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REGULAR ARTICLES

Virulent gene profile and antibiotic susceptibility pattern of Shiga toxin-producing *Escherichia coli* (STEC) from cattle and camels in Maiduguri, North-Eastern Nigeria

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Abstract

Prevalence and distribution of Shiga toxin-producing *Escherichia coli* (STEC) serogroups from the faecal samples of cattle and camels slaughter in Maiduguri abattoir and their antibiotic resistance profile of the isolates were determined. The highest prevalence (24%) was recorded in the month of September and more STEC isolates came from cattle than the camels. There was significant ($P < 0.05$) seasonal trend in the prevalence of STEC among cattle and camel with more cases recorded during the wet season. Although, the study did not demonstrate significant influence of sex from the various sources. The serogroups recorded in this study were O157, O26, O91, O103 and O111. There was no significant difference ($P < 0.05$) between the detection rates of serogroups. The serogroup O26 was significantly ($P < 0.05$) the most observed serogroup in both camels and cattle. None of the STEC isolates tested positive for the O45 serogroup. PCR assays shows that 50 (63.3%) of the 86 STEC isolates carried the *stx2* gene, 34 (43%) possessed the stx1 gene, and 14 (16.3%) carried both *stx1* and *stx2* genes. Other genes detected include *eae* and *ehlyA*. The antimicrobial resistance among the STEC O157 and non-O157 isolates from cattle and camels in Maiduguri abattoir were very high and the STEC isolates were resistant to at least one or more of the antimicrobial agents tested. There was also multidrug resistance with the most frequent occurring patterns been ampicillin/nalidixic acid and tetracycline/trimethoprim. However, all the 79 isolates were sensitive to chloramphenicol, ceftazidime and ceftriaxone; therefore, these drugs could be drugs of choice in the treatment of STEC infections.

Keywords Shiga toxin-producing *Escherichia coli* · Virulent gene · Antibiotic resistance · Cattle · Camels

Introduction

E. coli is regarded as a pathogen of worldwide importance in commercially produced beef, its presence can lead to significant economic losses (Rigobelo and de Ávila 2012). Most *E. coli* strains are harmless commensals, but some serotypes can cause serious food poisoning in humans (Hassan et al. 2014; CDC 2018). Shiga toxin, a potent cytotoxin, is mainly produced by *Escherichia coli* and *Shigella dysenteriae* type 1, and sporadically could be produced by *Citrobacter freundii*, *Enterobacter cloacae*, *Shigella flexneri*, *Shigella sonnei*, *Aeromonas hydrophila*, and *Aeromonas caviae* (Llorente et al. 2014). Shiga toxin (Stx)-producing *Escherichia coli* (STEC) are one of the most important groups of diarrhoeagenic *E. coli* that is associated with food-borne outbreaks (Paton and Paton 1998; Beutin et al. 2007). STEC have

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been identified to be pathogenic to humans and a major source of contamination for foods of animal origin constituting a major public health concern (Dastmalchi and Ayremlou 2012). Farm animals are asymptomatic carriers of STEC, a zoonotic enteric pathogen that causes human gastrointestinal illnesses and meat contaminated by STEC is an important means for zoonotic transmission from animals to humans (Ojo et al. 2010; Amézquita-López et al. 2014). Bovine *E. coli* strains can produce Shiga-like toxins (Stx), heat labile (LT) or heat-stabile (ST) enterotoxins, cytotoxic necrotizing factors (CNF1 and CNF2) and haemolysins (Rigobelo and de Ávila 2012).

STEC are characterized by the production of one or more types of Shiga toxins (stx1 or stx2 or their variants), which subdue the host cells' protein synthesis (Melton-Celsa 2014). Infection by these enterotoxins results in diarrhoeic secretions due to the action of one or more enterotoxins, and can lead to dehydration and death. (Rigobelo et al. 2006).

The most important natural reservoirs of STEC are cattle. Transmission to humans occurs through food, water, and direct contact with animals or their environment (Bonanno et al. 2015). Since cattle are the reservoir for STEC, studies have found an association between cattle population and the incidence of STEC infections in humans. The incidence of STEC infection in humans was higher in areas with high cattle population and in areas where manure was used for agricultural practices (Frank et al. 2008).

Three main routes of transmission have been identified by outbreak investigators as food borne infections which are frequently associated with consumption of contaminated undercooked beef and unpasteurized milk, person to person spread, and direct or indirect animal contact (Parry et al. 1998). Direct and indirect zoonotic transmission through contact with animals or their faeces has also been reported in several settings (Synge and Hopkins 1994).

The main virulence factors of STEC associated with human disease are potent cytotoxins also called shiga toxin (stx1 and stx2), which are encoded by the *stx1* and *stx2* genes. Two additional markers also play a major role in the pathogenesis of haemolytic colitis and haemolytic uremic syndrome (HUS), they include intimin, encoded by the *eae* gene, and enterohaemolysin, encoded by the *ehlyA* gene (Paton and Paton 1998 and Karmali et al. 2010). This genetic virulence characteristic is often used in epidemiological studies to correlate between strains from various sources (Askari et al. 2010)

Without any doubt, antimicrobial therapy is a major tool for infectious disease treatment in veterinary medical practice but resistance to antimicrobials is a cause of great concern (Beco et al. 2013). The global rise in the prevalence of antibiotic resistance is an important problem especially in developing countries where there is limited control of the quality, distribution and use of antibiotics in human medicine, veterinary medicine, and food-animal agriculture (Okeke et al. 1999; Kumarasamy et al. 2010).

Diseases caused by *E. coli* often require antimicrobial therapy; however, antibiotic-resistant strains of this bacterium cause longer and more severe illnesses than their antibiotic-susceptible counterparts (Shahrani et al. 2014).

Isolation of non-O157 STEC and some EPEC serotypes was reported from faeces of diarrheic calves collected from various farms in Zaria, North-central Nigeria (Sa'Ayinzat et al. 2015), but the prevalence and distribution of STEC serogroups O157, O26, O45, O91, O103 and O111 which are associated with public health risks are unknown in cattle and camel around Maiduguri, North-eastern Nigeria, in spite of the public health safety problems this poses. It is due to this prevalent background that this study was conducted to determine the prevalence and characteristics of Shiga toxin-producing *E. coli* (STEC) isolated from cattle and camels in Maiduguri, Nigeria.

The aims of this study were to determine the prevalence of STEC isolates from faeces of slaughtered cattle and camels in Maiduguri, Nigeria, to determine the serogroups of the STEC isolates, determine the virulent genes using polymerase chain reaction (PCR) and to evaluate the antimicrobial susceptibility patterns of the STEC isolates.

Material and methods

Study area

Maiduguri, our study area is located in the semi-arid zone of Borno State, situated in the North Eastern part of Nigeria, with an area of about 69,436 km^2 and lies within latitude 10 - 13°N and longitude 12 - 15°E. It lies within the savannah and Sahel vegetation, and receives little average annual rainfall of 613 mm. The wet season in the study area starts in April and extends into Mid-October while the dry season runs from November till Mid-March.

