Scientific Research Publishing

Advances in Microbiology, 2026, 16(9), 371-389
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Occurrence and Virulence Gene Profiles of Enterohaemorrhagic *Escherichia Coli* O157:H7 Isolated from Cattle at Abattoirs in the Federal Capital Territory, Nigeria

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How to cite this paper: Madugu, S.A., Ameh, J.A., Mailafia, S., Olabode, H.O.K., Egwu, G.O. and Shammah, V.B. (2026) Occurrence and Virulence Gene Profiles of Enterohaemorrhagic *Escherichia Coli* O157:H7 Isolated from Cattle at Abattoirs in the Federal Capital Territory, Nigeria. *Advances in Microbiology*, **16**, 371-389.
https://doi.org/10.4236/aim.2026.169021

Received: July 21, 2026
Accepted: September 11, 2026
Published: September 14, 2026

Abstract

Enterohaemorrhagic (EHEC) *Escherichia coli* O157:H7 is an important foodborne zoonotic pathogen carried asymptomatically by cattle and capable of causing severe disease in humans. This study investigated the occurrence and virulence gene profiles of (EHEC) *E. coli* O157:H7 isolated from cattle at selected abattoirs in the Federal Capital Territory (FCT), Nigeria. A total of 450 rectal swab samples were collected from cattle slaughtered at five major abattoirs (Dei-Dei, Gwagwalada, Kubwa, Karu, and Guzape). Isolation was carried out using standard bacteriological techniques, followed by biochemical confirmation and serological identification using O157 latex agglutination and H7 flagellar antigen tests. Genomic DNA was extracted from confirmed isolates using the QIAGEN DNA extraction kit, and multiplex polymerase chain reaction (PCR) was performed to detect the *eaeA*, *hlyA*, *rfbE*, and *fliCH7* virulence genes. Overall, *E. coli* was isolated from 175 (38.9%) samples. Of the 28 presumptive isolates selected for confirmation, 18 (64.3%) were biochemically confirmed as *E. coli*, while 8 (44.4%) possessed the O157 antigen. From the total *E. coli*, 18 (4%) were suspected O157 colonies on Sorbitol MacConkey Agar. Following O157 latex agglutination, 8 (1.78%) were positive, H7 flagellar agglutination shows 5 (1.11%) and EHEC result shows 2 (0.44%). Molecular characterization demonstrated the presence of the targeted virulence genes among the confirmed *E. coli* O157 isolates, confirming their pathogenic potential. The occurrence of EHEC *E. coli* O157:H7 did not differ significantly

among the sampled abattoirs (Fisher-Freeman-Halton exact test, $p > 0.05$). The findings demonstrate that cattle slaughtered in FCT abattoirs constitute a reservoir of pathogenic *E. coli* O157:H7 and underscore the need for strengthened abattoir hygiene, routine microbiological surveillance, and integrated One Health interventions to reduce contamination of beef and the risk of zoonotic transmission.

Keywords

EHEC *Escherichia coli* O157:H7, Cattle, Virulence Genes, Multiplex PCR, One Health

1. Introduction

Escherichia coli (*E. coli*) is a facultatively anaerobic, Gram-negative bacterium that colonizes the intestine of humans and warm-blooded animals as part of their normal gut flora. Although many strains are harmless commensals of the gut microbiome, several *E. coli* pathotypes have acquired virulence factors that enable them to cause intestinal and extra-intestinal infections [1] [2]. Based on pathogenic mechanisms, clinical presentations, and their genotypic traits, diarrheagenic *E. coli* (DEC) are classified into six major pathotypes: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC), and enterohaemorrhagic *E. coli* (EHEC) [3] [4]. Enterohaemorrhagic *E. coli* is considered one of the deadliest *E. coli* pathotypes due to its capacity to cause serious systemic sequelae [5].

Enterohaemorrhagic *E. coli* O157: H7 is a Shiga toxin-producing *E. coli* (STEC) serotype that represents a global food safety challenge. Pathogens of this group are characteristically Shiga toxin-producing (stx1 and/or stx2), harbingers of the locus of enterocyte effacement (LEE), and able to mediate intimate attachment to intestinal epithelial cells [6]-[8]. EHEC O157: H7 infections are foodborne and can occur after the ingestion of as few as bacteria. Domestic animals, particularly cattle, are widely known to be asymptomatically infected with EHEC O157: H7, contributing to their persistence as foodborne pathogens of public health concern [9].

Studies have shown cattle are the predominant reservoir host of EHEC O157:H7. In cattle, colonization of EHEC O157:H7 is prevalent at the terminal recto-anal junction (R-AnJ) and is shed intermittently in faeces as the bacteria colonize the large intestine [10]-[13]. Experimental studies have established that cattle can be easily colonized and persistently infected with EHEC O157:H7, thus serving as the primary source of contamination of the environment and the food chain. Several research works carried out locally and in different parts of the world have isolated EHEC O157:H7 from cattle faeces, hides, and carcasses [12].

