

Research Article

Isolation and Antimicrobial Susceptibility Profile of *Escherichia coli* O157:H7 from Raw Milk of Dairy Cattle in Holeta District, Central Ethiopia

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A cross-sectional study was conducted in small, medium, and large-scale dairy farms of Holeta district to isolate, identify, and antimicrobial susceptibility profile of *Escherichia coli* O157:H7 in raw milk of dairy cattle. A total of 210 lactating cows were selected for raw milk samples, and 19% (40/210) were found to be positive for *E. coli* whereas 5.2% (11/210) were confirmed as *E. coli* O157:H7 positive using the *Escherichia coli* O157 latex test. Accordingly, all *E. coli* was highly susceptible to Ciprofloxacin (100%), Gentamycin (100%), Oxytetracycline (100%), and Tetracycline (63.63%). Furthermore, the resistance of 72.73%, 54.54%, 54.54%, and 45.45% was developed to Cefoxitin, Sulphamethoxazole, Cloxacillin, and Streptomycin, respectively. Factors such as parity, age, body condition, herd size, milk yield, udder hygiene, and udder lesion showed a statistically significant ($p < 0.05$) association with the occurrence of *E. coli* infection in dairy cattle. In conclusion, in this study, a higher prevalence of *Escherichia coli* O157:H7 and its drug susceptibility profile is an alarm for the health of the public, and awareness creation to the farm owners and the community is recommended.

1. Introduction

Milk and dairy products are consumed by billions of people around the globe daily and have valuable nutritional factors such as proteins, lipids, minerals, and vitamins [1, 2]. Accordingly, it is important to ensure the microbial quality of milk and dairy products [3]. Foodborne diseases are an important challenge in public health; in particular, developing countries are largely affected because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, and lack of financial resources [4, 5]. Foodborne diseases that are caused by bacteria include pathogenic *Escherichia coli*, *Salmonella*, *Campylobacter*, *Listeria*, *Yersinia*, *Shigella*, and *Enterobacter*, whereas *Escherichia coli* and *Salmonella* species were recorded as a

major cause of foodborne diseases and food poisoning [6, 7].

Escherichia coli O157:H7 is the most important bacterial pathogens that cause life-threatening infections such as hemorrhagic colitis (HC), abdominal pain, bloody diarrhea, hemolytic uremic syndrome, and kidney failure particularly in humans worldwide [8, 9]. Milk and other dairy products are mostly contaminated with *E. coli* O157:H7 during direct exposure to feces due to poor handling systems and causes intestinal or extraintestinal disease [10, 11]. The high prevalence of *E. coli* O157:H7 in dairy products may be due to improper milking hygiene, poor house hygiene, lack of postmilking teat dipping and practicing of milk by contact labors use of lubricant, and absence of order in milking cows of different ages. Moreover, its occurrence was high in dairy farms without being noticeable in farm treatment [12].

Shiga toxin-producing *E. coli* (STEC), also called verotoxin producing *E. coli*, are those strains of *E. coli* that produce at least one member of a class of potent cytotoxins called Shiga toxin [13, 14]. Numerous sporadic infections and outbreaks caused by Shiga toxin-producing *Escherichia coli* O157:H7 (STECO157) have been reported in the United States and elsewhere worldwide. The majority of STEC O157 infections are foodborne; many are associated with bovine sources. STEC O157 was first linked to outbreaks of severe bloody diarrhea in 1982 and is often referred to as a “recently emerged” human pathogen [15]. Enterohemorrhagic *E. coli* (EHEC) strains are a subtype of the Vero (Shiga) toxin (Vtx or Stx)-producing *E. coli* (VTEC or STEC) [3, 16].

In Ethiopia, the consumption of raw (unpasteurized) milk is very traditional, and contaminated milk and milk products are the most common transmission pathway of *E. coli* O157:H7 from animals to humans. Even though the disease caused by *E. coli* is very important in the country and of great public health concern, *E. coli* O157:H7 has received very little consideration in many of the previous public health studies. Most of the previous studies circulated in limited areas and fail to represent the incidence of *E. coli* O157:H7 under different management and ecological situations. Therefore, this study was conducted to isolate and identify the antimicrobial susceptibility pattern of *E. coli* O157:H7 from raw milk of dairy cows in and around Holeta dairy farms.

