Infection and Drug Resistance

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Bacteriological Quality and Antimicrobial Susceptibility Patterns Among Raw Milk Producers and Vendors in Gomole District, Borena Zone, Southern Ethiopia

Alqeer Aliyo[1], Ayichew Seyoum[2], Zelalem Teklemariam[2]

[1]Department of Medical Laboratory Science, Institute of Health, Bule Hora University, Bule Hora, Ethiopia; [2]Department of Medical Laboratory Science, College of Health and Medical Sciences Haramaya University, Harar, Ethiopia

Correspondence: Alqeer Aliyo, Department of Medical Laboratory Science, Institute of Health, Bule Hora University, Bule Hora, Ethiopia, Tel +251 968-467458, Email alker438@gmail.com

Background and Aim: Milk consumption plays a great role in the nutrition of consumers and the income of producers as well as vendors, but their bacteriological quality causes loss of those benefits. Hence, the aim of this study was to determine the bacteriological quality, associated factors and antimicrobial susceptibility pattern among raw milk of producers and vendors in Gomole district, Borena zone, South Ethiopia, from March 1 to April 30, 2019.

Methods: A cross-sectional study was conducted on 130 purposively selected study participants. Pretested structured questionnaires and observation checklists were used to collect data. Then, 15 to 20 mL of milk samples were collected from producers and vendors for laboratory analysis. Standard plate count agar and eosin methylene blue agar were used for total bacterial count and total coliform count, respectively. Bacterial isolation from poor-quality milk was performed with biochemical tests and antimicrobial susceptibility tests using Kirby Bauer's disk diffusion method. After completeness checking, the data were analyzed by Statistical Package for the Social Sciences version 21. Chi-square (χ^2) was used to analyze association factors, and a p value <0.05 was considered statistically significant.

Results: The overall means ± standard deviation of the total bacterial count (TBC) and total coliform count (TCC) were 7.75 ± 0.882 and 6.69 ± 1.545 log10 CFU/mL, respectively. The mean TBC was significantly different between producers' and vendors' milks t = 2.1 (P < 0.001). The proportions of raw milk TBC and TCC of poor quality were 90% and 80%, respectively. Poor hand washing practices before milking, water source and cleanliness of milk containers were associated with poor milk quality. The isolated bacteria were *E. coli* (30.8%), *S. aureus* (17.9%) and *Salmonella* spp. Out of the bacterial isolates, 80.4% were extensive drug resistant, 14.3% were multidrug resistant, and 5.4% were resistant against all antimicrobials used in this study.

Conclusion: Ensuring proper hygienic practices during milking, storage and transportation to reduce milk contamination.

Keywords: raw milk, antimicrobial susceptibility pattern, producers, vendors

Introduction

Milk is an animal-derived food product that provides essential nutrients such as protein, energy, vitamins and minerals that promote the growth and maintenance of body tissue.[1] Biologically active compounds such as casein and whey proteins in milk have been found to be important for physiological and biochemical functions that have crucial impacts on human metabolism and health. Immunoglobulin in milk is important to protect newborns against a number of diseases.[2]

In Ethiopia, milk and milk products have a high value in feeding pastoral communities. Especially in the lowlands where livestock keeping is the main occupation, all groups of society consume milk.[3] Fresh milk is sold without pasteurization to the public either directly from small producers, via informal markets or through dairy farmers. These

Received: 1 March 2022
Accepted: 12 May 2022
Published: 20 May 2022

practices create milk contamination during production, handling and transportation and processing that acts as a vehicle for the transmission of milk-borne pathogens to humans.[4]

Bacterial contamination of milk is the major cause of food-borne diseases, which pose a serious threat to the health of millions of people worldwide. The magnitude of foodborne disease, death or other complications associated with milk contamination increases day-to-day and imposes a substantial burden on health care systems.[5] A major public health challenge is the increase in foodborne illness, which causes the death of millions of people. Including Salmonella, food poisoning is one of the most common and widely distributed diseases in the world, estimated to cause 1.3 billion cases of gastroenteritis and three million deaths worldwide.[6] Staphylococcal food poisoning (SFP) is among the most prevalent causes of gastroenteritis worldwide.[7] In addition, E. coli O157:H7, the most important foodborne pathogen, causes diarrhea, hemorrhagic colitis and hemolytic uremic syndrome in humans.[8]

In Africa, foodborne diseases are responsible for 33–90% of deaths in children and represent a serious problem for the continent.[9] Nevertheless, in developing countries such as Ethiopia, milk is a significant source of foodborne diseases and other infectious diseases. This happens where milk production and various dairy products take place under unsanitary conditions and poor production practices.[10]

Multidrug resistance (MDR) has increased worldwide and is considered a public health threat. Several recent investigations reported the emergence of multidrug-resistant bacterial pathogens from different origins, including humans, birds, cattle, and food, which increases the need for routine application of antimicrobial susceptibility testing to detect the antibiotic of choice as well as the screening of emerging MDR strains.[11–20] This is worsening the clinical scenario and is currently one of the greatest medical challenges. It is also the reason for low cure rates, loss of human life and animal and milk products.[21,22]

In Ethiopia, bacteriological quality control of raw milk and milk products is not usually conducted on a routine basis.[23] This situation results in the higher poor bacteriological quality of raw milk of pastoral communities in South Ethiopia.[24] Moreover, the raw milk producers and vendors in the Gomole district supply their milk directly to consumers without appropriate measures taken. No previous study has investigated bacteriological quality, factors contributing to the deterioration of raw milk quality and appropriate drugs for the treatment of individuals infected from milk-borne illness in Gomole district pastoral communities. Therefore, this study was carried out to determine the bacteriological quality, associated factors and antimicrobial susceptibility pattern among raw milk producers and vendors in Gomole district, Southern Ethiopia.

Methods

Study Area and Period

The study was conducted in Gomole district located in the Borena zone of Oromia Regional state from March 1 to April 30, 2019. The district is located 525 km from Addis Ababa along the Addis Ababa - Moyale highway. The Gomole district has 67,798 total human populations, and more than 90% of the populations are pastoralists. The district climate is arid and semiarid, and the annual mean daily temperature varies from 17 °C to 30 °C with a bimodal rain season. In 2018, animal statistics showed that there were 37,000 cattle, 14,890 camels and 108,222 goats in the district. Raw milk is the main product exported to neighboring districts and crosses the border to Kenya.

Study Design and Population

A community-based cross-sectional study was conducted on purposively selected raw milk producers and vendors in Gomole district.

Inclusion and Exclusion Criteria

Raw milk producers and vendors in four randomly selected kebeles of Gomole district during the study period were included in the study, while milk imported from outside of the study area and their producers or vendors were not included in the study.

