Shiga toxin-producing Escherichia coli *O157:H7 in milk and milk products in Ogun State, Nigeria*

Akhigbe Ivbade[1], Olufemi Ernest Ojo[2*] & Morenike Atinuke Dipeolu[1]

[1] *Department of Veterinary Public Health and Reproduction, College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Abeokuta, Nigeria.*
[2] *Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Abeokuta, Nigeria.*

** Corresponding author at: Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Abeokuta, Ogun State, Nigeria.*
Tel.: +234 803 5803 716, e-mail: oeoefemi@yahoo.com, ojooe@funaab.edu.ng.

Veterinaria Italiana 2014, **50** (3), 185-191. doi: 10.12834/VetIt.129.2187.1
Accepted: 21.05.2014 | Available on line: 30.09.2014

Keywords
Milk,
Milk products,
Multidrug resistance,
Nigeria,
STEC O157,
Virulence genes.

Summary
Shiga toxin producing *Escherichia coli* (STEC) O157 is a major cause of food-borne illnesses in humans. This study investigated the presence of STEC O157 in milk and milk products in Ogun State, Nigeria. Of a total of 202 samples 10 (5%) were positive for STEC O157 including 1 (2%) of 50 raw milk samples, 3 (6%) of 50 samples of fresh local cheese, 1 (2%) of 50 samples of fried local cheese and 5 (9.6%) of 52 fermented milk samples. There was no significant difference ($p>0.05$) in the prevalence of STEC O157 among the sample types. Of 10 isolates, shiga toxin 1 gene (stx_1) was detected only in 2 samples (20%), shiga toxin 2 (stx_2) was extracted only in 6 samples (60%), stx_1/stx_2 in 2 samples (20.0%), intimin gene (*eaeA*) in 5 samples (50%), and enterohaemolysin (*E-hlyA*) gene was isolated in 7 (70%) samples. Rates of resistance of the STEC O157 isolates were: amoxicillin/clavulanic acid 100%, ampicillin 100%, chloramphenicol 60%, nalidixic acid 20%, norfloxacin 10%, streptomycin 30%, sulphamethoxazole/trimethprim 20%, and tetracycline 90%. The isolates were all susceptible to ciprofloxacin and neomycin. The presence of virulent multidrug resistant *E. coli* O157 strains in milk and milk products as revealed by this study unveils a risk of human exposure to these potentially fatal pathogens following consumption of contaminated products.

Escherichia coli *O157:H7 e produzione di tossina shiga in latte e derivati nello stato di Ogun, Nigeria*

Parole chiave
Antibiotico resistenza,
Derivati del latte,
Escherichia coli (STEC) O157,
Latte,
Nigeria,
Tossina shiga,
Virulenza.

Riassunto
Escherichia coli (STEC) O157 produttore di tossina shiga è una delle maggiori cause di patologie di origine alimentare nell'uomo. Lo studio ha analizzato la presenza di STEC O157 in latte e derivati nello stato di Ogun, Nigeria. Su 202 campioni, 10 (5%) sono risultati positivi per STEC O157, in particolare, 1 (2%) dei 50 campioni di latte crudo, 3 (6%) dei 50 campioni di formaggio fresco locale, 1 (2%) dei 50 campioni di formaggio locale fritto e 4 (9,6%) dei 52 campioni di latte fermentato. Non è stata riscontata una differenza significativa ($p>0,05$) tra le prevalenze di STEC O157 nei diversi tipi di campione. Nei 10, il gene 1 della tossina shiga (stx_1) è stato rinvenuto in 2 campioni (20%), il gene 2 (stx_2) in 6 campioni (60%), stx_1/stx_2 in 2 campioni (20%), il gene *intimin* (*eaeA*) in 5 campioni (50%) e il gene *enterohaemolysin* (*E-hlyA*) in 7 campioni (70%). I tassi di antibiotico resistenza di STEC O157 sono stati: 100% per amoxicillina/acido clavulanico, 100% per ampicillina, 60% per cloramfenicolo, 20% per acido nalidixico, 10% per norfloxacina, 30% per streptomicina, 20% per sulfametossazolo/trimetoprim e 90% per tetraciclina. Gli isolati si sono dimostrati tutti sensibili a ciprofloxacina e neomicina. La presenza nel latte e derivati di ceppi di *Escherichia coli* O157 virulenti e resistenti a più farmaci, rilevata nello studio, ha evidenziato il rischio per il consumatore di esposizione ad agenti patogeni potenzialmente letali.

