RESEARCH Open Access

Occurrence of *Escherichia Coli* O157:H7 in lactating cows and dairy farm environment and the antimicrobial susceptibility pattern at Adami Tulu Jido Kombolcha District, Ethiopia

Frehiwot Mesele^{1,2*}, Samson Leta², Kebede Amenu² and Fufa Abunna²

Abstract

Background Food-borne pathogens are the foremost causes of food-borne human illness in the world. *Escherichia coli* O157:H7 (*E. coli* O157:H7) is one of the major food-borne pathogenic bacteria around the world. Though evidence is lacking; especially in developing countries like Ethiopia, the potential health impact of *E. coli* O157:H7 can be high where food production, handling and consumption is often taking place under unhygienic conditions. In Ethiopia, studies reported *E. coli* and *E. coli* O157: H7 from food of animal origin, mainly meat and milk, and also animal surfaces and feces. The objective of the present study was to investigate the occurrence of *E. coli* O157:H7 in raw milk and the dairy production farm environment and further assess the antimicrobial resistance pattern of the bacterium.

Methods Samples of milk from individual lactating cows' and dairy farm environmental samples (feces, water and manure) were collected at Adami Tulu Jido Kombolcha district (ATJKD) and analyzed for the presence of *E. coli* O157:H7. Standard microbiological techniques including culture, biochemical testing and serological test were performed to isolate and identify the bacterium. The bacterial isolates were evaluated for antimicrobial susceptibility patterns using disk diffusion method. A questionnaire was used to collect possible factors affecting *E. coli* O157:H7 occurrence.

Results The overall prevalence of *E. coli* O157:H7 was 4.7% (19/408) (95% CI: 2.6; 6.7). Out of 19 *E. coli* O157:H7 isolates, 4/50, 7/154, 2/50, and 6/154 were from water, milk, manure, and feces samples, respectively. From potential risk factors considered in this study area, floor type, cleaning of pens, milking location and hand washing during the time of milking were significantly associated with the occurrence of *E. coli* O157:H7. The antimicrobial susceptibility pattern indicated varying degrees of resistance. All of the isolates were found to be resistant ampicillin, cephalothin, and rifampin, and 100% susceptibility was observed against the drugs: chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, kanamycin, and tetracycline. Concerning streptomycin, 63.15% of the isolates were susceptible and 36.8% showed intermediate susceptibility.

Conclusions The occurrence of multi-drug resistance *E. coli* O157:H7 observed both in lactating cows and in dairy farm environments can sustain a continuous transmission of the bacteria. The occurrence of multidrug-resistant *E. coli* o157:H7could hamper the control and prevention efforts.

*Correspondence: Frehiwot Mesele frehiwotmesele2021@yahoo.com Full list of author information is available at the end of the article



Keywords Antimicrobial susceptibility test, Dairy farm environment, E. coli O157:H7, Lactating cows

Introduction

Foodborne pathogens are prominent causes of foodborne illness with huge public health and economic consequences [1]. They place a heavy burden costing billions of dollars in medical care, social costs, and overall economic and infrastructure effects on countries [2]. Foodborne diseases disproportionately affecting developing countries of the world due to major contributing factors such as overcrowding, poverty, changes in eating habits, mass catering, complex and lengthy food supply procedures with increased international movement, inadequate sanitary conditions, and poor general hygiene practices [3, 4]. The emergence of major foodborne pathogens such as Salmonella and E. coli has persisted as a public health concern of foodborne pathogens despite considerable efforts aimed at prevention and control [5]. Around 2 million people die per year due to diseases of foodborne pathogens [6]. Consumption of raw/undercooked meat, vegetables, and fruits are the main sources of infection that leads to public health significance of E. coli O157:H7 [7, 8].

E. coli O157:H7 are commonly found in farm animals in cattle gut [9, 10]. Contaminated feed and water, the immediate environment of the animal, and cattle feces have been considered the primary source of animal origin of E. coli O157:H7 in human infection [9, 11]. E. coli O157:H7 can cause from mild cases of diarrhea to severe illness in humans such as hemorrhagic colitis, hemolytic-uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP) leading to death [12, 13]. In Ethiopia, raw milk, minced raw meat [14, 15], cream, creamed fish, vegetables, and poultry and their products are regarded as high-risk commodities in respect of pathogen contents, natural toxins, and other possible contaminants and adulterant [16–18].

The farm environment is the main factor in sustaining a population of viable *E.coli* O157:H7 which can survive in feces, manure, pen surfaces, bedding, flooring, and water [10, 19–23]. Specifically, cattle manure could allow prolonged survival of the bacterium outside the host [24]. Contaminated drinking water may contribute to the dissemination and maintenance of *E. coli* O157:H7 on farms [21].

Antimicrobials are used as the therapeutic agents in humans and in animals as therapeutics or prophylaxis.. Inadequate selection and abuse of antimicrobials may lead to resistance in various bacteria and make the treatment of bacterial infections difficult [25]. Antimicrobial resistance in *E. coli* O157:H7 has been reported

worldwide [26]. Treatment for *E. coli* infection has been increasingly complicated by the emergence of resistance to most first-line antimicrobial agents [27]; including ampicillin, amoxicillin, ceftriaxone, chloramphenicol, ciprofloxacin, cotrimoxazole and tetracycline [28–30].

In Ethiopia, studies stated that the presence of multidrug resistance (MDR) in various livestock production systems to diverse antimicrobial drugs. In Ethiopia, the prevalence of MDR ranges from 5.7 to 100% according to Hiko et al. [31], Messele et al [32] Shecho et al [33], Abebe et al [34], Haile et al [35], Mohammed et al [16], Dejene et al [36], Haile [37], Tassew [38] and Taye et al [39] from raw meat, and dairy products at Bishoftu, Modjo, Addis Ababa, Haramaya, Tigray testing resistance of different human importance antibiotics including Ampicillin, cephalothin, gentamicin and tetracycline.

