Original Article

Sahel Journal of Veterinary Sciences

Article History
Received: 05-09-2020
Revised: 04-12-2020
Accepted: 05-12-2020
Published: 30-12-2020

Sahel J. Vet. Sci. Vol. 17, No. 4, pp 16-23 (2020)

Isolation, Molecular Detection and Antimicrobial Susceptibility Profile of *Escherichia coli* O157:H7 in Household - reared Small Ruminants in Zaria Metropolis, Kaduna State, Nigeria

***Yakubu, R. O., Lawan, M. K., Kwaga, J. K. P. and Kabir, J.**

Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

* *Author for Correspondence: yakubunumaramat@gmail.com*

ABSTRACT

Escherichia coli O157:H7 is a zoonotic enteric pathogen of public health significance worldwide. A cross-sectional study was carried out during which 384 faecal samples of household-reared small ruminants and water used in the various houses where the animals are reared were collected. The samples were enriched on tryptone soya broth and cultured on EMB and CT-SMAC to isolate *E. coli* and *E. coli* O157:H7 respectively; subjected to conventional biochemical tests and *E. coli* O157:H7 was confirmed using Wellcolex latex agglutination test kit. *E. coli* O157:H7 isolates were subjected to antimicrobial susceptibility test and multiplex PCR was carried out to detect the presence of virulence genes *stx*1, *stx*2, *eae*A and *hly*A. The results of the isolation showed isolation rate of *E. coli* O157:H7 of 4.69% (9/192), 0.52% (1/192) which were obtained from faeces and water samples respectively. The results of the characterisation showed that one of the *E. coli* O157:H7 isolated harboured the *eae*A and *hly*A genes but was negative for *stx*1 and *stx*2 genes. The highest number of isolates showed resistance to erythromycin (90.9%) while the least was to gentamicin (6.3%). About 97.7% (43/44) of the isolates had multiple antibiotic resistance index greater than 0.2. In conclusion, household-reared small ruminants in the study area were found to be reservoirs of *E. coli* O157:H7 and humans living within these households are at risk of infection. The multiple antibiotic resistance recorded in this study suggests wide spread use of antimicrobial drugs in the study area.

Keywords: *Escherichia coli* O157:H7, Household-reared small ruminants, Latex agglutination, Multiplex PCR, Antimicrobial sensitivity

INTRODUCTION

Vero toxin-producing *Escherichia coli,* particularly the serotype *Escherichia coli* O157:H7 has gained public health significance worldwide as a zoonotic enteric pathogen causing foodborne illnesses (La Ragione *et al*., 2008). Vero toxin-producing *Escherichia coli* (VTEC) is so named because it can produce one or more exotoxins (VT-1, VT-2 and their sub-variants) that have cytopathic effects on Vero cells (African green monkey kidney cells) and other cell lines (Karmali, 1989). They are also referred to as Shiga toxin-producing *Escherichia coli* (STEC) because the toxin (VT-1) produced have structural and functional similarity to the toxin produced by *Shigella dysenteriae* type 1 (Griffin, *et al*., 1988). Only a minority of the strains cause illness in humans and the ones that do are collectively known as enterohaemorrhagc *E. coli* (EHEC) and are the major cause of foodborne diseases (Croxen *et al*., 2013). Typical illness ranges from mild diarrhoea to life threatening infection such as haemorrhagic colitis (HC), haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Chileshe and Ateba, 2013) VTEC usually do not cause illness in animals with few exceptions such as diarrhoea in calves (Nataro and Kaper, 1998).

The pathogenicity of VTEC is due to the possession of the virulence genes which are located on chromosomes and plasmids (Bolton, 2011). VTEC are characterized by the production of cytotoxins that inhibit protein synthesis (Bolton, 2011), the VT1 and VT2 are two major toxin types that are associated with virulence for humans, the *vtx*1 is divided into three subtypes (*vtx*1a, *vtx*1c and *vtx*1d) while seven subtypes form the *vtx*2 group (*vtx*2a, *vtx*2b, *vtx*2c, *vtx*2d, *vtx*2e, *vtx*2f and *vtx*2g). Of the two groups, subtypes of *vtx*2 are associated with more severe HUS syndrome (Chattaway *et al*., 2011; Etcheverria and Padola, 2013). The *eae* gene that is necessary for the production of attaching and effacing lesions in the intestinal mucosa and *hly*A, also known as *ehx*A, encodes the EHEC enterohemolysin, which releases hemoglobin from red blood cells during infection are accessory virulence factors that lead to the severity of infection in humans (Beutin *et al*., 1989).

Ruminants were regarded as the primary reservoirs and a source of infections for humans (La Ragione *et al*., 2008).

Routes for human infection with *E. coli* O157:H7 are direct contact, person to person spread, contaminated food and contaminated water (Mahendra and Yodit, 2017). The use of Antibiotics for treatment of VTEC infections are of questionable value and have not shown to be of clear clinical benefit, however, increase in antibiotic resistance has been noted over the years (Lupindu, 2017).