Introduction

Some members of the shiga toxin-producing *Escherichia coli* (STEC) group have proved to be important food-borne pathogens of significant public health importance. These pathogenic STEC strains are widely associated with both outbreaks and sporadic cases of food-borne disease in humans, ranging from complicated diarrhoea to haemorrhagic colitis (HC) and haemolytic uraemia syndrome (HUS). Shiga toxin-producing *E. coli* serotype O157:H7 is considered one of the most important of all known food-borne pathogens because of the severity of associated illnesses and the apparent low infective dose of less than 10 cells (Bach *et al.* 2002, Blanco *et al.* 2003). At the same time, non-O157 STEC strains belonging to other serogroups including O26, O91, O103, O111, O128 and O145 are also known to cause fatal infections in humans (Johnson *et al.* 2006).

Shiga toxin producing *E. coli* O157:H7 primarily colonizes the large intestine. Intimate adhesion to the microvilli of the intestine is made possible by the presence of intimin, a major virulence factor contributing to the pathogenicity of STEC O157:H7 (Osek and Gallien *et al.* 2002). Intimin production is controlled by the *eaeA* gene. The growth of STEC O157:H7 in human intestinal tract leads to elaboration of large quantity of toxins, which can cause severe damage to the lining of the intestine and other vital organs of the body (Nataro and Kaper 1998, Osek and Gallien *et al.* 2002). These toxins are very similar to the toxins produced by *Shigella dysenteriae* hence the appellation 'shiga toxins' (Osek and Gallien *et al.* 2002). The 2 major shiga toxins recognised are shiga toxin 1 (Stx 1) and shiga toxin 2 (Stx 2) coded for by stx_1 and stx_2 respectively. The most virulent STEC O157:H7 also possesses an additional virulence gene, *E-hlyA*, which is responsible for the production of enterohaemolysin (Osek and Gallien *et al.* 2002).

The intestinal tract of asymptomatic ruminants is the main reservoir of STEC O157:H7. Zoonotic transmission to humans usually occurs through consumption of undercooked contaminated foods of bovine origin. Faecal contamination of other food products or direct contact with infected animals can also lead to human infection. Milk and milk products are among the most common sources of STEC O157:H7 infection mainly due to faecal contamination (Armstrong *et al.* 1996). The frequent epidemiological evidence of fresh milk as a source of human O157:H7 infection also suggests the mammary gland as a potential source of infection (Wells *et al.* 1991).

In Nigeria, there is a dearth of information on the role of milk and milk products as vehicle for the transmission of STEC O157:H7 to humans. Therefore, this study investigated the occurrence, virulence genes and antimicrobial resistance of STEC O157:H7 in raw milk and ready-to-eat milk products in Ogun State, Nigeria, with the goal to assess the possible risk of human exposure to STEC O157:H7 through consumption of milk and milk products. Samples were also screened for the detection and antimicrobial susceptibility of non-O157 *E. coli* strains.

Materials and methods

Samples and sampling procedures

Two hundred and two samples were collected and screened for the presence of shiga toxin–producing *E. coli* O157. Collected samples included raw milk (50), fermented milk called *nono* in local dialect (52), local fresh cheese called *wara* (50) and fried *wara* cheese (50). Sampling was conducted from May to August 2011. In the study area, this period of the year correspond to the peak of rainy season with high availability of pasture resulting in high milk yield and increase in the street hawking of milk products. There is also an increase in the possibility of food contamination from runoff and flood-water. An inspection of each sampling sites was conducted every week throughout the sampling period. Freshly expressed raw milk samples were collected from apparently healthy lactating cows in 5 cattle herds located in suburban areas of Ogun State, Nigeria. Fifty millilitres of raw milk were collected directly from the lactating cow into sterile universal bottles after cleaning the udder with warm disinfectant solution and ethanol soaked in cotton wool. Ready-to-eat milk products (fermented milk/*nono*, fresh cheese/*wara* and fried cheese/*wara*) were collected from vendors hawking these products along streets in 4 major towns in Ogun State, Nigeria. Fifty grammes of fresh *wara*, fried *wara* and 50 ml of *nono* were collected from different street vendors. Samples were collected into sterile containers held opened for the sellers and only 1 sample was obtained from an individual vendor on each visit. Samples were properly labelled and transported in cooler with ice-packs to laboratory for immediate microbiological analysis.

