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Research Article

Assessment of Post-Harvest Handling Practices, Quality and Safety of Milk and Antimicrobial Susceptibility Profiles of *Escherichia coli* O157:H7 Isolated From Milk in and around Asella Town, Oromia, Ethiopia

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Keywords

- *E. coli* O157:H7
- Handling practices
- Latex agglutination test
- Milk; Post-harvest

Abstract

Background: Unpasteurized raw milk consumption can contribute for the the risk of ingestion and transmission of food-borne pathogens and ingestion of potentially harmful toxins. Many microorganisms can get access to milk and products, among these *E.coli* is one of food born pathogen which is found in unpasteurized milk . Coliforms and *E. coli* are often used as marker organisms. The presence of *E. coli* in milk is consideredas a reliable indicator of fecal contamination and indicates a possible presence of enteropathogenic and/or toxigenic microorganisms which constitute a public health hazard.

Objectives: To assess post-harvest handling practices of milk in dairy farms, milk vendors, milk collection centers and restaurant/kiosk and isolate and evaluate antimicrobial susceptibility profiles of *Escherichia coli* O157:H7 isolated from milk in and around Asella town, Ethiopia.

Methods: A cross-sectional study was conducted from November 2016 to April 2017, a total of 112 samples (87 dairy farms, 16 restaurants/kiosks, 5 milk venders and 4 milk collectors) with a single visit were interviewed to collect the required information using a semi-structured questionnaire.

Results: The result shows (66%) farmers in the study areas were kept their cattle under closed type barn. More than (48%) farmer did not wash their hands between milking and did not practice udder drying. (37.8%) farmers did not wash udder before milking. More than (75%) respondents venders, milk collectors and milk restaurant uses plastic containers for raw milk handling and storage. Of 112 samples tested only 38 (33.9%) were found to be positive for *E. coli* and from 38 samples which were positive for *E. coli* only 10 (8.9%) were found *E. coli* O157:H7 on latex agglutination test. No positive *E. coli* O157:H7 isolate was isolated from both milk collection center and restaurant. All *E. coli* O157:H7 isolates were checked for their susceptibility pattern by 10 selected antibiotics. The isolated strains were found to be 100% susceptible to chloramphenicol, norfloxacin, oxytetracycline, tetracycline, sulfamethoxazole, trimethoprim, streptomycin, and 30% susceptible to Erytromycin. However, 100% resistance to amoxicillin and vancomycin and 60% for cloxacillin and 40% for erythromycin. Intermediate susceptibility was observed in cloxacillin (40%). The indiscriminate use of antimicrobial agents might account, at least in part, for such a high resistance.

Conclusion: In this study, unhygienic practices of milking and post-harvest handling along the dairy value chain possibly contributed to microbial contamination of milk. Detection of *E. coli* in milk is of public health importance due to its zoonotic potential. It is recommended that veterinary/extension services be provided to livestock farmers on proper animal husbandry and control of zoonotic animal diseases. Awareness creation to the dairy farmers and all stakeholders at different levels regarding to milk handling practices should be given so as to reduce the milk rejection rate because of spoiled milk and milk borne pathogens resulting from contamination of milk.

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ABBREVIATIONS

E. coli: *Escherichia coli*, STEC: Shiga Toxin-Producing *E. coli*; STEC O157: Shiga Toxin-Producing *Escherichia coli* O157

INTRODUCTION

Milk plays an important role in human nutrition throughout the world where it promotes growth and maintenance of body tissue [1-3]. It is the most comprehensive food product of animal origin providing more essential nutrients (protein, energy, vitamins and minerals) in significant amounts than any other single food [4]. It is the major source of regular income for smallholder milk producers because it is produced and sold daily [5]. Absence of organized marketing network has made the produced milk unable to reach the consumer. Together with the perishable nature of milk post-harvest losses is high due to spillages and spoilage. In some case studies losses of up to 20-35% have been reported from milking to consumption for milk and dairy products [6]. It is well established that consumers want clean, wholesome and nutritious food including milk that is produced in a sound, sanitary manner and is free from pathogens [7,8]. In developing counties, including Ethiopia, the hygienic levels exercised during milk production are key factors affecting the quality of milk mainly the microbial quality [9]. Not only milk producers but also handlers such as collectors and transporters, vendors and consumers play a key role in ensuring the microbial quality of milk across the milk supply chain [10]. Mishandling and disregard of hygienic measures by milk handling personnel may enable spoilage microbes to come into contact with milk and in some cases to survive and multiply in sufficient numbers to reduce the shelf-life of milk and cause spoilage of milk before it reaches to its final destination [11,12]. Effects of post-harvest losses of spillage and spoilage as result of poor handling practices. For instance, in recent studies by Food and Agriculture Organization (FAO), economic losses in the dairy sector in Eastern Africa are estimated at $ 90 million per year. Causes of losses in the milk value chain take route in every transaction from production to consumption [13,14]. Food borne diseases that are caused bacteria include pathogenic *Escherichia coli, Salmonella, Campylobacter, Listeria, Yersinia, Shigella* and *Enterobacter.* Food-borne bacterial diseases are a serious challenge to human and animal health [15].

