Original Research Article
DOI: https://doi.org/10.62418/ijvph.10.2.2024.58-64

Characterization of *Escherichia coli* Isolated from Raw Cow Milk from Shops in Asella Town, South East, Ethiopia

Asaminew Getu Ayele [(1)], Amana Feyisa Amesa [(2)], Leta Muleta Kisi [(3)], Tesfaye Rebuma Abdeta [(4)], Tamasgen Ragasa[(5)], Mahendra Pal [(6)]*

[(1)]Doctor of Veterinary Medicine, District Veterinary Clinic coordinator Barbare, SNNP Ethiopia, [(2)]Doctor of Veterinary Medicine, District Veterinary Clinic coordinator, Abuna Gindeberet, Oromia, Ethiopia, [(3)]Shaggar City Administration, Sebeta Sub-City administration Meta Woreda Agricultural office, Sebeta, Oromia, Ethiopia, [(4)]Shaggar City Administration, Sebeta Sub-City Administration Agricultural office, Sebeta, Oromia, Ethiopia, [(5)]East Wallaga Zone, Wayu Tuka District Agricultural Office, Nekemte, Oromia, Ethiopia, [(6)]Narayan Consultancy of Veterinary Public Health and Microbiology, Bharuch, Gujarat, India

(Received: 20th September 2024 | Accepted: 15th December 2024)

Abstract

Foodborne infections are an important challenge to public health and cause significant economic problems in many countries of the world. This research was conducted to assess the occurrence of *Escherichia coli* in raw milk samples from retail shops, to estimate effect of practices associated with milking and post-milking processes and to determine the antimicrobial susceptibility profile of *E. coli* isolates. A cross-sectional study was conducted from November 2018 to April 2019 from raw cow's milk shops in Asella town. A simple random sampling strategy was followed and a total of 384 raw milk samples were collected and immediately processed for *E. coli* isolation and identification by using selective media and biochemical tests. From 384 samples, 177 (46.09%) were positive for *E. coli*. Hygienic condition of the milk container, equipment washing practice and milk shop location was found to be significantly associated with the prevalence of *E. coli.* Forty two (10.94%) *E. coli* were from stainless steel and 135 (35.16%) from plastic milk containers from milk shops, with statistically significant differences ($P = 0.001$). However, the prevalence of isolated *E. coli* was 33.33% in poor hygienic sanitation and 12.76% in good hygienic sanitation, with a statistically significant difference (P-value = 0.006). Randomly selected 15 *E. coli* isolates were subjected to antimicrobial sensitivity test with seven commonly used antimicrobials disks. *E. coli* isolates showed susceptibility to tetracycline (73.33%) and sulfamethoxazole-trimethoprim (66.66%) and were resistant to kanamycin (100%), penicillin-G (100%), amoxicillin (100%) and erythromycin (86.66%). Hence, attention should be given to proper handling of the raw milk and to use susceptible antibiotics in the treatment of diseases both in humans and animals.

Keywords: Antimicrobial Disk, *E. coli,* Raw Milk, Susceptibility test

Introduction:

Foodborne infections are an important challenge to public health and cause significant economic problems in many countries (WHO, 2015). The crucial goal of all food safety programs is to prevent food products contaminated by potential pathogens from reaching the consumer. Milk is an excellent medium for bacterial growth, which not only spoils the milk and associated products but also can cause infections in consumers (Oliver et al., 2005). Because of the specific production, it is not possible to fully avoid contamination of milk with microorganisms; therefore, the microbial contamination of milk is an important tool in determining its quality (Torkar and Teser, 2008).

Huge numbers of microbes can get access to milk and various milk products, including *Escherichia coli*, which is an indicator of milk and milk product contamination disease that can be transmitted directly or indirectly between animals and humans (CDC, 2016).

E. coli is a zoonotic pathogen that causes morbidity and mortality in humans and animals in developing as well as in developed nations (Pal, 2007). This bacterium is common in milk and dairy products in developing countries such as Ethiopia because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment and lack of education for food handlers (Farzan et al., 2012).

