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ISOLATION, IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY TESTING OF *ESCHERICHIA COLI* ISOLETED FROM RAW COW MILKS OF BORENA BREED DAIRY FARMS IN HARAMAYA UNIVERSITY, EASTERN ETHIOPIA

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ABSTRACT

A number of *E. coli* strains are recognized as important pathogens of colibacillosis in food animals (poultry and ruminants) and some of them can cause severe human diseases such as hemorrhagic colitis and hemolytic uremic syndrome. *E. coli* are Gram-negative, facultative anaerobic, rod-shaped and highly motile bacteria. A cross-sectional study was conducted from December 2020 to February 2021 to isolation and identification of *E. coli as* well as the antimicrobial susceptibility of *E. coli* isolated from raw milk. Biochemical tests methods were used to confirm *E. coli* and isolates were subjected to antimicrobial susceptibility test using the agar disc diffusion method. Out of 28 raw milk samples collected, 15 (53.6%) were found to be positive for *E. coli*. The presence of *E. coli* was higher in adults (77.8%) followed by (50%) in old cattle and least in young (28.6%) but it was furthermore the variation in the prevalence among age groups was not statistically Significant (P>0.05). Statistically significant difference was not observed (P>0.05) in the presence of *E. coli* among those animals with different body condition scores but the presence of *E. coli* was higher proportion in poor body condition cattle 6(60%), then in good body condition cattle 9(50%). The highest percentages of *E. coli* isolates were isolated from cows which have many parities 9(69.2%) and the lowest in cows with few parities 2(33.3%) and from cows with early lactation stage 7(58.3%). The antimicrobial susceptibility profile showed that the most frequently encountered form of resistance in all samples was resistance to Ampicillin (93.3%), followed by resistance to Amoxicillin (80%), resistance to Tetracycline (73.3%), resistance Chloramphenicol (33.3%) and resistance to kanamycin (20%). Milk samples were produced and handled under poor hygienic conditions, stored, and transported in inappropriate containers and under temperature abuse conditions leading to high health risk to the consumers. Relevant intervention program and awareness creation on best practice of milk handling should be conducted to minimize contamination of milk and milk products with pathogens of public health importance in Haramaya University dairy farm is necessary.

1 INTRODUCTION

A number of *E. coli* strains are recognized as important pathogens of colibacillosis in food animals (poultry and ruminants) and some of them can cause severe human diseases such as hemorrhagic colitis and hemolytic uremic syndrome (Ferens and Hovde, 2011). The treatment of illnesses caused by this bacterium often requires antimicrobial therapy. The repeated and unsuitable use of antibiotics has led to an increasing rate of antimicrobial resistance (Mooljuntee *et al*., 2010). There is worldwide concern about the appearance and rise of bacterial resistance to commonly used antibiotics. The evolution, increasing prevalence and dissemination of pathogenic bacteria resistant to multiple antimicrobial agents is currently recognized as one of the most important problems in global public health (Bush, 2010). The rapid spread of antibiotic resistance genes, facilitated by mobile genetic elements such as plasmids and transposons, has led to the emergence of multidrug resistant (MDR) strains of many clinically important species that now frequently leave clinicians out of therapeutic options (Hawkey and Jones, 2009). Despite the extensive scientific progress and technological developments achieved in the past years in developed countries, food-borne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and medical and social costs (Ali *et al.,* 2010). Moreover, sporadic cases and outbreaks of human diseases caused by food-borne pathogens have been linked to ground beef, raw milk, meat and dairy products, vegetables, unpasteurized fruit juices and water. From those, contaminated raw milk is one of the primary sources of food-borne illnesses (Rahimi *et al.,* 2012). Furthermore, changes in eating habits, mass catering, complicated and long supply chains with increased international movement and poor hygiene practices are major contributing factors. Thus, it is essential to keep the health and hygiene of the cow, the environment in which the cow is housed and milked, hygiene during milking and storage equipment influence microbial contamination of milk (Javaid*et al.,* 2009).

Raw milk being as nutritious food; it serves as an ideal medium for the growth of various bacteria including pathogenic organisms which have a significant impact on public health (Popescu and Angel, 2009). Many microorganisms can get access to milk and milk byproducts among which species, *Escherichia coli* is recognized to be of primary concern (Thaker *et al.,* 2012). Moreover, many *E. coli* strains are harmless or even beneficial to the host. However, some strains of *E. coli* can be pathogenic to humans and are harbored in food animals. Among them *E. coli* is the best-known pathogenic strain (Riemann and Cliver, 2006; Kozub-Witkowski *et al.,* 2008). In Ethiopia dairy production depends mainly on indigenous livestock genetic resources; more specifically on cattle, goats, camels and sheep. Cattle has the largest contribution (81.2%) of the total national annual milk output, followed by goats (7.9%), camels (6.3%) and sheep (4.6%) (CSA, 2013).