Escherichia coli (E. coli) O157:H7 is one of the most important food borne pathogens, causing diarrhea, hemorrhagic colitis and haemolytic uremic syndrome in humans worldwide [16]. *Escherichia coli* are genetically heterogeneous group of bacteria whose members are typically non-pathogens that are a part of the normal microflora of the intestinal tract of humans and animals. However, certain subsets of this bacterial species have acquired genes that enable them to cause intestinal or extra intestinal disease [17,18]. *E coli* that cause enteric disease have been divided into pathotypes, based on their virulence factors and mechanisms by which they cause disease. One of these pathotypes, called Shiga toxin-producing *E. coli* (STEC), refers to those strains of *E. coli* that produce at least 1 member of a class of potent cytotoxins called Shiga toxin. The STEC are also called verotoxin producing *E. coli.* The name Shiga toxin (STX), derived from similarity to a cytotoxin produced by *Shigelladysenteriae* serotype 1 and verotoxin (VT), based on cytotoxicity for Vero cells are used interchangeably [19,20].

Shiga toxin-producing *Escherichia coli* O157:H7 (STEC O157) can cause severe enteric infections. Symptoms may include abdominal pain, bloody diarrhea, hemorrhagic colitis and haemolytic uremic syndrome (HUS) [21,22]. Numerous sporadic infections and outbreaks caused by STECO157 have been reported in the United States and elsewhere in worldwide. The majority of STEC O157 infections are food borne; many are associated with bovine sources. STEC O157 was first linked to outbreaks of severe bloody diarrhea in 1982, and is often referred to as a "recently emerged" human pathogen [23]. *E. coli* O157:H7 was first recognized in 1982 as a human pathogen and cattle have been identified as a major source of *E. coli* O157:H7 infection of human but it is not pathogenic in cattle and present in the feces of healthy cattle [24]. Moreover, *E. coli* isolation reveals fecal contamination in the combined-sewer outflows [25].

To protect milk from spoilage loss as well as consumers from milk-borne public health problems, there needs to be the availability of documented information on hygienic milk handling practices of actors (producers, collectors and transporters, vendors and consumers) across the supply chain. This is because, such information may be important for governmental, non-governmental and other development organizations to undertake relevant development interventions, which make milk producers, traders and consumers to have clear understanding on the hygienic practices essential for safe milk handling. This understanding may be important to ensure safety and suitability of raw milk for its intended use. Furthermore, it has not been determined well to what extent hygienic milk handling practices of actors (producers, collectors and transporters, vendors and consumers) serve as sources of *E. coli* O157: H7 to milk contamination. Thus, the objectives of this study was to assess the post-harvest handling practices, quality, safety and hygienic practice of cattle milk, to isolate and identify *E. coli* O157:H7 from farm, vendors, milk collection centers and restaurant from raw/ unpasteurized and boiled cow milk and to identify the antimicrobial susceptibility patterns of *E.coli* O157: H7in and around Asella town of Oromia, Ethiopia.

MATERIALS AND METHODS

Study area

The study was conducted in selected sites in central Ethiopia, Oromia regional state of Arsi zone, in and around Asella town which were selected purposively based on their accessibility and availability of high dairy cows population. Asella town is the administrative center of the zone, and located 175 km southeast of Addis Ababa. Arsi zone is one of the 18 administrative zones of Oromia regional state. It is found in the central part of the region. It is located at 6°79' and 8°49' N and 38°41' and 40°44' E. It has an area of 2,118,675 hectares, of which 39.7% is highland, 29.1% is lowland and 27.5% is mid-altitude. The altitude of the area is ranging between 500 (Awash and Wabe valley) and 4245 (Mount Kaka) meters above sea level. The annual temperature varies between 10°C and 25°C. The average annual rainfall ranges between 901mm and 1200mm, with some spatial and temporal variability in quantities and distribution. Its pattern is

of a bimodal type with 60% occurring in the long rainy season extending from June to September and the short rainy season from December to February. The other two seasons are the cool dry season extending from October to November and the major dry season from March to May [26].

Study animal and population

The study animals were apparently healthy dairy cows located in and around Asella town. The study hasinvolved different actors and nodes along the dairy value chain which is farmers, milk collection centers, milk vendors and milk restaurant/kiosk.

Study design

A cross sectional study was carried out from November 2016 to April 2017 toassess milk post-harvest handling practices of milk from dairy farms, vendor, milk collection center and restaurant/kiosk and isolation of *Escherichia coli* O157:H7 from raw/ unpasteurized and boiled cow milk and antimicrobial susceptibility profiles of *Escherichia coli* O157:H7 in and around Asella.

Sampling technique

The study area is selected purposively due to accessibility and willingness of the dairy farm owners to participate in this research. Lactating cows were included to collect raw milk samples from milk containers (storage milk containers after milking). First the study populations were divided according to their location as urban (Asella town) and peri-urban (the surrounding areas). Then the populations were classified according to their geographic location Peasant association (PA). Then PAs were selected using simple random sampling and the dairy farms located within the PAs were identified. Finally, 87 household farms were selected purposively based on the availability of lactating cows and the willingness of the owners. A list of households owning dairy farms was obtained from records maintained by the Asella town multipurpose dairy development and formal interview was made to locate the farms, obtain farmers consent and to give a brief description on the research objectives. Purposive sampling was made for 16 milk restaurants/kiosks, 5 milk vendors and 4 milk collection center. Prior to sampling, all the restaurants/kiosks and milk vendors were identified. Milk samples were collected and general questions focused on the type of milk sold and source of milk was administered to all vendors and restaurants (Table 1).

