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Molecular detection of virulence gene in *E. coli* 0157:H7 from raw meat sold in four different markets in Abuja, Nigeria

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Abstract

*Escherichia coli*are a leading cause of many human food-borne infections such as diarrheal, haemorrhagic colitis and haemolytic uremic syndrome. Isolation of *E.coli* from raw meat sold in selected market in FCT Abuja was carried out. A total of 120 meat samples of beef, pork and chevon were collected and *E.coli* isolated were identified using standard microbiological methods. The antibiotic susceptibility test for the isolates was carried out and interpreted in accordance with clinical and laboratory standard institute (CLSI) Protocol. The molecular detection of*E.coli* virulence genes was carried out using Polymerase Chain Reaction (PCR) method. Out of the 120samples the occurrence of *E. coli* was 28.3%. The highest percentage occurrence was observed from pork (35.0%) followed by beef (27.5 %) and the lowest was from chevon (22.5 %). *E. coli* isolated from beef were highly susceptible to imepenems (100%) followed by gentamicin (90.9%), amoxicillin-clavulanic acid and cefotaxime. (81.8%) but less susceptible to nitrofrantan (54.5%).*E. coli* isolated from goat meat were highly susceptible to imepenems, gentamicin, ciprofloxacin and cefotaxime (88.8%) followed by cefoxitin and, nitrofrantan (77.7%), ceftazidime and ampicillin (66.6%), but less susceptible to amoxicillin-clavulanic acid (55.5%).*E. coli* isolated from pork meat were highly susceptible to imepenems (85.7%)followed bygentamicin with (78.5%) but less susceptible to amoxicillin-clavulanic acid (42.8%).The *E. coli* pathotype genes that were detected from cow meat were *eltB* (33.3%) and *stx1* (66.6%). From chevon meat genes detected were *eltB*(66.6%) and *stx2* (100%). From pork the genes detected were *stx2*(100%) and *stx1*(66.6%). The isolates were resistance and different *E.coli*Pathotype genes were detected from the resistance *E. coli* Isolates. The results obtained suggest that some meat samples sold in markets in Abuja harboured *E.coli* 0157:H7 with various virulence genes. This constitutes a great risk to human health. There is therefore, the need to process the meat properly before consumption.

Keywords: food-borne, antibiotic, Pathotype, diarrheal, meat and detected

Introduction

Escherichia coli (*E. coli*) are a Gram-negative, facultative anaerobic, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded animals. Most of the *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination (Saba *et al.,* 2015). The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K2 (Vogt and Dippold, 2005) and by preventing the establishment of pathogenic bacteria within intestine (Hudault *et al.,* 2001) Nevertheless, the recognition of Shiga Toxin *E.coli* (STEC) as a distinct class of pathogenic *E. coli* resulted from some epidemiological observations, which were characterized by severe abdominal pain and watery diarrhoea and hemolytic-uremic syndrome (Dunn *et al.,* 2004). Most infections caused by *E. coli* O157 result from the consumption of food and water contaminated with faecal matter of infected animals (Smith *et al.,* 2014).*Escherichia coli* O157 are an important food-borne pathogen that can cause diarrhoea, haemorrhagic colitis (HC), and haemolyticuraemic syndrome (HUS) in humans (Caro *et al*. 2006). *E. coli* O157:H7 is the predominant serotype causing severe human infections. Cattle meat products are considered to be the main reservoir of *E. coli* O157 worldwide. STEC can be found in the faecal flora of a variety of animals, especially ruminants. Ruminant livestock such as cattle, deer, goats and sheep naturally carry *E. coli* O157:H7 in their systems. The cattle, however, are considered to be one of the primary sources of *E. coli* O157:H7 worldwide. Numerous studies have shown that *E. coli* O157:H7 prevalence is widespread in dairy and beef animals, and can be found in, on and around cattle in most parts of the world without causing any disease symptoms (Smith *et al.,* 2014). In most developing countries, especially Africa and India, cattle are allowed to roam freely among people at home and in the cities. The risk of contracting STEC from cattle dung is therefore higher in these regions since some of the remnants of the faeces finally get into streams through surface running water (Addo *et al,*. 2011). These sources of water are the last resort for some inhabitants to get water for their daily activities. Raw cow meat has an outstanding nutritional quality, but is also an efficient vehicle for transmission of diseases to humans. Pathogenic bacteria pose a serious threat to human health, and constitute a significant proportion of all dairy-related diseases (Donkor *et al.,* 2007). The emergence of new foodborne pathogens such as STEC in raw cow meat in recent studies may increase the threat of ingestion and transmission of food borne pathogens and ingestion of harmful toxins. The most important causes of foodborne diseases are Shiga toxin-producing *E. coli* among other serotypes of *E. coli*. Shiga Toxin Producing *E. coli* are widespread in the guts of humans and warm-blooded animals. STEC are thought to be reserved in livestock mainly cattle, sheep, goat and poultry birds although some domestic animals such as cats and dogs do harbor these bacteria. The pathogenic nature of STEC is attributed to a number of virulence factors including shiga toxins, intimin and haemolysin (Musa *et al*., 2013). STEC has surfaced as a major foodborne pathogen and has become a major public health concern causing diseases from mild diarrhoea to haemorragic colitis, haemorrhagicuraemic syndrome (HUS) and thrombocytopenic purpura (TTP) in humans. This study focus on isolation and molecular detection of virulence gene in *E. coli* 0157:H7 from raw meat sold in four different markets in Abuja, Nigeria.

