

Antimicrobial Profiles and Conventional PCR Assay of Shiga Toxigenic *Escherichia coli* O157:H7 (STEC) Isolated from Cattle Slaughtered at Bedele Municipal Abattoir, South West Ethiopia

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Background: Shiga toxin producing *Escherichia coli* O157:H7 (STEC) is considered the most prevalent food borne pathogen that has gained increasing attention worldwide in recent years.

Methods: A cross-sectional study was carried out at Bedele Municipal abattoir on cattle that were reported healthy from detailed ante-mortem inspections and having various body conditions scores. A total of 516 samples were collected and examined after enriched in modified peptone water. Following an enrichment, the samples were plated onto MacConkey agar and then onto Eosin methylene blue agar. Finally after a few similar procedures, 14 *E. coli* O157:H7 (STEC) isolates were confirmed through latex agglutination test. The collected data were analyzed using SPSS version 20 statistical software.

Results: This study finding revealed that the overall prevalence of *E. coli* O157:H7 out of 516 samples was found to be 2.7%. However, on sample type basis, the prevalence of *E. coli* O157:H7 from fecal samples, carcass swabs, butcher hand swabs and knife swabs were 4.7%, 3.3%, 1.1% and 1.1%, respectively. It was also found that that the prevalence of *E. coli* O157:H7 was significantly affected by age groups of slaughtered cattle ($p < 0.05$). Moreover, in vitro antimicrobial susceptibility test result on average showed that almost all of *E. coli* O157:H7 isolates were highly susceptible to kanamycin and no resistance was shown to ciprofloxacin and gentamicin. Finally, the conventional PCR detection of *stx1*, *st2* and *hlyA* genes revealed that only 21.4% and 14.3% were found to contain *stx1* and *hlyA* genes respectively.

Conclusion: To wrap up, this study showed that Shiga toxin producing *E. coli* O157:H7 (STEC) isolates were found with almost low overall prevalence rate from all sample sources in this study site. Therefore, improving abattoir facilities and slaughter house workers' personal hygiene are recommended to curtail *E. coli* O157:H7 meat contamination in this abattoir.

Keywords: antimicrobial susceptibility test, cattle, conventional PCR analysis, personal hygiene

Introduction

Escherichia coli (*E. coli*) is a normal part of the intestinal micro-flora of many healthy animals including humans.¹ Cattle have been identified as a major reservoir of the organism.² Both pathogenic and non-pathogenic *E. coli* strains reside in the gastrointestinal tract of their hosts. The non-pathogenic *E. coli* strains provide various host benefits such as vitamin K and B₁₂ whereas the pathogenic strains such as Verocytotoxigenic *E. coli* (VTEC) and Shiga toxin-producing *E. coli* O157:H7 (STEC) can cause severe, chronic, and potentially fatal illness related to their ability to produce one or more toxins known as verotoxin or Shiga-like toxin respectively in humans.³ Likewise, pathogenic *E. coli* strains can rarely cause the disease known as calf scours in calves.⁴

Pathogenic STEC (*E. coli* O157:H7) produce one or more Shiga toxin (*Stx*) which consist of two groups designated *Stx1* (consisting of the three variants *Stx1a*, *Stx1c* and *Stx1d*) and *Stx2* (composed of seven distinct variants *Stx2a*, *Stx2b*, *Stx2c*, *Stx2d*, *Stx2e*, *Stx2f*, and *Stx2g*).⁵ There also Non-O157 STEC serogroups, in particular, O26, O103, O111, and O145 recognized for their pathogenic potential and constitute together with *E. coli* O157:H7 the so called “top five” serogroups of human pathogenic STEC.⁶

Shiga toxin-producing *E. coli* O157:H7 (STEC) can infect humans via various routes; however, a large proportion of infections and human outbreaks have occurred following the consumption of contaminated food products of animal origin, such as raw milk, uncooked or poorly cooked meats.^{7–9} In Ethiopia, carcass contaminations occurring from abattoir environments and, skin-to-carcass or fecal-to-carcass transfer of the pathogen during slaughter process at abattoirs^{10,11} and widespread consumption of raw or undercooked meat (locally known as *Kiffo* and *Kurt*) and other traditional practices are potential contributing factors of exposure to *E. coli* O157:H7 human infection.¹²

Antibiotic use in *E. coli* O157:H7 infection is controversial because of the potential to increase production and secretion of Shiga toxins, thus promoting the onset of Haemolytic Uremic Syndrome (HUS) in humans.¹³ However, early administration of antibiotics was found to not stimulate the release of Shiga toxin from O157 and non-O157:H7 strains in vitro.¹⁴

Unfortunately, inappropriate ways of antimicrobial uses have contributed to the increase in antimicrobial resistance.¹⁵ Antibiotic resistance in *E. coli* O157:H7 (STEC) has been increasingly noted over the last 20 years.¹⁶ A recent study revealed that a higher incidence rate of *E. coli* O157:H7 drug resistance was observed in different parts of Ethiopia.^{17,18}

In spite of the higher risk of *E. coli* O157:H7 infection and a rising claim of antimicrobial resistance of *E. coli* O157:H7, scanty data was available regarding the occurrence and antimicrobial resistance profile of *E. coli* O157:H7 isolated from cattle faeces, carcass swabs, butcher hand swabs, knives and tap water in the abattoir of present study site.^{19,20} Furthermore, there was shortage of scientific evidences about virulence genes (*stx1*, *stx2* and *hlyA*) of *E. coli* O157:H7 (STEC) isolated from contaminated sample sources in the abattoir.²¹ Thus, the aims of this study were to isolate and identify, determine antimicrobial resistance profile, and detection of toxic and virulence genes of *E. coli* O157:H7 isolates in the study area.

