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Isolation, identification and antimicrobial susceptibility testing of *Escherichia coli* isolated from selected dairy farms in and around mekelle, Ethiopia

Abstract

A cross sectional study was conducted from November 2014 to April 2015 in selected dairy farms in and around Mekelle, Ethiopia with the objective of isolating and identifying *Escherichia coli* from raw cow's milk and determing its antimicrobial susceptibility pattern. From a total of 300 raw milk samples collected, 75 (25%) were found to be positive for *E. coli* organisms by culture method. Among the potential risk factors, stage of lactations $(x^2=149.677; P=0.000)$, age groups $(x^2=6.010; P=0.04)$ and parity numbers $(x^2=11.594; P=0.009)$ were found significantly associated with occurrence of *E. coli*. The highest percentage of *E. coli* isolates were found from cows' milk with early stage of lactation 53 (84.1%) and from cows with four and above parity number 43 (32.8%). On the other hand, the association between different farms were not statistically significant $(x^2=5.712; P=0.222.)$ with the occurrence of *E. coli* organisms. The antimicrobial susceptibility pattern revealed that all the isolates were found to be 100% susceptible to gentamicin followed by kanamycin (92%), sulphamethoxazole- trimthoprim (76%); were found to be 100% resistant to penicillin G followed by amoxicillin (84%) and tetracycline (60%). Therefore, gentamicin, kanamycin and sulphamethoxazole-trimethoprim were found to be the most effective drugs against *E. coli*. To safeguard the quality of raw milk, people engaged in milk and dairy production chain should be trained for hygienic practices.

Keywords: antimicrobial susceptibility*,* Escherichia coli, identification, isolation, milk

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List of abbreviations:
CLSI, Clinical and laboratory standards institute; CSA, Central Statistical Agency; CVM, College of Veterinary Medicine; EMB, Eosin Methyl Blue; Lbs, Libras; Ml, Milliliter; MR, Methyl Red; O-F, Oxidation-Fermentation; SPSS, Statistical Package for Social Sciences; TSI, Triple Sugar Iron

Introduction

Milk is the fluid normally secreted by female mammals for the nourishment of their young ones. It is a compulsory part of daily diet for the expectant mothers as well as growing children. Milk and milk products are ideal foods for all age groups in both rural and urban people all around the world.[1] Milk is defined to be the lacteal secretion, practically free from colostrums, obtained by the complete milking of one or more healthy cows, 5 days after and 15 days before parturition.[2] Raw untreated milk is still used by large number of farm families and workers and by a growing segment of the general population who believe that the milk is not only safe but also imparts beneficial health effects that are destroyed by pasteurization.[3] Milk is considered a complete and nutritious food; not only for the new-born mammal and for the human beings, but it is considered as a good medium for many microorganisms.[3,4] It starts its journey in the udder of a mammal as a sterile substance, but as it passes out of the teat, it is inoculated by the animal's normal flora.[5] Being a nutritionally balanced food stuff with a low microbial load when drawn from the udder of a healthy cow, milk gets contaminated at various stages including the cow itself, the milker's hand or milking equipment, storage vessels and water supply particularly when used for adulteration.[6–8] Milk being a major constituent of human diet, can serve as a good medium for the growth of many microorganisms especially bacterial pathogens, therefore its quality control is considered essential to the health and welfare of a community. The threat posed by diseases spread through contaminated milk is well known and the epidemiological impact of such diseases is considerable. The presence of these pathogenic microorganisms in milk has emerged as a major public health concern especially for those individuals who still drink raw milk.[9] Milk is virtually a sterile fluid when secreted into alveoli of udder. However, beyond this stage of production, microbial contamination might generally occur from three main sources; within the udder, exterior to the udder and from the surface of milk handling and storage equipments, but the surrounding air, feed, soil, feces and grass are also possible sources of contamination. Raw milk is usually colonized by a variety of many zoonotic pathogens such as *Campylobacter jejuni, Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureu s*and *Yersinia enterocolitica.* Therefore, they represent an important source of food borne pathogens. These pathogens in milk have been linked to the environment in the farm, mixing clean milk with mastitis milk and from livestock.[10] E.coli frequently contaminates food organism and it is a good indicator of fecal pollution.[11] Presence of *E. coli* in milk products indicates the presence of enters pathogenic microorganisms, which constitute a public health hazard. *E. coli* is among many pathogenic microorganisms which can access to milk and some of dairy products which considered a reliable indicator of contamination by manure, soil and contaminated water.[12] Therefore, the purpose of this study is to isolate and identify *E. coli* from raw cow`s milk samples taken from selected dairy farms in and around Mekelle and to determine the antimicrobial susceptibility pattern of *E. coli* isolates.

