Bacteriological quality, antibiotic susceptibility pattern of isolates and associated factors among raw cow's milk collected from cafeterias in Adama Town, Oromia Region, Ethiopia

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Abstract

Background: Milk is an excellent medium for the growth of microorganisms because it is composed of water, nutrients, and an almost neutral pH, and consuming such pathogens is hazardous to human health. Based on our knowledge, there is currently no published research available regarding the bacteriological quality of raw cow's milk sourced from cafeterias in Adama Town.

Methods: A cross-sectional study was conducted in Adama Town, Oromia Region, Ethiopia, from March 23 to June 6, 2019. The study included 115 randomly selected samples of raw cow's milk obtained from cafeterias. Questionnaire was used to collect data on handling practices. The milk samples were cultured on Eosin methylene blue agar, mannitol salt agar, and Salmonella-Shigella agar to determine the total and coliform count using serial dilutions. The antibiotic susceptibility testing of the bacterial isolates was assessed using the Kirby-Bauer disk diffusion method on Muller-Hinton agar. The data were analyzed using Statistical Package for the Social Sciences (SPSS) version 22. A logistic regression model was used to assess the association between predictors and milk quality and a p-value of less than 0.05 was considered statistically significant.

Results: The overall mean ± standard error of total bacterial and coliform counts of sampled milk were 6.600 ± 0.144 and 4.96 ± 0.10 log10 CFU/mL, respectively. Based on 2009 Ethiopian standards for the bacteriological quality of raw milk, 71.3% of the samples were of poor quality, and 67% of them also tested positive for total coliforms. *Staphylococcus aureus* (23.5%) and *Escherichia coli* (7.8%) strains were identified in the milk samples. All E coli isolates were found to be resistant to ampicillin, but susceptible to amoxicillin-clavulanic acid, ceftriaxone, gentamicin, cotrimoxazole, ciprofloxacin, tetracycline, chloramphenicol, cefoxitin . The majority of *Staphylococcus aureus* isolates showed resistance to one or more of the tested antibiotics.

Conclusions: In this study, more than seven out of ten of the milk samples had significant levels of bacterial contamination. Multidrug resistance was observed in nearly half of the total tested isolates. Adequate sanitary measures, hygienic practices, strict monitoring, and quality control measures should be in place to ensure the delivery of safe and quality milk to consumers.

Keywords: Raw Cow Milk, Handling Practices, Bacteriological Quality, Antibiotic Resistance, Susceptibility.

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Introduction

Milk is a nutritious organic colloid with a nearly neutral pH. It is a popular food for all human age groups, especially for children. In addition to water, milk contains a variety of macro- and micro molecules. However, it can also be a breeding ground for pathogenic microorganisms like *Salmonella* spp., *S. aureus,* and *E. coli*. These bacteria are often associated with milk-borne illnesses (Hassan *et al.,* 2015, Mahari and Yemane, 2016, Sarkar, 2016). Diarrheal diseases alone result in approximately 550 million cases and 230,000 deaths worldwide each year (WHO, 2015).

Raw milk is at risk of bacterial contamination from various sources, including sick animal udders, soil, animal feed, milking and storage equipment, and animal feces (Reta and Addis, 2015, Mahari and Yemane, 2016). The microbiological quality of raw milk primarily determines the quality of milk products. Therefore, it is crucial to assess the milk quality from farm to table. However, in Ethiopia, milk production is disorganized, and raw milk is typically distributed to customers without regular hygienic quality checks at any level (Dehinenet *et al*., 2013, Solomon *et al*., 2014).

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Numerous studies conducted in different parts of Ethiopia have revealed a high bacterial load in milk. For instance, the study from Oromia region found that 99% of raw milk samples collected from pastoral communities had a total bacterial count (TBC) of 7.64 log10 CFU/ml (Worku *et al*., 2012). Similarly, studin a selected sub-city of Addis Ababa, found that 98% of the collected raw milk had a higher TBC (8.6 ± 1.01 x 106) than the maximum recommended level of 2.0 ×106. (Bruktawit, 2016).