E. coli O157 is a major foodborne pathogen that causes illnesses ranging from mild diarrhea to hemorrhagic colitis and severe complications such as hemolytic uremic syndrome (HUS), a leading cause of acute kidney failure in children [14]. Globally, outbreaks have been linked to contaminated beef, unpasteurized milk, vegetables, and water, resulting in significant morbidity, mortality, and economic losses due to reduced consumer confidence in beef products [15]. Because antibiotic treatment may increase toxin release, managing EHEC O157 infections is challenging. Cattle serve as an important reservoir, and transmission to humans commonly occurs during slaughter through carcass contamination caused by poor hygiene, contaminated equipment, and improper handling practices [16]. These risks are further exacerbated in many low- and middle-income countries by inadequate slaughterhouse infrastructure, poor sanitation, insufficient training of meat handlers, and weak enforcement of food safety regulations, increasing the likelihood of contaminated meat reaching consumers [14].

Routine surveillance and monitoring for the presence of foodborne pathogens in abattoirs are not regularly conducted in Nigeria. Although several abattoir-based studies have been conducted within the country, sampling was restricted to specific geopolitical zones. These include South-Western Nigeria [17], South-Western Nigeria [18], South-Eastern Nigeria [19], and North-Central Nigeria [20] [21]. Importantly, these works focused only on the prevalence of bacteria, with limited molecular data on EHEC O157:H7 occurrence and virulence gene profiles available for Nigeria, especially in the Federal Capital Territory (FCT), Abuja. With this limited information, risk assessment and preparedness for outbreaks would be difficult. There is increasing demand for animal protein as Nigeria's population continues to grow. Abuja, being the seat of the government, welcomes more livestock traders from within and outside the country in search of markets. Therefore, there is a need for updated and locality-specific data.

2. Materials and Methods

2.1. Study Area

The study was conducted in the Federal Capital Territory (FCT), Abuja, Nigeria, which is located in the North-Central geopolitical zone of the country between latitudes 8˚25' and 9˚20'N and longitudes 6˚45' and 7˚39'E. The FCT covers an area of approximately 7315 km^2 and comprises six Area Councils: Abuja Municipal Area Council (AMAC), Gwagwalada, Kuje, Bwari, Abaji, and Kwali. The territory has a tropical savannah climate characterized by distinct wet and dry seasons, with average annual temperatures ranging from 25˚C to 30˚C. Livestock farming and cattle trading are important economic activities in the FCT, and several major abattoirs operate within the territory, supplying beef to residents and neighboring states. The high volume of cattle slaughtered and the extensive meat distribution network make the FCT an appropriate setting for investigating the occurrence and virulence gene profiles of *E. coli* O157 in slaughtered cattle, as well as assessing the potential public health risks associated with beef production and

consumption (see Figure 1) [22].

2.2. Study Design

A cross-sectional study was conducted to determine the occurrence and virulence gene profiles of *E. coli* O157:H7 isolated from cattle at selected abattoirs in the Federal Capital Territory (FCT), Nigeria. The specific aims were to isolate and identify *E. coli* and EHEC O157:H7 from cattle rectal swabs collected at abattoirs sampled, confirm the isolates using standard biochemical tests for *E. coli* and slide agglutination test for EHEC O157:H7, and determine the virulence genes associated with isolates using multiplex PCR.

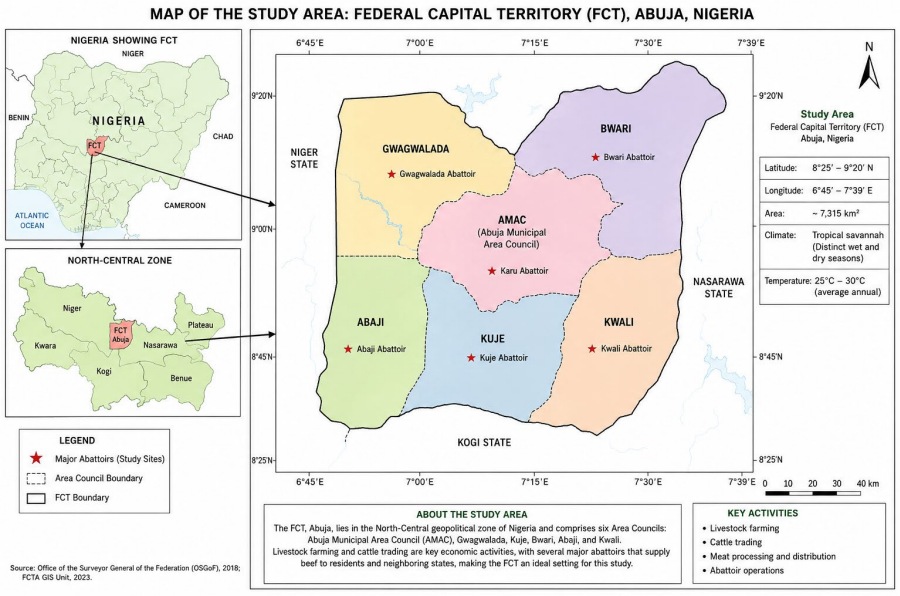

Figure 1. Map of the Federal Capital Territory (FCT), Nigeria, showing the study area and the six Area Councils. *Adapted from* Federal Capital Territory Administration (n.d.) [22].