Methods and Materials

Description of Study Area

The study was carried out at Bedele Municipal abattoir located in Bedele town. Buno-Bedele (Bedele) is a town and separate woreda in south western Ethiopia. The town is located in the Buno Bedele zone Oromia regional state and it is located, 468km away from Addis Ababa (Finfinne). The district has a longitude and latitude of 8° 27'N and 36° 21'E and an elevation between 2012 and 2162 metres above sea level. Based on figures from the central statistical agency in 2018, Bedele town has an estimated total population of 21,289 of whom 10,556 are men 10,733 are women. The average rain falls of the district including the town is 1361mm, with an average temperature of 30.5°C. *Woina-dega* and *kola* agro-ecology are known climate type of Bedele district.²²

Study Populations

The sample sources for this study were slaughtered cattles' faeces, carcass swabs, tap water and, butcher hand and knife swabs. In this study, the cattle were brought by cattle traders from different livestock markets of south western parts of Ethiopia to Bedele livestock market and then to Bedele Municipal abattoir after being purchased by meat house owners of Bedele town. Thus, as these cattle were brought from the whole parts of south west region of Ethiopia, we can assume the collected samples could represent the region. The study involved cattle of different age groups categorized as young, adult and old for cattle having 2≤3 years of age, 3–6 years of age and ≥6 years of age respectively. However, as a result of cultural barriers (cultural taboo) to consume calf meat below two years in Ethiopia in general and in this study area in particular, they were not included in this study. Furthermore, body condition scores of cattle were determined after detailed visual physical examination and rare palpations. Then, they were grouped as poor, medium and good body conditions for cattle having body condition score of (1≤3), (4≤7) and (8≤9) respectively based on schemes described in.^{23,24} These cattle were registered during ante-mortem inspection and identified according to their identification number that was given by Bedele town municipality to the owners of the cattle to be slaughtered.

Moreover, before slaughter activities were carried out, the cattle were inspected for judging their health status through measuring normal parameters such as body temperature, respiratory rate, heart rate and pulse rate at rest in addition to inspecting them for manifestations of clinical signs of disease. And any cattle with abnormal health parameters and manifested any signs of disease condition were not included in this study.

Study Design and Sampling Method

Cross-sectional study was carried out during the present study. Furthermore, in order to select the individual sampling units, the systematic random sampling technique was adopted in which the identification codes of the individual cattle to be slaughtered were employed. In this sampling technique, the number of cattle available during a particular day (N) was divided by the desired sample size (n) to get the sampling interval (k), ie $k=N/n$.

Sample Size Determination

The minimum sample size required for this study was calculated according to the formula given by.²⁵ Based on the previously described *Escherichia coli* O157:H7 prevalence estimate of 8.33% at Jimma town municipal abattoir, the minimum sample size calculated was 300 (150 fecal and carcass swab samples separately). In addition, 216 samples (93 hand and knife swab samples were separately and purposefully collected besides 30 tap water samples used in the abattoir). Thus, the total number of samples collected and analyzed was 516 samples.

In formula,

$$n = \frac{Z^2 * P_{exp} * (1 - P_{exp})}{d^2}$$

Where, n-sample size, d^2 -absolute precision, P_{exp} -expected prevalence and Z-statistic for level of confidence.

Laboratory Protocols

Procedures of Sample Collections

Before collection of a sample, the study cattle was identified according to their identification codes and then the carcass swabs, feces, hand swabs, knife swabs and water samples were collected according to their identification codes separately in different sample collection facilities in order to avoid sample blending. For labeling purposes of sample collection facilities, cecal content and cattle meat swab from each cattle were differentiated by CC and CMS which represent cecal content and cattle meat swab respectively in addition to writing the identification number of the cattle nearby these codes and they were also written on laboratory result sheets respectively. And carcasses were labeled consecutively as the de-skinning processes were completed. Furthermore, acronyms such as HS, KS and W were used to represent hand swabs, knife swabs and water samples respectively during sample collection procedures.

Isolation and Confirmatory Test Protocols of *E. coli* O157:H7

Aseptically collected fecal pellets from the cecum of the slaughtered cattle, carcass swabs, butcher hand swabs, knife swabs and water samples were suspended into modified peptone water. About 50 μ L of the samples suspended into modified peptone water were streaked onto MacConkey agar. From the colonies grown on MacConkey agar, single colony with pink appearance was taken and cultured onto Eosin methylene blue agar. In the same manner, from the colonies with distinctive metallic sheen appearance grown on Eosin methylene blue agar, again single colony was taken and cultured on sorbitol MacConkey agar (Oxoid Ltd., Hampshire, UK), and after all of the above laboratory procedures, the plates were incubated at 37°C for 24 hours. Finally, colonies with a pale periphery or that appeared colorless were tested for indole production by conducting indole test. Indole positive isolates were cultured on nutrient agar for serological confirmation by latex agglutination.

The *E. coli* O157:H7 latex agglutination test was carried out to confirm this strain by applying standard test procedures (Oxoid, Hampshire, UK). And the result of the latex agglutination test was recorded as positive when agglutination of the latex particles occurred within 1 minute and negative when no agglutination happened after 60 seconds in the test area.

