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Isolation and antibiogram of *Escherichia coli* O157: H7 from diarrhoeic calves in urban and peri-urban dairy farms of Hawassa town

Haben Fesseha | Mesfin Mathewos | Saliman Aliye | Endale Mekonnen

School of Veterinary Medicine, Wolaita Sodo University, Wolaita Sodo, Ethiopia

Correspondence
Haben Fesseha, School of Veterinary Medicine, Wolaita Sodo University, P.O. Box 138, Wolaita Sodo, Ethiopia.
Email: haben.senbetu@wsu.edu.et

Abstract

Background: Calf diarrhoea is the most serious issue in the livestock industry, resulting in significant financial losses.

Methods: A study was undertaken in 32 urban and peri-urban dairy farms of Hawassa town to isolate *E. coli* from diarrhoeic calves, assess associated putative factors related to the occurrence, and the evaluate antibacterial susceptibility patterns of isolates. A convenience sampling technique was performed for the selection of these dairy farms and calf samples. A total of 68 faecal samples were collected directly from the rectum of diarrhoeic calves. The faecal samples were confirmed as *E. coli* O157: H7 positive using the latex agglutination test.

Results: In this study, 47(69.1%) samples were positive for *E. coli*, of which 22 (46.8%) were identified as *E. coli* O157:H7 strains based on their latex agglutination character. Factors such as frequency of calf house cleaning, type of supplement provided, and method of colostrum feeding were significantly correlated ($p < 0.05$) with calf diarrhoea, while the other risk factors had no significant association. Antibiogram of *E. coli* O157:H7 isolates showed that the isolates were highly sensitive to gentamycin, ceftriaxone, trimethoprim-sulphamethoxazole, and ciprofloxacin and were found to be resistant to tetracycline, kanamycin and amoxicillin.

Conclusion: Our findings revealed that calf diarrhoea is still a major health problem of calves in the study area. Hence, improved calf and farm management practice, an ad libitum quantity of colostrum, and good farm hygienic practices should be ensured. This study also revealed that some antibiotic-resistant *E. coli* O157:H7 isolates need to be further investigated for their public health implications.

KEYWORDS
antibiogram sensitivity, calf diarrhoea, *E. coli* O157: H7, isolation, risk factors

1 | INTRODUCTION

Ethiopia is the largest livestock resourceful country on the African continent, with approximately 57.8 million cattle, 29.33 million sheep and 29.11 million goats (Central Statistical Agency, 2015). In addition, this sector contributes 16.5% of the national Gross Domestic Product (GDP) and 35.6% of the agricultural GDP (Behnke, 2010; Metaferia et al., 2011). Dairying is a form of livestock production that is practiced almost everywhere in the world, including Ethiopia, and involves a large number of small-, medium- or large-scale, subsistence or market-oriented farms, with herd size being the primary determinant. It is the primary source of income for dairy farmers in Ethiopia's urban and peri-urban settlements (Chagunda et al., 2006; Hadgu et al., 2021).

Newly born calves are a significant source of livestock production for beef and breeding worldwide. However, calves have encountered many complications, such as diarrhoea, pneumonia, joint disorders, umbilical infections, trauma and congenital abnormalities (Heinrichs & Radostits, 2001). Calf morbidity and mortality were ranked next to mastitis as the second largest problem for dairy production in Ethiopia (ILCA, 1994). The mean annual birth-to-weaning mortality in calves was reported to be in the range of 2–18.5% in the mixed crop-livestock system in different parts of Ethiopia (Fentie et al., 2016; Fentie et al., 2020; Hadgu et al., 2021). Non-infectious causes such as trauma to the different parts of the gastrointestinal tract (hardware disease due to metallic foreign bodies, oesophageal and bowel obstruction such as intussusception and volvulus due to various types of foreign bodies), metabolism (simple indigestion, ruminal acidosis and alkalosis) (Fubini & Divers, 2008), malnutrition, other non-specific or miscellaneous causes, and poor husbandry practices are responsible for calf diarrhoea as well as calf mortality and morbidity in general in different parts of Ethiopia (Hadgu et al., 2021; Romha, 2014).

One of the most common causes of calf mortality and morbidity in the dairy industry is neonatal calf diarrhoea or scour (Fentie et al., 2020; Hadgu et al., 2021; Tajik et al., 2012). Calf diarrhoea is caused by a variety of infectious (bacteria, viruses and parasites) and non-infectious agents (poor quality and quantity of colostrum, environmental stress due to extreme weather and poor husbandry) (Cho & Yoon, 2014), especially in calves under the age of 12 days (El-Seedy et al., 2016). Some of these infectious agents have been linked to food-borne disease zoonosis in humans (El Ayis et al., 2015). Bovine rotavirus (BoRV), *Cryptosporidium parvum*, Bovine coronavirus (BoCV), Salmonella, and *E. coli* are some of the most common enteropathogens that cause calf diarrhoea (Meganck et al., 2015). Although *E. coli* is a minor pathogen in most studies of developed countries, such as the United States and Australia, in Ethiopia, it is among the major pathogens in most dairy farms due to poor hygienic conditions and the handling of feeding utensils, calving areas and calf pens (Gebregiorgis & Tessema, 2016; Kidane, 2014; Mohammed et al., 2020; Yeshiwas & Fentahun, 2017).

E. coli is a cause of calf diarrhoea, also known as white scour (Kolenda et al., 2015), and causes diarrhoea, haemorrhagic colitis, and dysentery in weak, malnourished, debilitated and immunosuppressed calves, particularly calves that do not receive maternal antibodies through colostrum feeding (Ellaithi, 2004; Mailk et al., 2013; Mohamed, 2009). The incidence of diarrhoea in calves under 30 days of age varies between 10% and 20%. Calf diarrhoea has a detrimental effect on calves' health, herd survival and production, resulting in substantial financial losses (Bazeley, 2003).