2. Materials and Methods

2.1. Study Area. The study was conducted in selected dairy farms in Holeta town from December 2018 to June 2019. Holeta is located in central Ethiopia, in Oromia National Regional State, West Showa zone, at a distance of 29 km from Addis Ababa. Its astronomical location is latitude/longitude: 9° 00' N and 38° 30' E longitude and has an altitude of 2400 m above sea level. It has an experience of a bimodal rainfall pattern in which the main rainy season occurs between June and September and the Short rainy season from March to May. The average annual rainfall is about 1144 mm [17].

2.2. Study Animals. The study animals were lactating cows in selected farms and villages of Holeta town. The sampling units were individual lactating cows within a farm under study. Lactating local (indigenous) zebu, Holstein Friesian, and Holstein Friesian cross with local zebu breed in small, medium, and large-scale dairy farms of Holeta town and the surroundings were the study population based on their management systems and owner's willingness.

2.3. Study Design and Sample Size. A cross-sectional study was conducted in the Holeta district. The list of dairy owners was obtained from the concerned body, and the farms were then classified as small, medium, and large based on the number of cows and farms and households selected by simple random sampling from the list while keeping the

proportions close to each other as much as possible. All lactating dairy cows in the selected farms were included in the study and a total of 210 cows were sampled from sixteen dairy farms selected by a simple random sampling method.

2.4. Study Methodology

2.4.1. Method of Data Collection. A questionnaire was developed, and all information related to the study objectives was recorded. Data collected include address and are pertinent to cow-level factors, including dairy cows age, parity, breed, presence of a lesion on the skin of udder or teat, and milking practice, where the owners of cows wash their hands and udder before and after milking, wash hand and udder before milking, dry udder before milking, and wash hand only before milking; the type of milking and type of floor were obtained by observation and by interviewing the different farm attendants and owners. The age of the animals was determined from birth records and dentition characteristics and categorized as a young adult (3 to 6 years), adults (>6 to 9 years), and old (>10). Parity was categorized as few (1–3 calves), moderate (3–6 calves), and many (6 and above calves).

2.4.2. Milk Sample Collection and Culturing. A total of 210 fresh milk samples were collected in sterilized containers (bottles) and labeled based on temporary ID given to a cow, kept in a thermos flask icebox, and transported to Addis Ababa University College of the veterinary medicine microbiology laboratory to culture the milk samples for isolation and identification of *E. coli* O157:H7. The examination of milk samples was conducted within 5 hours after collection. All milk samples were processed bacteriologically, and different biochemical tests were performed according to the procedures employed by Quinn et al. [18].

2.4.3. Isolation and Identification of Bacteria. The isolation and identification of *E. coli* O157:H7 was performed using techniques recommended by Quinn et al. [18].

(1) Culturing. A sterile loop was dipped into a thawed milk sample and streaked onto MacConkey agar plates as differential media for the identification of *E. coli* and incubated at 37°C for 24 hours. The presumed well-selected typical and atypical colonies were again subcultured on selective medium Eosin Methylene Blue (EMB) Agar and incubated at 37°C for 24 hrs. Morphologically typical colonies were producing metallic sheen [19] and under the same conditions to get pure colonies of *E. coli*. After the next 24 hrs of incubation, a well-isolated colony was selected and subcultured further onto nutrient agar (NA) to be used for biochemical confirmation.

(2) Biochemical Confirmation of *E. coli*. For primary and secondary biochemical tests, pure cultures of a single colony from MacConkey agars were transferred onto a nutrient agar plate. Tests such as Oxidase, Catalase, Indole, Methyl red,

Voges-Proskauer (VP), and Citrate (IMViC) tests were done to confirm the presence of *E. coli* in the test samples. Colonies that are positive for tryptophan utilization (indole test) (red ring), positive for Methyl red, negative for citrate utilization (green slant), and negative for Voges-Proskauer (VP) test were considered to be *E. coli* positive. [18]. Moreover, the Gram staining of the bacterial colony was done on a sterile glass slide as described by Cheesbrough [20]. Isolates of presumptive *E. coli* for all biochemical tests were cultured on sorbitol MacConkey agar for further test on latex agglutination test.

(3) *Screening Test by E. coli O157 Latex Agglutination Test.* Latex agglutination test was employed using latex kit for the screening of *E. coli* O157:H7. Sorbitol-negative (clear) colonies exhibiting colony morphology typical for *Escherichia coli* O157:H7 per plate were picked and spread plated on Cefixime tellurite sorbitol MacConkey (CT-SMAC). Then, after 24 hours of incubation, a fresh single colony of nonsorbitol fermenter from sorbitol MacConkey agar was picked and subjected to latex agglutination using an *E. coli* O157 latex kit. Isolates of presumptive *E. coli* O157:H7 for all latex agglutination tests were cultured on nutrient agar (NA) for antimicrobial susceptibility testing.

