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Antibiotic resistance profile of β-lactamase-producing *Escherichia coli* O157:H7 isolated from ready-to-eat foods in Ekiti State, Nigeria

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ABSTRACT

The severity and treatment failure of infections caused by *Escherichia coli* are largely due to possession of multiple virulence factors and resistance to different classes of antibacterial agents. This organism is one of the most important public health concerns globally because it can easily be transmitted through food to human beings causing serious infection. The genetic responsibility of such genes accountable for most of its activities can be easily transmitted from one strain to another. Shiga-toxin production among *E. coli* from ready-to-eat (RTE) food, and presence of virulence factors and antibiotic resistance among the strains were investigated in this study using standard methods. Overall, 231 *E. coli* strains were recovered occurring mostly in pounded yam [n=36 (15.5 %)], and least in meat pie [n=16 (6.9 %)]. Shiga-toxin production was detected in 185 (80.1 %) with the highest [n=32 (88.9 %)] occurring in pounded yam and the least found in fish pie (1, 5.9 %). β-lactamase production was detected in 179 (ca. 71.4 %) while 26 (ca. 11.3 %) of these were identified as *E. coli* O157:H7 serotype.The susceptibility pattern of *E. coli* isolates showed that remarkable susceptibility to fluoroquinolone. Levofloxacin was most effective on the isolates with 27.7 % resistance. Among the cephalosporins, cefotaxime and cefoxitin effectively inhibited 84.4 % and 55.8 % of the isolates respectively. All the isolates were resistant to cefixime, cefpodoxime and cefuroxime. This study revealed the presence of antibiotic resistant β-lactamase producing *E. coli* and its pathogenic strains O157:H7 in RTE foods sold in Ekiti State.

Introduction

Food-borne illnesses still remain a global challenge with highest occurrence in resource poor nations of the world (Mersha*et al*., 2009; Akbar *et al*., 2014; Hemeg, 2018). Poor handling and sanitation practices, weak regulatory system, inadequate food safety laws coupled with a lack of education for food-handlers remain the major contributors to high rate of food intoxication and/or infection (Haileselassie *et al*., 2013; Shahreza *et al*., 2017; Liu *et al.*, 2018). *Escherichia coli* remains the most significant food-borne disease-causing bacterium that has gained increasing attention in recent years (Clarence *et al*., 2009; Chileshe and Ateba, 2013; Akbar *et al*., 2014; Shahreza *et al*., 2017; Mashak, 2018; Nfongeh*et al*., 2018). Among different pathotypes of the organism, shiga-toxigenic strain is a significant pathogenic group of the species (Rasheed *et al*., 2014; Nfongeh *et al.,* 2018).

Illnesses caused by this pathogen can be life-threatening, and associated with several symptoms including haemolytic colitis (HC), haemolytic-uremic syndrome (HUS), bloody and non-bloody diarrhoea, and thrombotic thrombocytopenia purpura (TTP) (Warren *et al*., 2008; Chileshe and Ateba, 2013; Hessain *et al*., 2015; Singh *et al.,* 2016,Somda *et al.,* 2018) and antibiotic resistance worsens the severity of the infections caused by the isolates (Akbar and Anal, 2014; Oje *et al*., 2016; Li and Webstar, 2018). The resistance has been partly blamed on widespread and improper usage of antimicrobial agents in agriculture and medicine (Akbar *et al*., 2014; Shaikh *et al*., 2015; Zaman *et al.,* 2016; Gaougaou *et al.,* 2018).

The ability of bacteria to produce β-lactamase has made them to resist the action of β-lactam antibiotics (Amador *et al*., 2009; Adebayo-Tayo *et al*., 2012; Akbar *et al*., 2014; Gaougaou *et al.,* 2018). The level of the antibiotic resistance among the organism is worrisome (Warren *et al*., 2008; Akinyemi *et al*., 2015; Bok *et al*., 2015; Zaman *et al*., 2016). *Escherichia coli* isolated from different sources such as human (Iroha *et al*., 2012), wild, domesticated or food animals (Platell *et al*., 2011;

Dobiasova *et al*., 2013; Akbar *et al*., 2014; Hessain *et al*., 2015; Mashak 2018), ready-to-eat foods (Amador *et al*., 2009; Akbar *et al*., 2014; Shahreza *et al*., 2017) has been reported to produce β-lactamase hence resistant to most β-lactams.