Treatment costs, time spent on medication and resulting chronic illness, thrift and reduced growth efficiency, loss of genetic capacity both from the loss of the calf and the farmer's unwillingness to invest in higher priced semen in the face of a calf mortality crisis and impair adequate heifer replacement are all factors that contribute to economic losses (Bazeley, 2003). Heifer substitution has a significant impact on dairy farmers' ability to maximise productivity by enabling them to cull low-producing cows selectively. Calf mortality is a major economic problem for all dairy farmers, especially those who farm intensively (Moran, 2011).

E. coli is a Gram-negative, rod-shaped, flagellated, non-sporulating and facultative anaerobic bacterium of the family Enterobacteriaceae. It is the most genetically versatile bacteria and supplies both plasmid and phage-mediated genes. *E. coli* produces septicaemia and diarrhoea in a wide range of hosts including humans and animals (Hemashenpagam et al., 2009). Diarrhoeagenic *E. coli* strains are classified into six main pathotypes based on their distinct virulence determinants (virulence mechanisms), namely, enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohaemorrhagic *E. coli* (EHEC)/Shiga toxin-producing *E. coli* (STEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffusively adherent *E. coli* (DAEC) (Xia et al., 2010). Among the six major diarrhoeagenic *E. coli* pathotypes, ETEC is the most common cause of diarrhoea in dairy and beef calves in the first few days of life. The other diarrhoeagenic classes, EHEC and EPEC, are important causes of disease in humans (Bartels et al., 2010; Kolenda et al., 2015; Moxley & Smith, 2010).

Escherichia coli O157: H7 is the most important bacterial pathogen that causes life-threatening infections such as haemorrhagic colitis (HC), abdominal pain, bloody diarrhoea, haemolytic uremic syndrome and kidney failure, particularly in humans worldwide (Mersha et al., 2010; Pal et al., 2016). Milk and other dairy products are mostly contaminated with *E. coli* O157: H7 during direct exposure to faeces due to poor handling systems and cause intestinal or extra-intestinal disease (Bacon et al., 2000; Bélanger et al., 2011). The high prevalence of *E. coli* O157: H7 in dairy products may be due to improper milking hygiene, poor house hygiene, lack of post milking teat dipping and practicing of milk by contact labour use of lubricants, and absence of order in milking cows of different ages. Moreover, its occurrence was high in dairy farms without noticeable farm treatment (Radostits et al., 2016).

Antibiotic-resistant strains of this bacterium cause longer and more serious diseases in susceptible animals. Numerous studies have shown that the impact of *E. coli* on antibiotic resistance increases over time for various antimicrobials (Cortés et al., 2010; Orden et al., 2000; Tadesse et al., 2012). Inappropriate or irrational use of antimicrobial drugs against diarrhoeal infection in humans and animal has presumptively assumed possible causes of antibiotic resistance. This may pose a

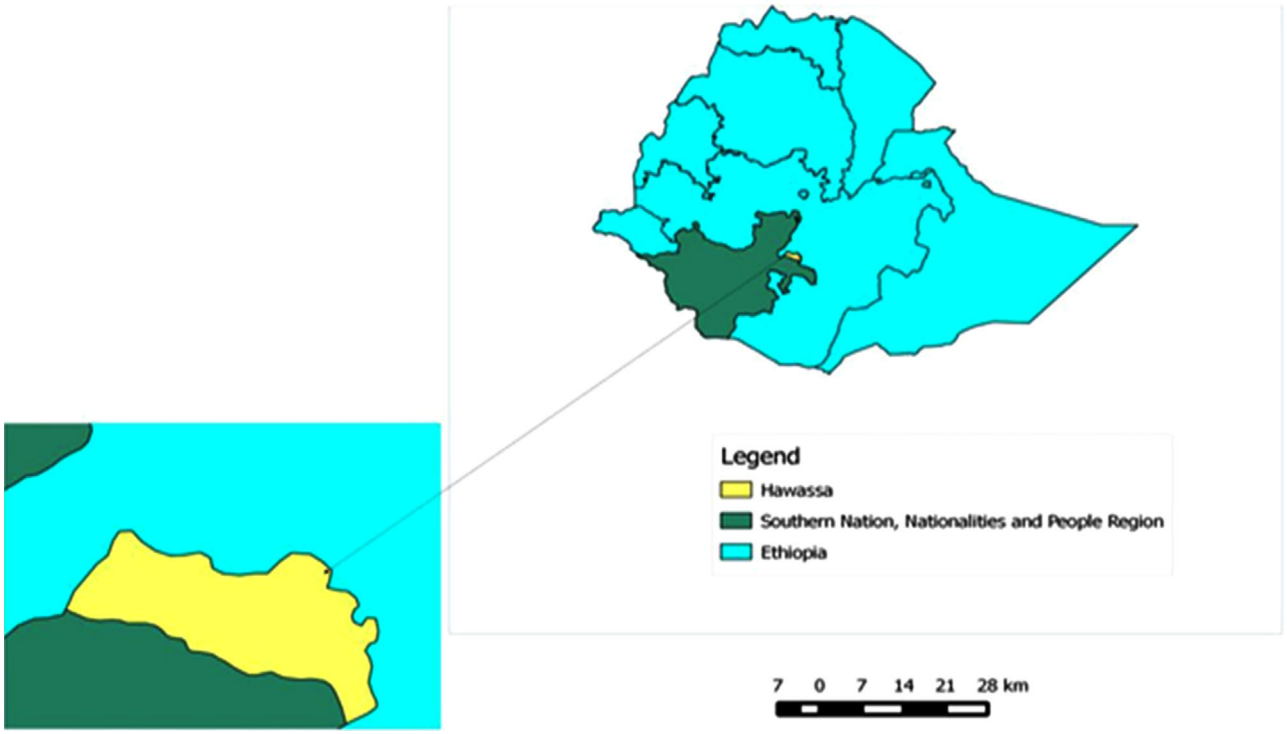

FIGURE 1 Geographical location of Hawassa, ArcGIS software, 2018

possible health danger to humans and animals with regard to the growth of resistant bacterial strains and drug residues. With certain scientific expertise and antibiotic profiles of various diarrhoeal bacterial isolates, effective therapeutic steps for managing diarrhoea of calves have to be regularly studied (Kaura et al., 1988).

