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ORIGINAL RESEARCH

Epidemiology and Antimicrobial Susceptibility Pattern of *E. coli* O157:H7 Along Dairy Milk Supply Chain in Central Ethiopia

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Background: Enterohemorrhagic *Escherichia coli* (O157:H7) is the primary cause of bloody diarrhea or hemorrhagic colitis. The study was carried out with to determine the epidemiology and antimicrobial resistance pattern of *E. coli* O157:H7 along the dairy supply chains in Akaki Kaliti sub-city of Addis Ababa, Bishoftu and Sululta towns of central Ethiopia.

Methods: A cross-sectional study design with random sampling methods was employed. Thus, a total of 450 raw cow milk (294), milker hand swab (65) and water (91) samples were collected from dairy farms, milk collection centers and Cafeterias and processed according to the standards to isolate and identify *E. coli* O157:H7. The samples were initially enriched in buffered peptone water, then plated onto Sorbitol MacConkey agar. Consequently, the suspected non-sorbitol fermenting colonies were confirmed as *E. coli* biochemically and serological test using latex agglutination tests.

Results: Out of the total 450 samples examined, 6.0% were found to be contaminated by *E. coli* O157:H7. Accordingly, 9.89% of water, 9.23% of milker hand swab and 4.08% of raw milk samples were contaminated by the pathogen. Furthermore, the prevalence of *E. coli* O157:H7 was 7.79%, 6.21% and 3.97% in Akaki kaliti sub-city, Sululta and Bishoftu towns, respectively. The result of Fisher exact analysis revealed a significant difference observed ($p < 0.05$) between the occurrence of the pathogen and the source of sample, sources of water used, sampled material and type of containers. The study also revealed that varying level of resistance of *E. coli* O157:H7 isolates against nine antimicrobial discs tested and 100% (n = 27) of the isolates showed multidrug-resistance comprising from two up to seven antimicrobial drugs.

Conclusion: In conclusion, this study has indicated the occurrence of *E. coli* O157:H7 and its multiple drug-resistant profiles in milk samples along the dairy supply chains and its risk to public health and food safety. Therefore, proper hygienic practices from dairy farms to fork and rational drug usage are recommended.

Keywords: antimicrobial resistance, critical control points, *Escherichia coli* O157:H7, Ethiopia, milk

Introduction

Food-borne pathogens are the leading cause of illness, despite the substantial scientific research and technical improvements in developed countries in recent years, and death in developing countries costs billions of dollars in medical care and medical and social costs.[1] Occasional cases and outbreaks of human diseases caused by food-borne pathogens have been linked to ground beef, raw milk, meat and dairy products, vegetables, unpasteurized fruit juices, and water. One of the most common causes of food-borne disease is contaminated raw milk.[2,3] In addition, it is an ideal medium for the growth of various bacteria.[4] *Escherichia coli* is one of the many microorganisms that can get access to milk and milk byproducts.[5] Moreover, many *E. coli* strains are harmless or beneficial to the host. Some strains of *E. coli*, however, are harmful to humans and are found in food animals. Among them, *E. coli* O157:H7 is the best-known pathogenic strain.[6,7]

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Enterohemorrhagic *Escherichia coli* (O157:H7) is the primary cause of bloody diarrhea or hemorrhagic colitis. Thus, the synthesis of verocytotoxins,[8] commonly known as Shiga toxins, is a distinguishing trait of this group (Stx).[9] Moreover, cattle are the primary reservoir of *E. coli* O157:H7 and an important source of human infection[10] and undercooked ground beef and unpasteurized milk being common vehicles of the pathogen transmission.[11] The food-borne *E. coli* O157:H7 is estimated to cause 2.8 million acute illnesses each year worldwide.[12] In addition, an estimated 73,480 infections caused by *E. coli* O157:H7 infection occur in the United States each year, resulting in an estimated 2168 hospitalizations and 61 deaths[13,14] and a 607 million US dollar economic burden.[15] It has emerged as an important global zoonotic food and water-borne pathogen.[16,17]

International food management organizations, particularly the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the International Hazard Analysis Critical Control Point (HACCP) alliance, have already provided guidelines to member countries on safe handling procedures such as HACCP and Good Manufacturing Practices (GMP) to reduce the risk of zoonotic infection transmission associated with contaminated food.[1,18,19] Furthermore, antibiotic resistance in *E. coli* has been found globally, and rising resistance rates in *E. coli* are a significant issue in both developed and developing countries. As a result, an increase in bacterial resistance to antibiotics complicates infection therapy.[20] A few studies have been carried out in Ethiopia to examine the prevalence and antibiotic resistance patterns of *E. coli* from various clinical sources.[21] Recent research findings[22–27] have proven that *E. coli* O157:H7 has developed resistance to a variety of routinely used medicines, including erythromycin, amoxicillin-clavulanic acid, sulfonamides, ampicillin, and tetracycline, and those certain strains have gained multidrug resistance.

Furthermore, little is known about the frequency, distribution, and related virulent genes of *E. coli* O157:H7 in humans, animals, or animal-derived foods in Ethiopia.[22,23,28] Also, it is unclear to what extent milk producers' and retailers' homes serve as a source of *E. coli* O157:H7 contamination for dairy products. As a result, research into this pathogenic organism contributes to a better understanding of its epidemiology by identifying transmission routes, vehicles, and sources of milk contamination. Therefore, the aims of the present study were to assess the prevalence of *E. coli* O157:H7 in raw cow milk, milker hand swabs, and water samples, as well as to determine the pattern of antimicrobial resistance along the dairy supply chain.