The study therefore aims at investigating the level of association of *E. coli* with RTE food and its resistance to third generation cephalosporin and fluoroquinolones. The rate of Shiga-toxin production among *E. coli* recovered from RTE foods in Ekiti State, Nigeria was also studied.

Materials and methods

Isolation and Identification of E. coli

A total of 211 non-repeat RTE food samples which included rice (n= 35), beans (n= 28), semovita (n= 32), pounded yam (n= 36), scotch-egg (n= 30), meat pie (n= 16), doughnut (n= 28) and fish pie (n= 26). The foods were obtained from 15 food outlets from different towns/cities which either serve as seat of government or have tertiary institution(s), across the three senatorial districts of Ekiti State. The samples were collected into sterile containers and stored in an ice pack and transported to the laboratory for processing within one to two hours of collection (Olutiola *et al*., 2000). The samples were serially diluted and inoculated on Eosine Methylene Blue Agar (LAB M, UK) and incubated at 37 ^{o}C for 24h. The plates were observed for greenish metallic sheen characteristic of *E. coli*. The identity of the isolates was determined using different biochemical methods which include Gram reactions, indole, Voges-Proskauer and Methyl-Red test, utilization of citrate, fermentation of carbohydrate (arabinose, fructose, galactose, inositol, mannitol, mannose, rhamnose, ribose, sorbose and xylose). The results were interpreted according to Holt *et al*. (1994).

Antibiotic susceptibility testing

The antibiotic susceptibility of the isolates was tested using the disk diffusion method (Bauer *et al*., 1966). The isolates were standardized and susceptibility of the using the method of CLSI (2016). The isolates were tested against ten commercial antibiotic disks (Oxoid, Basingstoke, Hampshire, UK) with different concentrations (in µg) which included: cefixime (5), cefpodoxime (10), cefotaxime (30), cefoxitin (30), cefuroxime (30), ciprofloxacin (5), levofloxacin (5), norfloxacin (10), pefloxacin (5) and moxifloxacin (5). The standardized isolates were seeded on sterile Mueller-Hinton agar and the antibiotic disc was gently placed on the agar. The plates were incubated at 37 ^{o}C for 24 h and the results read. The diameter of the zone of inhibition was taken and the result interpreted according to CLSI guideline (CLSI, 2016). Multiple antibiotic resistance was also determined among the isolates.

Detection of β-lactamase-production

Modified iodometric method of Esan *et al.* (2016) was used to test for β-lactamase production by *E. coli* isolates. A 0.1ml volume of penicillin solution (6 mg/ml penicillin and 0.5 M phosphate buffer at pH 6.0) was dispensed into each well of a 96-well microtitre plate and test organisms were separately inoculated to make an opaque, milky suspension. Two (2) drops of starch solution (1 g soluble starch boiled to dissolve in 100 mL distilled water) were added and agitated gently. This was incubated at room temperature (25±2 ^{o}C) for 1 hour. A drop of the iodine reagent, prepared by dissolving 2.03 g iodine and 53.2 g potassium iodine and 100 ml distilled water, was then added and left for 60 minutes. The preparations were observed for decolourization (to colourless) within 10 minutes which is indicative of positive reaction.

Shiga-toxin production assay

Sobitol fermentation by *E. coli* isolates was used to determine Shiga-toxin production. Pure culture of the *E. coli* isolates was aseptically cultured on Sorbitol MacConkey agar (Lab M, UK) and incubated at 37 ^{o}C for 24 hours. The plate was observed after incubation, for pink colouration noted for Shiga-toxin production as described by Pradel *et al.* (2000).

Serotyping of isolates

Latex agglutination test described by Lupindu *et al*. (2014) was used for serotyping all *E. coli* isolates using a commercially prepared serotyping kit for *E. coli* O157: H7 (Oxoid, Basingstoke, UK). Following the manufacturer's instruction, the smooth suspension was observed for visible agglutination within 60 seconds. This procedure was repeated simultaneously using positive and negative control latexes. The presence of agglutination was taken to be positive result.

