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Research Article

Isolation and Antibiogram of *Escherichia coli* Isolated from Selected Dairy Farm at Sebeta, Oromia, Ethiopia

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Abstract

Mastitis is the most prevalent disease of dairy animals, imparting huge economic losses to the dairy industry. *Escherichia coli (E. coli)* bacteria are among the common causes of mastitis in dairy animals. A cross-sectional study was carried out from March 2021 to August 2021 on lactating Cattle suffering from mastitis cases to isolate *Escherichia coli* and to assess their antimicrobial susceptibility pattern in Sebeta town, Oromia Special Zones, Ethiopia. Prevalence of mastitis by California Mastitis Test at cow level was found to be 87.14% (122/140), out of which 12.85% (18/140) and 74.28% (104/140) were clinical and subclinical, respectively. The study revealed that out of the 560 quarters examined, 24 (4.2%) of them were blind and 536 were functional. The *Escherichia coli* isolated from CMT positive was 8.07% (31/384); from this clinically and sub clinically affected udder were 1.04% (4/384) and 7.03% (27/384) respectively. Finally, the antimicrobial profiles of the 31 *E. coli* confirmed isolates were assessed using 10 different antimicrobials. Out of the 31 isolates tested, 7 (22.58%) isolates were found to be highly resistant to Ampicillin, 5 (16.12%) isolates were resistant to Trimethoprim+Sulphamethoxazole, and 3 (9.67%) and 4(12.9%) isolates were developed resistant to Amoxicillin+Cluvanate acid and Tetracycline respectively. However, all thirty-one isolates of *Escherichia coli* were found to be highly susceptible to five antimicrobials namely Ciprofloxacin (100%), Cefoxitin (100%), Meropenem (100%), Cefotaxime (100%) and Gentamycin (100%). In conclusion, this study determined the importance of *Escherichia coli* as one of mastitis causing bacterium of the dairy industry and investigated its antimicrobial resistance pattern. Thus, researchers would like to emphasize the need for urgent intervention to control the diseases and prevent the associated loss.

Keywords; Antimicrobial resistance; CMT; Sebeta; Mastitis; *Escherichia coli*

Introduction

Mastitis is an inflammation of the mammary glands. It causes a great loss or reduction in animal productivity than any disease of dairy cattle. It is the costliest disease and remains a series problem for dairy industry for its influences both on the quantity and quality of milk produced it causes a marked fall in milk yield and is a cause of culling of animals [1].

It is the disease that cause burden in dairy livestock all over the world, the persistency of the microbes leads to invasion of tissue that can opportunistically results in mastitis. Due to invasion and bacterial infection mammary glands release white blood cells leading to secretion of toxins in response to immune response that trigger mastitis infection in bovine. There are several bacteria involved in causing mastitis however most infections are caused by Gram-negative rods like *Escherichia coli* or Gram-positive cocci mainly *Staphylococcus* and *streptococci* depending on the mode of transmission and host condition [2].

Escherichia coli are a bacterium commonly found in gastrointestinal tract of animals and humans (Welch 2006). Most of the *Escherichia coli* are non-pathogenic or harmless [3] but some of them can be pathogenic if they are opportunistic [4] and commonly affect those individuals of immune-challenged. Pathogenic *Escherichia coli* are usually the types that can cause diarrhea when transmitted through contaminated water and foods [5].

E. coli frequently contaminates food organism and it is a good indicator of fecal pollution [6]. Presence of *E. coli* in milk products indicates the presence of enteropathogenic 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 [7]. In Ethiopia, the consumption of raw milk is very traditional and contaminated milk and milk products are the most common transmission pathway of *E. coli* from animals to humans. Even though the disease caused by *E. coli* is very important in the country and of great public health concern, *E. coli* has received very little consideration in many of the previous public health studies. Most of the previous studies circulated in limited areas and fail to represent the incidence of *E. coli* under different management and ecological situations.