2.3. Sample Size Determination

The minimum sample size for this study was determined using the formula for estimating a single population proportion, as described by Thrusfield [23]:

$$N = Z^2 pq / d^2$$

where:

N = Sample size;

Z = Standard normal deviation for a 95% confidence interval (1.96);

P = Prevalence 32.8% [24];

D = desired precision (0.05);

$q = 1 - p$.

$$N = \frac{(1.96)^2 \times 0.328 \times (1-0.328)}{(0.05)^2} = 339 .$$

The calculated minimum sample size was 339. However, to improve the preci-

sion and reliability of the study and to ensure adequate representation of cattle slaughtered across the selected abattoirs, the sample size was increased to 450 fresh cattle faecal samples. A total of 90 samples were collected from each of the five selected abattoirs during the study period. Samples were collected irrespective of the age, sex, or health status of the animals presented for slaughter.

2.4. Sample Collection

Approximately 5 - 10 g of fresh cattle rectal swabs were collected aseptically using sterile disposable gloves from the rectum of slaughtered cows into sterile, labeled universal sample containers. Samples were labeled with appropriate codes to indicate the location (abattoir) of origin and date of sampling. All samples were transported in ice boxes maintained at 4˚C to the laboratory. They were further processed immediately or stored temporarily at 4˚C for no more than 24 h prior to analysis.

2.5. Isolation of *E. coli* O157

Isolation of *E. coli* O157 was carried out using standard bacteriological procedures. Rectal swab samples collected from slaughtered cattle were aseptically inoculated into sterile, labelled sample bottles containing 8 - 9 mL of MacConkey broth (Oxoid, UK) and incubated at 37˚C for 24 hours for pre-enrichment. Following incubation, a loopful of each enriched culture was streaked onto Eosin Methylene Blue (EMB) agar (Oxoid, UK) and incubated at 37˚C for 24 - 48 hours. Colonies exhibiting a characteristic greenish metallic sheen on EMB agar were regarded as presumptive *E. coli* [25].

Representative colonies with a metallic sheen were aseptically picked using a sterile inoculating loop and subcultured onto Sorbitol MacConkey Agar (SMAC; Oxoid, UK), followed by incubation at 37˚C for 24 hours. Colourless (non-sorbitol fermenting) colonies on SMAC were considered presumptive *E. coli* O157, since most *E. coli* O157 strains are unable to ferment sorbitol. These presumptive isolates were subsequently subcultured onto nutrient agar slants, incubated at 37˚C for 24 hours, and stored at 4˚C until further characterization [24].

2.6. Biochemical Characterization of *E. coli* O157 Isolates

Presumptive *E. coli* O157 isolates were further confirmed by conventional biochemical tests as described by Mailafia *et al.* [24]. The isolates were tested for indole production and motility using Sulphide-Indole-Motility (SIM) medium (Merck, Germany), citrate utilization using Simmons citrate agar (Merck, Germany), methyl red and Voges-Proskauer (MR-VP) reactions using MR-VP medium (Merck, Germany), and urease production using urea agar (Oxoid, UK). Isolates exhibiting the characteristic biochemical profile of *E. coli*—motile, indole positive, methyl red positive, Voges-Proskauer negative, citrate negative, and urease negative—were considered confirmed *E. coli* isolates and were preserved for subsequent molecular characterization of *E. coli* O157 virulence genes.

2.7. Serological Identification of *E. coli* O157

Biochemically confirmed *E. coli* isolates were serologically identified using a slide latex agglutination test for the detection of O157 and H7 antigens. Briefly, a pure colony from each confirmed isolate was emulsified separately with O157 and H7 latex antisera on a clean glass slide according to the manufacturer's instructions. The reaction mixture was gently rocked for approximately 1 minute and examined for visible agglutination. Isolates that produced visible agglutination with both O157 and H7 antisera were regarded as *E. coli* O157, while those showing no agglutination or reacting with only one antiserum were considered negative for *E. coli* O157 [26] [27].

2.8. DNA Extraction

Genomic DNA was extracted from confirmed *E. coli* O157 isolates using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Briefly, a pure overnight bacterial culture was harvested and suspended in the appropriate lysis buffer containing Proteinase K to facilitate cell lysis. Following incubation, ethanol was added to the lysate, and the mixture was transferred onto a DNeasy spin column, where genomic DNA selectively bound to the silica membrane. The column was washed sequentially with the recommended wash buffers to remove proteins and other contaminants. Finally, purified genomic DNA was eluted with the elution buffer supplied in the kit and stored at −20˚C until used as the template for polymerase chain reaction (PCR) amplification of the virulence genes [28] [29].

2.9. Molecular Detection of Virulence Genes by Multiplex PCR

Multiplex PCR was used to detect virulence genes associated with enterohaemorrhagic *E coli* O157:H7. Primers targeting *eaeA* (intimin), *fliC* (H7 flagellin), *rfbE* (O157 somatic antigen), *hlyA* (HlyA) (enterohaemolysin), and stx1 (Shiga toxin 1) genes were used for detection. Genomic DNA extracted from confirmed *E. coli* isolates served as template for the PCR assay. Primers used for detection of each gene and the expected amplicon sizes are described below (**Table 1**).