Results

A total of 231 *E. coli* was isolated from 211 different RTE food samples obtained from different food outlets in the study areas. The distribution of these isolates in each of the RTE foods is depicted on Table 1. *Escherichia coli* was mostly recovered from pounded yam with about 15.5 %, followed by rice (ca. 15.2 %) while 13.9 %, 13.0 % and 11.3 % of the isolates were recovered from semovita, scotch-egg and fish-pie, respectively. Other food samples such as beans and doughnut investigated each account for ca. 12.1% of the isolates whereas the least (6.9 %) of the *E. coli* was from meat pie.

Table 2 shows the results of shiga-toxin and β-lactamase production among *E. coli* isolates. Of the 231 isolates, 185 (80.1 %) were shiga-toxigenic with the highest number of 32 (88.9 %) from pounded yam isolates and the least number of 14 (50.0 %) from doughnut. Furthermore, 179 (77.5 %) of the isolates produced β-lactamase with the highest 31 (86.1 %) from pounded yam and the least of 13 (46.4 %) from doughnuts. The isolates that produced the combination of shiga-toxin and β-lactamase among the 231 *E. coli* isolates from the RTE foods were 165 (71.4 %), with the highest number of occurrence 24/26 (85.7 %) among isolates from beans samples and the least 11/28 (39.3 %) from doughnut. Although 30/35 (85.7 %) of the isolates from rice produced shiga-toxin and β-lactamase separately, but 25/35 (71.4 %) produced shiga-toxin and β-lactamase simultaneously.

Table 3 represents the percentage distribution of *E. coli* O157:H7 detected among the total 185 Shiga-toxigenic *E. coli* isolates recovered from RTE food samples. Of these 26 (11.3 %) belong to the strain of O157:H7, with highest number [6 (17.1 %)] of the strains recovered from rice. Five (5) of the strains were seperately isolated from semovita (15.6 %) and pounded yam (13.9 %), three [3 (18.8 %)] were from meat pie, two (2) of them were also recovered seperately from beans, scotch egg (6.7 %) and doughnut (7.1 %), while only a strain (3.8 %) of the isolates was recorded for fish pie.

The highest occurrence of β-lactamase producing *E. coli* O157:H7 was recovered from rice [n=6 (20.00 %)], followed by semovita [n=5 (18.52 %)] while the least was recovered from fish pie [n=1 (5.88 %)]. From the eight different types of

food samples a total of 185 Shiga-toxigenic strain of *E. coli* were isolated out of which only 26 (14.05 %) were confirmed to be β-lactamase producers as shown in Table 3.

The antibiogram of all 231 *E. coli* isolates to the antibiotics tested is depicted on Table 4. We found that moxifloxacin was the most effective among the antibiotics, as 212 (91.8%) of the isolates were susceptible to it. Moreover, the susceptibility to fluoroquinolones shows 6.9%, 11.3%, 16.0% and 20.3% of the isolates were resistant to ciprofloxacin, pefloxacin, norfloxacin and levofloxacin, respectively. Of the cephalosporins tested, cefotaxime and cefoxitin showed a level of effectiveness. About 11.7% and 44.1% of the isolates were resistant cefotaxime and cefoxitin, respectively, whereas all the isolates (n=231) showed the highest level of resistance to cefixime, cefpodoxime and cefuroxime.

Multiple antibiotic resistance (MAR) among the isolates is shown in Table 5. Out of 102 (22.4 %) that were resistant to three (3) antibiotics and 36.4% of the isolates were resistant to 4 antibiotics with 6 different resistotypes. The highest number of combinations (11) was recorded for 35 (15.2 %) of the *E. coli* isolates that were resistant to 5 antibiotics.

