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Full Length Research Paper

Public health risk of O157 and Non-O157 Shiga toxin producing *Escherichia coli* strains in a semi-urban environment in Nigeria

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Vehicles of shiga toxin producing *Escherichia coli* (STEC) infections can be quite broad and under reported especially in the rural and semi-urban communities in the developing countries, suggestive of silent and high prevalence which is a public health risk. This may not be unconnected with acute renal failure cases in children and adolescents in the developing countries as STEC related infections have been associated with the few investigated among many un-investigated cases. In this study, multiplex PCR molecular profiling showed high prevalence (27.9%) of STEC strains in the environment studied, majority of which were the non-O157: H serotypes. Sixty-two (86%) of the *E. coli* 16SrRNA gene positive isolates were positive for *stx1*, *stx2* or both. Two O157: H serotypes were identified, twenty-five (40%) as non-O157: H^+ (motile) and 34 (54%) classified as non-O157: H^- (non-motile) serotypes. There was a marked heterogeneity in the STEC strains identified in this study, suggesting that the ecology of STEC seems more complex in the environment studied than elsewhere likely enhanced by commercial, social and cultural practices. The results of this study revealed an alarming prevalence of non-O157 serotypes and a few O157 serotypes in the environment studied which is a public health risk and could serve as a point of outbreak or sporadic cases globally as the world today is a global village.

Key words: Multiplex polymerase chian reaction (PCR), *Escherichia coli*, shiga toxin, serotypes, public health, prevalence.

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) is a zoonotic food and water borne pathogen associated with diarrhoea, haemorrhagic colitis (HC), haemolytic-uremic syndrome (HUS) and renal failure in children (Coombes et al., 2008). Although most studies have shown that outbreaks of HC and HUS are associated with *E. coli* O157:H7 (Pradel et al., 2001; 2008), some reports have shown a high prevalence of non-O157 and O157: H^-(non-motile) serotypes in human diseases and in animals and food products (Coombes et al., 2008; Pradel et al., 2001; Karch and Bielaszewska, 2001; Beutin et al., 2007). Non-O157 STEC serotypes have been associated with human illnesses, which were either severe or epidemic in many countries (Brooks et al., 2005; Tozzi et al., 2003; Caproli et al., 1997).

Recurrent urinary tract infections (RUTI) especially with consecutive new strains of *E. coli* have been demonstrated to endanger renal function (Koljalg et al., 2009). Reported increase in resistance to antibiotics by urinary tract *E. coli* strains is also a concern that further complicates and makes them a serious public health concern. This situation may be made worse by the circulation of substandard antibiotics in the human and

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veterinary health care systems especially in the developing countries.

End-stage renal disease (ESRD) and chronic kidney disease (CKD) are now major public health problems especially in Nigeria that are fast threatening to reach alarming proportions. Unlike in adults in whom diabetes and hypertension are responsible for the majority of ESRD/CKD, congenital causes are responsible for the greatest percentage of all cases seen in children in the developed countries and infectious causes are responsible for most cases in the developing countries (Warady and Chadha, 2007).

Acute renal failure is a common problem in children and adolescents especially in developing countries and mortality due to renal failure associated with HUS was up to 68% of cases in a report by Arora et al. (1997). Ten and eighteen years retrospective study of chronic renal failure cases in Nigeria by Alebiosu et al. (2006) and Anoche and Eke (2005) showed that it is a common cause of morbidity and mortality with urinary tract infections traced as accounting for 19% and 13.7% of cases respectively.

Akinsola et al. (1989) earlier reported that renal failure constituted 8% of hospital admissions in a specialist hospital in the South Western part of Nigeria. Although acute renal failure associated with STEC infections especially in children is mostly under reported in developing countries like Nigeria, vehicles of STEC infection are quite broad, suggestive of its silent prevalence.

Rural drinking water and recreational water are increasingly being recognised as important vehicles due to lack of portable drinking water especially in the rural and semi-urban areas in these countries. Many foods and animal products eaten are exposed and social and cultural practices largely encourage person to person contact.

Seasonal migratory cattle rearing practice where herds of cattle move from one region to another in search of greener pasture enhances the spread of STEC infections as cattle, sheep and goats have been pinned to be the main reservoirs of STEC strains (Pradel et al., 2000).

Research into such prevailing situation may be lacking and hence the prevalence of STEC related diseases especially renal failure is under reported or not reported at all.

The high prevalence of non-O157 STEC strains in a semi-urban environment in Nigeria in this study seems to point to a likely same or higher prevalence rate in the entire country which may be linked to increase in renal failure in children in the country.