The State shares boundaries with Chad to the North East, Cameroon to the East and Adamawa state to the South West. One of the main Agricultural activities in this area is livestock production.

Sampling techniques

Faecal samples were collected from cattle and camels slaughtered at the Maiduguri abattoir. The sample size was calculated using the formula $n = t^2 \times p\,(1-p) \div m^2$ from each source as described by FAO (1990). n represents the number of sample to be collected, t is the confidence level at 95% and standard value is 1.96; p is the estimated percentage prevalence from each sample source and m is the margin of error at 5% (standard value is 0.05).

Isolation and identification of STEC

Faecal samples were collected from cattle and camels slaughtered at Maiduguri abattoir. All samples were collected in sterile well-labelled containers and were transported to the University of Maiduguri Veterinary Diagnostic Microbiology Laboratory in ice packs to avoid putrefaction before analysis. At the laboratory, the samples were enriched in modified tryptone soya broth (mTSB), supplemented with novobiocin using the ratio of 1:9 (1 g of faeces in 9 ml of mTSB) and incubated at 37 °C for an initial period of 6 h and then for an extended period of 12 h as described by Savoye et al. (2011). From the Broth, a loopful of the culture was streaked on to MacConkey agar (Oxoid®, UK) plates. The plates were then incubated for 24 h at 37πC and bacterial growths were observed. Lactose fermenting colonies were picked and then streaked on eosin methylene blue (EMB) agar (Oxoid®, UK) plates. Typical *E. coli* colonies were picked as presumptive isolates (Plate 1). Plates with mixed cultures were subcultured to obtain a pure culture of *E. coli* and pure *E.coli* isolates were then transferred to Nutrient agar (Oxoid®, UK) slants, incubated at 37 °C for 24 h. After growth was observed, the presumptive *E. coli* colonies on nutrient agar (CM3, (Oxoid®,UK) slants were picked and streaked on to sorbitol McConkey agar cultures (SMAC) (CM813, Oxoid®, UK) and then incubated for 24 h at 37 °C. The identity was confirmed by motility and biochemical tests (indole, methyl red, Voges-Proskauer, and Simmons citrate tests) using standard procedures as described by Cheesbrough (2000). The confirmed *E. coli* isolates based on the results of the biochemical tests were selected for antimicrobial agent sensitivity testing. And these confirmed isolates were inoculated into new TSB tubes and incubated until the turbidity was 0.5 McFarland standard, this was done for 3 h.

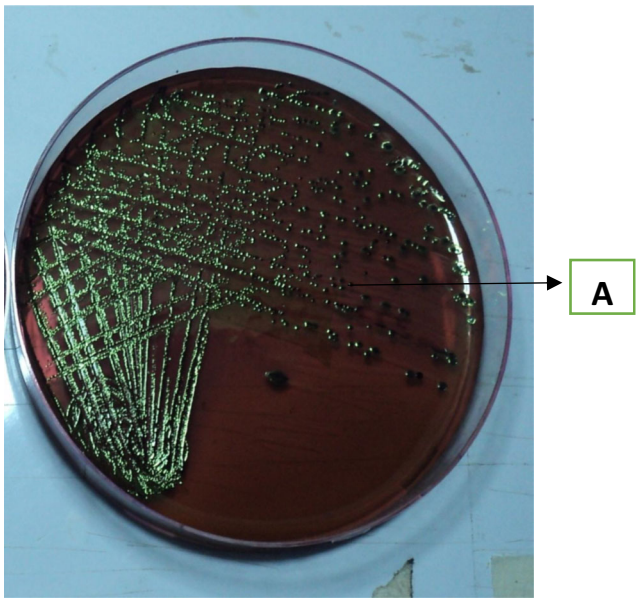

Plate 1 Greenish metallic sheen colonies (presumptive for *Escherichia coli* with discrete colonies labelled A on eosin methylene blue agar plate

Detection of *E. coli* O26, O103, O111, O91 and O45 serogroups

E. coli Non-O157 Identification Kit, Prolex™ (PL 1070) produced by Pro-Lab Diagnostics Round Rock, TX, USA, was used to determine the serogroup of the isolates from cattle and camel faecal samples. The test cards labelled with each of the serogroups O26, O103, O111, O191 or O45 were used in the detection.

PCR techniques

1 ml of phosphate buffer saline (PBS) was transferred to a 1.5-ml Eppendorf tube. Then a loop full of the bacterium was picked from a plate and transferred to the Eppendorf tube and centrifuged at 14,000 rpm for 5 min. Supernatant was discarded and the pellet was re-suspended in 100 μl TE buffer. The suspension was boiled at 95 °C for 10 min and then transferred directly to ice to form a lysate. The lysed DNA was diluted 10-fold in TE buffer from presumptive pure *E.coli* strains, grown overnight in nutrient broth (Sigma Chemical Co. USA) at a temperature of 37 °C, they were further pelleted by centrifugation. Lysate was centrifuged again as described above and 50 μl of the supernatants were used as DNA for the PCR assay. The PCR assay list of the primers, their sequence and lengths are shown in Table 1. All *E. coli* isolates from cattle and camel faecal samples were subjected to PCR. *Stx* 1, *stx* 2 and *eae* virulent genes were detected using the primers and PCR assay technique as described by China et al. (1998) and Scheutz et al. (2012).

Expression of enterohaemolysin A (EHlyA)

The *E. coli* isolates were observed for haemolytic activities on a blood agar plates containing phosphate buffered saline (PBS) made from nutrient agar containing washed and unwashed 7% sheep erythrocytes (RBC). Strains, which were haemolytic on both washed and unwashed 7% sheep blood agar within 4–6 h of incubation at a temperature of 37 °C with a clear, broad zone of alpha haemolysis were considered to have produced alpha-haemolysin (a-hly), while those that were only haemolytic on washed sheep blood agar with a narrow, turbid haemolytic halo after more than 8 h incubation duration at a temperature of 37 °C produced enterohaemolysin (ehly) (Plate 2). Expression of enterohaemolysin was determined based on the method described by Beutin et al. (1997).