Conventional PCR Analysis Protocol

The conventional PCR analysis was carried out to detect the presence of the virulence genes (*stx1*, *stx2* and *hlyA*) in *E. coli* O157:H7 isolates, which were already confirmed by latex agglutination, by using the methods, described in these studies.^{26,27}

Data Management and Analysis

The data that were collected after laboratory analysis were entered into MS-Excel spreadsheet (Microsoft Corp., Redmond, USA). Then, data from MS-Excel were transported to SPSS 20 statistical software (SPSS Inc., Chicago, IL, USA). The descriptive statistics such as percentages and statistical associations between the suggested risk factors were computed using a Pearson chi-square test. And statistical differences were considered significant at $P < 0.05$.

Results

Overall *E.coli* O157:H7 Prevalence

From the total collected samples (516), the overall prevalence of *E. coli* O157:H7 was found to be 2.7%. However, with regard to sample types, the prevalence of *E. coli* O157:H7 isolated from bovine feces, carcass swabs, hand swabs and knife swabs were 4.7%, 3.3%, 1.1% and 1.1%, respectively. Furthermore, in the present study, it was observed that there was relatively higher prevalence of *E. coli* O157:H7 from fecal and carcass sample than hand swabs and knife swabs (Table 1).

Furthermore, during the present study, the effect of some risk factors such as sex, age and body condition score to see if they bring the statistical difference on the prevalence rate of *E. coli* O157:H7 was considered. The result of the study indicated that there was a statistically significant variation among different age groups of slaughtered cattles ($p < 0.05$) while statistically insignificant difference was observed among the cattle of different body conditions, sexes as well with respect to prevalence of *E. coli* O157:H7 ($p > 0.05$) (Table 2).

Antimicrobial Sensitivity Test

The present study findings regarding antimicrobial susceptibility patterns of ten selected antimicrobial agents revealed that most of the isolates from fecal samples were susceptible to the selected antimicrobial agents at varying degree. All of the isolates from fecal samples were sensitive to ciprofloxacin (100%) despite few isolates had shown varying resistance rates against some antimicrobials such as Ampicillin (57.1%), Streptomycin (57.1%), Tetracycline (28.6%) and Nalidixic acid (14.3%). Similarly, four and five *E. coli* O157:H7 that were isolated from carcass swab samples had also shown susceptibility towards Ciprofloxacin, and Kanamycin at the rates of 80% and 100% respectively even though three and one *E. coli* O157:H7 isolates had shown resistance against Ampicillin with a rate of 60% and Tetracycline with a rate of 20% (Table 3).

Moreover, *E. coli* O157:H7 isolates from hand and knife swabs had also shown resistance to some of the antimicrobials. 50% of the *E. coli* O157:H7 isolates from these swab samples had developed resistance to Ampicillin. Similarly, 100% of isolates from hand and knife swab samples were resistant to Nalidixic acid in contrast to the isolates from carcass swab samples in which none of the isolate was resistant to Nalidixic acid (Table 3).

Table 1 Prevalence of *Escherichia coli* O157:H7 from Different Types of Samples

Types of Samples	Number of Samples	<i>E.coli</i> (%)	<i>E.coli</i> O157:H7 (%)
Feecal sample	150	23(15.3%)	7(4.7%)
Carcass swab	150	17(11.3%)	5(3.3%)
Hand swabs	93	7(7.5%)	1(1.1%)
Knife swabs	93	4(4.3%)	1(1.1%)
Water samples	30	1(3.3%)	–
Total(Overall)	516	52(10.1%)	14(2.7%)

Table 2 Statistical Association of Prevalence of *E. coli* O157:H7 with Different Risk Factors

Risk Factors	Number	<i>E. coli</i>	<i>E. coli</i> O157:H7 (%)	Chi-Square (χ^2)	P-value
Sex					
Male	137	36(26.3%)	11(8%)	10.32	0.07
Female	13	4(30.8%)	1(7.7%)		
Age					
Young (2≤3 years)	6	3(50%)	1(16.7%)	7.16	0.002
Adult (3–6 years)	121	28(23.1%)	9(7.4%)		
Old (≥6 years)	23	9(39.1%)	2(8.7%)		
Body condition					
Thin	9	2(22.2%)	2(22.2%)	18.23	0.103
Medium	111	31(27.9%)	7(6.3%)		
Good	30	7 (23.3%)	3(10%)		

Table 3 Antimicrobial Sensitivity Patterns of Serologically Positive Isolates of *E. coli* O157:H7

Anti. Used	Types of Samples									Aver. Antimicrobial Sensitivity Patterns from All Sample Types		
	Feacal Samples(n=7)			Carcass Samples(n=5)			Hand and Knife Swab Sample(n=2)					
	S	I	R	S	I	R	S	I	R	S	I	R
	No. %	No.%	No.%	No.%	No.%	No.%	No.%	No.%	No.%			
AMP	1(14.3%)	2(28.6%)	4(57.1%)	0(0%)	2(40%)	3(60%)	0(0%)	0(0%)	1(50%)	4.80%	22.9%	55.7%
CEP	5(71.4%)	1(14.3%)	1(14.3%)	3(60%)	1(20%)	0(0%)	1(50%)	0(0%)	0(0%)	60.5%	11.4%	4.80%
CIP	7(100%)	0(0%)	0(0%)	4(80%)	1(20%)	0(0%)	0(0%)	0(0%)	0(0%)	60.0%	6.70%	0.00%
C	5(71.4%)	2(28.6%)	0(0%)	3(60%)	1(20%)	1(20%)	0(0%)	0(0%)	0(0%)	43.8%	16.2%	6.70%
GEN	4(57.1%)	3(42.9%)	0(0%)	2(40%)	3(60%)	0(0%)	1(50%)	0(0%)	0(0%)	52.0%	34.3%	0.00%
KAN	6(85.7%)	1(14.3%)	0(0%)	5(100%)	0(0%)	0(0%)	0(0%)	1(50%)	0(0%)	61.9%	21.4%	0.00%
NA	3(42.9%)	3(42.9%)	1(14.3%)	2(40%)	3(60%)	0(0%)	0(0%)	0(0%)	2(100%)	27.6%	34.3%	38.1%
SXT	5(71.4%)	1(14.3%)	1(14.3%)	4(80%)	1(20%)	0(0%)	2(100%)	0(0%)	0(0%)	83.8%	11.4%	4.8%
S	2(28.6%)	1(14.3%)	4(57.1%)	2(40%)	2(40%)	1(20%)	0(0%)	1(50%)	0(0%)	22.9%	34.8%	25.7%
TE	2(28.6%)	3(42.9%)	2(28.6%)	1(20%)	3(60%)	1(20%)	0(0%)	0(0%)	0(0%)	16.2%	34.3%	16.2%
Aver. (%)	57.14%	24.3%	18.6%	52%	34%	12%	20%	10%	15%			