In Ethiopia, particularly in Hawassa city, the isolation, identification of *E. coli* from diarrhoeic calves and evaluation of antibacterial sensitivity patterns of *E. coli* have not been widely studied. As a result, the study aimed to isolate *E. coli*, assess antibiotic susceptibility patterns and determine the factors that contribute to the occurrence of *E. coli* from a diarrhoeic calf in Hawassa City's urban and peri-urban areas.

2 | METHODS

2.1 | Study area

The current study took place at selected dairy cattle farms in Hawassa town from November 2017 to March 2018. It is approximately 275 km south of Addis Ababa. Hawassa is located at an elevation of 1750 m above sea level and is roughly located between 6°83′ to 7°17′ N and 38°24′ to 38°72′ E. Hawassa has a total area of around 50 km^2 and is divided into eight sub-cities and 32 kebeles (kebeles are the smallest administrative unit below the sub-city/woreda level). It receives an average annual rainfall of 955 mm and has an average annual temperature of 20°C (Fesseha et al., 2020) (Figure 1).

2.2 | Study animals and sampling technique

The study animals were local and Holstein Friesian cross-breed calves of both sexes up to 3 months of age that were kept under different management systems (extensive, semi-intensive and intensive) and clinically affected with diarrhoea and exhibited indicators of systemic disease (e.g., low appetite, fever, dehydration, weakness and impaired suckling reflex) and excreted different types of diarrhoea having diverse colours. The faecal consistency or type of diarrhoea was classified into watery (presence of profuse water-logged faecal particles), bloody (faeces with blood or blood clots), mucoid (presence of viscous mucous within the faecal) and mixed (presence of blood or particles of undigested food, blood clots or pieces of intestinal tissue) (Graham et al., 2018; Renaud et al., 2020).

During the study, 32 dairy farms were selected using convenience sampling from total dairy farms in the study areas on the basis of accessibility and willingness of the farm owners to participate in the study and grouped into smallholder (≤5 heads of milk cow), medium-sized farms (6–50 heads of milk cow) and large-scale (>50 heads of milk cattle) farms depending on the number of cows available in the selected farms (Lema et al., 2001). In addition, 15 small, 15 medium and 2 large-scale dairy farms located in urban and peri-urban areas were involved in the study. The study farms practice semi-intensive and intensive management systems. The ages of diarrhoeic calves were categorised into five age groups: 1–7 days, 8–15 days, 16–30 days, 31–60 days and 61–90 days according to Gebregiorgis and Tessema (2016). Different factors related to the onset of diarrhoea, such as floor type, the

practice of having a separate pen and colostrum feeding time and its duration, were recorded before samples were collected.

2.3 | Study design and sample size determination

A cross-sectional study design was employed from October 2019 to May 2020 in large-, medium- and small-scale dairy farms in Hawassa city and its surrounding farms. Non-probability convenience and purposive sampling were used for the selection of farms and faecal samples from each diarrhoeic calf. Thus, a total of 68 diarrhoeic calves (aged between 1 and 90 days) were sampled based on convenience sampling. In addition, factors such as the proportion of the calf population on each farm, case availability and the willingness of the farm owners were considered during the study.

The health status of each calf was evaluated through a detailed clinical examination using different types of clinical signs and parameters. Calves that showed poor appetite, rough hair coat, fever, dehydration, sunken eye, reduced suckling reflex, non-treated, weakness and abnormal faecal consistency (diarrhoea) were considered for the present study.

2.4 | Study methodology

2.4.1 | Questionnaire survey

The dairy owners were given a semi-structured questionnaire to evaluate the overall husbandry of calves via face-to-face discussions. Generally, the questionnaire contains the following sorts of diarrhoea: calf health, hygiene, health issues, colostrum feeding times and periods. In addition to the surveys, housing, farm hygiene and barn floor direct observational evaluations were carried out. Housing hygiene was graded from 1 to 4: 1 – very clean, 2 – clean, 3 – poor and 4 – very poor according to the previous work of Yibrah and Tsega (2017).

2.4.2 | Sample collection and processing

Faecal samples were collected from both clinical and subclinical (mild) cases during farm visits based on the findings of clinical signs and parameters. Shortly after the onset of diarrhoea, approximately 32 g of faecal samples were collected straight from the rectum of a non-treated diarrhoeic calf by using a disposable latex glove. During the farm visit and an emergency call by the farm owners, faecal specimens were gathered. Faecal samples were placed into sterile wide-mouth screw-capped bottles, and the samples were cooled in an icebox containing ice packs and transported to the microbiology laboratory for sample processing. Until processing time, faeces were kept at 4°C. During sampling, the farm husbandry practices evaluated, including identification number, sampling date, age, breed, sex, farm type, faecal consistency and farm housing (separate housing, floor type, cleaning and disinfection), provision of supplement feedstuffs to calves, colostrum feeding and history of diarrhoea, were documented in recording format.

2.4.3 | Isolation and identification of *E. coli* from diarrhoeic calves

Bacteriological culturing and examination: Suspicious colonies were further subcultured in nutrient media (HiMedia, India) and aerobically incubated for 24–48 h at 37°C. Pure colonies were sub-cultured on MacConkey agar for 24–48 h at 37°C following a morphological evaluation with Gram staining features. In the isolation and identification of *E. coli*, growth on MacConkey agar was used as the primary criterion. In addition, MacConkey agar colonial features were employed to divide putatively isolated bacteria into two groups: lactose fermenter and non-lactose fermenter. *E. coli* colonies suspected of further sub-cultured on agar media with Eosin methyl blue (EMB) for selective identification of *E. coli*. The colonies that appeared green-black with a metallic sheen, which differentiates *E. coli* on EMB, were chosen and kept on nutrient agar for additional biochemical analyses after 24 h.