Materials and Methods

Study area

The study was carried out in markets within Abuja, Federal Capital Territory (FCT). Abuja falls within latitude 7°45E and at latitude 7°39N at the equator and 850m above the sea level. Federal Capital Territory (FCT) Abuja is approximately 58k from Keffi (FCDA, 2015).

Sample collection

Total of 120 meat samples comprising 30 beef, 30 pork and 30 chevon were purchased from different sellers of raw meat from each of the markets namely Wuse market; Garki market, Kubwa market and Utako market in FCT, Abuja and packaged with sterile sample container and transported in a cool box with ice pack within 6 hours to Department of Microbiology laboratory, Nasarawa State University Keffi, Nasarawa State.

Isolation of *E. coli* O157:H7

Isolation of *E. coli* O157:H7 from raw meat samples were carried out using the method described by Oloyede*et al*. (2016), 2.0g of each meat sample was ground and thoroughly homogenized in 18.0ml of sterile tryptone soya broth supplemented with 20mg/L novobiocin and incubated at 37℃ for 24 hours. The enrichment broth cultures were streaked onto Sorbitol-MacConkey agar. The plates were then incubated at 37℃ for 24 hours. Non- sorbitol fermenting colonies were picked and sub-cultured on sorbitol-MacConkey agar plates. The Non-sorbitol fermenting colonies were then serologically typed for O157:H7 antigens by slide agglutination test using polyvalent and monovalent anti- *E. coli* O and H sera.

Identification of *Escherichia coli*

The *Escherichia coli* isolates were further identified using standard microbiological procedures based on cultural, morphological and biochemical characteristics such as Indole Test, Methyl Red Test/Voges-Proskauer Test and Citrate Test

Molecular Detection of Virulence Genes

DNA extraction

Purification of DNA was achieved using a genomic DNA purification kit (Fermentas, GmbH, Germany) according to the manufacturer's instruction and the total DNA was measured at 260 nm optical density according to the method described by Ebrahim*et al.* (2012)

Detection of fliCh7 gene

All oligonucleotide primers was obtained from a commercial source (Cinna Gen, Iran). In order to determine the H7 (fliCh7) gene of *E. coli* O157:H7 strains, PCR analysis was used as authorised by Goncuoglu *et al*. (2010). The PCR was performed with primers as described previously15 (Table 1) in a final volume of 50 µL containing 1× Reaction Buffer (Fermentas, GmbH, Germany), MgCl2 (Fermentas, GmbH, Germany), each of the four deoxynucleoside triphosphates (dNTPs) (Fermentas, GmbH, Germany), Taq DNA polymerase (Fermentas, GmbH, Germany), 0.50 µM of primers and 10 µL DNA. DNA amplification reactions were carried out using a DNA thermal cycler (Master Cycler Gradiant, Eppendrof, Germany) with the following program: one cycle of 2 min at 94 °C, 35 cycles of denaturation at 94 °C for 20 s, annealing at 54 °C for 1 min, and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. The PCR products were stained with 1% solution of ethidium bromide and visualized under UV light after gel electrophoresis on 1.5% agarose.