Isolation and identification of *E. coli* including STEC serogroups

One millilitre of each fresh milk and fermented milk samples was inoculated into 9 ml of sterile tryptic soy broth (TSB, Oxoid®, Basingstoke, UK) in a universal bottle for pre-enrichment. The pre-enrichment culture was incubated at 37 °C for 8 hours. Ten grammes of each of the cheese samples were aseptically weighed and thoroughly homogenised

in 90 ml of sterile distilled water and 1 ml of the homogenate was inoculated into 9 mls of sterile tryptic soy broth for pre-enrichment. Following pre-enrichment, 1 ml of all the TSB culture was inoculated into 9 ml of modified tryptic soy broth (mTSB, Oxoid®, Basingstoke, UK) supplemented with novobiocin (Oxoid®, Basingstoke, UK) and incubated at 37 °C for 18 - 24 hours for selective enrichment. A loopful of the mTSB culture was streaked onto a plate of Sorbitol MacConkey agar containing 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (BCIG) (SMAC-BCIG, Oxoid®, Basingstoke, UK) into which cefixime and tellurite supplement (Oxoid®, Basingstoke, UK) has been incorporated for selective isolation of *E. coli* O157. The inoculated plates were incubated at 37 °C for 18 - 24 hours. The plates were subsequently examined for growth. Straw colour or pale yellow colonies representing non-sorbitol fermenter on SMAC-BCIG plates were tentatively identified as suspected *E. coli* O157:H7 colonies. On each plate, 5 non-sorbitol fermenting and 5 sorbitol fermenting colonies were tested for oxidase and catalase production. When the numbers of available colonies for each category were less than 5, all available colonies were tested. Oxidase negative, catalase positive colonies were subjected to further identification using a commercially available biochemical test kit for the identification of Gram-negative, oxidase negative bacilli (Microbact GNB 24E, Oxoid®, Basingstoke, UK) and the results interpreted using the accompanying computer software package (Microbact® 2000 version 2.03, Oxoid®, Basingstoke, UK). Isolates with characteristics consistent with those of *E. coli* irrespective of their sorbitol fermentation reactions were identified serologically using a latex agglutination test kit (*E. coli* O157 latex test, Oxoid®, Basingstoke, UK) according to manufacturer's instruction. Isolates producing positive reaction (agglutination) with *E. coli* O157 latex test were also tested with *E. coli* H7 antiserum (Difco®, Sparks, Maryland, USA) by a slide agglutination test. Isolates lacking the *E. coli* O157 antigen were further screened for group detection of STEC serogroups O26, O91, O103, O111, O128 and O145 using polyvalent latex agglutination test kit (Dryspot Seroscreen DR0300, Oxoid®, Basingstoke, UK).

Detection of virulence genes in *E. coli* O157:H7

Virulence genes, including shiga toxin 1 (stx_1), shiga toxin 2 (stx_2), intimin (*eaeA*) and enterohaemlysin (*E-hlyA*) as well as the *E. coli* O157 somatic antigen encoding gene ($rfbE_{O157}$), were detected in serologically identified isolates by polymerase chain reaction (PCR) using specific primers as previously described (Osek and Gallien 2002, Ojo *et al.* 2008).

Determination of antimicrobial susceptibility of *E. coli* O157 isolates

All identified *E. coli* O157:H7 isolates and additional 46 non-O157 *E. coli* isolates (1 isolate from each *E. coli*-positive sample) were tested for susceptibility to selected antimicrobial agents on Mueller Hinton Agar (Oxoid®, Basingstoke, UK) by the standard Kirby-Bauer disk diffusion method and the results interpreted in accordance with the recommendation of Clinical and Laboratory Standards Institute (CLSI)[1]. Selected antimicrobials included amoxycillin/clavulanic acid (Amc, 30µg), ampicillin (Amp, 10µg), chloramphenicol (Chl, 30µg), ciprofloxacin (Cip, 5µg), nalidixic acid (Nal, 30µg), neomycin (Neo, 30µg), norfloxacin (Nor, 10µg), streptomycin (Str, 10µg), sulphamethoxazole/trimethoprim 19:1 (Sul, 25µg) and tetracycline (Tet, 30µg).