Primary identification of bacterial isolates: Under the oil immersion objective, all of the isolates were stained with Gram stain to detect cell shape, Gram response, and purity (100× magnification). Primary biochemical tests were also conducted via, catalase test, triple sugar iron (TSI) test, potassium hydroxide (KOH), sulphur indole motility (SIM) test and oxidation-fermentation (O-F) test. Standard bacteriological procedures were used to identify suspected *E. coli* colonies and purified *E. coli* cultures were kept in nutrient broth for subsequent identification using biochemical testing as described in Quinn et al. (2002).

Secondary identification of bacterial isolates: Indole, methyl red (MR), Voges-Proskauer (VP) and citrate utilisation biochemical assays were used to identify *E. coli* isolates preliminarily after overnight incubation at 37°C on each of the four tests (Quinn et al., 2002). On the other hand, *E. coli* isolates were further cultured on Sorbitol MacConkey agar for 24–48 h at 37°C to identify pathogenic and non-pathogenic *E. coli* strains. Lactose is replaced by sorbitol in sorbitol MacConkey agar. *E. coli* bacteria that are not pathogenic ferment sorbitol to generate acid. Because pathogenic *E. coli* cells are unable to ferment sorbitol, this strain grows on peptone. This raises the medium's pH, allowing the pathogenic strain to be distinguished from other *E. coli* strains using the medium's pH indicator (Novicki et al., 2000).

Screening test by *E. coli* O157 Latex agglutination test: For the screening of *E. coli* O157:H7, a latex agglutination test was used with a latex kit. Sorbitol-negative (clear) colonies with colony morphology similar to *E. coli* O157:H7 were selected and spread plated on Cefixime tellurite sorbitol MacConkey plates (CT-SMAC). After a 24-h incubation period, a single colony of the non-sorbitol fermenter was selected from sorbitol MacConkey agar and treated with latex agglutination using an *E. coli* O157 latex kit. For all latex agglutination tests, an

isolate suspected of being *E. coli* O157:H7 was cultivated on nutrient agar (NA) for antibiotic susceptibility testing.

2.5 | Antibiotic susceptibility tests

Antibiotic susceptibility profiles were conducted for *E. coli* O157:H7 isolates using the disc diffusion method (Kirby–Bauer method) on Mueller–Hinton agar (Oxoid, England) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2012, 2014). Pure colonies on nutrient agar were taken with a wire loop and transferred to a tube containing 5 ml of saline water and emulsified. The broth culture was incubated at 37°C for 4 h until it achieved the 0.5 McFarland turbidity standards. A sterile cotton swab was dipped into the suspension and the bacteria were swabbed uniformly over the surface of a Muller–Hinton agar plate within a sterile safety cabinet. The plates were held at room temperature for 15 min to allow drying. Antibiotic discs with a known concentration of antibiotics were placed and the plates were incubated for 18–24 h at 37°C.

Each isolate was tested for a series of 10 antibiotic discs (Oxoid, England), namely, amoxicillin (AML; 25 μg), amikacin (AK; 30 μg), ceftriaxone (CRO; 5 μg), chloramphenicol (C; 30 μg), gentamycin (CN; 10 μg), streptomycin (S; 10 μg), kanamycin (K; 30 μg), ciprofloxacin (CIP; 5 μg), tetracycline (TE; 30 μg) and trimethoprim-sulphamethoxazole (SXT; 25 μg). A ring of discs (Oxoid, England) containing single concentrations of each antibiotic agent was then placed onto the inoculated surface using sterile forceps, and gently pressed with the point of the forceps to ensure complete contact with the agar surface. The discs were placed no greater than 24 mm (centre to centre), and the plates were then inverted. After 18–24 h of incubation at 35°C ± 2°C, aerobically, clear zones produced by antibiotic inhibition of bacterial growth were measured in mm using a measuring calliper and then classified as resistant, intermediate or sensitive according to the published interpretive chart of Clinical and Laboratory Standards Institute (CLSI) breakpoints (CLSI, 2014).

2.6 | Data analysis

Data describing the calves' sex, age, breed, management system, type of diarrhoea, method of colostrum feeding, time of first colostrum feeding, duration of colostrum feeding, calf house cleaning frequency, type of supplement, floor type and separate housing were coded and entered into a Microsoft Excel spreadsheet 2019. The data were then exported to STATA, version 13, for appropriate statistical analysis. The prevalence of *E. coli* isolates from the total diarrhoeagenic calves was determined by using descriptive statistics. In addition, multivariable logistic regression was used to determine the correlation between the occurrence of *E. coli* isolates and the associated risk factors. All results were reported as statistically significant if the *p* value was less than 0.05. The antibiotic efficacy of each drug was determined by comparing the zone of inhibition of each drug with the standard.

3 | RESULTS

3.1 | Distribution of *E. coli* isolates from different farms

During this study, the samples were collected from diarrhoeic calves in an aseptic manner. Almost all calves showed disease signs such as elevated temperature, depression, dehydration, reduced suckling reflex, rough hair coat, loss of weight, weakness, soiling of the hindquarter and tail with diarrhoeic faeces. Out of 68 faecal samples collected, 47 (69.1%) of the isolates were *E. coli* positive. Out of 47 positive isolates, 46.8% (22/47) were *E. coli* O157:H7 isolates since the isolates were not able to ferment sorbitol that showed colourless colonies, and the isolates were also tested for latex agglutination using a latex kit for the screening of *E. coli* O157:H7 (Figure 2).