Detection of virulence factors

The *E. coli* O157:H7/NM isolates were screened for the presence of stx1 (encoding for Shiga toxin 1), stx2 (encoding for Shiga toxin 2), eaeA (encoding for intimin),and ehlyA (encoding for enterohemolysin) genes using PCR method.14,15 The list of primers and the sizes of the expected PCR products are given in Table 1. According to Fratamico *et al*., multiplex PCR protocol was used to prepare the master mix with a total concentration of 50 µL containing incomplete 1× Reaction Buffer [160 mM (NH4)2SO4, 670 mMTris-HCl (pH 8.8); 0.1% Tween-20] (Fermentas, GmbH, Germany), 3.0 mM MgCl2 (Fermentas, GmbH, Germany), 400 µM each of the four deoxynucleoside triphosphates (dNTPs) (Fermentas, GmbH, Germany), 2.5 U Taq DNA polymerase (Fermentas, GmbH, Germany), 0.50 µM of all primers that were used.

Results

The cultural, morphological and biochemical characteristics of *E .coli* isolated from selected raw meat sold in markets in FCT, Abuja are as shown in Table 1 The pinkish colonis on MCA, greenish Mentallic sheen which were Gram Negative, rod- shape, indole Positive, Methyl red Positive and sugar fermentation test as shown in Table 4.1 were identified as *E .coli*

Percentage occurrence of the *Escherichia coli* isolates

The percentage occurrence of the *Escherichia coli* isolates is as given in Table 2. Out of 120 samples collected the percentage occurrence was 34(28.3%) and the highest percentage occurrence was observed from pork meat 14 (35.0%) followed by beef meat 11(27.5 %) and the lowest was from chevon meat 9 (22.5 %) respectively.

The occurrence of *Escherichia coli* isolated from raw meat in respect to different markets in FCT, Abujais as given in Table 4.3. It was recorded that from location A (Wuse market) chevon had highest occurrence (13.3%) while beef and pork had the least (6.6 %)each. From location B (Garki market) the occurrence from beef, chevon and pork were10.0 % respectively. From location C (Kubwa market) the highest occurrence was observed from pork (16.6 %) followed by beef (13.3 %) and the least was from chevon(6.6 %). From location D (Utako market) the highest occurrence was observed from pork (13.6 %) while beef recorded 6.6 % respectively. No *E coli* was recovered from chevon in location D. (Table 3)

Table 1: Cultural, morphological and biochemical characteristics of *Escherichia coli* isolated from selected raw meat sold in markets in Abuja,FCT Nigeria

.Cultural characteristics	Morphological characteristics		Biochemical characteristics								Inference
	Gram stain	Morphology	Ind	Mr	Vp	Ct	Lac	Glu	Gal	Suc	
Pinkish colonies on MCA and greenish metallic sheen colonies on EMB agar	-	Rod shape	+	+	-	-	+	+	+	+	*E. coli*

MCA= Mac Conkey Agar; EMB= Eosin Methylene blue; - =Negative; + =positive; Ind =Indole; Mr = Methyl Red;Vp =Voges Proskauer ;Ct =Citrate; Lac=Lactose ;Glu = Glucose; Gal =Galactose ;Suc=Sucrose.

Table 2: Percentage occurrence of *Escherichia coli* isolated from selected raw meat sold in markets in Abuja, FCT

Sample	No. of Samples	No. (%) isolated
Beef	40	11(27.5)
Chevon	40	9(22.5)
Pork	40	14(35.0)
Total	120	34(28.3)

Table 3 Occurrence of *Escherichia coli* isolated from selected raw meat sold in markets in Abuja, FCT

Location	No sample	Beef No. (%)	Chevon No. (%)	Pork No. (%)
A	30	2(6.6)	4(13.3)	2(6.6)
B	30	3(10.0)	3(10.0)	3(10.0)
C	30	4(13.3)	2(6.6)	5(16.6)
D	30	2(6.6)	0(0.0)	4(13.6)

Key; A- Wuse market; B- Garki market; C- Kubwa market and D- Utako market

Antibiotic susceptibility of *Escherichia coli* isolated from raw meat

The antibiotic susceptibility of *Escherichia coli* isolated from raw meat in respect to different meat type is as given in Table 4. The results revealed that *Escherichia coli* isolated from beef were more susceptible to imepenems (100%) followed by gentamicin (90.9%), amoxicillin-clavulanic acid and cefotaxime. (81.8%), ampicillin, ceftazidime and Cefoxitin(72.7%), ciprofloxacin and ofloxacxin (63.6%) but less susceptible toNitrofrantan(54.5%). *Escherichia coli* isolated from chevon were highly susceptible to imepenems, gentamicin, ciprofloxacin and cefotaxime (88.8%) followed by cefoxitin and nitrofrantan(77.7%), ceftazidime and ampicillin 66.6%, and the lowest was amoxicillin-Clavulanic acid (55.5%). It was also observed that *Escherichia coli* isolated from pork were highly susceptible to imepenems (85.7%)followed by gentamicin with (78.5%), ciprofloxacin with (71.4%), Cefoxitin (64.2%), ampicillin and Cefotaxime (57.1%) but less susceptible to Amoxicillin-Clavulanic acid, Ceftazidime and Nitrofrantan(42.8%) respectively.(Table 4)