Isolation, identification and antimicrobial susceptibility testing of Escherichia coli isolated from selected dairy farms in and around mekelle, Ethiopia

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Materials and methods

Study area

The study was carried out from November 2014 to April 2015 in and around Mekelle, Tigray, Ethiopia. Mekelle is the capital of Tigray region, located about 783 kilometers north of Addis Ababa. Its geographic location is 13º32`N and 39º33`E, with human population of about 215,546. It has an average altitude of 2200 meter above sea level, with a mean minimum and maximum monthly temperature of 8.7ºc and 26.8ºc respectively. The annual average rainfall in Mekelle is 600mm Hg and more than 70% of its falls between the months July and august. The long dry season extends from October to May.[13]

Study design and sample size

A cross sectional study was conducted to isolate, identify and determine antimicrobial susceptibility testing of *E. coli* from five selected dairy farms in and around Mekelle, Tigray, Ethiopia. Farms were selected purposively based on willingness of the farm owners. A total of 300 cows were sampled from five purposively selected dairy farms.

Study animals and sample collection

The study animals were cross breed lactating dairy cows that were purposively selected from five dairy farms. A total of 300 samples of raw cow`s milk were collected from selected dairy farms. The samples were collected in sterilized containers (bottles) and were brought in ice box to the microbiology laboratory of college of veterinary medicine, Mekelle University, immediately to culture the milk samples for isolation and identification of *E. coli*.

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 37ºc 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 Gram negative 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 colonies were sub-cultured onto the surface of the Eosin Methylene Blue agar (EMB agar) for purification. Biochemical analysis of *E. coli* isolates were 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.

Antimicrobial susceptibility pattern

The antimicrobial drugs used were amoxicillin, gentamicin, kanamycin, penicillin G, sulphamethoxazole trimthoprim and tetracycline. The antimicrobial susceptibility testing was done by the agar disc diffusion method as described by Clinical and Laboratory Standards institute.[14] In brief, a 0.5 Mac-Farland standardized suspension of the bacteria was prepared in 0.85% sterile normal saline solution. A sterile cotton swab was dipped into the standardized suspension of bacteria and then uniformly streaked over the entire surface of the Mueller- Hinton agar. Then, the paper discs impregnated with a fixed concentration of antibiotics were placed on the agar surface and incubated in an inverted position at 37ºc for 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 Table 1.[14]

Table I Antimicrobial drugs used and their concentrations

Antimicrobials	Disk concentrations
Amoxicillin	30 microgram
Gentamicin	10 microgram
Kanamycin	30 microgram
Penicillin	10 microgram
Sulpha*	23.75 and 1.25 microgram
Tetracycline	30 microgram

*, sulphamethoxazole-trimethoprim;

Data management and analysis

The data was entered into excel spread sheet and different statistical models were employed to analyze the data collected using Statistical Package for Social Sciences (SPSS) version 15 software. Descriptive statistics was used to describe the frequency and percentage of the results. Chi-square test was used to check the association of potential risk factors with the occurrence of *E. coli*. The association was taken as significant when p-value is less than 0.05 and not significant when p-value is greater than 0.05.

Results

Isolation and identification of *Escherichia coli*

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 300 raw cow milk samples collected, 75 (25%) 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 catalase positive, methyl red positive, indole positive and citrate negative. The isolates were also found to be fermentative or facultative anaerobes and having ability to utilize the three sugars (glucose, sucrose and lactose) on oxidation- fermentation test and triple sugar iron slant agar test, respectively.