Table 1. Primers used for molecular detection of *E. coli* O157:H7 virulence and serotype-specific genes.

Target gene	Primer	Primer sequence (5'-3')	Amplicon size (bp)	Target function	Reference
eaeA	Forward Reverse	GCAAATTTAGGTGCGGGTCAGCGTT GGCTCAATTTGCTGAGACCACGGTT	494	Encodes intimin (an adherence protein)	Wang *et al.* [30]
rfbE	Forward Reverse	CTACAGGTGAAGGTGGAATGG ATTCCTCTCTTTCCTCTGCGG	327	Encodes the O157 somatic antigen	Wang *et al.* [30]
fliCH7	Forward Reverse	TACCATCGCAAAAGCAACTCC GTCGGCAACGTTAGTGATACC	247	Encodes the H7 flagellar antigen	Wang *et al.* [30]
hlyA	Forward Reverse	AGCTGCAAGTGCGGGTCTG TACGGGTTATGCCTGCAAGTTCAC	569	Encodes enterohemolysin (EHEC-hlyA)	Wang *et al.* [30]

Each PCR reaction was performed in a final volume of 25 µL consisting of 12.5 µL of 2× PCR master mix (which contains Taq DNA polymerase enzyme, deoxynucleotide triphosphates [dNTPs], $MgCl_2$, and buffer), 0.5 µL each of forward and reverse primers, 5 µL of DNA template, and made up to volume with nuclease-free water. PCR was performed in a thermocycler under the following optimized conditions: initial denaturation at 94˚C for 5 minutes, followed by 35 cycles of denaturation at 94˚C for 30 seconds, annealing at 58˚C - 60˚C for 30 seconds, extension at 72˚C for 60 seconds, and a final extension at 72˚C for 7 minutes.

The PCR products were resolved on a 1.5% agarose gel prepared in 1× TBE buffer. Gels were stained with ethidium bromide and visualized using a gel documentation system under UV transillumination. Band sizes of the amplified fragments were determined by comparison with a 100 bp DNA molecular weight marker. Standard EHEC O157:H7 strains were used as positive controls while nuclease-free water was used as the negative control for each PCR run.

2.10. Data Collection and Analysis

Data were collected from laboratory investigations, including bacteriological isolation, biochemical identification, serological confirmation, and molecular detection of virulence genes in *E. coli* O157 isolates recovered from cattle rectal swab samples. Laboratory findings were entered into Microsoft Excel 2019 and analyzed using IBM SPSS Statistics version 26. Descriptive statistics were used to summarize the occurrence of *E. coli* and *E. coli* O157 as frequencies and percentages. Differences in the occurrence of *E. coli* among abattoirs were assessed using Pearson's Chi-square test, while the distribution of *E. coli* O157 across sampling locations was evaluated using the Fisher-Freeman-Halton exact test because of the small number of positive isolates. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Isolation and Identification of *E. coli* and *E. coli* O157:H7

A total of 450 cattle faecal samples were collected from five abattoirs in the Federal Capital Territory (FCT), Nigeria, and examined for the presence of *E. coli* and *E. coli* O157:H7. Of the 450 samples analyzed, 175 (38.9%) yielded *E. coli* isolates, while 8 (1.8%) were confirmed as *E. coli* O157:H7, representing 4.6% (8/175) of the confirmed *E. coli* isolates (Table 2).

The distribution of *E. coli* isolates varied slightly among the five abattoirs (Table 3). Dei-Dei recorded the highest prevalence of *E. coli* with 41 (45.6%) positive samples, followed by Karu with 37 (41.1%), Gwagwalada with 35 (38.9%), Kubwa with 32 (35.6%), and Guzape with 30 (33.3%). The prevalence of *E. coli* O157 was low across all sampling locations, with two isolates (2.2%) recovered from each of the Dei-Dei, Gwagwalada, Karu, and Guzape abattoirs, whereas no *E. coli* O157 isolate was detected in samples collected from the Kubwa abattoir.

Pearson's Chi-square test showed no statistically significant difference in the prevalence of *E. coli* among the five abattoirs ($\chi^2 = 2.11$, $p = 0.71$). Because the

occurrence of *E. coli* O157:H7 was low and several expected cell frequencies were less than five, the Fisher-Freeman-Halton exact test was used to compare the distribution of O157:H7 isolates across the abattoirs. The exact test showed no statistically significant difference in the occurrence of *E. coli* O157:H7 among the five sampling locations ($p = 0.69$).

Table 4 presents the sequential screening and confirmation of *E. coli* O157:H7 and EHEC among the 450 cattle faecal samples examined from abattoirs within the Federal Capital Territory. Of the 450 samples examined, 18 (4.00%) produced suspected O157 colonies on sorbitol MacConkey agar (SMAC) and were therefore subjected to further confirmation. Following latex agglutination testing, 8 (1.78%) samples were positive for the O157 antigen, indicating the presence of *E. coli* isolates with the O157 serogroup characteristic. Further serological testing for the H7 flagellar antigen identified 5 (1.11%) H7-positive isolates among the samples examined. Thus, the combined O157 and H7 findings indicate the presence of isolates with O157:H7-associated characteristics in the cattle population sampled. The result also shows 2 (0.44%) isolates as EHEC-positive. This represents the proportion of the 450 cattle faecal samples classified as EHEC in the study.