In countries where foodborne diseases were investigated and documented, the relative importance of pathogens like *S. aureus, Campylobacter jejuni*, *E. coli and Salmonella* species was recorded as a major cause of foodborne diseases (Pal, 2007; Rashid et al., 2013; Pal, 2017; Pal et al., 2022; Pal et al., 2024). These organisms were known to cause acute gastroenteritis and may cause more serious septicemic disease, usually in the very young, the elderly or immunocompromised individuals.

Currently, the other major problem to human health is the issue of antimicrobial resistance due to the use of antibiotics in livestock production as well as human disease conditions in developing countries. In Ethiopia, the major antibiotics used for the treatment of animal and human diseases include penicillin, streptomycin, gentamycin and oxytetracycline. Even though it needs a better understanding of antibiotic use in Ethiopia, the variation of drug resistance might be due to indiscriminate use of antimicrobials in animal production without prescription in the animal and human health sectors, which might favor selection pressure that increased the advantage of maintaining resistance genes in bacteria (Mekonnen et al., 2012).

So far, there are no studies conducted on the occurrence and drug sensitivity profile of *E. coli* from milk in Asella town, Arsi zone. Therefore, the objectives of this research were to assess the occurrence of *E. coli* in raw milk samples from shops, to estimate effect of practices associated with milking and post-milking processes and to determine the antimicrobial susceptibility profile of the *E. coli* isolates.

Materials and Methods:

Study Area:

The study was conducted from October 2018 to June 2019 in Asella town. Asella is the capital city of Arsi Zone, located about 166 km south-east of Addis Ababa, the capital city of Ethiopia, at a geographical coordinate of 38032'-40050' east longitude and 136045'-8050 'north latitude. The average altitude of the town is 2300–2700 M.a.s.l. with a mean annual rainfall. The town has eight kebeles and the population of the town is 101,739.

Sample Size and Sample Size Determination:

A simple random sampling strategy was followed to collect raw milk from individual milk shops. To calculate the total size, the sample size will be decided based on the formula described by Thrusfield (2007) with a 95% confidence interval at 5% desired absolute precision and the assumption of the expected prevalence of 50% since there are no more previous reports in this area.

$$N = \frac{1.96^2 \times p\,(1-p)}{d^2}$$

Where N= Sample size

P= expected value

d= desired absolute precision

Accordingly, the total sample size for this study was 384.

Study Design:

A cross-sectional study was conducted from October 2018 to June 2019 on raw cow milk samples collected from different sources of raw milk shops in Asella towns.

Sample collection

There were a total of 384 raw milk 5ml/10ml samples collected from milk shops from different sources in Asella town. Hygeinic condition and material of milk container, container washing practice and milk shop location were also recorded. After the milk samples are aseptically collected, samples are labeled and packed with sterile bottles and transported with an ice box to the Asella Regional Veterinary Laboratory for bacterial isolation and identification. Samples were processed immediately for bacterial identification to species level using culture media and then isolates were kept in the refrigerator at 4 °C.

Isolation and Identification E. coli

One ml of thoroughly mixed raw milk were aseptically taken and added to 9 ml of sterile nutrient broth and incubated overnight at 37^0C for 24 hrs. The mixture of nutrient broth and raw milk sample was subcultured on a sterile nutrient agar plate under aseptic conditions and incubated at 37^0C for 24 hrs. An enriched sample was taken from agar, then Gram-staining and KOH tests were done to differentiate gram-negative bacteria from gram-positive bacteria. After differentiation of gram-negative bacteria from gram-positive bacteria, gram-negative bacteria were inoculated on MacConkey Agar (Oxoid, UK), by quadrant streaking technique and plates were incubated at 37^0C for 24 hrs. Pink-colored colonies observed after incubation were considered presumed for *E. coli*. A single isolated colony was picked and streaked on Eosin methylene blue agar (EMB) medium (Oxoid, UK) and incubated at 37^0C for 24 hrs. Those that produced a characteristic metallic sheen on EMB agar medium were considered positive for E. coli. Such colonies were taken and tested for biochemical tests, such as catalase, triple sugar iron tests (TSI), indole production, citrate utilization and methyl red tests. Voges-Proskauer (VP) tests, oxidase tests and motility tests were carried out to identify the organisms that were isolated from the samples according to the standard procedure described by Quinn and others (2011) and Kanungo (2017).

