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Occurrence and antimicrobial susceptibility profile of *Escherichia coli* O157:H7 from food of animal origin in Bishoftu town, Central Ethiopia

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Abstract

Background: *Escherichia coli* O157:H7 (*E. coli* O157:H7) have frequently been associated with food borne illness and are considered as most serious of known food borne pathogens leading to severe illnesses and high mortality rates in humans. Most of outbreaks were traced to raw meat and raw milk consumption, as well as to dairy products such as yogurt and cheese derived from raw milk.

Results: Out of 200 samples examined, 40 (20%) and 7 (3.5%) of the samples were positive to *E. coli* and *E. coli* O157:H7 respectively. The highest isolation of *E. coli* was from cheese (40%), followed by raw milk (32%), yogurt (25.71%), beef (13.84%), and pasteurized milk (0%). Among *E. coli* O157:H7 isolates, the highest isolation was from raw milk (12%) followed by cheese (5.71%) and meat (3.07%). However, no *E. coli* O157:H7 was isolated from pasteurized milk and yogurt. Antibiotic susceptibility profile showed that *E. coli* was resistant for vancomycin (89.74%), ampicillin (76.92%) and streptomycin (69.23%). The analysis showed that, 92.5% of isolates showed multidrug resistance comprising 2–4 antimicrobials.

Conclusion: The occurrence of *E. coli* O157:H7 and its multiple antibiotic resistant profiles shows a risk for public health and food safety as well as animal production. These findings stress the need for an integrated control of *E. coli* O157:H7 from farm production to consumption of food of animal origin.

Keywords: Drug susceptibility, *E. coli*, *E. coli* O157:H7, Meat, Milk, Milk products

Background

Foodborne diseases and food poisoning are the widespread and great public health and well-being concerns of individuals and countries of the modern world. Especially, developing countries are largely affected by food-borne infections (Carbas et al. 2012). Among the major infectious agents, *Escherichia coli* O157:H7 has frequently been associated with foodborne illness. Particularly, over the past decade, *E. coli* O157:H7 has been reported increasingly from all parts of the world and in the worst case, it is "one of the most serious" foodborne pathogens leading to severe illnesses and high mortality rates in humans (Blanco et al. 2003; Jo et al. 2004). This consideration is in fact due to the small infectious dose of the organism because fewer than 40 cells of *E. coli* O157:H7 can cause illness in some people (Strachan et al. 2005).

It has been indicated that an estimated 74,000 cases and 61 deaths annually are attributable to *E. coli* O157:H7 in the USA, and many outbreaks (in the USA) related to foodborne illness have been connected to consumption of contaminated foods derived from cattle, especially meat and raw milk. In the 1980s, most outbreaks due to *E. coli* O157:H7 were associated with inadequately cooked hamburgers and raw milk. Later, outbreaks were traced to other dairy products such as yogurt and cheese (Doyle et al. 2006; Mora et al. 2007). More recently, in 2016 outbreak of *E. coli* O157:H7, slaughtered animals were the

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main sources of infection and led to illness of eleven people in the USA (CDC, 2016).

Escherichia coli O157:H7 has been found in the intestines of healthy cattle, deer, goats, and sheep. However, cattle have been identified as a major reservoir of *E. coli* O157:H7 and consumption of foods of bovine origin such as beef and dairy products have been associated with some of the largest food poisoning outbreaks in which this organism was identified as the etiologic agent (Acha and Szyfress, 2001; IFT (Institute of Food Technology), 2003; Perelle et al. 2007).

Due to an increased demand for animal protein, the animal production sectors in low and middle-income countries have been regularly using antimicrobials for therapy, disease prevention and growth (Van Boeckel et al. 2015). This practice could be responsible for antimicrobial resistance among commensals in the intestinal tracts of food animals, which may subsequently risk public health due to food animals' weak response to, or loss of response to, drug therapy. Hence, there should be isolation of pathogenic organisms and regular evaluation of their antimicrobial susceptibility profiles. In Ethiopia, some studies have been conducted to identify pathogenic *E. coli* from human and animal sources such as stool samples (Demisse, 2005), raw beef, sheep meat, goat meat (Hiko et al. 2008; Lula 2011), feces, skin of meat handlers (Mersha et al. 2010), yogurt and cheese (Tsegaye and Ashenafi 2005). However, recent and detailed information on the prevalence and multi-drug susceptibility profile of pathogenic *E. coli* is limited. Therefore, the present study was conducted to add current information pertaining to the occurrence and antibiotic susceptibility profiles of *E. coli* and *E. coli* O157:H7 from milk, milk products and meat in and around Bishoftu, Central Ethiopia, where food of animal origin is widely consumed.

