin. Resistance frequencies of isolates from various locations and contact surfaces to the tested antibiotics had statistically significant difference (x^2 = 670.49; p< 0.001).

3.5. Antibiotic resistance index of E coli isolates from various vending outlets

Table 6 shows the results of antibiotics resistivity test of *E. coli* isolates obtained from various vending outlets and their corresponding MDRI. For contact surfaces from street vendor, 29.19% of *E. coli* isolates were resistant to OFX^f, REF^f, STR^a, CEP^c, NAL^f, COT^s, AMP^p antibiotics group having the highest multidrug resistance index (MDRI) of 0.7. However, 40(34.78%) and 27 (25.23%) isolates from food hawkers and restaurants had highest resistance to OFX^f, REF^f, STR^a, AMP^p CEP^c and STR^a, CEP^c, NAL^f, SEP^s, AMP^p antibiotics group respectively with MDRI values of 0.5 each.

3.6. Molecular characterization of carbapenemase-producing E coli isolates

Figures 1, 2 and 3 showed the agarose gel electrophoresis results for the characterization of selected *E. coli* isolates based on the presence of the various carbapenemase genes. Among the 6 isolates randomly selected and screened, 4 were shown to harbor the bla-OXA-48 gene with 238bp (Figure 1) while all the isolates harboured Bla-VIM with 390bp (Figure 2) and bla-KPC with 498bp (Figure 3).

4. Discussion

Food vending outlets are highly patronized in Nasarawa State especially in Nasarawa Eggon and Lafia Metropolis which are proximal to Abuja, Nigeria's Capital City. However, these vending outlets has been implicated to be a source of microbial food contamination as found in this study. This study revealed a significantly higher level of *E.*

Table 2: Occurrence of *E. coli* isolates from various food vending outlets in various locations.

	Location					
	Nasarawa Eggon		Lafia		Total	
Vending outlets	N	n (%)	N	n (%)	N	n (%)
Restaurants	120	24 (20.00)	120	23 (19.17)	240	47 (19.58)
Street vendors	120	46 (38.33)	120	29 (24.17)	240	75 (31.25)
Hawkers	90	52 (57.78)	90	26 (28.89)	180	78 (43.33)
Hotels	72	-	120	-	192	-
Eateries	-	-	72	-	72	-
Total	402	122 (30.35)	522	78 (14.94)	924	200 (21.65)
	χ^2 = 73.11, P **<0.001**		χ^2 = 57.22, P **<0.001**		χ^2 = 136.51, P **<0.001**	

N = Total number of samples collected n = Number of positive samples

Table 3: Occurrence of *E. coli* isolates from various contact surfaces in both locations

	Location					
	Nasarawa Eggon		Lafia		Total	
Contact surfaces	N	n (%)	N	n (%)	N	n (%)
Plates	108	36 (33.33)	138	15 (10.87)	246	51 (20.73)
Cups	108	28 (25.93)	138	29 (21.01)	246	57 (23.17)
Spoons	108	20 (18.52)	138	6 (4.35)	246	26 (10.57)
Table tops	78	38 (48.72)	108	28 (25.93)	186	66 (35.48)
Total	402	122 (30.35)	522	78 (14.94)	924	200 (21.65)
	χ^2 = 21.06, P **<0.001**		χ^2 = 28.24, P **<0.001**		χ^2 = 39.26, P **<0.001**	

N = Total number of samples collected; n = Number of positive samples

Table 4: Potentials of vending outlets as risk factors for the isolation of *E. coli*

Location	Outlets	OP	O′N	ON	O′P	OR
Nasarawa Eggon	Restaurants	24	184	96	98	4416/9408 (0.47)
	Street vendors	46	206	74	76	9476/5624 (1.64)
	Hawkers	52	242	38	70	12584/2660 (4.73)
	Hotels	0	330	72	0	0
	Eateries	0	0	0	0	0
Lafia	Restaurants	23	347	97	55	7981/5335 (1.50)
	Street vendors	29	353	91	49	10237/4459 (2.30)
	Hawkers	36	380	64	52	13680/3328 (4.11)
	Hotels	0	402	120	0	0
	Eateries	0	450	72	0	0
Total	Restaurants	47	369	193	153	17343/29529(0.59)
	Street vendors	75	397	165	125	29775/20625(1.44)
	Hawkers	78	310	102	122	24180/12444(1.94)
	Hotels	0	522	192	0	0
	Eateries	0	522	72	0	0