In developing countries like Ethiopia, the production of animal source foods often takes place under unhygienic conditions and; consumption of raw products can be common. Due to the unhygienic handling, the foods might be contaminated by feces, manure, and poor quality water in dairy farms with further potential hazards and source of infection for humans [9].. Epidemiology of food-borne pathogens specifically that of *E*. coli O157: H7 is not well studied. But recently, considerable studies have been reported on the occurrence of E. coli O157: H7 from food of animal origin mainly raw meat from abattoirs, butcher shops, and restaurants according to these authors [8, 14, 15, 31, 32, 34, 35, 38-45]; Similarly, reports of the occurrence of the pathogen from dairy products from farms, milk vendors and supermarkets are available [15, 34–36, 46–49]. Some studies also covered isolation of the pathogen from feces, water, and contact surfaces such as farms and slaughterhouses [8, 36, 37, 40, 41, 44, 45] in different parts of Ethiopia. The isolation of the pathogen associated with common practices of raw animal products consumption in many circumstances increase the risk of E. coli O157:H7 infection. The close contact of humans with cattle feces and manure can be another direct source of infection to humans.

The prevalence estimates ranged from 0.01 to 13.4%. Assefa and Bihon [50] conducted a meta-analysis to estimate the pooled prevalence of *E. coli* and *E. coli* O157:H7 and reported a pooled prevalence of 15% (95% $\rm CI=13-17\%$) for *E. coli* and 4% (95% $\rm CI=3-5\%$) for *E. coli* O157:H7. Therefore studies revealed that *E.*

coli O157:H7 is an important food-borne pathogen in humans and MDR is a major problem and there is limited information on the occurrence of *E. coli O157:H7* and its antibiotic resistance pattern in lactating cow and dairy farm environment in the present study area. Thus, this study aimed to assess the occurrence of *E. coli* O157:H7 in lactating cows and dairy environments. In addition, risk factors associated with *E. coli* O157:H7 occurrence and antimicrobial resistance profile of the isolates were evaluated.

Materials and methods

Study area

Adami Tulu Jido Kombolcha district (ATJK) is found in the mid-Rift Valley at 7° 9′N latitude and 38° 7 'E longitude Livestock production is the dominant farming system and crop production is not common. Dairy cattle are mostly reared in small to medium scale dairy operations, in which animals are managed both intensively and extensively. They are often provided with some supplementary diet in addition to the natural pasture and agricultural by-products [51]. But there is no large-scale dairy farm in the area and herd size in the dairy farms ranges 3 to 31 heads of cattle.

Study design, sampling methods and sample size determination

The study population was lactating dairy cows in ATJK district and comprises exotic, crossbred and local breeds in small and medium scale dairy farms managed under intensive and extensive management conditions. At the animal level, raw milk and feces samples were collected from the teat and rectum of selected dairy cows, respectively. From the dairy farm environment, manure and water samples were also collected. Semi-structured questionnaire was used to interview the farm owners (supplementary file).

A stratified random sampling method was used to sample dairy farms. The farms were categorized based on their herd size into three strata (small-scale < 10 animals), medium-scale (10 to 50 animals) and large-scale (> 50 animals) using the classification made by Megersa et al [52]. Based on the data obtained from the district live-stock and fishery agency there are 22 medium, 68 small-scale and no large-scale dairy farm in the district. Then dairy farms and individual lactating cows were selected using simple random sampling method. Random sampling technique was used to recruit farmers and there was no compensation given to them and their participation was on voluntary basis with their full consent.

Sample size was determined by using the formula given by Thrusfield [53] with consideration of 95% confidence interval and 5% precision. The expected prevalence was set at 10.4% based on a previous study conducted by Abebe et al [34] resulting in 144.. Thus, 154 dairy cows in 50 dairy farms were sampled. The total sample size then became 408 (154 milk samples, 154 feces samples, 50 water and 50 manure samples).

Sample collection

All the samples after collection were tagged by animal ID, date of sampling and sample type. The samples were transported to the Veterinary Microbiology of Laboratory, of the College of Veterinary Medicine and Agriculture of Addis Ababa University using an icebox maintaining a cold chain for microbiological analysis. Upon arrival, the samples were stored in a refrigerator at 4°C for 24 hours until being processed for isolation as described by Quinn et al. [54].

Milk samples were collected directly from teats by sterile screw topped universal bottle. The fecal samples were collected using a sterile stomacher bag aseptically directly from the rectum and stored in an icebox until analysis (within 24 hours). Milk and feces samples were collected from the same animal. Water samples (10 ml) were collected using sterile capped universal bottles. Pooled manure samples were also collected from the selected dairy farms using sterile stomacher bags from different points including pen, floor surface and dung storage area. Isolation and identification of *E. coli* O157:H7 was made based on the colony morphology in different media, staining characteristics and biochemical properties [55].

Sample processing

Non-selective pre-enrichment was necessary for the effective recovery of low levels of stressed *E. coli* O157. All sorbitol non-fermenting colonies that reacted with O157 were considered presumptive E. coli O157:H7 for further analysis. Thus, enrichment was conducted according to OIE guidelines [56] All enriched samples were cultured on a sterilized Sorbitol MacConkey (SMAC) agar plate, (CM0813, Oxoid Basingstoke, England) and the confirmed pure cultures were transferred to nutrient agar to be used for additional biochemical and serological confirmation as described by Quinn et al. [54]. The non-sorbitol fermenting (NSF) E. coli (colorless or pale colonies) was considered as E. coli O157: H7 strains whereas pinkish-colored colonies (sorbitol-fermenters) were considered as non-O157:H7 E. coli strains. The NSF isolates were again subjected to latex *E*. coli O157: H7 agglutination test for confirmation.

Serological confirmation of *Escherichia* strains possessing the O157 serotype antigen was done using dry spot *E.coli* O157 latex agglutination test according to the manufacturer's instruction (Oxoid, DR120M).