Table 1: Biochemical characterization of *Escherichia coli*

Biochemical test	Reaction
Gram stain	Gram negative (small road shape)
KOH	+ve (gel formation)
Oxidase	-ve

Catalase	+ve
Simmon's	-ve
Indole- Production	+ve
Methyl Red	+ve
Voges- Proskauer	-ve
TSI test	A/A (yellow +gas) without H2S production
Motility test	Motile

TSI: Triple Sugar Iron; -ve: Negative; +ve: Positive

Antimicrobial Susceptibility Test:

An antimicrobial susceptibility test, through the Bauer - Kirby diffusion test, was performed for all *E. coli* isolates following the protocol in CLSI (2015). At least 15 isolated *E. coli* bacteria from a nonselective agar plate (nutrient agar); just the top of the colonies is touched and the growth is transferred to a tube containing 4-5 ml of nutrient broth. The inoculated broth was incubated at 37^0C for 2 hours until a visible turbidity appeared and the turbidity was compared to 0.5 McFarland standards and then the bacterial suspension was inoculated on Mueller Hinton agar (Oxoid, UK) with the sterile swab to cover the whole surface of the agar. The inoculated plates were left at room temperature to dry. Before using the antimicrobial disks, they were kept at room temperature for 1 hour and the antibiotic-impregnated disks were placed on the agar surface by the help of sterile forceps, in such a way that the distance between the centers of the two disks was not less than 24 mm. Following this, the plates were incubated aerobically at 37^0C for 24 hrs.

The diameters of the zone of inhibition around the disks were measured to the nearest millimeter using calibrated rulers and the isolates were classified as susceptible, intermediate and resistant according to the interpretation guidelines given by Jan (2013).

Data Management and Analysis:

All data were checked against the standards and methods used to perform the study. Data was entered in a Microsoft Excel spreadsheet and analyzed using STATA version 11. Descriptive statistics such as percentages and frequencies were computed to report desired outputs. The association between risk factors was tested by chi-square and logistic regression and the significant difference was perceived when the P-value was less than 0.05.

Table 2: Drug susceptibility interpretive zone of inhibition diameter

Antibiotics	Disc code	Potency	Zone of diameter		
			S	I	R
Tetracycline	TE	30µg	≥15	12-14	≤11
Streptomycin	S	100µg	≥15	12-14	≤11
Kanamycin	KAN	30µg	≥18	14-17	≤13
Penicilline- G	PG	10µg	≥15	12-14	≤11
Trimethoprim-sulphamethoxazole	T-SXY	1.25/23.75µg	≥16	11-15	≤10
Amoxacilin	AML	10/20µg	≥18	14-17	≤13
Erythromycin	ERY	15µg	≥23	12-14	≤11

Data Management and Analysis:

All data were checked against the standards and methods used to perform the study. Data was entered in a Microsoft Excel spreadsheet and analyzed using STATA version 11. Descriptive statistics such as percentages and frequencies were computed to report desired outputs. The association between risk factors was tested by chi-square and logistic regression and the significant difference was perceived when the P-value was less than 0.05.

Results and Discussion:

Isolation and Identification of *E. coli:*

Among the total 384 raw cow milk samples collected from different milk shops in Asella town, 177 (46.09%) samples were found to be positive for *E. coli* as per cultural characterless and biochemical tests. The Chi square test showed that the hygienic condition of the milk container, equipment washing practice and milk shop location were found to be significantly associated with the prevalence of *E. coli* in the raw milk (Table 3).

Table 3: Distribution of *E. coli* in raw cow's milk by the different variables

Variables	Category	No. of examined	No of positive (%)	X^2	P-value
Shop area	Hanqu	99	42(42.42)	7.8826	0.049
	Burqitu	137	76 (55.47)		
	Haliila	76	29(38.15)		
	Cilaalo	72	30(41.67)		
Hygiene condition of the milk container	Poor	250	128 (51.20)	7.5178	0.006
	Good	134	49 (36.6)		
Milk equipment Washing practice	Only water	260	129 (49.7)	4.0187	0.045
	Water and detergent	124	48 (38.7)		
Type of milk container	Steel	124	42 (33.87%)	11.0113	0.001
	Plastic	260	135 (51.9)		
Total		384	177 (46.09%)		

Univariable logistic regression analysis showed that the occurrence of *E. coli* in plastic containers is 2.11 times higher than in stainless steel (Table 4).