Sample Size Determination

The sample size was calculated by Epi Info Version 7.2.1.0 using the double population proportion formula,[25] with the following assumptions: power of the study=80%, 95% confidence level, the ratio of unexposed:exposed is 1:1 including 15% contingency. The final sample size was 132.

Sampling Technique

Out of 14 kebeles (locations) in Gomole district, initially, four kebeles were selected using a simple random method. Then, producers and vendors in Kebeles were proportionally allocated. Finally, 132 study participants (97 producers and 35 vendors) were selected using a purposive sampling approach (Figure 1).

Data and Sample Collections

Data were collected through face-to-face interviews with the study participants by pretested structured questionnaires. The questionnaire contains sociodemographic characteristics such as age and sex and other factors affecting the quality of raw milk; cleaning practice of udder, source and type of water used for cleaning purposes, preservation method, hand

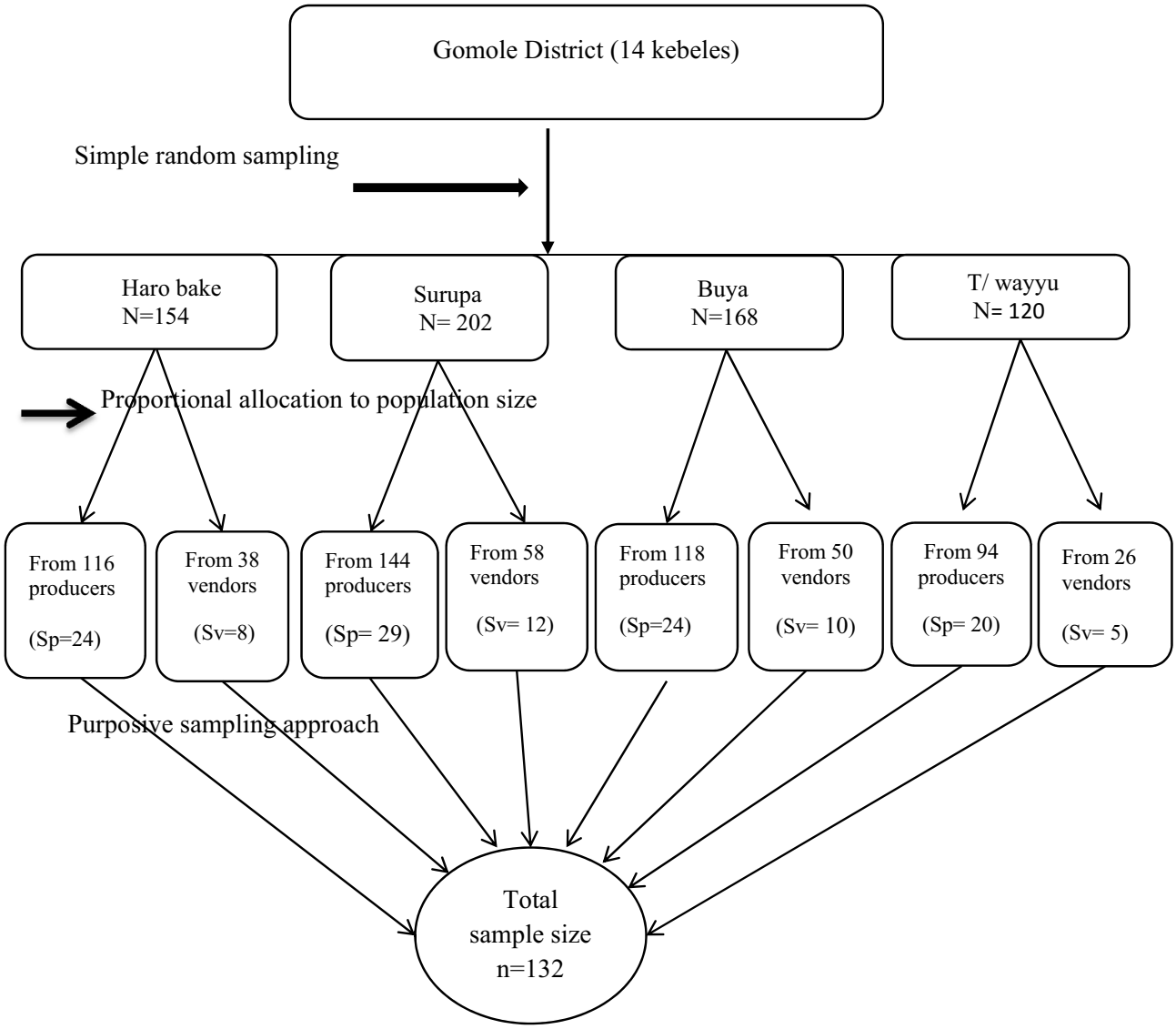

Figure 1 This figure shows schematic presentation of sampling techniques. In this figure N is the total number of producers and vendors in the selected kebeles, where Sp= the study sample size of producers in the selected kebeles; Sv=the study sample size of vendors from selected kebeles; and n= the total sample size.

washing practices before milking, types of milk containers and others. In addition, direct observation to assess participant personal hygiene, condition around milk and cleanliness of using containers was also noted in the checklists (S1 file). Then, approximately 15–20 milliliter (mL) raw milk samples were aseptically collected from producers' and vendors' containers placed into sterile screw cups. Consequently, the samples were labeled, placed in a 4°C ice box and transported to the laboratory within two hours for bacterial quality determination and antimicrobial susceptibility testing.

Laboratory Analysis

Total Bacteria and Coliform Enumeration

The total coliform count was determined by sterile standard plate count agar eosin methylene blue agar (EMBA). One milliliter of raw milk sample was added to a sterile test tube containing 9 mL of sterile peptone water. After thoroughly mixing, the suspension was serially diluted up to 10^{-7}. From all diluted tubes, 1 mL was taken and spread on sterile 15–20 mL standard plate count agar (Oxoid, Basingstoke, UK) and eosin methylene blue agar (EMB) media (Oxoid, Basingstoke, UK). The inoculated petri dishes were incubated at 35°C for 24 hours.[26] The plates with colonies ranging from 25–250 colony forming units per milliliter (CFU/mL) of sample were selected for determination of TBC.[27] TBC was determined as the total number of CFU per milliliter of milk sample, which was calculated using the formula. CFU/mL=Average number of colonies counted/ Dilution factor ×Volume plated. Total bacterial counts were judged according to East African standard milk samples and were considered good quality if the bacterial load was < 2×106 CFU/mL and poor if the bacterial load was > 2×106 (>6.3 log10) CFU/mL.[26] Regarding coliforms after incubation, green metallic sheen dark centered nucleated colonies appeared on the plates counted as total coliforms.[27] The results were interpreted as East African standard raw milk containing less than 50,000 (<4.7 $\log_{10}$) CFU/mL TCC, which is acceptable.[28]