The world is a global village and hence an outbreak of STEC infections in a remote part of the globe is a threat to global health. STEC-infected domestic animals normally do not show signs of disease and can be included in food. As a result, products of animal origin, such as meat and milk are at risk of contamination with STEC originating from animals (Beutin, et al., 2007).

Hawking and selling of foods along the streets is also a common practice in African developing countries which evidently will promote person to person spread of STEC infection. Since the natural reservoirs of STEC are domestic and wild ruminant animals, which shed the bacteria with their faeces into the environment (Caprioli et al., 2005) and contaminated foods and foodstuffs are major vehicles of STEC transmission to humans; we became interested in investigating STEC strains from humans, domestic animals and food samples for their role as vehicles of transmission to humans in a semi-urban society. While the prevalence of O157 and non-O157: H STEC strains have been investigated in many parts of the world (Pradel et al., 2008), it is to the best of our knowledge under reported in Nigeria. Renal failure in children and adolescents are being reported and many of these renal failure cases in children could be STEC related.

In this study, we report the prevalence of STEC infections and the public health risk in a semi-urban society in central Nigeria, hosting people from different regions of the country that could be on transit or are permanent residents. The study focused on the investigation of STEC *E. coli* from diarrhoea stool of gastroenteritis patients, domestic animals and food elements and the application of multiplex PCR for characterisation, aiming at the dynamics of STEC diseases in Nigeria with increasing cases of renal failure in children. Two primers sets (Table 1) were used to detect genes encoding virulence factors and serotyping. The study involved 5 virulence genes *Stx1, Stx2, Stx2d, EHEC hylA* and *eaeA*; genotype genes $rfbE_{O157}$ and $fliC_{H7}$ and an internal positive control for *E. coli 16S rRNA*.

MATERIALS AND METHODS

Sampling and bacterial isolation

A total of fifty-two *E. coli* isolates were collected from the Federal College of Veterinary and Medical Laboratory Technology Clinic Laboratory and the National Veterinary Research Institute (NVRI) Veterinary Clinic Laboratory. The isolates (pigs = 10 isolates; cattle = 16 isolates; goats = 8 isolates; sheep = 8 isolates; human = 10 isolates) were isolated from the diarrhoea stool of gastroenteritis patients and the faeces of infected animals over a 3-months period, between July and October, 2009. There was a total of 178 *E. coli* isolates in the registers of the 2 laboratories for the time period under review but only 52 were collected for this study due to limited availability of PCR reagents.

Twenty *E. coli* isolates were isolated from food samples often eaten without heat processing (10 isolates out of 71 raw cow milk samples and 10 isolates out of 96 “gari” samples - fried cassava grittings) within the 3 months period. A 20 g portion of each “gari” sample was soaked in 100 ml buffered peptone water in sterile 200 ml bottle (Duran®). One millilitre “gari” supernatant was mixed with 15 ml LB agar (Sigma) at 45°C in a petri dish in triplicates and incubated for 24 h at 37°C. One millilitre of milk was mixed with 15 ml LB agar (Sigma) at 45°C in a petri dish and incubated at 37°C for 24 h.

Table 1. List of primers sequences used in this study.

Primer set	Primer	Sequence (5' – 3')	Target gene	Size of amplicon (bp)	Reference
A	HylA-F	AGCTGCAAGTGCGGGTCTG	*EHEC hylA*	569	Wang et al., 2002
	HylA-R	TACGGGTTATGCCTGCAAGTTCAC			
	RfbE-F	CTACAGGTGAAGGTGGAATGG	*rfbE$_{O157}$*	327	Wang et al., 2002
	RfbE-R	ATTCCTCTCTTTCCTCTGCGG			
	FliC-F	TACCATCGCAAAAGCAACTCC	*fliC*	247	Wang et al., 2002
	FliC-R	GTCGGCAACGTTAGTGATACC			
	Stx2-F	TTAACCACACCCACGGCAGT	*stx2*	346	Pollard et al., 1990
	Stx2-R	GCTCTGGATGCATCTCTGGT			
B	Eae-F	GCAAATTTAGGTGCGGGTCAGCGTT	*eae*	494	Wang et al., 2002
	Eae-R	GGCTCAATTTGCTGAGACCACGGTT			
	E16S-F	CCCCCTGGACGAAGACTGAC	*16S rRNA*	401	Wang et al., 2002
	E16S-R	ACCGCTGGCAACAAAGGATA			
All	Stx1-F	GAAGAGTCCGTGGGATTACG	*stx1*	130	Pollard et al., 1990
	Stx1-R	AGCGATGCAGCTATTA			

About 3 - 5 colonies with a typical *E. coli* morphology were picked per plate and sub cultured in LB broth for multiplex PCR. The *E. coli* isolates collected from the human and veterinary clinics were characterised by biochemical tests: urease production, indole production, β-galactosidase activity and carbohydrate fermentation.