2.4.4. Antimicrobial Susceptibility Testing. The antibiotic susceptibility tests of the *E. coli* O157:H7 isolates were performed according to the National Committee for Clinical Laboratory Standards (NCCLS) [21] method using Kirby-Bauer disk diffusion test on Muller-Hinton agar. The agar disk diffusion method has been used to test common fast-growing bacterial pathogens and is recognized to work well with *E. coli* O157:H7. Reliable results can be obtained with disk diffusion tests that use a standardized methodology and zone diameter measurement correlated with minimum inhibitory concentration (MIC), and the inhibition zone was measured and interpreted as susceptible, intermediate, and resistant based on the standard inhibition zone given by clinical and laboratory standard institute (CLSI) [22].

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 hours 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 the Muller-Hinton agar plate within a sterile safety cabinet. The plates were held at room temperature for 15 minutes to allow drying. Antibiotic discs with a known concentration of antimicrobials were placed, and the plates were incubated at 37°C for 18–24 hours. Eleven different families of antibiotics (Oxoid, UK) were used based on the trend of using these antibiotics in the different farms of the study area (Table 1).

2.4.5. Data Management and Statistical Analysis. The data collected were entered into the Microsoft Office Excel 2016 spreadsheet and analyzed using STATA version 13. Descriptive statistics were used to summarize the generated

data, and the prevalence of *E. coli* O157:H7 related to specific risk factors (herd size, type of farm, udder lesion, milk yield, type of floor, parity, milking type, body condition score, age, farm hygiene, and udder hygiene) was determined as the proportion of affected cows out of the total sample. The association of these specific variables on the prevalence of *E. coli* O157:H7 was calculated using a chi-square (X^2) test. Besides, antibiotic efficacy was determined by comparing the zone of inhibition of each drug with the standard. A statistically significant association between variables is considered to exist if the p value is <0.05 .

3. Results

3.1. Isolation and Identification of E. coli O157:H7. *E. coli* isolates grown on MacConkey agar, Eosin Methylene Blue (EMB) Agar, and Sorbitol MacConkey Agar were identified based on different characteristics on biochemical tests [18]. Catalase, Simmon's citrate agar, sugar fermentation on Triple Sugar Iron Agar, Indole Production, Voges-Proskauer, and Methyl red tests were performed. The isolates of the above media were tested by Triple Sugar Iron Agar (TSI) slant culture by stab method, and after 48 hours of incubation at 37°C, yellow slant with yellow butt, presence of gas bubbles, and absence of black precipitate in the butt were observed which is indicative of *E. coli*. Performing Simon's citrate test, no color change from green to blue was observed, performing Methyl red test, red color indicative of acid production was observed, performing indole test, red ring at the top of culture broth was observed, and performing Voges-Proskauer test, no color change to pinkish was observed.

The serological test was also employed by using the latex agglutination test for screening of *E. coli* O157:H7, and then, from 40 *E. coli* positive samples, eleven isolates were agglutinated in both tests and taken as *E. coli* O157:H7. Finally, out of 210 raw milk samples of dairy cows examined for the presence of *E. coli* O157:H7 by detailed bacteriological and serological examinations, the results showed that the overall prevalence of *E. coli* was 19%, out of which 5.2% was *E. coli* O157:H7.

3.2. Risk Factors Associated with the Occurrence E. coli O157:H7. In this study, eleven factors were considered as potential risk factors for the occurrence of *E. coli* O157:H7 in the study area. These were herd size, type of farm, udder lesion, milk yield, type of floor, parity, milking type, body condition score, age, farm hygiene, and udder hygiene.

In the present study, age was taken into consideration, and the prevalence of *E. coli* O157:H7 was measured for different age groups of lactating cows. The highest prevalence (82.5%) was found in lactating cows of ages 3–6 years, followed by cows of ages 6–9 years (12.5%), and the lowest prevalence was recorded in cows of ages less than or equal to 9 years old. Parity is also considered as the main factor in the study, and a higher prevalence (87.5%) was recorded in cows that gave birth to 1–3 calves, followed by cows which gave birth to 4–6 calves (12.5%), and the lowest prevalence was

TABLE 1: Antibiotic disk used to test *E. coli* O157: H7 and their respective concentrations.

