**Detection of *uidA*, *stx1*, and *stx2* genes in *Escherichia coli* O157:H7 isolated from cattle faecal matter and river water**

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**ABSTRACT**

Cattle can harbour enterohaemorrhagic *Escherichia coli* O157:H7 serotype in their faecal matters. This study aimed to isolate *E. coli* O157:H7 from the intestinal digesta, cattle dungs and water; and assess the antibiotic susceptibility and production of shiga toxins by the isolates. The counts of viable bacteria and faecal coliforms in 13 each of intestinal digesta and cattle dung and the 12 water samples were determined using nutrient agar and Eosin methylene blue agar respectively. Sorbitol MacConkey agar (SMAC) was used to screen for *E. coli* O157:H7 among the 38 *E. coli* isolates. PCR amplification of *uidA* genes was used to authenticate the isolates as enterohaemorrhagic *E. coli* O157:H7 serotype while amplification of *stx1* and *stx2* showed the production of shiga toxins. The antibiotic susceptibility patterns of the isolates were determined using standard disk diffusion method. The count of viable bacteria and faecal coliform was highest in the intestinal digesta followed by cattle dungs. There was 100% susceptibility to ofloxacin coupled with 100% resistant to augmentin by all the 8 *E. coli* O157 and 10 non-O157 isolates. The *E. coli* O157 isolates were more susceptible to ciprofloxacin, gentamicin, ampicillin and cefuroxime than non-O157 isolates but were less susceptible to nitrofurantoin and ceftazidime than non-O157. Eight (44.4%) out of the 18 presumptive *E. coli* O157 on SMAC amplified *uidA* genes and were confirmed as *E. coli* O157. They were isolated only from intestinal digesta and cattle dung. The prevalence of *stx1* and *stx2* genes among the *E. coli* O157 was 37.5% and 12.5% respectively. It is concluded from this study that intestinal digesta and cattle dung harboured *E. coli* O157: H7 some of which possessed shiga toxin.

**Keywords:** Cattle faeces, *E. coli* O157:H7, Shiga toxins, *uidA* genes, amplification

**1. INTRODUCTION**

Cattle dungs are the undigested residues of food consumed by these animals. It is usually dark brown in colour. It harbours a rich microbial diversity, containing different species of bacteria, protozoa and yeasts (Randhawa and Kullar, 2011; Behera and Ray, 2021). Cattle are raised for many reasons including milk, cheese, beef, hide and skin etc.

 The extensive use of chemical fertilizers for replenishment of nutritional deficiencies to increase crop yield produce detrimental effects such as increase in soil acidity, mineral imbalance and soil degradation (Ayoola and Makinde, [2008](https://bioresourcesbioprocessing.springeropen.com/articles/10.1186/s40643-016-0105-9#CR12)). Cattle dung can be used as a substitute for chemical fertilizers as well as a conditioner for soil (Yadav *et al*., [2013](https://bioresourcesbioprocessing.springeropen.com/articles/10.1186/s40643-016-0105-9#CR167); Be´langer *et al*., [2014](https://bioresourcesbioprocessing.springeropen.com/articles/10.1186/s40643-016-0105-9#CR19)).

 *Escherichia coli* is a normal flora in the intestinal tract of both healthy animals and humans. It serves as a reliable indicator of faecal contamination. It is a diverse bacterial group with pathogenic strains classified into groups such as enteropathogenic, enteroinvasive, enterohaemorrhagic, enterotoxigenic, and enteroadherent/enteroaggregative. *E. coli* O157:H7 belongs to the enterohaemorrhagic group. It is present in the gastro-intestinal tract (GIT) of cattle, sheep, goat etc. It is transmitted through the consumption of food and water contaminated by faecal matter of animal carriers, from person to person, and direct contact with carrier animals or faeces or digesta from the intestinal tract (Cortes-Sanchez and Salgado-Cruz, 2017). Contamination of water sources may be from agricultural waste, animal excreta, and industrial effluents, and sewage disposal (Scraper *et al*., 2002). This strain has low infective dose and high pathogenicity. It has been incriminated in gastroenteritis. Accumulation of faecal materials can act as a collection basin for these pathogens which may enter water system by the direct contamination through the seepage or surface runoff (Avery *et al*., 2004).