Methods

Study area

The study was conducted in Bishoftu town. Bishoftu town is located at 9°N latitude and 40°E longitudes at an altitude of 1850 m above sea level in central high lands of Ethiopia. It has an annual rainfall of 866 mm of which 84% is in the long rainy season (June to September). The dry season extends from October to February. The mean annual maximum and minimum temperatures are 26 °C and 14 °C respectively, with mean relative humidity of 61.3% (ADARDO 2007). The livestock production system in the area is both intensive and extensive type (CSA 2015).

Study design and sampling strategy

A cross-sectional study was conducted from November 2016 to April 2017 to determine the occurrence and antimicrobial resistance profile of *E. coli* O157:H7 in/for milk, milk products (cheese and yogurt) and beef samples. In the present study 200 samples (milk = 65, cheese = 35, yogurt = 35 and meat = 65) were collected on a voluntary basis (owner's willingness to provide the samples). Cafeterias, restaurants, open markets and supermarket that had a high level of consumers were included in the study.

Collection and transportation of samples for laboratory analysis

About 20 ml of milk (both pasteurized and raw), cheese and yogurt samples were collected aseptically in sterile disposable corked plastic tubes. The pasteurized milk, cheese and yogurt obtained from the cafeterias, restaurants, and supermarket were kept under refrigerator until used for consumption by customers. The pasteurized milk was packaged using a disposable small plastic bag, whereas the cheese and yogurt were kept in silver/glass vessels until used for consumption. The raw milk samples were obtained from milk sellers found in open markets (the streets of the town). Milk found on the open markets was handled with a plastic container of up to 3 litters' capacity and with no cooling facility. About 25 g of beef meat sample was taken from carcass hanged inside the houses of restaurants and placed in a disposable plastic bag. The entire collected samples were labeled appropriately, placed in a box containing ice and transported immediately to Microbiology Laboratory, College of Veterinary Medicine and Agriculture, Addis Ababa University. Then the samples were placed in a refrigerator at +4 °C and subjected to culture within 24 h of sampling.

Isolation and identification of *Escherichia coli* and *Escherichia coli* O157:H7

Detection of *E. coli* and *E. coli* O157:H7 was carried out according to the protocol of ISO-16654: 2001 standard. A loopful of milk, cheese and yogurt aseptically taken from all of the sample bottles and a swab from the surface of about 25 g portions of meat dissected by sterilized blade from all of the meat samples collected were individually inoculated on MacConkey agar for primary isolation of *E. coli* (Difco laboratories, USA) and incubated aerobically at 37 °C for 24 h. The plates were observed for the growth of *E. coli* (pink colony; lactose fermenter). A single, isolated colony was picked and sub-cultured on Eosin Metyline Blue (EMB) agar for formation of metallic sheen. Simultaneously another single colony with similar characteristics was picked and stained with Gram's stain. The isolate was examined for stain and morphological characteristics using bright-field microscopy. KOH test was then employed to confirm the Gram's reaction (Quinn et al. 2004). Suspected colonies of *E. coli* (pinkish color appearance on MacConkey agar and metallic sheen on EMB) (Figs. 1 and 2) were then sub-cultured onto blood agar to appreciate colony characteristics and then pure colonies taken from blood agar were inoculated on nutrient agar (OXOID) (non-