Bacterial Isolation and Identification

Escherichia coli were isolated from coliform-contaminated eosin methylene blue agar (EMBA) plates, and one or two typical greenish metallic sheen suspected colonies were subjected to Gram staining. The presence of Gram-negative rod isolates was confirmed by biochemical tests.[29] For *Salmonella* spp. identification, 1 mL of milk was preenriched in 9 mL of lactose broth at 37°C for 24 hours. Then, 1 mL of preenrichment sample was inoculated into 10 mL of selenite F broth and incubated at 37°C for 24 hours. A loop full of selective enrichment was streaked on xylose-lysine decarboxylase (XLD) (Oxoid, Basingstoke, UK) and incubated at 37°C for 24 hours. All suspected nonlactose-fermenting Salmonella colonies were picked from plate agar, streaked onto nutrient agar plates and then incubated at 37°C for 24 hours. From plate agar, pure isolate single colonies were picked for biochemical tests.[24] *Staphylococcus aureus* was isolated by placing 0.1 mL aliquots from dilutions of poor quality graded samples into properly labeled mannitol salt agar (MSA) plates. The plates were spread and incubated at 37°C for 24 to 48 hours. For confirmation, one or two yellowish suspected colonies were transferred from MSA plates into nutrient broth (NB) tubes and incubated at 35°C for 48 hours. Following the incubation period, a loop full of NB was streaked on the nutrient agar plates and incubated at 35°C for 48 hours. The pure isolate colonies were subjected to Gram staining, catalase tests and coagulase tests. Those forming clumping by the coagulase test were considered positive.[30]

Antimicrobial Susceptibility Test

The antimicrobial susceptibility test (AST) of the bacterial isolate was performed by the Kirby-Bauer disk diffusion technique as modified by the Clinical and Laboratory Standard Institute (CLSI). From a pure culture, 3–5 selected colonies of bacteria were taken and transferred to a tube containing 4–5 mL sterile normal saline and mixed gently to make a homogenous turbid suspension adjusted to a McFarland 0.5 standard. A sterile cotton swab was dipped into the standardized suspension of bacteria and then uniformly streaked over the entire surface of Mueller-Hinton agar (Oxoid Ltd., UK) as per the guidelines of the Clinical and Laboratory Standards Institute.[31] Then, paper discs impregnated with a fixed concentration of the antimicrobial discs obtained from Himedia (Mumbai, India) were used in the study. The criteria used to select five antimicrobial agents, ampicillin, chloramphenicol, ciprofloxacin, tetracycline and gentamicin, were based on the availability and frequency of prescription for the management of bacterial infections in animals as well as for humans in Ethiopia.[32] Antimicrobial discs were placed on the agar surface and incubated in an inverted position at 37°C for

24 hours. After incubation for 24 hours, clear zones of inhibition were produced by the bacterial growth and diffusion of the antibiotics, which were measured in millimeters using a ruler and interpreted as susceptible, intermediate and resistant.[31]

Quality Control

Qualities of the obtained data were ensured by following a standard procedure in each step of the work. The questionnaire for the study was adopted from prior studies and developed according to the local context. The questionnaire was pretested for completeness and appropriateness to the local context on 5% of producers and vendors in Fichwa district. Based on the results of the pretest, some questions were modified. Every day, questionnaires were checked in the field after data collection by data collectors, supervisors and principal investigators. The calibrated equipment was used for the measurement of reagents and other materials before employing the process. The quality of the reagents, antibiotic disks and disinfectant solutions was ensured following the manufacturer's direction, and the expiration date was checked. Before dispensing, the antimicrobial disks were kept at room temperature for one hour. The culture media were prepared and sterilized based on the manufacturer's instructions, after which the sterility test of the media was checked by incubating 3–5% of the batch at 35–37°C overnight. American Type Culture Collection (ATCC) reference strains, such as *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *S. typhimurium* ATCC 14028, were used for the quality control of culture.

Data Processing and Analysis

The total bacterial and coliform count results were interpreted as direct and converted into Log_{10} CFU/mL by a logarithm calculator. The data were analyzed by SPSS version 21 for descriptive statistics, frequency, percentage and chi-square (χ^2) for association tested between dependent and independent variables, and a *t*-test was used for mean comparison. For all analyses, a 95% confidence level and p value <0.05 were considered statistically significant. The information is presented using tables and graphs.

Results

Questionnaire and Observational Survey Results

Out of a total, 130 study participants were enrolled in this study, with a response rate of 98.5%. Among the study participants, 95 (73.1%) were producers, and the remaining 35 (26.9%) were vendors. The age of the study participants ranged from 19 years to 67 years with a mean and standard deviation of 38.1 ±11.4. In terms of sex, the majority 108 (83.1%) of study participants were female. Regarding education status, 114 (87.7%) were unable to read and write, and only 5 (3.8%) attended primary school. On the other hand, among the study participants, only 21 (16.2%) trained on milk handling, and 101 (77.7%) did not know the diseases transmitted through contaminated raw milk (Table 1). Out of the study participants interviewed, the majority 106 (81.5%) of them used plastic containers, more than half of them 69 (53.1%) used motorcycles as a transportation means, and 43.1% transported milk for less than two hours (Table 2).

Regarding observations of hygienic practices, approximately 88.4% of participants used containers with poor cleanliness, and 54.7% observed good personal hygiene. Approximately 38.5% of the milk was kept under cool conditions (Table 3).

Total Bacteria and Coliform Counts

The overall total bacterial count (TBC) mean was 7.75±0.882 $\log_{10}$ (5.6×10^7) CFU/mL, while the total coliform count (TCC) mean was 6.69±1.545 $\log_{10}$ (4.89×10^6) CFU/mL. The mean of TBC was significantly different between producers' and vendors' milks t=2.1 (P<0.001); however, no TCC mean significantly differed t= 1.4 (P >0.062) (Table 4).

Raw Milk Quality and Bacterial Isolates

The overall TBC and TCC poor-quality milks were 117 (90%) and 104 (80%), respectively (Figure 2). The proportion of poor quality was higher among milk collected from vendors, but the difference was not statistically significant (*P*>0.05). Bacteria isolated from poor-quality milk were *Salmonella* spp. (2.6%), *S. aureus* (17.9%), and *Escherichia coli* (30.8%).