The antibiotics are routinely practiced as therapeutic measures of this malady but indiscreet use of antibiotics develops resistance in animal body which lowers cure rate. That all can lead to low therapy success and increased risk to human health due to uncontrolled

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use of antibiotics [8]. The development of resistance of the bacteria to antimicrobial agents makes mastitis more difficult to control. Antimicrobial resistance has become a huge public health issue worldwide [9]. Moreover, in the country both veterinary and medical drugs are often misused, creating ideal conditions for t he development of resistant strains, thus better understanding of the antimicrobial susceptibility/resistance/patterns of pathogens isolated from animal source foods like milk is needed. There was no recent study with regard to the prevalence of mastitis, isolation of *E. coli* and antibiogram pattern of the isolates among dairy cattle at Sebeta town. Therefore, this study was conducted with the following objectives

- To isolate and determine antibiogram profile of *E. coli* isolates from selected dairy farms in the study area.

Materials and Methods

Study Area

The study was conducted in Sebeta town. Sebeta is an urban set up located about 26 kilometers southwestern of Addis Ababa (Figure 1). Its geographical location is 08°9200 North and 38°6200 east. The mean annual rainfall and temperature of the town are 1073 milliliters and 17.40°C, respectively. The altitude ranges from 2356-2405 m above sea level. The conurbation was purposely selected mainly due to presence of many commercial and semi-commercial farms.

Study Design

A cross sectional study was conducted to generate the desired data from March 2021 to August 2021.

Sample Size Determination

The required sample size of this study was determined by the formula given by [10] based on the 4.3% mean expected previous prevalence [11]; 95% confidence interval and 5% desired precision. The sample size was calculated as follows.

$$n = \frac{1.96^2 * P_{exp}(1 - P_{exp})}{d^2}$$

Where, N: required sample size; P_{exp}: expected prevalence, and d^2; desired absolute precision of 0.05. Therefore, the calculated sample size was 63, but to increase the precision of the study and to increase the number of *E.coli* isolates, from a total of 140 dairy cows, 384 milk samples was collected from dairy farms in Sebeta Town.

Study Population

The study was carried out on 140 lactating exotic, local and crossbreed dairy cows kept under intensive, semi-intensive and extensive farming system in the study area. Nineteen dairy farms located in Sebeta Town were included in the study. Then simple random sampling was employed to select individual cows. The study involved physical examination of sampled cows and collection of milk samples following standard procedures. The samples from each functional udder of cows were screened for sub-clinical mastitis using the California Mastitis Test (CMT).

Milk Sample Collection and Transportation

During milk sample collection, cows were restrained in standing position and sampling began with teat cleaning by scrubbing thoroughly using cotton balls moistened with 70% alcohol. Milk samples were collected first from the closest teats followed by those at the far side of the udder by maintaining universal bottle at approximately 45° angles. The fore strip milk was discarded and 10-15 ml of midstream milk sample was taken. The universal bottles were labeled for information such as date of collection, name of farm and cow identification number [12]. Milk Samples were transported using cold chain (Ice box) from dairy farms to Animal Health Institute (AHI) located in Sebeta Town.

Sample Storage and Processing

Most mastitis causing microorganisms survive under refrigeration for several days or freezing for several weeks. The milk samples were stored in the laboratory at +4°C until laboratory examination is conducted. CMT positive milk samples were inoculated on media and incubated at 37°C for 24–48 hours.

Bacterial Isolation and Identification

Bacterial isolation and identification were conducted using the guideline prescribed by [13]. Since the primary and main step of any bacteriological isolation starts with preparation of media, the study started by preparing different selective, differential and indicator media that were needed for the research. Brain Heart Infusion Broth, Brain Heart Infusion agar, MacConkey agar, Eosin Methylene Blue (EMB) agar, Nutrient agar, and Muller-Hinton agar media were prepared whenever they were needed to do colony characterization and to perform further biochemical, confirmatory and susceptibility tests.Secondary biochemical test of *E. coli* isolates was performed using gram staining, IMViC and Triple Sugar Iron (TSI) slant agar test.