Potential risk factors

In this study, the occurrence of *E. coli* was significantly associated with different stage of lactations (x^2=149.677; P=0.000) and with different parity numbers (x^2=11.594; P = 0.009) Table 2. Isolation of *E. coli* has also be significantly associated with age groups *(x^2*=6.010; P=0.04). The highest percentages of *E. coli* isolates were isolated from early stage of lactation 53 (84.1%) and from cows with four and above parity number 43 (32.8%). On the other hand, the present finding revealed that the association between different farms with the occurrence of *E. coli* organisms were not statistically significant (x^2=5.712; P=0.222) (Table 2).

Isolation, identification and antimicrobial susceptibility testing of Escherichia coli isolated from selected dairy farms in and around mekelle, Ethiopia

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Table 2 *Escherichia coli* isolates association with potential risk factors

Risk factors		Positive	Negative	Total	P-value CI 95 %
Parity	1	5(13.5%)	32(86.5%)	37	
	2	8(13.1%)	53(86.9%)	61	X^2 = 11.594
	3	19(26.8%)	52(73.2%)	71	P = 0.009
	≥4	43(32.8%)	88(67.2%)	131	
Age	3-6 years	46(22.1%)	162(77.9%)	208	X^2 = 6.010
	7-10 years	29(31.5%)	63(68.5%)	92	P = 0.04
Farms	Kalamino	30(30%)	70(70%)	100	
	CVM	5(14.3%)	30(85.7)	35	X^2 = 5.712;
	Hidasse	2(13.3%)	13(86.7)	15	P = 0.222
	Kuaha	10(20%)	40(80%)	50	
	Aga'azi	28(28%)	72(72%)	100	
Stage of lactation	Early	53(84.1%)	10(15.9%)	63	X^2 = 49.677
	Mid	15(11.9%)	111(88.1%)	126	P = 0.000
	Late	7(6.3%)	104(93.7%)	111	
	Total	75(25%)	225(75%)	300	

Antimicrobial susceptibility pattern

A total of 75 *E. coli* isolates were tested against 6 antimicrobials following CLSI guidelines. All *E. coli* isolates were found to be 100% susceptible to gentamicin followed by kanamycin (92%), sulphamethoxazole-trimthoprim (76%). On the other hand, all *E. coli* isolates were found to be 100% resistant to penicillin G followed by amoxicillin (84%) and tetracycline (60%).Therefore, gentamicin, kanamycin and sulphamethoxazole-trimethoprim were the most effective drugs against *E. coli* (Figure 1).
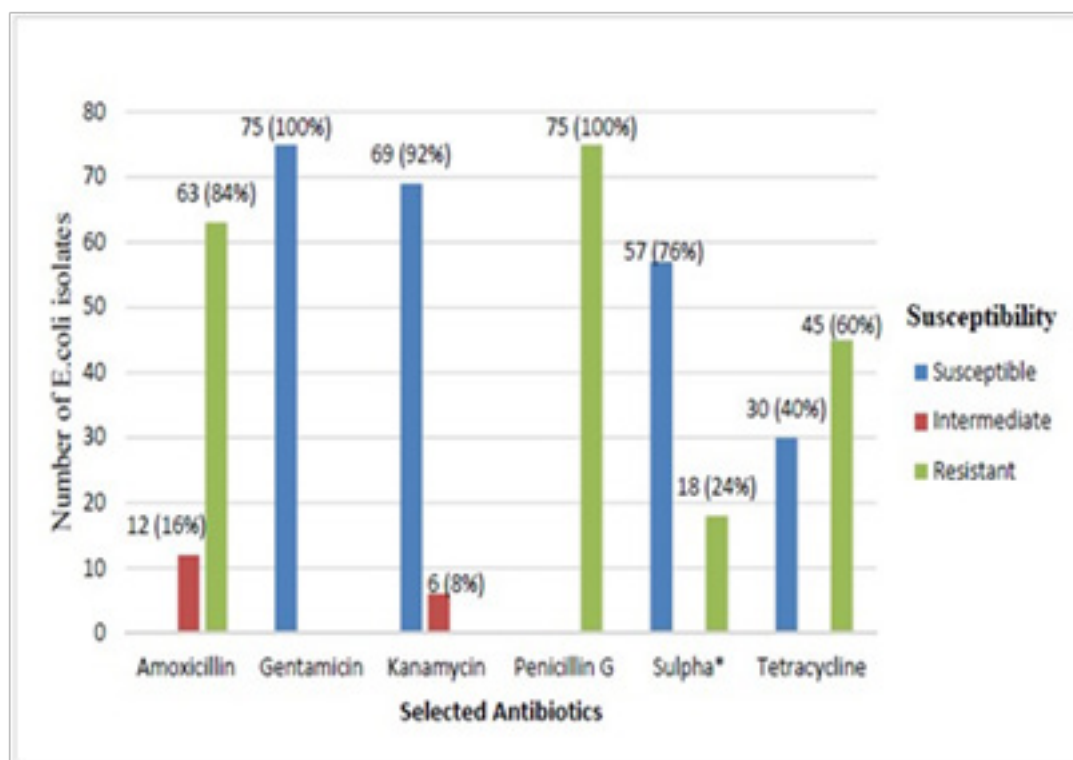