 The *uidA* genes found in *E. coli* 0157:H7 has a G residue rather than the T residue found in wild type *E. coli* at position 92 of its structural gene. This highly conserved base change in the *uidA* allele is a powerful marker of O157:H7 strains. The pathogenicity of *E. coli* O157:H7 is due to the presence of a 60-MDa plasmid (Bosilevac and Koohmaraie (2011) and the production of toxins including the shiga toxins, *stx1* and *stx2* (Jamshidi *et al*., 2016).

 The development of antibiotics resistance in some bacterial pathogens is rampant because of the clinical use of antibiotics. The excessive use of antibiotics in farm animals’ management to treat diseases, suboptimal dosage, use of antibiotics in the animal feeds have led to the incidence of antibiotic resistance bacteria, which can be transferred to human through the food chain (Cortes-Sanchez and Salgado-cruz, 2017).

The objectives of this research were to determine the counts of viable bacteria, total and faecal coliform in the faeces, intestinal digesta and river waters; characterize the isolates as *E. coli*; screen the possible isolates as being *E. coli* O157:H7 strain, detect the presence of *uidA*, *stx1* and *stx2* genes in the positive isolates; and determine the antibiotic susceptibility patterns of the isolates.

**2. MATERIALS AND METHODS**

**2.1 Collection of faecal and water samples**

 Thirteen samples each of the cattle dungs and the intestinal digesta of the slaughtered cattle were collected into sterile polythene bags using sterile handtrowel (Grauke *et al.*, 2002). Furthermore, 12 water samples were collected into the sterile sampling bottle using standard method (Sule and Bello, 2021).

**2.2 Bacteriological analysis of samples**

Ten grammes (10g) of the cattle dung/intestinal digesta was weighed aseptically into 90ml sterile distilled water in a conical flask to obtain10-1 dilution. Then, 1ml from 10-1 dilution was added to 9ml of sterile distilled water in a test tube to obtain 10-2 dilution. The process of serial dilution was repeated up to 10-6 dilution. For the water sample, 10ml of the raw water was used for the 10-1 dilution in place of the faecal material.

Viable bacteria and faecal coliform were isolated and enumerated using nutrient agar (NA) and eosin methylene blue agar medium (EMB) respectively. Aliquot (0.1ml) from different dilutions was introduced on the surface of sterile set plates of EMB. The inoculum was spread on the plate with the aid of sterile L-shaped glass spreader. The viable bacterial count was determined using pour plate technique. The plates were incubated inverted at 37oC for 48 hours. The colonies formed were counted and expressed in cfu/g or ml. The colonies (*E. coli*) with greenish metallic sheen on EMB were subcultured and kept in a refrigerator at 4 – 8oC until they were needed (Alalade *et al.*, 2018).

**2.3 Characterization of isolates**

The putative *E. coli* were confirmed through Gram staining and biochemical tests such as oxidase, catalase, methyl red, voges-proskauer, indole, citrate utilization, oxidation- fermentation and sugar fermentation (lactose and glucose). The isolates that were positive as *E. coli* were further screened if they were *E. coli* O157:H7 by cultivating them on Oxoid Sorbitol MacConkey agar (SMAC) supplemented with Cefixime-tellurite where they produced white colonies (Muller and Ehlers, 2005).

**2.4 Antimicrobial susceptibility test**

 The colonies were standardized using 0.5 McFarland’s standard. The standardized culture was streaked on sterile Mueller Hinton agar using sterile swab stick. Rapid labs multiple antibiotic discs, CM-128NR100 were placed on the agar and pressed firmly on the plate and subsequently incubated at 37oC for 24 hours before the diameter of inhibition was taken. The susceptibility of the isolates to each antibiotic was interpreted as susceptible, intermediately susceptible and resistant using the guidelines of CLSI (2020). The antibiotics used were Ceftazidime (CAZ) 30µg, Cefuroxime (CRX) 30µg, Gentamicin (GEN) 10µg, Ciprofloxacin (CPR) 5µg, Ofloxacin (OFL) 5µg, Amoxycillin/Clavulanate (AUG) 30µg, Nitrofurantoin (NIT) 300µg and Ampicillin (AMP) 10µg.

**2.5 Multiple antibiotic resistance (MAR) index**

It was obtained by dividing the number of antibiotics each isolate showed resistance by the total number of antibiotics tested (Sule *et al*., 2020).