MALDI-TOF Biotyper Identification System

Final confirmation was done by MALDI-TOF, according to the manufacturer's guidelines [14]. A single young colony of sub cultured bacteria was directly deposited on a MALDI-TOF plate (Bruker Daltonik GmbH, Karlsruhe, Germany). At the end of sample 0.5 µl BTS were added to the plate. The sample was mixed with 0.5 µl of matrix solution and placed on the steel surface of the target plate to dry. The matrix solution (cinnamic acid or a benzoic acid derivate) co-crystallizes with the sample on the target plate. The loaded target plate is inserted into the machine where it was then transported to the measuring chamber. Within the mass spectrometer, a high vacuum has to be continuously maintained. However, upon insertion of the loaded target plate, air is introduced into the system and the vacuum must be reestablished before sample analysis can be performed. Once a sufficient vacuum has been created, the individual samples were exposed to short laser pulses. The laser's energy vaporizes the microorganism together with the matrix, leading to ionization of the (ribosomal) proteins. An electromagnetic field, created by a potential of about 20 kV, accelerates the ions before they enter the flight tube. The Time of Flight (TOF) of the analytes to reach the detector at the end of the flight tube was precisely measured. The degree of ionization as well as the mass of the proteins determines their individual TOF. Based on this TOF information, a characteristic spectrum was recorded and constitutes a specific sample fingerprint, which was unique for a given species. This virtual gel view represented all of the peaks in a spectral file and was used to compare the spectra of the *E. coli* isolates that were tested. Clusters with similar protein expression were identified by the Principal Component Analysis (PCA) [15]. Overall, thirty-one isolates were confirmed as *Escherichiacoli* by

MALDI-TOF tests and were preserved in a brain heart infusion broth with glycerol broth to conduct antimicrobial susceptibility tests.

Antimicrobial Susceptibility Test

Disc Diffusion Susceptibility Test: Antimicrobial Susceptibility test of the isolates *Escherichia coli* was analyzed for ten different antimicrobials namely Amoxicillin and Cluvanate acid (30µg), Tetracycline (30µg), Meropenem (10µg), Ciprofloxacin (10µg), Ceftriaxone (30µg), Cefoxitin (30µg), Sulphamethoxazole+Trimethoprim (25µg), Ampicillin (10µg), Cefotaxime (30µg) and Gentamycin (10µg). The antimicrobials were selected based on their availability at the laboratories.

The test was carried out by using the agar disc diffusion method, first 5ml of 0.85% saline water was dispensed in a test tube labeled for 31 isolates and the colonies from a brain heart infusion agar media was then taken by a disposable plastic loop and put in the saline water and each suspension with the organism was mixed well and measured for a turbidity of 0.5 McFarland standards and cultured thoroughly on Muller- Hinton Agar (MHA) media of 4mm depth and 90mm diameter. After that the ten different antimicrobial discs listed above were taken from their corresponding containers by using forceps and diffused in the respective MHA Media of the thirty-one isolates and incubated at 37^0C for 18 hours. After incubation, measurement of the diameter of the clear Zones of Inhibition (ZOIs) around and including the antimicrobial discs of each isolate were conducted and interpreted to categorize as susceptible, intermediate and resistant according to the performance standards given by Clinical and Laboratory Standards Institute [16].

Data Analysis

Descriptive statistics such as frequency, percentage, and/or proportion were used for prevalence, antimicrobial resistance test. Chi-squire test (χ^2) was used to assess significant differences by Statistical Package for Social Science (SPSS) of version 20 software. The results with less than P-value of 0.05 were considered statistically significant.

Results

California Mastitis Test

The prevalence of mastitis in this study was found to be 87.14% (122/140) using CMT. From this 12.85% (18/140) were clinical and 74.28% (104/140) were subclinical cases. Out of the 560 quarters 24 of them were blind (4.2%) and 536 were functional. From a total of 536 quarters, 33 of them were showing clinical and 503 were subclinical forms of mastitis. Hence the overall prevalence of mastitis in our study was confirmed to be 384. From a total of 536 quarters tested for mastitis by CMT Test, 6.15% (33/536) were clinical and 65.48% (351/536) subclinical (Table 1).

The above table indicates that overall prevalence at quarter level found to be 73% in selected dairy farm in Sebeta town.