Statistical analysis

Data were expressed in absolute values and in percentages and the compared by Chi-square test at $p<0.05$ probability level using Statistical Software Package for Social Sciences[2].

Results

Escherichia coli O157:H7 was isolated from 10 (5.0%) of the 202 samples examined. The organism was detected in 1 (2.0%) of the 50 samples of raw milk, 3 (6.0%) of the 50 samples of fresh cheese, 1 (2.0%) of the 50 samples of fried cheese, and 5 (9.6%) of the 52 samples of fermented milk (Table I). The differences in prevalence of *E. coli* O157:H7

Table I. *Prevalence of* Escherichia coli *in milk and milk products in Ogun State, Nigeria.*

Sample types	Number sampled	Number (%) positive for STEC O157:H7	Number (%) positive for non-O157 *E. coli*
Fresh raw milk	50	1 (2.0)	15 (30)
Fresh cheese (*wara*)	50	3 (6.0)	12 (24)
Fried cheese	50	1 (2.0)	2 (4)
Fermented milk (*nono*)	52	5 (9.6)	17 (32.7)
Total	**202**	**10 (4.9)**	**46 (22.8)**

[1] Clinical and Laboratory Standards Institute (CLSI). 2008. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved standard. 3th ed, CLSI document M31-A3. Clinical and Laboratory Standards Institute, 940 West Valley Road, Wayne Pennsylvania, USA, ***28***(8), 1-99.

[2] Statistical Package for Social Sciences version 16 (SPSS). 2007. SPSS Inc. 233 South Wacker Drive, 11th floor Chicago, Illinois 60606. http://www.spss.com.

Table II. *Rates of antimicrobial resistance of* Escherichia coli *isolates from milk and milk products in Ogun State, Nigeria.*

Antimicrobial agents	STEC O157:H7 (n=10)		non-O157 E. coli (n=46)	
	Number (%) sensitive	Number (%) resistant	Number (%) sensitive	Number (%) resistant
Amoxycillin/clavulanic acid	5 (50.0)	5 (50.0)	27 (58.7)	19 (41.3)
Ampicillin	0 (0.0)	10(100.0	3 (6.5)	43 (93.5)
Ciprofloxacin	10 (100.0)	0(0.0)	40 (87.0)	6 (13.0)
Chloramphenicol	4 (40.0)	6 (60.0)	35 (76.1)	11 (23.9)
Nalidixic acid	8 (80.0)	2 (20.0)	37 (80.4)	9 (19.6)
Neomycin	10(100.0)	0(0.0)	42 (91.4)	4 (8.7)
Norfloxacin	9 (90.0)	1(10.0)	40 (87.0)	6 (13.0)
Streptomycin	7 (70.0)	3 (30.0)	15 (32.6)	31 (67.4)
Sulphamethoxazole/trimethoprim	8 (80.0)	2(20.0)	37 (80.4)	9 (19.6)
Tetracycline	1 (10.0)	9 (90.0)	7 (15.2)	39 (84.8)

Table III. *Antimicrobial resistance patterns and virulence gene profiles of STEC O157:H7 isolates from milk and milk products in Ogun State, Nigeria.*

S/NO	Source of isolate	Antimicrobial resistance patterns	Virulence genes				
			stx$_1$ only	*stx*$_2$ only	*stx*$_1$/*stx*$_2$	*eaeA*	*E-hlyA*
1	Raw Milk	AmpTet	+	-	-	-	-
2	*Wara*	AmcAmpChlStrSulTet	+	-	-	-	+
3	*Wara*	AmpChlTet	-	+	-	+	+
4	*Wara*	AmcAmpChlNalNorSulTet	-	+	-	-	+
5	Fried *Wara*	AmpChlTet	-	+	-	+	+
6	*Nono*	AmpStrTet	-	-	+	+	+
7	*Nono*	AmcAmpTet	-	+	-	+	+
8	*Nono*	AmcAmpChlNalTet	-	-	+	-	+
9	*Nono*	AmpAmpChlStrTet	-	+	-	+	-
10	*Nono*	Amp	-	+	-	-	-
Total number (%) positive for virulence genes			2 (20)	6 (60)	2 (20)	5 (50)	7 (70)