Method of data collection

A single-visit-multiple-subject formal survey technique [27] was used to collect data through interviews. Data obtained from respondents was on milking system, milking frequency, milking hygienic practices (washing of millers' hand, milk utensils and udder before milking), farmers', sources of farm water, housing management. Structured questionnaires was used which focused on all selected farmers with lactating cattle to obtain information regarding animal management, milk production, milking and milk handling and source of water. In addition, milk vendors and processors and owners of milk restaurants was interviewed on the quality of milk they handle, possible sources of microbial contamination and type of container they use for handling and storage of milk . Lastly a checklist of questions was administered to workers at the milk collection centers. The questionnaire was made of pre-coded closed ended questions with very few open ended questions.

The questionnaire is administered through face to face conversation. While administering questionnaires, direct observation on general cleanliness and hygienic practices with regard to milk also done and noted. Upon finishing of the administration of questionnaires, milk sample was collected for laboratory analysis. All milk samples were collected from all the actors along the dairy value chain. In that aspect, milk samples was collected from farmers, restaurants /milk kiosks/ milk selling points, milk vendors, and milk collection centers. At farm level, a pooled milk sample was obtained directly from the containers used for storage. About 25 ml of milk sample was collected and put in a sterile tube and placed in a cool box with ice packs. Thereafter the samples was transported to Asella regional laboratory and stored at 20ºC until microbiological analysis. Types of milk samples intended to be collected are raw milk and boiled milk.

The *Escherichia coli* 0157:H7 organisms isolated from the milk sample, in the present study was tested for their antibiotic susceptibility. The antibiotic susceptibility test was performed on 10 isolates of *E. coli* O517:H7. The isolates were tested for 10 commonly used commercially available antimicrobials using the Kirby-Bauer disk diffusion method by 0.5 McFarland standards on Muller Hinton agar plats (Table 2).

Microbiological analysis

Media preparation:

Nutrient agar: Nutrient agar (OXOID® Ltd.,Oxoid, England) containing 1 g/l of '*lab-lecmo*' powder, 2 g/l of yeast extract, 5 g/l of peptone, 5 g/l of sodium chloride and 15 g/l of agar was prepared according to the manufacturer's instructions. Briefly, 28 g of the powder was dissolved in 1 liter of distilled water. The solution was boiled to dissolve completely and sterilized by autoclaving at 121 ºC for 15 minutes. Before use, the media was cooled up to 45 ºC poured onto sterile Petri dishes. The plates were left at room temperature for two hours for the media to solidify then put upside down in the incubator for 24 hours at 37°C to check for sterility and to dry the condensed vapor on the plate cover.

MacConkey agar: MacConkey agar REF (76875(MM011)) composed of 17 g/l of peptic digest animal tissue, 10 gm/l of lactose, 5 gm/l of sodium chloride, 0.03 gm/l of neutral red and 13.5 g/l of agar was prepared according to the manufacturer's instructions where 50.03 gm of the powder was dissolved in 1000 ml of distilled water. The solution was heated to dissolve and sterilized by autoclaving at 121 ºC for 15 minutes. Before use the media was cooled to 45°C and poured onto sterile Petri dishes. The plates were left at room temperature for two hours for the media to solidify then put upside down in the incubator for 24 hours at 37°C to check for sterility and to dry the condensed vapor on the plate cover.

Eosin methyl blue (EMB): The presumed well-selected typical and atypical colonies was again sub-cultured on selective

medium Levine Eosin Methylene Blue (EMB) Agar and incubated at 37ºC for 24 h Morphologicallytypical colonies was producing metallic sheen (Harrigan and MacCance, 1976) and under the same conditions in order to get pure colonies of *E. coli*. After the next 24 hrs of incubation, well-isolated colony was selected and sub-cultured further onto Nutrient agar (NA) so as to be used for biochemical confirmation.

Isolation and identification of bacteria

Stage 1: Culturing of milk samples: Petri dishes with MacConkey agar media was labelled and divided into two equal halves. A sterile loop will dipped into a thawed milk sample and streaked onto MacConkey agar plates as a differential media for identification of *E. coli*. Then, the plates was inverted and incubated at 37ºC for 24 hours. After incubation period, the plates was examined for typical and atypical colonies. Typical colonies of *E. coli* grown on MacConkey agar are dry, medium in size, pink in colour and appeared singular or in groups. Atypical colonies was small red colonies in singular or group form.