Isolation and Identification of E. coli

The results of the present study revealed that out of 384 CMT positive milk samples, 31 samples were found to be positive for *E. coli*. Isolates were characterized as bright pink color on MacConkey agar plates and showed blue-greenish metallic sheen on EMB agar plate. Upon Gram's staining of the isolates under 100x using light

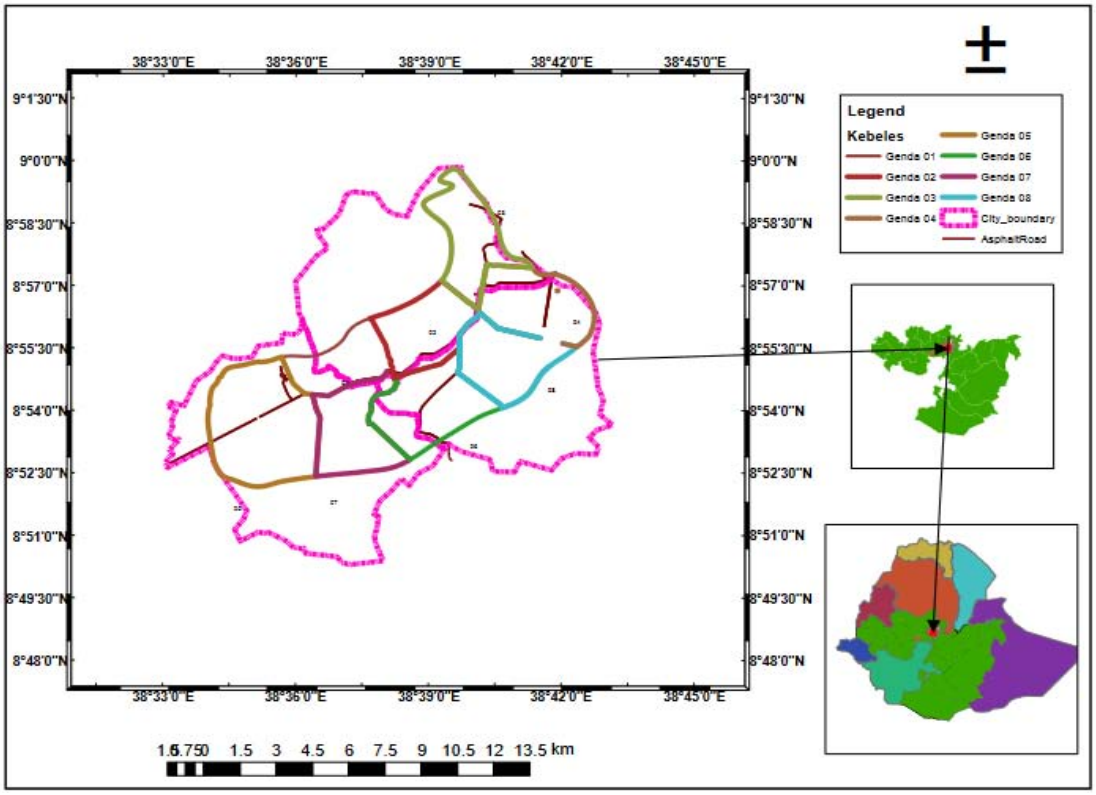

Figure 1: Map of the Study Area.

Table 1: Prevalence of mastitis at quarter level in selected dairy farms of Sebeta Town.

Farm	Blind quarter	Negative quarter	Positive quarter	Total Examined	Prevalence %
1	1	8	19	28	71.42%
2	0	12	16	28	57.14%
3	3	17	28	48	64.58%
4	1	15	40	56	73.21%
5	4	16	48	68	76.47%
6	0	12	20	32	62.50%
7	0	8	16	24	66.66%
8	3	13	24	40	67.50%
9	1	11	8	20	45.00%
10	2	6	16	24	75.00%
11	2	2	24	28	92.85%
12	0	4	28	32	87.50%
13	0	0	12	12	100%
14	1	3	16	20	85.00%
15	2	10	20	32	68.75%
16	2	6	12	20	70.00%
17	0	2	18	20	90.00%
18	1	5	10	16	68.75%
19	1	2	9	12	83.33%
Total	24	152	384	560	73%

Table 2: A table shows the similarity of Organism best match and Organism second best match.