Antibiotic-resistant bacteria in food indicate a significant public health threat (Merhawit *et al*., 2014). Consuming milk contaminated with antibiotics can cause allergic reactions, disrupt the balance of intestinal bacteria, and contribute to the emergence of antibiotic-resistant pathogenic bacteria, thereby posing risks to public safety (Malek *et al.,* 2015). The uncontrolled use of antimicrobials in the environment is causing an increase in antimicrobial resistance rates (Senthilkumar and Prabakaran, 2005). Numerous studies have shown that *Staphylococcus aureus* and *Escherichia coli* are becoming resistant to a wide range of antimicrobials, which is concerning for their use in clinical treatment (Khan *et al.,* 2014, Reta *et al*., 2016). However, there is limited research on antimicrobial resistance in food animals despite its importance in detecting changes in resistance patterns, implementing control measures for antimicrobial use, and preventing the spread of multidrug-resistant bacteria (Khan *et al*., 2014).

Materials and Methods

Study area, design, and period

This study was conducted in Adama town, which is located in the East Shoa Zone, approximately 99 km southeast of Addis Ababa. Adama town was established in 1916 and sits at an altitude of 1600 to 1700 meters above sea level. The town experiences an average annual temperature of 21.9OC and an annual rainfall of 847.14 millimeters. It has 152 hotels and 278 cafeterias, with a health service coverage of 78.6% (Adama City, 2016). For this study, a cross-sectional study was conducted on milk samples collected from randomly selected cafeterias between March 23 and June 6, 2019.

Sample Size Determinations and Sampling Techniques

The sample size was determined using a single proportion formula considering bacteriological quality using a 98% prevalence of bacterial contamination based on the study conducted in Addis Ababa (Bruktawit, 2016), a Z score of 95% confidence level', and a margin of error of 5%. The sample size for the associated risk factors was calculated by double population proportion formula using Epi Info version 7 statistical software, with an outcome of 59.5% in the unexposed group and 84.6% in the exposed group, and a power of 80%, 95% CI (Solomon *et al.,* 2014). A maximum sample size of 115 raw milk samples was taken into consideration for this study. Cafeterias that served raw milk at the time of the data collection and retailed cow's milk were included randomly.

Data Collection Methods

Data were collected by the following method;

Face-to-face interviews; were conducted using a standardized questionnaire survey on workers directly involved in the handling of milk in a cafeteria to assess data related to cafeterias' milk handling practices.

Observational assessment; was done using a checklist to assess milk handling practices.

Sample collection, transportation, and preparation; 10 to 15 mLs of raw milk was collected into a sterile falcon tube in an aseptic procedure and kept in an ice box at a temperature below 5 ºC. Then, it was immediately transported to the Oromia Public Health Research Capacity Building and Quality Assurance Laboratory (OPHRCBQAL) in Adama town for bacteriological quality analysis. In the laboratory after thoroughly mixing each sample, it was diluted in 0.85% sterile normal saline by pouring one ml of milk into 9 ml of diluent in a test tube to get a dilution of 1:10. All the samples were further serially diluted up to 10^{-7} before plating. All Petri plates were labeled with a water-proof marker concerning the dilution factor and sample number (Healy *et al.,* 2014, Senthilkumar *et al.,* 2014).

Determining total bacterial count and milk quality; The conventional standard plate count method was used for the enumeration of total viable bacteria. One ml sample from each serial dilution of the test sample was pipetted into empty sterile petri dishes in duplicate, and then molten, cooled (47^{OC}) nutrient agar (Oxoid) was added and incubated at 37^{OC} for 48 hours.

Plates in duplicate with colonies between 25 and 250 were selected, and the average count was calculated and expressed as Coliform Count per milliliters (CFU/ml) (Solomon *et al*., 2014).

$$\text{CFU/mL} = \frac{\text{Average number of colonies from duplicate}}{\text{Dilution factor x volume plated}}$$

Milk samples were considered good quality or acceptable if their bacterial load was ≤ 2 x 106 CFU/mL and poor if it was > 2 x 106 CFU/mL.

Total Coliform Count (TCC) and isolation of *E. coli:* Using a sterile cotton swab and a 10^{-3} dilution, 0.1 mL of the sample was spread in duplicate on Eosin Methylene Blue (EMB) agar (Park Scientific, U.K.). After that, the plates were incubated for 24 hours at 37°C. On both plates, the typical purplish to dark red coliform colonies produced by the fermentation of lactose were counted, and the average count was recorded. Colonies on EMB that had a greenish metallic sheen were assumed to be *E. coli*, and they were gradually purified by streaking on the same medium before being recognized by their Gram staining properties and biochemical characterization (Abbas *et al*., 2013, Banik *et al.,* 2014, Agunbiade *et al.*, 2015). Other gram-negative coliform bacteria were determined by performing a panel of biochemical tests including oxidase, triple sugar iron agar (sugar fermentation, gas, H2S production, and gas), indole, urease, citrate utilization, lysine iron agar, methyl red/Voges Proskauer (MR/VP), and motility test.