**2.6 Extraction of DNA**

The isolates were cultivated in sterile nutrient broth at 37oC for 48 hours. The bacterial genomic DNA was extracted from the broth culture using the Quick-DNA Fungal/Bacterial Miniprep Kit ([Zymo Research, Catalogue No. D6005](https://www.zymoresearch.com/products/quick-dna-fungal-bacterial-miniprep-kit)).

**2.7 Synthesis of primer**

The primers synthesized have oligonucleotide sequences as presented in Table 1 according to Cebula *et al*. (1995) and Feng and Monday (2000). Inqaba Biotechnical Industry (Pty) Ltd., Pretoria, South Africa synthesized the primers used for PCR amplification with quotation number NG2019/19366.

**Table 1**: Oligonucleotide sequences and sizes of amplified fragments

|  |  |  |
| --- | --- | --- |
| Genes | Oligonucleotide sequences (5’ – 3’) | Fragment sizes |
| *stx1* (F) | CAGTTAATGTGGTGGCGAAGG | 348bp |
| *stx1* (R) | CACCAGACAATGTAACCGCTG |  |
| *stx2* (F) | ATCCTATTCCCGGGAGTTTACG | 548 bp |
| *stx*2 (R) | GCGTCATCGTATACACAGGAGC |  |
| *uidA* (F) | GCGAAAACTGTGGAATTGGG | 252 bp |
| *uidA* (R) | TGATGCTCCATCACTTCCTG |  |

**2.8 PCR amplification of genes**

 The extracted genomic DNA was used for PCR. The cocktail of the PCR was as follows: 2.5ul of 10x PCR buffer, 1ul of 25mM MgCl2, 1ul each of forward and reverse primer, 1ul of DMSO, 2ul of 2.5mM DNTPs, 0.1ul of 5u/ul Taq DNA polymerase, 3ul of 10ng/ul DNA and 13.4ul nuclease free water. The cycling parameters of the PCR was set up as follows: initial denaturation at 94oC for 5 minutes, thereafter 36 cycles of denaturation at 94oC for 30 seconds, annealing at 55oC for 30 seconds, elongation at 72oC for 45 seconds, final elongation step at 72oC for 7 minutes and held at 10oC. Viewing of the amplified fragments were observed on safe view stained 1.5% agarose electrophoresis gels. The ladder used for loading was 50bp from NEB and the hyper ladder from Bioline (Sule *et al*., 2022).

**3. RESULTS**

**3.1 Bacteriological loads of faecal materials and water samples**

 The count of heterotrophic bacteria (HB) and faecal coliform (FC) isolated from the intestinal digesta ranged from 4.7 x 105 - 1.3x 107cfu/g and 1.6 x 105 – 2.2 x 106cfu/g respectively. For the cattle dungs, HB and FC ranged from 7.5 x 104 – 4.8 x 106cfu/g and 5.0 x 104 – 1.0 x 106cfu/g respectively (Table 2). Furthermore, the HB and FC of the river water ranged from 4.4 x 104 – 9.6 x 106 cfu/ml and 1.0 x 101 – 1.0 x 103cfu/ml respectively (Table 3).

**3.2 Characterization of isolates**

 All the 38 *E. coli* isolates were Gram negative, oxidase positive, catalase positive, indole and methyl red positive, voges-proskauer and citrate negative, ferment lactose and glucose with the production of acid and gas, and fermentative utilization of glucose when cultivated in Hugh and Leifson medium. The *E. coli* obtained from the intestinal digesta and cattle dung was 13(34.2%) each whereas 12(31.6%) was obtained from the water samples. Eighteen of the *E. coli* isolates produced white colonies on SMAC and were putatively identified as *E. coli* O157:H7. These were 8 (44.4%) from intestinal digesta and 5 (27.8%) each from cattle dung and water sample.

**3.3 Antibiotic susceptibility patterns and MAR index of isolates**

The antibiotic susceptibility patterns of the 8(44.4%) *E. coli* O157 and the 10(56.6%) non-O157 is presented in Table 4. All the *E. coli* O157 and non-O157 were susceptible and resistant to ofloxacin and augmentin respectively.