Drugs	Disc code	Concentration (μg)	Resistant	Intermediate	Susceptible
Streptomycin	S	10	≤ 11	12–14	≥ 15
Sulfamethoxazole	RL	100	≤ 12	13–16	≥ 17
Cefoxitin	CF	30	≤ 14	15–17	≥ 18
Tetracycline	TE	30	≤ 11	12–14	> 15
Oxytetracycline	OT	30	≤ 11	12–14	≥ 15
Vancomycin	VA	30	≤ 15	—	> 15
Ciprofloxacin	CIP	5	≤ 15	16–20	≥ 21
Trimethoprim	TR	5	≤ 10	11–15	≥ 16
Cloxacillin	OB	5	≤ 10	11–12	> 13
Gentamycin	GEN	10	≤ 12	13–14	> 15
Chloramphenicol	C	30	≤ 12	13–17	≥ 18

TABLE 2: Association of risk factors with the occurrence of *E. coli* O157:H7 in the study area.

Variables	Category	No. examined	Positive	Ch-square (X^2)	<i>p</i> value
Herd size	<10	114	5 (12.5%)	63.242	0.0001
	10–42	67	15 (37.5%)		
	≥ 42	29	20 (50%)		
Type of floor	Good concrete	68	4 (10%)	11.304	0.001
	Bad concrete	100	17 (42.5%)		
	Soil	42	19 (47.5%)		
Parity	1–3	159	35 (87.5%)	4.078	0.044
	4–6	46	5 (12.5%)		
	> 6	5	0 (0%)		
Milking type	Hand	181	35 (87.5%)	0.071	0.0001
	Machine	29	5 (12.5%)		
Body condition	Poor	57	22 (55%)	19.555	0.0001
	Moderate	130	16 (40%)		
	Good	23	2 (5%)		
Age in years	3–6	114	33 (82.5%)	15.918	0.0001
	6–9	62	5 (12.5%)		
	≥ 9	34	2 (5%)		
Farm hygiene	Poor	129	35 (87.5%)	14.175	0.0001
	Good	81	5 (12.5%)		
Udder lesion	Yes	49	20 (50%)	19.642	0.0001
	No	161	20 (50%)		
Udder hygiene	Washing/drying	161	3 (7.5%)	6.925	0.007
	Washing only	49	37 (92.5%)		
Milk yield	Low	60	4 (10%)	25.002	0.852
	Medium	89	31 (77.5%)		
	High	61	5 (12.5%)		
Type of farm	Intensive	61	30 (75%)	51.262	0.0001
	Semi-intensive	130	10 (25%)		
	Extensive	19	0 (0%)		

recorded in cows which gave birth to greater than 6 calves (Table 2).

In the current study, milking practices such as washing and drying of udder also have a significant role in the highest prevalence of *E. coli* O157:H7. There was more likelihood occurrence (92.5%) in poor udder hygiene cows as compared to those who wash their cows regularly (7.5%). *E. coli* O157:H7 prevalence was also significantly higher in cows with poor body condition (55%) than cows with moderate (40%) and good body condition (5%). The effect of udder lesion on the current prevalence of *E. coli* O157:H7 was also

studied, and the result revealed that 50% of cows having infected udder were found to be infected with this bacteria. Herd size also played a vital role in the occurrence of *E. coli* O157:H7 in the study area, and the prevalence was also significantly higher in farms having more than 42 herd size (50%) than farms with 10–42 herd sizes (37.5%) and less than 10 herd sizes (12.5%) (Table 2).

As indicated in Table 2, herd size, age, type of farm, udder or teat lesion, type of floor, parity, milking type, body condition score, farm hygiene, and udder hygiene were found statistically significant with a *p* value less than 0.05.

A total of eleven *E. coli* serotype O157:H7 isolates were tested with eleven available antibiotics with a disc diffusion method. From these isolates, all were highly susceptible to Ciprofloxacin (100%), Gentamycin (100%), and Oxytetracycline (100%) and there was lower susceptibility to Tetracycline (63.635%), Vancomycin (27.27%), Chloramphenicol (27.27%), Streptomycin (9.09%), and Trimethoprim (9.09%). However, a different proportion of resistance was observed to Cefoxitin (72.73%), Sulphamethoxazole (54.54%), Cloxacillin (54.54%), and Streptomycin (45.45%) (Table 3).