VTEC prevalence of 100% and 89.3% was reported from sheep and goats kept on city farms in southern Germany (Schilling *et al*., 2012). A VTEC prevalence of 8.7% was reported from healthy cattle in Spain (Orden *et al*., 2002). The prevalence of VTEC in small domestic ruminants is less well documented than in cattle (La Regionne *et al*., 2008). There is also a paucity of information regarding the epidemiology of *E. coli* O157:H7 in developing countries (Abdissa *et al*., 2017) hence the need to determine the prevalence of *Escherichia coli* O157:H7 in household reared small ruminants and water sourced from these households in Zaria metropolis, Kaduna state, Nigeria. The study also showed the antimicrobial susceptibility profile of the isolates.

MATERIALS AND METHODS

Study Area

This study was conducted in Zaria metropolis, Zaria metropolis consists of two Local Governments, Zaria and Sabon Gari. Twelve (12) wards, six (6) from each of the local government were selected for the study. Zaria is a Local Government Area and a major city in Kaduna State in Northern Nigeria. There are twelve (12) wards in Zaria Local Government and eleven (11) wards in Sabon Gari Local Government. Zaria and Sabon Gari economy are primarily based on agriculture.

Sample Size

The sample size was determined according to the formula described by Thrusfield (1997) using a 95% level of significance. A 50% expected prevalence was employed in the determination of the sample size.

Study Design

A cross-sectional study was carried out and three hundred and eighty-four (384) samples were collected in this study, one hundred and ninety-two (192) faecal and water samples each. Twelve (12) wards were selected, six (6) wards from each Local Government Area using simple random sampling. Sixteen households were sampled in each ward and thirty-two samples were collected (sixteen water samples and sixteen faecal samples). Households, where small ruminants were kept, were located using the snowballing method of sampling.

Sample Collection

Faecal samples were collected from small ruminants (sheep and goats) in the various households where small ruminants were kept in Zaria and Sabon Gari Local Government of Kaduna State. Faecal samples were collected from the rectum using sterilized polythene bags wrapped around the finger, freshly voided faeces were also taken from the environment where available. Water samples were collected from drinking water containers within the households where these small ruminants were reared, the source of the water was noted, and they included water collected from Wells, Taps, Borehole and Rain. Twenty millilitre of water samples was collected in sterile sample bottles. The samples were properly labelled and kept in a flask containing an ice pack and transported to the Bacterial Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria for analysis.

Enrichment of Samples and Isolation of Verotoxin *E. coli* O157:H7

One gram of each faecal sample was enriched in 5ml of Tryptone soya broth and incubated at 37^{o}C for 24 hours. Five ml of water sample was centrifuged at 2000 rpm for 15 minutes and was enriched in 5ml of Tryptone soya broth and incubated at 37^{o}C for 24 hours. A loopful of the enriched samples were inoculated on Eosin methylene-blue (EMB) agar, and then incubated at 37^{o}C for 24 hours. The colonies with a greenish metallic sheen on EMB agar which is a typical feature of *E. coli* were transferred to sorbitol MacConkey agar to check for the presence of non-sorbitol fermenters, colourless colonies on Sorbitol MacConkey agar were presumed to be *E. coli* O157:H7. The presumptive *E. coli* O157:H7 isolates were transferred to nutrient agar to be used for additional tests (Dissasa *et al*., 2017).

Biochemical Analysis

The presumptive *E. coli* O157:H7 isolates were biochemically characterized according to the method described by Barrow and Feltham (2003). The isolates were subjected to 10 different biochemical tests which included Triple sugar ion, MR-VP (Methyl Red and Voges Proskauer), Simmons citrate, SIM (Sulphide, Indole, Motility) medium, Urea agar test, Sucrose, Maltose, Mannitol, Lactose and Sorbitol.

Serological Test.

The non-sorbitol fermenting *E. coli* colonies were serologically confirmed by using Wellcolex rapid latex agglutination kit for O157:H7 (Oxoid Ltd., Basingstoke, UK). Identification of VTEC was carried out following the Manufacturer's instructions for the presence of O157 antigens. The suspected *E. coli* O157:H7 isolates were sub-cultured on EMB and incubated at 37^{o}C for 24 hours. The culture was further enriched in tryptone soya broth at 37^{o}C for 24 hours. Fifty microlitre of the enriched culture was dropped onto the slide, and one drop of Wellcolex rapid latex agglutination kit for O157:H7 was added and mixed using sterile application sticks, the mixture was rocked for one minute, and samples that agglutinated were identified as *E. coli* O157:H7.