Table 4: Univariable logistic regression analyses of risk factors associated with *E. coli* isolated

Variables	Category	OR	95 % CI	P-value
Hygiene condition of milk container	Poor	1.00	0.357-0.844	0.006
	Good	0.549		
Milk equipment Washing practice	Only water	1.00		
	Water and Soap	0.641	0.414-0.991	0.046
Type of milk container	Stainless steel	1.00	1.352-3.288	0.001
	Plastic	2.109		

Antimicrobial Susceptibility Profile of *Escherichia coli:*

The antimicrobial susceptibility profiles of *E. coli* isolates from raw cow milk samples (Table 5) showed susceptibility to tetracycline (73.33%) and sulfamethoxazole-trimethoprim (66.66%). The isolates were resistant to kanamycin (100%), penicillin-G (100%), amoxacillin (100%) and erythromycin (86.66%). In general, the antimicrobial susceptibility test revealed sulfamethoxazole-trimethoprim and tetracycline were the antimicrobials indicated as sensitive against *E. coli* isolated from this study.

Table 5: Antimicrobial Susceptibility of *E. coli* isolated from raw milk sample

Antibiotics	Zone of diameter		
	Susceptible Number (%)	Intermediate Number (%)	Resistant Number (%)
Tetracycline	11(73.33)	2 (13.33)	2 (13.33)
Streptomycin	0	7 (46.66)	8 (53.33)
Kanamycin	0	0	15 (100)
Penicillin- G	0	0	15 (100)
Trimethoprim-sulphamethoxazole	10 (66.66)	4 (26.66)	1(6.66)
Amoxacillin	0	0	15 (100)
Erythromycin	0	2 (13.33)	13 (86.66)

The isolation rate of *E. coli* in the present study was found to be lower (46.09%) as compared to other research outputs, which included 100% by Swai and Schoon (2011) from Arusha, Tanzania, 90.67% (Robert et al., 2014), 51.66% (Soomro et al., 2002) from milk vending shops and 58% (Reta et al., 2016) from raw cow's milk in Ethiopia. On the other hand, the present finding was higher than the report of Abrha and co-workers (2014), who reported 26.6% prevalence of *E. coli* from milk samples from cafeterias in Ethiopia. The variation seen in prevalence of *E. coli* between the present study and the previous studies may be due to differences in sample size, farming system, farm size, milking equipment type, milking technique, geography, ecology and way of washing equipment and hygienic conditions of the milking containers and personal hygienic condition of milk handlers. In addition, contaminants coming from unclean environmental conditions and poor udder preparation might expose raw milk to bacterial contamination.

In the present study, the prevalence of *E. coli* was higher in plastic milk containers than stainless steel. This may be because the plastic containers have a greater risk of milk contamination. The result showed that there is a statistically significant difference (P< 0.05 p-value = 0.001) between milk samples taken from plastic milk containers and stainless steel. A similar finding was also reported by Disassa and co-investigators (2017) in other parts of Ethiopia. Other researchers reported higher *E. coli* isolates in the raw milk value chain from plastic containers in shops (90.0%) in Tanzania (Robert et al., 2014).

In this study, the prevalence of *E. coli* from milk contained in containers with poor hygienic conditions was 33.33% higher than in milk containers with good hygienic conditions (12.76%). This may be due to the variation in milk storage equipment hygiene. In good hygienic sanitation, all utensils and equipment were cleaned and rinsed using water and detergents immediately after using the milk container so as to reduce milk contamination. However, the result showed that there is a statistically significant difference (P-value = 0.006) between the poor hygienic sanitation and good hygienic sanitation of the studied samples. A similar finding was obtained by Disassa and others (2017), in which a higher prevalence of *E. coli* was observed in poor hygienic conditions than in good hygienic conditions.

In this study, the prevalence of *E. coli* in Burqitu (19.79%) was higher than Haliila (7.55%), Cilaalo (7.81%) and Hanqu (10.94%). This may be due to the lack of awareness about hygiene; the site was at the periphery of Asella town and most of the settlement of Kebeles came from the ruler area.