In the present study, about 49%, 45% and 6% of the *E. coli* isolates were isolated from diarrhoeic calves located in medium-, small- and large-scale dairy farms, respectively. The isolation of *E. coli* was not significantly correlated with either the medium- ($p = 0.804$) or large farm type ($p = 0.331$). However, there was a higher negative correlation between the isolation of *E. coli* O157: H7 and medium ($r = -0.03$, 95% CI: −0.27 to −0.21) and large-sized farm types ($r = -0.16$, 95% CI: −0.50 to −0.17), while small-sized farms held constant (Table 1).

3.2 | Major risk factors related to the occurrence of *E. coli* in diarrhoeic calves

In the present study, factors such as sex, age, breed, management system, type of diarrhoea, method of colostrum feeding, time of first colostrum feeding, duration of colostrum feeding, frequency of cleaning calf house, type of supplement, floor type and separate housing were analysed for their influence on the occurrence of *E. coli* from diarrhoeic calves. The frequency of calf house cleaning, type of supplement provided and method of colostrum feeding were significantly correlated ($p < 0.05$) with the occurrence of *E. coli* from diarrhoeic calves.

The cleaning of the calf's house every day was significantly correlated with the isolation of *E. coli* from diarrhoeic calves ($p \leq 0.001$). However, there was a significantly higher negative correlation between the isolation of *E. coli* and cleaning of the calf house every day ($r = -0.47$, 95% CI: −0.71 to −0.23). About 68% of the dairy farm owners provided milk for their calf using hands or buckets and this had a significantly positive correlation with the occurrence of *E. coli* ($r = 0.28$, 95% CI: 0.054–0.51). Moreover, calves that were provided with a mixture of milk and other concentrate feedstuffs were infected with *E. coli* (74.5%) compared with claves only provided with milk (25.5%). The type of feed supplement had a significantly positive correlation with the occurrence of *E. coli* ($r = 0.43$, 95% CI: 0.16–0.69).

According to the current study, about 40% of the *E. coli* isolates were recovered from 1-week-old calves compared with calves between 16- and 30-day old (21.3%) and 8- and 15-day-old (17.02%). Based on this study finding, isolation of *E. coli* was not statistically correlated

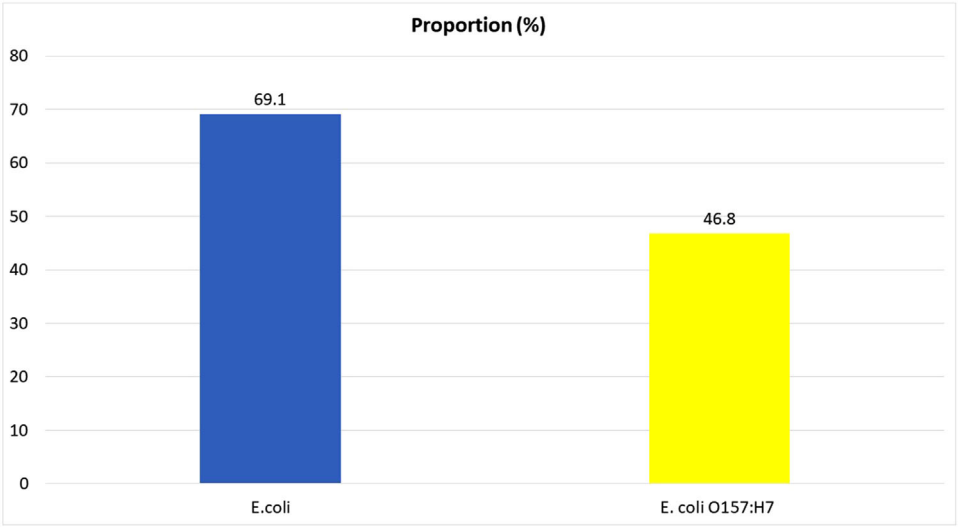

FIGURE 2 Proportion of *E. coli* and *E. coli* O157:H7 isolates from diarrhoeic calves

TABLE 1 Distribution of *E. coli* isolates from different farms

Farm type	No. calves examined	No. +ve for *E. coli*	Prevalence (%)	RR (95% CI)	*p* Value
Small-sized farm	31	12	44.7	Ref	Ref
Medium-sized farm	28	23	48.9	−0.03 (−0.27 to −0.21)	0.804
Large-sized farm	9	3	6.4	−0.16 (−0.50 to −0.17)	0.331
Total	**68**	**47**	**69.1**		

($p > 0.05$) with age, sex, type of diarrhoea, breed, management system, method of colostrum feeding, duration of colostrum feeding, floor type, separate housing and first colostrum feeding time. Additionally, factors such as duration of colostrum feeding, floor type and separate housing were found to have very little association with the isolation of *E. coli* from diarrhoeic calves. However, other factors, such as age, sex, type of diarrhoea, breed, management system, method of colostrum feeding and first colostrum feeding time, were not directly associated with the occurrence of *E. coli* from calves with diarrhoea (Table 2).

3.3 | Antibiotic susceptibility patterns

In the present study, 22 *E. coli* O157:H7 isolates were tested against 10 commonly used antibiotics using the disc diffusion method (Kirby–Bauer method). The antibiotic susceptibility profiles of the isolates showed that all isolates were 100% sensitive to gentamycin. In addition, ciprofloxacin (95.5%), ceftriaxone (86.4%), trimethoprim-sulphamethoxazole (81.8%) and streptomycin (59.1%) were effective against isolates of *Escherichia coli* O157: H7. On the other hand, *Escherichia coli* O157: H7 isolates were 86.4%, 77.3% and 72.7% resistant to tetracycline, kanamycin and amoxicillin, respectively (Table 3).

4 | DISCUSSION

In this research, the isolation and identification of *Escherichia coli* O157: H7 from diarrhoeic calves were performed using standard bacteriological procedures and different precipitating factors that were responsible for the occurrence of the disease were assessed through a questionnaire survey. Furthermore, the isolates of *E. coli* O157: H7 were tested against different antibiotic discs. According to various study findings, calf diarrhoea was found to be a major health issue in dairy farms, causing high mortality and morbidity in calves. Calf diarrhoea has also resulted in significant economic losses, including money invested in care/treatment, loss of genetic potential due to the loss of calves and farmers' inability to invest in higher-priced semen in the face of calf mortality. It has also hampered proper heifer replacement, which affects dairy farmers' ability to increase productivity by encouraging them to cull low-producing cows selectively (Fubini & Divers, 2008; Hadgu et al., 2021; Radostits et al., 2007; Svensson et al., 2003).