Sample Name	Sample ID	Organism (best match)	Score Value	Organism (second-best match)	Score Value
A1 (+++) (A)	19214 (Standard)	*Escherichia coli*	2.29	*Escherichia coli*	2.29
A2 (+++) (A)	17856 (Standard)	*Escherichia coli*	2.38	*Escherichia coli*	2.33
A3 (+++) (A)	17867 (Standard)	*Escherichia coli*	2.18	*Escherichia coli*	2.15
A4 (+++) (A)	19188 (Standard)	*Escherichia coli*	2.38	*Escherichia coli*	2.37
A5 (+++) (A)	19184 (Standard)	*Escherichia coli*	2.40	*Escherichia coli*	2.37
A6 (+++) (A)	19200 (Standard)	*Escherichia coli*	2.41	*Escherichia coli*	2.31
A7 (+++) (A)	19191 (Standard)	*Escherichia coli*	2.33	Escherichvia coli	2.30

Result overview table--continued on next page

microscope, pink-colored, small rod-shaped organisms arranged in single, pairs or short-chain were identified. The biochemical characteristics of 31 *E. coli* isolate showed positive for catalase, Methyl red and Indole test but negative for Voges-Proskauer, Urease, and Citrate. In addition, reactions in TSI agar slant revealed yellow but with gas and production of hydrogen sulfide was observed. Almost all the isolates of *E. coli* fermented lactose, sucrose and glucose with the production of both acid and gas. Based on the results from the biochemical test further identification was done MALDI-TOF to confirm the bacteria.

MALDI -TOF Identification

The 31 isolates that were identified as *E.coli* by biochemical test were again subjected to MALDI-TOF Biotyper for confirmation. A total of 31 (8.07%) isolates were confirmed as *Escherichia coli* by MALDI-TOF identification system (Table 2 and 3). Out of these 1.04% clinical and 7.03% were subclinical. The *E. coli* isolated at cow level was 19.67% and out of this 12.12% were clinical and 7.55% were subclinical. All isolates were analyzed for 10 different antimicrobials to assess their antibiotic profiles and categorize them as susceptible, intermediate or resistant.

Antimicrobial Resistance Test

A total of 31 isolates were subjected to the 10 antibacterial agents for AMR test. The AMR test results showed that *E. coli* is 100% susceptible to six antimicrobial agents and showed different percentage of resistance to five antimicrobials (Table 4).

As shown in Table 3, all 31 isolates of *Escherichia coli* were found to be highly susceptible to five antimicrobials namely Ciprofloxacin (100%), Meropenem (100%), Ceftriaxone (100%), Cefotaxime (100%) and Gentamicin (100%). However, 7 isolates were also found to be highly resistant to Ampicillin (22.58%) followed by 5 isolates resistant to Trimethoprim+sulphamethoxazole (16.12%) and also 4, 3 and 2 isolates were found to be resistant to Tetracycline (12.9%),

Table 3: A table shows rank, matched pattern and score value of *E. coli*.