Antimicrobial susceptibility testing

Antimicrobial susceptibility tests were performed by standard disc diffusion technique using commercially available antimicrobial disks. Antimicrobial disks containing ampicillin ($10\,\mu g$), cephalothin ($30\,\mu g$), ciprofloxacin ($5\,\mu g$), chloramphenicol ($30\,\mu g$), gentamicin ($10\,\mu g$), kanamycin ($30\,\mu g$), nalidixic acid ($30\,\mu g$), rifampin ($5\,\mu g$), streptomycin ($10\,\mu g$) and tetracycline ($30\,\mu g$) (HI media, India) were used. Subsequently, the diameter of the inhibition zone created around each disk was measured using a digital caliper. The results were classified as sensitive, intermediate and resistant according to the standard supplied by CLIS [57] and multidrug resistance refers to the resistance of a single isolate against more than two drugs.

Questionnaire survey

A semi-structured questionnaire was used to collect additional data on demographic characteristics, milking system, milking and hygienic practices (washing of milkers' hands, milk utensils and udder before milking), farmers' awareness of cattle and milk-borne zoonoses, transmission routes, sources of farm water, housing management. The questionnaire was pretested on five dairy farm owners. The interview was made in local language (Afaan Oromo or Amharic). All 50 farm owners were interviewed through face-to-face conversation.

Data management and statistical analysis

Data were entered into a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA). Descriptive statistics (determination of proportions) were used to summarize the data. The overall prevalence of E. coli O157: H7 in milk, feces and environmental samples was estimated using the standard formula. R statistical software Version 3.3.2 [58] was used to analyze the data. Pearson chi-square, Pearson's Chisquared test with Yates' continuity correction and fisher exact tests were used to assess the association of different risk factors with the occurrence of E. coli O157:H7. Univariable and multivariable binary logistic regression analysis were performed to quantify crude and adjusted effect of the risk factors on the occurrence of E. coli O157: H7. The step AIC function in 'MASS' package was used to select the final multivariable logistic regression model using backward variable elimination process. Likelihood ratio test was used to compare models during model selection. P-value less than 5% (P < 0.05) was considered statistically significant.. In cases of estimating the effect of different risk factors in terms of Odds ratio (OR) with corresponding 95% confidence interval, statistical significance was assumed if the confidence interval did not include one among its value.

Results

Occurrence of E. coli O157:H7

Out of 408 samples collected and processed, 19 were positive for *E.coli* O157:H7. The prevalence of *E. coli* O157:H7 in lactating cows (milk and feces) and dairy farm environment (water and manure) at ATJK district were found to be 4.7%. (95% CI: 2.6; 6.7). Of these positive cases, the isolation of *E. coli* O157:H7 was the highest in water sample 4(8%), followed by milk samples 7 (4.5%), in manure 2(4%) and 6 (3.9%) in feces as presented in Table 1. Prevalence of *E. coli* O157:H7 was isolated in 11 (7.1%) individual cows with the reference of feces and milk and 14 (28.0%) at the farm level based on all types of samples.

Univariable analysis of the association of *E. coli* O157:H7 with different risk factors

The effect of potential risk factors on the occurrence of $E.\ coli$ O157:H7 was assessed and from the risk factors considered, cleaning of pens, milking location, use of towels and hand washing during the time of milking had a statistically significant impact on the occurrence of $E.\ coli$ O157:H7 (P<0.05) using univariable logistic regression analysis. On the contrary, factors such as the breed of the animal, herd size, area, floor type, use of detergent and history of mastitis did not show significant differences (p>0.05) Table 2.

Multivariable analysis of the association of *E. coli* O157:H7 with different risk factors

From potential risk factors considered in this study (Table 3), area, floor type, cleaning of pens, milking location and hand washing during the time of milking were significantly associated (P<0.05) with the occurrence of E. coli O157:H7. As shown in Table 3, the odds ratio of E. coli O157:H7 occurrence was 9.32 times higher in urban areas than in rural areas. In pens

Table 1 Occurrence of *E. coli* O157:H7 in different sample type

Sample type	Total sample examined	E. coli isolates	E. coli O157:H7 strains	
Milk	154	15 (9.7)	7 (4.5)	
Feces	154	16 (10.4)	6 (3.9)	
Water	50	6 (12)	4 (8)	
Manure	50	5 (10)	2 (4)	
Overall	408	42 (10.3)	19 (4.7)	

Mesele et al. BMC Veterinary Research (2023) 19:6 Page 5 of 10

Table 2 Univariable logistic regression analysis of *E.coli* O157:H7 occurrence with various risk factors

Risk factors	E. coli 0157:H7						
	No. examined	No. of positive	X ²	P-value	Crude OR	95% CI OR	
Area							
Rural	120	8	0.61	_	_	_	
Urban	269	11		0.306	0.61	0.24-1.60	
Breed							
Exotic	101	1	*	-	-	-	
Cross	183	13		0.059	7.17	0.93-55.65	
Local	105	5		0.155	4.81	0.55-41.89	
Cleaning of pens							
No stay overnight	133	13	7.81 ^a	_	_	_	
Stay overnight	256	6		0.005**	4.17	1.61-12.5	
Herd size (Farm scale)							
Medium scale	137	3	2.23	_	_	_	
Small scale	252	16		0.095	2.90	0.94-12.62	
Milking location							
In barn	151	13	5.43 ^a	_	_	_	
Anywhere	238	6		0.005**	3.45	1.32-10.00	
Hand wash							
Before and after milking	377	15	*				
Only before milking	12	4		0.000***	8.37	2.15-27.49	
Floor type							
Earthen	214	8	0.75 ^a	_	_	_	
Concrete	175	11		0.275	1.68	0.67-4.42	
Use of towel							
No use of towel	96	8	*	_	_	_	
Before milking	174	9		0.310	0.60	0.22-1.61	
Before and after milking	138	2		0.023	0.16	0.03-0.78	
Use of detergent							
No	52	2	*	_	_	_	
Yes	356	17		0.767	1.25	0.35-8.06	
History of mastitis							
No	104	8	*	_	_	_	
Yes	304	11		0.096	0.45	0.18-0.19	

OR Odds Ratio

where the feces stay overnight, the odds ratio of *E. coli* O157:H7 occurrence was nearly 50 times higher. Animals that were milked anywhere on the farm had 16.67 times higher risk compared to animals that are milked in a milking barn. Hand washing practice had also a significant impact on the occurrence of *E. coli* O157:H7, the odds ratio of *E. coli* O157:H7 occurrence in farms where hand washing is practiced only before milking was 8.51 times higher when compared with farms where before and after milking hand wash is experienced. Farms with concrete floor were 48.74 times at

higher risk when compared with farms with ordinary floor type (earthen floor).