Occurrence of pathotypes genes in Antibiotic-resistant *Escherichia coli*from selected raw meat sold in different markets in Abuja, FCT

The pathotypes genes detected in some antibiotic resistant *Escherichia coli* isolated from selected raw meat sold in markets in Abuja, FCT is as given in Table 4.5.The results revealed that *eltB* pathotype base pair of 322bp, *stx2* pathotpye base pair of 320bp and *stx1*pathotype base pair of 322.(plate 1, 2, 3 respectively)

The occurrence of *E. coli* pathotype genes that were detected from cow meat were *eltB* (33.3%) and *stx1* (66.6%). From goat meat genes detected were *eltB*(66.6%) and *stx2* (66.6%). From pork the genes detected were *stx2*(100%) and *stx1*(66.6%) each(Table 5).

Table 4 Antibiotics susceptibility of *Escherichia coli* isolated from selected raw meat sold in markets in Abuja, FCT

		Susceptible (%)		
Antibiotics	Disc Content(µg)	Beef n= 11	Chevon n=9	Pork n=14
Ampicillin	30	8(72.7)	6(66.6)	8(57.1)
Amoxicillin-Clavulanic acid	30	9(81.8)	5(55.5)	6(42.8)
Ceftazidime	30	8(72.7)	6(66.6)	6(42.8)
Cefotaxime	30	9(81.8)	8(88.8)	8(57.1)
Cefoxitin	30	8(72.7)	7(77.7)	9(64.2)
Ciprofloxacin	5	7(63.6)	8(88.8)	10(71.4)
Gentamicin	10	10(90.9)	8(88.8)	11(78.5)
Imepenems	5	11(100)	8(88.8)	12(85.7)
Ofloxacxin	30	7(63.6)	6(66.6)	7(50.0)
Nitrofrantan	30	6(54.5)	7(77.7)	6(42.8)

Table 5:Occurrence of pathotypes genes in Antibiotic-resistant *Escherichia coli* from selected raw meat sold in markets in Abuja, FCT

Pathotypes genes	Frequency (%) (n=10) Beef (n=3)	Chevon (n=3)	Pork (n=3)
eltB	1(33.3)	2(66.6)	0(0.0)
stx2	0(0.0)	2(66.6)	3(100)
stx1	2(66.6)	0(0.0)	2(66.6)

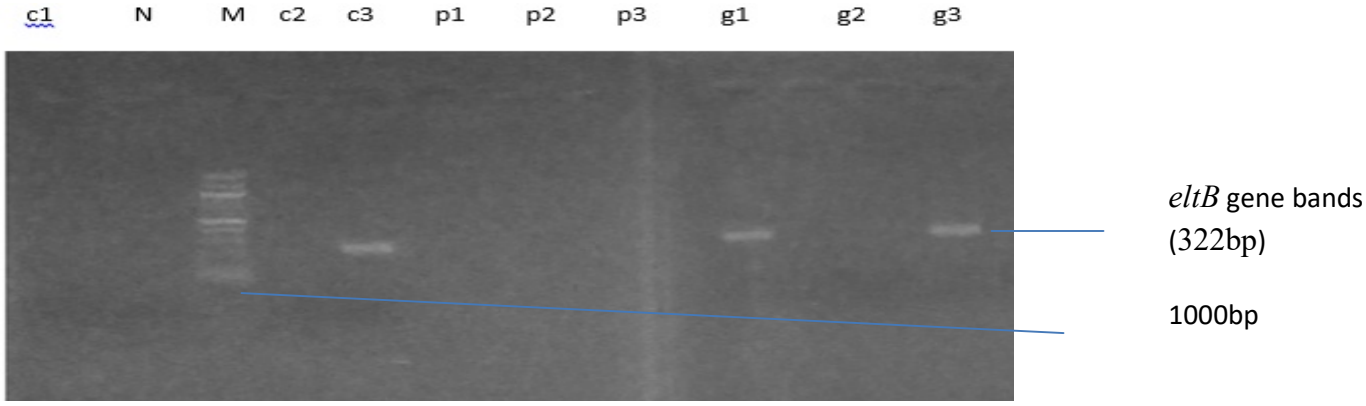

Plate 1: Agarose gel electrophoresis of *eltB* (322bp) gene of the bacterial isolates. Lane L represents a 1000bp molecular ladder