In the current study, out of 68 diarrhoeic sampled calves, the isolation of *Escherichia coli* was 69.1% (47/68). This result was comparable with other previous research findings of Yeshiwas and Fentahun (2017) who reported 53 (70.7%) of 75 diarrhoeic sampled calves in Debre-Zeit, Majeed et al. (2011) 64% in Kuwait and Dawit (2012) 64%

TABLE 2 Multivariate logistic regression analyses of isolation rate of *E. coli* with host and management factors

Variable	Category	No. of sample	Frequency of *E. coli O157: H7* N (%)	RR (95% CI)	*p* Value
Sex	Male	15	13 (27.7)	Ref	Ref
	Female	53	34 (72.3)	−0.12 (−0.36 to −0.11)	0.295
Age	1–7 days	23	19 (40.4)	Ref	Ref
	8–15 days	8	8 (17.02)	−0.02 (−0.34 to −0.29)	0.880
	16–30 days	15	10 (21.3)	−0.09 (−0.35 to −0.17)	0.488
	31–60 days	8	4 (8.5)	−0.15 (−0.50 to −0.20)	0.391
	61–90 days	14	6 (12.8)	−0.19 (−0.46 to −0.06)	0.137
Breed	Local	47	34 (72.3)	Ref	Ref
	Cross	21	13 (27.7)	−0.13 (−0.37 to −0.12)	0.300
Management system	Intensive	26	19 (40.4)	Ref	Ref
	Semi-intensive	27	22 (46.8)	−0.08 (−0.36 to −0.19)	0.536
	Extensive	15	6 (12.8)	−0.16 (−0.44 to −0.11)	0.239
Type of diarrhoea	Watery	49	34 (72.3)	Ref	Ref
	Bloody	6	4 (8.5)	−0.08 (−0.42 to −0.26)	0.630
	Mucoid	4	2 (4.3)	−0.19 (−0.63 to −0.24)	0.378
	Mixed	9	7 (14.9)	0.09 (−0.19 to −0.38)	0.485
Method of colostrum feeding	Suckling	33	15 (31.9)	Ref	Ref
	Hand/bucket feeding	35	32 (68.1)	0.28 (0.054 to −0.51)	0.016
Time of first colostrum feeding	< 12 h	53	37 (78.7)	Ref	Ref
	12–24 h	9	6 (12.8)	−0.14 (−0.47 to −0.19)	0.390
	>24 h	6	4 (8.5)	0.16 (−0.19 to −0.50)	0.372
Duration of colostrum feeding	<12 h	44	28 (59.6)	Ref	Ref
	12–24 h	18	15 (31.9)	0.12 (−0.12 to −0.35)	0.318
	24–48 h	6	4 (8.5)	0	–
Calf's house cleaning frequency	Once per week	20	18 (38.3)	Ref	Ref
	Every other day	20	17 (36.2)	−0.05 (−0.31 to −0.21)	0.705
	Every day	28	12 (25.5)	−0.47 (−0.71 to −0.23)	0.0001
Type of supplement	Milk	28	12 (25.5)	Ref	Ref
	Mixed*	40	35 (74.5)	0.43 (0.16 to 0.69)	0.002
Floor-type	Concrete	50	33 (70.2)	Ref	Ref
	Soil	18	14 (29.8)	0.02 (−0.21 to −0.24)	0.890
Separate housing	Yes	27	17 (36.2)	Ref	Ref
	No	41	30 (63.8)	0.004 (−0.23 to −0.23)	0.974

*Represents calves that receive milk together with different concentrate feed stuffs.

in Addis Ababa and Debre Zeit, Ethiopia and Nazir and Hussain (2007), who reported 60%.

However, our study finding was higher than previous reports of Masud et al. (2012), who reported 44%, Dereje (2012) 43.1% and Gebregiorgis and Tessema (2016) 36.8%. Additionally, the current research was higher than the report of Razzaque et al. (2006) who reported 24%, Megersa et al. (2009), who reported 37%, Darsema (2008) 13.5%, Lanz Uhde et al. (2008) 5.5%, Aggernesh (2010) 38% in Debre Zeit and Demissie (2007) 43.1% from Addis Ababa dairy farms.

On the other hand, this study result was lower than previous research findings of Mohammed et al. (2020), who reported 93.75% of 16 diarrhoea cases, Paul et al. (2010) who reported 76% of 100 samples, and Dubie et al. (2014) 82% of 100 diarrhoeic calves' samples. This heterogeneity in *E. coli* prevalence may be due to differences in climatic environments, sample size, age groups tested, colostrum feeding

TABLE 3 Antibiotic susceptibility profiles of *E. coli* isolates from diarrhoeic calves

Antibiotics discs	Disc potency (μg)	Antibiotic susceptibility patterns		
		S (%)	I (%)	R (%)
Amikacin (AK)	30	17 (77.3)	1 (4.5)	4(18.2)
Amoxicillin (AML)	25	2 (9.1)	4 (18.2)	16 (72.7)
Ceftriaxone (CRO)	5	19 (86.4)	0 (0.00)	3 (13.6)
Chloramphenicol (C)	30	11 (50.0)	7 (31.8)	4 (18.2)
Ciprofloxacin (CIP)	5	21 (95.5)	0 (0.00)	1 (4.5)
Gentamycin (CN)	10	22 (100.0)	0 (0.00)	0 (0.00)
Kanamycin (K)	30	2 (9.1)	3 (13.6)	17 (77.3)
Streptomycin (S)	10	13 (59.1)	7 (31.8)	2 (9.1)
Tetracycline (TE)	30	2 (9.1)	1 (4.5)	19 (86.4)
Trimethoprim-sulphamethoxazole (SXT)	25	18 (81.8)	3 (13.6)	1 (4.5)

*S (Sensitive), I (intermediate), R (resistance).

methods, environmental quality and inadequate sanitation, which also causes pathogenic strains to grow up in the ecosystem of young animals. Furthermore, a high dose of pathogenic *E. coli* could be enough to suppress colostral immunity (Quinn et al., 2002; Radostits et al., 2007).