Table 1 Sociodemographic Characteristics and Knowledge of Raw Milk Producers and Vendors in Gomole District, Borena Zone, South Ethiopia, 2019

Factors	Category	Producers (n=95) No (%)	Vendors (n=35) No (%)	Total (n=130) No (%)
Sex of participants	Male	18(19)	4(11.4)	22(16.9)
	Female	77(81)	31(88.6)	108(83.1)
Age group	19–34 years	39(41.1)	15(42.9)	54(41.5)
	35–50 years	40(42.1)	15(42.9)	55(42.3)
	51–67 years	16(16.8)	5(14.2)	21(16.2)
Educational status	Cannot read and write	84(88.4)	30(85.7)	114(87.7)
	Can read and write	7(7.4)	4(11.4)	11(8.5)
	Primary school	4(4.2)	1(2.9)	5(3.8)
Train on milk handling	Yes	13(13.7)	8(22.9)	21(16.2)
	No	82(86.3)	27(77.1)	109(83.8)
Know milk borne diseases	Yes	21(22.1)	8(22.9)	29(22.3)
	No	74(77.9)	27(77.1)	101(77.7)

Table 2 Hygienic Practices, Environment, Transportation and Milk Container Characteristics Among Raw Milk Producers and Vendors in Gomole District, Borena Zone, South Ethiopia, 2019

Factors	Category	Producers (n=95) No (%)	Vendors (n=35) No (%)	Total (n=130) No (%)
Types of milk equipment	Plastic	72(75.8)	34(97)	106(81.5)
	Aluminum/Steel	3(3.2)	–	3(2.3)
	Wood	12(12.6)	–	12(9.2)
	Traditional pot	8(8.4)	1(3)	9(7)
Source of water for cleaning	Tap water	63(66.3)	19(54.3)	82(63.1)
	Well	18(18.9)	11(31.4)	29(22.3)
	Spring	13(13.7)	4(11.4)	17(13.1)
	Pond	1(1.5)	1(2.9)	2(1.5)
Type of water used to wash milk containers	Cold water	20(21)	5(14.3)	25(19.2)
	Cold water +soap	–	1(2.9)	1(0.8)
	Hot water only	66(69.5)	23(65.7)	89(68.5)
	Hot water + soap	9(9.5)	6(17.1)	15(11.5)

(*Continued*)

Table 2 (Continued).

Factors	Category	Producers (n=95) No (%)	Vendors (n=35) No (%)	Total (n=130) No (%)
Mode of transportation	On foot	35(36.8)	11(31.4)	46(35.4)
	By motorcycle	52(54.7)	17(48.6)	69(53.1)
	By animal	3(3.2)	–	3(2.3)
	By vehicle	5(5.3)	7(20)	12(9.2)
Transportation hours	Less than 1 hour	32(33.7)	11(31.4)	43(33.1)
	Less than 2 hours	46(48.4)	10(28.6)	56(43.1)
	3 hours and above	17(17.9)	14(40)	31(23.8)
Mix milk from different sources	Yes	80(84.2)	34(97.1)	114(87.7)
	No	15(15.8)	1(2.9)	16(12.3)
Milk Preserving method	By smoking	94(98.9)	35(100)	129(99.2)
	By fridge	1(1.1)	–	1(0.8)
Clean barn practice	Daily	6(6.3)	–	6(6.3)
	Once a week	32(33.7)	–	32(33.7)
	Once a month	57(60)	–	57(60)
Hand wash before milking	Yes	10(10.5)	–	10(10.5)
	No	85(89.5)	–	85(89.5)
Udder wash practice	Yes	1(1.1)	–	1(1.1)
	No	94(98.9)	–	94(98.9)

Table 3 Observational Findings Among Raw Milk Producers and Vendors in Gomole District, Borena Zone, South Ethiopia, 2019

Factors	Category	Producers (n=95) No (%)	Vendors (n=35) No (%)	Total (n=130) No (%)
Personal hygiene	Looks poor	52(54.7)	10(28.6)	62(47.7)
	Looks good	43(45.3)	25(71.4)	68(52.3)
Cleanliness of milk container	Looks poor	84(88.4)	27(77.1)	111(85.4)
	Looks good	11(11.6)	8(22.9)	19(14.6)
Conditions around milk	Clean	13(13.7)	4(11.4)	17(13.1)
	Dusty	16(16.8)	5(14.3)	21(16.2)
	Cool conditions	41(41.2)	9(25.7)	50(38.5)
	Hot conditions	25(26.3)	17(48.6)	42(32.3)

Table 4 Total Bacteria and Coliform Counts Among Raw Milk of Producers and Vendors in Gomole District, Borena Zone, South Ethiopia, 2019

Bacterial Count log_{10} CFU/mL	Producers' Milk Mean ± SD	Vendors' Milk Mean ± SD	Overall Milks Mean ± SD	t-test	
				t value 95% CI	P value
TBC	7.57±0.83	8.25±0.838	7.75±0.882	2.1(1.00, 3.5)	0.001
TCC	6.54±1.53	7.11±1.54	6.69±1.545	1.4(0.028, 1.17)	0.062

Abbreviations: TBC, total bacteria count; TCC, total coliform count; SD, standard deviation; CI, confidence interval.

However, the high prevalence of *S. aureus, Salmonella* spp. and *E. coli* isolated from vendors' raw milk was not statistically significant (p>0.05) (Table 5).

Factors Associated with Raw Milk Bacteriological Quality

In the present study, the source of water for cleaning, handwashing practices before milking and cleanliness of milk containers were significantly associated with poor bacteriological quality of raw milk (p<0.05) (Table 6).

Antimicrobial Susceptibility and Resistance Profile of Bacterial Isolates

The bacterial isolates were mostly susceptible to ciprofloxacin. All isolated bacteria showed resistance against ampicillin, but higher resistance was observed against *E. coli* (81.2%). *Salmonella* spp. was the only bacterial isolate that showed high resistance against all antimicrobials used in this study (Table 7). The overall multidrug resistance (MDR) and pandrug resistance (all tested antimicrobial resistant) of all bacterial isolates were 14.3% and 5.4%, respectively (Table 8).