Abbreviations: S, sensitive; I, intermediate; R, resistance Anti; used, Antimicrobials used; No, Number; Env't, Environment; Aver., Average; for abbreviated drug name, see the abbreviation page.

The susceptibility patterns of all *E. coli* O157:H7 isolates from feacal, carcass samples, and hand and knife swabs against ten selected antimicrobials indicated that few isolates were highly susceptible to Sulfamethoxazole-Trimethoprim whereas few isolates less susceptible to Gentamicin, Kanamycin and Ciprofloxacin (Figure 1).

Detection of Virulence Genes of *E. coli* O157:H7

Out of fourteen confirmed *E. coli* O157:H7 isolates, the conventional PCR analysis result revealed that only three isolates (21.4%) contained *stx1* genes as it was shown in Figure 2 whereas two isolates (14.3%) were shown to contain *hlyA* genes as it had been indicated in Figure 3 and none of these isolates was revealed to contain *stx2* genes.

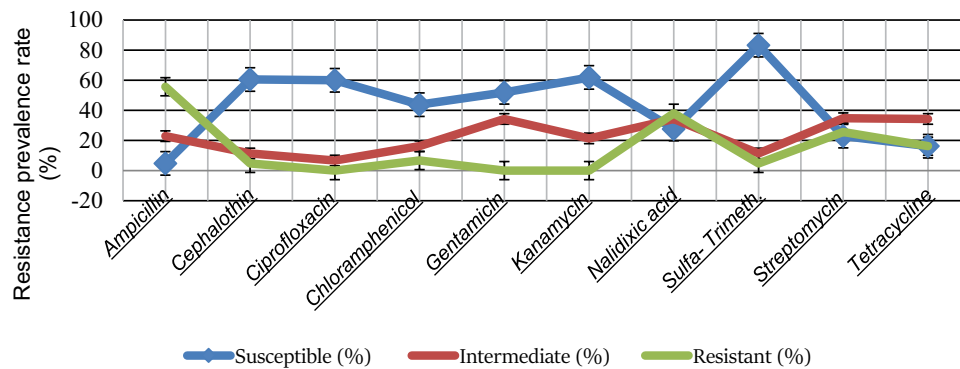


Figure 1 Bar-chart showing the susceptibility patterns of *E. coli* O157:H7 from different sample types.

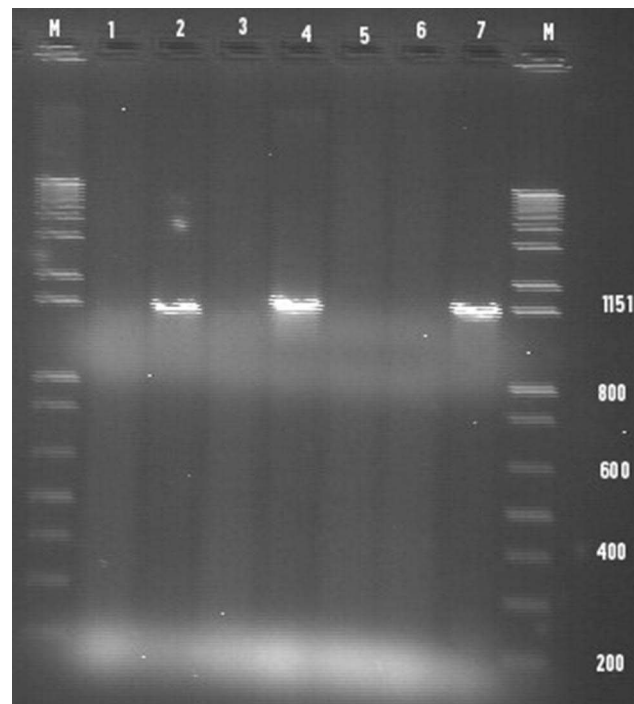


Figure 2 Conventional PCR products of *hlyA* gene with an amplicon size of 1151 bp run on the gel electrophoresis for *E. coli* O157:H7 isolates.
Notes: M = 100 bp DNA marker, lane=2 and 4 are positive result for *hlyA* gene, lane =6 and 7 negative and positive control for *hlyA* gene respectively.

Discussion

In the present study, it was revealed that the overall prevalence of *E. coli* O157: H7 from different types of samples (feaces, carcass swabs, water sample, hand swabs and knife swabs) which was 2.7% is more or less in line with the finding of Robi and Gelalcha²³ who reported 2.4% overall prevalence of *E. coli* O157: H7 from Hawassa. On the contrary, the overall prevalence of the present study was highly contradicted by the finding of Beyi et al¹¹ in which they reported an overall prevalence of 8.3%. This considerable variation could be attributed to the prolonged storage of the isolated *E. coli* samples with glycerol suspension at low temperature. The sample type based prevalence of *E. coli* O157: H7 from feaces also is in agreement with the findings from these studies^{28,29} from Hawassa and Canada respectively.