Methods

Description of the Study Areas

The study was carried out at Akaki Kaliti sub-city of Addis Ababa, Bishoftu and Sululta towns of central Ethiopia. Samples were collected from dairy farms, milk collection centres and cafeterias located at the aforementioned towns. The study areas are known for their high potential for dairy production and located in the central highlands of Ethiopia. Bishoftu and Sululta towns are located in Oromia National Regional State located at a distance of 47 km southeast and 23 km northwest of the capital Addis Ababa, respectively. Bishoftu town is situated between 8°44′4.74″ North latitude and 39°0′30.72″ East longitude. The altitude of the town ranges from 1900 to 1995 meters above sea level. Sululta is situated between 9°11′00″ North latitude and 38°44′59.99″ East longitude. It lies at an altitude of 2500 meters above sea level.[29] Akaki Kaliti sub-city is one of the ten sub-cities of Addis Ababa. It is situated at a latitude of 8°53′40.92″ North and 38°46′23.52″ longitude of East. It lies at an altitude of 2140 meters above sea level.[30]

Study Design

A cross-sectional study design was carried out to determine the epidemiology and antimicrobial sensitivity pattern of *E. coli* O157:H7 from farm to fork along the dairy supply chains in central Ethiopia.

Sample Size Determination

The required sample size was calculated by considering a previously published prevalence estimate of 6.9% by[31] reported from a study on the bacterial isolates from raw cow milk in Holeta district. Therefore, the required sample size was calculated as 98 based on the prevalence estimate described above (6.9%). Accordingly, a total of 294 (226 from farmers, 42 from milk collection centers and 26 from cafeterias) raw cow milk samples were collected from the study areas.

Sampling Strategy

First, a baseline survey was done in the research areas to determine the total number of farms, farm size, farming system, and the status and number of milk collection centers and cafeterias. According to the survey results, dairy farms were divided into three categories based on herd size (small, medium, and large-scale commercial dairy farms) for the collection of raw cow milk samples from dairy farms. Furthermore, as explained by Dohoo et al,[32] the proportionate stratified random sampling method was used to allocate the number of samples to be gathered from each stratum in proportion to the total number in the stratum. A simple random sampling technique was employed to collect milk from dairy farms, collection centers and cafeterias. Furthermore, water and swab samples were collected from each dairy farm, milk collection centers and cafeterias.

Sample Collection and Transportation

A sterile syringe was used to collect 5 mL of milk samples aseptically from bulk milk buckets and tanks on dairy farms, as well as tanks in milk collecting centers and cafeterias, into a sterile screw-capped falcon tube. Similarly, 5 mL of water was collected from a water tank for routine cleanliness of equipment and staff in dairy farms, milk collection centers, and cafeterias. For sampling and transferring the milker hand swab samples, sterile cotton swabs with breakable sticks were immersed in a tube containing 10 mL of dilution fluid, buffered peptone water.[33] Following that, the samples were maintained in a refrigerator at 4°C until analysis, and culturing was completed within 24 hours as stated by.[34]

Laboratory Investigation

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

Each raw milk and water sample (1 mL) was supplemented with 9 mL of buffered peptone and incubated for 18–24 hours at 37°C. Similarly, a swab sample in 10 mL BPW was incubated for 18–24 hours at 37°C. After carefully mixing the enrichment, ten microliters (10 µL) of the enriched samples were placed onto Sorbitol MacConkey agar and incubated at 37°C for 24 hours. Following that, typical colonies on SMAC agar (pale, unable to ferment sorbitol) were moved to nutrient agar for further confirmatory biochemical tests (indole and KIA tests) and serological tests, as reported by.[34]

Serological Test

The serological confirmation of *E. coli*-sorbitol-negative colonies was performed using an *E. coli* 57:H7 latex agglutination assay using latex particles coated with antibodies specific for the *E. coli* O157 and *E. coli* H7 antigens. Identification of *E. coli* O157:H7 was carried out following the manufacturer's instructions, colonies that agglutinated were assumed to be *E. coli* O157:H7.

Antimicrobial Susceptibility Testing

The Clinical and Laboratory Standards Institute's Kirby Bauer disc diffusion method on Mueller Hinton agar was used to determine the antimicrobial susceptibility pattern of *E. coli* O157:H7 isolates.[35] The isolates of *E. coli* O157:H7 were evaluated for sensitivity to the most commonly used antimicrobials, including ampicillin (AMP/10 µg), cefoxitin (FOX/30 µg), streptomycin (S/10 µg), tetracycline (TE/30 µg), ciprofloxacin (CIP/5 µg), gentamicin (GEN/10 µg), sulfamethoxazole (RL/100 µg), trimethoprim (TR/25 µg) and doxycycline (DO/30 µg). Furthermore, isolates that showed resistance to two or more antimicrobials were labeled as multidrug-resistant.[36]

Data Management and Statistical Analysis

The data obtained was classified, filtered and coded using Microsoft Excel® 2010. The data was then be exported to STATA version 14 (Stata Corp., Texas, USA) for appropriate statistical analysis. Then, the Fisher's exact test was used to measure the association of *E. coli* O157:H7 occurrence with incriminated categorical risk factors. Also, logistic regression analysis was utilized to measure the degree of association between the risk factors and *E. coli* O157:H7 occurrence. The data were interpreted as significant when the *p*-value is less than 0.05. Meanwhile, for antimicrobial susceptibility test, the results were interpreted according to Clinical and Laboratory Standards Institute[35] interpretive criteria for *Enterobacteriaceae.*

Results

Overall Prevalence of *Escherichia coli* O157:H7

Out of the total of 450 samples examined in the present study, *Escherichia coli* O157:H7 was isolated from 27 (6.0%) of the samples based on latex agglutination assay. Accordingly, 12 (7.79%), 9 (6.0%) and 6 (3.97%) of the samples were found to be contaminated by the pathogen in Akaki kaliti sub-city, Sululta and Bishoftu towns, respectively. On the other hand, from the total 294 milk samples examined at the different sampling point, *E. coli* O157:H7 was contaminates 9 (34.62%) of the milk samples from cafeterias and 3 (1.33%) of the milk samples from dairy farms (Table 1).