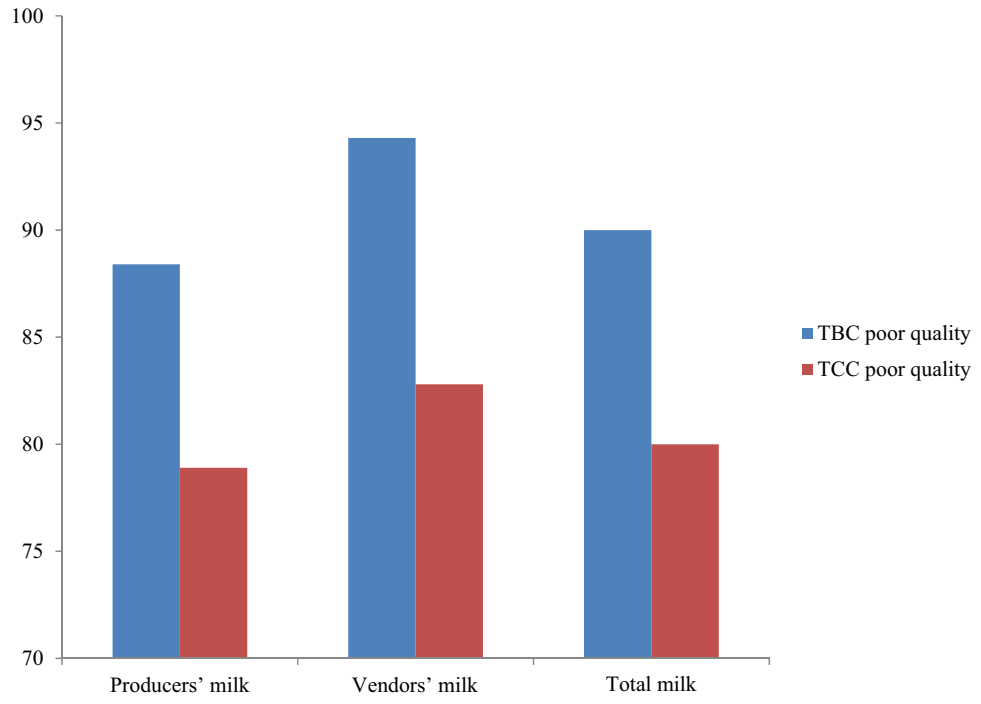

Figure 2 This figure shows poor quality proportion of total bacteria count (TBC) and total coliforms count (TCC) among producers' milk, vendors' milk and both milks.

Table 5 Proportion of Bacteriological Quality and Bacterial Isolates Among Raw Milk Producers and Vendors in Gomole District, Borena Zone, South Ethiopia, 2019

Poor Quality	Producers' Milk No (%)	Vendors' Milk No (%)	Overall Milks No (%)	p value
TBC	84(88.4)	33(94.3)	117(90)	0.323*
S. aureus	14(16.7)	7(21.2)	21(17.9)	0.566
Salmonella spp.	1(1.2)	2(6.1)	3(2.6)	0.135*
TCC	75(78.9)	29(82.8)	104(80)	0.621
E. coli	23(30.7)	9(31)	32(30.8)	0.971

Note: *Fisher's exact value.
Abbreviations: TBC, total bacteria count; TCC, total coliform count.

Table 6 Factors Associated with the Bacteriological Quality of Raw Milk from Producers and Vendors in Gomole District, Borena Zone, South Ethiopia, 2019

Factors	Category	Poor Quality (No. %)	Good Quality (No. %)	χ^2	P value	df
Gender	Male	21(95.4)	1(4.6)	0.875	0.349*	1
	Female	96(88.9)	12(11.1)			
Age group	19–34	48(88.9)	6(11.1)	0.765	0.682*	2
	35–50	49(89.1)	6(10.9)			
	51–67	20(95.2)	1(4.8)			
Educational status	Not read and write	104(91.2)	10(8.8)	1.560	0.213*	2
	Can read and write	9(81.8)	2(18.2)			
	Primary grade 1–8	4(80)	1(20)			
Source of water	Tape water	70(85.4)	12(14.6)	5.299	0.021*	1
	Nontape water	47(97.9)	1(2.1)			
Transportation hours	<1 hour	36(83.7)	7(16.3)	3.299	0.199*	2
	<2 hours	53(94.6)	3(5.4)			
	3<hours	28(90.3)	3(9.7)			
Mix milk from different sources	Yes	102(89.5)	12(10.5)	0.285	0.593*	1
	No	15(93.8)	1(6.2)			
Train on milk handling	Yes	18(85.7)	3(14.3)	0.511	0.475*	1
	No	99(89)	10(11)			
Know disease transmit by contaminated milk	Yes	24(82.8)	5(17.2)	2.175	0.140	1
	No	93(92.1)	8(7.9)			
Hand wash before milking	Yes	4(40)	6(60)	25.59	0.001*	1
	No	80(94.1)	5(5.9)			

(*Continued*)

Table 6 (Continued).

Factors	Category	Poor Quality (No. %)	Good Quality (No. %)	χ^2	P value	df
Personal hygiene	Looks poor	57(91.9)	5(8.1)	0.493	0.482	1
	Looks good	60(88.2)	8(11.8)			
Cleanliness of milk containers	Looks poor	110(99.1)	1(0.9)	69.86	0.001*	1

Notes: *Fischer's exact value; good quality milk ≤$6.3_{\log 10}$; poor quality milk>$6.3_{\log 10}$ CFU/mL.
Abbreviations: χ^2, Chi-square; df, degrees of freedom.

Table 7 Antimicrobial Susceptibility Pattern of Bacterial Isolates from Raw Milk

Antimicrobials	*E. coli* (n=32)		*S. aureus* (n=21)		*Salmonella* spp. (n=3)	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Ampicillin	6(18.8)	26(81.2)	8(38.1)	13(61.9)	1(33.3)	2(66.7)
Ciprofloxacin	32(100)	–	21(100)	–	2(66.7)	1(33.3)
Chloramphenicol	32(100)	–	15(71.4)	6(28.6)	1(33.3)	2(66.7)
Gentamicin	32(100)	–	19(90.5)	2(9.5)	2(66.7)	1(33.3)
Tetracycline	32(100)	–	16(76.2)	5(23.8)	1(33.3)	2(66.7)

Abbreviations: S, Susceptible; I, intermediate; R, resistance.

Table 8 Antimicrobial Resistance Profile of Bacterial Isolates

Bacteria Strains	No. of Isolates	Extensive Drug Resistant (XDR) No. (%)	Multidrug Resistant (MDR) No. (%)	Pandrug Resistant (PDR) No. (%)
E. coli (n=32)	32	26(81.2)	–	–
S. aureus (n=21)	21	16(76.2)	6(28.6)	–
Salmonella spp.	3	3(100)	2(66.7)	1(33.3)
Total	56	45(80.4)	8(14.3)	3(5.4)

Notes: XDR ≤2 drugs resistant. MDR ≥3 drug resistant.
Abbreviation: PDR, all drug resistant.