Stage 2: Sub-culturing of presumed E. coli colonies: The presumed well-selected typical and atypical colonies was again sub-cultured on selective medium Levine Eosin Methylene Blue (EMB) Agar and incubated at 37ºC for 24 h Morphologically typical colonies was producing metallic sheen [28] (Harrigan and MacCance, 1976) and under the same conditions in order to get pure colonies of *E. coli*. After the next 24 hrs of incubation, well-isolated colony was selected and sub-cultured further onto Nutrient agar (NA) so as to be used for biochemical confirmation

Stage 3: Biochemical confirmation of *E. coli*: Tests such as Gram staining and biochemical reactions like Oxidase, Catalase, Indole, Methyl red, Voges Proskouer (VP) and Citrate (IMViC) tests were done on well-isolated colony from nutrient agar plates to confirm the presence of *E. coli* in the test samples. Colonies producing, positive for tryptophan utilization (indole test) (red ring), positive for Methyl red, negative for citrate utilization (green slant) and negative for Voges-Proskauer (VP) test were considered to be *E. coli* positive [29] (Quinn et al., 2004). Isolate presumptive of *E. coli* for all biochemical tests were cultured on sorbitol MacConkey agar for further test on Latex agglutination test**.**

Gram staining technique: The Gram staining of the bacterial colony was done on a sterile glass slide as described by [30] (Cheesbrough, 2000).A drop of normal saline was placed on a glass slide and loop full of well-isolated bacteria colony was beadded and made a smear which was dried in air and fixed by gently flaming. A fixed smear was covered with crystal violet stain for about 2 minutes then, rapidly washed with slowly running tap water and again the smear was covered with Lugol's iodine for about 2 minutes and washed again with tap water. Thereafter,acetone-alcohol was used to decolorize the fixed smear and washed for the third time. Then, the fixed smear was covered with counter stain neutral red that stayed for about 2 minutes then washed off with running tap water. The slide with smear was placed on a draining rack for the smear to dry. A drop of oil immersion was added on the smear and examined under the light microscope with 100X objective to visualize the morphology of the bacteria. Gram positive bacteria appeared spherical or cocci in shape with pale to dark purple colour while Gram negative bacteria appeared rod or coccobacilli with pale to dark red colour.

Catalase test: Colonies that demonstrate the Gram's reaction identical with the E coli species was further tested for the presence of catalase enzyme. Pure colonies of the isolates were picked from the nutrient agar using a sterile loop and mixed with a drop of hydrogen peroxide (H_2O) on a clean microscope glass slide. Positive reaction indicated by the liberation of bubbles of oxygen within few seconds and those with negative reaction did not produce bubbles the catalase positive isolates were considered as *E coli*

Oxidase Test: The test was performed as described by Oxoid® Ltd., Basingstoke, Hampshire, England, Ref MB0266A, Lot 1284539. The well-isolated colonies was stickled and streaked onto the moistened oxidase detection strips using a sterile plastic loops, and then the strips was observed for colour change within 10 seconds. If the deep blue or purple colour appeared, confirming an oxidase positive reaction.

Indole Test: Peptone water was prepared and about 3 ml of it dispensed in test tubes using a sterile pipette. Then, fresh sterile loops was used to pick a well-isolated colony of bacteria and inoculated into bijou tubes, thereafter, the tubes was incubated at 37ºC for 48 hours. After incubation period, 0.5 ml of Kovac's Indole Reagent (Loba Chemie Pvt. Ltd, Lot LM01131303) was added to the inoculated test tubes. The tubes was subjected to gentle shaking and examined for red colour in the surface layer within 10 minutes [30] (Cheesbrough, 2000). A red ring on top of the tube indicated indole positive reaction

M**ethyl red test:** The standard buffered glucose MR-VP broth used for the MR and VP tests was modified by substituting sodium chloride (NaCl) for dipotassium phosphate and add- ing 1% agar. The composition of the medium per liter was as follows: proteose peptone, 7 g; glucose, 5 g; NaCl, 5 g; agar, 10 g. The medium was dissolved by steaming and sterilized at 121°C for 15 min. Final pH was 6.3. For performance ofthe MR test, 5 drops of MR indicator solution were added to bacterial growth on the agar surface [31].

Voges-Proskauer: The VP test for the production of acetylmethylcarbinol was performed on the same modified (unbuffered) medium used for the MR test. Growth on the agar surface was flooded with 0.6 ml of a -naphthol (5% in absolute ethyl alcohol) followed by 0.2 ml of creatineKOH reagent. The creatine-KOH reagent was stored at 3°C for a maximum of 21 days.

Citrate agar test: Citrate utilization was determined on conventional Simmons citrate agar. The medium was dissolved by steaming and steri- lized at 121°C for 15 min. No reagents were used for this test [31].

Stage 4: Screening Test by *E. coli* O157 Latex agglutination test: Latex agglutination test was employed using latex kit for the screening of *E. coli* O157:H7. Sorbitol-negative (clear) colonies exhibiting colony morphology typical for *Ehscherichia* coli O157:H7 per plate was picked and spread plated on CT-SMAC. Then after 24 hour of incubation, a fresh single colony of non-sorbitol fermenter from sorbitol MacConkey ager was picked and

subjected to latex agglutination using an *E. coli* O157 latex kit. Isolate presumptive of *E. coli* O157:H7 for all Latex agglutination tests were cultured on Nutrient Agar (NA) for antimicrobial susceptibility testing

Antimicrobial susceptibility: The *Escherichia coli* 0157:H7 organisms isolated from the milk sample, in the present study was tested for their antibiotic susceptibility. The antibiotic susceptibility test was performed on 10 isolates of *E. coli* O517:H7. The isolates were tested for 10 commonly used commercially available antimicrobials using the Kirby-Bauer disk diffusion method by 0.5 McFarland standards on Muller Hinton agar plats.