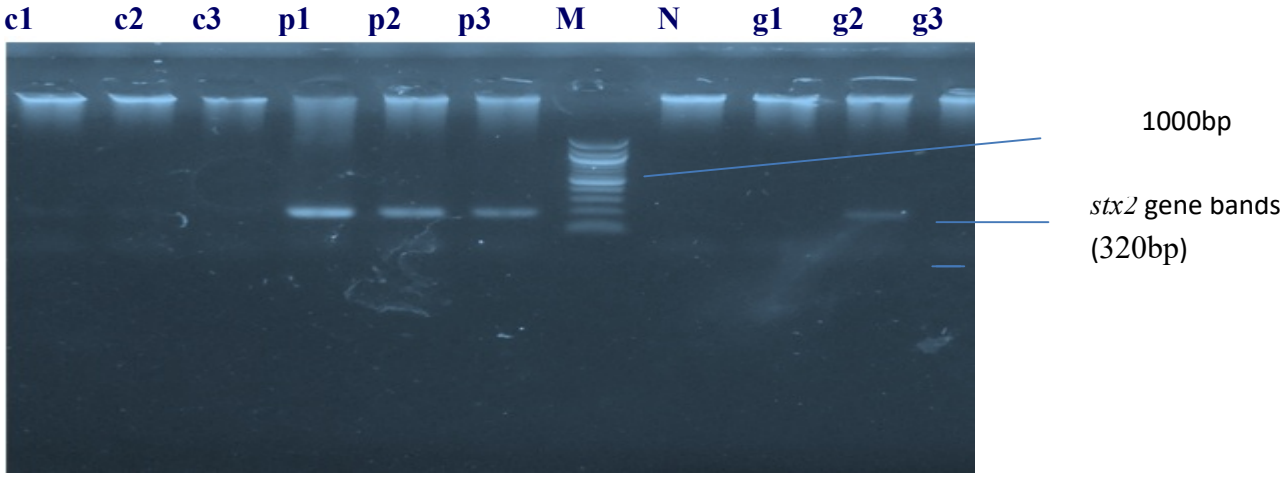

Plate 2 :Agarose gel electrophoresis of *stx2* (320bp) gene of the bacterial isolates. Lane L represents a 1000bp molecular ladder

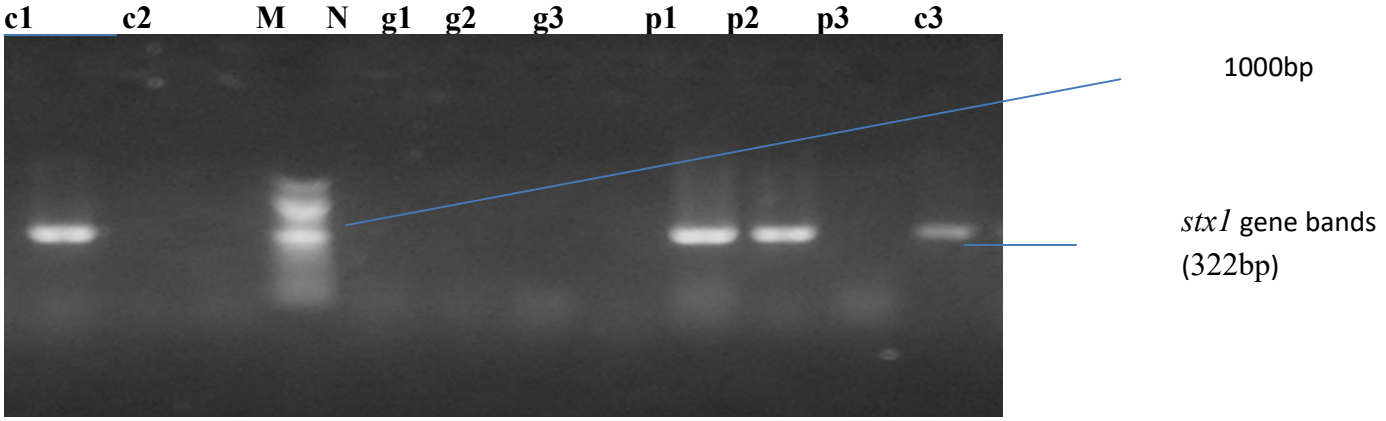

Plate 4.3: Agarose gel electrophoresis of *stx1* (298bp)gene of the bacterial isolates. Lane L represents a 1000bp molecular ladder