OP = Examined outlet positive; O′N = Other outlets negative, ON = Examined outlet negative;

Table 5: Antibiotic resistivity test of *E. coli* isolates from restaurants

		Intermediate	Resistant	Susceptibility		
Antibiotics	N	n (%)	n (%)	n (%)	χ^2	P-value
Tarvid	47	36 (76.60)	8 (17.02)	3 (6.38)	395.88	**<0.001**
Reflacine	47	38 (80.85)	6 (12.77)	3 (6.38)		
Ciproflox	47	5 (10.64)	-	42 (89.36)		
Augmentin	47	2 (4.26)	-	45 (95.74)		
Gentamycin	47	4 (8.51)	-	43 (91.49)		
Streptomycin	47	16 (34.04)	17 (36.17)	14 (29.79)		
Ceporex	47	32 (68.89)	6 (12.77)	9 (19.15)		
Nalidixic acid	47	29 (61.70)	7 (14.89)	11 (23.40)		
Cotrimoxazole	47	8 (17.02)	37 (78.72)	2 (4.26)		
Ampicillin	47	19 (40.43)	26 (55.32)	2 (4.26)		

Table 6: Antibiotic resistivity test of *E. coli* isolates from street vendors

		Intermediate	**Resistant**	**Susceptibility**		
Antibiotics	N	n (%)	n (%)	n (%)	χ^2	p-value
Tarvid	75	40 (53.33)	22 (29.33)	13 (17.33)	598.77	**<0.001**
Reflacine	75	46 (61.33)	6 (8.11)	23 (30.67)		
Ciproflox	75	-	-	75 (100.00)		
Augmentin	75	1 (1.33)	-	74 (98.67)		
Gentamycin	75	1 (1.33)	-	74 (98.67)		
Streptomycin	75	9 (12.00)	40 (53.33)	26 (34.67)		
Ceporex	75	32 (42.67)	12 (16.00)	31 (41.33)		
Nalidixic acid	75	35 (46.67)	31 (41.33)	9 (12.00)		
Cotromoxazole	75	17 (22.67)	58 (77.33)	-		
Ampicillin	75	20 (267)	55 (73.33)	-		

Table 7: Antibiotic resistivity test of *E. coli* isolates from hawkers

		Intermediate	**Resistant**	**Susceptibility**		
Antibiotics	N	n (%)	n (%)	n (%)	χ^2	P-value
Tarvid	78	48 (61.54)	13 (16.67)	17 (21.79)	670.49	**<0.001**
Reflacine	78	55 (70.51)	3 (3.85)	20 (25.64)		
Ciproflox	78	2 (2.56)	-	76 (97.44)		
Augmentin	78	-	-	78 (100.00)		
Gentamycin	78	3 (3.85)	-	75 (96.15)		
Streptomycin	78	50 (64.10)	2 (2.56)	26 (33.33)		
Ceporex	78	64 (82.05)	1 (1.28)	13 (16.67)		
Nalidixic acid	78	39 (50.00)	13 (16.67)	26 (33.33)		
Cotrimoxazole	78	50 (64.10)	28 (35.90)	-		
Ampicillin	78	23 (29.49)	55 (70.51)	-		

Table 8: Antibiotic resistance index of *E. coli* isolates from various vending outlets