Colonies isolated from pure culture were transferred into a test tube of 5 ml tryptone soya broth. The turbidity of the broth incubated was adjusted by adding sterile saline or more isolated colonies to obtain turbidity visually comparable with that of 0.5 McFarland standards. Muller- Hinton Agar (MHA) plate was prepared using a sterile cotton swab dipped into tryptone soya broth culture, and then the surface of MHA plate was swabbed.

Later the antibiotic discs ampicillin (10 µg), bacitraicin (10 µg), tetracycline (10 µg), chloramphenicol (30 µg), cloxacillin (5 µg), erythromycin (15 µg), norfloxacin (10 µg), sulphamethoxazole (100 µg), chloramphenicol (30 µg) and stereptomycin (25 µg) were placed on the agar plate using sterile forceps, and pressed gently to ensure complete contact with the agar surface. Antibiotic discs used were from Oxoid, (Hampshire, England). The plates were incubated for 24 hours at 37^0C under aerobic condition. Inhibition zones were measured and interpreted as susceptible, intermediate and resistant according to NCCLS guidelines [32] (NCCLS, 2012).

Method of data analysis

All the quantitative and qualitative data were summarized on Microsoft excel spread sheet and analyzed. The analysis was carried by STATA version 11.0. Association isolation frequency and considered variables (sample types, sample origin,) determined by Chi-square tests. The significance level was set at $p< 0.05$.

RESULTS

In this study, 112 individuals were requested for an interview and accepted to participate. Several practices were undertaken at farm level as according to the information obtained from the respondents which were consideredtobe the factors which predispose raw milk to microbial contaminations, such as animal house floor, cleanliness of the animal house, washing hands between milking, washing udder and/or teats before milking, cloning milking utensils, source of water for cleanliness (hands and milk equipment's), use of separate and shared towel for draying teats,themain source of water for sanitary activities associated (Table 3-6).

Of the total 112 milk samples, 38 (33.9%) samples were positive for *E. coli* with the highest percentage in raw milk from collected from dairy farms (Table 7).

Of the 38 milk samples which were positive for *E.coli* subsequently tested for *E. coli* O157:H7 and 10(8.9%) showed positive with highest percentage observed in raw milk collected from farmers and venders in both districts (Table 8).

The *E. coli* O157: H7 isolates were subjected to antimicrobial susceptibility test, using 10 selected antimicrobials. The isolated strains were 100% susceptible to sulfamethoxazoletrimithoprim

Table 1: Type of milk sample collected for laboratory analyses.

Type of milk	Source	No. Sample
Raw milk	Farmer	87
	Venders	5
	Milk collection center	4
Boiled Milk	Restaurants/kiosks	16
Total		**112**

Table 2: Antibiotic disks used to test *E. coli* O157:H7 and their respective concentrations.

No	Antibiotic disks	Disccode	Concentration (µg)	Diameter of Zone of inhibition in mm		
				Resistance	Intermediate	Susceptible
01	Oxytetracycline	OT	30	≤11	12-14	>15
02	Tetracycline	TE	10	≤11	12-14	>15
03	Chloramphenicol	C	30	≤16	13-17	>18
04	Streptomycin	S	25	≤11	12-14	>15
05	Trimethoprim-sulfamethoxazole	TR	100	≤10	11-15	>16
06	Cloxacillin	OB	5	≤10	11-12	>13
07	Norfloxacin	NOR	10	≤12	13-16	>17
08	Vancomycin	VA	30	≤15		>15
09	Ampicillin	AM	10	≤13	14-16	>15
10	Erythromycin	E	15	≤13	14-22	>23

Table 3: General hygienic management practices made by farmers (n=87).

Variables	Category	Total % (87)
Animal breed	Crossed	89.4
	Local	10.4
Breeding system	AI	76.4
	Natural	14.1
	Both	9.5
Feeding system	Stall feed	85.5
	Grazing	7.5
	Both	7
Types of animal barn	Open	10.5
	Semi-open	23.5
	Closed	66
Floor	Concurrent/cement	54.1
	Stone	7
	Mud/earth	36.1
Drainage	Good	15.2
	Satisfactory	36.5
	Poor	48
Source of water	Tap	77.5
	River	21.5
	Well	1.1
Hand wash	Before milking	100
	Between milking	50.9
	No wash between milking	49.1
Udder wash	before milking	94.12
	no wash	5.88
Towel used for drying teat	Individual towel	0
	Shared towel	55.29
	No towel used	44.71
Cleaning of milk utensil	Yes	100
	No	0

Table 4: Equipment used for milk handling and storage in milk collection center (n=4).