Similarly, higher prevalence rates of *E. coli* O157: H7 isolated from fecal samples were also reported in these studies^{30,31} from Jimma and Addis Ababa and they reported the prevalence rate of 10% and 6.4% respectively. Furthermore, Iweriebor et al³² from South Africa reported prevalence rate of 31.7% which is by far greater than the present report. The marked difference observed in prevalence rates between the present finding and those reported by

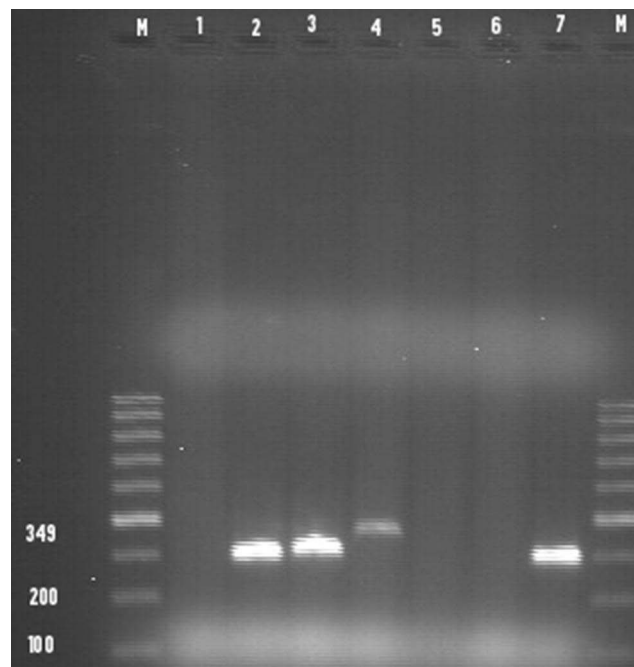


Figure 3 Conventional PCR products of *stxI* gene with an amplicon size of 349 bp run on gel electrophoresis for *E.coli* O157:H7 isolates.
Notes: M= 100 bp DNA marker, lane=2, 3 and 4 positive results for *stxI* gene, lane =6 and 7 negative and positive controls for *stxI* gene respectively.

various investigators might be related to variation in laboratory procedure applied, animal management system, sampling techniques and, geographical and climatic factors.²⁸

The prevalence rates of *E. coli* O157: H7 from carcass samples and, hand and knife swab samples were lower than that of the fecal samples. The possible explanation behind these findings could be the collected cecal contents (feces) from the cecum of slaughtered cattle were highly contaminated with *E. coli* O157: H7 since the recto-anal junction (RAJ) of cattle is the principal site of colonization for *E. coli* O157: H7 relative to other livestock species.^{33,34}

Regarding the prevalence of *E. coli* O157: H7 in association with risk factors such as age, sex and body condition scores, during the present study it was found that there was significant variation among different slaughtered cattle age groups (young 16.7%, adult 7.4% and old 8.7%) of the study cattle with respect to the prevalence of *E. coli* O157: H7 ($p < 0.05$). This finding is inline with the findings of Abreham et al²⁸ who reported the prevalence of *E. coli* O157: H7 can significantly vary in relation to the age of the cattle. However, the finding by Mekonnen et al³⁵ showed insignificant statistical difference ($p > 0.05$) between the age groups regarding the prevalence of *E. coli* O157: H7 which contradicts the present finding which could be related to the underdeveloped immune status of young cattle. On the other hand, during the present study the prevalence of *E. coli* O157: H7 was not significantly affected by the sexes and body condition scores of study cattle ($p > 0.05$). This finding is in agreement with the report of Abreham et al²⁸ from Hawassa even though it is argued by³⁵ in which they reported the presence of significant statistical association between body condition scores of slaughtered cattle and prevalence of *E. coli* O157: H7.

In the present study, the antimicrobial resistance patterns of *E. coli* O157:H7 isolates were mainly observed in drugs that have been in use in human and some of veterinary clinics for prolonged years for therapeutic and prophylactic purposes since during this long course they always look for ways to survive, perpetuate their generations and eventually develop resistance.³⁶ Hence, in this study, many *E. coli* O157:H7 isolates were found to have developed resistance against the older drugs such as Ampicillin, Nalidixic acid, Streptomycin and tetracycline with the resistance rates of 55.7%, 38.1%, 25.7% and 16.2% respectively. The study report by Messele et al³⁷ in Bishoftu and Addis Ababa supports the present finding. Similarly, on the basis of resistance rate of *E. coli* O157:H7 isolates against Ampicillin, tetracycline, Nalidixic acid and Streptomycin, the finding of Messele et al³⁷ in Addis Ababa and Bishoftu is consistent with the present report.

This study also pointed out that more than 50% of the isolates were sensitive to few rarely used antimicrobials in veterinary clinics such as Gentamicin and Ciprofloxacin with a sensitivity rate 52% and 60% respectively which is in agreement with the report by Natvig et al,³⁸ who reported the sensitivity rate of Gentamicin and Ciprofloxacin to be 81.0% and 74.7% respectively despite the presence variation in degree of sensitivity. Moreover, Dejene³⁹ reported that higher number of the *E. coli* O157:H7 isolates were susceptible to antimicrobials such as Ciprofloxacin (92.5%) and Gentamicin (59.26%). Likewise, Reuben and Owuna from Nigeria⁴⁰ Alam et al from Bangladesh⁴¹ and Hamid et al from Bishoftu³¹ have reported 89.5% and 78.9%; 50% and 66.67%, and 100% and 85.7% susceptibility of *E. coli* O157:H7 isolates to Gentamicin and Ciprofloxacin, respectively and all of these findings are consistent with the current report. However, there are findings which are in contradiction to the present finding. For instance, Mesele from Adami Tulu Jido Kombolcha⁴², and this study⁴³ from Italy reported 100% and 36.6% sensitivity rates respectively and these observed variations could be attributed to the difference in degree of utilization of these drugs in the mentioned localities.