Antibiotics	**No. of isolates (%)**	**MDRI**
		Street vendors
OFX^f, REF^f, STR^a, CEP^c, NAL^f, COT^s, AMP^p	67 (29.91)	0.7
OFX^f, STR^a, NAL^f, COT^s, AMP^p	60 (26.79)	0.5
AMP^p, STR^a, COT^s	57(25.44)	0.3
SEP^s, AMP^p	40(17.85)	0.2
		Hawkers
OFX^f, REF^f, STR^a, AMP^p CEP^c	40 (34.78)	0.5
OFX^f, STR^a, NAL^f, COT^s,	25 (21.74)	0.4
AMP^p, STR^a, NAL^f	31 (26.96)	0.3
COT^s, OFX^f	19 (16.52)	0.2
		Restaurants
STR^a, CEP^c, NAL^f, COT^s, AMP^p	27 (25.23)	0.5
AMP^P, STR^A, COT^S	26 (24.30)	0.3
COT^s, AMP^p, CEP^C	37 (34.58)	0.3
OFX^f, STR^a,	17 (15.89)	0.2

MDRI= Multidrug resistance index, OFX=Tarivid, REF=Reflacine, CPX=Ciprolox, AUG=Augmentin, GEN=Gentamycin, STR=Streptomycin, CEP= Ceporex, NAL= Nalidixic Acid, COT= Cotrimoxazole, AMP=Amplicin, f = Flouroquinolones, c= Cephalosporin, p= Penicillin, s= Sulfonamides, a= Aminoglycosides

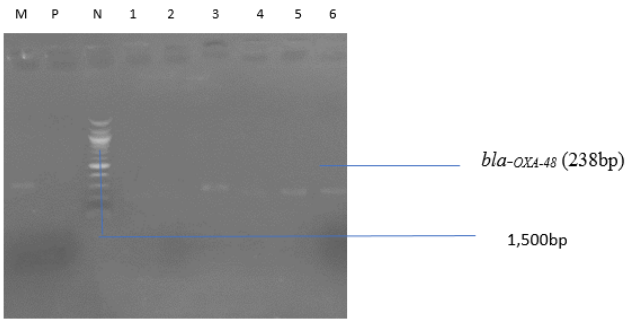

Fig. 1: Agarose gel electrophoresis ofcarbapenemase genes from *E. coli* isolates from Lafia and Nasarawa Eggon indicating positive bands in line 3, 4, 5, and 6 for bla$_{-OXA-48}$ gene. M represents a 1,500bp DNA molecular ladder. N represents negative control; P represents positive control.

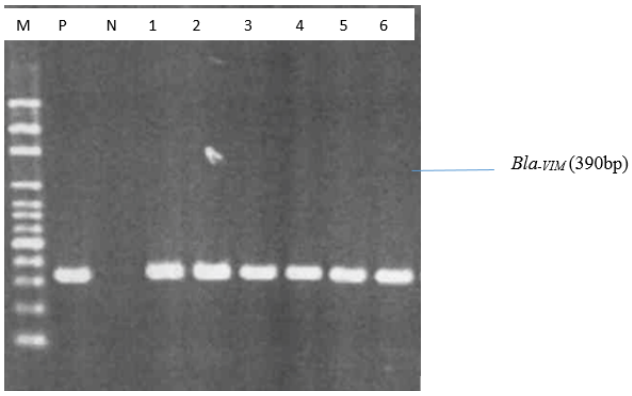

Fig. 2: Agarose gessl electrophoresis of carbapenemase genes from *E. coli* isolates from Lafia and Nasarawa Eggon indicating positive bands in lane 1 - 6 for bla$_{-VIM}$ gene. M represents a 1,500bp DNA molecular ladder. N represents negative control; P represents positive control.

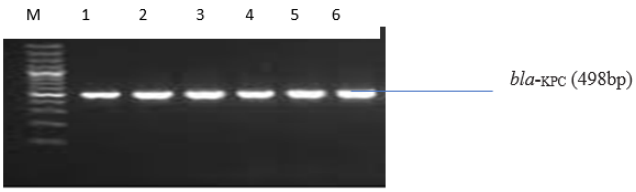

Fig. 3: Agarose gel electrophoresis ofcarbapenemase genes from *E. coli* isolates from Lafia and Nasarawa Eggon indicating positive bands in lane 1 - 6 for bla$_{-KPC}$ gene. M represents a 1,500bp DNA molecular ladder.

coli contamination in Nasarawa Eggon (48.72%) compared to Lafia (25.93%). This could be attributed to the existence of limited water availability in Nasarawa Eggon Community as seen during the study. Workers of most food vending outlets bought water from cart pushing water vendors and using the limited water to wash all utensils throughout the day compared to those in Lafia, the State Capital, where most vending outlets had potable water supply. A similar *E. coli* prevalence of 26 % was also observed in a study carried out from various vending outlets in Kaduna State, Nigeria.[37] In another finding, a slightly lower prevalence of 20.3% was also reported for *Escherichia coli* from different vending outlets in Kano Metropolis.[38] The lower prevalence seen in major towns may be related to the higher educational status and hygienic awareness of the vendors.