+ = positive reaction; - = negative reaction.

among the sample types were not statistically significant (p>0.05). Furthermore, non-O157 *E. coli* was detected in 46 (22.8%) of the 202 samples. The prevalence of non-O157 *E. coli* was 30% in fresh raw milk, 24% in fresh cheese, 4% in fried cheese and 32.7% in fermented milk (Table I). The prevalence was significantly lower (p<0.05) in fried cheese than in other sample types. None of the tested sorbitol-fermenting *E. coli* isolates was positive for *E. coli* O157 antigen. Moreover, all the samples were negative for non-O157 STEC serogroups O26, O91, O103, O111, O128 and O145.

All *E. coli* O157:H7 isolates detected in this study were resistant to ampicillin but susceptible to ciprofloxacin and neomycin. Some isolates also showed resistance to amoxicillin/clavulanic acid (50.0%), chloramphenicol (60.0%), nalidixic acid (20.0%), norfloxacin (10.0%), streptomycin (30.0%), sulphamethoxazole/trimethoprim (20.0%) and tetracycline (90.0%) (Table II). Resistance to chloramphenicol was higher among STEC O157 than non-O157 *E. coli* isolates, while resistance to streptomycin was higher among non-O157 *E. coli* than STEC O157 isolates. Nevertheless, resistance to other tested antimicrobials was comparable between the 2 groups of *E. coli* strains (Table II). Eight of 10 STEC O157:H7 isolates showed resistance to more than 3 antimicrobial agents from different classes (multidrug resistance). Two of the isolates had similar resistance pattern (AmpChlTet), but all other isolates had diverse resistance patterns (Table III). Among the 46 non-O157 *E. coli* isolates, 3 were susceptible to all tested antimicrobials, while 31 demonstrated multidrug resistance (Table IV).

Virulence genes were detected in all the *E. coli* O157:H7 isolates. In more details, *stx*$_1$ was detected in 2, *stx*$_2$ in 6, *stx*$_1$/*stx*$_2$ in 2, *eaeA* in 5 and *E-hlyA* in 7 of 10 *E. coli* O157:H7 isolates. The virulence gene profile of each of the isolates is presented in Table III.

Table IV. *Resistance patterns of non-O157* Escherichia coli *isolates from milk and milk products in Ogun State, Nigeria.*

Resistance patterns	Number of isolates by sample types				Total
	Raw milk	*Wara*	Fried *Wara*	*Nono*	
AmcAmpCipChlNalNeoNorStrSulTet	2	1	-	1	**4**
AmcAmpCipChlNalNorStrSulTet	1	-	-	1	**2**
AmcAmpChlNalStrSulTet	1	2	-	1	**4**
AmcAmpChlStrTet	-	1	-		**1**
AmcAmpStrTet	4	1	-	3	**8**
AmpStrTet	3	3	1	5	**12**
AmpTet	2	1	1	4	**8**
Amp	1	2	-	1	**4**
Susceptible to all	1	1	-	1	**3**
Total	**15**	**12**	**2**	**17**	**46**

Discussion

The present study confirmed the presence of STEC O157 in milk and milk products in Ogun State, Nigeria. Among the 4 milk products examined, the prevalence of *E. coli* O157 was higher in *nono* (9.6%), followed by fresh *wara* (6.0%), raw milk (2.0%) and fried *wara* (2.0%). However, the observed differences in prevalence were not statistically significant ($p>0.05$).