Isolation of *Salmonella* species: *Salmonella* species pre-enrichment was carried out as previously described by study (Reta *et al*, 2016). One mL of milk was pre-enriched with 9 ml of buffered peptone water and incubated at 37 °C overnight. A portion of 0.1 ml was transferred to 10 mL of selenite-cysteine F-broth (Oxoid), and incubated for another 24 hours at 37OC. Finally, a 0.1 m1 sample from the selective enrichment was inoculated on *Salmonella-Shigella* Agar (Hi-Media, India) and cultured for 24 hours at 37^{OC} (Reta *et al.,* 2016).

Isolation of *Staphylococcus aureus:* was screened by surface spreading 0.1 ml from a 10-12 dilution on Mannitol Salt Agar (Biomark Laboratories, India). The plates were incubated at 37OC for 24–48 hours before being checked for bacterial growth (Worku *et al*., 2012, Malek *et al.,* 2015). The identification was based on colony characteristics, gram staining properties, and biochemical characterization.

Antimicrobial Susceptibility Testing (AST): AST of the targeted isolates were done by Kirby-Bauer's disk diffusion technique as recommended by the Clinical and Laboratory Standards Institute (CLSI). From a pure culture, 3-5 selected colonies of bacterial isolates were gently mixed with 4-5 ml of sterile normal saline in the tube to make a homogenous turbid suspension adjusted to a McFarland 0.5 standard. The bacterial suspension was uniformly spread on Mueller Hinton Agar (Oxoid) plates using a sterile cotton swab (CLSI, 2015). The following commonly used antibiotic discs were tested: ampicillin (10µg), amoxicillin clavulanic acid (20µg), erythromycin (15µg), cotrimoxazole (25µg), gentamicin (10µg), tetracycline (30µg), ciprofloxacin (5µg), chloramphenicol (30µg), cefoxitin (30µg), ceftriaxone (30µg) and clindamycin (2µg) (CLSI, 2015).).

Quality Control

The questionnaire was pretested, and training was given to data collectors. Each questionnaire was checked daily for completeness. *Escherichia coli* (American type culture collection (ATCC) 25922), *S. aureus* (ATCC 25923), and *Salmonella enterica serovar Typhimurium* (ATCC 13311) strains were obtained from the Oromia Public Health Research Capacity Building and Quality Assurance Laboratory to ensure the quality of culture media. Contamination of media and diluents was checked by incubating selected media and diluent inoculated plate at 37°C for 48 hrs.

Data Processing and Analysis

Data were initially checked for completeness, entered into Epi Info version 7 statistical software, and exported to Statistical Package for Social Sciences (SPSS) version 22 computer software for analysis. The associations between milk quality and various factors were predicted using bivariate and multivariable logistic regression. In the bivariate logistic regression, variables with a p-value of less than 0.25 were further analyzed in multivariable logistic regression. A p-value less than 0.05 at the 95% CI was considered to be statistically significant.

Ethical Consideration

Ethical clearance was obtained from the Institutional Health Research Ethics Review Committee (IHRERC)

of the College of Health and Medical Science, Haramaya University. After ethical clearance, a formal letter was written to the Oromia Regional Health Bureau (ORHB) notifying approval of the research proposal was obtained. After having permission from ORHB, the Adama town health office communicated a letter in local languages to the enrolled study cafes. Then data collection was commenced by explaining the objectives and procedures of the study to each study participant. Written and signed consent was obtained from respondents during the time of the interview. Information obtained during the data collection period were kept confidential.

Results

Descriptive characteristics of the study population

A total of 115 employees who are directly engaged in the processing of milk from the selected cafeterias were interviewed. The majority of them (55.7%) were women. Eighty-five-point two percent of the cafeteria's workers did not have awareness of zoonotic transmission of diseases by consuming raw milk, and 66.1% did not provide employees with routine health checks. All of them did not receive milk that was transported under the cold chain. The majority (91.3%) stored milk in plastic containers. In this study 100% of study participants used pipe water for cleaning of equipment and did not obtained training cafeteria services handling practice (Table 1).