Table 1 Primer sequences and lengths of PCR amplification products

Primer product sizes (bp)	Target gene	Oligonucleotide sequence (5′–3′)
LP30 *E. coli* 348 O157	stx1	5′CAG TTA ATG TGG TGG CGA AGC 3′
LP31 *E. coli* 348 O157	stx1	5′CAC CAG ACA ATG TAA CCG CTG 3′
LP43 *E. coli* 584 O157	stx2	5′ATC CTA TTC CCG GGA GTT TAC G 3′
LP44 *E. coli* 584 O157	stx2	5′GCG TCA TCG TAT ACA CAG GAG C 3′
SK1 *E. coli* 863 O157	eae	5′CCC GAA TTC GGC ACA AGC ATA AGC 3′
SK2 *E. coli* 863 O157	eae	5′CCC GGA TCC GTC TCG CCA GTA TTC G 3′

Antimicrobial susceptibility testing

Antibiotic susceptibility testing of all the *E.coli* isolates was done with Mueller-Hinton agar (CM337, Oxoid® UK) plates with an agar depth of 4 mm, were prepared according to manufacturer's instructions and prior to use were visually examined to ensure that plates were free of visible contamination before poured to a uniform depth of approximately 4 mm. Within 15 min of preparing the adjusted inocula, a sterile cotton swabs were used to streak the inocula evenly on the surface of the agar. The swabs were rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inocula from the swabs. Any excess moisture on the agar surface was allowed to be absorbed prior to application of the antimicrobial disks. The lid of the plates were left slightly opened for 5 min to allow excess moisture to be absorbed before applying disks Antimicrobial disks containing ampicillin 10 μg/disk; tetracycline 30 μg/disk; ceftazidime 10 μg/disk; streptomycin 10 μg/disk; gentamycin 10 μg/disk; nalidixic acid 30 μg/disk; ceftriaxone 30 μg/disk; ciprofloxacin 5 μg/disk; cefotaxime 30 μg/disk; chloramphenicol 30 μg/disk and trimethoprim 5 μg/disk (Oxoid, UK®) and nutrient agar (CM3, Oxoid®) plates Oxoid, UK®. Disk diffusion method of antibiotic susceptibility testing as described by the National Committee for Clinical Laboratory Standards (Reller et al. 2009) was used. The values were interpreted using the prescribed standards by CLSI (2006).

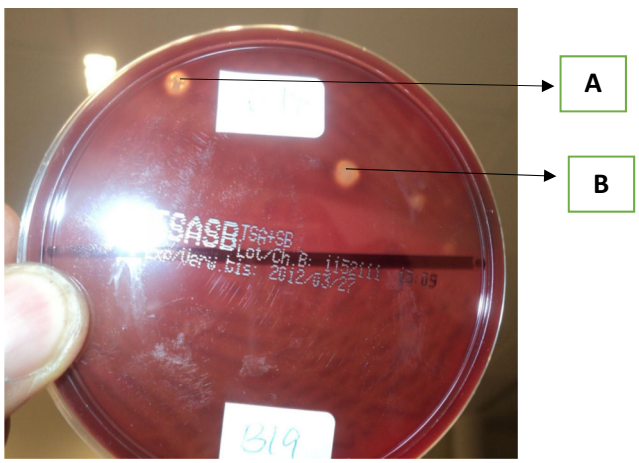

Plate 2 Sheep blood agar plate showing zones of haemolysis A and B by *Escherichia coli*

Statistical analysis

The Student *t* test was used to analyse the difference among the various serogroups, virulence genes properties and seasonal trend. Duncan's multiple range test and Pearson correlation analysis were used for testing associations between virulent genes.

Results

Prevalence of STEC organisms from faecal samples of cattle and camel

Out of the 600 faecal samples from cattle, 9.3% ($n = 56$) were positive for the presence of STEC (Table 2). The highest, 24.0% ($n = 12$) prevalence was recorded in the month of September, this was followed by 22% ($n = 11$) and 14.0% ($n = 7$) for the months of October and June, respectively. The months of July, December and January had 12.0% ($n = 6$) each. No STEC was isolated during the months of January and March. There was significant ($P < 0.05$) seasonal influence on prevalence of STEC among faeces of cattle tested (Table 3). The highest isolation rates 20.0% ($n = 29$) and 12.8% ($n = 14$) were recorded in wet season for female and male cattle, respectively. The prevalence in dry season was 3.6% ($n = 8$) and 3.8% ($n = 5$) for female and male cattle, respectively. There was no significant difference in the prevalence rate of STEC between male and female cattle.

Out of the 600 faecal samples collected from camels, 3.8% ($n = 23$) of the samples were found to be positive for STEC (Table 4). The highest 10.0% ($n = 5$) prevalence rate was recorded in the month of September, this was followed by 8.0%

Table 2 Prevalence of STEC isolates from faeces of cattle slaughtered in Maiduguri, Borno State

Month	No. tested	No. STEC+ve	Percentage (%) + ve
June	50	7	14
July	50	6	12
August	50	2	4
September	50	12	24
October	50	11	22
November	50	4	8
December	50	6	12
January	50	0	0
February	50	1	2
March	50	0	0
April	50	3	6
May	50	3	6
Total	600	56	9.3
Mean		4.5	

Table 4 Prevalence of STEC isolates from faeces of camels slaughtered in Maiduguri, Borno State

Month	No. tested	No. STEC+ve	Percentage (%) + ve
June	50	4	8.0
July	50	3	6.0
August	50	0	0
September	50	5	10.0
October	50	2	4.0
November	50	3	6.0
December	50	3	6.0
January	50	0	0
February	50	0	0
March	50	0	0
April	50	2	4.0
May	50	1	2.0
Total	600	23	3.6
Mean		1.9	

($n = 4$), in the month of June, and 6.0% ($n = 3$) each in the months of July, December and January. No STEC was isolated during the months of August, February and March. There was significant ($P < 0.05$) seasonal trend in the isolation of STEC from camel faeces (Table 4). The highest isolation rate 6.9% ($n = 17$) was recorded in wet season. This was significantly lower than the rate 1.7% ($n = 6$) obtained in the dry season. There was also no significant ($P < 0.05$) difference in the prevalence of STEC among the male and female camels tested. The prevalence of STEC among the male and female camels were 4.7% ($n = 11$) and 3.3% ($n = 12$), respectively (Table 5).

Biochemical characteristics of STEC isolates

Results of the bacteriological studies showed that, out of the 1200 faecal samples collected from camels and cattle ($n = 600$ each), 27.0% ($n = 162$) and 36.6% ($n = 220$) samples respectively showed typical growths of *Escherichia coli* as shown in Fig. 1. Out of the 382 biochemically presumptive *E. coli* isolates, 9.91% ($n = 119$) were non-sorbitol fermenters (NSF), with 33.8% ($n = 73$) from cattle and 30.1% ($n = 41$) from camels. Motility test showed that 27.7% ($n = 33$) of the confirmed non-sorbitol fermenting *E. coli* isolates were motile (Table 6).

Serogrouping of STEC isolates from cattle

In cattle, the 56 STEC isolates belonged to 5 different serogroups. The results as shown in Table 7 revealed that 12.5% ($n = 7$) were O157 and 87.5% ($n = 49$) were non-O157 serogroup. Serogroup O26 accounted for 39.3% ($n = 22$) of the isolates, which was significantly ($P < 0.05$) the most frequently detected serogroup among the non-O157 (Table 7). There was no significant difference between the detection rates of serogroups O103, 10.7% ($n = 6$) and O157, 12.5% ($n = 7$). However, serogroup O91 accounted for 32.1% ($n = 18$) of the STEC isolates from cattle.

