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

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Abstract: Food borne infections are an important challenge to public health and cause significant economic problem in many countries. This research was conducted, to assess the occurrence of *E. coli* in raw milk sample from shops, to estimate associated risk factor of isolates bacteria and to determine the antimicrobial susceptibility profile of *E. coli* isolates. A cross sectional study was conducted from November 2021 to April 2022 from shops of raw cow's milk at Asella town. Simple random sampling strategy was followed to collect raw milk from individual milk shops. A total of 384 samples of raw milk were collected and processed immediately for bacterial isolation and identification to species level by using culture media and biochemical test. From 384 samples, 177 were positive for *E. coli*. The overall prevalence of *E. coli* was (46.09%). Of which 42 (10.94%) were from stainless steel and 135 (35.16%) from plastic milk container from milk shops, with statically significant differences (= 0.001). However the prevalence of isolated *E. coli* was (33.33%) in poor hygienic sanitation and (12.76%) in good hygienic sanitation with statically significant difference (P-value = 0.006). A 15 *E. coli* isolates were subjected to antimicrobial sensitivity test to seven commonly used antimicrobials Disk. *E.coli* showed susceptible to Tetracycline (73.33%) and sulfamethoxazole-trimethoprim (66.66%). The isolates were resistance to some antibiotics like kenamycine (100%), penicillin -G (100%), amoxacilline (100%) and erythromycin (86.66%). Hence, attention should be given to proper handling of the food items and using recent antibiotics in the treatment of diseases in both humans and animals. Therefore, Comprehensive training should be given to owners sell milk and shop distributor improves the hygienic practices, Awareness should be created on the risk of raw milk consumption in the area and Physicians in the area should consider tetracycline and sulfamethoxazole-trimethoprim as first choice drugs in the treatment of clinical diseases associated with *E. coli*.

Key words: Antimicrobial Disk · Asella Town · *E. coli* · Raw Milk · Susceptibility Test · Prevalence

INTRODUCTION

Food borne infections are an important challenge to public health and cause significant economic problem in many countries [1]. 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 [2]. 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 [3].

Huge numbers of microbes can get access to milk and various milk products including *E. coli*, which is an indicator of milk and milk product contamination disease that can be transmitted directly or indirectly between animals and humans [4].

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Escherichia coli is common 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 [5].

In countries where food borne diseases were investigated and documented, the relative importance of pathogens like *S. aureus, Campylobacter, E. coli* and *Salmonella* species was recorded as a major cause food borne diseases [6]. These organisms were known to cause acute gastroenteritis and may cause a more serious septicemic disease, usually in the very young, the elderly or immuno-compromised individuals [7].

Currently, the other major problem to human health is the issue of antimicrobial resistance due to use of antibiotics in livestock production as well as human diseases conditions in developing countries. In Ethiopia, the major antibiotics used for treatment of animal and human diseases include penicillin, streptomycin, gentamycin and oxytetracycline. Even though it needs a better understanding of antibiotics 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 sector, which might favor selection pressure that increased the advantage of maintaining resistance genes in bacteria [8].

So far, there are no studies conducted on the occurrence and drug sensitivity profile of *E. coli* from milk in Asella town, Arsi zone. There for the objectives of this research were to assess the occurrence of *E. coli* and estimate associated risk factor of isolates bacteria in raw milk sample from shops and further to determine the antimicrobial susceptibility profile of the *E. coli* isolates

MATERIALS AND METHODS

Study Area: The study was conducted from October 2021 to June 2022 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 geographical coordination of 38°32'- 40°50' east longitude and 136°45'-8°50 '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: 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 [9] with 95% confidence interval at 5% desired absolute precision and assumption of the expected prevalence 50% since no more previous report in this area.