Genomic DNA Extraction

Five millilitres of each broth culture was centrifuged at 2,700 × g for 10 minutes to pellet the cells. The cells were washed twice with 1 ml PBS pH 7.4 and suspended in 200 µl PBS pH 7.4. The genomic DNA was extracted using ZR Genomic DNA™ Miniprep Kit (ZYMO Research Corp. USA) according to the manufacturer's instructions.

Briefly, 100 µl suspended *E. coli* cells was mixed with 95 µl 2 x Digestion Buffer and 5 µl proteinase K and incubated at 55°C for 20 min in a block heater (Staurt®). 700 µl Lysis Buffer was added to the digest and mixed thoroughly by vortexing in a vortex mixer (Thermolyne®). The genomic DNA was collected in a spin column and washed twice with wash buffers by centrifugation at 14,000 × g for 1 min in a micro centrifuge (Eppendorf-5417R). The DNA was eluted into 1.5 ml micro centrifuge tube using the elution buffer at maximum speed (20,000 × g) for 30 s. The extracted DNA samples were stored at -20°C until needed for PCR.

Shiga toxin producing *Escherichia coli* (STEC) polymerase chain reaction (PCR)

Stx1 and Stx2 primers sets as designed by Pollard et al. (1990) were used to detect *Stx1* and *Stx2* genes. The PCR reaction mixture was a total of 25 µl containing 5.0 µl DNA extract, 2.5 µl of 10 × buffer (Fermentas®), 0.5 µl of 10 mM dNTPs mix (Fermentas®), 0.5 µl of 20 µM primers (Table 1), 1.5 µl of 25 mM $MgCl_2$ and 2.5 U of Taq Polymerase (Fermentas®). PCR amplification was performed in GeneAmp 9700 (Applied Biosystems) with initial denaturation at 94°C for 5 min. This was followed by 40 cycles of denaturation at 94°C for 1 min; annealing at 53°C for *Stx1* and at 55°C for *Stx2* for 1 min; and extension at 72°C for 1 min.

Ten microlitres of the PCR product was electrophoresed in an agarose gel (1.5%) containing 5 µl of 10 mg/ml ethidium bromide at 80 V for 60 min. 50 bp DNA marker (Fementas) was used as molecular size marker. DNA amplifications were examined under U.V transilluminator and results documented using Gel Documentation System (Synegene®).

Multiplex polymerase chain reaction (PCR)

Two sets of primer mixtures were used to run the Multiplex PCR (Table 1). In general the reagents mixture was as follows: 5 µl of genomic DNA extract was used as template with Mg^{2+} free 2.5 µl 10xPCR reaction buffer (Fermentas®); 0.5 µl of 10 mM dNTPs; 1.5 µl of 25 mM $MgCl_2$; and 2.5 units of Taq polymerase (Fementas®). Set A contained 0.1 µM E16S 0.6 µM HlyA; 1.5 µM Stx2d; 0.4 µM of FliC; 0.9 µM of RfbE primers and set B mixture was 0.75 µM EAE and 0.1 µM E16S primers. PCR amplification was performed in GeneAmp 9700 (Applied Biosystems) with initial denaturation at 95°C for 8 min.

This was followed by 30 cycles of denaturation at 95°C for 30 s; annealing at 58°C for 30 s; and extension at 72°C for 30 s. Final extension was at 72°C for 7 min. Ten microlitres of the PCR product was electrophoresed in an agarose gel (1.5%) containing 5µl of 10 mg/ml ethidium bromide at 80 volts for 60 min. 50 bp DNA marker (Fementas®) was used as molecular size marker. DNA amplifications were examined under U.V transilluninator(Sigma) and results documented using Gel Documentation System (Synegene®).