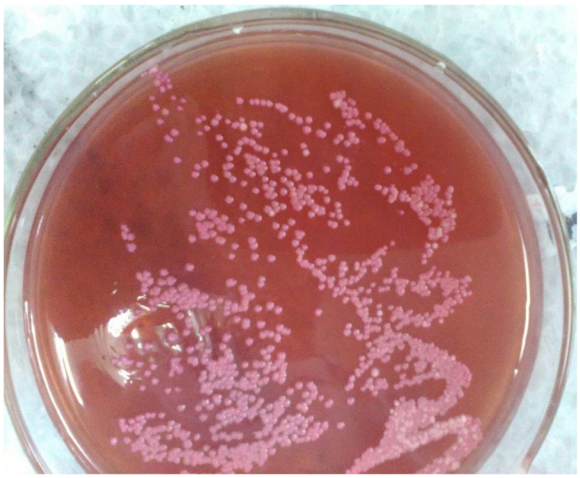

Fig. 1 *E. coli* on MacConkey agar. Note the pinkish colonies

selective media). Biochemical tests were performed to confirm the *E. coli* using catalase test, Indole Production test, Methyl red test, Voges proskaur test, and Simmon's Citrate test on tryptone broth, MR-VP medium and Simon citrate agar respectively (ISO 2003). Then the bacterium that was confirmed as *E. coli* was subcultured onto Sorbitol MacConkey agar (SMA) (OXOID, England) from nutrient agar (OXOID). SMA (OXOID, England) and plates were incubated at 35 °C for 20 to 22 h. *E. coli* O157:H7 does not ferment sorbitol and, therefore, produces colorless colonies (Fig. 3). In contrast, most other *E. coli* strains ferment sorbitol and form pink colonies (Soomro et al. 2002) (Fig. 4). All non-sorbitol fermenting colonies from the Sorbitol MacConkey agar were subjected to slide agglutination with

Fig. 2 Characteristics of *E. coli* on EMB. The metallic sheen appearance is characteristics for the organism

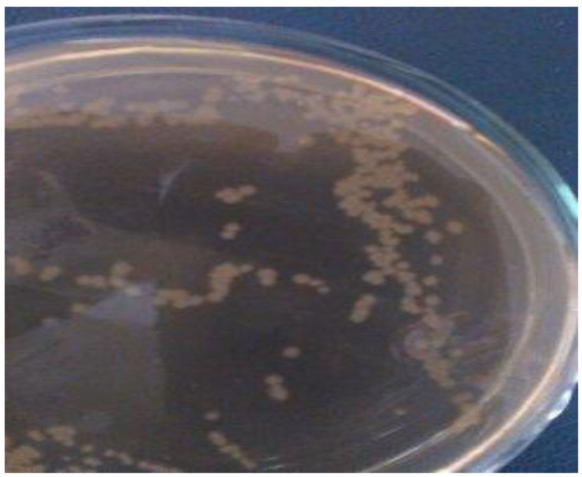

Fig. 3 Characteristics of pathogenic *Escherichia coli* on Sorbitol MacConkey Agar. Note the whitish colonial appearance (non-sorbitol fermenters) of *E. coli* O157:H7

the *E. coli* O157:H7 latex test kit (OXOID). The latex beads were coated with antibodies which bind to any O157 or H7 antigens on the test organisms, forming a visible antigen antibody precipitate (DeBoer and Heuvelink 2000). Colonies giving a precipitation reaction were confirmed as *E. coli* O157:H7 positive.

Antimicrobial susceptibility test of *Escherichia coli*

Antibiotic susceptibility tests of all *E. coli* isolates were performed following the standard agar disk diffusion method according to (CLSI (clinical and laboratory standards institute), 2012) using commercially available antimicrobial disks. Isolates were screened for susceptibility

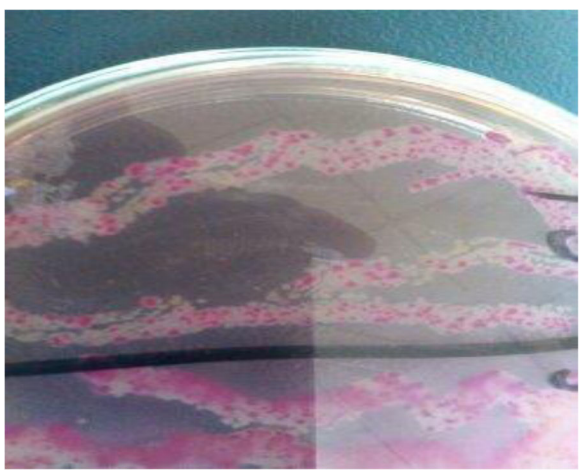

Fig. 4 Characteristics of *Escherichia coli* on Sorbitol MacConkey Agar. Note the pinkish colonies formed by most strains of *E. coli* other than *E. coli* O157:H7