Discussion

The present study revealed that the total bacterial count (TBC) overall mean was 5.6×10^7 (7.75±0.8824 $\log_{10}$) CFU/mL. This finding is in line with a study conducted in Borena Yabello (8.149 ± 0.043 $\log_{10}$), South Ethiopia.[33] However, this finding was higher than that of a study conducted in Iran (1.03×10^6 ±0.0022 CFU/mL),[34] Dire Dawa (6.76 Log_{10} CFU/mL).[35] In addition, the overall total coliform count (TCC) mean of raw milk was 6.69±1.545 $\log_{10}$ (4.89×10^6) CFU/mL. This result is comparable with the study in Borena Yabello (6.323 ± 0.028 $\log_{10}$),[33] Hawassa (6.52 $\log_{10}$ CFU/mL),[25] and Eastern Ethiopia (7.32±0.07 $\log_{10}$).[36] However, this finding is higher than that of Tanzania ($2.8\text{x}10^4$ CFU/mL),[37] Kenya (4.00±0.66 $\log_{10}$ CFU/mL),[38] and Mersa Ethiopia (5.15 $\log_{10}$ CFU/mL).[39] The differences in studies might be attributed to variations in the surrounding temperature, geographical area, seasonal variations, fecal contamination cleanliness of milk containers, hygienic practices during milking, and transportation.[10]

The overall proportion of TBC poor-quality milk was 90%. This finding is similar to a study conducted in Tanzania (90%)[37] but higher than a study from Adigrat Ethiopia (60%)[40] and lower than a study from Borena Abaya, Ethiopia

(99%).[24] A higher TBC can reduce milk quality, shelf life and the nutritional quality of milk and threatens the health of consumers due to toxic metabolites produced by different organisms growing in it.[30]

Enserchеia coli isolated was 30.8%, which was higher than that in a study conducted in Zambia (13%),[41] Tanzania (3.5%),[37] Mekele, Ethiopia (17.6%),[42] Hawassa, Ethiopia (8.8%),[43] and Borena Abaya South Ethiopia (12.9%).[24] However, lower than Iran (69%),[34] Arusha Tanzania (90.67%),[44] two district Tanzania (3.5%),[37] Isiolo Kenya (38.2%),[38] and Mekele Ethiopia (44.4%).[45] The number of *Staphylococcus aureus* isolates was 21 (17.9%), which was lower than that in studies conducted in Iran (41.66%),[34] Zambia (22%),[41] Ethiopia Hawassa (35.2%),[43] Mekele (27.5%),[42] and Mekele (26.7%).[45] In contrast, the prevalence was higher than that in a study in Tanzania (0.9%)[37] and Borena Abaya, Ethiopia (7.29%).[24] In addition to *E. coli* and *S. aureus, Salmonella* spp. were the third most common isolates, with a 2.6% prevalence, which was lower than that in a study in Arusha Tanzania (37.4%)[44] and Borena Abaya District, South Ethiopia (11.5%).[24] The difference in prevalence might vary with the geographic region of the area samples collected, sample size and high proportion of those bacteria in milk related to the level of poor hygienic practices.

In this study, factors such as hand wash practice before milking, source of water for cleaning and cleanliness of milk containers showed statistically significant ($p<0.05$) associations with poor bacterial quality of raw milk. This finding agrees with studies conducted in Hawassa, Ethiopia,[43] Yabelo Borena, Ethiopia[33] and Kampala Uganda.[46]

Antimicrobial susceptibility tests showed that *E. coli* isolates were 100% susceptible to gentamicin, ciprofloxacin and chloramphenicol. This finding is similar to studies conducted in Adigrat, Ethiopia,[40] Tanzania[47] and Egypt[48] but higher sensitivity than studies in Bangladesh[49] and Jigjiga Ethiopia.[32] However, *E. coli* was highly resistant to ampicillin (81.2%). This study is similar to other studies.[40,47,49] *Staphylococcus aureus* was highly sensitive to ciprofloxacin, gentamicin, tetracycline and chloramphenicol (100%, 90.5%, 76.2% and 71.4%, respectively), which was comparable to a study conducted in Adigrat, Ethiopia,[40] Tanzania,[47] Egypt,[48] and Bangladesh.[49] In contrast, *S. aureus* was highly resistant to ampicillin (61.9%), which was similar to a study conducted in Bangladesh[49] and Egypt[48] and lower than a study from Adigrat Ethiopia.[40]

In terms of *Salmonella* spp. isolates, 33.3% were sensitive to chloramphenicol and tetracycline, and 66.7% were sensitive to gentamicin and ciprofloxacin, which is lower than the results of studies conducted in Bangladesh,[49] Tanzania[47] and Jigjiga Ethiopia.[32] In the present study, *Salmonella* spp. showed 66.7% resistance to ampicillin, which is comparable to a study conducted in Bangladesh[49] and lower than that in Tanzania[47] but higher than that in Jigjiga, Ethiopia.[32] This high level of resistance of *E. coli, S. aureus* and *Salmonella* spp. to ampicillin in this study might be a result of suboptimal, prolonged, indiscriminate and use of inappropriate antibiotics for the treatment of diseases and excessive use of antimicrobial therapeutic and prophylactic treatment.

The present study obtained 14.3% overall multidrug resistance. The finding of the study was lower than that of a study conducted in Jigjiga (55.2%).[32] *S. aureus* isolates were found to be 4.8% multidrug resistant (≥3 drugs). This finding is lower than that of a study conducted in Egypt, which found *S. aureus* 34.8% and *E. coli* 94.9%.[48] Although MDR *S. aureus* has a low prevalence in raw milk samples from the study area, it is hurdling to develop a healthy and safe living environment for humans. On the other hand, *Salmonella* spp. isolates demonstrated a high level of multidrug resistance (66.7%), especially among commonly used drugs such as ampicillin, chloramphenicol and tetracycline.

Limitations of the Study

This study did not identify the virulence genes and antimicrobial resistance genes of isolated bacteria due to the inability of resources. The correlation between phenotypic and genotypic MDR was not assessed.

Conclusion and Recommendations

The findings of this study showed that both producers' and vendors' raw milk was highly contaminated with total bacteria and coliforms. The overall bacteria isolated from raw milk with poor bacteriological quality were 32 (30.8%) *Escherichia coli*, 21 (17.9%) *Staphylococcus aureus* and 3 (2.6%) *Salmonella* spp. Antimicrobial-resistant bacteria isolated from milk may be a great challenge for the healthcare system. Based on the findings, milk producers and vendors should practice cleaning the milk container properly and washing their hand before milking. Local, regional and national governments

should establish diagnostic centers for the identification, antimicrobial resistance surveillance and prevention of zoonotic transmission of pathogens.