Association of Risk Factors with *Escherichia coli* O157:H7 Occurrence

There was statistically significant difference observed ($p < 0.05$) between the occurrences of *E. coli* O157:H7 with the source of sample, sources of water used, sampled material and type of containers. However, there was no statistically significant difference observed ($p > 0.05$) between the occurrence of the pathogen with the study area, type of sample and scales of dairy farms (Table 2).

Association *Escherichia coli* O157:H7 Occurrence in Milk Sample

Out of the total 294 milk samples examined, 4.08% (n = 12) of the samples were contaminated by *E. coli* O157:H7. Also, it indicated that there was no statistically significant difference observed ($p > 0.05$) between the occurrences of *E. coli* O157:H7 with the study area and dairy farm scales. There was statistically significant difference observed ($p < 0.05$) between the occurrence of the pathogen with the source of samples, sampled materials and source of water used (Table 3).

Association *Escherichia coli* O157:H7 Occurrence in Water Sample

On the other hand, out of the total 91 water samples examined, 9.89% (n = 9) of the samples were found to be contaminated by *E. coli* O157:H7. There was no statistically significant difference observed ($p > 0.05$) between the occurrence of the pathogen with the study areas and type of containers used. However, there was statistically significant difference observed ($p < 0.05$) between the occurrence of the pathogen with the sources of samples and sources of water used. Also, the occurrence of *E. coli* O157:H7 was significantly ($p = 0.004$) higher (27.59%) in the well water with 23.2 (95% CI 2.74, 196.9) times more likely to occur than pipe water sources (Table 4).

Association of *Escherichia coli* O157:H7 Occurrence in Swab Sample

Out of the total 65 milker hand swab samples examined, 9.23% (n = 6) of the samples were positive for *E. coli* O157:H7. There was no statistically significant difference observed ($p > 0.05$) between the occurrences of *E. coli* O157:H7 in the study areas, dairy farm scales, and water sources. However, the occurrence of the pathogen was 1.94 (95% CI 0.29, 12.95) times more likely to occur in the milker's hand from dairy farm in Sululta than dairy farms in Akaki Kaliti sub-city dairy (Table 5).

Antimicrobial Susceptibility Profile of *E. coli* O157:H7

The study of antimicrobial sensitivity of *E. coli* O157:H7 recovered from different sample types revealed that, of the total 27, all isolates were resistant to ampicillin and cefoxitin. On the other hand, 70.37% (n = 19), 59.26% (n = 16), 37.04% (n = 10), 25.93% (n = 7), 22.22% (n = 6) and 14.81% (n = 4) were found to be resistant to streptomycin, tetracycline, doxycycline, gentamicin, sulfamethoxazole and trimethoprim, respectively. None of the isolates was resistant to ciprofloxacin (Table 6). In addition, multidrug resistance analysis showed that 27/27 (100%) of tested *E. coli* O157:H7 isolates were resistant to different combinations of two or more antimicrobials. A multidrug-resistance pattern consisting of seven drugs was seen in 3/27 (11.11%) isolates (Table 7).

Table 1 Prevalence of *E. coli* O157:H7 from Different Sample Types in Dairy Supply Chains, Central Ethiopia

Sources	Sample Types	Study Areas								Total		
		Akaki Kaliti Sub-City			Bishoftu			Sululta				
		No of Examined	No of Positive	Percentage	No of Examined	No of Positive	Percentage	No of Examined	No of Positive	Percentage	No of Examined	No of Positive	Percentage
Farm	Milk	78	1	1.28	70	0	0.0	78	2	2.56	226	3	1.33
	Water	22	2	9.09	19	1	5.26	17	0	0.0	58	3	5.17
	Milker hand swab	24	2	8.33	21	1	4.76	20	3	15	65	6	9.23
Collection center	Milk	12	0	0.0	18	0	0.0	12	0	0.0	42	0	0.0
	Water	2	0	0.0	3	0	0.0	2	0	0.0	7	0	0.0
Cafeteria	Milk	8	4	50	10	2	20	8	3	37.50	26	9	34.62
	Water	8	3	37.50	10	2	20	8	1	12.5	26	6	23.08
Total		**154**	**12**	**7.79**	**151**	**6**	**3.97**	**145**	**9**	**6.21**	**450**	**27**	**6.00**

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Table 2 Association of Risk Factors with *E. coli* O157:H7 Occurrence Along Dairy Supply Chains