Results from Table 2 showed that table top swabs in both locations had highest occurrence of *E. coli* with 48.72% and 35.48% for Nasarawa Eggon and Lafia Metropolis respectively. This may be due to the large surface area of the tables often used in most food vending outlets which exposes them to soil, air, water and human contamination. Also, majority of the table tops in Nasarawa Eggon community had wooden surfaces which might have favored growth and biofilm formation as reported by Dantas et al.[19] Many studies have also reported significant levels of *E. coli* contaminations of food processing surfaces.[6,7] A similar study carried out in Makkah city, obtained 17.7% for *E. coli* contamination of food contact surfaces with 21% of washed serving dishes being contaminated with this pathogen. They also reported the isolation of the pathogen in at least one sample from each of the sixteen different contact surfaces.[7]

This study reported no occurrence of *E. coli* in food contact surfaces from the Hotels and Eateries surveyed while the pathogen was isolated from the other outlets with values of 43.33%. 31.25% and 19.58% for hawkers, street vendors and restaurants respectively. This could be attributed to a general lack of tap water, inefficient sewage disposal system, inappropriate storage conditions and the preparation and presentation of these food in the open or in crude structures as was the case with some restaurants and most street vendors and food hawkers. The mesophilic temperature and corresponding high relative humidity prevailing in both locations must have also contributed significantly to the unacceptable level of contamination.[39] This unfavorable environmental conditions are drastically reduced in hotels and eateries with the provision of air conditioned environments. This study agrees with a similar study carried out in Owerri, Nigeria that reported high prevalence of *E. coli* at various vending outlets.[40] A study in Kano State, Nigeria, had relatively lower values for vending outlets with the highest contamination level of 9.8% and least value of 2.8% though not acceptable.[38,39]

In both Nasarawa Eggon and Lafia, food hawkers' swabs had highest contamination risk (odd ratio) of 4.73 and 4.11 respectively with an overall value of 1.94 while overall values for Street vendors and Restaurants were 1.44 and 0.59 respectively. These values clearly suggest that the risk of cross-contamination from hawkers was higher relative to street vendors and restaurant outlets. More so, the hygienic status of the food hawkers was generally poor couple with the fact that they were always mobile without any specific sales point thereby exposing their utensils to air and human contamination. Oranusi et al.[40] and Nawawee et al.[41] reported that the nature of food vending sites may greatly affect the safety of food products

Resistance exhibited by *E. coli* to a number of antimicrobials including Aminoglycosides, Carbapenems, Penicillin, Cephalosporins, Fluoroquinolones, Sulfonamides, Tetracycline, and Trimethoprim have all been described in previous studies.[42–44] This study however obtained the resistivity profile for *E. coli* isolates from restaurants to be 78.72 % with highest resistance to cotrimoxazole while 8 (17.02%) had intermediate resistance and 2 (4.26 %) were susceptible. All isolates were not resistant to ciprofloxacin, augmentin and gentamycin. Resistant potentials of the isolates to the various antibiotic was significantly different ($x^2 = 395.88$; $p< 0.001$). Similar results were obtained from street vendors and hawkers isolates.The high level of resistance shown in this study can be attributed to the uncontrolled use of broad-spectrum antimicrobials for therapeutic purposes by clinicians in treating infections and over-the-counter prescriptions.[45] The sale of these drugs by drug hawkers (non-professionals) in the Nigerian community has encouraged the practice of self-medication, leading to misuse or abuse of drugs, which has also contributed to these observed resistance patterns.[44] This study agrees with other findings carried by Eyitayo et al.[46] in Lagos, Nigeria where *E. coli* isolates showed resistance to different antibiotics such as sulfamethoxazole (61.1%), Gentamicin (7.7%), Ampicillin (59.1%), Tetracycline (73.7%), Aztreonam (7.7%), Ciprofloxacin (13.0%). Other findings conducted by Ogbolu et al.[42] from different food vending outlets in Kaduna, Nigeria showed high *E. coli* resistance profile to different antibiotics such as Cotrimoxazole (46.2%), Amoxicillin (53.8%) and Sparfloxacin (27.2%) which is in agreement with the findings of this study.