to Gentamycin (GN) (10 μg), Ampicillin (AMP) (10 μg), Tetracycline (TE) (30 μg), Chloramphenicol (C) (30 μg), Ciproflocxazilin (CIP) (5 μg), Vancomycin (VA) (30 μg), Streptomycin (S) (10 μg) and Ceftriaxone (CRO) (30 μg) by the disk diffusion assay (Becton Dickinson BBL Diagnostics) in Mueller-Hinton agar. Each isolated bacterial colony from pure fresh culture was transferred into a test tube of 5 ml Tryptone Soya Broth (TSB) (OXOID, England) and incubated at 37 °C for 6 h. The test broth was adjusted to McFarland 0.5 turbidity to obtain desired bacterial population. Mueller-Hinton agar (Bacton Dickinson and Company, Cockeysville, MD, USA) plates were prepared according to the manufacturer guidelines. A sterile cotton swab was immersed into the inoculum suspension and rotated against the side of the tube to remove the excess fluid and then swabbed in three directions uniformly on the surface of Mueller-Hinton agar plates. After the plates dried, antibiotic disks were placed on the inoculated plates using sterile forceps. The antibiotic disks were gently pressed onto the agar to ensure firm contact with the agar surface, and incubated at 37 °C for 24 h. Following this the diameter of inhibition zone formed around each disk was measured using a black surface, reflected light and transparent ruler by lying it over the plates. The results were classified as sensitive, intermediate or resistant according to the standardized table supplied by CLSI (clinical and laboratory standards institute) (2012) (Table 1).

Statistical analysis

The collected data for bacterial contamination analysis were entered and analyzed using SPSS version 17 computer software. Accordingly, descriptive statistics such as percentages and frequency distribution were used to describe/present bacterial isolates and antimicrobial susceptibility which was expressed as percent of resistant, intermediate or susceptible.

Table 1 Guidelines for antibiotic discs used for antimicrobial susceptibility test of *E. coli* with their respective concentrations

Antimicrobial agent	Symbol	Disc content	Zone of inhibition in millimeters (mm) with its interpretation		
			Susceptible	Intermediate	Resistant
Ceftriaxone	CRO	30 μg	≥ 23	20–22	≤ 19
Streptomycin	S	10 μg	≥ 15	12–14	≤ 11
Tetracycline	TTC	30 μg	≥ 15	12–14	≤ 11
Gentamycin	GN	10 μg	≥ 15	13–14	≤ 12
Ciprofloxacilin	CIP	5 μg	≥ 21	16–20	≤ 15
Vancomycin	VA	30 μg	≥ 12	10–11	≤ 9
Chloramphenicol	C	30 μg	≥ 18	13–17	≤ 12
Ampicillin	AMP	10 μg	≥ 14	12–13	≤ 11

Source: (CLSI (clinical and laboratory standards institute), 2012)

Results

Occurrence of *E. coli* and *E. coli* O157:H7 from milk, milk products and meat

In the present study, out of 200 bacteriologically examined samples, 40 (20%) were harboring *E. coli*. The highest isolation was from cheese (40%), followed by raw milk (32%), yogurt (25.71%), meat (13.84%) and pasteurized milk (0%). Out of 200 samples, 7 (3.5%) were contaminated by *E. coli* O157:H7. The highest isolation rate of *E. coli* O157:H7 was from raw milk (12%) followed by cheese (5.71%) and meat (3.07%), whereas it was not isolated from pasteurized milk and yogurt were (Table 2).

Antimicrobial susceptibility patterns

The study of antimicrobial sensitivity of *E. coli* recovered from different sample types revealed a varying degree of susceptibility to antimicrobial agents used. Accordingly, *E. coli* was highly susceptible to Ceftriaxone (100%), Tetracycline (97.5%), Ciprofloxacillin (97.5%), Chloramphenicol (92.5%), and Gentamycin (82.5%). Furthermore, resistance of 90%, 80% and 77.5% was developed to Vancomycin, Ampicillin and Streptomycin respectively (Table 3 & Fig. 5).

Multidrug resistance analysis showed that, 37/40 (92.5%) of tested *E. coli* isolates were resistant to different combinations of two or more antimicrobials (Table 4) and the proportion was higher in milk and milk products (28.4%) than meat samples (15.4%) (Table 5). A multidrug resistance pattern consisting of four drugs was seen in 3/40 (7.5%) isolates. Moreover, the majority of the isolates 16/40 (40%) showed multidrug resistance to Ampicillin, Vancomycin and Streptomycin. All isolates of *E. coli* O157:H7 were resistance to at least two drugs and 14.4% of them showed resistance to Ampicillin, Vancomycin, Streptomycin and Tetracycline.