In the current study, different practices of washing milk container without detergent and washing milk container with detergent showed a statistically significant difference (P-value = 0.049) in prevalence of *E. coli*. A similar report was also made by Disassa et al. (2017) in which a higher prevalence of *E. coli* was observed in washing equipment within only water (82.5%) than in water and detergent (27.4%).

Antimicrobial resistance emerges from the use of antimicrobials in animal and human medicine and the subsequent transfer of resistance genes in bacteria among animals, humans, animal products and the environment. In Ethiopia, there have been reports on the drug resistance of *E. coli* isolates from animal-derived food products (Mohammed et al., 2014).

In the present study, *E. coli* showed high resistance to penicillin-G (100%), kenamycin (100%) and erythromycin (86.66%), as well as high sensitivity to tetracycline (73.33%) and trimethoprim-sulphamethoxazole (66.66%). Different researchers reported antimicrobial resistance of *E. coli* isolates from raw milk in their previous studies from Ethiopia. Reports from other researchers had also indicated *E. coli* isolates' resistance to kenamycin (50%), erythromycin (60%), in Tigray, Ethiopia (Haftay et al., 2018).

The present study was similar to the above study; this might be due to high antimicrobial use for individual cows to treat various diseases affecting the dairy sector. In the present study, *E. coli* isolates showed high sensitivity to tetracycline (73.33%) and trimethoprim-sulphamethoxazole (66.66%). Similarly, Geta (2019) reported 60% of *E. coli* isolates to be susceptible to tetracycline from a study done in Ethiopia. The results of this investigation were in agreement with the findings of other studies conducted in different parts of the world (Briscoe et al., 2005).

Conclusion:

The current study revealed the occurrence of *E. coli* in raw cow milk from milk shops in Asella town. The pathogenicity of isolates were not the scope of work in the current study but occurrence of *E. coli* in milk samples suggests a potential zoonotic risk of raw milk consumption in the area. Milking equipment washing practice, type of milk container and factors related to hygienic practices (poor and good) were the main factors that caused the occurrence of *E. coli* in the raw milk from the shop. *E. coli* isolates manifested absolute drug resistance to penicillin, kanamycin, amoxicillin and mostly to erythromycin. Antibiotics such as tetracycline and sulfamethoxazole-trimethoprim could be considered as first-choice drugs, as the isolates were mostly susceptible to these drugs. Hence, attention

should be given to proper handling of the milk and dairy items and the treatment of diseases both in humans and animals should include susceptible antibiotics.

Conflict of interest:

There was no conflict of interest among the authors.

Contribution of authors:

All the authors contributed equally in the preparation of the manuscript.

Acknowledgement:

The authors provide sincere gratitude to District Veterinary Clinic coordinator, Ethiopia.

References:

Abrha B, Abebe M, Hailelule AB. Antibiogram of Escherichia coli strains isolated from food of bovine origin in selected Woreda of Tigray. Veterinary World. 2014; 6(3): 17-22.

Briscoe D, Rubowitz A, Assia E. Changing bacterial isolates and antibiotic sensitivities of purulent dacryocystitis. Orbit. 2005; 24 (2): 95-8.

Centers for Disease Control and Prevention. Enterohaemorrhagic *Escherichia coli* and other E. coli causing hemolytic uremic syndrome. Iowa State University, Institute for International Cooperation in Animal Biology; 2016.

CLSI. Performance standards for antimicrobial susceptibility testing; Twenty-fifth informational supplement. CLSI document M100-S25. Wayne, PA: Clinical and Laboratory Standards Institute; 2015. p. 32-94.

Disassa N, Sibhat B, Mengistu S, Muktar Y, Belina D. Prevalence and antimicrobial susceptibility pattern of Escherichia coli O157:H7 isolated from traditionally marketed raw cow milk in and around Asosa Town, Western Ethiopia. Veterinary Medicine International Research. 2017; Article ID: 431376.

Farzan R, Rahimi E, Momtaz H. Virulence properties of Shiga toxin-producing *Escherichia coli* isolated from Iranian raw milk and dairy products. Veterinary Research. 2012; 49(4):159-66.

Geta G, Kebede A, Chemedissa M. Microbiological safety of fruit juices consumed in cafes and restaurants of Debre-Markos Town, North Western Ethiopia.World News of Natural Sciences. 2019; (24): 287-98.