Table 1: Cafeterias' milk handling practices and other assumed associated factors, Adama town, Ethiopia 2019 (n=115)

Variable	Categories	No (%)
Gender of interviewed	Men	51 (44.3)
	Women	64(55.7)
Type of storage equipment	Aluminum Vessel/bucket	10 (8.7)
	Plastic Vessel/bucket	39 (33.9)
	Plastic Jerry can	66 (57.4)
Transport milk to Cafeterias under cold chain	Yes	-
	No	115 (100)
Use of fridge in Cafeterias	Yes	84(73)
	No	31(27)
Equipment washing practices (How to wash equipment)	With boiled water and soap	30(26.1)
	With cold water and soap	67(58.3)
	With Cold water and soap and smoking	18(15.7)
Cafeterias that have regular health checkups for their workers	Yes	39(33.9)
	No	76(69.1)
Awareness of zoonotic transmission of some diseases by consuming raw milk	Yes	17 (14.8)
	No	98 (85.2)
Cafeterias that sold raw milk	Yes	41(35.7)
	No	4(64.3)

Bacteriological quality of raw cow milk

Total viable bacterial count (TVBC) and quality of raw cow milk

A total of 82 (71.3%) of the milk samples were analyzed as poor or unacceptable quality and had a high load of TVBC (3.98 x10^6). The overall mean ± standard error (SE) of TVBC was 6.60 Log10 CFU/ml ± 0.14 Log10 CFU/mL (3.98 x10^6 CFU/ml ± 1.39 CFU/mL) (95% CI, 6.31- 6.88 Log10 CFU/ml (3.73x10^6 – 4.2 x10^6 CFU/ml).

Total coliforms count and other bacterial isolates

Out of 115 raw milk samples, 67% were positive for coliforms, and the overall mean ± SE was 4.96±0.10 Log10 CFU/ml (9.1 x 104 ± 1.27 CFU/ml) (95% CI, 4.75–5.17 log10 CFU/ml (6.61 x 104 – 11.59 x 104 CFU/ml). *Escherichia coli* isolates constitute 7.8% (95% CI, 1.81–17.41%) of the total coliforms, and a number of other total coliforms were identified. *Staphylococcus aureus* was detected in 23.5% (95% CI: 8.31–38.69) of the samples, but no samples tested positive for *Salmonella* spp. (Table 2).

Table 2: Prevalence of coliforms isolated from raw cow's milk collected from cafeterias in Adama town, Ethiopia 2019

Bacterial isolates	No. (%)
Klebsiella pneumoniae	25 (32.4)
Staphylococcus aureus	27 (23.5)
Enterobacter cloacae	13 (16.9)
Citrobacter freundii	13 (16.9)
Pantoea agglomerans	11 (14.3)
Klebsiella oxytoca	8 (10.4)
Escherichia coli	6 (7.8)
Enterobacter aerogens	1 (1.3)

Factors contributing to the quality of raw milk

In this study, none of the possible studied associated factors (equipment used for milk storage, source of milk, use of refrigerator for storage of milk, raw milk sold in cafés, methods of cleaning milk containers, awareness of zoonotic transmission of diseases from raw milk, regular health checkups of cafeterias' workers, physical sanitary of the working environment (cafeteria's room (table, floor, roof, wall)) were associated with poor quality of milk ($p > 0.05$) (Table 3).

Antibiotic susceptibility pattern of targeted bacterial isolates

All *E. coli* isolates (n =6) were resistant to ampicillin but sensitive to all other antibiotics tested. All the isolates of *S. aureus* (n =27) were sensitive to ciprofloxacin, while 15 isolates were resistant to ampicillin (Table 4).

Table 3: Factors associated with total bacterial counts in CFU/mL of cow milk in Adama town, 2019 (n=115)

Associated Factors	Catagories	Poor	Good	COR (95% *CI*)	AOR (95%*CI*)	*P* value
Type of Equipment used for milk storage	Metallic Vessel	5(50.0)	5(50)	1	1	
	Plastic Vessel	30(76.9)	9(23.1)	0.300 (0.07, 1.27)	0.25 (0.05,1.15)	0.07
	Jerry can	47(71.2)	19(28.8)	0.40 (0.11, 1.56)	0.31 (0.07, 1.34)	0.12
Source of Milk	Center of Collection & Distribution	21 (65.6)	11(34.4)	1	1	
	Direct From Producers	61 (73.5)	22(26.5)	0.689 (0.29, 1.66)	0.79 (0.32, 1.99)	0.62
Storage of Milk in fridge before selling	Yes	59(70.2)	25(29.8)	1	1	
	No	23(74.2)	8(25.2)	0.821(0.32, 2.02)	0.673 (0.24, 1.88)	0.45
Methods of cleaning milk containers	Boiled Water + Soap	21(70)	9(30)	1	1	
	Water +Soap	47(71.2)	19(28.8)	0.993 (0.39, 2.54)	0.92 (0.34, 2.52)	0.87
	Water + Soap +Smoking	14(73.7)	5(26.3)	0.667 (0.17, 2.59)	0.56 (0.13, 2.46)	0.44
Awareness of zoonotic transmission of diseases from raw milk	Yes	12(63.2)	7(36.8)	1	1	
	No	70 (72.9)	26(27.1)	0.637 (0.23,1.79)	0.535 (0.18, 1.61)	0.27
Regular health checkup of Cafeterias' Workers	Yes	31(79.5)	8(20.5)	1	1	
	No	51(67.1)	76(32.9)	1.900 (0.76,4.73)	2.157 (0.83, 5.63)	0.116
Physical sanitary of working environment	Looks clean	59(71.9)	23(28.1)	1	1	
	Looks dirty	23(69.7)	10(30.3)	1.115 (0.46, 2.70)	1.094 (0.42,2.84)	0.853
Total		**82(71.3)**	**33(28.7)**			