The phenotypic detection of *E. coli* from most other serotypes was based on its inability to ferment sorbitol sugar on sorbitol MacConkey (SMAC) agar and a latex agglutination test using a latex kit for the screening of *E. coli* O157:H7. The present study also revealed that out of positive *E. coli* O157:H7 isolates, 46.8% (22/47) were not able to ferment sorbitol, which showed colourless colonies. The current study finding was much higher than the previous study report of Lee and Choi (2006), who reported 4% (8/200) from hamburger samples, Tadese et al. (2021) who reported 9.1% (18/197) from raw beef samples and Ababu et al. (2020), who reported 5.2% (11/210) from raw milk samples. This variation in the occurrence of pathogenic *E. coli* could come from the sample type and the number of samples examined and the laboratory protocol used to isolate and identify *E. coli*.

In the present study, some factors were investigated for their association with the occurrence of *E. coli* from calves showing diarrhoea. Accordingly, factors such as the frequency of calf house cleaning, type of supplement provided, and method of colostrum feeding were significantly correlated ($p < 0.05$) with the occurrence of *E. coli* from diarrhoeic calves. However, isolation of *E. coli* was not directly significantly correlated ($p > 0.05$) with age, sex, type of diarrhoea, breed, management system, method of colostrum feeding and first colostrum feeding time.

The current study revealed that the cleaning of the house of calves every day was significantly correlated with the isolation of *E. coli* from diarrhoeic calves ($p \leq 0.001$). However, there was a significantly higher negative correlation between the isolation of *E. coli* and the cleaning of the house of calves every day ($r = -0.47$). Radostits et al. (2007) also indicated that the different stressing factors and the type of infection strain they face right after birth are responsible for numerous types of neonatal infections that are more common during their early years. In addition, young neonates below 1 week of age are the most vulnerable as their intestinal flora is not fully established compared with older age (Charles et al., 2003).

The study also revealed that the highest percentage (40.4%) of *E. coli* isolates were recovered from newly born calves (1–7 days age group) compared with other age categories. This study finding is in agreement with previous reports of different studies that revealed that younger calves were mostly clinically affected (Gebregiorgis & Tessema, 2016; Lorino et al., 2005; Maddox-Hyttel et al., 2006; Muktar, 2014; Muktar et al., 2015; Santın et al., 2004). This study finding was also supported by reports of Villarroel (2009), who noted that as the age of the calves increased, the incidence of calf diarrhoea decreased. This could be due to the days-old calves' immune system's inability to fight disease-causing agents compared to older calves (Darsema, 2008). This finding was also consistent with the findings of Wudu (2004), Aggernesh (2010) and Dereje (2012), who reported that calves aged between 0 and 30 days were at great risk of calf diarrhoea, particularly during the first week. In contrast to the aforementioned research findings, Gebremedhin (2014) reported that as the age of the calves increased, the occurrence of *E. coli* had no significant association with neonatal calf diarrhoea (NCD). This disparity in research results may be attributed to sample size differences, poor husbandry practice or the study area's environmental conditions.

In the present study, a high isolation rate of *E. coli* was recorded in female calves (72.3%) than in male calves (27.7%). However, breed and sex do not correlate with the occurrence of calf diarrhoea due to *E. coli*. This agreed with the report of Yenehiwot (2008) and Gebremedhin (2014). Gebregiorgis and Tessema (2016) also reported that sex does not correlate with the occurrence of calf diarrhoea. Male calves, according to the author, do not receive much attention or care because their position on the farm is regarded as irrelevant, especially as replacement stock. In contrast with our findings, Debnath et al. (1995) and Mansour et al. (2014) reported that the sex of the calves has a significant effect on the calf mortality rate.

In the present study, most (68.1%) dairy owners used hand or bucket systems to provide milk for their calf, and there was a significant

positive correlation with the occurrence of *E. coli* ($r = 0.28$, $p = 0.016$). Moreover, most (74.5%) dairy owners provide a mixture of milk with other concentrate feedstuffs as a feed supplement for their calves. This had a significantly positive correlation with the occurrence of *E. coli* ($r = 0.43$, $p = 0.002$). This might have contributed to the decreased colostrum transfer that provides better passive immunity. In their first week of life, calves have a passive immune system as well as receptors for *E. coli* adhesions (Villarroel, 2009). As Stoltenow and Vincent (2003) stated that the high risk of diarrhoea in calves could be due to inadequate passive transfer of colostral immunity. The research findings of Trotz-Williams et al. (2007) and Lorino et al. (2005) also revealed that delayed colostrum intake, especially in the first 6 h of age, predisposes the calf to a higher risk of *E. coli* prevalence.

Furthermore, Olsson et al. (1993) found that with every hour of delay in the first 12-h colostrum feeding, the risk of calf infection increased by 10%. In contrast, in our investigation, the calves that obtained colostrum within the first 12 h (78.7%) had a higher degree of calf diarrhoea than calves who obtained colostrum between 12 and 24 h (12.8%). This variation might be due to calves feeding on colostrum through hand and bucket, poor sanitation of the equipment and barns. This finding was supported by Shiferaw et al. (2002), who reported that the microenvironment hygiene of the farm has a great effect on the occurrence of calf mortality and morbidity in Holeta, Ethiopia and Bendali et al. (1999), who stated that unclean calf barns might be linked with a higher risk of calf scour in southwest, France. Additionally, calves with irregular bedding changes, inadequate living conditions and insufficient sanitation have an elevated chance of morbidity (Charles et al., 2003; Perez et al., 1990).