The safety of dairy products with respect to food borne disease is of great concern around the world and it is especially true in developing countries where production of milk and various milk products takes place under unsanitary conditions and poor production practices (Mogessie,1990). Milk from a healthy udder contains few bacteria but it picks up many bacteria from the time it leaves the teat of the animal depending on the hygienic level exercised during milking, cleanliness of the milk utensils, condition of storage, manner of transport for further processing until it is used for human consumption. These microorganisms are indicators of both manner of handling milk from milking till consumption and the quality of the milk (Ahmed, 2009; Fatine *et al*., 2012; Lunder and Brenne, 1996; Shunda *et al.,* 2013).

In Haramaya area there is high consumption of raw cow milk along with local food. However, although there is risk associated with the consumption of raw cow milk, there is lack of information on the extent of raw milk contamination by bacteria in this area. In addition, there has been no established milk quality control system. Therefore, the Objectives of present study were initiated

- To isolate and identify *E. coli* from samples taken from lactating Borena breed cattle of Haramaya University dairy farm.
- To determine the antimicrobial susceptibility pattern of *E. coli* isolates from milk sample taken from Haramaya University dairy farm.

3 MATERIAL AND METHODS

3.1Description of the Study Area

The study was conducted in Haramaya University dairy farm of Oromia Regional State, Estern Ethiopia, from December, 2020 to February, 2021. East Haramaya University is located at 520 km East of Addis Ababa; it is about 17km from the city of Harar, 40km from Dire Dawa (where there is regional airport). and has an altitude of 8 ^{0}50-9 ^{0}15`N and longitude of 9 $^{0}$36`N41 ^{0}52`E at altitude of 1850m above the sea level. The annual rainfall of the area is between 834-1300mm and annual temperature of minimum and maximum, 21-26 ^{0}C, respectively. The mean annual rainfall is about 596mm.Rainy season occurs with bimodal distribution 70% of which occurs during the main rainy season (June-September) and 30% during the small rainy season (March- April) and relative humidity of 50.4%(CSA,2015).

3.2 Study design and sample size

A cross sectional study was conducted to isolate, identify and determine antimicrobial susceptibility testing of *E. coli* from Borena breed dairy caws in Haramaya university farm, Oromia, Ethiopia. Farms were selected purposively based on the availability of lactating animals. A total of 28 cows were purposively selected from 60 animal population in the dairy farms.

3.3 Study Animals and Sample collection

The study animals were cross breed lactating dairy cows that were purposively selected from Borena breed dairy farms. A total of 28 samples of raw cow`s milk was collected from dairy farm. The samples were collected in sterilized containers (test tubs) and were brought in ice box to the Microbiology Laboratory of College of Veterinary Medicine, Haramaya University, immediately to culture the milk samples for isolation and identification of *E. coli*.

3.4 Isolation and identification of *Escherichia coli*

The milk samples were streaked on the surface of nutrient Agar plates. The plates were incubated in an inverted position at 37^{0}C for 24 hours. After incubation for 24 hours, Gram's staining was made by picking a well isolated colonies and prepared a thin smear on a clean glass slide from the culture for differentiation of Gram positive and Gram-negative bacteria and, only those Gramnegative bacteria were transferred and streaked onto the surface of the Mac Conkey agar plates for differentiation of lactose fermenting and non-lactose fermenting bacteria and, only those lactose fermenting bacteria having a pinkish color colony were sub-cultured onto the surface of the Eosin Methylene Blue agar (EMB agar) for purification. Biochemical analysis of E. coli isolates was performed using catalase test, oxidation-fermentation (O-F) test, indole test, methyl red (MR) test, citrate test and triple sugar iron (TSI) slant agar test.