Antimicrobial susceptibility pattern of isolates

As indicated in Fig. 1, there was a varying degree of resistance in which; 100% resistance was observed for ampicillin, cephalothin and rifampin and on the other hand 100% susceptibility was observed for chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, kanamycin and tetracycline. Concerning streptomycin, 63.15% of the isolates were susceptible and 36.8% were intermediate.

^{***} Significant level (P < 0.001), **Significant level (P < 0.01), *Computed using fisher exact test, — reference

^a Pearson's Chi-squared test with Yates' continuity correction

Table 3 Multivariable logistic regression analysis of *E. coli* O157:H7 occurrence with various risk factors

Risk factors	Adjusted OR	95% CI OR	<i>p</i> -value	
Area				
Rural	-	_	-	
Urban	9.32	1.79-48.60	0.008**	
Cleaning of pens				
No stay overnight	-	-	-	
Stay overnight	50	7.69-50.0	0.000***	
Milking location				
In barn	-	-	-	
Anywhere	16.67	3.03-83.33	0.001**	
Hand wash				
Before and after milking	=	-	-	
Only before milking	8.51	1.88-38.49	0.005**	
Floor type				
Earthen	=	-	-	
Concrete	48.74	3.49-680.66	0.004**	

^{***} Significant level (P < 0.001), ** Significant level (P < 0.01), - reference

All isolates showed the presence of MDR referring to the resistance of a single isolate against more than two drugs.

Discussion

In Ethiopia, *E. coli* O157:H7 is considered to be an important challenge for the dairy industry and public health development [47]. This study also indicates *E. coli* O157:H7 to be the major dairy development challenge in the study area. The overall prevalence of *E. coli* O157:H7 was 4.7%. The result is in line with the result reported by

Haile et al. [35] who reported a prevalence of 3.5% in food of animal origin in Addis Ababa Ethiopia. This result also supports previous studies [9, 59–62] that reported cattle to be asymptomatic carriers and major reservoirs of *E. coli* serotype O157:H7.

Raw milk can be a vehicle of transmission for E. coli O157:H7 [63] and the risk of consumption of raw milk is high; even though the prevalence detected is relatively low [64]. The isolation rate of E. coli O157:H7 from raw milk samples were similar to that recorded in the current study (4.54%) was slightly in agreement with the report of 2.9% by Disassa et al. [48]. But, the prevalence is far lower when compared to the reports of Abebe et al. [34] who reported 10.4% from Tigray, Ethiopia. The variations could be because of the differences in animal management, milking systems, and milk hygiene and handling practices from farm to farm and the location of study areas. In the previous studies milking system, milk hygiene and handling practices are more modernized because the farms are intensified with high labor and capital that minimize contact of one milker to many cows, and access to water and other equipment. The detection of E. coli O157:H7 from milk is not only a reliable indicator of fecal contamination but also an indicator of poor hygiene and sanitary conditions during milking and handling.

In the present study, a 3.9% isolation rate of *E. coli* O157:H7 was recorded from feces samples. This is in agreement with the prevalence reported by Atinafie et al. [41] and Mersha et al. [45] both reported a prevalence of 4.7% in Hawasa and Modjo, respectively. Isolation of *E. coli* O157:H7 from feces is regarded as important

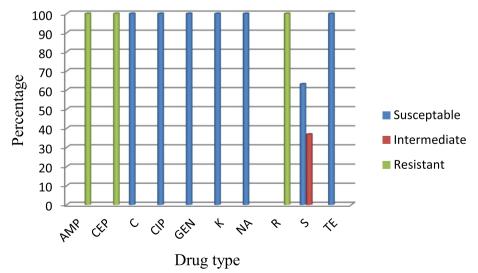


Fig. 1 Antimicrobial susceptibility pattern of *E.coli* O157:H7 to ten antimicrobials. Key: AMP: Ampicillin, CEP: Cephalothin C: Chloramphenicol, CIP: Ciprofloxacin, GEN: Gentamicin K: Kanamycin, NA: Nalidixic acid, R: Rifampin, S: Streptomycin, TE: Tetracycline

epidemiological information. Inhabited cattle could shed 10^1 to 10^7 CFU of *E. coli* O157:H7 per gram of feces. Given that typical cattle excrete 20 to 50kg of feces per day, this provides large inoculums of *E. coli* O157:H7 for the farm environment and could contaminate dairy products in the presence of poor hygienic practices [65].

Animal drinking water was also identified as one source of *E. coli* O157:H7 in dairy farms [36]. In this study, a prevalence of 8% was reported from water samples. The presence of *E. coli* O157:H7 in water samples may contribute to the prevalence of infection in cattle, a factor directly related to the contamination of dairy products and the environment. Contaminated water can serve as a vehicle for *E. coli* O157:H7 transmission in cattle, although there was variation among animals in the doses necessary to initiate shedding [36, 66].