Risk Factors	No. Examined	No Positive (%)	Fisher's Exact p-value
Study areas			0.357
Akaki Kaliti	154	12 (7.79)	
Bishoftu	151	6 (3.97)	
Sululta	145	9 (6.21)	
Farm scales			0.193
Large	85	1 (1.18)	
Medium	145	4 (2.76)	
Small	119	7 (5.88)	
Source of samples			0.000
Cafeterias	52	15 (28.85)	
Collection centers	49	0 (0.0)	
Farm	349	12 (3.44)	
Type of samples			0.057
Milk	294	12 (4.08)	
Swab	65	6 (9.23)	
Water	91	9 (9.89)	
Source of water			0.000
Pipe	62	1 (1.61)	
Well	29	8 (27.59)	
Sampled materials			0.000
Bucket	226	3 (1.33)	
Collection containers	68	9 (13.24)	
Type of container			0.001
Plastic	217	19 (8.76)	
Stainless steel	168	2 (1.19)	

Table 3 Univariable Logistic Regression Analysis Based on Prevalence of *E. coli* O157:H7 in the Milk Samples and Associated Risk Factors

Risk Factors	No. Examined	No of Positive (%)	OR (95% CI)	p-value
Study areas				
Akaki Kaliti	98	5 (5.10)	Ref.	
Bishoftu town	98	2 (2.04)	0.39 (0.07, 2.05)	0.264
Sululta town	98	5 (5.10)	1 (0.28, 3.57)	1.000
Farm scales				
Medium	98	1 (1.02)	Ref.	
Large	63	0 (0.0)	–	
Small	65	2 (3.10)	3.1 (0.27, 34.67)	0.363
Sampled materials				
Bucket	226	3 (1.33)	Ref.	
Collection containers	68	9 (13.24)	11.34 (2.98, 43.21)	0.000
Source of samples				
Cafeterias	26	9 (34.62)	Ref.	
Collection centers	42	0 (0.0)	–	
Farm	226	3 (1.33)	0.03 (0.006, 0.10)	0.000
Source of water				
Pipe	62	3 (4.84)	Ref.	
Well	29	9 (31.03)	8.85 (2.18, 35.95)	0.002
Type of container				
Plastic	168	12 (7.14)	Ref.	
Stainless steel	126	0 (0.0)	–	

Table 4 Univariable Logistic Regression Analysis Based on Prevalence of *E. coli* O157:H7 in the Water Samples and Associated Risk Factors

Risk Factors	No. Examined	No of Positive (%)	OR (95% CI)	p-value
Study areas				
Akaki Kaliti sub-city	32	5 (15.63)	Ref.	
Bishoftu town	32	3 (9.38)	0.56 (0.12, 2.57)	0.454
Sululta town	27	1 (3.70)	0.21 (0.02, 1.9)	0.164
Source of samples				
Cafeterias	26	6 (23.10)	Ref.	
Collection centers	7	0 (0.0)	–	
Farm	58	3 (5.20)	0.2 (0.04, 0.79)	0.024
Source of water				
Pipe	62	1 (1.61)	Ref.	
Well	29	8 (27.59)	23.2 (2.74, 196.9)	0.004
Type of container				
Plastic	49	7 (14.28)	Ref.	
Stainless steel	42	2 (4.76)	0.3 (0.06, 1.53)	0.148

Table 5 Univariable Logistic Regression Analysis Based on Prevalence of *E. coli* O157:H7 in the Milker's Hand Swab Samples and Associated Risk Factors

Risk Factors	No. Examined	No of Positive (%)	OR (95% CI)	p-value
Study areas				
Akaki Kaliti sub-city	24	2 (8.33)	Ref.	
Bishoftu town	21	1 (4.76)	0.55 (0.05, 6.54)	0.636
Sululta town	20	3 (15)	1.94 (0.29, 12.95)	0.493
Farm scales				
Large	14	1 (7.14)	Ref.	
Medium	24	2 (8.33)	1.2 (0.09, 14.35)	0.896
Small	27	3 (11.11)	1.63 (0.15, 17.24)	0.687
Source of water				
Pipe	50	3 (6.0)	Ref.	
Well	15	3 (20.0)	3.92 (0.7, 21.9)	0.120

Table 6 Overall Antimicrobial Susceptibility Profile of *E. coli* O157:H7

Antimicrobials	Resistance	Intermediate	Susceptible
	N (%)	N (%)	N (%)
Ampicillin	27 (100)	0 (0.0)	0 (0.0)
Cefoxitin	27 (100)	0 (0.0)	0 (0.0)
Ciprofloxacin	0 (0.0)	2 (7.41)	25 (92.59)
Doxycycline	10 (37.04)	6 (22.22)	11 (40.74)
Gentamicin	7 (25.93)	4 (14.81)	16 (59.26)
Streptomycin	19 (70.37)	5 (18.52)	3 (11.11)
Sulfamethoxazole	6 (22.22)	6 (22.22)	15 (55.56)
Tetracycline	16 (59.26)	7 (25.93)	4 (14.81)
Trimethoprim	4 (14.81)	0 (0.0)	23 (85.19)

Table 7 The Fortitude Demonstration of Multidrug-Resistance Pattern of *E. coli* O157:H7 Isolates

Drug Types	Number of Isolates	%
Resistance to one drug	0	0.0
Resistance to two drugs	4	14.81
AMP*FOX	4	14.81
Resistance to three drugs	5	18.52
AMP*FOX*TE	2	7.41
AMP*FOX*S	2	7.41
AMP*RL*FOX	1	3.7
Resistance to four drugs	8	29.63
AMP*RL*FOX*S	3	11.11
AMP*FOX*DO*S	1	3.7
AMP*FOX*TE*S	3	11.11
AMP*FOX*GEN*TE	1	3.7
Resistance to five drugs	2	7.41
AMP*FOX*DO*TE*S	2	7.41
Resistance to six drugs	5	18.52
AMP*FOX*GEN*DO*TE*S	4	14.81
AMP*RL*FOX*TR*TE*S	1	3.7
Resistance to seven drugs	3	11.11
AMP*RL*FOX*DO*TR*TE*S	1	3.7
AMP*FOX*GEN*DO*TR*TE*S	2	7.41
Total	**27**	**100.0**

Note: *Indicates the word "and" (combination of resistant drugs).