Antimicrobial Susceptibility Testing of Isolates

The suspected *E. coli* O157:H7 isolates were sub-cultured from nutrient agar onto EMB agar incubated at 37^{o}C for 24 hours. Mueller-Hinton agar media was used for susceptibility testing according to the criteria of the Clinical and Laboratory Standards Institute (CLSI, 2018). Colonies from each fresh culture were suspended into 5ml of normal saline (0.9% w/v NaCl) using a sterile wire loop until the turbidity was

equivalent to 0.5 McFarland standard, incubated for about 20-30 minutes at 37ºC and streaked uniformly onto the Mueller-Hinton agar using sterile swab sticks. Each antimicrobial was dispensed onto the plates using an antimicrobial susceptibility disk dispenser and incubated for 24 hours at 37ºC. Zones of inhibition were measured using a ruler. The presumed *E. coli* O157:H7 isolates were tested for sensitivity to the most commonly used antimicrobials which included, Cefoxitin (30ug), Gentamicin (10ug), Erythromycin (15ug), Perfloxacin (5ug), Streptomycin (10ug), Trimethoprim-Sulfamethoxazole (25ug), Tetracycline (30ug), Ampicillin (10ug), Amoxicillin (30ug), Chloramphenicol (30ug), Nitrofurantoin (300ug) And Ciprofloxacin (5ug) (Schroeder *et al*., 2002). The antimicrobials used for the study was sourced from Oxoid Ltd., Basingstoke, UK. The zones of inhibition were measured in millimetres and interpreted as sensitive, intermediate and resistant according to the criteria by CLSI (2018). MAR index was determined for each isolate according to the formula by Krumpermann (1983).

Detection of *stx*1, *stx*2, *eae*A and *hly*A Genes Using Multiplex PCR

DNA extraction: DNA was extracted by boiling method as described by Diana *et al*. (2012) and Mondour *et al*. (2016). The DNA was quantified using a nano drop spectrophotometer.

***PCR amplification and gel electrophoresis*:** PCR amplification and gel electrophoresis were carried out according to the methods described by Paton and Paton (1998). Positive samples were identified based on the presence of bands of appropriate sizes compared to the VTEC O157:H7 positive control (EDL 933). Expected amplicon sizes are listed in Table 1.

Virulence genes primer sequences: The primers used for detection of *E. coli* O157:H7 are listed in Table 1.

Table 1: Multiplex PCR Primer Sequences and Expected Amplicon Sizes

Primer	Primer Sequence (5'- 3')	Target Gene	Amplicon Size (bp)	References
Stx1F	ATAAATCGCCATTCGTTGACTAC	*stx*1	180	Paton and Paton, 1998
Stx1R	AGAACGCCCACTGAGATCATC			
Stx2F	GGCACTGTCTGAAACTGCTCC	*stx*2	255	Paton and Paton, 1998
Stx2R	TCGCCAGTTATCTGACATTCTG			
EaeAF	GACCCGGACAAGCATAAGC	*eae*A	384	Paton and Paton, 1998
EaeAR	CCACCTGCAGCAACAAGAGG			
HlyAF	GCATCATCAAGCGTACGTTCC	*hly*A	534	Paton and Paton, 1998
HlyAR	AATGAGCCAAGCTGGTTAAGCT			

Data Analysis

The data obtained were analyzed using Statistical Package for the Social Sciences (SPSS) Version 17 and presented using tables, charts, percentages and frequencies. Fischer's exact test was used to test for association between wards sampled, species of animal, type of water source and isolation $p > 0.05$ was considered significant.

Ethical Statement

All experimental protocols were subjected to the approval of the Ahmadu Bello University Committee on Animal Use and Care.

RESULTS

A total of nine faecal samples agglutinated with the Wellcolex rapid latex agglutination kit for *E. coli* O157:H7 resulting in an isolation rate of *E. coli* O157:H7 of 4.69% from faeces of household-reared small ruminants in Zaria (Table 2). One out of the one hundred and ninety-two water samples collected was positive for *E. coli* O157:H7 with an isolation rate of 0.52%.

The study also shows that 7(5.5%) sheep and 2(3.1%) goats were positive for *E. coli* O157:H7 and based on the species of animal sampled an isolation rate of 5.5% and 3.1% was obtained from sheep and goats reared in households in Zaria respectively (Table 3).

The antimicrobial sensitivity test conducted on the 44 positive isolates following biochemical tests revealed the number and percentage of *E. coli* O157:H7 isolates resistant or susceptible to the antimicrobial tested in the study (Table 4).

The antibiograms were used to calculate the multiple antibiotic resistance indices. From the study only one isolate (T2W) had MAR index below 0.20, and four isolates had between 0.20-0.39, twenty-five had between 0.40-0.59, thirteen had values between 0.60-0.79 and one isolate had above 0.80. The study showed that all except one of the forty-four isolates had MAR index greater than 0.20 (Table 5).

The ten positive isolates by latex agglutination were subjected to multiplex PCR for the detection of *stx*1, *stx*2, *eae*A and *hly*A genes. One of the isolates was positive for *eae*A and *hly*A genes (lane 1) as presented on Figure 1.

DISCUSSION

An isolation rate of *E. coli* O157:H7 of 4.69% was recorded from faeces of household-reared small ruminants in the study area with isolation rate varying amongst the wards and Local Government Areas sampled. The isolation rate recorded in this study is similar to the work of other researchers (Ogden *et al*. 2005; Mersha *et al*., 2010; Akanbi *et al*., 2011). This work, varies with the report of Keen *et al*. (2006); Oporto *et al*., (2008); Jacob *et al*. (2013) who reported higher isolation rates than that obtained in this study.