From the manure samples, a prevalence of 4% was recorded. Farm manure may disseminate, transmit, or propagate E. coli O157:H7 and it could be a good vehicle of E. coli O157:H7 [59]. Manure sewages from cattle houses could result in contamination of the surrounding land, with cattle keepers and their household members being at increased risk. The survival of E. coli O157:H7 in manure depends on many variables, including the level of pathogen shedding by animals, conditions, and duration of manure storage, extraneous microbial interactions within stored manure, and interactions with water [67]. Several researchers have investigated the survival of *E.* coli O157:H7 in manure from various animals, under different conditions such as temperature or aeration, presence of different manure amendments and at a range of manure-to-soil ratios [68]. Kudva et al. [24] found that E. coli O157:H7 survived for more than 21 months in ovine manure at levels ranging up to 10⁶ CFU/g manure. Experiments with artificially inoculated bovine feces have also confirmed the survival of *E. coli* O157:H7 for greater than 40 days, dependent on initial inoculums and holding temperature [69].

Many factors were tested for associations with *E. coli* O157:H7, yet relatively few were significant in the final model. Factors such as area (urban, rural), floor type, cleaning of pens, milking location and hand washing during the time of milking were found to be significantly associated with the occurrence of *E. coli* O157:H7. *E. coli* O157:H7 shedding in cattle and its survival in the environment could be multifactorial. No single factor could stand out as the major risk factor for shedding. But, factors related to poor hygienic practices were found to affect the occurrence of the bacteria [63]. It is important to note that, the use of towels and detergents were not significantly associated with the occurrence of *E. coli* O157:H7. This suggests

that the use of towels and detergent alone is unlikely to prevent the presence of *E. coli* O157:H7 while the other hygienic practices are poorly practiced. Thus, general hygienic practices might represent a critical control point for reducing the transmission of *E. coli* O157:H7 in dairy farms [49].

In this study, 100% MDR was observed. All isolates were resistant to ampicillin, cephalothin and rifampin. This is in agreement with the report of Bekele et al. [43] and Atinafie [41]. Multidrug resistance occurred due to the misuse of antimicrobial agents or due to genetic mutation [70]. On contrary, all isolates were susceptible to the most commonly used antimicrobials including chloramphenicol, ciprofloxacin, gentamicin [71] and tetracycline. However, Hiko et al. [31], Bekele et al. [43] and Haile et al. [35] were reported resistance to tetracycline which is the most commonly used antimicrobials in Ethiopia, which is contrary to the present study. But, Mohammed et al. [16] reported susceptibility to tetracycline which is in line with the present study.

This study has limitation on the size of a sample that might affect exactness of estimates and it minimizes the power of conclusion given from this study. There is also resource limitation in characterizing and identifying antimicrobial resistance and resistance genes in detail; because lack of materials and equipment needed to perform their procedures such as PCR, DNA microarray, etc.

Conclusion

The current study revealed a substantial occurrence of E. coli O157:H7 in lactating cows and dairy farm environments in ATJK district. E. coli O157:H7 was isolated from feces, manure, milk and water designating a sustaining transmission of the bacteria. The occurrence of E. coli O157:H7 in milk samples suggests a potential zoonotic risk of raw milk consumption in the area. Factors related to poor hygienic practices such as cleaning of pens, milking location and hand washing were the main factors that backed the occurrence of E. coliO157:H7 in the dairy farms. E. coli O157:H7 isolates manifested a MDR; 100% resistance to ampicillin, cephalothin and rifampin was observed. Antimicrobials such as chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, kanamycin and tetracycline may be used as a means to reduce these strains in dairy cattle in ATJK. Comprehensive training should be given to farm owners, milkers and other personnel involved in dairying activity to improve the hygienic practices. Strict animal and environment level hygienic practices should be practiced to break a sustained transmission of the bacteria.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-023-03565-9.

Additional file 1.
Additional file 2.

Acknowledgements

We thank Oromia Agricultural Research Institute and Addis Ababa University and also Thematic research project "Post-harvest loss -PHL" for the provision of all the necessary financial support and laboratory ingredients.

Authors' contributions

FM takes part in study design, sample collection, laboratory work, data analysis and interpreting and writing the first draft of the manuscript. FA, SL and KA participated in study design, data analysis and interpreting and write up of the draft of the manuscript. All authors contributed to the final version of the manuscript and approved the submission.

Funding

This research was funded by Addis Ababa University and Oromia Agricultural Research Institute.

Availability of data and materials

All relevant data are within the paper. Raw data are available with the corresponding author upon request.

Declarations

Ethics approval and consent to participate

This research was approved by Addis Ababa University, collage of veterinary medicine animal research ethical committee with reference number VM/ ERC/28/05/10/2018. All methods were performed by skilled experts with relevant guidelines and regulation listed by ethical committee of the University. Safety, welfare and wellbeing of the study animals were secured during the study. Informed consent for study participation was obtained from all farm owners for questionnaire interview and to collect milk and fecal samples from the animals.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Adami Tulu Agricultural Research Center, P.O. Box 35, Ziway, Ethiopia. ²Addis Ababa University, Collage of Veterinary Medicine and Agriculture, P.O.Box 34, Bishoftu, Oromia, Ethiopia.

Received: 9 August 2021 Accepted: 2 January 2023 Published online: 11 January 2023

References

- Agueria DA, Terni C, Baldovino VM, Civit D. Food safety knowledge, practices and attitudes of fishery workers in Mar del Plata, Argentina. Food Control. 2018;91:5–11.
- Fratamico PA, Bhunia AK, Smith JL. Foodborne pathogens: microbiology and molecular biology. Wymondham, Norfolk, UK: Caister Academic Press; 2005. p. 273.
- Bhandare S, Sherikarv A, Paturkar A, Waskar V, Zende R. A comparison of microbial contamination on sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. Food Control. 2007;18:854–68.