Table 1: Percentage distribution of *Escherichia coli* isolates in RTE food samples

		Distribution of isolates	
Food samples	**No. Examined**	**No. of isolates**	**Percentages**
Rice	33	35	15.2
Beans	30	28	12.1
Semovita	28	32	13.9
Pounded yam	28	36	15.5
Scotch-egg	28	30	13.0
Meat pie	15	16	6.9
Doughnut	27	28	12.1
Fish pie	22	26	11.3
Total	**211**	**231**	

Table 2: Production of pathogenic factors among *E. coli* isolates from RTE foods

		No. of producers (%)		
Food samples	**No. Examined**	**β-lactamase**	**Shiga-toxin**	**β-lactamase and Shiga-toxin**
Rice	35	30 (85.7)	30 (85.7)	25 (71.4)
Beans	28	26 (92.9)	27 (96.4)	24 (85.7)
Semovita	32	25 (78.1)	27 (84.4)	23 (71.9)
Pounded yam	36	31 (86.1)	32 (88.9)	29 (80.6)
Scotch-egg	30	24 (80.0)	24 (80.0)	25 (83.3)
Meat pie	16	13 (81.3)	14 (87.5)	12 (75.0)
Doughnut	28	13 (46.4)	14 (50.0)	11 (39.3)
Fish pie	26	17 (65.4)	1 (3.9)	16 (61.54)
Total	**231**	**179 (77.5)**	**169 (73.2)**	**165 (71.4)**

Table 3: Prevalence of β-lactamase producing *E. coli* O157:H7 among shiga-toxigenic *E. coli* isolates from RTE foods

	Occurrence (%) n= 231	
Food samples	**Shiga-toxigenic**	***E. coli* O157:H7**
Rice	30	6 (20.00)
Beans	27	2 (7.41)
Semovita	27	5 (18.52)
Pounded yam	32	5 (15.63)
Scotch-egg	24	2 (8.33)
Meat pie	14	3 (21.43)
Doughnut	14	2 (14.29)
Fish pie	17	1 (5.88)
Total	185 (80.1)	26 (14.05)

Table 4: Susceptibility pattern of *E. coli* isolates from RTE foods to cephalosporins and fluoroquinolones antibiotics

		Disc	Susceptibility (n=231)		
S/N	**Antibiotics**	**Content (µg)**	**Sensitive No (%)**	**Intermediate No (%)**	**Resistant No (%)**
		Cephalosporins			
1	CFM	5	0 (0.0)	0 (0.0)	231 (100)
2	CPD	10	0 (0.0)	0 (0.0)	231 (100)
3	CTX	3	195 (84.4)	9 (3.9)	27 (11.7)
4	FOX	30	129 (55.8)	27 (11.7)	102 (44.1)
5	CXM	30	0	0	231 (100)
Fluoroquinolones					
6	CIP	5	208 (90.1)	7 (3.0)	16 (6.9)

7	LEV	5	167 (72.3)	17 (7.4)	47 (20.3)
8	NOR	10	173 (74.9)	21 (9.1)	37 (16.0)
9	PEF	5	191 (82.7)	14 (6.0)	26 (11.3)
10	MXF	5	212 (91.8)	6 (2.6)	13 (5.6)

Keys: *CFM- Cefixime; CPD- Cefpodoxime; CTX- Cefotaxime; FOX- Cefoxitin; CXM- Cefuroxime;CIP- Ciprofloxacin; LEV- Levofloxacin; NOR- Norfloxacin; PEF- Pefloxacin; MXF- Moxifloxacin*

Table 5: Phenotypic pattern of multiple resistant *E. coli* isolated from RTE foods