Table 2: Isolation rates of *E. coli* O157:H7 from selected wards in Sabon Gari and Zaria Local Government Areas using rapid latex agglutination kit

S/No.	Ward Sampled	No. of Samples Collected	Number (%) Positive**
1	Samaru	16	1 (6.20)
2	Basawa	16	0 (0.00)
3	Bomo	16	1 (6.20)
4	Hanwa	16	0 (0.00)
5	Dogarawa	16	2 (12.50)
6	Jushin Waje	16	2 (12.50)
7	Tundu Wada	16	1 (6.20)
8	Kwarbai A	16	0 (0.00)
9	Gyallesu	16	0 (0.00)
10	Kaura	16	1 (6.20)
11	Unguwan Fatika	16	1 (6.20)
12	Unguwan Juma	16	0 (0.00)
	TOTAL	192	9 (4.69)

P value = 0.823 is greater than 0.05 hence no statistically significant difference. **= positive for *E. coli* O157:H7 by latex agglutination test.

Table 3: Isolation rate of *E. coli* O157:H7 from household-reared sheep and goats in Sabon Gari and Zaria Local Government Areas using rapid latex agglutination kit

S/No	Species of Animals Sampled	Number of Animals Sampled	Number (%) positive**
1	Sheep	128	7 (5.50)
2	Goat	64	2 (3.10)
	Total	192	9 (4.69)

P value = 0.702 is greater than 0.05 hence no statistically significant difference.
**= positive for *E. coli* O157:H7 by latex agglutination test.

Table 4: Antimicrobial Resistant profile of *E. coli* O157:H7 Isolates from sheep and goats in Sabon Gari and Zaria Local Government Areas

Antimicrobials, Code and Concentration (µg)	Number (%) of Resistant Isolates.
Perfloxacin (PEF), 5	38 (86.40)
Ciprofloxacin (CIP), 5	5 (11.00)
Cefoxitin (FOX), 30	15 (34.10)
Erythromycin (E), 15	40 (90.90)
Tetracycline (TE), 30	21 (47.70)
Gentamicin (CN), 10	3 (6.80)
Ampicillin (AMP), 10	29 (65.90)
Nitrofurantoin (F), 300	34 (77.30)
Trimethoprim-Sulfamethoxazole (SXT), 25	31 (70.50)
Amoxicillin (AMC), 30	18 (40.90)
Chloramphenicol (C), 30	31 (77.30)
Streptomycin (S), 10	25 (56.30)

Table 5: Multiple Antimicrobial Resistance Indices of *E. coli* O157:H7 Isolates from Sheep and Goats in Sabon Gari and Zaria Local Government Areas

S/No	MAR index/range	No of Isolates
1	< 0.20	1
2	0.20-0.39	4
3	0.40-0.59	25
4	0.60-0.79	13
5	>0.80	1
	Total	44

MAR = Multiple Antibiotic Resistant

The differences in the isolation rate reported in this study when compared to other reports could be due to differences in geographical location, sampling technique and methodologies used in the laboratory. The presence of *E. coli* O157:H7 in small ruminants in the study area is of public health significance as the faeces of these animals could contaminate food, water and the environment and in turn put inhabitants of these households at a greater risk of infection with *E. coli* O157:H7.

From this study, sheep were found to relatively harbour more *E. coli* O157:H7 than goats, although the difference was not statistically significant. This is in line with the findings of other researchers who reported a higher prevalence of *E. coli* O157:H7 from the faeces of sheep than goats (Keen *et al*.,

2006; Akanbi *et al*., 2011; Pralhad *et al*., 2018; Abreham *et al.,* 2019). Cornick *et al*., (2000) stated that VTEC is better adapted to persist in the alimentary tract of sheep than other pathotypes of *E. coli.* This could be responsible for the relatively higher prevalence in sheep in this study.

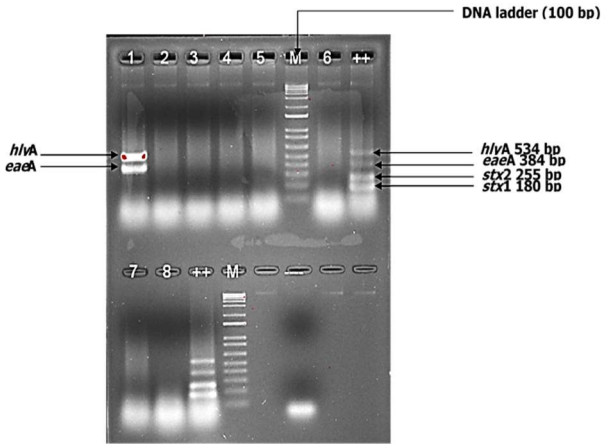

Figure 1: Multiplex PCR amplification of *stx*1, *stx*2, *eae*A and *hly*A genes (lane 1-8 were isolates of *E. coli* O157:H7 positive by latex agglutination test, lane M=100bp molecular ladder, lane ++ = positive control, lane ---- = negative control, isolate in lane 1 was positive for *eae*A and *hly* genes