Serogrouping of STEC isolates from camels

Out of the 23 STEC isolates from camels, 17.4% ($n = 4$) belonged to serogroup O157, while 82.6% ($n = 19$) were

Table 3 Influence of sex and season on prevalence of STEC isolated from faeces of cattle in Maiduguri

Seasons Means	Sex				Total STEC + ve	
	Females		Males		(%)	
	No. tested	STEC+ve	No. tested	STEC+ve		
Wet	138	29 (20)	109	14 (12.8)	43 (7.4)	21.5^a
Dry	221	8 (3.6)	132	5 (3.8)	13 (3.6)	6.5^b
Total ($n = 600$)	359	37 (10.3%)	231	19 (8.22%)	56 (9.5%)	
Mean		18.5^a		10.4^a		

Means with the different letter superscript were significantly different ($P < 0.05$)

Table 5 Influence of sex and season on prevalence of STEC isolated from faeces of camel in Maiduguri

Seasons Means	Sex				Total STEC + ve	
	Females		Males		(%)	
	No. tested	STEC+ve	No. tested	STEC+ve		
Wet	149	10 (6.7)	98	7 (6.5)	17 (6.9)	4.0[a]
Dry	219	2 (0.9)	134	4 (2.3)	6 (1.7)	1.5[b]
Total ($n = 600$)	368	12 (3.3%)	232	11 (4.7%)	23 (3.8%)	
Mean		5.2[a]		4.0[a]		

Means with the different letter superscript were significantly different ($P < 0.05$)

non-O157. Among the non-O157 serogroups, 43.5% ($n = 10$) belonged to serogroup O26, which was significantly ($P < 0.05$) the most observed serogroup. 17.4% ($n = 4$) isolates belonged to serogroup O103. However, serogroups O111 and O91 accounted for 13.0% ($n = 3$) and 8.7% ($n = 2$) isolates, respectively. None of the STEC isolates tested positive for the O45 serogroup (Table 8).

Detection of stx1, stx2, eae genes and enterohaemolysin A

The results of the PCR assays show that 63.3% ($n = 50$) of the 86 STEC isolates carried the *stx2* gene, 43% ($n = 34$) possessed the *stx1* gene, and 16.3% ($n = 14$) carried both *stx1* and stx2 genes (Table 9). *Stx1* genes were more prevalent in camel (43.5%) than cattle (42.9%) although not significantly different, whereas *stx2* strains were more commonly recovered from cattle (71.4%) and camels (43.5%). Isolates carrying both *stx1*- and *stx2* genes were more frequently isolated from cattle (23.2%) than from camels (4.3%). The 79 STEC isolates were investigated for the presence of the *eae* genes, only 38% ($n = 30$) of 79 STEC isolates were positive for the *eae* gene (Fig. 3).The *eae* genes were significantly ($P < 0.05$) higher in STEC from camels (69.6%) than those in cattle (25.0%), respectively (Table 9). The result for haemolytic activities of the 79 STEC isolates is also presented in Table 9. Out of the 79 STEC isolates, 38% ($n = 30$) produced clear narrow zones of haemolysis on nutrient agar containing 7% washed sheep blood, typical of enterohaemolysin production. Some of the plates are shown in Plate 1. 69.6% ($n = 16$) of the isolates that produce enterohaemolysin were from camels and were significantly ($P < 0.05$) higher than those from cattle (25.0%). Figures 1, 2 and 3 show the photographs of Agarose gel electrophoresis analysis results of PCR assays of *stx1*, *stx2* and *eae* genes of the *E. coli* isolates from the faecal samples of the cattle and camels slaughtered at the Maiduguri Abattoir.

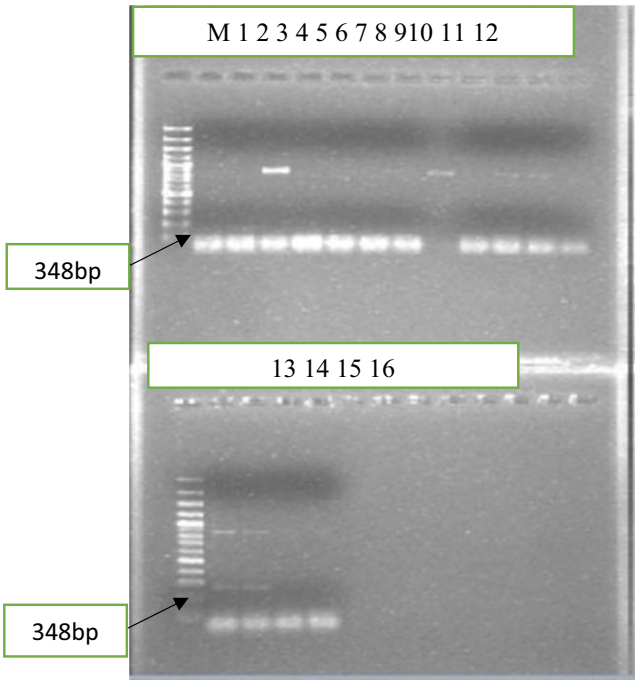

Fig. 1 Agarose gel electrophoresis of PCR amplification of *Stx1* genes of *Escherichia coli*. Lane M: 100 bp Molecular weight marker/ladder. Lane1–6: PCR product of *Vtx1* positive isolates of *E. coli.* Lane 7: positive control. Lane 8: negative control. Lane 9–16: PCR product of *Stx1* positive isolates of *E. coli*

Pattern of distribution of virulence genes in the 79 STEC isolates from cattle and camels in Maiduguri

The results of virulence gene (*stx1, stx2, eae* and *ehlyA*) characteristics of the 79 STEC isolates from the various sources showed that 100% of the isolates had at least one virulence genes (Table 10). The results revealed eleven pattern of distribution of the virulence genes from the 79 STEC isolates. There were significant differences ($P < 0.05$) among the combinations from the cattle and camel faecal sources. The most frequent pattern among the isolates from camels was stx1/eae/ehlyA 34.2% ($n = 8$) which was significantly more than what was obtained in cattle 8.9% ($n = 5$). Gene stx2 was significantly higher among cattle isolates (32%), than for the camels (21.7%). Overall results did show that, there was significant ($P < 0.05$) positive correlation between the *eae* and *ehlyA* genes among the isolates. However negative correlation exist among the isolates for *stx1* and stx2 genes, Also, there was significant ($P < 0.05$) positive correlation between the *eae* and *stx2* genes.