Discussion

The present study revealed that *E. coli* was isolated from 20% of ready to eat foods of animal origin (milk, milk products and meat). Meanwhile, the study confirmed that *E. coli* and *E. coli* O157:H7 were not found in pasteurized milk. The presence of *E. coli* in pasteurized milk doesn't reflect the survival of the organism to the appropriate level of pasteurizing temperature. Rather, it

Table 2 Frequency of *E. coli* and *E. coli* O157:H7 isolated from meat, milk and milk products

Food type	*E. coli* positive	*E. coli* O157:H7 positive
Pasteurized milk	0/40 (0%)	0/40 (0%)
Raw milk	8/25 (32%)	3/25 (12%)
Meat	9/65 (13.84%)	2/65 (3.07%)
Cheese	14/35 (40%)	2/35 (5.71%)
Yogurt	9/35 (25.71%)	0/35 (0%)
Total	40/200 (20%)	7/200 (3.5%)

Table 3 Antimicrobial susceptibility profile of *E. coli* isolated from meat, milk and milk products ($n = 40$)

Type of drugs	Number (%) of:					
	E. coli[a]			*E. coli O157:H7*		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Gentamycin	33 (82.5%)	6 (15%)	1 (2.5%)	7 (100)	0	0
Ampicillin	5 (12.5%)	4 (10%)	31 (77.5%)	2 (28.6)	0	5 (71.4)
Ciprofloxacillin	39 (97.5%)	1 (2.5%)	0 (0%)	6 (85.7)	1 (14.3)	0
Streptomycin	2 (5%)	10 (25%)	28 (70%)	0	1 (14.3)	6 (85.7)
Tetracycline	39 (97.5%)	0 (0%)	1 (2.5%)	6 (85.7)	0	1 (14.3)
Cloramphenicol	37 (92.5%)	2 (5%)	1 (2.5%)	7 (100)	0	0
Vancomycin	4 (10%)	0 (0%)	36 (90%)	1 (14.3)	0	6 (85.7)
Ceftriaxone	40 (100%)	0 (0%)	0 (0%)	7 (100)	0	0

[a]= includes all isolates

might be due to poor hygienic handling after the milk is pasteurized, which contributes to milk contamination (Ali and Abdelgadir 2011).

Similar with the present finding, Mekuria et al. (2014) showed that 23.7% samples from food of bovine origin harbored *E. coli*. Furthermore, 32% of raw milk samples were found to harbor *E. coli*, which is somewhat in agreement with the report of 33.9% by Disassa et al. (2017). However, the prevalence is far lower when compared to the reports of Shunda et al. (2013) from Mekelle town (44%) and far higher when compared to 26% prevalence reported by Farhan et al. (2014) and 23.3% by Elbagory et al. (2016). In the present study, the isolation rate of *E. coli* O157:H7 from raw milk was 12%, which is comparable to prevalence report of 10.4% by Mekuria and Beyene (2014). Whereas, the highest occurrence of *E. coli* O157:H7 were found by Chye et al. (2004) (33.5%) and Lye et al. (2013) (18.75%) in Malaysia. This might be due to differences in animal management, milking systems, and milk handling practices among different countries.

In the present study, 5.71% isolation rate of *E. coli* O157:H7 was recorded from cheese sample. This rate is slightly higher than the report of Sancak et al. (2015) with 2% prevalence. In the study of Zelalem et al. (2015), *E. coli O157:H7* was found to survive the manufacturing of *Ayib* (Ethiopian cottage cheese). In Ethiopian cottage cheese, complete inactivation of the organism occurred after 20 and 40 min of cooking at 70 °C, indicating that if there is under treatment of heat, the cheese can act as source of *Escherichia coli O157:H7* (Zelalem et al. 2015). Furthermore, Spano et al. (2003) stated that, cheese could be free of *E. coli* O157:H7 if high temperature is used during milk processing. Furthermore, in some types of cheese like Cheddar cheese, *E. coli* O157:H7 has the ability to grow during the manufacture of the cheese and it could be detected by enrichment after 60 days of