The prevalence of STEC O157 in raw milk is lower than the 30.9% reported in raw unpasteurized milk in Syria (Nazih 2007). In the Syrian study (Nazih 2007), raw milk samples were bought at the local market, which could introduce contamination by handling along the food chain; while for our study, fresh milk samples were collected directly from lactating cows after disinfection of the udder so as to prevent extraneous contamination of the samples and ensure that bacteria present in the raw milk came directly from the mammary gland. This facilitated identification of the likely sources of bacterial contaminants in milk products along the processing and marketing chains. Furthermore, in the Syrian study 10 colonies were tested, against the 5 considered in the present study, which could possibly lead to higher prevalence. Besides, differences in farming practices between Syria and Nigeria could also account for the differences in the prevalence of STEC O157 in their cattle populations. A 3.0% prevalence of STEC O157 in raw milk, similar to the finding reported in this article, has been detected in Plateau State, Nigeria (Itelima and Agina 2010). However, it is noteworthy that STEC O157 was not found in raw milk in Egypt (Abd El-Atty and Meshref 2007). The detection of STEC O157:H7 in raw milk could be due to secretion of the pathogen in infected udder. Raw milk obtained from a healthy udder is usually sterile; however, coliform mastitis caused by *E. coli* can lead to the presence of *E. coli* in milk expressed from mastitic udder. Shiga toxin producing *E. coli* has been associated with coliform mastitis in cow (Kobori *et al.* 2004). Although the examined cows were apparently healthy, the only positive cow could be suffering subclinical mastitis. Bacterial contaminants from the skin and faeces of cow can also lead to the presence of *E. coli* O157 in raw milk. *Escherichia coli* O157 has been detected in the faeces and hide of ruminants especially cattle (Elder wt al. 2000, Ojo *et al.* 2010).

Itelima and Agina reported 2.9% prevalence of STEC O157 in fermented milk/*nono* samples in Plateau State Nigeria (Itelima and Agina 2010), this is a lower value than the 9.6% prevalence observed in the present study. This could be due to difference in the levels of hygiene along *nono* processing and marketing chain between the 2 locations. The 2.0% prevalence of STEC O157 in fresh and fried *wara* observed is similar to the 2.0% prevalence reported in Kareish cheese in Egypt (Abd El-Atty and Meshref 2007).

The presence of *E. coli* O157 in milk products especially fermented cheese/*nono*, fresh cheese/*wara*, and fried cheese/*wara* observed, could be the result of faecal contamination from cattle, the environment, or water used for processing the milk. Unhygienic handling during processing and marketing can lead to contamination. To avoid the risk of such a contamination, the samples were collected from vendors hawking these products along the streets with little regards to sanitary measures. Apart from STEC O157, other *E. coli* strains were found. The overall detection of *E. coli* in the samples could have been higher but for the selective agents (novobiocin and cefixime-tellurite) incorporated into culture media to promote the recovery of STEC at the expense of other bacteria which are inhibited by the selective agents. Therefore, the detected *E. coli* strains were only those resistant to the selective agents. The presence of *E. coli* in samples is generally used as an indication of faecal contamination.

The reported rate of detection of STEC O157 of both stx_1/stx_2 genes in association is lower than reported in a previous study (Ojo *et al.* 2010), but the rate of detection of either stx_1 or stx_2 alone is higher. The higher detection of stx_2 than stx_1 in STEC O157, as observed, is similar to the one reported of other studies (Paton and Paton 1998a, Sasaki *et al.* 2012, Wagner*et al.* 2004). Although the mechanism of action of stx_1 and stx_2 are the same, they produce different degrees and types of tissue damage (Lee *et al.* 2007). Strains of *E. coli* O157 positive for stx_2 are more frequently encountered in STEC-associated diseases in humans than stx_1-positive strains (Wagner *et al.* 2004). Moreover, *E. coli* O157 strains that possessed stx_2 are generally more implicated in severe infections and are more likely to cause HUS that those possessing stx_1 (Lee *et al*. 2007). Similar to the findings presented in this article, Ojo *et al*. (2010) also reported 54.7% and 70.8% detection rates for *eaeA* and *hlyA* respectively in *E. coli* O157. The detection of virulence genes in STEC O157 isolates from milk and milk products suggests that these isolates are potentially pathogenic and may induce illness when transmitted to humans.