Table 6 Biochemical test characteristics of STEC isolates from cattle and camels in Maiduguri

Sample number (%)	*E. coli* + ve (%)	Indole + ve (%)	NSF (%)	Motility
Cattle (600)	220 (36.6)	216 (98.1)	78 (33.8)	21 (28.8)
Camels (600)	162 (27)	160 (98.7)	41 (25.6)	12 (29.3)
Total (1200)	382 (31.83)	376 (98.4)	119 (31.1)	33 (27.7)

NSF non-sorbitol fermenters

Antibacterial susceptibility testing of STEC

The antimicrobial resistance among the STEC O157 and non-O157 isolates from cattle and camels in Maiduguri abattoir are shown in Table 11. Whereas, camels STEC O157 isolates were more resistant to tetracycline than isolates from cattle (Table 12). Only one STEC non-O157 was resistant to cefotaxime which was isolated from camels slaughtered at the Maiduguri abattoir (Plate 3). None of the isolates was resistant to chloramphenicol, ceftazidime and ceftriaxone. Cattle STEC O157 isolates showed a higher number of resistance 42.9% ($n = 3$), to ampicillin and streptomycin each than the number of resistance 28.6% ($n = 2$) to gentamycin and nalidixic acid 28.6% ($n = 2$), this is shown in Table 13. Ampicillin, and tetracycline 41.7% ($n = 5$) each, were the most commonly resistant antimicrobials, followed by streptomycin 33.3% ($n = 4$), gentamycin, nalidixic acid and trimethoprim 25% ($n = 3$) each and cefotaxime 8.3% ($n = 1$). None of the isolates were resistant to chloramphenicol, ciprofloxacin, ceftazidime and ceftriaxone. Cattle non-O157 STEC showed a higher number of resistant strains than camel to ampicillin (65.3%) and gentamycin (26.5%).

Multidrug resistance patterns of STEC isolates from camels and cattle

The multidrug resistance profile of STEC isolated from camels and cattle is shown in Table 14. STEC isolates that were resistant to at least one or more of the antimicrobial agents tested. The most frequent occurring patterns were ampicillin/nalidixic acid and tetracycline/trimethoprim, 10.4% ($n = 7$) each. This is followed by gentamycin/trimethoprim 7.4% ($n = 5$). All the most frequent resistance profiles were identified among STEC isolates from camels and cattle.

Discussions

The results of this study suggest that camel and cattle faeces are important sources of meat contamination with STEC O157 and non-O157 STEC in Maiduguri. In developing countries, high level of carcass contamination with faeces from gastrointestinal tracts of food animals is common in abattoir and slaughter slabs. This is specifically true in Nigeria, where butchering of meat are done on concrete floor under unhygienic slaughtering and meat processing conditions with lack of potable water (Olatoye 2010).

The results of this study showed that the prevalence of STEC from camel faeces in Maiduguri was 3.8%. The values obtained concur with the findings reported by Rahimi et al. 2010, pp. 179–185 and 2012, pp. 559–564 from Iran and Bosilevac et al. 2015, pp. 89–96, although the latter reported a higher prevalence of 10.7% in Riyadh, Saudi Arabia. However, this finding is at variance with previous reports on camel faecal samples from the United Arab Emirates, where *E. coli* O157:H7 was not identified (Moore et al. 2002, pp. 283–287) and East African countries (El-Sayed et al. 2008, pp. 469–473).

The differences observed may be due to differences in geographical locations and laboratory techniques used for the detection of STEC organisms. However, those studies targeted

Table 7 Distribution of the 56 STEC isolates from cattle to various serogroups

Serogroup (%)	No. of isolates ($n = 23$)	Percentage
O157	7	12.5
O26	22	39.3
O45	0	0
O91	18	32.1
O103	6	10.7
O111	3	5.4
Total	56	100

Table 8 Distribution of the 23 STEC isolates from camels to various serogroups

Serogroup (%)	No. of isolates ($n = 23$)	Percentage
O157	4	17.4
O26	10	43.5
O45	0	0
O91	2	8.7
O103	4	17.4
O111	3	13.0
Total	23	100

Table 9 Distribution of virulence factor-encoding genes in the STEC strains of the isolates from cattle and camels in Maiduguri

Virulent gene	Isolates from cattle *n* (%) Total = 56	Isolates from camel *n* (%) Total = 23
Stx_1	24 (42.9%)[b]	10 (43.5%)[b]
Stx_2	40 (71.4%)[a]	10 (43.5)[b]
Stx_1/Stx_2	13 (23.2%)[a]	1 (4.3%)[b]
Eae	14 (25%)[b]	16 (69.6%)[a]
EhlyA	14 (25%)[b]	16 (69.6%)[a]

Percentages with the same superscript were not significantly different ($P < 0.05$)

only serogroup O157 and one of the reasons advanced for failure to detect STEC in camels around the desert could be that, the sedentary life pattern of camels in the desert minimizes the contact of camels with other animal species and therefore the transmission of STEC (El-Sayed et al. 2008). In Maiduguri, during the dry season, camel population increases because of movements of camel nomads from deserts in search of feeds, where camels and cattle come in contact during grazing. Cattle have been reported to be reservoirs for STEC in Maiduguri (Moses et al. 2012), for this reason there could be interspecies transfer of STEC from cattle to camels.

In this study, 9.3% of the cattle faeces tested were positive for STEC. Although, ruminants have been reported as the main natural reservoir for human STEC infections (Caprioli et al. 2005; Gyles 2007 and Hussein 2007), the figure obtained in this study was rather low compared to previous reports of 19% in India by Das et al. 2005, pp. 617–626, in Bangladesh by Islam et al. 2008, pp. 5414–5421 and in Nigeria by Ojo et al. 2009, pp. 23–30, reported 10%. This discrepancy in prevalence might be due to the regional differences and management system. Caprioli et al. 2005, pp. 289–311, reported that the use of specific immunoconcentration procedures for STEC O157 as used by (Islam et al. 2006a, b) strongly

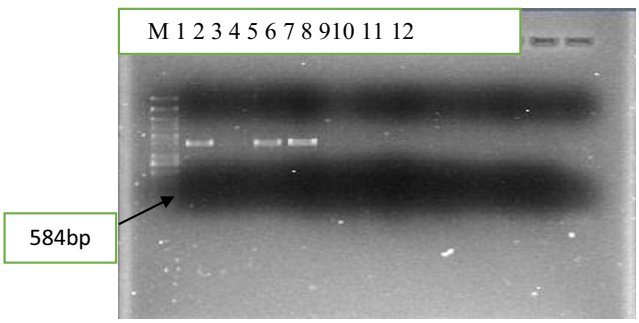

Fig. 2 Agarose gel electrophoresis analysis of PCR amplification of *stx2* genes of *Escherichia coli*. Lane M: 100 bp Molecular weight marker/ladder. Lane 1: positive control. Lane 2: negative control. Lane 3 and 4: PCR product of *stx2* positive isolates of *E. coli*

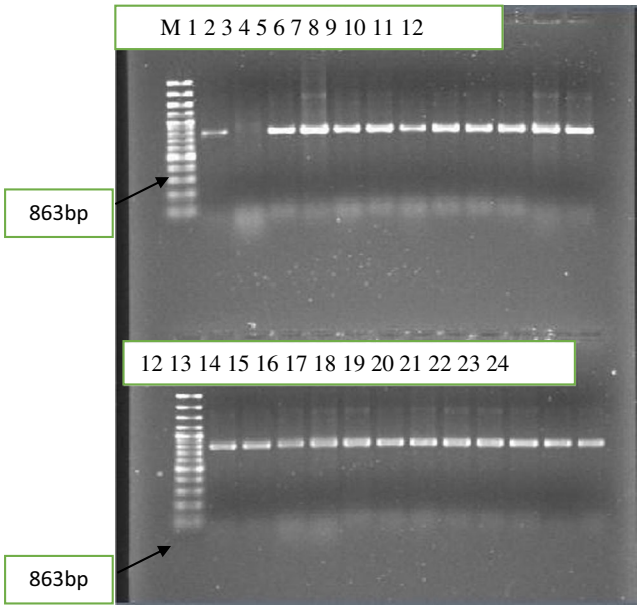

Fig. 3 Agarose gel electrophoresis of PCR amplification of *eae* genes of *Escherichia coli*. Lane M: 100 bp Molecular weight marker/ladder. Lane 1: positive control. Lane 2: negative control. Lane 3–24: PCR product of *eae* positive isolates of *E. coli*

enhanced the sensitivity of the isolation methods and higher rates of recovery.