The phenotypic and biochemical characteristics of the confirmed *E. coli* O157:H7 isolates are summarized in Table 5. All isolates produced characteristic greenish metallic sheen colonies on Eosin Methylene Blue (EMB) agar and were oxidase negative. The isolates were uniformly motile and produced acid and gas during glucose fermentation on Triple Sugar Iron (TSI) agar. In addition, all isolates were methyl red positive, Voges-Proskauer negative, indole positive, and urease negative. These biochemical characteristics were consistent among all isolates examined and supported their identification as *E. coli* O157:H7.

The molecular characterization revealed considerable variation in the distribution of virulence-associated genes among the eight *E. coli* O157:H7-associated isolates (as shown in Table 6). The *hlyA* gene, with an expected amplicon size of 596 bp, was detected in G228 and G263, while G288 showed a faint band interpreted as a possible *hlyA* detection. The *eaeA* gene (494 bp) was detected in D18 and K138, whereas the *rfbE* gene (327 bp) was detected only in D18. The *fliC* gene (247 bp) was detected in G201, D18, K121 and K138.

Among the isolates, D18 demonstrated the most extensive virulence-associated profile, with simultaneous detection of *eaeA*, *rfbE*, and *fliC*. This profile indicates the presence of markers associated with intimin, the O157 antigen, and H7 flagellar characteristics, respectively. K138 also carried *eaeA* and *fliC*, giving it an *eaeA*/*fliC*-associated profile. G201 and K121 each showed detection of the *fliC* marker alone among the four virulence-associated genes examined.

Table 2. Prevalence of *E. coli* isolates from cattle in FCT abattoir.

To Sample examined	Number of Positive *E. coli*	Number of Positive *E. coli* O157:H7
	E. coli isolate	*E. coli* O157:H7 strains
	Number of positives (%)	Number of positives (%)
N = 450	175 (38.88)	8 (1.77)

Table 3. Prevalence of *E. coli* and *E. coli* O157:H7 in different abattoirs within the Federal Capital Territory, Nigeria.

Location of abattoir	No. of samples examined	*E. coli* No. positive (%)	*E. coli* O157:H7 No. positive (%)
Dei-Dei	90	41 (45.56)	2 (2.22)
Gwagwalada	90	35 (38.89)	2 (2.22)
Kubwa	90	32 (35.56)	0 (0.00)
Karu	90	37 (41.11)	2 (2.22)
Guzape	90	30 (33.33)	2 (2.22)
Total	**450**	**175 (38.89)**	**8 (1.78)**
Statistical test		Pearson's $\chi^2 = 2.11$, $p = 0.71$	Fisher-Freeman-Halton exact test, $p = 0.69$

Table 4. Screening and confirmation of *E. coli* O157:H7 and EHEC among cattle faecal samples.

Detection/confirmation stage	Number positive (n)	Prevalence (%)
Cattle faecal samples examined	450	100.00
Suspected O157 colonies on SMAC	18	4.00
O157 positive by latex agglutination	8	1.78
H7 positive by flagellar agglutination	5	1.11
EHEC positive	2	0.44

Table 5. Phenotypic and biochemical reactions of suspected isolates of *E. coli* O157:H7.

Isolate	EMB	GS	O	M	TSI	VP	MR	CI	I	U
G201	GMS	−Ve	−Ve	+ve	A/A+ gas	−Ve	+ve	Green	+ve	−Ve
G228	GMS	−ve	−ve	+ve	++	−ve	+ve	Green	+ve	−ve
G263	GMS	−ve	−ve	+ve	++	−ve	+ve	Green	+ve	−ve
G288	GMS	−ve	−ve	+ve	++	−ve	+ve	Green	+ve	−ve
D18	GMS	−ve	−ve	+ve	++	−ve	+ve	Green	+ve	−ve
D55	GMS	−ve	−ve	+ve	++	−ve	+ve	Green	+ve	−ve
K121	GMS	−ve	−ve	+ve	++	−ve	+ve	Green	+ve	−ve
K138	GMS	−ve	−ve	+ve	++	−ve	+ve	Green	+ve	−ve

Key: EMB = Eosine Methylene Blue, GMS = Greenish Metallic Shine, O = Oxidase, M = Motility, TSI = Triple Sugar Iron, VP = Voges Proskauer, MR = Methyl Red, CI = Citrate, I = Indole, U = Urease, −ve = Negative, +ve = Positive

Table 6. Canonical molecular characteristics of *E. coli* O157:H7-associated isolates from FCT abattoirs.