The emergence and dissemination of antimicrobial resistance in bacteria is becoming worrisome, *E. coli* O157 isolates from milk and milk products showed very high (90% and above) rates of resistance to ampicillin and tetracycline, moderate (50-60%) resistance rates to chloramphenicol and amoxicillin/clavulanic acid, low (10%-30%) resistance rates to streptomycin, nalidixic acid, sulphamethoxazole/trimethoprim and norfloxacin but no resistance to ciprofloxacin and neomycin. High resistance rates to ampicillin (82.5%) were previously reported in Nigerian *E. coli* O157 isolates (Ojo *et al.* 2008, Ojo *et al.* 2010), it is noteworthy that the observed 90% resistance of STEC O157 to tetracycline is much higher than 16.4% resistance reported in Syria (Nazih 2007). The rate of STEC O157 resistance to quinolones is lower than those reported by other authors (Orden *et al.* 2001). Furthermore non-O157 *E. coli* isolates showed antimicrobial resistance rates comparable with those observed in STEC O157 except for the higher chloramphenicol and lower streptomycin resistance rates observed in STEC O157 than in the non-O157 *E. coli* isolates. The isolates displayed multidrug resistance to 3 or more classes of antimicrobials. This may be a consequence of the use of these antimicrobials in the prevention and treatment of diseases in animals. The development of antimicrobial resistance in bacteria has been attributed to the use of these agents in animals[3]. Herdsmen involved in our research had unrestricted access to antimicrobial agents and used these drugs indiscriminately in livestock production. Antimicrobial resistance in pathogenic bacteria implies non-effectiveness of antimicrobial therapy in cases of infection with these organisms leading to protracted morbidity, increased mortality and economic loss. Antimicrobial resistant bacteria resident in the gut of carrier animals contribute significantly to environmental contamination and spread of antimicrobial resistant bacterial strains in the environment and in edible animal products. Although STEC O157:H7 infections are generally not treated with antibiotics, resistant strains identified may play important roles in the maintenance and dissemination of resistant traits in the community. *Escherichia coli* strains can share resistance-encoding genetic materials among themselves and with other pathogenic and non-pathogenic members of the family *Enterobacteriaceae* thereby widening the antimicrobial resistance niche. The transmission of multidrug resistance STEC O157 from animals to humans through food especially milk products can cause food-borne infection refractory to antimicrobial therapy leading to protracted illness and possibly death.

This study revealed that milk and milk products in Ogun State are contaminated with potentially virulent, multidrug resistant STEC O157. Thus, milk and milk products are potential vectors for zoonotic STEC O157 transmission from cattle to humans in the study area. Subclinical mastitis, unhygienic handling during processing and marketing may contribute to the presence of STEC O157 in milk and milk products. There is a need for stricter regulatory measure to prevent STEC contamination in animal-source foods including milk and milk products. Routine tests should be conducted on dairy cows to detect subclinical mastitis before milking. Regular public enlightenment and education programmes on food safety for food vendors will help in minimising food contamination and thus reduce the risk of human infection with STEC O157:H7. Overdependence on antimicrobial and misuse of antimicrobial agents in animals can be prevented by policy formulation and enforcement to curb the continuous emergence of antimicrobial resistance in bacteria.

[3] World Health Organization (WHO). 1998. Use of quinolones in food animals and potential impact on human health. Report of a WHO meeting Geneva, Switzerland. WHO/EMC/ZDI/98.10.http://whqlibdoc.who.int/HQ/1998/WHO_EMC_ZDI_98.10.pdf.

References

Abd El-Atty N.S. & Meshref A.M.S. 2007. Prevalence of *Salmonella* and *Escherichia coli* O157 in some foods. *BS Vet Med J, 5th Scientific Conference*, 73-78.

Armstrong G.L., Hollingswort. J. & Morris J.G. 1996. Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of Entry of a new pathogen into the food supply of the developed world. *Epidemiol Rev*, **18**(1), 414-419.

Bach S.J., McAllister T.A., Veira D.M., Gannon V.P.J. & Holley R.A. 2002. Transmission control of *Escherichia coli* O157:H7: A review. *Can J Anim Sci*, **2**, 475-490.

Blanco M., Blanco J.E., Mora A., Rey J., Alonso J.M., Hermoso M., Hermoso J., Alonso M.P., Dhabi G., Gonzalez E.A., Bernardez M.I. & Blanco J. 2003. Serotypes, virulence genes and intimin types of shiga toxin (verotoxin)-producing *E. coli* isolates from healthy sheep in Spain. *J Clin Microbiol*, **41**, 1351-1356.

Elder R.O., Keen J.E., Siragusa G.R., Barkocy-Gallagher G.A., Koohmaraie M. & Laegreid W.W. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in faeces, hides, and carcasses of beef cattle during processing. *Proc Natl Acad Sci U S A*, **97**, 2999-3003.