Variables	Category	No(%) respondent
Containers used for milk storage	Plastic container	50
	wide necked aluminum vessel	50
Milk quality parameter	Alcohol test	50
	Milk Lactometer	50
Vehicle used to transport milk	Bajaj	25
	Donkey	75
Chilling and cooling machine	Refrigerator	50

(SXT25µg), stereptomycin (S25µg), oxytetracycline (OT30µg), chloramphenicol (C30µg), Tetracycline (TE10µg) norfloxacin. From all antimicrobials used ampicilin (Aml25µg) and vancomycin (VA30µg) (100%) resistance to all isolates followed by (60%), cloxacillin (OB5µg) and erythromycin (40%). Intermediate susceptibility was observed in (70%) erythromycin (E30) and (40%) cloxacillin (OB5µg) (Table 9 and Figure 1).

Table 5: Equipment used for milk selling and sanitary practices performed by venders (n=4).

Variables	Category	No(%) of vender respondent
Type of milk sold	Raw milk	100
	Boiled milk	0
	Fermented milk	0
Customers	Households	75
	Restaurant/Kiosk	25
Type of container for selling milk	Wide necked-aluminum vessels	25
	Narrow necked plastic containers	25
	Used water bottles	50
Time to finish milk	3 hrs. after collection	50
	6hrs after collection	50
	9 hrs. after collection	0
	12 hrs. after collection	0
Cleaning routine for the milk containers	Cleaning just before putting the milk	50
	Cleaning after delivery of milk	0
	Twice a day (before putting in milk and after delivery of milk	50
Cleanliness of the environment	Very clean	0
	Clean	25
	Dirty	75

Table 6: Source of milk, preparation of milk and equipment used for handling practices by restaurant/kiosks (n=16).

Variables	Category	No(%) of restaurant respondent
Type of milk sold	Raw milk	13.33
	Boiled milk	92.33
	Fermented milk	13.33
Milk Source	A recognized vendor(s) in the area	13.33
	Famer(s) in the neighboring village	40
	Farmer(s) from the same village	40
	From their own farm	6.64
Containers used for milk storage	Wide necked-aluminum vessels	13.33
	Wide necked-plastic vessels	
	Narrow necked plastic containers	80
	Sieve and boil	80
Preparation of milk for consumption	Boil	20
How milk is served	Hot from a thermal flask in a cup	93.33
	Hot from a cooking pan in a cup	6.67

Table 7: Isolation and distribution of *E coli* in Area, sample source and sample type.

Variables		N	Number of positive	%	Chi2(5)	OR (95%)	p-value
District	Asella	64	18	28.1	4.63	Ref	Ref
	Bilalo	7	2	28.5		1.02	0.980
	Gorasilingo	12	6	5		2.56	0.143
	Gonde	11	6	54.5		3.0	0.093
	Scabeti	7	2	28.5		1.02	0.980
	Kallicho	11	4	36.3		1.46	0.581
Total		112	38	33.9			
Sample Source	Collection center						
	Farm	4	1	25	2.84	Ref	Ref
	Restaurant	87	33	37.9		1.8	0.06
	Venders	16	3	18.7		0.69	0.781
		5	1	2		0.75	0.858
Total	Boiled	112		82.9			
Sample type	milk	16	3	18.75	1.9	Ref	Ref
	Raw milk	96	35	36.45		2.48	0.177
Total		112		55.2			

Table 8: Isolation frequency of *E. coli* O157:H7 and its association with sample types and sample.

Variables		N	Number of positive	%	Chi2(5)	p-value
District	Asella	64	4	6.25	3.54	0.617
	Bilalo	7	1	14.2		
	Gorasilingo	12	2	16.6		
	Gonde	11	2	18.1		
	Scabeti	7	0	0		
	Kallicho	11	1	9		
Total		112	10	8.9		
Sample Source	Collection center	4	0	0	2.9	0.403
	Farm	87	9	10.4		
	Restaurant	16	0	0		
	Venders	5	1	20		
Total		112	10			
Sample type	Boiled milk	16	0	0	1.83	0.176
	Raw milk	96	10	10.4		
Total		112	10			

Table 9: Antimicrobial susceptibilities amongst 10 isolates of *E. coli* O157: H7.

Antibiotic disks	Susceptible	Intermediate	Resistance
	No. (%)	No. (%)	No. (%)
Oxytetracycline	10(100%)	0	0
Tetracycline	10(100%)	0	0
Chloramphenicol	10(100%)	0	0
Streptomycin	10(100%)	0	0
Trimethoprim- sulfamethoxazole	10(100%)	0	0
Cloxacillin	0	*4(40%)*	6(60%)
Norfloxacin	10(100%)	0	0
Vancomycin	0	0	10(100%)
Ampicillin	0	0	10(100%)
Erythromycin	3(30%)	7(70%)	0

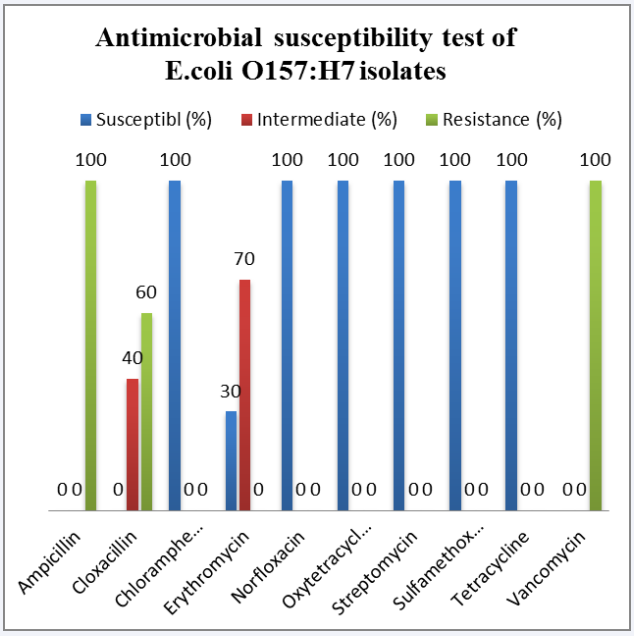

Figure 1 Antimicrobial susceptibility pattern of *E. coli O157: H7* isolated from milk.