RESULTS

Polymerase chain reaction (PCR) characterisation of shiga toxin producing *Escherichia coli* (STEC) isolates

A total of fifty-two *E. coli* isolates were collected from the Federal College of Veterinary and Medical Laboratory Technology Clinic Laboratory and the National Veterinary

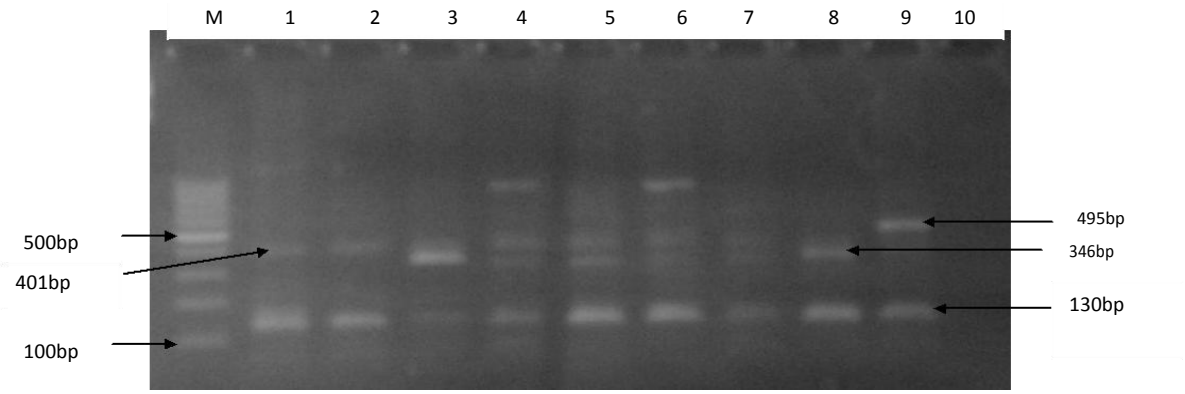

Figure 1: PCR amplification of characteristic genes of STEC isolates.

Figure 1. PCR amplification of characteristic genes of STEC isolates. Representative amplification of characteristic genes from selected STEC isolates from humans, cattle and pigs. Lane M – 100 bp DNA marker (Fermentas®), lanes 1 – 3 human isolates, lanes 4 – 6 cattle isolates; lanes 7 – 9 pig isolates. Stx 1-F and stx1-R amplified ≈130 bp; stx2-F and stx2-R amplified ≈ 346 bp; Eae-F and Eae-R primers set amplified ≈ 495 bp; E16S-F and E16S-R amplified 401bp. E16S primers set was used to amplify *E.coli* 16S rRNA and as control line 10 – negative control (nuclease free water).

Research Institute (NVRI) Veterinary Clinic Laboratory. Both the human and animal isolates (pigs = 10 isolates; cattle = 16 isolates; goats = 8 isolates; sheep = 8 isolates; human = 10 isolates) were isolated from the diarrhoea stool of gastroenteritis patients and faeces of infected animals over a 3-months period, between July and October, 2009.

The Stx1 and Stx2 primers amplified the expected gene segments (130bp and 346bp respectively) after optimization of the reaction conditions (Figure 1). The PCR using *Stx1*, *Stx2* and the *E .coli* 16SrRNA primers (E16S) gave distinct and sharper bands after optimization of the reaction conditions. The reaction conditions reported in this paper are the optimum conditions obtained in this study. Seventy-one (98%) isolates were *E. coli 16SrRNA* gene positive which indicates that all except one were *E. coli* isolates; E16S primers were designed specifically to amplify *E. coli* 1*6SrRNA* gene. Sixty-two (86%) of the 1*6SrRNA* gene positive isolates were positive for either *Stx1,* or *Stx2* gene which implies that they are STEC isolates. Thirty-five (56%) were *Stx1* gene positive and 27 (43%) *Stx2* gene positive; the presence of either or both has been related to the ability of STEC strains to cause serious disease in humans. Ironically, only cattle isolates (3) were positive for *Stx2d* gene.

The non STEC isolates unexpectedly were more in the human, milk and "gari" isolates than the animal isolates and have been associated with diarrhoea as the isolates were from diarrhoea stool from gastroenteritis patients who visited Federal College of Veterinary and Medical Laboratory Technology Clinic Laboratory for medical attention or laboratory diagnosis. Generally, the results showed that majority of the *E. coli* isolates studied were STEC strains, indicative of their prevalence in the environment considered in this study.