ItelimaU.J. & Agina S.E. 2010. Occurrence of *Escherichia coli* O157:H7 in raw and locally fermented milk ('*Nono*') in Plateau state Nigeria. *Glob J Agric Sci*, **2**, 31-36.

Johnson K.E., Thorpe C.M. & Sears C.L. 2006. The emerging clinical importance of non-O157 shiga toxin-producing *Escherichia coli*. *Clin Infect Dis*, **43**, 1587-1595.

Kobori D., Rigobelo E.C., Macedo C., Marin J.M. & Avila F.A. 2004. Virulence properties of shiga toxin-producing *Escherichia coli* isolated from cases of bovine mastitis in Brazil. *Rev Elev Med Vet des Pays Trop*, **57**(1-2), 15-20.

Lee J.E., Reed J., Shields M.S., Spiegel K.M., Farrell L.D. & Sheridan P.P. 2007. Phylogenetic analysis of shiga toxin 1 and shiga toxin 2 genes associated with disease outbreaks. *BMC Microbiology*, **7**, 109.

Nataro J.P. & Kaper J.B. 1998. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev*, **11**, 142-201.

Nazih D. 2007. Detection and antimicrobial susceptibility of *Escherichia coli* O157:H7 in raw bovine milk, some of dairy products and water samples. *Damascus University Journal for Basic sciences*, **23**, (1)m, 217-219.

Ojo O.E., Ajuwape A.T.P., Otesile E.B., Owoade A.A., Oyekunle M.A. & Adetosoye A.I. 2010. Potentially zoonotic shiga toxin-producing *Escherichia coli* serogroups in the faeces and meat of food-producing animals in Ibadan, Nigeria. *Intl J Food Microbiol,* **142**, 214-221.

Ojo O.E., Oyekunle M.A., Ogunleye A.O. & Otesile E.B. 2008. *Escherichia coli* O157:H7 in food animals in parts of South Western Nigeria: Prevalence and *in vitro* antimicrobial susceptibility. *Trop Vet*, **26**, 23-30.

Orden J.A., Ruiz-Santa-Quiteria J.A., Cid D., Díez R., Martínez S. & de la Fuente R. 2001. Quinolone resistance in potentially pathogenic and non-pathogenic *Escherichia coli* strains isolated from healthy ruminants. *J Antimicrob Chemother*, **48**, 421-424.

Paton J.C. & Paton A.W. 1998a. Pathogenesis and diagnosis of shiga toxin-producing *Escherichia coli* infections. *Clin Microbiol Rev*, **11**, 450-479.

Paton A.W. & Paton J.C. 1998b. Detection and characterisation of shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx_1, stx_2, *eaeA*, enterohemorrhagic *Escherichia coli hlyA*, rfb_{O111}, and rfb_{O157}. *J Clin Microbiol*, **36**, 598-602.

Osek J. & Gallien P. 2002. Molecular analysis of *Escherichia coli* O157 strains isolated from cattle and pigs by the use of PCR and pulsed-field gel electrophoresis methods. *Vet Med Czech*, **47**(6), 149-158.

Sasaki Y., Usui M., Murakami M., Haruna M. Kojima A., Asai T. & Yamada Y. 2012. Antimicrobial resistance in shiga toxin-producing *Escherichia coli* O157 and O26 isolates from beef cattle. *Jpn J Infect Dis*, **65**, 117-121.

Wagner M., Allerberger F., Manafi M., Lindner G., Friedrich A.W., Sonntag A.-K. & Foissy H. 2004. Characterization of pathogenic *Escherichia coli* isolated from humans in Austria: phenotypes, toxin gene types and epidemiology. *J Vet Med B*, **51**, 288-292.

Wells J.G., Shipman L.D., Greene K.D., Sower E.G., Green J.H., Cameron D.N., Downes F.P., Martin M.L., Griffin P.M., Ostroff S.M., Potter M.E., Tauxe R.V. & Wacchsmuth I.K. 1991. Isolation of *Escherichia coli* serotype O157:H7 and other shiga-toxin-producing *Escherichia coli* from dairy cattle. *J Clin Microbiol*, **29**, 985-89.