In the present study, an isolation rate of *E. coli* O157:H7 of 0.52% was gotten from water sourced from households where small ruminants were reared. This work is in contrast with the finding of Garba *et al*. (2009); Fashina *et al*. (2018); Aboh *et al*. (2015); Adzitey *et al*., (2015) who reported higher prevalence from different sources of water. Although, *E. coli* was present in borehole and well water samples, but *E. coli* O157:H7 was not isolated from these samples in this study. This finding is in line with the report from Brij region of Uttar Pradesh, India where they recorded the presence of *E. coli* in groundwater but no *E. coli* O157:H7 was isolated (Sharma *et al*., 2017). The variability in the occurrence of *E. coli* O157:H7 in this study compared with the findings from other studies cited above may be due to differences in sample size, source of the sample. Coliforms such as *E. coli* have been widely used as indicators of the microbiological quality of surface and ground waters (Ahmed *et al*., 2005). The isolation of coliforms, especially *E. coli* from water sources is attributable to faecal contamination of human and animal origin. According to Wanke (1990), water for human consumption should be free of *E. coli*. Consequently, the presence of *E. coli* in water samples encountered in this study suggests faecal contamination and renders it unsafe for drinking.

Development of antibiotic resistance among bacteria such as *E. coli calls* for an important public health concern. This study revealed resistance by isolates to several antimicrobials tested. This is in line with the work of other authors (Shittu *et al*., 2007; Adefarakan *et al*., 2014; Onifade *et al*., 2015; Urumova *et al*., 2015; Bukar-Kolo *et al.*, 2016) who reported similar percentages of resistance to various antimicrobials. The variation in the percentage of resistance to the antimicrobials tested in this study compared to other work may be attributed to different rates at which antimicrobials were used in different study areas and the distinction in the type of samples from which *E. coli* was isolated.

The present study shows that 97.7% of the *E. coli* O157:H7 isolates had multiple antibiotic resistance indices greater than 0.2, which is in agreement with the findings of Bahiru *et al*. (2013), who reported that 96.3% of *E. coli* isolates had multiple antibiotic resistance index greater than 0.2. It also agrees with the work of Olowe *et al*. (2008), who reported that 90% of *E. coli* isolates had multiple antibiotic-resistant phenotypes. The multiple resistance to antimicrobials observed in this study suggest that there may be an abuse of antimicrobials in the study area which may be due to incorrect use and improper dosage of antimicrobials by quacks, farmers and frequent application in animal feeds as a supplement and in the treatment of veterinary related diseases. The multiple antibiotic-resistant *E. coli* in this study may probably be plasmid-mediated as it has been demonstrated by White and McDermott (2011) that the majority of multiple antimicrobial-resistant phenotypes are due to acquisition of external genes that provide resistance to an entire class of antimicrobials. Studies on antimicrobial-resistance of *E. coli* from different animal species showed an increase in the incidence of resistance over the years as a result of the wide-spread use of antimicrobial drugs in animals (Cid *et al.,* 1996); this increase in resistance to antimicrobials may serve as a threat to future animal and human disease management (Adesokan *et al*., 2015).

Although the main virulence feature of VTEC is associated with the production of verocytoxins, this cannot be solely responsible for full pathogenicity. VTEC strains seem to be pathogenic for humans only if they possess other virulence factor such as intimin, encoded by the *eae* gene on the locus of enterocyte effacement (LEE), which is responsible for intimate attachment to intestinal epithelial cells (Kaper *et al*., 1998) and *hly*A gene which releases hemoglobin from red blood cells during infection, thus providing a source of iron for the bacteria (Beutin *et al*., 1989).

In this study intimin gene (*eae*A) and the entero-haemolysin gene (*hly*A) were detected by multiplex PCR from one of the *E. coli* O157:H7 isolates, however, *stx*1 and *stx*2 gene were not detected. This is in agreement with the findings from previous studies where virulence genes were detected in only few strains (Lenahan *et al*., 2007; Mersha *et al*., 2010; Lawan *et al*., 2015; Lawan *et al*., 2017). The absence of *stx*1 and *stx*2 genes in this finding disagrees with the report of Mersha *et al*. (2010); Lawan *et al*. (2017) and Fashina *et al*. (2018), who reported the presence of *stx*1 and *stx*2 in *E. coli* O157:H7 isolates. The absence of the *stx* genes in this study may be attributed to the loss of the stx-phages during storage as *stx* genes are temperate phage-mediated that may be lost from the bacterial genome (Karch *et al*., 1992; Lawan *et al*., 2015). The presence of *eae*A and *hly*A gene in the study is in agreement with the report of previous studies (Sharaf and Shabana, 2016; Fashina *et al*., 2018). The presence of the *eae*A and *hly*A gene in this study is of public health importance as these genes could confer enhanced virulence during infection.

Conclusion

This study established that household-reared small ruminants in Zaria metropolis harbour *E. coli* O157:H7 and the water used in households where small ruminants are reared may be contaminated with *E. coli* and *E. coli* O157:H7. The study shows that the *E. coli* O157:H7 isolates had varying resistance to the antimicrobials tested with 97.7% of the isolates showing multiple antibiotic resistance (MAR) index greater than 0.2. One of the isolates harbour *eae*A and *hly*A genes but lacked the *stx*1 and *stx*2 genes.