Discussion

Food-borne diseases are one of the most serious public health issues in developing countries, including Ethiopia. Human infections with *E. coli* O157:H7 have largely been linked to animal-sourced meals.[37] Therefore, information on the incidence of these infections and their susceptibility to antimicrobials help the policy makers to develop appropriate strategies regarding prevention, treatment and control protocols.

Upon this study, from the different sample types examined the prevalence of *E. coli* O157:H7 was higher in the water samples (9.89%) when compared with milkers' hand swab (9.23%) and raw cow milk (4.08%) samples. Also, there were no statistically significant differences observed ($p = 0.057$) between the occurrence of the pathogen and source of samples.[38] This study showed a slightly higher rate of raw milk contamination by *E. coli* O157:H7 compared with the reports of[26] in and around Asosa western Ethiopia who have reported as 2.90%. Similarly, the current finding from raw cow milk was higher when compared with the reports from abroad by[39] in Libya (3.5%),[40] in Nigeria (2%),[41] in Greece (0.74%),[42] in Egypt (0.5%),[43] in Turkey (0.0%),[44] in Tanzania (0.0%) and[45] in Ghana (0.0%). The greater finding in this study could be attributed to variations in dairy farming type, such as the emphasis on livestock systems in other countries, geographic location, and the level of sanitary precautions.

Meanwhile, the current finding from raw cow milk was lower when compared with the reports of[46] in Iraq,[31] in Holeta and[27] in Bishoftu towns who have reported prevalence estimates of 4.67%, 6.9% and 12%, respectively. The variation in *E. coli* O157:H7 contamination frequency reported in different research could be attributed to differences in sample size, dairy farming system, milking techniques, location, and sanitary procedures applied across different regions and study areas. As a result, the presence of *E. coli* O157:H7 does not necessarily indicate direct faecal contamination of milk, but it is an indicator of poor hygiene and unclean practices during milking and subsequent milk handling, and it poses a potential risk to people who consume such products.[47]

On the other hand, in this study, *E. coli* O157:H7 was isolated from 9.89% of the water samples. Also, from the different sampling points, a relatively higher contamination frequency of water by the pathogen was observed in water

samples collected from cafeterias (23.10%) when compared with water collected from dairy farms (5.20%) and the variation was not statistically significant (p = 0.198).[38] However, this study showed a higher rate of water contamination (9.89%) by the pathogen compared with the reports of[28] in Modjo and[48] in Batu towns who have reported 4.3% and 3.3% contamination frequency, respectively. The discrepancies seen could be attributed to variances in methodologies used, the absence of well water treatment or disinfection, carelessness in water usage, sample size, and geographical regions where the investigations were done. Consequently, the bacteriological quality of water destined for sanitation and domestic uses is affected by quality status at the source and water handling practices during collection, transportation, and storage; comparable thoughts were reflected by.[49]

Similarly, in the current study, *E. coli* O157:H7 was isolated from 9.23% of the milkers' hand swab in the dairy farms. In addition, a higher contamination frequency of the pathogen was observed from dairy farms in Sululta town (15%) when compared with dairy farms in Akaki Kaliti sub-city (8.33%) and Bishoftu town (4.76%), by which the variation was not statistically significant (p > 0.05).[38] However, compared to this study,[50] Msolo et al in South Africa have reported 7% isolation frequency and this was lower than the present finding. The observed variation could be attributed to differences in hygiene procedures, sample size, geographical location, and water supply used between the various locations and study sites. In the course of the study, from the total 27 isolates tested ≥50% susceptibility to the antimicrobials was found to be ciprofloxacin (92.5%), trimethoprim (85.19%), gentamicin (59.26%) and sulfamethoxazole (55.56%). The current finding was in line with the reports of[51] in Nigeria,[52] in Bangladesh and[27] in Bishoftu town who 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. However, the current finding was in contrast to the reports of[26] in and around Asosa town who have reported 36.4% to gentamicin. The observed variance could be attributed to the pathogen's expression of resistant gene code, which is related with emerging and re-emerging characteristics of the isolates in terms of diverse agro-ecology.[51]

On the other hand, the finding revealed that ≥50% of *E. coli* O157:H7 isolates were found to be resistant to ampicillin, cefoxitin, streptomycin and tetracycline. This was in agreement with the findings of[26] in and around Asosa and[27] in Bishoftu towns who have reported ≥50% of resistance rate to ampicillin, streptomycin and cefoxitin. Similarly,[26,51,52] Disassa et al, Reuben et al and Alam et al have reported ≥50% resistance rate to tetracycline. As a result, the emergence of resistance could be attributed to the inappropriate or indiscriminate use of antibiotics for therapeutic and preventive purposes in both *E. coli* and other illnesses. In the present study, 27/27 (100%) of *E. coli* O157:H7 isolates exhibited resistance to at least two or more of the nine antimicrobial agents tested. Compared to this finding,[27] in Bishoftu town has reported 100% resistance of all the isolates to more than two drugs from milk. Also,[53] and[54] have also reported 100% and 73.3% resistance rate of the isolates to two or more drugs, respectively, in an abattoir-based study conducted in Ethiopia and this was in agreement with this finding. Likewise, in this study, the higher rate of multidrug-resistance was observed for four drugs (29.63%) followed by six (18.52%), three (18.52%) and two (14.81%) drugs. In contrast to this, the resistance of (57.1%) to two (28.6%) to three and (14.3%) to four drugs were reported by Bedasa et al.[27] Also, the resistance of 71.4% and 28.6%, and 40% and 13.3% for three and four drugs were also reported by Bekele et al[24] and Atnafie et al,[54] respectively, from an abattoir-based investigation, Ethiopia. As a result, the emergence of antibiotic resistance in bacteria such as *E. coli* is a major public health concern. The variation in multidrug resistance development for bacteria may be due to variations in dose, route of administration, regimen, and continuous and indiscriminate use of antimicrobials for treatment and feed additive in various study areas, as well as level of awareness and geographic location for studies abroad. Because of the rapid development of multidrug resistance, the efficiency of treatments and the ability to manage infectious diseases in both animals and people may be significantly limited.[5]