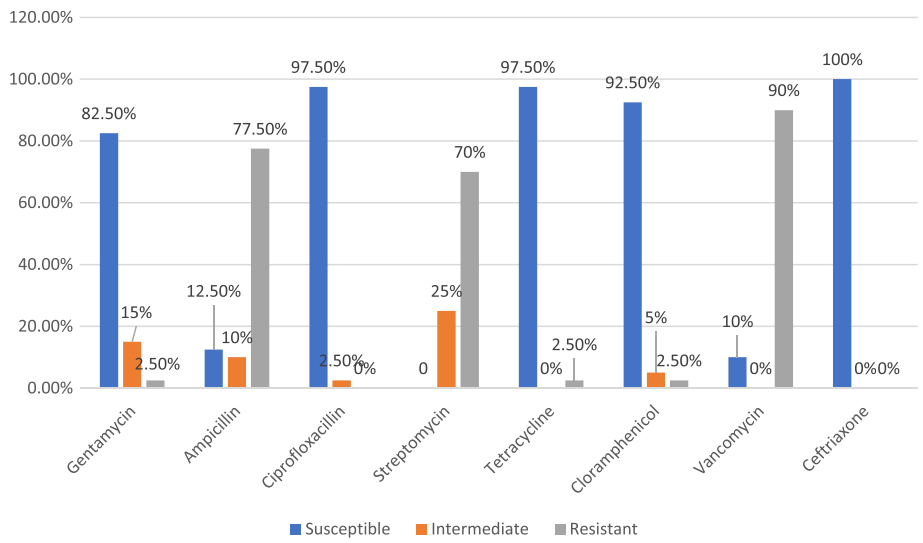

Fig. 5 In-vitro antimicrobial susceptibility of *E. coli* isolates

Table 4 Multidrug resistance patterns of *E. coli* isolates ($n = 40$)

Antimicrobial	Resistant to drug combination	Number (%) of resistant isolates	
		E. coli	*E. coli O157:H*
One drug	V	2 (5)	–
Two drugs	AMP, ST	3 (7.5)	1 (14.4)
	AMP, V	9 (22.5)	1 (14.4)
	V, ST	6 (15)	2 (28.4)
Three drugs	AMP, V, ST	16 (40)	2 (28.4)
Four drugs	AMP, V, ST, CHL	1 (2.5)	–
	CN, AMP, V, ST	1 (2.5)	–
	AMP, V, ST, T	1 (2.5)	1 (14.4)
None	Resistance to none	1 (2.5)	–
Total		40 (100)	7(100)

ripening (Reitsma and Henning 1996). In addition, Ramsaran et al. (1998) observed a significant increase in the number of *E. coli* O157:H7 during the manufacture of Camembert cheese, and stabilized number of colony forming units can be found after 75 days, indicating the potential for survival in this type of cheese.

The other finding of the present study is that *Escherichia coli* O157:H7 was not isolated from yogurt (Ethiopian naturally fermented milk) samples. Contrarily, Vahedi et al. (2013) reported 9% prevalence of *Escherichia coli* O157:H7 in yogurt samples and Zelalem et al. (2015) indicated that *E. coli* O157:H7 was found to survive the manufacturing of Ergo (Ethiopian naturally fermented milk). However, the absence of *E. coli* O157:H7 from yogurt is partly supported by the study of Osaili et al. (2013), who found that *E. coli* O157:H7 increased during fermentation and the population of *E. coli* O157:H7 decreased slightly during cooling. In connection to this, Osaili et al. (2013) indicated that lowering the temperature during cooling may lead to the increased susceptibility of *E. coli* O157:H7 to an acid environment and the population of *E. coli* O157:H7 during storage at +4 oC decreased sharply. It was evident that almost all cafeterias in the study area had refrigeration, and this could partly contribute for the absence of the isolates in the yogurt samples. Overall, the variation in the prevalence reports of the organism from cheese and yogurt samples could be due to differences in procedures followed during preparation of the dairy products, as well as improved enrichment and isolation procedures.