Two (25%) *E. coli* O157 were susceptible to ciprofloxacin while 6 (75%) were intermediately susceptible to it. For the non- O157, 2(20%), 7(70%) and 1(10%) were susceptible, intermediately susceptible and resistant to ciprofloxacin. Seven (87.5%) of *E. coli* O157 and 1(12.5%) were susceptible and intermediately susceptible to gentamicin respectively. Non-*E. coli* O157 isolates had 6(60%), 1(10%) and 3(30%) susceptible, intermediately susceptible and resistant to gentamicin. The MAR index of *E. coli* O157 and non-O157 isolates ranged from 0.13 to 0.50 and 0.38 to 0.75 respectively (Tables 4).

**3.4 PCR amplification of genes**

Only 8 (44.4%) of the 18 tentatively identified *E. coli* O157 on SMAC amplified *uidA* genes at 252bp and were confirmed as *E. coli* O157. These isolates were E1, E4, E6, E7 and E8 from intestinal digesta as well as E9, E11 and E12 from cattle dung (Figure 1).

Out of the 8 *E. coli* O157, only 3 (37.5%) showed amplification for *stx1* and produced the expected band at 348bp (Figure 2). These isolates were E9, E11 and E12 gotten from cattle dung. Only 1 (12.5%) of the *E. coli* O157 (E11) amplified *stx2* gene and produced the expected band at 548bp (Figure 3).

**Table 2:** Bacteriological counts of the intestinal digesta and cattle dung

|  |  |
| --- | --- |
| Locations | Count (cfu/g) |
|  Intestinal digesta |  Cattle dung |
| HB | FC | HB | FC |
| A | 1.4 x 106 | 1.1 x 106 | 3.7 ×105 | 2.5 × 105 |
| B | 4.7 x 105 | 2.7 x 105 | 7.2 ×105 | 5.0 × 105 |
| C | 8.1 x 105 | 2.2 x 105 | 4.0 ×106 | 7.0 × 105 |
| D | 1.0 x 106 | 2.9 x 105 | 4.5 ×105 | 4.0 × 105 |
| E | 3.0 x 106 | 2.2 x 106 | 9.0 ×105 | 6.0 × 105 |
| F | 2.7 x 106 | 1.6 x 105 | 4.8 $×10$6 | 1.9 × 105 |
| G | 3.8 x 106 | 2.2 x 106 | 3.5 ×105 | 2.5 × 105 |
| H | 3.0x 106 | 1.2 x 106 | 8.5 ×104 | 6.0 × 104 |
| I | 8.1 x 106 | 1.3 x 106 | 9.0 ×105 | 5.0 × 105 |
| J | 2.6 x 106 | 1.2 x 106 | 9.2 ×105 | 9.0 × 105 |
| K | 1.3 x 107 | 2.0 x 106 | 1.1 ×106 | 1.0 × 106 |
| L | 2.3 x 106 | 2.0 x 106 | 7.5 ×104 | 5.0 × 10 4 |
| M | 1.3 x 106 | 9.7 x 105 | 1.6 ×105 | 1.5 × 105 |

HB- Heterotrophic bacteria; FC- Faecal coliform; A- Wara Village I, Ilorin; B- Tipper Garage I, Ilorin; C- Akerebiata I, Ilorin; D-Oja-Tuntun I, Ilorin; E- Akerebiata II, Ilorin; F- Oja-Tuntun II, Ilorin; G-Wara Village II, Ilorin; H-Tipper Garage Ilorin, II; I- Mandate I, Ilorin; J- Mandate II, Ilorin; K-Tipper Garage III, Ilorin; L -Wara Village III, Ilorin; M -Akerebiata III, Ilorin**Table 3:** Bacteriological counts of water from river at different locations in Ilorin, Nigeria

|  |  |
| --- | --- |
| Sample sites |  Counts (cfu/ml) |
| Bacteria | Faecal coliform |
| Oja-Tuntun | 2.7 x 106 | 1.0 x 101 |
| Surulere | 2.6 x 106 | 1.0 x 101 |
| Abata-Shuban | 2.9 x 106 | 1.0 x 103 |
| Oke-Foma | 4.4 x 104 | 1.0 x 101 |
| Oloje | 1.5 x 105 | 1.0 x 102 |
| Opomalu | 8.4 x 106 | 1.0 x 102 |
| Unity | 6.6 x 105 | 1.0 x 102 |
| Coca cola | 6.0 x 104 | 1.0 x 102 |
| Amilegbe | 2.3 x 106 | 1.0 x 103 |
| Akerebiata | 1.8 x 106 | 1.0 x 103 |
| Shao-Garage | 5.6 x 105 | 1.0 x 101 |
| Ita-Amodu | 9.6 x 106 | 1.0 x 101 |