- 4. Podpecan B, Pengov A, Vadnjal S. The source of contamination of ground meat for production of meat products with bacteria Staphylococcus aureus. Slov Vet Res. 2007;44:25–30.
- Diane GN, Marion K, Linda V, Erwin D, Awa AK, et al. Foodborne diseases. Int J Food Microbiol. 2010;139:3–15.
- WHO, Food safety and foodborne diseases and value chain management for food safety. "Forging links between Agriculture and Health" CGIAR on Agriculture and Health Meeting in WHO/HQ, Accessed June 2007.
- Getaneh DK, Hordofa LO, Ayana DA, Tessema TS, Regassa LD. Prevalence of Escherichia coli O157:H7 and associated factors in under-five children in eastern Ethiopia. PLoS One. 2021;16(1):e0246024. https://doi.org/10. 1371/journal.pone.0246024.
- Mengistu S, Abayneh E, Shiferaw D. E. coli O157:H7 and Salmonella Species: Public Health Importance and Microbial Safety in Beef at Selected Slaughter Houses and Retail Shops in Eastern Ethiopia. J Vet Sci Technol. 2017;8:468. https://doi.org/10.4262/2157-7579.1000468.
- Fairbrother JM, Nadeau E. Escherichia coli: on-farm contamination of animals. Rev Sci Tech. 2006;25:555–69. https://doi.org/10.20506/rst.25.2.1682.
- Money P, Kelly AF, Gould SW, Denholm-Price J, Threlfall EJ, Fielder MD, et al. Cattle, weather and water: mapping *Escherichia coli* O157:H7 infections in humans in England and Scotland. Environ Microbiol. 2010;12:2633–44. https://doi.org/10.1111/j.1462-2920.2010.02293.x.
- Caprioli A, Morabito S, Brugere H, Oswald E, et al. Enterohaemorrhagic *Escherichia coli* emerging issues on virulence and modes of transmission. Vet Res. 2005;36:289–311. https://doi.org/10.1051/vetres:2005002.
- Cobbaut K, Houf K, Buvens G, Habib I, De Zutter L. Occurrence of nonsorbitol fermenting, verocytotoxin-lacking *Escherichia coli* O157 on cattle farms. Vet Microbiol J. 2009;138:174–8.
- Earley B, Leonard N. The characterisation of E. coli O157: H7 isolates from cattle faeces and feedlot environment using PFGE. Vet Microbiol J. 2006:114:331–6.
- Beyi AF, Fite AT, Tora E, Tafese A, Genu T, Kaba T, et al. Prevalence and antimicrobial susceptibility of *Escherichia coli* O157 in beef at butcher shops and restaurants in Central Ethiopia. BMC Microbiol. 2017;17:49. https:// doi.org/10.1186/s12866-017-0964-z.
- Asfaw Geresu M, Regassa S. Escherichia coli O157: H7 from food of animal origin in Arsi: occurrence at catering establishments and antimicrobial susceptibility profile. SCI World J. 2021;2021:7. https://doi.org/10.1155/ 2021/6631860.
- Mohammed O, Shimelis D, Admasu P, Feyera T, et al. Prevalence and antimicrobial susceptibility pattern of *E. coli* isolates from raw meat samples obtained from abattoirs in Dire Dawa City, eastern Ethiopia. *International journal of.* Microbiol Res. 2014;5:35–9. https://doi.org/10.5829/idosi.ijmr. 2014.5.1.82306.
- Abayneh E, Nolkes D, Asrade B, et al. Review on common foodborne pathogens in Ethiopia. Afr J Microbiol Res. 2014;8:4027–40. https://doi. org/10.5897/AJMR2014.7015.
- Mesele F, Abunna F. Escherichia coli O157: H7 in foods of animal origin and its food safety implications. Adv Biol Res. 2019;13(4):134–45.
- Awadallah MA, Ahmed HA, Merwad AM, Selim MA, et al. Occurrence, genotyping, Shiga toxin genes and associated risk factors of *E. coli* isolated from dairy farms, handlers and milk consumers. Vet J. 2016;217:83– 8. https://doi.org/10.1016/j.tvjl.2016.09.014.
- Davis MA, Cloud-Hansen KA, Carpenter J, Hovde CJ, et al. Escherichia coli O157:H7 in environments of culture-positive cattle. Appl Environ Microbiol. 2005;71:6816–22. https://doi.org/10.1016/j.tvjl.2016.09.014.
- LeJeune JT, Besser TE, Rice DH, Hancock DD, et al. Methods for the isolation of water-borne *Escherichia coli* O157. Lett Appl Microbiol. 2001;32:316–20. https://doi.org/10.1046/j.1472-765x.2001.00905.x.
- 22. LeJeune JT, Wetzel AN. Preharvest control of *Escherichia coli* O157 in cattle. J Anim Sci. 2007;85:73–80. https://doi.org/10.2527/jas.2006-612.
- Sargeant JM, Sanderson MW, Smith RA, Griffin DD. Associations between management, climate, and *Escherichia coli* O157 in the faeces of feedlot cattle in the Midwestern USA. Prev Vet Med. 2004;66:175–206. https://doi. org/10.1016/j.prevetmed.2004.09.007.
- Kudva IT, Blanch K, Hovde CJ, et al. Analysis of *Escherichia coli* O157: H7 survival in ovine or bovine manure and manure slurry. Appl Environ Microbiol. 1998;64:3166–74. https://doi.org/10.1128/AEM.64.9.3166-3174.1998.
- Kolar M, Urbanek K, Latal T, et al. Antibiotic selective pressure and development of bacterial resistance. Int J Antimicrob Agents. 2001;17:357–63. https://doi.org/10.1016/S0924-8579(01)00317-X.