Number of Antibiotics	**Resistotypes**	**Number of Occurrence No (%)**
3	CFM-CPD-CXM	102
	Total	102 (22.4)
4	CFM-CPD-FOX-CXM	51
	CFM-CPD-CXM-LEV	10
	CFM-CPD-CTX-CXM	9
	CFM-CPD-CXM-NOR	8
	CFM-CPD-CXM-PEF	4
	CFM-CPD-CXM-CIP	2
	Total	84 (36.4)
5	CFM-CPD-FOX-CXM-LEV	9
	CFM-CPD-CTX-FOX-CXM	8
	CFM-CPD-FOX-CXM-NOR	7
	CFM-CPD-CXM-LEV-PEF	3
	CFM-CPD-FOX-CXM-PEF	2
	CFM-CPD-FOX-CXM-NOR	1
	CFM-CPD-CXM-LEV-NOR	1
	CFM-CPD-CTX-CXM-NOR	1
	CFM-CPD-CXM-LEV-PEF	1
	CFM-CPD-CXM-CIP-NOR	1
	CFM-CPD-CXM-LEV-MXF	1
	Total	35 (15.2)
6	CFM-CPD-FOX-CXM-LEV-NOR	4
	CFM-CPD-FOX-CXM-LEV-PEF	3
	CFM-CPD-FOX-CXM-LEV-MXF	1
	CFM-CPD-FOX-CXM-NOR-PEF	1
	CFM-CPD-CXM-CIP-NOR-PEF	1
	CFM-CPD-FOX-CXM-CIP-NOR	1
	CFM-CPD-FOX-CXM-CIP-LEV	1
	Total	12 (5.2)
7	CFM-CPD-FOX-CXM-CIP-NOR-MXF	1
	CFM-CPD-FOX-CXM-CIP-LEV-NOR	1
	CFM-CPD-FOX-CXM-LEV-NOR-MXF	1
	CFM-CPD-CTX-FOX-CXM-CIP-NOR	1
	CFM-CPD-CTX-FOX-CXM-LEV-PEF	1
	CFM-CPD-CTX-CXM-LEV-PEF-MXF	1
	Total	6 (2.6)
8	CFM-CPD-FOX-CXM-CIP-LEV-PEF-MXF	1
	CFM-CPD-FOX-CXM-CIP-LEV-NOR-PEF	1
	Total	2 (0.9)
9	CFM-CPD-FOX-CXM-CIP-LEV-NOR-PEF-MXF	1
	CFM-CPD-CTX-FOX-CXM-LEV-NOR-PEF-MXF	1
	Total	2 (0.9)
10	CFM-CPD-CTX-FOX-CXM-CIP-LEV-NOR-PEF-MXF	5
	Total	5 (2.2)
Total		**231 (100)**

Keys: *CFM- Cefixime; CPD- Cefpodoxime; CTX- Cefotaxime; FOX- Cefoxitin; CXM- Cefuroxime;CIP- Ciprofloxacin; LEV- Levofloxacin; NOR- Norfloxacin; PEF- Pefloxacin; MXF- Moxifloxacin*

Discussion

The risk of food poisoning particularly associated with the consumption of street-vended foods remains a serious threat in many parts of the world, with microbial contamination being one of the major problems, especially in low resource Nations. It is recognized that food-borne pathogens represent a serious health hazard, with the risk mainly depending on the type of food and the way of processing. The ignorance of street food sellers and or handlers as the cause of food-borne illnesses is a risk factor that must never be ignored (FAO, 2007; Singh *et al.*, 2016; Bengtsson-Palme *et al.,* 2018). *Escherichia coli* continues to take a centre stage are the first germs of faecal

contamination indicators. The presence of *E. coli* is an indicator of lack of proper hygiene in RTE production process. Consumption of vended RTE foods proved to be a major route of infection and an important role in the epidemiology of food-borne infections. Antimicrobial-resistant bacteria have been recovered from both humans (Iroha *et al*., 2012; Arzanlou *et al.,* 2017) and a wide variety of foods, which include vegetables, confectionaries (Pinegar and Cooke, 1985; Oje *et al*., 2016; Shahreza *et al*., 2017), meat, meat products and poultry products (Schroeder *et al*., 2004; Dobiasova *et al*., 2013; Hemeg, 2018; Mashak 2018) and others. There is therefore a need for surveillance of emerging antimicrobial-resistant organisms in RTE foods, because there is steadily accruing evidence from across the world indicating RTE foods as sources of antimicrobial-resistant organisms (Schroeder *et al*., 2004; Oluyege *et al*., 2009; Oje *et al*., 2016; Somda *et al.*, 2018). This study therefore, also shows that antibiotic-resistant bacteria are associated with RTE foods in Ekiti State.