The present study also shown that only 14.3% out of fourteen *E. coli* O157:H7 isolates were found to contain *hlyA* gene. Likewise, three isolates, 21.4% were found to contain only *stx1* genes. However, none of the isolates were found to contain *stx2* genes and this finding agrees with result of Tassew⁴⁴ who reported *stx2* genes were absent from the analyzed samples.

On the other hand, the study report⁴⁵ indicated that the probability of finding almost all of the virulence genes within a single isolate is low and this finding also supports the current result. Similar observations had been reported by Frydendahl⁴⁶ and Hornitzky et al⁴⁷ but the present finding contradicts the finding that all of virulence genes of *E. coli* O157:H7 exist together.^{48–50}

As to the limitations of this study, we have only focused only on *E. coli* O157:H7 (STEC) strain, but the work could be very complete and attractive if it also included other pathogenic but non *E. coli* O157:H7 serogroups causing illnesses in infected individuals.

Conclusions

In this study, *E. coli* O157:H7 was isolated from cecal content, carcass, hand and knife swab samples in almost low overall prevalence rate. However, this low prevalence can also pose various public health threats as their availability indicates degree of food contamination. On the other hand, statistical analysis result of this study showed that there is no significant statistical difference between sexes, body condition scores in contrast to the statistically significant association among the cattle age groups as to the prevalence of *E. coli* O157:H7. Moreover, the result of in vitro antimicrobial sensitivity test showed that few antimicrobials had inhibited the growth of *E. coli* O157:H7 isolates with varying quantity. However, some *E. coli* O157:H7 isolates developed resistance against antimicrobials such as Ampicillin, Nalidixic acid and streptomycin. Furthermore, the conventional PCR analysis of fourteen latex agglutination test positive isolates showed that only three isolates (21.4%) contained *stx1* genes while the other two isolates (14.3%) were found to contain *hlyA* genes. Thus, it is recommended that government should provide comprehensive and sustainable training regarding sanitary handling of meat to the workers of the abattoir particularly to the butchers to curtail meat contaminations in the abattoir.

Abbreviation

E. coli, *Escherichia coli*; WHO, World health organization; AMP, Ampicillin; CEP, Cephalotin; CIP, Ciprofloxacin; GEN, Gentamycin; KAN, Kanamycin; C, Chloramphenicol; NA, Nalidixic acid; SXT, Sulfamethoxazole trimethoprim; S, Streptomycin; TE, Tetracycline.

Research Animal Ethics Approval

Before starting sample collections, oral informed consent was obtained from meat shop owners who brought the cattle to the Bedele Municipal abattoir for slaughter after purchasing these cattle from local farmers. The ethical clearance certificate for this research was obtained from Wollega University, Research Animal Ethics and Welfare Committee (Reference RCITTVP035/01/03/2021).

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Author Contributions

All authors made a significant contribution to this research work, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