3.5 Antimicrobial susceptibility pattern

The antimicrobial susceptibility test was performed according to the Kirby-Bauer (1966) disc diffusion method using 6 commercially available antimicrobial disks. Mueller Hinton (MHI) agar media (Oxoid, Ltd., Hampshire, England) and a 0.5 Mac-Farland standardized suspension of the bacteria were prepared in 0.85% sterile normal saline solution. Sterile saline solutions were used as a culture media and for inoculum preparation, respectively. All the isolated E. coli were tested for sensitivity to most commonly used antimicrobials including, gentamicin (GEN) (10µg), ampicillin (AMP) (25µg) (Himedia, India), sulfamethoxazole (RL) (100µg) (Oxoid, UK), trimethoprim (TR) (25µg) (Himedia, India), kanamycin (c) (10µg) (Oxoid, UK), tetracycline (TE), (10µg) (Oxoid, UK), Penicillin G (p) and the inoculums were uniformly streaked on each respective agar plates. Then antimicrobial discs were applied and plates were incubated at 35^{o}C for 18-24 hours. After incubation for 24 hours, clear zones of inhibition were produced by the bacterial growth and diffusion of the antibiotics and these were measured in millimeter using a caliper and interpreted as susceptible, intermediate and resistant according to Clinical and Laboratory Standards Institute (CSLI, 2013) 'interpretive criteria for Enterobacteriaceae.

3.6. Data management and analysis

The coded data was entered in MS Excel and then analyzed using the SPSS (statistical package for the social sciences) version 20 software (SPSS Inc., Chicago, IL, USA). P-values were calculated using Chi-square and Odds ratio (OR) analysis to determine significant relationships between various criteria and distribution of antibiotic resistance properties of *E. coli* isolated from cow milk. A P-value less than 0.05 were considered statistically significant.

4. RESULTS

4.1. Isolation and identification of Escherichia coli and associated risk factors

Isolation and identification of E. coli organisms were conducted on raw cow's milk samples using conventional culture and biochemical analysis. From a total of 28 raw cow milk samples collected, 15 (53.6%) were found to be positive for *E. coli* organisms by culture on Eosin Methylene Blue agar medium. Based on biochemical analysis, *E. coli* isolates were found to be voges-prosker negative methyl red positive, indole positive and citrate negative. The presence of *E. coli* was higher in adults followed by 50% in old Cattle and least in young's but it was ; furthermore the variation in the prevalence among age groups was not statistically Significant (P>0.05). Statistically significant difference was not observed (P>0.05) in the presence of *E. coli* among Those animals with different body condition scores but the presence of *E. coli* was higher proportion in poor body condition cattle 6(60%), then in good cattle 9(50%) .The highest percentages of *E. coli* isolates were isolated from cows which have many parities 9(69.2%) and the lowest in cows with few parities 2 (33.3%) and from cows with early lactation stage 7 (58.3). On the other hand, the present finding revealed that the association between different risk factors with the occurrence of E. coli organisms were not statistically significant (Table 1).

Table 1: Microbiological Isolation and Identification of *E. coli* and associated risk factors.

Risk factors	No of Examine	d No of Positive	Percentage (%)	P-Value
Age Young	7	2	28.6	
Adult	9	7	77.8	0.139
Old	12	6	50	
Parity Few	6	2	33.3	
Medium	9	4	44.4	0.276
Many	13	9	69.2	
Body condition Poor	10	6	60	0.611

Good	18		9	50	
Stage of lactation Early	12	7		58.3	
Medium	8	5		62.5	0.550
Late	8	3		37.5	

4.2 Antibacterial susceptibility pattern of *E. coli*.

In this study results the most frequently encountered form of resistance in all samples was resistance to Ampicillin (93.3%), followed by resistance to Amoxicillin (80%), resistance to tetracycline (73.3%), resistance Chloramphenicol (33.3%) and resistance to kanamycin (20%) (Table 2).

Table 2: Antimicrobial resistance profiles of isolated E. coli

Antimicrobial agents	Disc potency (µg)	Number of isolates	Susceptible N (%)	Intermediate N (%)	Resistant N (%)
Amoxicillin	20	15	6.67	13.3	80
Ampicillin	10	15	0	6.67	93.3
kanamycin	30	15	73.3	6.67	20
Chloramphenicol	30	15	60	6.67	33.3
tetracycline	30	15	13.3	13.3	73.3

5. DISCUSSION

Present study reveals that from a total of 28 raw cow milk samples collected, 15 (53.6%) were found to be positive for *E. coli*. This finding is closely related to the studies by Fadaei, (2014); Ali *et al, (2011)* and Lubote *et al, (2014)* who reported 69%, 63 % and 90.67% from Iran, Khartoum and Tanzania, respectively. However current result is disagreed with the report by (Mohanty *et al. (2013*) who found (21%) from India, Lye *et al (2013)* and Addo *et al*., (2011) who reported 8.75% and 11.2% from Malaysia and Ghana, respectively. The variation that was seen in prevalence in different studies may be due to difference in sample size, farming system, farm size, milking equipment, milking technique, geography, ecology, duration of milk transportation, and hygienic conditions Lubote R*et al.* (2014).