The results of this study showed a significant seasonal trend. For cattle and camels, the prevalence of STEC was significantly higher in the wet season than in the dry season. These results corroborate with a study by Persson et al. 2007, pp. 516–524, reported high prevalence of *E. coli* O157:H7/NM in meat sampled in summer and fall. However, in a study of non-O157 STEC performed on sheep, the prevalence did not follow the seasonal trend previously reported for STEC O157:H7, with the highest prevalence rates (up to 26.0%) reported during winter and spring (Pierard et al. 1997, pp. 531–540). Kudva et al. 1997, pp. 892–899, hypothesized that changes in diet or environment influenced the seasonal variation in the prevalence of STEC O157:H7. A higher prevalence rate (10.7%) of *E. coli* O157 was reported when cattle hides were tested in the summer at Midwestern beef processing plants (Elder et al. 2000). This agrees with the findings of the present study. These different prevalence rates could be explained by sampling time. It is important to note that quantitative faecal shedding of STEC is considered a more important factor than prevalence in influencing the risk of human exposure and infection with STEC. Interestingly, the high prevalence recorded in camel and cattle during the wet season in this study corresponded with the human incidences recorded in the same season as recorded by Adamu et al. (2017). This agrees with the reports of Ogden et al. 2004, pp. 297–300, that the prevalence of *E. coli*o157 in beef cattle at slaughter was found to be greater ($P < 0.05$) during the cooler

Table 10 Pattern of distribution of virulence genes according to the origin of the 7 STEC strains tested

Virulent gene cattle	Isolates from camel *n* (%) Total = 23	Isolates from cattle *n* (%) Total = 56	Total *n* (%) Overall total = 79
Stx_1	1 (4.3%)	2 (3.6%)	3 (3.8%)
Stx_2	5 (21.7%)	18 (32.1%)	23 (29.1%)
Stx_1, *Stx2*	1 (4.3%)	6 (10.7%)	7 (8.9%)
Stx_1, *ehlyA*	0 (0%)	3 (5.4%)	3 (3.8%)
Stx_2, *ehlyA*	0 (0%)	7 (12.5%)	7 (12.5%)
Stx_1, *Stx2 ehlyA*	0 (0%)	6 (10.7%)	6 (7.6%)
Stx_1, *eae*	0 (0%)	1 (1.8%)	1 (1.3%)
Stx_1, *eae, ehlyA*	8 (34.8%)	5 (8.9%)	13 (16.5%)
Stx_2, *eae, ehlyA*	4 (17.4%)	2 (3.6%)	6 (7.6%)
Stx_1, *stx2, eae, ehlyA*	0 (0%)	1 (1.8%)	1 (1.3%)
Eae, ehlyA	4 (17.4%)	5 (8.9%)	9 (11.4%)

n number of positive samples, *NB* total number of positive isolates

months (11.2%) than during the warmer months (7.5%) which explain increased human infections at that time. This was the reverse of the known seasonality of human infections with STEC (WHO 1998).

Investigations on the prevalence of STEC are most commonly based on detection of the O157 serogroup. The prevalence in cattle faeces varies widely from 0 to over 50% (Rhoades et al. 2009). However, the results of STEC serogrouping reported in this study revealed that all the STEC isolates were within the six STEC serogroups that were tested for. Therefore, it is not surprising to have 56 STEC isolates from cattle of which all tested positive for at least one of the six STEC serotypes tested. Studies have shown that there are numerous STEC serotypes with 174 O antigens and 53 H antigens (Scheutz et al. 2004). Similarly, Blanco et al. 2003, pp. 345–351, reported about 200 serotypes of STEC. Therefore, the prevalence of the STEC serogroups for cattle differs depending on different factors. Different prevalence rates were reported for both E coli O157 and non-O157 *E. coli*, depending on the type of animal tested the season and the animal production system (Hussein 2007).

E. coli O26 was the most commonly isolated serogroup of those tested in camel faeces. Forty percent of *E. coli* O26 isolates carried *stx2, eae,* and *ehly*. This result contradicts the findings of several other studies which found that *E. coli* O26 strains typically carry *stx1* and eae but not *stx2* (Burnens et al. 1995; Blanco et al. 1997). However, high rates of carriage of *stx2* among *E. coli* O26 isolates from cattle have been reported (Geue et al. 2002), which agrees with the results of this work. It could be seen that, in most of the serogroup O26 strains isolated during this study, the carriage of stx1 was significantly ($P < 0.001$) associated with the carriage of *eae*, and *ehly*. In strains of *E. coli* O26 associated with diarrhoea and HUS in humans, it is common to find *stx1, eae,* and *ehly* (Zhang et al. 2009; Schmidt et al. 1999). Some strains carrying vtx2 have also been associated with diarrhoea and HUS (York et al. 2010). In addition, the *stx1, eae,* and *ehly* genes were much more common in *E. coli* O26 than in other serogroups. This may play an important role in facilitating colonization in the gut.

Table 11 Distribution of the 56 STEC isolates from cattle to various serogroups

Isolate source	Antimicrobial resistance		Total
	O157 *n* = 11	Non-O157 *n* = 70	
Camel	3 (75%)	17 (89.5%)	20 (86.9%)
Cattle	5 (71.4%)	41 (83.7%)	46 (82.1%)
Total	8 (72.7%)	58 (82.8%)	66 (81.4%)

E. coli O103 and O157 were the second most commonly isolated serogroup of those tested in camel faeces. Half of the *E. coli* O103 isolates carried *stx2*. This result is in contrast to the findings of a number of other studies in which STEC O103 possessed stx1 only (Wells et al. 1991, pp. 985–989; Zhang et al. 2009, pp. 449–454). Unlike serogroup O103, and serogroup O157 which were isolated at the same frequency, all but one isolate carried *stx2* and *ehly*. This finding is significant, since *stx2* and *ehly* are regarded as important virulence factors found in *E. coli* O157 strains associated with HC and HUS in humans (Serna and Boedeker 2008).