DISCUSSION

The present study was conducted to assess cattle milk quality and handling practices and determining presence of selected milk-borne zoonotic pathogen along the dairy value chain and determine antimicrobial susceptibility profile of *E. coli* O157:H7 in and around Asella districts of Arsi zone, Oromia Regional State of Ethiopia. Possible risk factors for microbial contaminations along the dairy value chain were explored and the involvement of *E. coli* O157:H7 as important milk-borne pathogens was elucidated by using latex agglutination test using anti O157 and H7 serum (Oxoid). *E. coli* O157:H7 was detected in 10 (8.9%) of 112 analyzed samples including nine bulk milk sample taken from farm and one from venders. None of milk sample that are taken from milk collection centers and restaurants contain *E. coli O157:H7*. There are a number of studies from different countries concerning the incidence of *E. coli O157:H7* isolation on a variety of foods [33-39]. Also reported that 6% of raw cow's milk samples examined in Egypt were contaminated with *E. coli O157:H7* [40]. Reported 3% of the milk samples tested in Austria to be positive for *E. coli O157:H7* and [41] found that 1% of 500 analyzed samples including two diced meat, one minced meat and two raw-milk cheese also [42] found that only 0.3% of the milk analyzed in Germany was contaminated with this serotype. Similar studies on raw cow's milk performed in the USA analyzing 42 samples [34] and in the Netherlands analyzing 1011 samples [43] resulted in no *E. coli O157:H7* isolation. Our study is lined with some reports from previous works 8% from Ethiopia [44], 8.3% from Iran [45] and 9.6% from Iran [46] at abattoir level. The reasons for this high isolation of *E.coli* 0157:H7 in the study area could be due to unhygienic practices during milking and poor milk handling.

The unhygienic manner of animal house floor and milking procedures might have contributed for environmental contamination of milk with fecal and infected animal wastes.

The farmers in the study areas were used 66% closed type barn following 23.5 % and, 10.5% semi-open and open type barn (Table 3). Result similar to [47] 80.4% of the respondents were used house type barn in central highland of Ethiopia and [48] 180(100%) in Adeaberga and Ejerie districts of west shoa zone, Ethiopia [49]. Farmers milking in open air exposure to contaminants enter from the environment [50]. Also who reported farmers milked their animals from undesignated poorly maintained milking hades/parlors predisposing milk to contamination and spoilage. Maintaining the sanitary condition of milking area is important prerequisite for clean milk production [47]. The milker can be an important source of milk contamination. Therefore, keeping good personal hygiene and milkers should be in good health during milking operation [47]. Most of the interviewed dairy producers 87(100%) washed their hands before milking additionally 50.9% washed their hand between milking while the rest 49.1% did not wash their hands between milking (Table 3).

The finding of the present study is higher than [48] reported 69.4% producer in Shoa zone Adeaberga and Ejerie districts wash their hand before milking. Cleaning of the udder of cows before milking is one of the most important hygienic practices required to ensure clean milk production. This is important since the udder of the milking cows could have direct contact with the ground, urine, dung and feed refusals [47]. As observed in this study 94.12% of the dairy farmers washed their cow's udder before milking and 37.8% did not wash udder before milking (Table 3) and simply allowed their calves to suckle before milking. Calf suckles and milking follows without cleaning the teats, Saliva from the calf mouth and unwashed teats increase bacterial counts [51]. The current result was lined with than [52] reported that 82.5% of the small size farm owning households in Hawassa city practice pre milking udder washing. But our result is higher than same previous study [48] who 62.2% respondents washed their cows udder from Shoa zone Adeaberga and Ejerie districts Conversely to this result [53] who reported that all respondents in Gurage Zone of Ezha district, do not have the experience of udder washing before milking

The use of individual towel and following essential cleaning practices during milking is important for the production of quality milk [47]. However, there was no practical application of the use individual towels for udder dryingamongst the respondents, (55.29%) used common towel and 47.1 % reported they did not practice udder drying (Table 3).The current study is higher than Saba (2015) reported that 15.6% of the study participants used common towel. This study also agree with [48] who reported that 46.7% of the smallholder households did not use towels for udder drying. Milking in dry condition significantly reduces bacterial count; thereby reduces milk rejection due to bacterial contamination. It is because no water droplets remains in the surface of the udder to drip into the milk and due to less chance of leaching dirt and bacteria from udder, teats and hands into milk [54] .