Figure 1 Antimicrobial susceptibility pattern of escherichia coli isolates.

Isolation, identification and antimicrobial susceptibility testing of Escherichia coli isolated from selected dairy farms in and around mekelle, Ethiopia

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Discussion

Escherichia coli are not only regarded as an indicator of fecal contamination but more likely as an indicator of poor hygiene and sanitary practices during milking and further handling. In this study, a total of 300 raw milk samples were studied and from these 300 raw milk samples, 75 (25%) milk samples were contaminated with *E. coli*. This is in agreement with the report by Mohanty et al.,[15] who found (21%) from India. The current finding was relatively higher as compared to the studies by Lye et al.,[16] & Addo et al.,[17] who reported 8.75% and 11.2% from Malaysia and Ghana, respectively. On the other hand, the present study was relatively lower as compared to the studies by Fadaei,[18] Ali et al.,[19] & Lubote et al.,[20] who reported 69%, 63 % and 90.67% from Iran, Khartoum and Tanzania, respectively. This finding difference in the present study from previous studies might be attributed to differences in environmental conditions, management and hygienic practices. In the present study, the occurrence of *E. coli* has been found significantly associated with different stage of lactations (x^2 = 149.677; P=0.000). The organism was found to be higher in milk taken from cows with early stage of lactation as compared to those in mid and late lactation. The highest percentage of *E. coli* isolates (84.1%) was isolated from early stage of lactation which is relatively higher as compared to the study by Waller et al.[21] 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.[22] 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.[23] A total of 75 *E. coli* isolates were tested against 6 antimicrobials based on CLSI guidelines and all *E. coli* isolates were found to be 100% susceptible to gentamicin followed by kanamycin (92%), sulphamethoxazole-trimthoprim (76%). On the other hand, all *E. coli* isolates were found to be 100% resistant to penicillin followed by amoxicillin (84%) and tetracycline (60%). Relatively similar findings have been reported by Bagre et al.,[24] who found all *E. coli* isolates were 100% susceptible to gentamicin, 75% resistant to amoxicillin and 15% resistant to sulphamethoxazole-trimthoprim from Burkinafaso. Reuben et al.,[25] were 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.,[26] 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.

Conclusion

The findings obtained in this study revealed that raw cow's milk were found to be highly contaminated with the *E. coli* isolates. Since many people still drink raw milk without further heat processing, it is a serious public health problem as milk is a vehicle for food borne diseases. Risk factors like parity number, stage of lactation and age has significant association with occurrence of *E. coli*; whereas there was no significant difference among different farms. Based on the antimicrobial susceptibility pattern, *E. coli* isolates were found to be highly susceptible to gentamicin, kanamycin and sulphamethoxazole-trimthoprim whereas greatly resistant to penicillin, amoxicillin and tetracycline. However, to safeguard the quality of raw milk, people engaged in milk and dairy production chain should be trained for hygienic practices.

Acknowledgment

Declaration

I declare that this research presents the work carried out by myself and does not incorporate without the acknowledgement of any material previously submitted for a degree or diploma in any university; and to the best of my understanding, it does not contain any materials previously published or written by another person except where due reference is made in the text; all substantive contributions by others to the work presented including jointly authored publications, is clearly acknowledged. Name of the Candidate: Dr. Gebremedhin Yohannes.

Conflicts of interest

The author declares that there are no conflicts of interest.

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Isolation, identification and antimicrobial susceptibility testing of Escherichia coli isolated from selected dairy farms in and around mekelle, Ethiopia

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