References

- Hajian S, Rahimi E, Mommtaz H, et al. A 3 year study of *Escherichia coli* O157: h7 in cattle, camel, sheep, goat, chicken and beef minced meat. *Int Conf Food Eng Biotechnol*. 2011;9:163–165.
- Battisti A, Lovari S, Franco A, et al. Prevalence of *Escherichia coli* O157 in lambs at slaughter in Rome, Central Italy. *Epidemiol Infect*. 2016;134:415–419. doi:10.1017/S0950268805005236
- Kolenda R, Burdukiewicz M, Schierack P, et al. A systematic review and meta-analysis of the epidemiology of pathogenic *Escherichia coli* of calves and the role of calves as reservoirs for human pathogenic *E. coli*. *Front Cell Infect Microbiol*. 2015;5:23. doi:10.3389/fcimb.2015.00023
- Spano L, Cunha K, Monfardini M, et al. High prevalence of diarrheagenic *Escherichia coli* carrying toxin-encoding genes isolated from children and adults in south eastern Brazil. *BMC Infect Dis*. 2017;17:773. doi:10.1186/s12879-017-2872-0
- Fuller C, Pellino C, Flagler J, et al. Shiga toxin subtypes display dramatic differences in potency. *Infect Immun*. 2011;79:1329–1337. doi:10.1128/IAI.01182-10
- European Food Safety Authority (EFSA). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks. *EFSA J*. 2015;14:4634.
- Brichta-Harhay D, Guerini MN, Arthur TM. Salmonella and *Escherichia coli* O157:H7 contamination on hides and carcasses of cull cattle presented for slaughter in the United States: an evaluation of prevalence and bacterial loads by immunomagnetic separation and direct plating methods. *Appl Environ Microbiol*. 2008;74(20):6289–6297. doi:10.1128/AEM.00700-08
- Mohammed O, Shimelis D, Admasu P, et al. Prevalence and antimicrobial susceptibility pattern of *E. coli* isolates from raw meat samples obtained from abattoirs in Dire Dawa City, eastern Ethiopia. *Int J Microbiol Res*. 2014;5:35–39.
- Mesele F, Abunna F. *Escherichia coli* O157: H7 in foods of animal origin and its food safety implications: review. *Adv Biol Res*. 2019;13(4):134–145.
- Arthur T, Brichta-Harhay DM, Bosilevac JM. Super shedding of *Escherichia coli* O157:H7 by cattle and the impact on beef carcass contamination. *Meat Sci*. 2017;86(1):32–37. doi:10.1016/j.meatsci.2010.04.019
- Beyi A, Fite A, Tora E, et al. Prevalence and antimicrobial susceptibility of *Escherichia coli* O157 in beef at butcher shops and restaurants in central Ethiopia. *BMC Microbiol*. 2017;17:49. doi:10.1186/s12866-017-0964-z
- Aklilu F, Daniel K, Ashenafi K, et al. Prevalence and antibiogram of *Escherichia coli* O157 isolated from bovine in Jimma, Ethiopia: abattoir based survey; Ethiopia. *J Vet. sci*. 2017;21:109–120.
- Colello R, Etcheverri A, Di Conza J, et al. Antibiotic resistance and integrons in Shiga toxin-producing *Escherichia coli* (STEC). *Braz J Microbiol*. 2015;46:1–5. doi:10.1590/S1517-838246120130698
- Ochoa T, Chen J, Walker C, et al. Rifaximin does not induce toxin production or phage-mediated-lysis of Shiga toxin-producing *Escherichia coli*. *Antimicrob Agents Chemother*. 2013;51(8):2837–2841. doi:10.1128/AAC.01397-06
- Wong C, Jelacic S, Habeeb R, et al. Risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157: H7 infections. *N Engl J Med*. 2000;342(26):1930–1936. doi:10.1056/NEJM200006293422601
- Tadesse D, Zhao S, Tong E, et al. Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950–2002. *Emerg Infect Dis*. 2015;18(5):741–749. doi:10.3201/eid1805.111153
- Bedasa S, Shiferaw D, Abraha A, et al. Occurrence and antimicrobial susceptibility profile of *Escherichia coli* O157: H7 from food of animal origin in Bishoftu town, Central Ethiopia. *Int J Food Contamination*. 2018;5. doi:10.1186/s40550-018-0064-3
- Sebsibe M, Asfaw E. Occurrence of multi-drug resistant *Escherichia coli* and *Escherichia coli* O157: H7 in meat and swab samples of various contact surfaces at abattoir and butcher shops in Jimma town, Southwest district of Ethiopia. *Infect Drug Resist*. 2020;13:3853–3862. doi:10.2147/IDR.S277890
- Abdissa R, Haile W, Feyisa A, et al. Prevalence of *E. coli* O157: H7 in beef cattle at slaughter and beef carcass at retailer shop in Ethiopia. *BMC Infect Dis*. 2017;17:277. doi:10.1186/s12879-017-2372-2
- Atnafie B, Paulos D, Abera M, et al. Occurrence of *E. coli* O157: H7 in cattle feces and carcass contamination in abattoir and butcher shops, Hawassa, Ethiopia. *BMC Microbiol*. 2017;17:18–24.
- Tassew A. *Isolation, Identification, Antimicrobial Profile and Molecular Characterization of E. coli O157: H7 at Debrezeit Elfora Export Abattoir and Addis Ababa Abattoirs Enterprise, Ethiopia* [MSc dissertation]; 2015.
- BDAO. *Bedele District Agricultural Office Report*. Bedele, Ethiopia: BDAO; 2018.