The source of water used for hygienic practices across the milk supply chain is presented in (Table, 2). For production of quality milk a good supply of clean water is essential. Water used

for washing and rinsing milk equipment and containers during milk handling must be the same safety and purity as drinking water [55]. Smallholder producers in and around Asella districts used different water sources for cleaning purpose i.e. tap water (77.5%), river water (21.4%) well water (1.1%), respectively. Water from non-tap sources used for different purposes can definitely contribute to poor quality milk and milk products .our study agree with [43] which is (19 %,) Smallholder producers in Ejerie district use river water. The finding is higher than finding of [56] who reported majority (64.4%) of respondent milk producers in Ezha district, Ethiopia were using water from non-tap sources for hygienic practices. It is important that producers should at least filter and heat treat it before use [47]. In the present study, almost all of the dairy producers 100 % washed milking utensils after every use (Table 3). 50% venders were washed milking utensils after every use and 50% were cleaned their milking utensil before and after usage (Table 4). Milking and milk storage utensils are properly cleaned and maintained.

Therefore, cleaning and disinfections of equipment after each milking is important for reduction of milk contamination from the equipment [57]. Producers should pay particular attention for the type as well as cleanliness of milk equipment. The equipment used for milk handling and the sanitary practices related to milk handling equipment across the supply chain are presented in Table 4-6. The results of this study revealed that (80%), of milk restaurants (50%) milk collectors and transporters and (75%) vendors in the study area were using plastic containers for raw milk handling and storage. [58,59] also mentioned in their study that plastic jar is the main milking equipment in the studied districts. Plastic jerry cans for milk handling is practiced by the majority of milk producers and almost all small-scale agents (collectors and transporters as well as vendors) is used in Kiambu County in Kenya [10]. The use of plastic containers is not advisable as it is sensitive to heat. Moreover, its surface is easily scratched by nature with the common cleaning systems. As a result, after some time the surface will contain a number of scratches, which can hardly be seen but are nearly impossible to clean with the common cleaning systems and provide hiding places for bacteria during cleaning and sanitization [60]. According to [61] use of plastic can be potential source of contamination of milk by bacteria. Because these equipment allow multiplication of bacteria on milk contacted surface. In connection with this, some researchers had reported that aluminum or stainless steel is preferred to other containers for milk handling [9,61]. Therefore plastic jar used for milk processing and storage determine the quality of milk and milk products. Venders' milk collector and restaurant therefore pay particular attention for the type as well as cleanliness of milk equipment should be easy to clean.

After milking proper milk cooling method is essential to maintain the quality of milk. 50% of Milk collection centers used refrigerators during collection, storage and transportation to processing plant and 50% did not have cooling facilities for raw milk to preserve. Milk processor and dairy cooperative union used vehicles for milk transportation. The vehicles were not appropriate for raw milk transportation because its lacks cooling facilities (Table 4).

Antibiotic resistant bacteria pose a growing problem of concern, worldwide since the bacteria can be easily circulated in the environment. Effectiveness of current treatments and ability to control infectious diseases in both animals and humans may become hazardous. A relatively high number of strains are resistant to the antimicrobial commonly used in the therapeutic protocol of many humans and animal infections [62] Food contamination with antibiotic-resistant bacteria can also be a major threat to public health, as the antibiotic resistance determinants can be transferred to other pathogenic bacteria, potentially compromising the treatment of severe bacterial infections. The prevalence of antimicrobial resistance among food-borne pathogens has increased during recent decades [63].

Antimicrobial resistance pattern of *E. coli* O157:H7 isolates from animal and human sources have been reported in Ethiopia by [39]. In the present study, all of the 10 isolates were highly susceptible to tetracycline, oxytetracycline, sulfamethoxazole-trimethoprim, chloramphenicol and norfloxaclin followed by relatively lower susceptible by erythromycin (30%) the result of this study almost comparable with work of [44,64]. However, the study conducted in Saudi Arabia [65], revealed that there was resistant strain to the drugs such as tetracycline, sulfamethoxazole-trimethoprim, and chloramphenicol. This variation probably attributed to the expression of resistant gene code by the pathogen which associated with emerging and re-emerging aspects of the isolates with the regards of different agro ecology [66]. On the other side, the current study revealed that all isolates were highly resistant to Amoxicillin (AML25µg) and vancomycin (VA30 µg). Similar findings were reported by many researchers [67-69]. This might be due to the use of inappropriate antibiotics for treatment of diseases [70] and also excessive use of antimicrobials for therapeutic and prophylactic treatment [71].

CONCLUSION

In the study area, unhygienic practices of milking and post-harvest handling along the dairy value chain possibly contributed to microbial contamination of milk. Detection of *E.coli* in milk is of public health importance due to its zoonotic potential. It is recommended that veterinary/extension services be provided to livestock farmers on proper animal husbandry and control of zoonotic animal diseases.

Awareness creation to the dairy farmers and all stakeholders at different levels regarding to milk handling practices should be given so as to reduce the milk rejection rate because of spoiled milk and milk borne pathogens resulting from contamination of milk.

Most human diseases are caused by pathogens from animal and/or animal products like milk and milk products. However, the contaminated one acts as source of *E.coli* O157: H7 which needs preventive actions at any point in the food production chain.

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