**Table 4:**  Antibiotic susceptibility patterns of *E. coli* O157 and non-O157

|  |  |  |  |
| --- | --- | --- | --- |
| Isolates  | Source | Zone of inhibition (mm) | MARIndex |
| NIT | AMP | CAZ | CRX | GEN | CPR | OFL | AUG |
| E1 | D | 29(S) | 12(R) | 25(S) | 20S) | 20(S) | 25(I) | 24(S) | 12(R) | 0.25 |
| E2\* | D | 17(S) | 11(R) | 22(S) | 13(R) | 21(S) | 22(I) | 25(S) | R | 0.38 |
| E3\* | D | 21(S) | R | 31(S) | R | 20(S) | 22(I) | 24(S) | R | 0.38 |
| E4 | D | 29(S) | 6(R) | 13(R) | 18(S) | 14(I) | 22(I) | 20(S) | R | 0.38 |
| E5\* | D | R | R | 12(R) | R | 20(S) | 22(I) | 24(S) | R | 0.63 |
| E6 | D | 29(S) | 15(I) | 25(S) | 20(S) | 15(S) | 24(I) | 29(S) | 12(R) | 0.13 |
| E7 | D | R | 15(I) | 14(R) | 20(S) | 18(S) | 22(I) | 18(S) | 12(R) | 0.38 |
| E8 | D | 30(S) | R | 12(R) | R | 20(S) | 26(S) | 22(S) | R | 0.5 |
| E9 | C | 15(I) | 12(R) | R | 13(R) | 16(S) | 25(I) | 23(S) | R | 0.5 |
| E10\* | C | 27(S) | 13(R) | 14(R) | 12(R) | 21(S) | 27(S) | 27(S) | 13(R) | 0.5 |
| E11 | C | 16(I) | 10(R) | R | 20(S) | 16(S) | 26(S) | 26(S) | 12(R) | 0.38 |
| E12 | C | 21(S) | 12(R) | R | 21(S) | 18(S) | 22(I) | 22(S) | 12(R) | 0.38 |
| E13\* | C | 24(S) | 11(R) | 15(R) | R | 14(I) | 25(I) | 23(S) | 7(R) | 0.5 |
| E14\* | W | 25(S) | R | 25(S) | 20(S) | 12(R) | 26(S) | 26(S) | 7(R) | 0.38 |
| E15\* | W | 24(S) | R | 20(I) | 21(S) | 11(R) | 24(I) | 24(S) | R | 0.38 |
| E16\* | W | 16(S) | R | R | R | 18(S) | 24(I) | 22(S) | R | 0.5 |
| E17\* | W | 25(S) | R | R | R | 20(S) | 13(R) | 22(S) | R | 0.63 |
| E18\* | W | 10(R) | R | R | R | R | 22(I) | 22(S) | R | 0.75 |

R – Resistance, S – Susceptible, I – Intermediately susceptible, NIT – Nitrofurantoin (300µg), AMP – Ampicillin (30µg), CAZ-Ceftazidime (30µg), CRX – Cefuroxime (30µg), GEN – Gentamicin (10µg), CPR – Ciprofloxacin (5µg), OFL – Ofloxacin (5µg), AUG – Augmentin (30µg). \* - Non- *E. coli* O157, D – Intestinal digesta, C- Cattle dung, W- Water

 M E1 E4 E6 E7 E8 E9 E11 E12

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 252bp

**Figure 1:** Agarose gel electrophoresis image of the amplified *uidA* genes

M=50bp Molecular ladder; E= *E. coli* 0157:H7

 M E9 E11 E12



348bp

**Figure 2:** Agarose gel electrophoresis image of the amplified *stx1* genes

 M=50bp Molecular ladder; E*= E. coli* isolates

 M E11

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548bp

**Figure 3:** Agarose gel electrophoresis image of the amplified *stx2* gene

M= 50bp Molecular ladder; E= *E. coli* isolates

**4. DISCUSSION**

 The range of counts of viable bacteria and faecal coliform obtained from the intestinal digesta of slaughtered cattle was higher than those isolated from cattle dungs. This is expected as the gastro-intestinal tract of cattle and other ruminants are known to harbour *E. coli*. In a similar study, Grauke *et al.* (2002) obtained count of *E. coli* O157:H7 as high as 105cfu/g in cattle faeces. Water samples from the river produced the least counts since these isolates would need to survive in the aquatic environment after being released or discharged through the sewage, manure contamination and defaecation.