Table 4: Antibiotic susceptibility pattern of *E. coli* and *S. aureus* isolates from raw milk samples collected from Adama town cafeterias, 2019

Antibiotics	*E. coli* (n=6)			*S. aureus* (n=27)		
	S (%)	I (%)	R (%)	S No (%)	I (%)	R (%)
Ampicillin	-	-	100	12 (44.4)	-	15(55.6)
Amoxicillin-Clavulanic acid	100	-	-	ND	ND	ND
Ceftriaxone	100	-	-	ND	ND	ND
Gentamicin	100	-	-	22(81.5)	-	5(18.5)
Cotrimoxazole	100	-	-	26(96.3)	-	1 (3.7)
Ciprofloxacin	100	-	-	27(100)	-	-
Tetracycline	100	-	-	14(51.9)	-	13(48.1)
Chloramphenicol	100	-	-	23(85.2)	-	4(14.8)
Cefoxitin	100	-	-	23(85.2)	-	4(14.8)
Erythromycin	ND	ND	ND	21(77.8)	-	6(22.2)
Clindamycin	ND	ND	ND	18 (66.7)	-	9(33.3)

ND: not done

Multi-drug resistance pattern of *S. aureus*

Most (66.67%) *S. aureus* isolates were resistant to two or more antibiotics. The overall MDR rate of the *S. aureus* isolates was 25.9% (95% CI: 24.43-27.57%) (Table 5).

Eleven S. aureus strains were resistant to two different antibiotics and seven of those isolates showed resistance to more than two commonly used antibiotics.

Table 5: Multiple drug resistance pattern of *S. aureus* isolates from raw milk samples collected from cafeterias in Adama town, 2019 Concentration of Mercury in Water Bodies

Antibiotics combination	**No (%)**
Ampicillin and Tetracycline	7 (25.9)
Ampicillin and Chloramphenicol	1 (3.7)
Clindamycin and Erythromycin	2 (7.4)
Clindamycin and Tetracycline	1(3.7)
Ampicillin, Chloramphenicol and Tetracycline	1(3.7)
Ampicillin, Gentamycin and Clindamycin	1(3.7)
Ampicillin, Clindamycin and Tetracycline	1(3.7)
Cefoxitin, Gentamycin and Clindamycin	1(3.7)
Ampicillin, Cefoxitin, Gentamycin and Tetracycline	1(3.7)
Ampicillin, Cefoxitin, Chloramphenicol, Clindamycin, Erythromycin,	2 (7.4)
MDR	7(25.9)
Total	18(66.7)