Isolate ID	*hlyA* (596 bp)	*eaeA* (494 bp)	*rfbE* (327 bp)	*fliC* (247 bp)	Molecular interpretation
G201	−	−	−	+	H7-associated marker detected
G228	+	−	−	−	*hlyA* detected

Continued

G263	+	−	−	−	*hlyA* detected; resistance genes detected
G288	+*	−	−	−	Possible *hlyA* detection; band is faint
D18	−	+	+	+	*eaeA/rfbE/fliC*-associated profile
D55	−	−	−	−	No clearly resolved target band in this gel
K121	−	−	−	+	H7-associated marker detected
K138	−	+	−	+	*eaeA/fliC*-associated profile

Key: + = gene detected/present; − = gene not detected/absent; * = faint band requiring confirmation; *hlyA* = enterohaemolysin-associated gene; *eaeA* = intimin gene; *rfbE* = O157-associated marker; *fliC* = H7 flagellin-associated marker; *blaTEM* = *β*-lactam resistance gene; *tetA* = tetracycline resistance gene.

3.2. Serological Confirmation of *E. coli* O157:H7

Serological identification using slide agglutination tests confirmed the presence of O157 and H7 antigens among selected *E. coli* isolates. A proportion of the isolates reacted positively with O157 antisera**,** while subsequent testing confirmed O157:H7 serotypes, thereby validating the presence of EHEC O157:H7 strains in cattle rectal swab samples from FCT abattoirs Figure 2 and Figure 3.

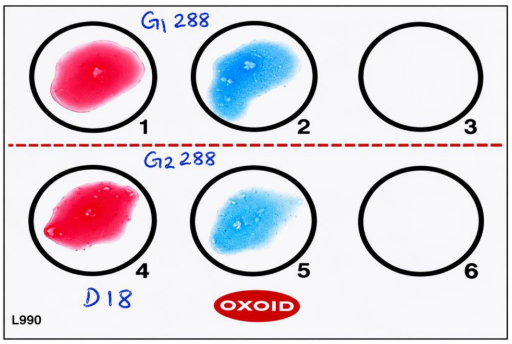

Figure 2. Circle 1 and 4 show positive results to the latex agglutination test to *E. coli* O157.

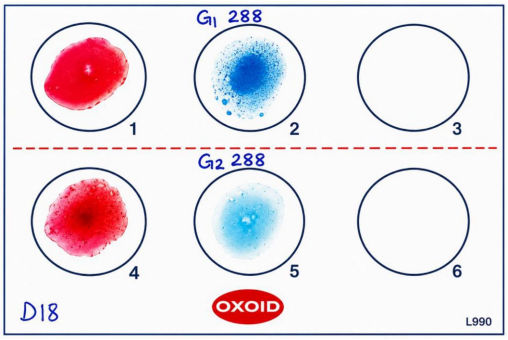

Figure 3. The Second two circles (2 and 5) show positive results to flagella test to confirm *E. coli* O157:H7.

3.3. Molecular Identification of *E. coli* O157 Virulence Genes

The multiplex polymerase chain reaction (PCR) assay successfully amplified the target virulence genes in the confirmed *E. coli* O157 isolates (as shown in Figure 4). The DNA molecular weight marker (100 bp ladder) was used to estimate the sizes of the amplified products. Specific amplification products corresponding to the hlyA (596 bp), eaeA (494 bp), rfbE (327 bp), and fliC H7 (247 bp) genes were detected in the isolates.

The electrophoretic profiles revealed variations in the distribution of virulence genes among the isolates. While some isolates harboured all four virulence genes, others lacked one or more target genes, resulting in different banding patterns across the lanes. Amplification of the rfbE gene confirmed the presence of the O157 serogroup, whereas amplification of the fliC H7 gene confirmed the H7 flagellar antigen. Similarly, the detection of the eaeA and hlyA genes demonstrated the presence of important virulence determinants associated with the pathogenicity of *E. coli* O157.

The positive control produced the expected amplification bands for the target genes, confirming the validity of the PCR assay, whereas no amplification was observed in the negative control, indicating the absence of contamination. The multiplex PCR assay confirmed the molecular identity of the isolates and demonstrated variability in their virulence gene profiles.

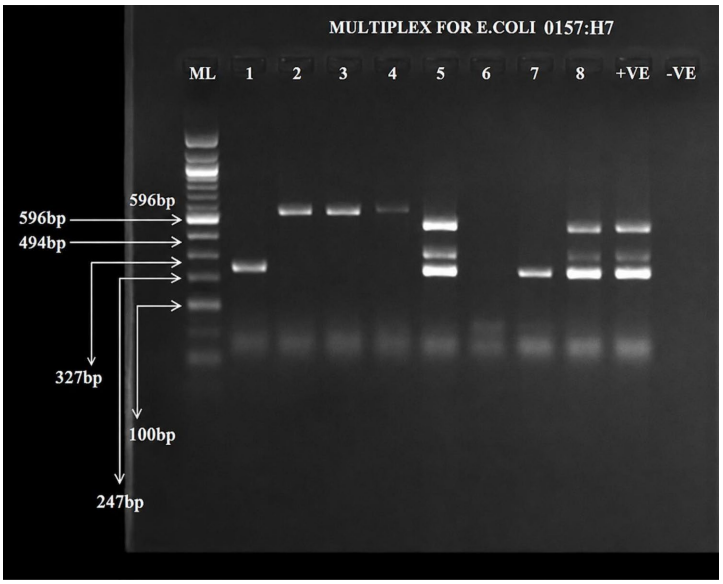

Figure 4. Multiplex PCR Gel Electrophoresis for *E. coli* O157:H7 Isolates.