In the current study the organism was found to be higher in milk taken from cows with medium stage of lactation as compared to those in early and late lactation. The highest percentage of *E. coli* isolates (62.5%) was isolated from early stage of lactation which is relatively higher as compared to the study by Waller *et al. (*2008) who found 66% from Swedish. The occurrence of more E. coli in milk during earlier lactation stage may be due to absence of dry cow therapy and birth related influences in addition to the difference due to management and hygienic practices. The amount of milk ejected is also higher during earlier lactation periods and this cause increased in patency of the teats and decreased local defense factors (thaker, 2012).

In this study, the occurrence of *E. coli* has also been found significantly associated with parity numbers and different age groups. The highest percentages of E. coli isolates were isolated from cows with four or above parity number (32.8%) and from cows with age group from seven to ten years (31.5%). This could be due to multiple parturition stresses and this ultimately down regulates their immunity, and immunity normally decreases as the animal gets older making more prone to E. coli infection. According to current study a total of 28 milk samples 17*E. coli* isolates were tested against 6 antimicrobials based on CLSI guidelines and all E. coli isolates were found to be susceptible to kalamaycine (73.3%) followed by chloramphinicol (60%), On the other hand, all *E. coli* isolates were found to be 100% resistant to ampicillin (93.3%followed by tetracyclin (73.3%). Relatively similar findings have been reported by Bagre *et al*. (2009) who foundall *E. coli* isolates were 100% susceptible to gentamicin, 75% resistant to amoxicillin and 15% resistant to sulphamethoxazole-trimthoprim from Burkinafaso. Reuben *et al. (2013)* was also reported all E. coli isolates were 100% resistant to penicillin and tetracycline, 84.2% to amoxicillin and sulphamethoxazole-trimethoprim and 89.5% of the isolates were susceptible to gentamicin from Nigeria. Salehi *et al. (*2006) were also reported all E. coli isolates were 100% susceptible to gentamicin, 53% resistant to amoxicillin, 77% resistant to kanamycin, 80% resistant to sulphamethoxazole-trimethoprim and 94% resistant to tetracycline from Iran. The high level of resistance of penicillin G (100%), amoxicillin (84%) and tetracycline (60%) obtained in this study might be as a result of suboptimal, prolonged and interrupted use of antimicrobials for prophylaxis and treatment of infection Therefore, in this study gentamicin, kanamycin and sulphamethoxazole-trimthoprim were found to be the most effective drugs against *E. coli* infection.

6.CONCLUSION AND RECCOMENDATIONS

In the current study, considerable proportions of milk samples were found contaminated with E. coli. The presence of these pathogens in the milk of animals indicated that it might be contaminated from either infected animal or unhygienic conditions during milking and handling at sample collection level. This is particularly important in causing several health impacts in consumers who have habit of eating raw or undercooked milk and milk products. Moreover, the higher drug resistant E. coli was observed on Ampicillin and milk of dairy animals is especially alarming which can have a significant public health risk.

Therefore, based on the present study the following recommendations are forwarded:

- Relevant intervention program and awareness creation on best practice of milk handling should be conducted to minimize contamination of milk and milk products with pathogens of public health importance.
- Education on the control and surveillance program of antimicrobial usage in animals are hereby recommended to ensure consumers' safety.
- Further investigation should be conducted in order to further characterize the pathogens and identify their serotypes.

LIST OF ABBREVIATION

CLSI	Clinical Laboratory Standard International
CSA	Central Statics Agency
E. coli	*Escherichia coli*
EMB	Eosin Methyl Blue
MDR	Multi Drug Resistance
MR	Methyl Red
SPSS	Statistical Package of Social Science
UK	United Kingdom
USA	United States of America

Ethical Approval

Ethical approval for this study was obtained from Haramaya University, College of Veterinary Medicine, Minutes of Animal Research Ethics and Review Committee.

Consent

Verbal consent was also obtained from the farm managers of Haramaya University to take samples from their cattle and for further research use of the samples.

Conflicts of Interest

The authors declare that they have no conflicts of interest.
Data Availability: The data used to support the findings of this study are available from the corresponding author upon request.

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