Multiplex polymerase chain reaction (PCR) detection of virulence and O157 serotype genes

The primers utilized were designed by Wang et al.(2002), but reaction conditions were empirically determined and optimized to give optimum results. The primers were designed to target the coding regions of the virulence genes (Table 1) and the concentrations of the primers in reaction mixtures were higher for those with weak loci and lower for those with strong loci to give satisfactory amplification. The multiplex PCR sensitivity and specificity tests were as carried out by Wang et al. (2002), who designed the primers used for multiplex PCR in this study. The multiplex PCR amplification gave distinct and clear bands for the virulence and serotype genes detected (*eaeA, hlyA, rfbE, fliC* genes). Typical amplification results are shown in Figures 1, 2 and 3.

The PCR product sizes obtained for the various genes (*eaeA* – 495bp, *hlyA* – 569bp, *fliC* – 247bp, *rfbE* – 327bp) were the same with the ones obtained by Wang et al. (2002). Three repeats of the amplification process with the same reagents mix and reaction conditions gave the same amplification pattern, which indicates the reproducibility of the protocol. However, the *E. coli E16S rRNA* gene product was more amplified in each repeat than any of the genes studied, indicative of high copy number of 16SrRNA genes in the genome.

The STEC isolates were identified as serotypes O157: H^+ (1), O157: H^- (1), non - O157: H^+ (25) and non-O157: H^- (34) isolates using the *rfbE*$_{O157}$ and *fliC* serotype gene specific primers. The majority of the STEC isolates were the non-O157: H serotypes many of which were also the non motile types (Table 2). Twenty-five (40%) were classified as non – O157: H^+ (motile) and 34 (54%) were classified as non – O157: H^- (non-motile) serotypes (Table 2). The only two O157: H serotypes were from

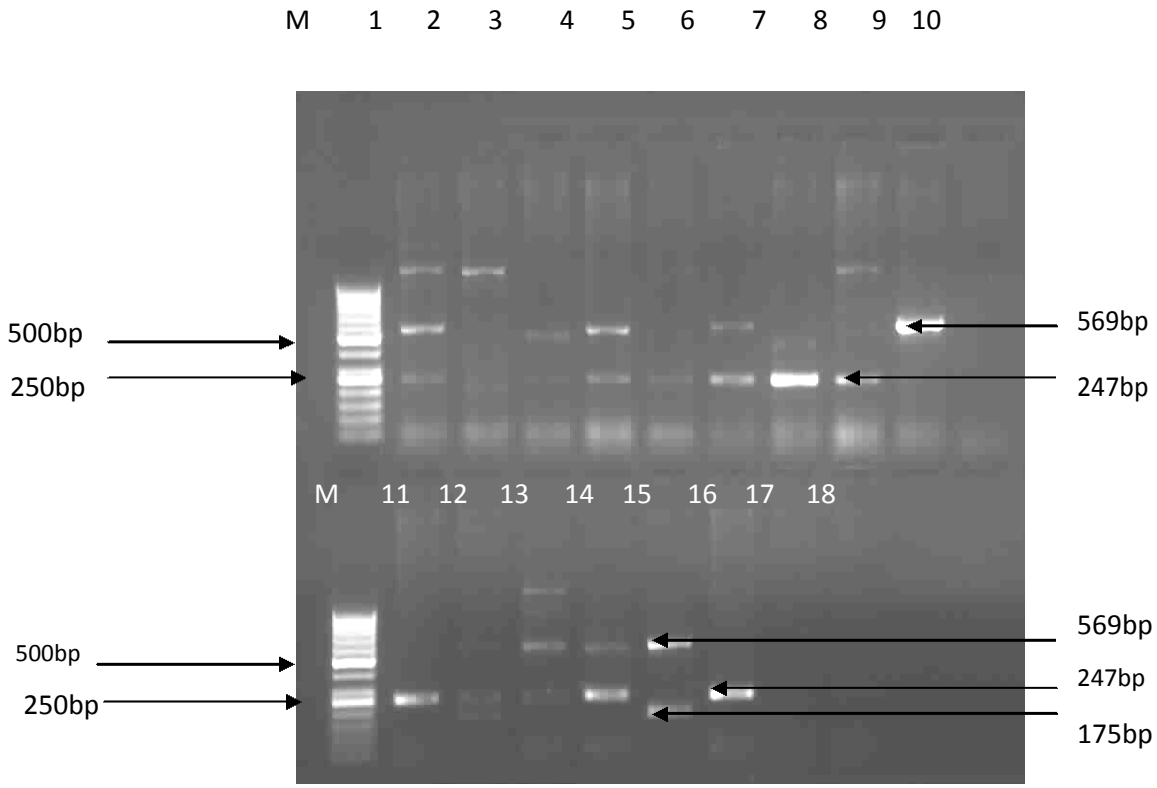

Figure 2. Multiplex PCR of virulence genes in STEC isolates from pigs and cattle faecal samples. Lane M – 50bp marker (Fermentas®); lanes 1-10 are pigs isolates; lanes 11-17 are cattle isolates. HlyA-F and HlyA-R primers set amplified **2**HlyA: Multiplex gene fragment PCR (569bp); of virulence FliC-F and FliC-R primers set amplified FliC gene fragment (247bp); Stx2d-F and Stx2d-R primers set amplified Stx2d gene fragment (175bp). Lane 18 – negative control (nuclease free water).