This gel electrophoresis image displays the results of Multiplex PCR for the detection of specific *E. coli* virulence genes (Figure 4). The Molecular Ladder (ML) on the left serves as a reference, with marked bands at 596 bp (Hyla), 494 bp (Eae), 327 bp (RfbE), and 247 bp (Flic). Lanes 1-8 represent different *E. coli* isolates, each showing distinct bands corresponding to these target genes. The +VE (Positive Control) exhibits multiple expected bands, confirming successful amplification,

while the −VE (Negative Control) shows no visible bands, ensuring no contamination. The presence of multiple bands in different sample lanes indicates the detection of various virulence genes in the *E. coli* isolates.

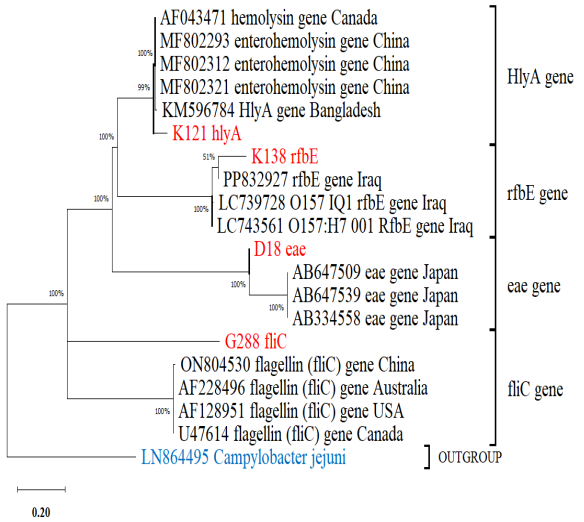

Figure 5. Maximum likelihood phylogenetic tree of *E. coli* O157:H7 virulence genes.

The phylogenetic analysis of the sequences of 4 *E. coli* virulence genes (fliC, HlyA, rfbE and eae) generated in this study (**Figure 5**) showed that, each of the 4 virulence genes sequences obtained in this study cluster with the ones obtained from the genbank with bootstrap values of 46% - 100%. Sequences generated in this study had 98% similarity with those in the genbank. The sequences produced in this study are the red-colored taxa. While the blue-colored taxon is the sequence of *Campylobacter jejuni* stain MTG14 from the Genbank, which was used as an outgroup, the black-colored taxa display the sequences obtained from the genbank.

4. Discussion

The overall *E. coli* prevalence of 38.88% is comparable with reports from other African countries indicating cattle as reservoirs of potentially pathogenic *E. coli* strains [31]-[33]. Detection of EHEC O157: H7 from cattle rectal swabs analyzed in this study (0.44%) is consistent with previous reports from Nigeria that cattle are reservoirs of this emerging foodborne pathogen of public health concern [24]. Isolation of EHEC O157: H7 from apparently healthy cattle used for meat production corroborates previous evidence that cattle asymptomatically shed this pathogen into the environment and contaminate meat and water products [34] [35].

The prevalence of EHEC O157:H7 isolates observed in this study is similar to other abattoir-based prevalence surveys conducted across Nigeria which report low but epidemiologically relevant detection rates [24] [36]. Similar prevalence rates of EHEC O157:H7 have been documented among cattle sampled from abat-

toirs in northern Nigeria [37] and south-south Nigeria [38]. Prevalence rates ranging from 1% to 8% have been attributed to differences in sampling design, sample sizes, and methods of detection. Similar observations have been reported in abattoir-based studies in Ethiopia [39]. These observations suggest that the prevalence of EHEC O157:H7 may be relatively low among cattle populations in Nigeria but is consistent across regions.

Molecular characterization of the isolates demonstrated the presence of important virulence genes, including *eaeA*, *fliC* H7, *rfbE*, and *hlyA*, confirming the pathogenic potential of the recovered *E. coli* O157 strains. The *eaeA* gene encodes intimin, an outer membrane adhesin that mediates intimate bacterial attachment to intestinal epithelial cells and promotes the formation of attaching-and-effacing lesions, which are characteristic of enterohaemorrhagic *E. coli* infections [40]-[42]. Similarly, the *hlyA* gene encodes enterohaemolysin, a pore-forming cytotoxin that contributes to host cell damage and enhances bacterial virulence [42] [43]. The *rfbE* and *fliC* H7 genes serve as highly specific molecular markers for the O157 somatic antigen and H7 flagellar antigen, respectively, and are widely employed for the molecular identification of *E. coli* O157 [30]. The detection of these virulence determinants confirms that the isolates recovered in this study possess genetic characteristics commonly associated with enterohaemorrhagic *E. coli* strains capable of causing severe human disease [42]-[44]. Further confirmation of the O157: H7 serotype was provided by the detection of *rfbE* and *fliC* genes, which are biomarkers for lipopolysaccharide (O antigen) and flagellin (F antigen) adhesins, respectively. Expression of *hlyA* mediates enterohemolysin production, which contributes to cytotoxicity in infected individuals. Concomitant detection of multiple virulence genes among single isolates recovered in this study has been observed in surveillance studies conducted globally [36] [45].