Considering the results obtained from this study, there is an indication that most food samples were contaminated by *E. coli*, an indicator of faecal contamination (Tambekar *et al.,* 2011; Ibrahim *et al.,* 2013; Oranusi *et al.,* 2013; Olawale *et al.*, 2014; 2015). Similarly, Okonkwo *et al.* (2008), Ajao and Atere (2009) and Oje *et al*. (2016) isolated the bacterium from RTE food samples. Oluyege *et al*. (2009) reported *E. coli* to be most abundant among the organisms isolated from foods sold on a university campus in Nigeria.

The results of this study support the finding of Moyo and Baudi (2004) that attributed the presence of indicator organisms, pathogens or high bacterial counts in food stuffs, food contact surfaces, equipment and utensils to poor personal and environmental hygiene. The results of this study fall below acceptable and tolerable levels of bacteria in RTE (ICMSF, 1996). The high level of unhygienic practices, use of contaminated instruments and materials in food processing could be the major factors that contributed to high bacterial load in food samples (Wilfred *et al*., 2012; Akbar and Anal, 2014).

We found that 80.1 % of the *E. coli* recovered from the RTE foods in the study areas are Shiga-toxigenic. Shiga-toxin producing *E. coli* has earlier been described as one of the most recognised important human pathogens of animal origin (Rani *et al*., 2005). Human infections due to Shiga-toxin producing *E. coli* are primarily associated with the consumption of faecally contaminated foodstuffs (Shahreza *et al*., 2017); this is evidenced in the findings in the present study showing abundant shiga-toxin production among the *E. coli* isolates from RTE foods. This is also in agreement with the study of Kalantar *et al*. (2013) that reported the incidence of shiga-toxigenic *E. coli* strains in foods.

Escherichia coli O157:H7 are mostly associated with food materials, especially those of bovine origin, ground meat and raw milk (La Ragione *et al*., 2008; Lye *et al*., 2013; Bhutani *et al*., 2015). Several studies have reported the prevalence of this organism in different food sources from around the world, including vegetables (Bhutani *et al*., 2015), poultry meats (Akbar *et al*., 2014), Domestic ruminant like cattle, sheep and goat (La Ragione *et al*., 2008, Ajayi *et al.*, 2012; Mashak 2018)) and some RTE foods (Amador *et al*., 2009; Strahilevitz *et al*., 2009; Platell*et al*., 2011; Liu *et al.,* 2018; Somda*et al.,* 2018). FAO/WHO/OIE (2007); Lavigne *et al*. (2007) and Cavaco *et al*. (2008) proposed that commensal microflora could represent a long-term reservoir of resistance genes that could be transferred horizontally to other bacteria.

In this study, we describe the high prevalence of resistance to third generation cephalosporins in *E. coli* isolates from RTE foods as an attribute of the inherent pathogenic factors such as shiga-toxin and β-lactamase production. Our results show that and 77.5 % produced β-lactamase making their presence in food particularly more worrisome. Production of β-lactamase among the isolates signifies their ability to resist β-lactams (Amin *et al*., 2009; Shaikh *et al*., 2015; Dobiasova *et al*., 2013; Bhutani *et al*., 2015; Gaougaou *et al.,* 2018). In developing nations, the abuse of antibiotic has largely contributed bacterial resistance to antibiotics (Adebayo-Tayo *et al*., 2012). This is consistent with the findings of Schroeder *et al*. (2002), Ajao and Atere (2009), Clarence *et al*. (2009), Hessain *et al*. (2015) and Kaushik *et al.* (2018) that attributed the multiple antibiotic resistance and virulence of *E. coli* isolated from meat and meat products to the presence of shiga-toxins and β-lactamase maker genes. Resistance development also might be related to exchange of resistance factors between related bacteria (Tenover, 2006; Zaman *et al.,* 2016; Bengtsson-Palme *et al.*, 2018; Blau *et al.,* 2018).

Conclusion

In conclusion, the presence of virulence factors and high resistance to antibiotics of *E. coli* isolated from RTE foods sold at food outlets particularly those included in this study in Ekiti State present an unacceptable situation that raises serious public health concerns. This is potentially hazardous to the health of consumers and producers alike. New strategies are hence needed to reduce the level of contamination to acceptable minimum.

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