Discussion

Escherichia coli is a leading cause of many human food-borne infections such as diarrhea, haemorrhagic colitis and haemolytic uremic syndrome. The strain can be transmitted to humans through consumption of contaminated foods including meats. The threat posed by enterohaemorrhagic *E. coli* diseases spread via contaminated and improperly cooked meat has been well recognized (Oloyede *et al.,*2016). Infections caused by *E. coli* have been a significant public health (Luna-Guevara *et al.,* 2019).

The percentage occurrence of *E. coli from different raw meat sold in* markets in Abuja, FCT *was* 37.7%, higher than the study reported by Oloyede *et al.,* (2016) in Abeokuta but lower than study reported by Bako *et al*. (2018) in Kaduna with a prevalence rate of 47.6%. The occurrences of *E. coli* from different raw meat samples was in agreement with the studies reported by different authors such as Reuben and Makut (2014), Kabiru *et al*. (2015) in Zaria, in Lafia and Karaye *et al*. (2019) in Zaria. The high occurrence of *E.coli* as observed in this research could be attributed to high *E. coli* on raw meat especially those that were collected from slaughter houses where there was no much water to wash the floor and tables to reduce the microbial load on the those surfaces as reported by (Karaye *et al*. (2019). Kabiru *et al*. (2015) also opined that using the same water that was used to clean or wash the gut of the slaughter animals contaminate the flesh as frequently done in most slaughter houses in markets in Abuja, FCT due to shortage of water in the study area. This could be another reason for this finding in which isolates were confirmed to be *E. coli*.

It was observed in this study in (Table 4.3) that *E. coli* were isolated more from pork followed by beef from different locations studied or sampled; this may be due to nature of feacal matter excreted by these groups of animals and this is similar to study reported by Reuben and Makut(2014). The isolation of the pathogen *E. coli* from raw meat sold in markets in Abuja, FCT suggests that contamination of beef, chevon and pork exposes the public that patronize these products to food-borne hazards. The socio-economic implication is that resources and time are wasted on medication. The effects of food-borne infections are also greatly felt by immune-compromised individuals such as, pregnant women, children and diabetic patients (Bako *et al*. 2018).

The antibiotic susceptibility of *E. coli* isolated from raw meat sold in markets in Abuja, FCT showed that the *E. coli* from different meat type were more susceptible to gentamicin, imepenems and ciprofloxacin amoxicillin-clavulanic acid, ampicillin, cefoxitin, ceftazidime but amoxicillin-clavulanic acid, ceftazidime and nitrofrantan

were less effective to *E. coli* isolated from pork as observed in this study. This is not in agreement with studies reported by Nwinyi and Nduchukwuka (2016). This observation presents a direct challenge to the public health, where these *E. coli* could serve as gene pool for horizontal gene spread of antibiotic resistance genes to other when they come in contact with them.

Three *E. coli* strains from the beed harbored both*eltB*and*stx1* genes, Three *Escherichia coli* strains from the chevon harbored both *eltB* and and *stx2* genes while *E. coli* strains from the pork harbored both *stx1* and *stx2* genes. None of the isolates carried the three genes screened for as observed in this study. Thus, those eight isolates could be classified as Shiga toxin-producing *E. coli*, but not enterohaemorrhagic. The predominance of shiga toxin-producing *E. coli* could become a serious risk to public health since this strain carrying stx1 and stx2genes have been associated with such serious illness as haemolytic uremic syndrome (Liua *et al*., 2007 and Oloyede *et al.,* 2016). However, the results of this study suggest that consumption of improperly cooked meat as well as cross-contamination of raw meat with other foods or food utensils could be major sources of shiga toxin-producing *E. coli* strains

Conclusion

The findings of this research have indicated the occurrence of *Escherichia coli* in different raw meat samples namely beef, chevon and pork sold in different markets in Abuja, FCT. The percentage occurrence was higher in pork samples than in the other two raw meat samples. The *Escherichia coli* isolated were more susceptible to imepenems, ciprofloxacin and gentamicin but less susceptible to ceftazidime and nitrofrantan**.** Eight isolates could be classified as Shiga toxin-producing *E. coli*.

Conflict of Interest statement

The authors declare no conflicts of interest

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