Abattoirs play a critical role in the transmission and environmental dissemination of enterohaemorrhagic *E. coli* (EHEC) O157, particularly when hygienic slaughtering and carcass handling practices are inadequate. Cattle transported from different parts of Nigeria to slaughterhouses in the Federal Capital Territory (FCT) may introduce the pathogen into the abattoir environment, resulting in contamination of carcasses, equipment, wastewater, and surrounding areas. These findings underscore the importance of improving sanitation, waste management, routine microbiological surveillance, and hygienic meat processing practices to reduce the risk of contamination and subsequent foodborne transmission [45] [46].

The phylogenetic analysis of the four *Escherichia coli* O157:H7-associated genes (*fliC*, *hlyA*, *rfbE*, and *eae*) provided evidence of close genetic relationships between the sequences obtained from cattle isolates in Abuja, Nigeria, and corresponding sequences deposited in GenBank. The sequences generated in this study clustered with reference *E. coli* sequences and showed approximately 98% sequence similarity, indicating that the detected gene sequences were closely related to previously reported *E. coli* O157:H7-associated sequences. The clustering observed for *rfbE* and *fliC* is particularly relevant because these genes are associated

with the O157 somatic and H7 flagellar characteristics, respectively, and have been used as molecular markers for identification of the O157:H7 serotype [30]. Similarly, *eae* and *hlyA* are important virulence-associated genes; *eae* encodes intimin, which contributes to intimate attachment of the organism to intestinal epithelial cells, whereas *hlyA* is associated with enterohaemolysin production [30] [43]. The bootstrap values observed in the tree (46% - 100%) indicate varying levels of support for individual branches, with branches receiving higher bootstrap values providing stronger evidence for the observed clustering. Thus, the phylogenetic relationships support the genetic similarity of the Abuja sequences to previously characterized *E. coli* O157:H7-associated genes circulating in other geographical regions.

In the phylogenetic tree, the sequences generated in the present study are indicated in red and are positioned within clusters containing homologous *E. coli* sequences retrieved from GenBank, whereas the black taxa represent reference sequences used for comparison. The inclusion of *Campylobacter jejuni* strain MTG14 as an anoutgroup provides an evolutionary reference for rooting the tree and separates the *Campylobacter* sequence from the *E. coli* sequences. The observed clustering of the Abuja isolates with geographically diverse GenBank sequences suggests that the virulence-associated genes examined in this study share substantial sequence conservation with homologous genes reported elsewhere. Similarity at individual virulence loci is expected because genes such as *rfbE* and *fliC* are used as serotype-associated markers, while *eae* and *hlyA* contribute to important pathogenic characteristics of enterohaemorrhagic *E. coli* [30] [43]. However, because the phylogenetic analysis was based on individual gene sequences rather than whole-genome data, the observed clustering should be interpreted as evidence of relatedness at the respective gene loci rather than definitive evidence of clonal or epidemiological relationships among the isolates. Whole-genome sequencing would provide stronger resolution for determining the evolutionary relationships and possible transmission links among the Abuja isolates and strains from other geographical locations.

The recovery of virulent *E. coli* O157 isolates carrying important virulence genes highlights the potential public health risk associated with contaminated meat and water. Because this pathogen has a low infectious dose and can cause severe illnesses such as haemorrhagic colitis and haemolytic uraemic syndrome, particularly among children and immunocompromised individuals, strengthened meat inspection, proper meat handling, thorough cooking of beef, and public health education are essential. Furthermore, the findings support the adoption of an integrated One Health approach involving the animal, human, and environmental health sectors to strengthen surveillance, improve disease prevention, and facilitate coordinated control of EHEC O157 in Nigeria [47].

5. Conclusion

This study demonstrated that cattle slaughtered in abattoirs within the Federal Capital Territory, Nigeria, serve as reservoirs of potentially pathogenic EHEC *E.*

coli O157:H7 carrying important virulence genes, underscoring the need for improved abattoir hygiene, routine molecular surveillance, and a coordinated One Health approach to reduce the risk of foodborne transmission and to protect public health.

Author Contributions

Shuaibu Aliyu Madugu: Conceptualization, methodology, investigation, data curation, formal analysis, and writing original draft preparation.

James Agbo Ameh: Supervision, methodology, project administration, and writing review and editing.

Samuel Mailafia: Supervision, methodology, project administration, and writing review and editing.

Hamza Olatunde K. Olabode: Supervision, methodology, project administration, and writing review and editing.

Godwin Onyemaechi Egwu: Supervision, resources, validation, and writing review and editing.

Victor Bitrus Shammah: Data analysis, visualization, interpretation of results, writing original draft preparation, and writing—review and editing.

All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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