the human and the "garri" STEC isolates suggesting that the non – O157: H serotypes are more prevalent in the environment under study. This is also expected as "garri" is a food item consumed mainly by humans in Nigeria.

The results do not show a distinct pattern of occurrence of the serotypes at animal species level, but cut across all the animal species, animal and plant products investigated in this study. Two other virulence genes (*hlyA* and *eae* genes) were also detected in the non – O157: H serotypes of the STEC isolates studied. *EHEC hlyA* gene was detected in 11 (15%) of the non – O157: H serotypes and *eae* gene was detected in 12 (17%) of the non – O157: H serotypes. These genes were not detected in the O157: H serotypes or not detected in this virulence gene test. A typical Multiplex PCR results showing the detection *EHEC a hlyA* and *eae* gene is shown in Figures 1and 3. These results showed diversity in the occurrence of these major virulence genes in the STEC isolates studied, however the sporadic nature of their occurrence across the animal species is not explainable. The diversity of STEC isolates and serotypes characterised in this study brings out the prevalence of STEC in this environment and by extension in Nigeria.

DISCUSSION

Multiplex PCR was used to assess the occurrence of STEC strains, both the O157 and non-O157 serotypes in domestic animals and humans diagnosed to have gastroenteritis based on clinical presentations. The primers used amplified the expected segments of the genes sought and amplifications were specific for the genes in question, presence of which were used to characterise the *E .coli* isolates. Multiplex PCR as used in this study, has been found to be an important diagnostic technique to identify STEC infections as the clinical presentation of STEC disease is sometimes confused with other conditions such as inflammatory bowel disease, *Clostridium difficile* infection and appendicitis. It is a rapid and sensitive diagnostic technique to be used in the laboratory diagnosis of STEC disease which can prevent unnecessary invasive, expensive surgical and investigative prognosis or misadministration of antibiotic therapy. The success of any effective preventive measure is dependent on the availability of rapid, sensitive, simple and reproducible method(s) for the detection of STEC strains.