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

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 2021 to June 2022 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 samples collected from milk shops from different source in Asella town. After the milk samples are aseptically collected, samples were labeled and packed with sterile bottles and transported with an icebox to 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 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°C for 24 hrs. The mixture of nutrient broth and raw milk sample was sub cultured on sterile nutrient agar plate under aseptic condition and incubated at 37°C for 24 hrs. An enriched sample was taken from blood agar then Gram staining and KOH test was 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), a dual purpose - selective and differential medium for *E. coli*, by quadrant streaking technique and plates were incubated at 37°C for 24 hrs. Pink colored colonies observed after incubation were considered as presumptive for *E. coli*. A single isolated colony was picked and streaked on Eosin methylene blue agar (EMB) medium (Oxoid, UK) and incubated at 37°C for 24 hrs. Those, which produced a characteristic metallic-sheen on EMB agar medium were considered as positive for *E. coli*. Such colonies were taken and added into nutrient broth for further

Table 1: Biochemical characterization of *E. 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 H_2S production
Motility test	Motile

Table 2: Drug susceptibility interpretive zone of inhibition diameter

			Zone of diameter		
Antibiotics	Disc code	Potency	S	I	R
Tetracycline	TE	30µg	≥15	12-14	≤11
Streptomycin	S	100µg	≥15	12-14	≤11
Kenamycine	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
Amoxaciline	AML	10/20µg	≥18	14-17	≤13
Erythromycine	ERY	15µg	≥23	12-14	≤11

biochemical tests, such as catalase, Triple sugar iron tests (TSI), indole production, citrate utilization and methyl red tests, Voges-Proskauer (VP) test oxidase test, motility test were carried out to identify the organisms that were isolated from the samples according to standard procedure described by Thaker *et al*. [10].

Antimicrobial Susceptibility Test: Antimicrobial susceptibility test, through Kirby diffusion test, was performed for all *E. coli* isolates following the protocol in CLSI [11]. At least 15 well isolated colonies of the same morphological type were selected from a non selective agar plate (nutrient agar); just the top of the colonies is touched and the growth transferred to a tube containing 4 -5ml of nutrient broth. The inoculated broth was incubated at 37°C for 2hrs until a visible turbidity appeared and the turbidity was compared to with 0.5 McFarland standards and then the bacterial suspension was inoculated on to 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 hr and the antibiotic impregnated disks were placed on the agar surface by the help sterile forceps, in such away the distance between the centers of the two disks was not less than 24mm. Following this, the plates were incubated aerobically at 37°C 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 [12].

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

RESULTS

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* (Table 3).

The Chi square test showed that, 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 4).

Table 3: *E. coli* isolated from raw cow milk samples

Type of Container	No of examined No (%)	No of positive No (%)	Chi^2	P-value
Stainless steel	124	42((33.87%)	11.0113	0.001
Plastic	260	135(51.92%)		
Total	384	177(46.09%)		

Table 4: Presence 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	30 (41.67)			
Hygiene condition of the milk container	Poor	250	128 (33.33)	7.5178	0.006
	Good	134	49 (12.76)		
Milk equipment Washing practice	Only water	260	129 (33.59)	4.0187	0.045
	Water and detergent	124	48 (12.5)		
Type of milk container	Steel	124	42 (33.87%)	11.0113	0.001
	Plastic	260	135 (35.15)		
Total		384	177 (46.09%)		

Table 5: 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 detergent	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		

Table 6: Antimicobial Susceptibility of *E. coli* isolated from raw milk sample

	Zone of diameter		
Antibiotics	Susceptible No (%)	Intermediate No (%)	Resistant No (%)
Tetracycline	11(73.33)	2(13.33)	2(13.33)
Streptomycin	0	7 (46.66)	8 (53.33)
Kenamycine	0	0	15 (100)
Penicilline- G	0	0	15 (100)
Trimethoprim-sulphamethoxazole	10 (66.66)	4 (26.66)	1(6.66)
Amoxaciline	0	0	15(100)
Erythromycine	0	2(13.33)	13(86.66)

Univariable logistic regression analysis showed that the occurrence of *E. coli* in plastic container is 2.11 times higher than in stainless steel (Table 5)

Antimicrobial Susceptibility Profile of *E. coli*: The antimicrobial susceptibility profiles of the *E. coli* isolates from raw cow milk samples (table 6) showed susceptibility to Tetracycline (73.33%) and sulfamethoxazole-trimethoprim (66.66%). The isolates were resistant to kenamycine (100%), penicillin-G (100%), amoxacilline (100%) and erythromycine (86.66%) antibiotics.

In general, the antimicrobial susceptibility test revealed sulfamethoxazole-trimethoprim and tetracycline were the antimicrobial indicated as active against *E. coli* isolated from this study.