Abbreviations

AST, Antimicrobial Susceptibility Test; ATCC, American Type Culture Collection; CFU/mL, Colony Forming Units per Milliliter; CLSI, Clinical and Laboratory Standard Institute; MDR, Multi-Drug Resistance; TBC, Total Bacteria Count; TCC, Total Coliform Count; TSI, Triple Sugar Iron; XLD, Xylose-Lysine Decarboxylase.

Data Sharing Statement

Data essential for the conclusion are included in this manuscript. Additional data can be obtained from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

Ethical clearance was obtained from Haramaya University College of Health and Medical Sciences, Institutional Health Research Ethics Review Committee (IHRERC). After ethical clearance, a formal letter was written from Haramaya University to Gomole district administration. After obtaining permission from the district administration, selected kebeles were informed by letter in the local language. The data collection was commenced by explaining the objectives and procedures of the study to each study participant. Written, informed and signed consent was obtained from each volunteer. Confidentiality of information was kept. No references were made in oral or written reports that could link participants to research.

Acknowledgments

We would like to express our deepest gratitude to Haramaya University, College of Health and Medical Sciences, for allowing us to conduct this study. We also thank Gomole districts and Bule Hora University for their support. Finally, our earnest thanks are extended to Mr Mohammed Aliyo for sharing information about the study area.

Author Contributions

All authors made a significant contribution in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

Haramaya University has funded the research. The funder has had no role in the study design, data collection, analysis, publications, or preparation of the manuscript.

Disclosure

The authors report no conflicts of interest in relation to this work and do not have any conflicts of interest related to any activities pertaining to this research work.

References

1. Pandey GS, Voskuil GC. *Manual on Milk Safety, Quality and Hygiene*. Vol. 52. Zambia: Golden Valley agricultural Research Trust; 2011.
2. Gobbetti M, Stepaniak L, De Angelis M, Corsetti A, Di Cagno R. Latent bioactive peptides in milk proteins: proteolytic activation and significance in dairy processing. *Crit Rev Food Sci Nutr*. 2002;42(3):223–239. doi:10.1080/10408690290825538
3. Godefay B, Molla B. Bacteriological quality of raw cow's milk from four dairy farms and a milk collection center in and around Addis Ababa. *Berl Munch Tierarztl Wschr*. 2000;113:1–3.
4. Gebrewold A, Alemayehu M, Demeke S, Dediye S, Tadesse A. Status of dairy research in Ethiopia. In: *Small Holder Dairy Development*. Addis Ababa: Ministry of Agriculture (MOA); 2000.
5. Food and Agricultural Organization of the United Nations. *FAO Stats*. Food and Agricultural Organization of the United Nations; 2006.

6. Ohud M, Eman MH, Hayam SA. Detection of Salmonella strains in clinical samples from Saudi Arabia by invA and hilA Polymerase Chain Reaction (PCR)-based assays. *African J Microbiol Res*. 2012;6:5410–5416.
7. Wang S, Duan H, Zhang W, Li JW. Analysis of bacterial foodborne disease outbreaks in China between 1994 and 2005. *FEMS Immunol Med Microbiol*. 2007;51:8–13. doi:10.1111/j.1574-695X.2007.00305.x
8. Mersha G, Asrat D, Zewde BM, Kyule M. Occurrence of *Escherichia coli* O157:H7 in feces, skin and carcasses from sheep and goats in Ethiopia. *Lett Appl Microbiol*. 2010;50:71–76. doi:10.1111/j.1472-765X.2009.02757.x
9. Flint J, Duynhoven Y, Angulo F, et al. Estimating the burden of acute gastroenteritis, food-borne diseases and pathogens commonly transmitted by food. *J Clin Infect Dis*. 2005;41:698–704. doi:10.1086/432064
10. Yilma Z, Loiseau G, Faye B. Manufacturing efficiencies and microbial properties of butter and Ayib—Ethiopian cottage cheese. *Livestock Res Rural Dev*. 2007;19(7):2004.
11. Makharita RR, El-Kholy I, Hetta HF, et al. Antibiogram and genetic characterization of carbapenem-resistant gram-negative pathogens incriminated in healthcare-associated infections. *Infect Drug Resist*. 2020;13:3991–4002. doi:10.2147/IDR.S276975
12. Algammal AM, Enany ME, El-Tarabili RM, Ghobashy MOI, Helmy YA. Prevalence, antimicrobial resistance profiles, virulence and enterotoxins-determinant genes of MRSA isolated from subclinical bovine mastitis in Egypt. *Pathogens*. 2020;9(5):362. doi:10.3390/pathogens9050362
13. Algammal AM, Hetta HF, Batiha GE, et al. Virulence-determinants and antibiotic-resistance genes of MDR-E. coli isolated from secondary infections following FMD-outbreak in cattle. *Sci Rep*. 2020;10(1):19779. doi:10.1038/s41598-020-75914-9
14. Enany ME, Algammal AM, Shagar GI, Hanora AM, Elfeil WK, Elshaffy NM. Molecular typing and evaluation of Sidr honey inhibitory effect on virulence genes of MRSA strains isolated from catfish in Egypt. *Pak J Pharm Sci*. 2018;31(5):1865–1870.
15. Algammal AM, Hashem HR, Alfifi KJ, et al. atpD gene sequencing, multidrug resistance traits, virulence-determinants, and antimicrobial resistance genes of emerging XDR and MDR-*Proteus mirabilis*. *Sci Rep*. 2021;11(1):9476. doi:10.1038/s41598-021-88861-w
16. Abolghait SK, Fathi AG, Youssef FM, Algammal AM. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from chicken meat and giblets often produces staphylococcal enterotoxin B (SEB) in nonrefrigerated raw chicken livers. *Int J Food Microbiol*. 2020;328:108669. doi:10.1016/j.ijfoodmicro.2020.108669
17. Algammal AM, Hetta HF, Elkelish A, et al. Methicillin-Resistant *Staphylococcus aureus* (MRSA): one health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. *Infect Drug Resist*. 2020;13:3255–3265. doi:10.2147/IDR.S272733
18. Algammal AM, Hashem HR, Al-Otaibi AS, et al. Emerging MDR-*Mycobacterium avium subsp. avium* in house-reared domestic birds as the first report in Egypt. *BMC Microbiol*. 2021;21(1):237. doi:10.1186/s12866-021-02287-y
19. Algammal AM, Mabrok M, Ezzat M, et al. Prevalence, antimicrobial resistance (AMR) pattern, virulence determinant and AMR genes of emerging multi-drug resistant *Edwardsiella tarda* in Nile tilapia and African catfish. *Aquaculture*. 2022;548:737643. doi:10.1016/J.AQUACULTURE.2021.737643
20. Batiha GE, Hussein DE, Algammal AM, et al. *Corrigendum to Application of Natural Antimicrobials in Food Preservation: Recent Views [Food Control 126 (2021) 108066] (Food Control (2021) 126, (S0956713521002048), (10.1016/J.foodcont.2021.108066))', Food Control*. Elsevier Ltd; 2021. doi:10.1016/J.FOODCONT.2021.108324
21. Centers for Disease Control and Prevention. *Antibiotic Resistance Threats in the United States (U.S. Department of Health and Human Services)*. Atlanta, GA, USA: CDC; 2019.
22. Costelloe C, Metcalfe C, Lovering A, Mant D, Hay AD. Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis. *Biomed J*. 2010;340:c2096. doi:10.1136/bmj.c2096
23. Alehegn W. *Bacteriological Quality of Bovine Milk in Small Holder Dairy Farms in Debre Zeit, Ethiopia. MSc Thesis*. Ethiopia: Addis Ababa University; 2004.
24. Worku T, Negera E, Nurfeta A, Welearegay H. Microbiological quality and safety of raw milk collected from Borana pastoral community, Oromia Regional State. *African J Food Sci Technol*. 2012;3:213–222.
25. Welearegay H, Yilma Z, Teklegiorgis Y. Incidence of pathogenic and indicator bacteria in raw and pasteurized milk in Hawassa city, rift valley of southern Ethiopia. *African J Anim Biochem Sci*. 2012b;7:100–107.
26. Murphy SC. *Sources and Causes of High Bacteria Count in Raw Milk*. Ithaca, New York, USA: Cornell University; 1996:1–34.
27. Kiiyukia C. Laboratory manual of food microbiology for Ethiopian health and nutrition research institute. UNIDO project (YA/ETH/03/436/11-52). 2003:1–197.
28. East African Standard (EAS). EAS 67:2000: raw cow milk specification: 1–14; 2006. Available from: https://law.resource.org/pub/eac/ibr/eas.67.2006.pdf. Accessed November 15, 2016.
29. Cheesbrough M. *District Laboratory Practice in Tropical Countries Part 2*. 2nd ed. Cambridge University Press; 2006:137–141.
30. Yousef AE, Carlstrom C. *Food Microbiology: A Laboratory Manual*. Hoboken, New Jersey: John Wiley and Sons, Inc.; 2003:277.
31. Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing*. 28th ed. CLSI supplement M100; 2018:1-56238-838.
32. Reta MA, Bereda TW, Alemu AN. Bacterial contaminations of raw cow's milk consumed at Jigjiga City of Somali Regional State, Eastern Ethiopia. *Int J Food Contamin*. 2016;3(1):2–9. doi:10.1186/s40550-016-0027-5
33. Gurmessa T. Microbiological quality and impact of hygienic practices on raw cow's milk obtained from pastoralists and market. The case of Yabello District, Borana zone, Ethiopia. *Globl J Food Sci Technol*. 2015;3:153–158.
34. Fadaei A. Bacteriological quality of raw cow milk in Shahrekord, Iran. *Vet World*. 2014;7(4):240–243. doi:10.14202/vetworld.2014.240-243
35. Mesfine S, Teka F, Ousman M. Microbiological quality of raw cow's milk from four dairy farms in Dire Dawa City, Eastern Ethiopia. *World J Dairy Food Sci*. 2014;10(1):09–14.
36. Tadele A, Ameha K, Yoseph M, Mitiku E. Microbiological quality of raw cow milk across the milk supply chain in Eastern Ethiopia. *East African J Sci*. 2016;10(2):119–132.
37. Emil H. *Evaluation of Microbial Contamination Along the Milk Value Chain in Two Districts of Tanzania. Master Thesis*, Morogoro, Tanzania: Sokoine university of Agriculture; 2015.