Acknowledgement

The authors appreciate the contributions and guidance of the staff and postgraduate students of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

Author Contribution

YRO, LMK. and KJKP designed the research work. LMK., KJKP and KJ supervised the work. YRO conducted the research, analysed the data and developed the draft manuscript. All authors have read and approved the final manuscript.

REFERENCES

Abdissa, R., Haile, W., Fite, A. T., Beyi, A. F., Getahun, E., Agga, G. E., Edao, B. M., Tadesse, F., Korsa, M. G., Beyene, T., Beyene, T. J., De Zutter, L., Cox, E. and Goddeeris B. M. (2017). Prevalence of *Escherichia coli* O157:H7 in beef cattle at slaughter and beef carcasses at retail shops in Ethiopia. *BMC Infect Dis*, 17:277

Aboh, A. E., Giwa, J. F. and Giwa, A. (2015). Microbiological assessment of well waters in Samaru, Zaria, Kaduna, State, Nigeria. *Ann. Afr. Med.*, 14 (10):32-3

Abreham, S., Teklu, A., Cox, E. and Tessema, T. S. (2019). *Escherichia coli* O157:H7: Distribution, molecular characterization, antimicrobial resistance patterns and source of contamination of sheep and goat carcasses at an export abattoir, Mojdo, Ethiopia. *BMC Microbiol*., 19:215. https//doi.org/10.1186/s12866-0199-1590-8

Adefarakan, T. A., Oluduro, A. O., David, O. M., Ajayi, A. O., Ariyo, A. B. and Fashina, C. D. (2014). Prevalence of Antibiotic Resistance and Molecular Characterization of *Escherichia coli* from Faeces of Apparently Healthy Rams and Goats in Ile-Ife, Southwest, Nigeria. IJS, 16 (3):447-460

Adesokan, H. K., Akanbi, I. O., Akanbi, I. B. and Obaweda, R. A. (2015). Pattern of antimicrobial usage in livestock animals in south-western Nigeria: the need for alternative plans. *Onderstepoort J. Vet. Res*., 82 (1):01-06

Adzitey, F., Sumaila, N. and Saba, C. K. S. (2015). Isolation of *E. coli* from drinking water sources for humans and farm animals in Nyankpala community of Ghana. *Res. J. Microbiol*, 10 (3):126-131

Ahmed, W., Neller, R. and Katouli, M. (2005). Host species specific metabolic finger print Database for enterococci and *Escherichia coli* and its application to identify source of faecal contamination in surface waters. *Appl. Environ. Microbiol*. 71 (8):4461-4468.

Akanbi, B. O., Mbah, I. P. and Kerry, P. C. (2011). Prevalence of *Escherichia coli* O157:H7 on hides and faeces of ruminants at slaughter in two major abattoirs in Nigeria. Lett. *Appl. Microbiol*., 53:336–340

Bahiru, A. A., Emire S. A. and Ayele, A. K. (2013). The prevalence of antibiotic resistant *Escherichia coli* isolates from faecal and water sources. *Acad. J. Microbiol. Res*., 1 (1):001- 010 DOI: http://dx.doi.org/10.15413/ajmr.2012.0109 ISSN: 2315-7771

Barrow, G. I. and Feltham, R. K.A. (2003). Cowan and Steels Manuel for the Identification of Medical Bacteria. (Paperback edition). Cambridge University Press, Cambridge, 128

Beutin, L, Montenegro, M. A., Orskov I, Oskov, F., Prada, J., Zimmermann, S. and Stephen, R. (1989). Close association of verotoxin (shiga like toxin) production with enterohemolysin production in strains of *Escherichia coli*. *J. Clin. Microbiol*., 27:2553–2561

Bolton, D. J. (2011). Verocytotoxigenic (Shiga toxin-producing) *Escherichia coli*: virulence factors and pathogenicity in the farm to fork paradigm, *Foodborne Pathog. Dis*., 8(3):357-65 DOI: 10.1089/fpd.2010.0699. Epub 2010 Nov 29.

Bukar-kolo, Y. M., Peter, I. D., Bukar, M. M., Muhammad, A. A., and Ayok, I. J. (2016). Prevalence and Antibiotic Susceptibility of Aerobic Bacterial Flora of the Skin, Wound and Anterior Vagina of Sheep, Goats and Dogs in Maiduguri, Nigeria. *Alex. J. Vet. Sci*., 49(2):6-12 ISSN 1110-2047, DOI: 10.5455/ajvs.218617

Chattaway, M. A., Dallman, T., Iruke, N., Okeke, I. N. and Wain, J. (2011). Enteroaggregative *E. coli* O104 from an outbreak of HUS in Germany 2011, could it happen again? *J.Infect. Dev. Ctries*, 5(6):425-436

Chileshe, J. and Ateba, C. N. (2013). Molecular identification of *Escherichia coli* 0145:H28 from Beef in the North-West Province, South Africa. *Life Sci. J.*, 10(4):1171-1176

Cid, O., Piriz, S., Riuz-Santa-Quiteria, J. A., Vadillo, S. and de la Fuente, R. (1996). *In vitro* susceptibility of *Escherichia coli* strains isolated from diarrhoeic lambs and goat kids to 14 antimicrobial agents. *J. Vet. Pharmacol. Ther*, 19:397-401

CLSI (2018). Performance Standards for Antimicrobial Susceptibility Testing. Clinical and Laboratory Standards Institute. 28th edition.