DISCUSSION

The isolation rate of *E. coli* in the present study was found to be lower (46.09%) as compared to other research outputs which include 100% by Swai and Schoon [13], from Arusha, Tanzania, 90.67% Robert [14] 51.66% Soomro [15] from milk vending shops and 58% Reta [16] from raw cow's milk in Ethiopia. On the other hand, the present finding was higher than the report of Abraha [17] who reported 26.6% prevalence of *E. coli* from milk samples from cafeteria in Ethiopia. The variation seen in prevalence of *E. coli* between the present study and the previous studies may be due to difference 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 out of 384 samples, 177 (46.09%) were found to be positive for *E. coli*. Of which 42 (10.94%) were from stainless steel and 135 (35.16%) from plastic milk container from milk shops. Therefore, the prevalence of *E. coli* was higher in plastic milk containers than stainless steel. This may be because the plastic containers having greater risk to milk contamination. The result showed that there is statistically significant (P< 0.05 p-value = 0.001) between from milk sample taken from plastic milk containers than stainless steel. A similar finding was also reported by Disassa [18] in other part of Ethiopia. Other researchers reported higher *E. coli* isolates in raw milk value chain from plastic container in shops (90.0%) in Tanzania [14].

In this study, the prevalence of *E. coli* from milk contained in container with poor hygienic condition was (33.33%) than in from milk container with good hygienic condition (12.76%). This may be due to the variation milk storage equipment hygiene. In Good hygienic sanitation, all utensils and equipment were cleaned and rinsed using water and detergents immediately after use milk container so as to reduce milk contamination. However, the result showed that there is statistically significant (P-value=0.006) between the poor hygienic sanitation and good hygienic sanitation of the study populations. A similar finding was obtained by Disassa [18] in which higher prevalence of *E. coli* was observed in poor hygienic condition than good hygienic condition.

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 periphery of Asella town and most of settlement of kebeles were comes from ruler area.

In the current, study the prevalence of isolated *E. coli* from Washing equipment without detergent (33.59%) and Washing equipment within detergent (12.5%). The result showed that there is statistically significant (P-value=0.049) between milk sample taken from washing equipment without detergent and within detergent was recorded. A similar report was also made by Disassa [18] in which higher prevalence of *E. coli* was observed in washing equipment within only water (82.5%) than water and detergent (27.4%).

Antimicrobial resistance emerges from the use of antimicrobials in animals and human and the subsequent transfer of resistance genes and 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 [19].

In present study *E. coli* isolated showed high resistance to penicillin-G (100%), Kenamycine (100%) and Erythromycin (86.66%) high sensitivity to Tetracycline (100%) and Trimethoprim-sulphamethoxazole (66.66%) reported by Kindu [20] in Ethiopia. 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 kanamycin (50%), erythromycin (60%), by Haftahy [21] in Tigray, Ethiopia.

The present study was in similar with above study, this might 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 (100%) and Trimethoprim-sulphamethoxazole (66.66%) similarly Kindu [20] has reported 60% of *E. coli* isolates to susceptible to tetracycline from study done in Ethiopia. The results of this study were in line with the finding of other study conducted in different parts of the world [22].

CONCLUSION AND RECOMMENDATIONS

The current study revealed occurrence of *E. coli* in raw cow milk from milk shop at Asella town. The 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 condition (poor and good) washing only water, water and detergent used for cleaning of equipments and hands were the main factors that the occurrence of *E. coli* in the raw milk from shop. *E. coli* isolates manifested a drug resistance; 100% resistance to Penicillin, kenamycine, Amoxicillin and 86.66% erythromycin was observed. Antibiotics such as tetracycline and sulfamethoxazole-trimethoprim could be considered as first choice drugs as the isolates are susceptible to these drugs. Hence, attention should be given to proper handling of the food items and using recent antibiotics in the treatment of diseases in both humans and animals. Therefore, Comprehensive training should be given to owners sell milk and shop distributor

improves the hygienic practices and Awareness should be created on the risk of raw milk consumption in the area. In addition to this Physicians in the area should consider tetracycline and sulfamethoxazole-trimethoprim as first choice drugs in the treatment of clinical diseases associated with *E. coli*

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