Haftay AT, Natsenet BG, Kidane WH, Hailu SG, Abraha B, Habtamu T. Antimicrobial resistance profile of *E. coli* isolated from raw cow milk and fresh fruit in Mekelle, Tigray region, Ethiopia. Veterinary Medicine international, Research. 2018; Article ID: 431378.

Jan H. Kirby-Bauer disk diffusion susceptibility test protocol [Internet]. American Society for Microbiology; 2013. Available from: http://www.microbelibrary.org.

Kanungo R. Ananthanarayan and Paniker's Textbook of Microbiology. 10 th Ed. Universities Press, Hyderabad, India. 2017.

Mekonnen H, Habtamu T, Kelali A. Contamination of raw and ready-to-eat foods and their public health risks in Mekelle City, Ethiopia. ISABB Journal of Food and Agriculture Sciences. 2012; 2(2): 20-9.

Mohammed M, Shimelis D, Admasu P, Feyera T. Prevalence and antimicrobial susceptibility pattern of E. coli isolates from raw meat samples obtained from abattoirs in Dire Dawa City, Eastern Ethiopia. International Journal of Microbiological Research. 2014; 5(1): 35-9.

Oliver SP, Jayarao BM, Almeida RA. Foodborne pathogens in milk and the farm environment: Food safety and public health implications. Foodborne and Disease. 2005; 2(2):115-29.

Pal M, Ketchakmadz D, Durglishvili N, Ketchakmadz I.Staphylococcus aureus: A major pathogen of food poisoning. Nutrition and Food Processing. 2022; 5: DOI:10.31579?2637-8914/074.

Pal M, Ragasa T, Rebuma T, Zendre R. Salmonellosis remains the hidden menace in our global food.

Pal M, Ragasa T, Rebuma T, Zendre R. Salmonellosis remains the hidden menace in our global food supply. A comprehensive review. American Journal of Medical and Biological Research. 2024; 12 (1): 1-12.

Pal M. Campylobacter jejuni: An emerging foodborne pathogen of global significance. Journal of Experimental Chemistry. 2017; 4: 1-4.

Pal M. Zoonoses. Second Edition, Satyam Publishers, Jaipur, India. 2007.

Quinn PJ, Markey BK, Leonard FC, Hartigan P, Fanning S, FitzPatrick ES. Veterinary Microbiology and Microbial Disease. Second Edition. Wiley and Blackwell, USA. 2011.

Rashid M, Kotwal SK, Malik MA, Singh M. Prevalence, genetic profile, virulence determinants

and multidrug resistance of *Escherichia coli* isolates from animals. Veterinary World. 2013; 6(3): 139-42.

Reta MA, Bereda TW, Alemu AN. Bacterial contaminations of raw cow's milk consumed in Jigjiga City, Somali Regional State, Eastern Ethiopia. International Journal of Food Contamination. 2016; 3(1):1.

Robert L, Francis S, Athanasia M. Prevalence of Salmonella spp. and Escherichia coli in raw milk value chain in Arusha, Tanzania. American Journal of Research Communication. 2014; 2(9):1-13.

Soomro AH, Arain MA, Khaskheli M, Bhutto B. Isolation of *Escherichia coli* from raw milk and milk products sold under market conditions in Tandojam, Pakistan. Pakistan Journal of Nutrition. 2002; 1(3):151-2.

Swai ES, Schoonman L. Microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania. Asian Pacific Journal of Tropical Biomedicine. 2011; 1(3):217-22.

Thrusfield M. Veterinary Epidemiology. USA: Blackwell Science Ltd; 2007. P. 181.

Torkar KG, Teger SG. The microbiological quality of raw milk after introducing two-day milk collecting system. Acta agricultural Slovenica. 2008; 92(1): 61-74.

WHO, (2015). First ever global estimates of foodborne diseases [Internet]. 2015. Available from: http://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.100192.

***Corresponding author's email ID:** palmahendra2@gmail.com

Citation: Ayele AG, Amesa AF, Kisi LM, Abdeta TR, Ragasa T, Pal M. Characterization of Escherichia coli Isolated from Raw Cow Milk from Shops in Asella Town, South East, Ethiopia. Indian Journal of Veterinary Public Health. 2024; 10(2): 58-64.

DOI: https://doi.org/10.62418/ijvph.10.2.2024.58-64