MDR; Multi Drug Resistance

Discussion

The mean total bacterial count (TBC) among the sampled raw milk was 6.60 Log10 CFU/ml ± 0.144 Log10 CFU/ml (3.98 x10^6 ± 1.39 CFU/ml) (95% CI, 6.31-6.88 Log10 CFU/ml (3.73x10^6 – 4.2 x10^6 CFU/ml), which exceeds the minimal quality limits of raw milk set by the Ethiopian Standards Agency (ESA) (ESA, 2009), of 2 x10^6. The current result was higher than the mean total viable bacterial count (TVBC) found in Ethiopia (mean TVBC of 1.07 x 10^6 CFU/ml) (Solomon *et al*., 2014) and Iran (mean TBC of 1.03 x 10^6 ±2.9 x 103 CFU/ml) (Fadaei, 2014). However, the current report is lower than the values reported in various regions of Ethiopia: (7.58 Log10 CFU/ml) (Asaminew and Eyassu, 2011), (9.82 Log10 CFU/ml) (Bereda *et al*., 2012), (7.64 Log10 CFU/mL) (Worku *et al*., 2012) (1.1x108 CFU/ml) (Dehinenet *et al*., 2013), (7.07 Log10 CFU/mL) (Simenew *et al.,* 2013), (7.13 Log 10 CFU/mL) (Gemechu *et al.,* 2014), (8.6 Log10 CFU/ml) (Bruktawit, 2016), and (8.2 Log10 CFU/ml) (Alganesh Tola and Gemechu, 2016); Bangladesh (1.3×107 to 5.2×10^8 CFU/ml) (Banik *et al.,* 2014), Egypt (Cairo and Giza) (8.8×10^7±1.5×10^7 and 4.4×10^8 ± 1.7×10^8 CFU/ml) , respectively (Hassan *et al.,* 2015). These differences may be attributed to variations in the study area, sample size, knowledge of foodborne illness transmission, use of milking utensils, cleanliness of the milking area or person milking, milk handler contamination, or the cow's udder, and transportation of milk from the harvesting area to the market.

Coliforms are usually found in fecal matter or the environment. Therefore, the presence of total coliforms or specific pathogens in a food item typically means poor hygienic practices at some point during food production (Healy *et al*., 2014, Fadaei, 2014). In this study, the average ± standard error for total coliform count (TCC was 4.96±0.10 Log10 CFU/mL (9.1 x 104 ± 1.27 CFU/mL) (95% CI, 4.75–5.17 log10 CFU/mL (6.61 x 104 – 11.59 x 104 CFU/mL). This result is similar to a published study from Ethiopia that reported a TCC of 5.0 Log10 CFU/mL (Gemechu *et al.,* 2014). However, it is higher than previous studies conducted in Ethiopia, which reported TCC of 4.49 ± 0.11 Log10 CFU/ml (Asaminew and Eyassu, 2011), 4.03 Log10 CFU/ml (Bereda *et al*., 2012), 3.0x10^4 CFU/mL (Dehinenet *et al*., 2013), and 1.82 Log10 CFU/mL (Simenew et al., 2013). A high coliform count in raw milk (>100 CFU/mL) indicates poor production practices, including inadequate pre-milking hygiene, the use of dirty equipment, and, in some cases, coliform mastitis (Healy *et al*., 2014). On the other hand, the current finding was lower than previous findings in different regions of Ethiopia, which reported TCC of 7.33 Log10 CFU/ml (Worku *et al*., 2012), 6.1 ± 0.92 Log10 CFU/ml (Bruktawit, 2016), 8.58 Log10 CFU/mL (Alganesh Tola and Gemechu, 2016), and in Iran, where a count of 1.4 x 105 ± 2.2 x 103 CFU/ml was reported (Fadaei, 2014). The variation in findings may be due to differences in hygienic practices during the milking process, storage, and transportation.

The current study found that 35.7% of cafeterias sold raw milk directly to consumers. The increasing popularity of raw milk can be attributed to customers' perceived nutritional benefits. However, it is essential to recognize that this practice also raises the risk of consuming milk and dairy products contaminated with harmful pathogens. Healy *et al*. (2014) stated that, the total coliform count should be below 10 CFU/ml for raw milk to be considered safe for consumption. Unfortunately, none of the milk samples tested met this quality standard, indicating that they are unsafe to consume in their raw form.

Enterobacteriaceae is a family of bacteria commonly found in nature and the normal gut bacteria of humans and animals. Some members of this family, such as Escherichia, *Klebsiella, Enterobacter, Citrobacter, and Pantoea agglomeran*s, can cause various infections (Alves *et al.,* 2015). These bacteria have been detected in different amounts in raw milk samples, which is a significant concern for the general population and individuals with weakened immune systems.

Escherichia coli is indicates the possible presence of *entero*-pathogenic and toxigenic microorganisms, which could constitute a public health hazard (Bouazza *et al.,* 2012). In this study, it was isolated at a lower percentage (7.8%; 95% CI: 1.81–17.41%), which is consistent with a report from Hawassa town, Ethiopia (8.2%) (Solomon *et al*., 2014). It was lower than previous studies conducted in Ethiopia (20%) (Merhawit *et al*., 2014), Jigjiga, Ethiopia (58%) (Reta et al., 2016), Tanzania (37.3%) (Lubote *et al*., 2014), Egypt (33%) isolated from Cairo and 38% from Giza (Hassan *et al*., 2015). The low isolation observed in this study might be due to different methods employed to isolate *E.coli* or the better handling practices in the study area during production and storage compared to the rest.