Antibiotic use is an important factor in maintaining human and animal health worldwide (World Health Organization, 2014). Recently, the development of antibiotic resistance in most bacterial species has become a serious threat to global public health (Acar & Moulin, 2013; Heuer et al., 2006; Merle et al., 2012).

According to the current study, *E. coli* O157:H7 isolates were susceptible to gentamycin, ciprofloxacin, ceftriaxone, trimethoprim-sulphamethoxazole, streptomycin and chloramphenicol. The sensitivity of the isolates to gentamycin was comparable with that of the Ababu et al. (2020), who stated that all isolates were highly susceptible to the list of antibiotic discs. The isolates' chloramphenicol sensitivity matched the findings of Guerra et al. (2006), who found that most bacteria isolated from diarrhoeal calves were chloramphenicol susceptible. In contrast, Abdullah et al. (2013) and Ahmad et al. (1986) reported that *E. coli* O157:H7 isolates were resistant to chloramphenicol. In this study, *E. coli* isolates were also sensitive to ciprofloxacin, which was comparable with the findings of Ababu et al. (2020), Muktar et al. (2015), Werckenthin et al. (2002), Aksoy et al. (2007) and Yenehiwot (2008), whose isolated bacteria were highly susceptible to ciprofloxacin.

The current research revealed that *E. coli* O157:H7 isolates were susceptible to streptomycin, which was in contrast to the report of Ababu et al. (2020), who stated that *E. coli* O157:H7 isolates were highly resistant to streptomycin. In the present research, *E. coli* isolates were resistant to tetracycline, kanamycin and amoxicillin. The presence of resistance against kanamycin is in agreement with the previous findings of Hiko et al. (2008), Minda Asfaw and Shimelis (2021) and Joon and Kaura (1993), whose isolated bacteria were less sensitive to kanamycin. Nonetheless, this was against the report of Tassew et al. (2010) and Taye et al. (2013), in which all the *E. coli* isolates were found to be susceptible to kanamycin. The resistance of the isolates to tetracycline was comparable with the report of Ababu et al. (2020), who stated that isolated *E. coli* species were resistant to tetracycline.

This variation might be due to sample size variation, sample type used, laboratory procedures and the number of antibiotics ($n = 10$) used during the current study compared to antibiotics used (7–8) in other studies conducted in Ethiopia. The difference may be because of the expression of the resistance gene code via the pathogen, which is correlated with existing and emerging isolated features of various agroecological aspects (Reuben & Owuna, 2013).

The finding of high resistance of *E. coli* O157:H7 isolates to amoxicillin agreed with the previous results of Abdullah et al. (2013), Abd-Elrahman (2011), Ansari et al. (2014), Edrington et al. (2006) and Nazir and Hussain (2007). The high resistance of these drugs in Gram-negative bacteria might be due to the transfer of resistance genes from Gram-positive bacteria of β-lactamase genes. Al-Assil et al. (2013) also explained that among the 25 *E.coli* isolates, the most prevalent β-lactamase gene was βlaCTX-M, which was detected in all of the isolates, whereas the βlaTEM gene was found in eight isolates of *E. coli*. This may also be attributed to the unregulated and improper use of these agents in veterinary clinics and farms and throughout the world. This is supported by the lack of policy on antibiotic use and the accessibility of antibiotics in the region. Since *E. coli* is an integral part of normal faecal flora, it is a potential indicator of resistance trends in humans and animals (Werckenthin et al., 2002).

5 | CONCLUSION

In the present study, the occurrence of *E. coli* O157:H7 from diarrhoeic calves was high in the dairy farms of the study area. Of the 69.1% *E. coli*-positive isolates, 46.8% were *E. coli* O157:H7 strains that could cause calf diarrhoea. Factors such as the method of colostrum feeding, hygiene of calves' barn and type of feed supplement provided were found to be significantly ($p < 0.05$) correlated with the occurrence of *E. coli* in calves. Observational and questionnaire surveys revealed that simply being aware of the benefits of colostrum feeding is insufficient; the cleanliness of the material used for colostrum administration as well as the hygiene of the calves' barn are critical for the ultimate success of *E. coli* O157:H7 control. Antibiotic susceptibility results revealed that most *E. coli* O157:H7 isolates were highly sensitive to gentamycin, chloramphenicol, ceftriaxone, ciprofloxacin trimethoprim-sulphamethoxazole and streptomycin. However, some *E. coli* isolates were found to be resistant to tetracycline, kanamycin and amoxicillin.

In conclusion, further study on the usefulness of the strain identification approach for *E. coli* O157:H7 strains should be carried out in comparison with PCR and serotyping. Special emphasis should be given to the time, method, and duration of colostrum feeding to the newborn

calves (colostrum should be provided before 6 h in an aseptic manner). Antibiotics that are sensitive to *E. coli* isolates should be the drugs of choice. The treatment of this disease should be designed based on the antibiotic susceptibility pattern of the isolates.

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CONFLICT OF INTEREST

All authors declare that there are no conflicts of interest in this work.

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

As the study was not experimental, no ethical approval was needed. However, before collecting samples, verbal consent was also pursued from the cattle owners to take faecal samples from their cattle and adopt strict hygienic measures.

AUTHOR CONTRIBUTIONS

HF was involved in conceptualisation, data analysis, preparing an original draft and review & editing; MM contributed by searching resources, data collection, data analysis and manuscript review & editing; SA involved in data collection, laboratory investigation, methodology, searching resources and review & editing the manuscript; EM involved in the data collection, investigation and article review & editing. All authors have read and approved the revised version of the manuscript.

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The data will be provided upon the request of the corresponding author.

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ORCID

Haben Fesseha https://orcid.org/0000-0001-6516-3036

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