Conclusion

In conclusion, the presence of E. coli O157:H7 in raw cow milk, milker hand swabs, and water samples collected along the dairy supply chain indicates a possible concern to public health and food safety. The pathogen was more prevalent in samples collected from the Akaki Kaliti sub-city. The presence of several antibiotic-resistant *E. coli* O157:H7 strains poses a risk to public health and food safety, as well as animal health and production. In general, this study provided an initial baseline of *E. coli* O157:H7 occurrence in dairy supply chains. Dairy farms should therefore undertake hygienic

methods like as hand cleaning between milking, udder washing before and after milking, and the application of teat dip with iodine tincture.

Abbreviations

BPW, Buffered peptone water; SMAC, Sorbitol MacConkey agar.

Data Sharing Statement

All data generated during this study are available upon request of the correspondence author.

Ethical Approval and Consent to Participate

Before any attempt to collect sample, the protocol was approved by Addis Ababa University College of Veterinary Medicine and Agriculture animal research ethical committee with reference number VM/ERC/21/05/10/2018.

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Author Contributions

All authors made a significant contribution to the work reported, 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.

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Disclosure

The authors have no conflicts of interest to declare. This manuscript is a part of an MVSc thesis entitled as "Epidemiology and assessment of critical control points of E. coli O157:H7 along dairy supply chains in central Ethiopia" and you can get online access to the full thesis paper in the repository directorate of Addis Ababa University.

References

1. Rahimi E, Momtaz H, Anari M, Alimoradi M, Momen M, Riahi M. Isolation and genomic characterization of *Escherichia coli* O157: NM and *Escherichia coli* O157: H7 in minced meat and some traditional dairy products in Iran. *African J Biotechnol*. 2012;11:2328–2332. doi:10.5897/AJB11.2167
2. Ali N, Farooqui A, Khan A, Khan A, Kazmi S. Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan. *J Infect Dev Countries*. 2010;4:382–388. doi:10.3855/jidc.599
3. Javaid S, Gadahi J, Khaskeli M, Bhutto M, Kumbher B, Panhwar A. Physical and chemical quality of market milk sold at Tandojam, Pakistan. *Pak Vet J*. 2009;29:27–31.
4. Popescu A, Angel E. Analysis of milk quality and its importance for milk processors. *Lucrări Tiinþifice Zootehnie Biotechnol*. 2009;42:501–506.
5. Thaker H, Brahmbhatt M, Nayak J. Study on occurrence and antibiogram pattern of *E. coli* from raw milk samples in Anand, Gujarat, India. *Vet World*. 2012;5:556–559. doi:10.5455/vetworld.2012.556-559
6. Riemann P, Cliver O. *Foodborne Infection and Intoxication*. 3rd ed. San Diego, California, USA: Academic Press; 2006:205–258.
7. Oliver S, Boor K, Murphy S, Murinda S. Food safety hazards associated with consumption of raw milk. *J Foodborne Pathogens Dis*. 2009;6:793–806. doi:10.1089/fpd.2009.0302
8. Bandyopadhyay S, Lodh C, Rahaman H, et al. Characterization of shiga toxin-producing (STEC) and enteropathogenic *Escherichia coli* (EPEC) in raw yak (*Poephagus grunniens*) milk and milk products. *J Res Vet Sci*. 2012;93:604–610. doi:10.1016/j.rvsc.2011.12.011
9. Gordillo R, Cardoba J, Andrade M, Luque M, Rodriguez M. Development of PCR assays for detection of *Escherichia coli* O157: H7 in meat products. *J Meat Sci*. 2011;88:767–773. doi:10.1016/j.meatsci.2011.03.011
10. Varela-Hernández J, Cabrera-Diaz E, Cardona-Lopez M, et al. Isolation and characterization of Shiga toxin-producing *Escherichia coli* O157: H7 and non-O157 from beef carcasses at a slaughter plant in Mexico. *Int J Food Microbiol*. 2007;113:237–241. doi:10.1016/j.ijfoodmicro.2006.06.028
11. Su H, Ma Q, Shang K, Liu T, Yin H, Ai S. Gold nanoparticles as colorimetric sensor: a case study on *E. coli* O157: H7 as a model for Gram-negative bacteria. *Sensors Actuators B Chem J*. 2012;161:298–303. doi:10.1016/j.snb.2011.10.035