Staphylococcus aureus is a common bacterium that can be found in various environments. It is a significant cause of bovine mastitis, which can contaminate raw milk and its products (Jahan *et al*.; 2015, Rola *et al*., 2016). In the current study, 23.5% (95% CI: 8.31–38.69) of the samples examined were found to be contaminated with *S. aureus*. This finding is consistent with previous studies conducted in Ethiopia, which reported contamination rates of 16.2%, 20%, and 24.2%, respectively (Abebe *et al*., 2013; Merhawit *et al*., 2014; Reta *et al.,* 2016). However, it is lower than the rates reported in India (68%) (Khan *et al*., 2014) and Egypt (100%) (Hassan *et al.,* 2015).This difference could be attributed to variations in the isolation methods used or handling practices during production and storage in the study area compared to other regions.

Staphylococcal food poisoning is a prevalent foodborne illness that occurs globally. It is caused by the consumption of foods that are contaminated with staphylococcal enterotoxins, primarily produced by *S. aureus*. This disease can be particularly severe and potentially fatal for infants, the elderly, and individuals with weakened immune systems (Rola *et al.,* 2016).

There was no significant association between any of the factors mentioned (such as equipment for milk storage, source of milk, raw milk sold in the café, storage of milk in the fridge before selling, methods of cleaning containers, awareness of zoonotic transmission diseases from raw milk, regular health checkups of café workers, and the sanitation of the working environment) and poor-quality milk ($p > 0.05$). The high count of contaminants could be attributed to initial contamination during production and transportation to cafeterias or based response of study participants. The survey results showed that all the examined cafeterias received milk transported at ambient temperature. The lack of a cooling system and poor handling practices during transportation could lead to increased microbial contaminants in the milk (Gemechu *et al*., 2014).

The study found that 91% (n = 105) of cafeterias received milk from the farm in plastic containers. However, using narrow-mouthed plastic containers can increase contamination levels as they are difficult to clean properly. Moreover, non-food-grade plastic containers present a challenge for cleaning and disinfection, making them a significant source of milk contamination and, consequently, poor quality (Omore *et al*., 2005; Kurwijila, 2006). In addition, none of the respondents surveyed had received formal training on cafeteria handling practices. Furthermore, the majority (85.2%) were unaware of the risks of consuming poor quality milk. This lack of knowledge likely contributed to the careless handling practices of workers, resulting in a high bacterial count and lower-quality milk (Kurwijila, 2006).

All *E. coli* isolates displayed resistance to ampicillin but were susceptible to amoxicillin-clavulanic acid, ceftriaxone, gentamicin, cotrimoxazole, ciprofloxacin, tetracycline, chloramphenicol, and cefoxitin, with a 100% susceptibility rate. Previous studies reported a high resistance rate to ampicillin in India (98%) (Khan *et al*., 2014) and Bangladesh (64.8 %) (Marjan *et al.,* 2014). In contrast, one study from Ethiopia found a low resistance rate (30%) (Reta *et al*., 2016). A study conducted in India found higher resistance rates for chloramphenicol, ceftriaxone, cotrimoxazole, tetracycline, and gentamicin (95%, 95%, 95%, 92%, and 80%, respectively) (Khan *et al*., 2014). In Bangladesh, a resistance rate of 64.6% was observed for ciprofloxacin (Marjan *et al*., 2014), while Ethiopia showed a resistance rate of 33.4% for tetracycline (Reta *et al*., 2016). The varying resistance patterns against antibiotics in different study areas may be attributed to prolonged and indiscriminate usage of these antimicrobials. In our study area, all antibiotics, except ampicillin, can be potentially used in veterinary services and human medicine to treat *E. coli* infection.

All *S. aureus* exhibited increasing resistance to all tested antimicrobials except for ciprofloxacin and, to a lesser extent, cotrimoxazole. However, they displayed alarming levels of resistance to ampicillin (55.6%), tetracycline (44.4%), and clindamycin (33.3%). High rates of tetracycline resistance were reported in Addis Ababa (73.2%) (Abebe *et al.,* 2013), Jigjiga city (69 %), (Reta *et al*., 2016), and Kombolcha town (77.4%) (Asmelash *et al.,* 2016).

In contrast to our study, others studies from Ethiopia and Bangladesh reported that high resistance rates to cefoxitin (42.7%) (Asmelash *et al.,* 2016) and to erythromycin (75%) respectively (Jahan *et al*., 2015). Similarly the Indian study indicate that high resistance to

ampicillin (98%) (Khan *et al*., 2014).The combination of ampicillin and tetracycline was the most commonly resistant antibiotic, accounting for approximately 26% of all resistance cases observed in this study.