- Erb A, Stürmer 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(2):83–90.
- Sabate M, Prats G, Moreno E, Balleste E, Blanch AR, Andreu A, et al. Virulence and antimicrobial resistance profiles among *Escherichia coli* strains isolated from human and animal wastewater. Res Microbiol. 2008;159:288–93. https://doi.org/10.1016/j.resmic.2008.02.001.
- Constable PD, Hinchcliff KW, Done SH, Grundberg W. Veterinary Medicine: A textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats. UK: Elsevier; 2017. p. 1591. ISBN: 9780-7020-5246-8
- Vijayarani K, Parthiban M, Raja A, Kumanan K, et al. Occurrence and characterization of *Escherichia coli* 0157:H7 and other serotypes in goat and sheep meat in India. Indian J Anim Sci. 2010;80(1019–21):20103344443.
- Berhe DF, Beyene GT, Seyoum B, et al. Prevalence of antimicrobial resistance and its clinical implications in Ethiopia: a systematic review. Antimicrob Resist Infect Control. 2021;10:168. https://doi.org/10.1186/s13756-021-00965-0.
- Hiko A, Asrat D, Zewde G. Occurrence of *Escherichia coli* O157: H7 in retail raw meat products in Ethiopia. J Infect Dev Countries. 2008;2:389–93. https://doi.org/10.3855/jidc.203.
- Messele YE, Abdi RD, Yalew ST, et al. Molecular determination of antimicrobial resistance in *Escherichia coli* isolated from raw meat in Addis Ababa and Bishoftu, Ethiopia. Ann Clin Microbiol Antimicrob. 2017;16:55. https://doi.org/10.1186/s12941-017-0233-x.
- Shecho M, Thomas N, Kemal J, Muktar Y, et al. Cloacael carriage and multidrug resistance *Escherichia coli* O157: H7 from poultry farms, Eastern Ethiopia. J Vet Med. 2017. https://doi.org/10.1155/2017/8264583.
- Abebe M, Hailelule A, Abrha B, Nigus A, Birhanu M, Adane H, et al. Antibiogram of *Escherichia coli* strains isolated from food of bovine origin in selected Woredas of Tigray, Ethiopia. African. J Bacteriol Res. 2014;6:17–22. https://doi.org/10.5897/JBR2014.012.
- Haile AF, Alonso S, Berhe N, Atoma TB, Boyaka PN, Grace D. Prevalence, Antibiogram, and multidrug-resistant profile of *E. coli* O157: H7 in retail raw beef in Addis Ababa, Ethiopia. Front Vet Sci. 2022;9. https://doi.org/ 10.3389/fyets.2022.734896.
- Dejene H, Abunna F, Tuffa AC, Gebresenbet G. Epidemiology and Antimicrobial Susceptibility Pattern of *E. coli* O157:H7 Along Dairy Milk Supply Chain in Central Ethiopia. Vet Med (Auckl). 2022;13:131–42. https://doi.org/10.2147/VMRR.S366888 PMID: 35706602; PMCID: PMC9191832.
- Haile, W. Prevalence and Sources of Contamination of Cattle Meat at Municipal Abattoir and Butcheries as well as its Public Health Importance in Addis Ababa, Ethiopia [dissertation]. AAU Institutional Repository 2017:25–56. http://etd.aau.edu.et/handle/123456789/21782.
- Tassew A. Isolation, Identification, Antimicrobial Profile and Molecular Characterization of Enterohaemorrhagic E. coli O157: H7 Isolated From Ruminants Slaughtered at Debre Zeit ELFORA Export Abattoir and Addis Ababa Abattoirs Enterprise [dissertation]. AAU Institutional Repository 2015. http://etd.aau.edu.et/handle/123456789/4135.
- Taye M, Berhanu T, Berhanu Y, Tamiru F, Terefe D, et al. 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 Veterinar Sci Techno. 2013;4:2. https://doi. org/10.4172/2157-7579.100013.
- Abdissa R, Haile W, Fite AT, Beyi AF, Agga GE, Edao BM, et al. Prevalence of *Escherichia coli* O157: H7 in beef cattle at slaughter and beef carcasses at retail shops in Ethiopia. BMC Infect Dis. 2017;17:277. https://doi.org/10. 1186/s12879-017-2372-2.
- Atnafie B, Paulos D, Abera M, Tefera G, Hailu D, Kasaye S, et al. Occurrence of *Escherichia coli* O157: H7 in cattle feces and contamination of carcass and various contact surfaces in abattoir and butcher shops of Hawassa, Ethiopia. BMC Microbiol. 2017;17:24. https://doi.org/10.1186/s12866-017-0938-1.
- Balcha E, Kumar A, Tassew H. Evaluation of Safety of Beef Sold in and around Mekelle with Special Reference to Enterohemorrhagic *Escherichia* coli O157:H7. Global Vet. 2014;4:569–72. https://doi.org/10.5829/idosi.gv. 2014.12.04.8375.
- Bekele T, Zewde G, Tefera G, Feleke A, Zerom K, et al. Escherichia coli
 O157: H7 in raw meat in Addis Ababa, Ethiopia: prevalence at an abattoir
 and retailers and antimicrobial susceptibility. Int J Food Contamination.
 2014;1:4. https://doi.org/10.1186/s40550-014-0004-9.
- 44. Dulo F. Prevalence and antimicrobial resistance profile of *Escherichia coli* O157: H7 in goat slaughtered in Dire Dawa municipal abattoirs as well as