12. Majowicz S, Scallan E, Jones-Bitton A, et al. Global incidence of human Shiga toxin-producing *Escherichia coli* infections and deaths: a systematic review and knowledge synthesis. *J Foodborne Pathogens Dis*. 2014;11:447–455. doi:10.1089/fpd.2013.1704
13. Rangel J, Sparling P, Crowe C, Griffin P, Swerdlow D. Epidemiology of *Escherichia coli* O157: H7 outbreaks in United States, 1982–2002. *Emerg Infect Dis J*. 2005;11:603–609. doi:10.3201/eid1104.040739
14. Scallan E, Hoekstra R, Angulo F, et al. Foodborne illness acquired in the United States-major pathogens. *Emerg Infect Dis J*. 2011;17:7–15. doi:10.3201/eid1701.P11101
15. Scharff R. Economic burden from health losses due to foodborne illness in the United States. *J Food Prot*. 2012;75:123–131. doi:10.4315/0362-028X.JFP-11-058
16. Chekabab S, Paquin-Veillette J, Dozois C, Harel J. The ecological habitat and transmission of *Escherichia coli* O157: H7. *FEMS Microbiol Lett*. 2013;341:1–12. doi:10.1111/1574-6968.12078
17. Pal M, Mulu S, Tekle M, Pintoo S, Prajapati J. Bacterial contamination of dairy products. *J Beverage Food World*. 2016;43:40–43.
18. Sikora T, Niewczas M, Prusak A. *Ethics and Trust in Quality Assurance*. In: Proceedings of the 57th EOQ Congress. 17-20 June 2013. Tallinn, Estonia; 2013:1–8.
19. Bruno A, Carolissen-Mackay V. Antimicrobial resistance and the activities of the Codex Alimentarius Commission. *Revue Scientifique Et Technique OIE*. 2012;31:317–323. doi:10.20506/rst.31.1.2122
20. Erb A, Sturmer T, Marre R, Brenner H. Prevalence of antibiotic resistance in *Escherichia coli*: overview of geographical, temporal and methodological variations. *Eur J Clin Microbiol Infect Dis*. 2007;26:83–90. doi:10.1007/s10096-006-0248-2
21. Gebre-Sealsssie S. Antimicrobial resistance patterns of clinical bacterial isolates in southwestern Ethiopia. *Ethiop Med J*. 2007;45:363–370.
22. Hiko A, Asrat D, Zewde G. Occurrence of *Escherichia coli* O157: H7 in retail raw meat products in Ethiopia. *J Infect Dev Countries*. 2008;2:389–393. doi:10.3855/jidc.203
23. Taye M, Berhanu T, Berhanu Y, Tamiru F, Terefe D. Study on carcass contaminating *Escherichia coli* in apparently healthy slaughtered cattle in Haramaya University slaughter house with special emphasis on *Escherichia coli* O157: H7, Ethiopia. *J Vet Sci Technol*. 2013;4:1–4.
24. Bekele T, Zewde G, Tefera G, Feleke A, Zerom K. *Escherichia coli* O157: H7 in raw meat in Addis Ababa, Ethiopia: prevalence at an abattoir and retailers and antimicrobial susceptibility. *Int J Food Contamin*. 2014;1:4–9. doi:10.1186/s40550-014-0004-9
25. Mohammed O, Shimelis D, Admasu P, Feyera T. 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.
26. Disassa N, Sibhat B, Mengistu S, Muktar Y, Belina D. Prevalence and antimicrobial susceptibility pattern of *E. coli* O157: H7 isolated from traditionally marketed raw cow milk in and around Asosa town, western Ethiopia. *J Vet Med Int*. 2017;2017:1–7.
27. Bedasa S, Shiferaw D, Abraha A, Moges T. Occurrence and antimicrobial susceptibility profile of *Escherichia coli* O157: H7 from food of animal origin in Bishoftu town, central Ethiopia. *Int J Food Contamin*. 2018;5:1–8. doi:10.1186/s40550-018-0064-3
28. Mersha G, Asrat D, Zewde B, Kyule M. Occurrence of *Escherichia coli* O157: H7 in faeces, skin and carcasses from sheep and goats in Ethiopia. *Lett Appl Microbiol*. 2010;50:71–76. doi:10.1111/j.1472-765X.2009.02757.x
29. CSA (Central Statistical Agency). *Central Statistical Agency of the Federal Democratic Republic of Ethiopia: Agricultural Samples Enumeration Statistical Abstract*. Addis Ababa, Ethiopia: CSA (Central Statistical Agency); 2015:446–539.
30. NMSA (National Meteorological Service Agency). *Rainfall and Temperature Data*. Addis Ababa, Ethiopia: NMSA (National Meteorological Service Agency); 2017.
31. Ayano A, Hiriko F, Simyalew A, Yohannes A. Prevalence of subclinical mastitis in lactating cows in selected commercial dairy farms of Holeta district. *J Vet Med Animal Health*. 2013;5:67–72.
32. Dohoo I, Martin W, Stryhn H. *Veterinary Epidemiologic Research*. Charlotte town, Prince Edward Island, Canada: AVC Inc; 2003:27–52.
33. ISO (International Organization for Standardization) 18593. *Microbiology of Food and Animal Feeding Stuffs: Horizontal Methods for Sampling Techniques from Surfaces Using Contact Plates and Swabs*. 1st ed. Geneva: ISO (International Organization for Standardization) 18593; 2004:1–14.
34. Quinn P, Carter M, Markey B, Carter G. *Clinical Veterinary Microbiology*. London, UK: Mosby Ltd; 2004:95–101.
35. CLSI (Clinical and Laboratory Standards Institute). *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI Document M100-S25*. Vol. 35 No. 3. Wayne, PA: CLSI (Clinical and Laboratory Standards Institute); 2015:32–194.
36. Hill D, Rose B, Pajkos A, et al. Antibiotic susceptibilities of *Pseudomonas aeruginosa* isolates derived from patients with cystic fibrosis under aerobic, anaerobic, and biofilm conditions. *J Clin Microbiol*. 2005;43:5085–5090. doi:10.1128/JCM.43.10.5085-5090.2005
37. Jo M, Kim J, Lim J, et al. Prevalence and characteristics of *Escherichia coli* O157 from major food animals in Korea. *Int J Food Microbiol*. 2004;95:41–49. doi:10.1016/j.ijfoodmicro.2004.01.016
38. Dejene H. *Epidemiology and Assessment of Critical Control Points of E. coli O157: H7 Along Dairy Supply Chains in Central Ethiopia. MVSc thesis*. Addis Ababa University College of Veterinary Medicine and Agriculture. Bishofu, Ethiopia. 2018. Available from: http://etd.aau.edu.et/handle/123456789/12299.
39. Garbaj A, Awad E, Azwai S, et al. Enterohemorrhagic *Escherichia coli* O157 in milk and dairy products from Libya: isolation and molecular identification by partial sequencing of 16S rDNA. *Vet World*. 2016;9:1184–1189. doi:10.14202/vetworld.2016.1184-1189
40. Ivbade A, Ojo O, Dipeolu M. Shiga toxin-producing *Escherichia coli* O157: H7 in milk and milk products in Ogun state, Nigeria. *Vet Ital*. 2014;50:185–191. doi:10.12834/VetIt.129.2187.1
41. Solomakos N, Govaris A, Angelidis A, et al. Occurrence, virulence genes and antibiotic resistance of *Escherichia coli* O157 isolated from raw bovine, caprine and ovine milk in Greece. *Int J Food Microbiol*. 2009;26:865–871. doi:10.1016/j.fm.2009.06.002
42. Ahmed A, Shimamoto T. Isolation and molecular characterization of *Salmonella enterica, Escherichia coli* O157: H7 and *Shigella* spp. from meat and dairy products in Egypt. *Int J Food Microbiol*. 2014;168:57–62. doi:10.1016/j.ijfoodmicro.2013.10.014
43. Sancak Y, Sancak H, Isleyici O. Presence of *E. coli* O157 and O157: H7 in raw milk and van herby cheese. *Bull Vet Inst Pulawy*. 2015;59:511–514. doi:10.1515/bvip-2015-0076
44. Swai E, Schoonman L. Microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania. *Asian Pac J Trop Biomed*. 2011;1:217–222. doi:10.1016/S2221-1691(11)60030-0
45. Addo K, Mensah G, Aning K, et al. Microbiological quality and antibiotic residues in informally marketed raw cow milk within the coastal Savannah zone of Ghana. *J Trop Med Int Health*. 2011;16:227–232. doi:10.1111/j.1365-3156.2010.02666.x