Samples tested	Rank (Quality)	Matched Pattern	Score Value	NCBI Identified
1	(+++)	*Escherichia coli* DSM 1578 DSM	2.29	562
2	(+++)	*Escherichia coli* DSM 682 DSM	2.38	562
3	(+++)	*Escherichia coli* DH5alpha BRL	2.18	562
4	(+++)	*Escherichia coli* RV412 A1 2010 06a LBK	2.38	562
5	(+++)	*Escherichia coli* BM11464 1 CHB	2.40	562
6	(+++)	*Escherichia coli* ATCC 25922 CHB	2.41	562
7	(+++)	*Escherichia coli* ESBL EA 1528T CHB	2.33	562
8	(+++)	*Escherichia coli* ATCC 35218 CHB	2.24	562
9	(+++)	*Escherichia coli* DSM 1103 QC DSM	2.29	562
10	(+++)	*Escherichia coli* ESBL 1528T CHB	2.35	562
11	(+++)	*Escherichia coli* ATCC 25922 THL	2.29	562
12	(+++)	*Escherichia coli* Nissl VML	2.36	562
13	(+++)	*Escherichia coli* ATCC 25922 THL	2.38	562
14	(+++)	*Escherichia coli* DH5alpha BRL	2.34	562
15	(+++)	*Escherichia coli*DH5alpha BRL	2.37	562
16	(+++)	*Escherichia coli* DSM 682 DSM	2.32	562
17	(+++)	*Escherichia coli* RV412 A1 2010 06a LBK	2.27	562
18	(+++)	*Escherichia coli* MB 11464 1	2.38	562
19	(+++)	*Escherichia coli*DSM 1576 DSM	2.31	562
20	(+++)	*Escherichia coli* RV412 A1 2010 06a LBK	2.28	562
21	(+++)	*Escherichia coli* DSM 1576 DSM	2.34	562
22	(+++)	*Escherichia coli* DH5alpha BRL	2.22	562
23	(+++)	*Escherichia coli* DH5alpha BRL	2.33	562
24	(+++)	*Escherichia coli* DH5alpha BRL	2.25	562
25	(+++)	*Escherichia coli* DSM 682 DSM	2.35	562
26	(+++)	*Escherichia coli* DSM 1576 DSM	2.33	562
27	(+++)	*Escherichia coli* DSM 1576 DSM	2.16	562
28	(+++)	*Escherichia coli* MB 11464 1	2.21	562
29	(+++)	*Escherichia coli* DSM 1576 DSM	2.23	562
30	(+++)	*Escherichia coli* RV412 A1 2010 06a LBK	2.32	562
31	(+++)	*Escherichia coli* DSM 1576 DSM	2.31	562

Amoxicillin+clavulanate acid (9.67%) and Cefoxitin 2(6.45%) respectively. The isolates were also indicating an intermediate resistance for Amoxicillin + clavulanate acid,Cefoxitin 1(3.2% for each) and Ampicillin 2(6.45%) (Table 4). Two isolates were resistance to more than two antibiotics.

Risk Analysis

In this study, the occurrence of *E. coli* was significantly associated with different parity numbers (χ^2=7.7706; P=0.021) (Table 5). The highest percentages of *E. coli* isolates were isolated from cows with eight and above parity number 2 (20%) and from poor body condition 8 (12.30%). On the other hand, the present finding revealed that the association between different groups of age, lactation stage, breed, body condition and quarter with the occurrence of *E. coli* organisms were not statistically significant (x^2=2.4077; P= 0.300; (x^2=2.4012; P= 0.301);x^2=0.7449; P= 0.689; χ^2=1.9610; P= 0.375; χ^2= 0.6859; P= 0.877 respectively (Table 5).

As the above table indicates, the occurrence of mastitis by *E. coli* were higher in poor body condition of animal (12.30%) when compared to those medium and very good body condition (6.49%) and (7.34%) respectively. Based on the parity of the animal, the highest percentages of *E. coli* isolates were isolated from cows with eight and above parity number (20%) when compared to those of parity number greater than or equal to three and less than or equal to seven (moderate) (11.11%)and less than three parity number (few) (4.32%). A high prevalence of *E. coli* was detected in right hind (9.57%) and left-fore quarter (8.24%) followed by left hind (8.16%) and right-fore quarter (6.31%).

Discussion

In our study the CMT test result showed the prevalence of

Table 4: Antibiogram Pattern of *E. coli* isolates to 10 different antimicrobials.