In this study, molecular profiling of the prevalence of

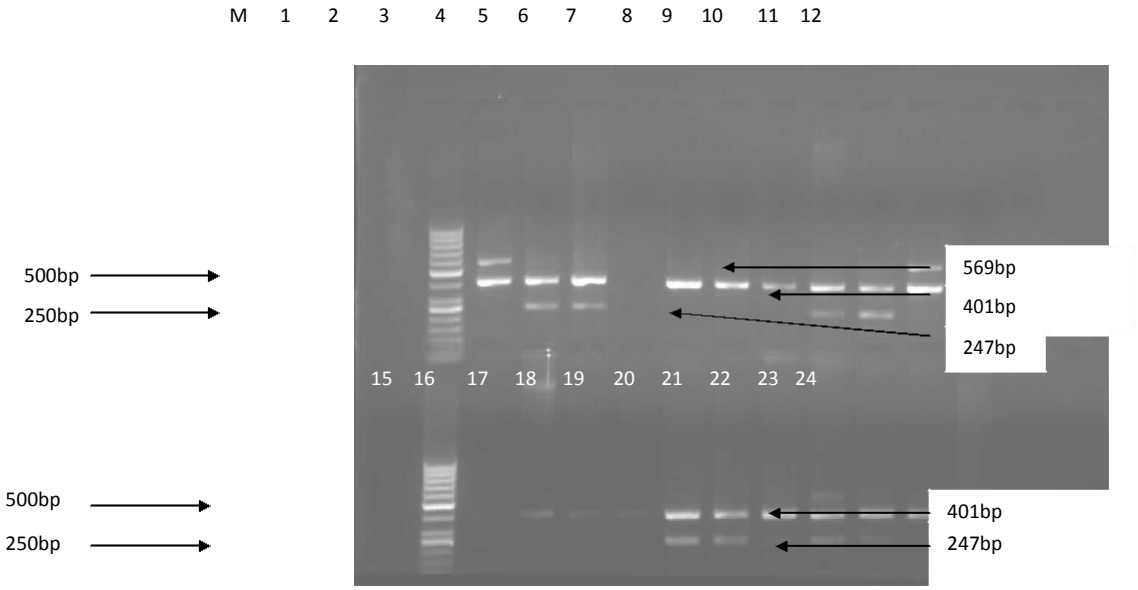

Figure 3. Multiplex PCR of virulence genes in STEC isolates from cattle, goats and sheep faecal samples and milk**Figure**.Lane**3**: MMultiplex–50bpDNAPCRmarkerofvirulence(Fementas®);genes inlanesSTEC1andisolates2cattlefromisolates;cattle,lanesgoats3 - and11goatssheepisolates; lanes 12 – 16 milk isolates; lanes17 – 23 sheep isolates. HlyA-F and HlyA-R primers set amplified HlyA gene fragment (569bp); FliC-F and FliC-R primers set amplified FliC gene fragment (247bp); E16S-F and E16S-R amplified E. coli 16SrRNA gene (401bp). E16S primers set was used to amplify *E.coli* 16S rRNA and as control. Lane 24 – negative control (nuclease free water).

Table 2. Major virulence genes detected by multiplex PCR.

Animal species	No. isolates	E. coli 16SrRNA	*stx1*	*stx2*	*stx2d*	fliC	hlyA	eae	rfbE	Serotype
Pigs	10	10	10	6	_	8	5	3	_	Non- O157: H^+ (8); Non- O157: H^- (2)
Cattle	16	16	9	8	3	5	4	5	_	Non- O157: H^+ (5); Non- O157: H^- (5)
Goats	8	8	5	6	_	4	1	_	_	Non- O157: H^+ (4); Non- O157: H^- (4)
Sheep	8	8	5	5	_	6	1	3	_	Non- O157: H^+ (6); Non- O157: H^- (2)
Milk	10	10	3	2	_	_	_	_	_	Non-O157: H^+ (0); Non-O157: H^- (10)
Gari	10	10	2	_	_	_	_	_	1	O157: H^- (1); Non-O157:H^-(1); Non-STEC (8)
Human	10	10	1	_	_	3	_	_	1	O157: H^+ (1); Non- O157: H^+ (2); Non-STEC (7)
Total	72	71	35	27	3	26	11	11	2	

O157 and non – O157 STEC strains in a semi-urban environment in Central Nigeria, hosting the only veterinary research institute in the country was carried out. Also as the only veterinary research institute, with 20 Outstation Research Laboratories spread across the entire nation, many samples treated in these laboratories are received from different parts of the country. Domestic animals are brought from different parts of the country for treatment of different types of animal diseases.

In addition, the host state (Plateau State) is unique in its geopolitical position, hosting people from different parts of the country who could be on transit or are permanent residents. This study gives a glimpse of the prevalence in the country because in addition to the unique factors above, most animals and food stuffs trade movement between the Southern and Northern parts of the country pass through the North Central Region of Nigeria.

The prevalence of STEC based on this study was 27.9 and 3.7% in the animal and human/food items respectively. These results showed that there was more prevalence of STEC strains in the domestic animals (pigs, cattle, goats, and sheep) which are the main sources of meat and milk across the country. The high incidence in the domestic animals may be explained by the fact that free range animal rearing is the most common animal husbandry in the country where same grazing grounds are used by farmers within a community.

The same streams, ponds and rivers are also used as common sources of drinking water for both humans and animals.

Domestic animals have been identified as natural reservoirs for STEC infections which shade the bacteria with their faeces into the environment (Al-Gallas et al., 2002; Sonntag et al., 2005; Caproli et al., 2007). Seasonal migratory cattle rearing practice in Nigeria where herds of cattle move from one region to another in search of greener pasture enhances the spread of STEC infections as cattle, sheep and goats have been pinned to be the main reservoirs of STEC strains (Pradel et al., 2000).

The marked heterogeneity of the STEC strains identified in this study showed that the ecology of STEC seems much more complex in Nigeria than elsewhere. Commercial, social and cultural practices contribute in no small measure to the spread of STEC strains from animal reservoirs to humans and from person to person. For instance hooking is a common practice in Nigeria which evidently will promote person to person spread of STEC infection. The results also showed that both *stx1* (56%) and *stx2* (43%) genes were markedly high and the presence of either or both has been related to the ability of STEC strains to cause serious disease in humans.