4. Discussion

The present study was conducted to assess the prevalence of *E. coli* O157:H7 from raw milk of dairy cattle and to determine the antimicrobial susceptibility profile of *E. coli* O157:H7 in and around Holeta districts of West Shewa zone, Oromia Regional State of Ethiopia. The potential risk factors associated with the occurrence of *E. coli* O157:H7 were explored, and the contribution of *E. coli* O157:H7 as important milk-borne pathogens was illustrated using latex agglutination test using anti-O157 and H7 serum.

Out of 210 raw milk samples collected directly from the udder, 19% (40/210) of raw milk samples were contaminated with *E. coli* strains whereas 5.2% (11/210) of the isolates were positive for *E. coli* O157:H7. The finding of *E. coli* O157:H7 was higher than that of Klie et al. [23] (3.9%) in Germany, Disasa et al. [24] (2.9%) in Asossa, and Allerberger et al. [25] (3%) in Austria. However, the result of the present finding is lower than that of Bedasa et al. [26] (12%) in Bishoftu, Mekuria and Beyene [27] (10.4%) in Tigray, Lye et al. [28] (18.75%) in Malaysia, and Abunna et al. [29] (8.9%) in Asella.

According to the report of Ranjbar et al. [30] in Iran, the prevalence of *E. coli* strains in raw milk and traditional dairy product samples was 30.16%. The report of Ombarak et al. [31] in Egypt also showed that the prevalence of *E. coli* strains in various types of dairy products had a range of 21 to 77% and both were higher than our findings. The overall prevalence of *E. coli* strains in raw milk samples of studies conducted on Switzerland [32], Iran [33], Italy [34], Egypt [35], Turkey [36], China [37], and Spain [38] had ranged between 1% and 27% which were comparable with our reported prevalence rate.

There were some probable explanations for the high prevalence of *E. coli* and *E. coli* O157:H7 from raw milk of dairy cattle in our study. At first, Ethiopian farmers especially smallholders used their hands and also traditional equipment for milking procedures which increase the risk of transmission of bacteria into the milk and dairy products [29, 39, 40]. Second, the lack of maintenance of raw milk samples at temperatures below 4°C facilitates the survival and proliferation of bacteria [24]. Thirdly, the occurrence of the disease, unhygienic manner of animals, and house floor might have contributed to the environmental contamination of milk with fecal and infected animal wastes [41]. The transmission of pathogenic agents from the infected staffs of

TABLE 3: Antimicrobial susceptibility pattern of *E. coli* O157:H7 isolates.

Drugs	Resistant	Intermediate	Susceptible
Streptomycin	5 (45.45%)	5 (45.45%)	1 (9.09%)
Sulphamethoxazole	6 (54.54%)	5 (45.45%)	0 (0%)
Cefoxitin	8 (72.73%)	3 (27.27%)	0 (0%)
Tetracycline	1 (9.09%)	3 (27.27%)	7 (63.63%)
Oxytetracycline	0 (0%)	0 (0%)	11 (100%)
Vancomycin	2 (18.18%)	6 (54.54%)	3 (27.27%)
Ciprofloxacin	0 (0%)	0 (0%)	11 (100%)
Trimethoprim	4 (36.36%)	6 (54.54%)	1 (9.09%)
Gentamicin	0 (0%)	0 (0%)	11 (100%)
Chloramphenicol	4 (36.36%)	4 (36.36%)	3 (27.27%)
Cloxacillin	6 (54.54%)	5 (45.45%)	0 (0%)

the milking halls and also farms is also another potential risk factor [30].

Among the potential risk factors considered, age, parity, body condition, udder hygiene, herd size, and udder lesion found a statistically significant effect $p < 0.05$. The prevalence of various livestock infections generally increases with increasing the herd size. Besides, this indicates the lack of knowledge of the community regarding animal husbandry and the large size can affect the health of animals. Age was also found to be associated with the occurrence of *E. coli* infection, which is higher in animals of old age (≥ 9) than in younger ones. This could probably associate with the ability of the immune system of an animal to defend against infection-causing agents. The finding of this study was also assessed for the number of parity as a predisposition to *E. coli* infections [42].

In this study, the prevalence of *E. coli* O157:H7 was higher in cows with dirty udder than that of cows with cleaned udder. Dirty udder contributes to a favorable condition for the multiplication of both contagious and environmental pathogens. Udder hygiene was also found to be statistically significant with the incidence of *E. coli* infection. The other important factor for the occurrence of *E. coli* infection in this study was the body condition of the animals. Poor body condition was the major risk factor for increased *E. coli* O157:H7 prevalence, and this factor remained statistically significant ($p < 0.05$). This could be associated with the reduced defense status of the animals. Several factors may contribute to poor body conditions including malnutrition or parasitic infection and old age compounded with reproduction stresses [2, 12, 29].