38. Wanjala NW, Wafula JM, Masani JN. Antimicrobial effect of smoking milk handling containers' inner surfaces as a preservation method in pastoral systems in Kenya. *Res Policy Pract*. 2016;6:17.
39. Oumer E, Tsegaye S, Damtew A, Feleke A. Hygienic practices and bacteriological quality of cow raw milk from selected smallholder dairy farms of Mersa Town, North Wollo, Ethiopia. *Eur J Exp Biol*. 2017;7(4):22.
40. Abrha B, Reda M, Taddele H, Berihun A. Bacteriological quality assessment of milk in dairy farms, cafeterias and wholesalers in Adigrat, Tigray, Ethiopia. *Eur J Biol Sci*. 2014;6(4):88–94.
41. Theodore JD, Knight-Jones M, Bernard H, et al. Microbial contamination and hygiene of fresh cow's milk produced by smallholders in Western Zambia. *Int J Environ Res Public Health*. 2016;13:737. doi:10.3390/ijerph13070737
42. Faisal SD, Ahmed AF. Bacteriological quality assessment of milk in College of Veterinary Medicine (CVM) dairy farm and kalamino dairy farm in Mekelle, Tigray, Ethiopia. *Dairy Vet Sci J*. 2018;8(2):555734. doi:10.19080/JDVS.2018.08.555734
43. Solomon M, Alemayehu R, Rahmeto A, Amene F, Berihun D. Bacteriological study on rawmilk collected from hawassa smallholder dairy farms. *Adv Biol Res*. 2014;8(5):194–200.
44. Lubote R, Shahada F, Matemu A. Prevalence of *Salmonella spp*. and *E. coli* in raw milk value chain in Arusha, Tanzania. *Am J Res Commun*. 2014;2(9):1–13.
45. Endale B, Shunda D, Habtamu T. Assessment of bacteriological quality of raw cow milk at different critical points in Mekelle, Ethiopia. *Int J Livestock Res*. 2013;3(3b):42–46.
46. Mbabazi P. Milk industry in Uganda, 1st (edn), Fountain Publishers Kampala, medicine. 10th (edn), A textbook of the diseases of cattle, horses, sheep, pigs and goats. Meetig proceedings. 2005:11.
47. Brown H. *Assessment of Microbiological Quality of Raw Milk and Antimicrobial Susceptibility of Selected Milk-Borne Bacterial in Kilosa and Mvomero Districts Tanzania. Master's Thesis*. Sokoine University of Agriculture; 2015.
48. Sadek OA, Sayed SM, Berbawy E, Mariam F, Mansy F, Hussien MF. Some antibiotic resistant bacteria of public health hazard isolated from raw milk sold in some Assiut City markets. *Assiut Univ Bull Environ Stud*. 2014;17(1):97–107.
49. Marjan S, Kanta DK, Munshi SK, Noor R. Drug-resistant bacterial pathogens in milk and some milk products in Dhaka Bangladesh. *Nutr Food Sci*. 2014;44(3):241–248. doi:10.1108/NFS-05-2013-0061