The absence of *E. coli* O45 in this study was not a surprise since this serogroup has not been found frequently in either camel or cattle (York et al. 2010; El-Sayed et al. 2008). However, the low prevalence of *E. coli* O91 serogroup is consistent with the findings of other studies (Krüger et al.

Table 12 Comparison of antimicrobial resistance profile of the O157 STEC and non-O157 STEC isolates from camels

Antimicrobials	Resistant STEC 0157 isolate strains from camel Total no. = 3 *n* (%)	Resistant STEC non-0157 isolate strains from camel Total *n* = 17 *n* (%)
AMP	1 (25)	6 (31.6)
CTX	0 (0)	1 (5.3)
TET	2 (75)	9 (47.4)
GEN	0 (0)	4 (21.1)
STP	0 (0)	7 (36.8)
CHL	0 (0)	0 (0)
NAL	1 (25)	6 (31.6)
TMP	1 (25)	5 (26.3)
CIP	0 (0)	2 (10.5)
CAZ	0 (0)	0 (0)
CRO	0 (0)	0 (0)

AMP ampicillin 10 μg/disc, *TET* tetracycline 30 μg/disc, *CAZ* ceftazidime 10 μg/disc, *STP* streptomycin 30 μg/disc, *GEN* gentamycin 10 μg/disc, *NAL* nalidixic acid 30 μg/disc, *CRO* ceftriaxone 30 μg/disc, *CIP* ciprofloxacin 5 μg/disc, *CTX* cefotaxime 5 μg/disc, *CHL* chloramphenicol 30 μg/disc, *TMP* trimethoprim 5 μg/disc, *n* number of STEC tested

2011, pp. 73–79). Concurrent shedding of more than one serogroup was uncommon and, with the exception of *E. coli* O26 and O91, we found no tendency for two serogroups to be isolated simultaneously more than could be expected by chance. Although *E. coli* O91 was detected in two samples, in one of these it occurred simultaneously with *E. coli* O26.

All the eleven virulence gene profiles were observed among isolates from cattle and six among isolates from camels. There were significant differences ($P < 0.05$) among the virulence gene profiles from the various sources. The most frequent virulence gene profile among the isolates was *stx1/eae/ehlyA*, which was significantly more among camels than the cattle isolates. Accordingly, these strains should be considered pathogenic for humans; however, their ability to cause severe disease and their potential to cause outbreaks could be questioned because most outbreak of STEC infection was associated with *stx2*-positive strains (EFSA 2009). Also isolates from cattle with the same profile or carrying stx1 genes in this study could be potential pathogens for humans (Gyles and Fairbrother 2004).

Isolates with *stx2* only in this study were significantly more among cattle than the camel isolates. Also it was observed that significant ($P < 0.05$) positive correlations between the *eae* and *stx2* genes exist among the isolates in this study. In

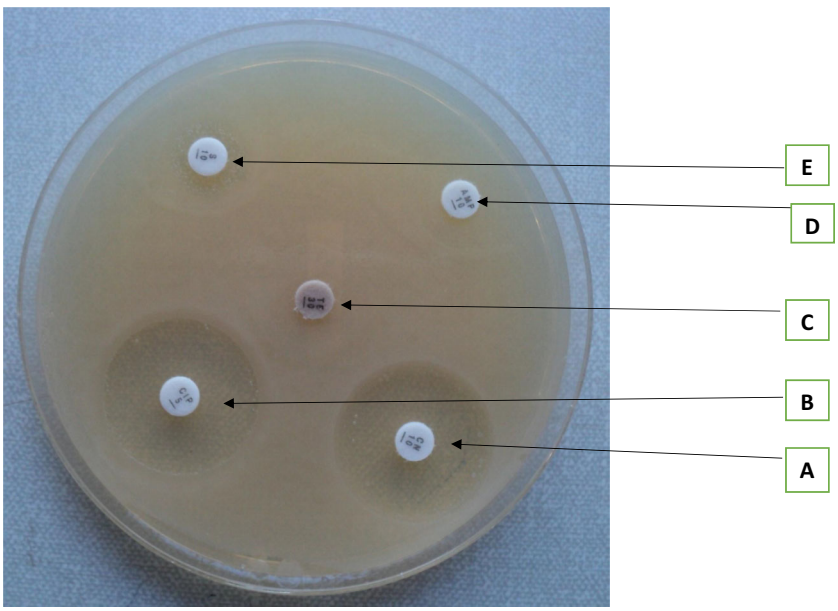

Plate 3 Disk diffusion showing zones of inhibition of growth of an isolate from camel susceptible to A = Gentomycin (GN), B = Ciprofloxacillin (CIP), E = Streptomycin (S) Resistance to, C = Tetracycline (TC) and D = Ampicillin (AMP)

Table 13 Comparison of antimicrobial resistance profile of the O157 STEC and non-O157 STEC isolates from cattle

Antimicrobials	Resistant STEC 0157 isolate strains from cattle Total no. = 5 *n* (%)	Resistant STEC non-0157 isolate strains from cattle Total no. = 41 *n* (%)
AMP	3 (42.9)	32 (65.3)
CTX	0 (0)	0 (0)
TET	2 (28.6)	17 (34.7)
GEN	2 (28.6)	13 (26.5)
STP	3 (42.9)	16 (32.7)
CHL	0 (0)	0 (0)
NAL	2 (28.6)	13 (26.5)
TMP	2 (28.6)	16 (32.7)
CIP	0 (0)	4 (8.2)
CAZ	0 (0)	0 (0)
CRO	0 (0)	0 (0)

AMP ampicillin 10 μg/disc, *TET* tetracycline 30 μg/disc, *CAZ* ceftazidime 10 μg/disc, *STP* streptomycin 30 μg/disc, *GEN* gentamycin 10 μg/disc, *NAL* nalidixic acid 30 μg/disc, *CRO* ceftriaxone 30 μg/disc, *CIP* ciprofloxacin 5 μg/disc, *CTX* cefotaxime 5 μg/disc, *CHL* chloramphenicol 30 μg/disc, *TMP* trimethoprim 5 μg/disc, *n* number of STEC isolates tested

agreement with the present study, *stx2* was the dominant Shiga toxin type in some other studies (Beutin et al. 1997, pp. 2175–2180 and Zahraei et al. 2006). In contrast, several studies have also shown that *stx1* was the major Shiga toxin type among STEC isolates from calves (Orden et al. 1998, pp. 239–248 and Leomil et al. 2003, pp. 103–109; Akter et al. 2016, pp. 63–68). The important criteria for these types of variations could be the geographical area where the animals originated. Five profiles that were found among isolates from cattle were not found in camels and six were not found in human. All profiles found in isolates from camels and humans are also present in isolates from cattle. This finding reconfirmed cattle as the reservoir for STEC.

According to Blanco et al. 2003, pp. 345–351 and Luga et al. 2007, pp. 205–211, non-O157 STEC isolated from calves with diarrhoea showed that the majority (77–81%) of STEC were resistant to at least one of the antimicrobial agents tested, with numerous isolates being resistant to streptomycin, tetracycline, and sulphonamide.