The study area showed a shocking level of resistance of *S. aureus* to commonly used antimicrobials, such as ampicillin and tetracycline. The high rates of *S. aureus* resistance found in various studies indicate this is a widespread problem. However, the overall resistance of *S. aureus* isolates to gentamycin, chloramphenicol, cefoxitin, and erythromycin was less than 25%, which aligns with a study conducted in Jigjiga, Ethiopia (Reta *et al.,* 2016). These antimicrobials were less resistant because they are not frequently used in the study area in veterinary services or human medicine.

This study, found that 26% (95% CI: 24.43-27.57%) of *S. aureus* isolates were resistant to three or more antibiotics, indicating the presence of multi-drug resistance (MDR). The most common form of MDR observed was the combination of ampicillin and tetracycline, along with one or more additional antibiotics. This combination accounted for seven instances of MDR among all reported cases. A similar prevalence of 25% was reported in a study conducted in Bangladesh (Jahan *et al*., 2015). On the other hand, our study found a lower prevalence than previously reported rates in different regions of Ethiopia: 47.6%. The present study showed a lower finding compared to 47.6%, as reported from various parts of Ethiopia by (Abebe *et al.* 2013), 82.8% (Asmelash *et al*. 2016), and 45.3% (Reta *et al*. 2016). The significant level of resistance observed across multiple drugs is a major public health concern as it poses challenges in treating foodborne outbreaks. Generally, the different resistance patterns to antibiotics reported from various study areas could be attributed to indiscriminate and repeated antibiotic usage in both animal and human treatment (Abebe *et al.* 2013; Reta *et al.* (2016)).

Strengths and Limitations of the Study

This study's cross-sectional design focused solely on point prevalence, which means it could not address periodic variations. Furthermore, the study only examined the counts of mesophilic bacteria. Other types of bacteria, such as psychrotrophic, thermoduric, and lactic acid, were not included in the investigation because they require specific mediums or incubation conditions. Similarly, pathogenic bacteria like *Campylobacter spp., Listeria spp.,* and *Mycobacterium bovis*, known to contaminate milk, were not isolated due to the need for specific media or biochemical/serological identification and testing. Additionally, AST was not conducted for all *Enterobacteriaceae* isolates due to the limited availability of sensitivity test drugs.

Conclusion

The study showed that over 70% of the analyzed milk samples had significant bacterial contamination. All *E. coli* isolates were found to be resistant to ampicillin but susceptible to other tested antibiotics. Staph*ylococcus aureus* bacteria were susceptible to ciprofloxacin and cotrimoxazole but had a high resistance rate to ampicillin and tetracycline. Collaborative efforts between agricultural and health agencies are crucial to ensure a safe and high-quality milk supply. Adequate sanitary measures, hygienic practices, strict monitoring, and quality control measures should be in place to ensure the delivery of safe and quality milk to consumers. Raising awareness about proper milk handling practices in the farm and cafeterias is also essential. Cefoxitin, cotrimoxazole, chloramphenicol, and Ciprofloxacin can be used empirically to treat *E. coli* and *S. aureus* infections in veterinary. Further studies are needed to investigate milk quality throughout the value chain, including contamination and antibiotic susceptibility testing for psychrotrophic, thermoduric, and lactic acid bacteria, as well as other pathogenic bacteria transmitted to humans through milk consumption.

Competing Interests

The authors declare that they have no competing interests.

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Authors' Contributions

All authors made a significant contribution to the work reported, whether that is 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

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List of Abbreviations

APC; Aerobic Plate Count, AST; Antimicrobial Susceptibility Test, ATCC; American Type Culture Control, CDC; Centers for Disease Control and Prevention, CFU/mL; Colony Forming Units per milliliter, CLSI Clinical and Laboratory Standard Institute,CSA; Central Statistical Agency ,EAS; East African Standard ,EMBA; Eosin Methylene Blue Agar, MDR; Multi Drug Resistance,MSA; Mannitol Salt Agar, OPHRC-BQAL; Oromia Public Health Research Capacity Building and Quality Assurance Laboratory , SPC; Standard Plate Count, Spp. Species, SSA; Salmonella-Shigella Agar,TCC; Total Coliform Count ,TPC Total Plate Count,TVBC; TBC Total Viable Bacterial Counts, WHO; World Health Organization

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