Cornick, N. A., Booher, S. L., Casey, T. A. and Moon, H. W. (2000). Persistent colonization of sheep by *Escherichia coli* O157:H7 and other E. *coli* pathotypes. *Appl. Environ. Microbiol*., 66:4926–4934

Croxen, M. A., Law, R. J., Scholz, R., Keeney, K. M., Wlodarska, M. and Finlay, B. B. (2013). Recent advances in understanding enteric pathogenic *Escherichia coli*. *Clin. Microbiol. Rev*. 26(4):822–880

Diana, J. E., C. F. Pui, and Son, R. (2012). Enumeration of Salmonella spp., Salmonella Typhi and Salmonella Typhimurium in fruit juices. *Int. Food Res. J*., 19:51-56

Disassa, N., Sibhat, B., Mengistu, S., Muktar, Y., and Belina, D. (2017). Prevalence and Antimicrobial Susceptibility Pattern of *E. coli* O157:H7 Isolated from Traditionally Marketed Raw Cow Milk in and around Asosa Town, Western Ethiopia. *J. Vet. Med. Int*. https://doi.org/10.1155/2017/7581531

Etcheverria A. I. and Padola N. L. (2013). Shiga toxin producing *Escherichia coli* factors involved in virulence and cattle colonization. *Virulence*, 4(5):366-372

Fashina, C. D., Babalola, G. O. and Osunde, M. O. (2018). Prevalence and Molecular Characterization of *E. coli* O157:H7 Isolated from Water Bodies in Ile-Ife and Environs. *J. Bacteriol. Parasitol*., 9 (4):340 doi:10.4172/2155-9597.1000340

Garba, I., Tijjani, M. B., Aliyu, M. S., Yakubu, S. E., Wada-Kura, A and. Olonitola, O. S. (2009). Prevalence of *Escherichia coli* in Some Public Water Sources in Gusau Municipal, North-Western Nigeria, BAJOPAS, 2(2):134 - 137

Griffin, P. M., Ostroff, S. M., Tauxe, R. V., Greene, K. D., Wells, J. G., Lewis, J. H., Blake, P. A. (1988). Illnesses Associated with *Escherichia coli* O157:H7 Infections. A Broad Clinical Spectrum. *Ann. Intern. Med*., 109:705- 712

Jacob, M. E., Foster, D. M., Rogers, A. T., Balcomb, C. C. and Sanderson, M. W. (2013). Prevalence and Relatedness of *Escherichia coli* O157:H7 Strains in the Faeces and on the Hides and Carcasses of U.S. Meat Goats at Slaughter. *Appl Environ. Microbiol*, 79(13): 4154–4158

Kaper, J. B., Elliot, S., Sperandio, V., Perna, N. T., Mayhew, G. F. and Blattner, F. R. (1998). Attaching and effacing intestinal histopathology and the locus of enterocyte effacement. In: *Escherichia coli* O157:H7 and Other Shiga Toxin Producing *E. coli* Strains. Kaper, J. B. and O'Brien, A. D. (Eds.). ASM Press Washington *DC,* 163–182

Karch, H., Meyer, T., Russmann, H. and Heesemann, J. (1992). Frequent loss of shiga-like toxin genes in clinical isolates of *Escherichia coli* upon subcultivation. *Infect. Immun.* 60:3464–3467

Karmali, M. A. (1989). Infection by verocytotoxin producing *Escherichia coli*. *Clin. Microbiol. Rev*., 2:15–38

Keen, J. E., Wittum, T. E., Dunn, J. R., Bono, J. L., and Durso, L. M. (2006). Shiga-toxigenic *Escherichia coli* O157 in agricultural fair livestock, United States. *Emerg. Infect. Dis*., 12:780–786

Krumperman, P. H. (1983). Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Appl. Environ. Microbiol.*, 46:165-170

La Ragione, R. M. Best, A., Woodward, M. J. and Wales, A. D. (2008). *Escherichia coli* O157:H7 colonization in small domestic ruminants. *FEMS Microbiol*. Rev., 33:394–410

Lawan, M. K., Bello, M., Lawan, J., Grande, L. and Morabito, S. (2015). Detection of Pathogenic *Escherichia coli* in Samples Collected at an Abattoir in Zaria, Nigeria and at Different Points in the Surrounding Environment. *Int. J. Environ. Res*. Pub. He., 12:679-691 doi:10.3390/ijerph120100679

Lawan, M. K., Bello, M., Lawan, J. and Morabito, S. (2017). Detection of Verocytotoxin-Producing *Escherichia coli* and Characterization of Sub-Variant of *stx* Genes Isolated from Zango Abattoir and Its Surrounding. NJSR, 16(4):412-419.