The present study documents for the first time in North Eastern, Nigeria, the antimicrobial resistance of a large collection of STEC O157 and non-O57 strains from cattle and camel. In the present study, increasing resistance was observed.

Among the 79 STEC isolated in this study, a higher level of resistance was detected in camel (87%) and cattle (82%). This is in accordance with data obtained by Sayah et al. 2005, pp. 1394–1404, Aibinu et al. 2007, pp. 22–33 and Shittu et al. 2007, pp. 164–170, they obtained a high percentage of resistance in isolates from animals and man.

The incidence of antimicrobial resistance among *E. coli* O157 and non-O157 isolated in this study indicated that 82% of the isolates were resistant to at least one antimicrobial agent.

Isolates from cattle showed more multidrug resistance than isolates from camels. Similar findings on multiple drug resistance of *E. coli* strains have been reported from Nigeria and other parts of the world (Aibinu et al. 2007; Ojo et al. 2009, Islam et al. 2016). The high level resistance to tetracycline obtained in this study also agrees with the report of Daini and Adesemowo 2008, pp. 397–400, who reported resistance in 54 and 88% STEC strains against gentamicin and tetracycline, respectively.

Aibinu et al. 2007, pp. 22–33, also reported 94.4% resistance to tetracycline in isolates from animals and man. It is interesting that isolates from cattle and camels showed resistance to nalidixic acid and ciprofloxacin which are quinolones and fluoroquinolones, respectively, in addition to most of the frequently resisted antibiotics such as ampicillin, tetracycline trimethoprim and gentamycin.

The high prevalence of antibiotic resistant bacteria in Nigeria and other developing countries has been associated with several factors including indiscriminate use of antimicrobials due to unregulated access of non-professionals to different classes of antimicrobials over-the-counter (Okeke et al. 1999; Kabir et al. 2002). Tetracycline is one of the most commonly available antibiotics for use as growth promoter and routine chemoprophylaxis in livestock production in our country, Nigeria. They are readily available in different dosage forms and in combination with other antibiotics and vitamins, hence the high levels of resistance observed in this study.

The public health significance of these findings is that antimicrobial resistant bacteria from food animals may colonize

Table 14 Multidrug resistance patterns of STEC isolates from camels and cattle

Antimicrobials resistance patterns	No. of isolates showing resistance	No. per source	
	n (%)	Cattle *n* (%)	Camel *n* (%)
AMP	2 (3)	–	2 (4.3)
TET	3 (4.4)	3 (15)	–
STP	1 (1.5)	1 (5.0)	–
NAL	1 (1.5)	1 (5.0)	–
AMP/NAL	7 (10.4)	1 (5.0)	6 (13.0)
AMP/STP	2 (3)	–	2 (4.3)
AMP/GEN	1 (1.5)	–	1 (2.2)
AMP/TET	2 (3)	–	2 (4.3)
TET/GEN	1 (1.5)	–	1 (2.2)
GEN/TMP	5 (7.4)	1 (5.0)	4 (8.7)
TET/TMP	7 (10.4)	2 (10)	5 (10.9)
TET/STP	1 (1.5)	1 (5.0)	–
GEN/NAL	1 (1.5)	1 (5.0)	–
STP/NAL	1 (1.5)	1 (5.0)	–
AMP/TET/CIP	1 (1.5)	1 (5.0)	–
AMP/TET/STP	3 (4.5)	1 (5.0)	2 (4.3)
AMP/TET/NAL	3 (4.5)	–	3 (6.5)
TET/GEN/TMP	1 (1.5)	–	1 (2.2)
AMP/STP/CIP	2 (3)	1 (1.5)	1 (2.2)
AMP/GEN/TMP	2 (3)	–	2 (4.3)
AMP/STP/NAL	3 (4.5)	2 (10)	1 (2.2)
TET/GEN/TMP	1 (1.5)	1 (5.0)	–
AMP/STP/CIP	2 (3)	1 (5.0)	1 (2.2)
AMP/GEN/TMP	2 (3)	–	2 (4.3)
AMP/TET/NAL	3 (4.5)	2 (10)	1 (2.2)
CTX/GEN/STP	1 (1.5)	1 (5.0)	–
AMP/GEN/STP	3 (4.5)	–	3 (6.5)
AMP/GEN/STP/TMP	3 (4.5)	1 (5.0)	2 (4.3)
AMP/TET/GEN/TMP	1 (1.5)	–	1 (2.2)
AMP/TET/STP/NAL	2 (3)		2 (4.3)
AMP/TET/NAL/TMP	3 (4.5)	1 (5.0)	2 (4.3)
AMP/TET/STP/TMP	1 (1.5)	–	1 (2.2)
AMP/TET/STP/CIP	2 (3)	–	2 (4.3)

AMP ampicillin 10 μg/disc, *TET* tetracycline 30 μg/disc, *CAZ* ceftazidime 10 μg/disc, *STP* streptomycin 30 μg/disc, *GEN* gentamycin 10 μg/disc, *NAL* nalidixic acid 30 μg/disc, *CRO* ceftriaxone 30 μg/disc, *CIP* ciprofloxacin 5 μg/disc, *CTX* cefotaxime 5 μg/disc, *CHL* chloramphenicol 30 μg/disc, *TMP* trimethoprim 5 μg/disc

the human population via the food chain by consuming improperly cooked enteral (visceral offal).

In conclusion, the findings of this study showed that more STEC isolates came from cattle compare to camels. There was significant ($P < 0.05$) seasonal trend in the prevalence of STEC among cattle and camel with more cases recorded during the wet season. Although, the study did not demonstrate significant influence of sex from the various sources. The serogroups recorded in this study were O157, non-O157 such as O26, O91, O103 and O111. This study documented the prevalent presence of *stx*2 genes, followed by *stx*1. Other genes detected in this study were *eae* and *ehlyA*. The antimicrobial susceptibility study showed high resistance profile to the agents tested. However all the 79 isolates were sensitive to chloramphenicol, ceftazidime and ceftriaxone. This is the first report of a large collection of antimicrobial resistance of STEC O157 and non-O157 strains in North Eastern, Nigeria.

The presence of STEC from faeces of these animals are alarm bells indicating food safety problems. We therefore recommend improvement in slaughter house hygiene to

minimize the risk of human infections. Furthermore, a systematic research be conducted on the meat of these animals to evaluate the level of contamination by STEC.

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Author contributions J.A Ameh conceived the idea, M.S. Adamu and Y.A. Kwabugge carried out the research, C.I.I. Ugochukwu wrote the manuscript while S.I. Idoko, C.I.I. Ugochukwu and N.S. Abubakar did the statistical analysis. All authors read and approved the manuscript for submission.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical considerations The ethics governing the use and conduct of experiments on animals were strictly observed, and the experimental protocol was approved by the University of Maiduguri Senate committee on Medical Research ethics. Proper permit and consent were obtained from the Maiduguri abattoir management, before the faecal samples of the cattle and camels slaughtered in this abattoir were used for this experiment.

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