46. Basil A, Khudor M, Abid-Smeasem O. Detection of verotoxigenic *E. coli* O157: H7 in raw milk using duplex PCR in Basra city, Iraq. *Mirror Res Vet Sci Animals*. 2012;1:25–33.
47. Arafa M. Bacteriological quality and safety of raw cow's milk and fresh cream. *Slovenian Vet Res*. 2013;50:21–30.
48. Mekonnen M, Assefa F, Lemma B, Von-haaren C, Haaren P. Assessing the occurrence of waterborne pathogens in Lake Ziway and drinking water system of Batu (Ziway) town, Ethiopia. *Ethiop J Health Dev*. 2014;28:116–125.
49. Rufener S, Mausezahl D, Mosler H, Weingartner R. Quality of drinking water at source and point of consumption drinking cup as a high potential recontamination risk: a field study in Bolivia. *J Health Popul Nutr*. 2010;28:34–41. doi:10.3329/jhpn.v28i1.4521
50. Msolo L, Igbinosa E, Okoh A. Prevalence and antibiogram profiles of *Escherichia coli* O157: H7 isolates recovered from three selected dairy farms in the Eastern Cape Province, South Africa. *Asian Pacific J Trop Dis*. 2016;6:990–995. doi:10.1016/S2222-1808(16)61170-2
51. Reuben R, Owuna G. Antimicrobial resistance patterns of *Escherichia coli* O157: H7 from Nigerian fermented milk samples in Nasarawa state, Nigeria. *Int J Pharm Sci Invent*. 2013;2:38–44.
52. Alam M, Akther S, Sarwar N, Morshed S, Debnath G. Prevalence and antimicrobial susceptibility of *E. coli* O157 isolated from raw milk marketed in Chittagong, Bangladesh. *Turk J Agric Food Sci Technol*. 2017;5:214–220. doi:10.24925/turjaf.v5i3.214-220.976
53. Dulo F, Feleke A, Szonyi B, Fries R, Baumann M, Grace D. Isolation of multidrug-resistant *Escherichia coli* O157 from goats in the Somali region of Ethiopia: a cross-sectional, Abattoir-based study. *PLoS One*. 2015;10:e0142905. doi:10.1371/journal.pone.0142905
54. Atnafie B, Paulos D, Abera M, et al. Occurrence of *Escherichia coli* O157: H7 in cattle faeces and contamination of carcass and various contact surfaces in abattoir and butcher shops of Hawassa, Ethiopia. *J BMC Microbiol*. 2017;17:24–30. doi:10.1186/s12866-017-0938-1