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

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**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 EM 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 Wellcolox 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 stx1, stx2, eaeA and hlyA. 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 eaeA and hlyA genes but was negative for stx1 and stx2 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 widespread 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 enterohaemorrhage *E. coli* (EHEC) and are the major cause of foodborne diseases (Croxon 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 vt1 is divided into three subtypes (vt1a, vt1c and vt1d) while seven subtypes form the vt2 group (vt2a, vt2b, vt2c, vt2d, vt2e, vt2f and vt2g). Of the two groups, subtypes of vt2 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 hlyA, also known as ehxA, 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°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°C for 24 hours. A loopful of the enriched samples were inoculated on Eosin methylene-blue (EMB) agar, and then incubated at 37°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°C for 24 hours. The culture was further enriched in tryptone soya broth at 37°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°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 37°C 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 37°C. 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), Perflloxacin (5ug), Streptomycin (10ug), Trimethoprim-Sulfamethoxazole (25ug), Tetracycline (30ug), Ampicillin (10ug), Amoxicillin (30ug), Chloramphenicol (30ug), Nitrofurantoin (300ug) And Ciprofloxacine (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 stx1, stx2, eaeA and hlyA 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>
<thead>
<tr>
<th>Primer</th>
<th>Primer Sequence (5'-3')</th>
<th>Target Gene</th>
<th>Amplicon Size (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stx1F</td>
<td>ATAAATCGCACCCTTGTGACTAC</td>
<td>stx1</td>
<td>180</td>
<td>Paton and Paton, 1998</td>
</tr>
<tr>
<td>Stx1R</td>
<td>AGAACGCCCTAGTCTACATTC</td>
<td>stx2</td>
<td>255</td>
<td>Paton and Paton, 1998</td>
</tr>
<tr>
<td>Stx2F</td>
<td>GGCACCTGTCGAAACTGCTCC</td>
<td>eaeA</td>
<td>384</td>
<td>Paton and Paton, 1998</td>
</tr>
<tr>
<td>Stx2R</td>
<td>TCGCCAGTTAATCTGACATTCTG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EaeAF</td>
<td>GACCCGGACAAGCATAAGC</td>
<td>hlyA</td>
<td>534</td>
<td>Paton and Paton, 1998</td>
</tr>
<tr>
<td>EaeAR</td>
<td>CCACCTGCAGCAACAAAGG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HlyAF</td>
<td>GCATCTACAGGGCTACGGTCC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HlyAR</td>
<td>AATGAGCCAAAGCCTGGTTAAGCT</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### 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 table 1 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 stx1, stx2, eaeA and hlyA genes. One of the isolates was positive for eaeA and hlyA 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., 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

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

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

<table>
<thead>
<tr>
<th>S/No</th>
<th>Species of Animals Sampled</th>
<th>Number of Animals Sampled</th>
<th>Number (%) positive**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sheep</td>
<td>128</td>
<td>7 (5.50)</td>
</tr>
<tr>
<td>2</td>
<td>Goat</td>
<td>64</td>
<td>2 (3.10)</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td>192</td>
<td>9 (4.69)</td>
</tr>
</tbody>
</table>

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

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

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

<table>
<thead>
<tr>
<th>S/No</th>
<th>MAR index/range</th>
<th>No of Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; 0.20</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>0.20-0.39</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>0.40-0.59</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>0.60-0.79</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>&gt;0.80</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><strong>TOTAL</strong></td>
<td><strong>44</strong></td>
</tr>
</tbody>
</table>

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., 19...
may be attributed to different rates at which antimicrobials similar percentages of resistance to various antimicrobials. Urumova et al study revealed resistance by isolates to several antimicrobials. 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. 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 verocytotoxins, 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 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 (eaeA) and the entero-haemolysin gene (hlyA) were detected by multiplex PCR from one of the E. coli O157:H7 isolates, however, stx1 and stx2 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 stx1 and stx2 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 stx1 and stx2 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 eaeA and hlyA 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 eaeA and hlyA gene in this study is of public health importance as these genes could confer enhanced virulence during infection.

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).

Figure 1: Multiplex PCR amplification of stx1, stx2, eaeA and hlyA 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 eaeA and hly genes.
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 *eaeA* and *hlyA* genes but lacked the *stx1* and *stx2* genes.

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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.

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