This study showed most of the *E. coli* isolates to be multi-resistant to the commonly used antimicrobials agents such as Tarivid, Reflacine, Nalidixic acid, Amplicin, Cotrimoxazole, Streptomycin and Ceporex. These resistance profiles were common and could be accounted for by a number of known acquired resistance genes. The high MDRI values obtained from all the food outlets may suggest that the materials used in cleaning the contact surfaces such as water may have been highly contaminated with antibiotics due to their indiscriminate usage. Similar studies on multidrug resistant *E. coli* from drinking water sources in Ghana reported 63% of the isolates having MDRI values > 0.2.[43] Transfer of drug resistant genes among the isolates through horizontal gene transfer may be another possible reason for the high MDRI values.

Resistance of *E. coli* isolates against carbapenem antibiotics has been widely reported.[47] In the present study, it was found that bla-$_{OXA-48}$ (238bp) carbapenemase genes was harbored by 4 out of the 6 screened *E. coli* isolates while bla-$_{VIM}$ (390bp) and bla-$_{KPC}$ (498bp) genes were present in all the isolates. This is in agreement with Mahmoud et al.[48] who reported the presence of carbapenemase genes in 28% of *E. coli* isolates from domestic drinking water in Khartoum, Sudan. They detected the presence of bla-$_{OXA-48}$ (15.5%), bla-$_{SPM}$(8.8%) and bla-$_{KPC}$ (44.4%). The similarities in two carbapenemase genes may suggest the fact that most of the *E. coli* isolates in this study must have been introduced to contact surfaces through domestic water. However, contrary to their findings, this study did not find the bla-$_{SPM}$ gene among the isolates while their study did not also identify bla-$_{OX-48}$ as was done in this study. The variations of the results may be due to the differences in the geographical location, duration of sampling and large difference in the sample sizes. Fewer isolates were used in this study which may not be representative and hence serve as a limitation.

The observed multidrug resistance and carriage of carbapenemase genes by *E. coli* isolates from food surface contacts as reported in this study is significantly of public health importance as there is a poor drug regulatory frame work in Nigeria and the administration of carbapenem is usually reserved as a last resort against life-threatening illnesses.

5. Conclusion

Food contact surfaces from various vending outlets in Nasarawa Eggon and Lafia Metropolis have been shown to be potential vehicles for the transmission of *E. coli* to human population. Highest overall frequency of isolation was obtained from table tops swab samples from hawkers, street vendors and restaurants while the eateries and hotels had no isolate. Multidrug resistance among the isolates was high with majority having a multidrug resistance index (MDRI) of > 0.4. Some isolates were shown to harbor bla-$_{OXA-48,}$ bla-$_{VIM,}$ and bla-$_{KPC}$ carbapenemase genes which may account for the high MDRI values.

Further analysis is required to ascertain the carbapenemase genes frequencies using larger population of *E. coli* isolates and to develop an epidemiological framework geared towards limiting its transmission. Prevention of possible community spread of multidrug resistant *E. coli* through food contact surfaces is imperative

and will require a multi-disciplinary approach involving all stakeholders.

6. Authors' Contributions

This research was carried out with the total collaboration of all the authors. Author JFN conceived, designed and wrote the protocols and first draft of this study. Authors NTD and HUK compiled the necessary literature and statistical analyses while authors NSH and PA contributed to the laboratory analyses. All authors reviewed and approved the first manuscript.

7. Conflict of Interest

All items used in this research were obtained locally and mainly used in our area of research. There is therefore no conflict of interest between the authors and producers of such items.

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