23. Robi D, Gelalcha B. Epidemiological classification of brucellosis in breeding female cattle under traditional breeding system of Jimma zone in Ethiopia. *Vet Anim Sci.* 2020;9. doi:10.1016/j.vas.2020.100117
24. Patrick A, Richard J. *Summarized Body Condition Scoring of Cattle, Western Beef Resource Committee.* Fourth ed. University of Idaho; 2020.
25. Thrusfield M. *Veterinary Epidemiology.* 3rd ed. Oxford, UK: Blackwell Science Ltd; 2005.
26. Mora A, Blanco J, Blanco M, et al. Antimicrobial resistance of Shiga toxin (verotoxin)-producing *Escherichia coli* O157: H7 and non-O157 strains isolated from humans, cattle, sheep and food in Spain. *J Res Microbiol.* 2015;156:793–806.
27. Inat G, Siriken B. Detection of *Escherichia coli* O157 and *Escherichia coli* O157: H7 by the immunomagnetic separation technique and *stx1* and *stx2* genes by multiplex PCR in slaughtered cattle in Samsun Province. *Turkey J Vet Sci.* 2010;11:321–326. doi:10.4142/jvs.2010.11.4.321
28. Abreham S, Teklu A, Cox E, et al. *Escherichia coli* O157: H7: distribution, molecular characterization, antimicrobial resistance patterns and source of contamination of sheep and goat carcasses at an export abattoir, Mojo, Ethiopia. *BMC Microbiol.* 2019;19:215. doi:10.1186/s12866-019-1590-8
29. Abayneh M, Asfaw E. Occurrence of multi-drug resistant *E. coli* O157: H7 in meat and swab samples at abattoir and butcher shops in Jimma town, Ethiopia. *BMC Infect Dis.* 2014. doi:10.21203/rs.3.rs-33376/v1
30. Ben A, Amir F, Ann M, et al. A risk assessment model for *E. coli* O157: H7 in ground beef and beef cuts in Canada: evaluating the effects of interventions. *La Tunisie medicale.* 2014;92:284–285.
31. Hamid M, Tefera T, Eguale T, et al. *E. coli* O157: H7: prevalence, Identification and antimicrobial resistance at Addis Ababa Municipal abattoir, Ethiopia. *Int J Adv Res Biol Sci.* 2018;10:136–146.
32. Iweriebor B, Iwu C, Obi L, et al. Multiple antibiotic resistance among Shiga toxin producing *E. coli* O157: H7 in faeces of dairy cattle farms in Eastern Cape of South Africa, Iweriebor. *BMC Microbiol.* 2015;15:195–213. doi:10.1186/s12866-015-0553-y
33. Naylor S, Low J, Besser T, et al. Lymphoid follicle dense mucosa at the terminal rectum is the principal site of colonization of Enterohemorrhagic *Escherichia coli* O157: H7 in the bovine host. *J Infect Immun.* 2003;71:1505–1512. doi:10.1128/IAI.71.3.1505-1512.2003
34. Sheng H, Lim J, Knecht H, et al. Role of *Escherichia coli* O157: H7 Virulence factors in colonization at the Bovine Terminal Rectal Mucosa, University of Idaho, Department of Molecular Biology, and Biochemistry, Moscow, Idaho. *Infect Immun.* 2016;74:83844.
35. Mekonnen H, Habtamu T, Kelali A, et al. Food safety knowledge and practices of abattoir and butchery shops and the microbial profile of meat in Mekelle City, Ethiopia. *Asian Pac J Trop Biomed.* 2013;3(5):407–412. doi:10.1016/S2221-1691(13)60085-4
36. Tadesse D, Zhaos S, Tong E, et al. Antimicrobial drug resistance in *Escherichia coli* from human and food animals, United States. *J Emerg Infect Dis.* 2012;2012:1950–2002.
37. Messele Y, Abdi R, Yalew S, et al. Molecular determination of antimicrobial resistance in *Escherichia coli* isolated from raw meat in Addis Ababa and Bishoftu, Ethiopia. *Ann Clin Microbiol Antimicrob.* 2017;16(55). doi:10.1186/s12941-017-0233-x
38. Natvig E, Ingham S, Ingham B, et al. *Salmonella enteric* serovar *Typhimurium* and *Escherichia coli* contamination of root and leaf vegetables grown in soils with incorporated bovine manure. *Appl Environ Microbiol.* 2012;68:2737–2744. doi:10.1128/AEM.68.6.2737-2744.2002
39. Dejene H. *Epidemiology and Assessment of Critical Control Points of E. coli O157: H7 along Dairy Supply Chains in Central Ethiopia College of Veterinary Medicine and Agriculture, Department of Clinical studies [MSc Thesis]; 2018.*
40. Reuben R, Owuna G. Antimicrobial resistance patterns of *Escherichia coli* O157: H7 from Nigerian fermented milk samples in Nasarawa state, Nigeria. *Int J Pharma Sci Invent.* 2014;2:38–44.
41. Alam M, Akther S, Sarwar N, et al. Prevalence and antimicrobial susceptibility of *E. coli* O157 isolated from raw milk marketed in Chittagong, Bangladesh. *Turk J Agric.* 2017;5:214–220. doi:10.24925/turjaf.v5i3.214-220.976
42. Mesele F. *Occurrence of Escherichia Coli O157: H7 in Lactating Cows and Dairy Farm Environment and Its Antimicrobial Susceptibility Pattern at Adami Tulu Jido Kombolcha District, Mid Rift Valley, Ethiopia [MSc dissertation]; 2018.*
43. Ahemed A, Shiloach Y, Robbins J, et al. Safety and immunogenicity of *Escherichia coli* O157 O-specific polysaccharide conjugate vaccine in 2–5-year-old children. *J Infect Dis.* 2005;193:515–521. doi:10.1086/499821
44. Tassew A. *Isolation, Identification, Antimicrobial Profile and Molecular Characterization of E. coli O157: H7 at Debrezeit Elfora Export Abattoir and Addis Ababa Abattoirs Enterprise, Ethiopia [MSc dissertation]; 2015.*
45. Sharaf E, Shabana I. Prevalence and molecular characterization of Shiga toxin-producing *Escherichia coli* isolates from human and sheep in Al-Madinah Al-Munawarah. *AlMunawarah Infect.* 2016;21. doi:10.22354/in.v21i2.651
46. Frydendahl K. Prevalence of sero-group and virulence genes in *E. coli* associated with post weaning diarrhea and oedema disease in pigs and comparison of diagnostic approaches. *Vet Microbiol.* 2013;85:169–182. doi:10.1016/S0378-1135(01)00504-1
47. Hornitzky M, Walker B, Bettelheim K, et al. Virulence properties and serotypes of Shiga toxin producing *Escherichia coli* from healthy Australian cattle. *Appl Environ Microbiol.* 2015;68:6439–6445. doi:10.1128/AEM.68.12.6439-6445.2002
48. Pradel N, Livrelli V, DeChamps C, et al. Prevalence and characterization of Shiga toxin producing *Escherichia coli* isolates from cattle, food and children during a one-year prospective study in France. *J Clin Microbiol.* 2010;38:1023–1031. doi:10.1128/JCM.38.3.1023-1031.2000
49. Johnsen G, Yngvild W, Heir E, et al. *Escherichia coli* O157: H7 in faeces from cattle, sheep and pigs in the southwest part of Norway during 1998 and 1999. *Int J Food Microbiol.* 2011;65:193–200. doi:10.1016/S0168-1605(00)00518-3
50. Omisakin F, Macrae M, Ogden I, et al. Concentration and prevalence of *Escherichia coli* O157 in cattle faeces at slaughter. *Environ Microbiol.* 2003;69:2444–2447. doi:10.1128/AEM.69.5.2444-2447.2003

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