Lenahan, M., O'Brien, S., Kinsella, K., Sweeney, T. and Sheridan, J. (2007). Prevalence and molecular characterization of *Escherichia coli* O157:H7 on Irish lamb carcasses, fleece and in faeces sample. *J. Appl. Microbiol*., 103:2401–2409

Lupindu, A. M. (2017). Epidemiology of Shiga toxin-producing *Escherichia coli* O157:H7 in Africa in *review*. *S. Afri. J. Infect. Dis*., 1(1):1–7

Mahendra, P. and Yodit, A. (2017). Public health significance of verotoxin-producing *Escherichia co li* O157:H7. *EC Microbiol*, 11(6):257-263.

Mandour, H. A., Hinawi A. M. H. and Xiulan S, (2016). Comparative Study of Rapid DNA Extraction Methods of Pathogenic Bacteria, American J. Biosci. Bioeng., 4(1):1-8 Doi: 10.11648/j.bio.20160401.11

Mersha, A. G. D, Zewde, B. M. and Kyule, M. (2010). Occurrence of *Escherichia coli* O157:H7 in faeces, skin and carcasses from sheep and goats in Ethiopia. Lett. *Appl. Microbiol*., 50: 71–76

Nataro, J. P. and Kaper, J. B. (1998). Diarrhoeagenic *Escherichia coli. Clin. Microbiol. Rev*., 11 (1):142-201

Ogden, I. D., MacRae, M., and Strachan, N. J. C. (2005). Concentration and prevalence of *Escherichia coli* O157:H7 in sheep faeces at pasture in Scotland. *J. Appl. Microbiol.,* 98: 646–651

Olowe, O., Okanlawon, B., Olowe, R. and Olayemi, A. (2008). Antimicrobial resistant pattern of *Escherichia coli* from human clinical samples in Osogbo, south western Nigeria. *Afri. J. Microbiol*. Res., 1(2):8-11

Onifade, A. K., Oladoja, M. A., and Fadipe, D. O. (2015). Antibiotics Sensitivity Pattern of *Escherichia coli* isolated from Children of School Age in Ondo State, Nigeria. http://www.sciencepub.net/researcher. 7.

Oporto, B., Esteban, J. I., Aduriz, G., Juste, R. A. and Hurtado, A. (2008). *Escherichia coli* O157:H7 and non O157 Shiga toxin producing *E. coli* in healthy cattle, sheep and swine herds in northern Spain. *Zoonosis Public Hlth*, 55:73–81

Orden, J. A., Cid, D., Ruiz-Santa-Quiteria, J. A., Garcı´a, S., Martı´nez S. and de la Fuente R. (2002). Verotoxin producing *Escherichia coli* (VTEC), enteropathoge nic *E. coli* (EPEC) and necrotoxigenic *E. coli* (NTEC) isolated from healthy cattle in Spain. *J. Appl. Microbiol*., 93:29–35

Paton, J. C. and Paton, A. W. (1998). Pathogenesis and diagnosis of shiga toxin producing *Escherichia coli* infections. *Clin. Microbiol. Rev*., 11:450–459

Pralhad, B. A., Ramesh, B. K., Patil, N. A., Kumar, P., Rao, J. B., Vinay, P.T., Mallinath, K. C., Kharate, A., Suryakanth, P. and Revappayya, M. (2018). Occurrence of *Escherichia coli* O157:H7 in Faecal Sample of Sheep and Goats in North East Karnataka. *Int. J. Curr. Microbiol*. Appl. Sci., 7(12):242-250

Schilling, A., Hotzel, H., Methner, U., Sprague, L. D., Schmoock, G., El Adawy, H., Wohr, A., Erhard, M. H. and Geue L. (2012). Zoonotic Agents in Small Ruminants Kept on City Farms in Southern Germany. *Appl. Environ. Microbiol*.,78(11):3785-93 Doi:10.1128/Aem.07802-11

Sharaf, E. F. and Shabana, I. I. (2016). Prevalence and molecular characterization of Shiga toxin-producing *Escherichia coli* isolates from human and sheep in *Al-Madinah Al Munawarah. Infection*. http://dx.doi.org/10.22354/in.v21i2.651

Sharma, B., Parul, Verma, A. K., Jain, U., Yadav, J. K., Singh, R. and Mishra, R. (2017). Occurrence of multidrug resistant *Escherichia coli* in groundwater of Brij region (UP) and its public health implications, *Vet. World*, 10(3):293-301

Shittu, O. B., Nwagboniwe, C. A. K. and George, O. O. (2007). Antibiotic Resistance Patterns of *Escherichia coli* Isolates from Human, Pet, Livestock and Poultry Living in Close Contact. *ASSET* Series B, 6 (2):164-170

Thrusfield, M. (1997*). Veterinary Epidemiology.* Wiley, pp. 584.

Urumova V., Lyutzkanov M. and Petrov V. (2015). Investigations on Prevalence and Antimicrobial Resistance of Enterohaemorrhagic *Escherichia coli* (EHEC) Among Dairy Farms in the north part of the Republic of Bulgaria. *Mac. Vet. Rev*., 38(1):21-29

Wanke, C. A., Croven, S. and Goss, C. (1990). Characterization of binding of *E. coli* strains which are enteropathogens to small bowel mucin. *Infect. Immun*., 58:794-800

White, D. and Mcdermott, P. (2011): Emergence and transfer of antibacterial resistance. *J. Dairy Sci*., 84: E151-155