 The isolates were either susceptible, intermediately susceptible or resistant to each antibiotic. There was 100% susceptibility to ofloxacin coupled with 100% resistant to augmentin by all the 8 *E. coli* O157 and 10 non-O157 isolates. The *E. coli* O157 isolates were more susceptible to ciprofloxacin, gentamicin, ampicillin and cefuroxime than non-O157 isolates but were less susceptible to nitrofurantoin and ceftazidime than non-O157. In a study, Bakhtiari *et al*. (2020) found that 96.5, 87.6 and 45.1% of their isolates were susceptible to nitrofurantoin, gentamicin, and ciprofloxacin respectively. Wardoyo *et al*. (2021) reported that 92.6, 86.5, and 81.4 % of their *E. coli* isolates were resistant to ampicillin, cefuroxime and augmentin respectively. Joseph *et al*. (2017) reported resistance of 77.2 and 51.7% to cefuroxime and augmentin respectively. Majority of the isolates (94.4%) in this study were resistant to at least 2 antibiotics. Their MAR index was greater than 0.2.

 The use of selective medium (SMAC) in this study prior to PCR amplification of *uidA* has allowed for 44.4% confirmation of the 18 *E. coli* isolates as *E. coli* O157. More *E. coli* O157 was detected in the intestinal digesta than from the cattle dung. This can be explained by the fact that after the faecal matters are expunged from the animal’s rectal, it is more prone to environmental contamination from soil and water. More of the *E. coli* O157 isolates amplified *stx1* (37.5%) than *stx2* (12.5%). Fagan *et al.* (1999) in their study of isolation of *E. coli* 0157:H7 from cattle faeces got 19.4% (35 of 180) and 6.7% (12 of 180) amplification for *stx1* and *stx2* genes respectively. Gholami-Ahangaran *et al.* (2021) isolated *E. coli* in the intestinal content and meat of Turkey but none of them carried *stx1* or *stx2* genes. Alalade *et al.* (2018) reported the detection of *E. coli* 0157:H7 possessing *stx2* in two wells and one stream, representing 3.75% of their water samples. However, in this study, none of the putative *E. coli* from the river water samples amplified the *uidA* gene; they were confirmed as non- 0157:H7. The non-amplification of *uidA* gene by the *E. coli* isolates from water is not unexpected as the level of *E. coli* O157: H7 in cattle intestinal material or dung is the region of 103 (Stein and Katz, 2017)and become diluted to negligible or non- detection level when excreted into the water body. Muller and Ehlers (2005) have reported water borne transmission of *E. coli* O157:H7 from recreational and drinking water. *E. coli* O157:H7 producing shiga toxins has been reported as the causative agent of haemolytic uremic syndrome (Jamshidi *et al*., 2016). According to Cebula *et al.* (1995), the *uidA* primer used in this study will not allow amplification of wild *E. coli* because of the double mismatch it has in it design.

Fode-Vaughan *et al*., 2003 used similar *stx*1 forward primer but a different *stx*1 reverse primer and obtained band at 513 base pair instead of at 348bp. This observation supported the fact that *stx* 1 can be amplified by different arrays of *stx* 1 reverse primers and at different regions. Hence, in our study the multiple bands seen can be attributed to multiple amplifications and the PCR conditions used in this study. Inspite of this, Cebula *et al*. (1995) and Feng and Monday (2000) have reported that amplification at 348bp are due to amplification of *stx*1 genes

**5. CONCLUSION AND RECOMMENDATION**

In conclusion, the high incidence of enterohaemorrhagic *E. coli* O157:H7 in 44.4% of the isolated *E. coli* is of public health significance since these animals can be a source of transfer to other healthy animals and humans. Shiga toxins, *stx1* and *stx2* genes were detected in some of the *E. coli* O157 isolates. There was 100% susceptibility and resistant to ofloxacin and augmentin respectively. It is recommended that faecal materials and intestinal digesta of cattle should be handle with care since they harboured *E. coli* 0157:H7. There is need for proper hygiene in the management of these wastes.

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