Table 5 Multidrug resistance profile of *E. coli* isolates based on type of food samples

Drugs	Number (%) of resistant *E. coli* isolates	Number in:	
		Milk and milk products ($n = 95$) [a]	Meat ($n = 65$)
AMP, ST	3 (7.5)	3	0
AMP, V	9 (22.5)	7	2
V, ST	6 (15)	4	2
AMP, V, ST	16 (40)	11	5
AMP, V, ST, CHL	1 (2.5)	1	0
CN, AMP, V, ST	1 (2.5)	0	1
AMP, V, ST, T	1 (2.5)	1	0
	Total	27 (28.4%)	10 (15.4%)

n = total number of samples tested; [a] sample didn't include pasteurized milk

As shown in Table 2, 3.07% of meat samples were harboring *E. coli* O157:H7, which is comparable to Hiko et al. (2008), Mersha et al. (2010), Jacob et al. (2014) and Zarei et al. (2013) who reported 4.2% (from Modjo and Debre zeit), 5.1% (from Modjo), 2.86% (from China) and 2.8% (from Iran), respectively. However, in Ethiopia, far higher prevalence was reported by Lula 2011 (11.2%), Mekuria and Beyene 2014 (10.4%) and Bekele 2012 (10.2%) from Dire Dawa, Tigray region and Addis Ababa, respectively. These variations could be due to differences in the hygienic conditions of meat preparation, processing, as well as storage.

The use of antibiotics in the treatment of *E. coli* O157:H7 infection is controversial, since antimicrobial therapy may increase the risk of development of hemolytic uremic syndrome (Molbak et al. 2002). Although some studies do not advise antibiotic treatment for infections caused by such bacteria, others suggest that disease progression may be prevented by administrating antibiotics during the early stage of infection (Schroeder et al. 2002). Thus, for the better response, an antimicrobial susceptibility test is necessary (Quinn et al. 2011). Hence, on the basis of this necessity, antimicrobial susceptibility testing was conducted on the isolates recovered from all the samples.

The present study showed that *E. coli* isolates were highly sensitive to ceftriaxone, gentamicin, ciprofloxacin, chloramphenicol and tetracycline. Meanwhile, the majority of the isolates were resistant to ampicillin, streptomycin and vancomycin. Similarly, Hiko et al. (2008) and Bekele (2012) from Ethiopia and Magwira et al. (2005) from Botswana revealed that the resistance of *E. coli* does exist mainly to ampicillin and streptomycin. However, various authors reported that *E. coli* is resistant to tetracycline (Hiko et al. 2008; Bekele 2012; Mude et al. 2017), which is contrary to the results of the present study. But in Dire Dawa, Mohammed et al. (2014) reported that *E. coli* was susceptible to tetracycline, which is in line with the present study finding.

Multidrug resistance analysis showed that 37/40 (92.5%) of tested isolates were resistant to different combinations of two to four tested antibiotics. This is in agreement with the report of Mude et al. (2017), who showed 92.3% of isolates were multidrug resistant. Moreover, various authors (Bekele et al. 2014; Iweriebor et al. 2015; Atnafie et al. 2017) from the country and abroad reported multidrug resistance patterns. Moreover, the present study revealed that the prevalence of multidrug resistant isolates was

higher in milk and milk products (28.4%) as compared to meat (15.4%) samples. This higher occurrence in dairy products could be related to the greater emphasis given to dairy production compared to beef production in the study district. Multidrug resistance usually occurred either due to indiscriminate utilization of antimicrobial agents or genetic mutation, which was difficult to elucidate with the present study methodology.

Conclusion

The presence of *E. coli* O157:H7 in foods of animal origin may originate from infected animals or unhygienic conditions during processing, handling and distribution. Importantly, the occurrence of *E. coli* O157:H7 and its multiple antibiotic resistant profiles shows a risk for public health and food safety, as well as animal health and production (Ulukanli et al. 2006). The higher prevalence of multidrug resistant *E. coli* isolates in dairy products is especially alarming. Proper handling and cooking foods of animal origin are probably as important in preventing *E. coli* O157:H7 infections.

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Availability of data and materials
All necessary data supporting our findings can be found in the repository.

Authors' contributions
SB, DS and AA developed the research concept and designed the methodology, data analysis and interpretation and preparation of the manuscript for publication. TM provided critical comments on the proposal methodology and reviewed the manuscript for publication. SB and TM carried out the sample collection, laboratory work and revision of the manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate
There was no involvement of animals or humans for sample taking, as this study was conducted on milk samples taken from containers which were ready for sale in non-standardized market systems.

Consent for publication
In our study, we don't have any images or videos, etc. of individual participants.

Competing interests
The authors declare that there is no financial or non-financial competing interest from any person or institute. We did not receive any technical assistance for developing the research concept or preparing the manuscript.

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