STEC strains producing stx2 only have been shown to be more commonly associated with serious human diseases such as HUS, than those producing stx1 alone or stx1 and stx2 possibly because the level of transcription of stx2 in vivo is higher than that of stx1 (Sung et al., 1990). The production of Shiga-toxin by STEC is the primary virulence trait responsible for HUS and the STEC strains are the major cause of gastroenteritis, haemorrhagic colitis(HC) and are also associated with renal failure in children (Banatvala et al., 2001; Todd and Dundas, 2001). Majority of the STEC isolates were the non-O157 serotypes many of which were the non-motile types.

Several reports have shown a high prevalence of non-O157 and O157: H^- serotypes in human diseases and in animals and food products (Pradel et al., 2001; Karch and Bielaszewska, 2001; Beutin et al., 2007; Coombes et al., 2008). The detection of more non-O157 serotypes in the domestic animals than in humans and food products may be explained by the type of animal husbandry practiced where grazing grounds and water sources in a community are open to all herds encouraging infections and re-infections. Similar serotypes prevalence pattern in both humans and animals may evidence animals to humans spread. More than 200 non-O157 STEC serotypes have been associated with human illnesses, occasionally severe or epidemic in Canada, the United States, Australia, Latin America and Europe (Brooks et al., 2005; Tozzi et al., 2003; Caproli et al., 1997). In this study, only one STEC strain of serotype O157: H^- (non-motile) was detected and it was in the “gari” food item.

Two other virulence genes (*hlyA* and *eae* genes) were also detected in the non – O157: H serotypes of the STEC isolates studied. Intimin (eae gene product), is responsible for intimate attachment of STEC to the intestinal epithelial cells, causing attaching and effacing lesions in the intestinal mucosas. However, a significant minority of Human STEC isolates, including those from patients with HC and HUS, do not contain eaeA, indicating that intimin is not essential for human virulence (Barrett et al., 1992). Enterohemolysin production (hlyA gene product) might have a direct association with the capacity of a given STEC strain to cause more serious disease (Paton and Paton, 1998). The results of this study showed diversity in the occurrence of these genes in the STEC isolates studied, however, the sporadic nature of their occurrence across the species studied is not explainable.

On the whole, this study has revealed the prevalence of STEC infections, particularly the non-O157 serotypes in this environment and by extension in Nigeria. This is a public health concern in the country and the world in general considering the fact that the world today is global village especially because of human movements through national and international air and sea ports.

The World Health Organisation (WHO) has called the identification of virulent non-O157 STEC, a public health priority especially due to its emergence in severe epidemic human disease (Coombes et al., 2008). The study has also shown the relevance of multiplex PCR as a new tool that can be used to assess the public health risks associated with STEC infections especially virulent non-O157 serotypes from foods, animals and the environment.

Based on the results of this study it is logical to suggest that the common incidences of renal failure in children and adolescents in Nigeria (Alebiosu et al., 2006; Anoche and Eke, 2005) may not be unconnected with STEC infections. They reported that 13.7 and 19% morbidity and mortality in 2 hospitals in Nigeria were traced to urinary tract infections.

A study by Warady and Chadha (2007) showed that infectious causes are responsible for the greatest percentage of all cases of end-stage renal disease (ESRD) and chronic kidney disease (CKD) in children in the developing countries. Recurrent urinary tract infections (RUTI) considered mostly as reinfections of the urinary tract with consecutive new strains of *E. coli* endangers renal function (Koljalg et al., 2009). This is further complicated by antimicrobial therapy for RUTI with increased resistance to antibiotics among urinary tract *E. coli* strains.

In Nigeria, the situation is made worse by the circula-tion of substandard antibiotics in our health care system. Acute renal failure cases associated with STEC infections especially in children is most likely under reported in Nigeria because vehicles of STEC infections are quite broad, suggestive of its silent high prevalence in the country.

In conclusion, multiplex PCR was successfully applied to assess the prevalence of STEC strains in a semi-urban environment in Central Nigeria and was identified as an important diagnostic technique to be used along with clinical diagnosis.

The results of this study revealed a moderately high prevalence of O157 and non-O157 serotypes STEC infection in the semi-urban environment studied which is a public health risk and could serve as a point of outbreak or sporadic cases globally as the world today is a global village.

The results also showed a higher prevalence of STEC in all the domestic animal groups particularly the non-O157 serotypes which may be explained by free range animal husbandry practice in the environment where same grazing grounds and water sources are used by farmers within a community constantly exposing the human population to STEC infections.

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