- food safety knowledge, attitude and hygiene practice assessment among slaughter staff, Ethiopia. CGSpaceA Repository of Agricultural Research Outputs 2014. https://cgspace.cgiar.org/handle/10568/43770.
- Mersha G, Asrat D, Zewde BM, Kyule M, et al. Occurrence of Escherichia coli O157: H7 in faeces, skin and carcasses from sheep and goats in Ethiopia. Lett Appl Microbiol. 2010;50:71–6. https://doi.org/10.1111/j. 1472-765X.2009.02757.x.
- Dadi S, Lakew M, Seid M, Koran T, Olani A, et al. Isolation of Salmonella and E. coli (E. coli O157:H7) and its Antimicrobial Resistance Pattern from Bulk Tank Raw Milk in Sebeta Town, Ethiopia. J Anim Res Vet Sci. 2020:4:021.
- Ababu A, Endashaw D, Fesseha H. Isolation and antimicrobial susceptibility profile of Escherichia coli O157: H7 from raw milk of dairy cattle in Holeta district, Central Ethiopia. Int J Microbiol. 2020;2020. https://doi.org/10.1155/2020/6626488.
- Disassa N, Sibhat B, Mengistu S, Muktar Y, Belina D, et al. Prevalence and antimicrobial susceptibility pattern of *E. coli* O157: H7 isolated from traditionally marketed raw cow Milk in and around Asosa town, Western Ethiopia. Vet Med Int. 2017. https://doi.org/10.1155/2017/758153.
- Shunda D, Habtamu T, Endale B. Assessment of bacteriological quality of raw cow milk at different critical points in Mekelle, Ethiopia. Int J Livestock Res. 2013;3:42–8. https://doi.org/10.1155/2017/826458.
- Assefa A, Bihon A. A systematic review and meta-analysis of prevalence of *Escherichia coli* in foods of animal origin in Ethiopia. Heliyon. 2018;4(8):e00716. Published 2018 Aug 6. https://doi.org/10.1016/j.heliyon.2018.e00716.
- Jergefa T, Kelay B, Bekana M, Teshale S, Gustafson H, Kindahl H, et al. Epidemiological study of bovine brucellosis in three agro-ecological areas of Central Oromia, Ethiopia. Rev Sci Tech Off Int Epiz. 2009;28:933–43. https://doi.org/10.20506/rst.28.3.1939.
- 52. Megersa M, Feyisa A, Wondimu A, Jibat T, et al. Herd composition and characteristics of dairy production in Bishoftu town, Ethiopia. J Agric Extension Rural Dev. 2011;3:113–7.
- Thrusfield M. Veterinary epidemiology. UK: Blackwell Science Ltd; 2005. p. 233–50.
- Quinn P, Carter M, Markey B, Carter G, et al. Clinical veterinary microbiology. UK: Wild life Publisher; 2004. p. 101.
- 55. ISO (International Organization for Standardization) 16654. Microbiology of Food and Animal Feeding Stuff-Horizontal method for the detection of *E. coli* O157. 2001 (en). https://www.iso.org/obp/ui/#iso:std:iso:16654: ed-1.v1:en.
- OIE (world Organization for Animal Health). Manual for diagnostic tests and vaccines for terrestrial animals. Verocytoxigenic *Escherichia coli* 2016. https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3. 10.09 VERO E COLI.pdf.
- CLSI. Performance Standards for Antimicrobial Susceptibility Testing;
 Twenty-Fifth Informational Supplement. Clinical and Laboratory Standards Institute (CLSI) document Wayne, PA, M100-S25. 2015;35. https://file.qums.ac.ir/repository/mmrc/CLSI2015.pdf.
- The R Core team is: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. 2016; Vienna, Austria. https:// www.R-project.org/.
- Ferens WA, Hovde CJ. Escherichia coli O157:H7:animal reservoir and sources of human infection. Foodborne Pathog Dis. 2011;8:465–87. https://doi.org/10.1089/fpd.2010.0673.
- Hancock DD, Besser TE, Rice DH, Ebel ED, Herriott DE, Carpenter LV, et al. Multiple sources of *Escherichia coli* O157 in feedlots and dairy farms in the northwestern USA. Prev Vet Med. 1998;35:11–9. https://doi.org/10.1016/ S0167-5877(98)00050-6.
- Sami M, Firouzi R. Prevalence of Escherichia coli O157: H7 on dairy farms in shiraz, Iran by immunomagnetic separation and multiplex PCR. Iranian journal of. Vet Res. 2007;8:319–24 https://www.sid.ir/en/journal/ViewP aper.aspx?id=101459.
- Wells JG, Shipman LD, Greene KD, Sowers EG, Green JH, Cameron DN, et al. Isolation of *Escherichia coli* serotype O157: H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. J Clin Microbiol. 2007;29:985–9. https://doi.org/10.1128/JCM.29.5.985-989.
- 63. USDA APHI. An update: Escherichia coli O157: H7 in humans and cattle. Centers for epidemiology and animal Health. 1997. https://books.google.com.et/books?id=JLRcNQJuoVkC&printsec=frontcover&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false.

- 64. Lye YL, Afsah-Hejri L, Chang WS, Loo YY, Puspanadan S, Kuan CH, et al. Risk of *Escherichia coli* O157: H7 transmission linked to the consumption of raw milk. Int Food Res J. 2013:20. https://www.proquest.com/openview/903d8da7c1445d728dbb634bcab43151/1?pq-origsite=gscholar&cbl=816390.
- Matthews R, Sapers M, Gerba P. The produce contamination problem Causes and Solutions. 2nd (eds) Elsevier, UK. 2014.ISBN-13:978–0–12-404611-5. https://eprints.mdx.ac.uk/15760/1/MATTHEWS_9780124046 115.pdf.
- Shere JA, Kaspar CW, Bartlett KJ, Linden SE, Norell B, Francey S, et al. Shedding of *Escherichia coli* O157: H7 in dairy cattle housed in a confined environment following waterborne inoculation. Appl Environ Microbiol. 2002;68:1947–54. https://doi.org/10.1128/AEM.68.4.1947-1954.2002.
- Ziemer CJ, Bonner JM, Cole D, Vinje J, et al. Fate and transport of zoonotic, bacterial, viral, and parasitic pathogens during swine manure treatment, storage, and land application. J Anim Sci. 2010;88:84–94. https://doi.org/ 10.2527/jas.2009-2331.
- Duffy G. Verocytoxigenic *Escherichia coli* in animal feces, manures and slurries. J Appl Microbiol. 2003:94–103. https://doi.org/10.1046/j.1365-2672 94 s1.11 x.
- Wang G, Zhao T, Doyle MP. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces. Appl Environ Microbiol. 1996;62:2567–70. https://doi.org/10.1128/AEM.62.7.2567-2570.
- 70. Mendelson M, Matsoso MP. The World Health Organization global action plan for antimicrobial resistance. S Afr Med J. 2015;105(5):325.
- Kibret M, Abera B. Antimicrobial susceptibility patterns of E. coli from clinical sources in Northeast Ethiopia. Afr Health Sci. 2011;11:40–5. https://doi.org/10.4314/ahs.v11i3.70069.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- $\bullet\,$ thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