Antibiotic-resistant bacteria pose a growing problem of concern worldwide. The development of antibiotic resistance among bacteria such as *E. coli* poses an important public health concern. The effectiveness of treatments and the ability to control infectious diseases in both animals and humans may be severely hampered since the bacteria can be easily circulated in the environment. A relatively high number of strains are resistant to the antimicrobial commonly used in the therapeutic protocol of many humans and animal infections [43]. Food contamination with antibiotic-resistant bacteria can also be a major threat to public health, as the antibiotic resistance determinants can be transferred

to other pathogenic bacteria, potentially compromising the treatment of severe bacterial infections. The prevalence of antimicrobial resistance among foodborne pathogens has increased during recent decades [44].

In the present study, all of the eleven isolates were highly susceptible to Oxytetracycline, Ciprofloxacin, and Gentamycin followed by relatively lower susceptibility to Tetracycline, Vancomycin, and Trimethoprim. On the other side, the current study revealed that all isolates were highly resistant to Cefoxitin, Sulphamethoxazole, Cloxacillin, and Streptomycin with a proportion of 72.73%, 54.54%, 54.54%, and 45.45%, respectively. The result of this study was almost comparable with the work of Bedasa et al. [26]. Momtaz et al. [3] reported that resistance against Tetracycline (86.88%) and Streptomycin (54.91%) was the most commonly reported antibiotic resistance-based finding of STEC strains of diarrheic patients which were comparable with the current finding.

According to Ranjbar et al. [30] in Iran, *E. coli* strains (6.72%) were prevalent in hospital food samples whereas Shiga toxin-producing *E. coli* (STEC) strains were resistant against Tetracycline. In contrast to our finding, Gentamycin and Ciprofloxacin were found resistant to STEC. On the other hand, Stewardson et al. [45] reported that Extended-Spectrum-Beta-lactamase-producing-Enterobacteriaceae-producing *E. coli* (ESBL-PE) was the most commonly detected (44.77%) in food samples, and ESBL-PE strains were susceptible to Gentamicin and Ciprofloxacin. Similarly, the finding of Mahanti et al. [46] in India [28] revealed that a high prevalence of resistance against Gentamicin was seen by STEC, Kang et al. in Korea [30] also showed that a high prevalence of resistance against Tetracycline and Streptomycin was detected by STEC, and Castillo et al. in Mexico [47] also showed a high prevalence of resistance against Trimethoprim and sulfamethoxazole by STEC. The variation in resistance for a single drug may be due to the expression of resistant gene coded by the pathogen which is associated with emerging and reemerging aspects of the isolates with regard to different agroecology [48]. This might be due to the inappropriate use of antibiotics for the treatment of diseases or irrational use of antimicrobials for therapeutic and prophylactic treatment [49].

5. Conclusion

In the present study, *E. coli* O157:H7 was the major contaminant of raw milk of dairy cattle in and around Holeta. In the study area, factors such as age, parity, body condition, herd size, udder hygiene, and lesion contributed to microbial contamination of dairy milk. Detection of *E. coli* in milk is of public health concern due to its zoonotic potential. All tested isolates were susceptible to Ciprofloxacin, Gentamycin, and Oxytetracycline whereas the isolates were found to be resistant to Cefoxitin, Cloxacillin, Tetracycline, Vancomycin, Chloramphenicol, Streptomycin, Sulphamethoxazole, and Trimethoprim. In conclusion, awareness creation to the dairy farmers and all stakeholders at different levels regarding milk handling practices should be given to reduce the milk rejection rate because of spoiled milk and milk-

borne pathogens resulting from contamination of milk. It is recommended that veterinary/extension services be provided to livestock farmers on proper animal husbandry and control of zoonotic animal diseases. Most human diseases are caused by pathogens from animal and/or animal products like milk and milk products. However, the contaminated one acts as a source of *E. coli* O157:H7 which needs preventive actions at any point in the food production chain. Besides, different epidemiological factors that interplay in *E. coli* occurrence should be studied routinely. Furthermore, there should be regular antibiotic sensitivity testing to *E. coli* O157:H7 to select effective antibiotics and also help to reduce the problem of drug resistance development toward commonly used antibiotics.

Data Availability

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

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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