Antimicrobial agents	Potency	Interpretation of results (zone diameter in mm)		
	µg/disk	S (%)	I (%)	R (%)
Ciprofloxacin	5µg	31 (100%)	0 (0%)	0 (0%)
Cefotaxime	30µg	31 (100%)	0 (0%)	0 (0%)
Cefoxitin	30µg	28 (90.32%)	1 (3.2%)	2 (6.45%)
Amoxicillin + Cluvanate acid	30µg	27 (87.1%)	1 (3.2%)	3 (9.6%)
Trimethoprim + Sulphamethoxazole	25µg	26 (83.87%)	0 (0%)	5 (16.12%)
Meropenem	10µg	31(100%)	0 (0%)	0 (0%)
Gentamycin	10µg	31 (100%)	0 (0%)	0 (0%)
Ampicillin	10µg	22 (70.96%)	2 (6.45%)	7 (22.58%)
Tetracycline	30µg	27 (87.09%)	0 (0%)	4 (12.9%)
Ceftriaxone	30µg	31 (100%)	0 (0%)	0 (0%)

%=Percent, S=Sensitive, I=Intermediate, R=Resistant

Table 5: Prevalence of *E. coli* occurrence with host and environment related factors.

Variables	Categories	No. of samples examined	No. of positive (%)	Chi-square (χ2)	P-Value
Body condition	Good	242	18 (7.34%)	1.9610	0.375
	Medium	77	5 (6.49%)		
	Poor	65	8 (12.30%)		
Age	Young	27	2 (7.40%)	2.4077	0.300
	Adult	332	29 (8.73%)		
Breed	Exotic	325	26 (8%)	0.7449	0.689
	Cross	22	1(4.54%)		
	Local	37	4 (10.81%)		
Parity	Few	185	8 (4.32%)	7.7706	0.021
	Moderate	189	21 (11.11%)		
	Many	10	2 (20%)		
Quarter	RH	94	9 (9.57%)	0.6859	0.877
	RF	95	6 (6.31%)		
	LH	98	8 (8.16%)		
	LF	97	8 (8.24%)		
Lactation	Early	21	1 (4.76%)	2.4012	0.301
	Medium	276	26 (9.42%)		
	Late	87	4 (4.59%)		

Key: RH= Right Hind; RF= Right Front; LH= Left Hind and LF= Left Front

mastitis in to be 87.14% (122/140). Our finding was similar to [17] who observed 86.2% cases of mastitis through CMT screening of in dairy cows in the study conducted at Kampala, Uganda in 2014 but it was lower in comparison with finding of [18], and [19] who reported that the overall mastitis prevalence in the farm was 66.6% and 71.0% in Assella Dairy farm and Holeta town respectively. This finding was not in agreement with those of [20] and [21] who reported the prevalence of 52.78% in Ethiopia and 52% in Nigeria, respectively.

In the current study, a total of 384 raw milk samples taken from 560 quarters were examined bacteriologically and biochemical tests were performed to detect *Escherichia coli*. All *E. coli* isolates were able to produce bright pink-colored colonies on MacConkey agar, characteristic metallic sheen colonies on the EMB agar.

The overall *E. coli* isolated among mastitis positive cattle in this study 19.67% was in agreement with the finding of [22] who reported 18.6% prevalence of *E. coli* in the study conducted at Benchi Maji Zone, Southwest Ethiopia in 2015.Similarly, our result was in agreement with [23] who reported 20.9% prevalence of *E. coli* in raw milk in Iran in 2021. However, our finding was lower than the finding of [24] who reported 40.7% prevalence of *E.coli* among dairy cows in Pakistan in 2004. Our finding was higher than the previous reports of [25] at Holeta (4.6%) and [19] in and around Sebeta (0.75%). The prevalence of *E. coli* is probably due to the fact that *E. coli* is the commonest environmental contaminants which are closely associated with hygiene. It becomes pathogenic whenever the hygienic conditions of the animal or environment become poor. In addition, the existence of high concentration of *E. coli* in milk also indicates the relatively poor quality of milk, related with substandard hygiene of the farm management.

Out of the total *E.coli* isolates (19.67%) in this study, the specific occurrence of *E.coli* in clinical and sub clinical cases was known to be 12.12% and 7.55%respectively. This finding was in agreement with [26,27] who reported 11.5 and 3.64% prevalence of *E.coli* from clinical and sub clinical cases respectively. The current finding was lower when compared to previous reports of [28,29] who observed 34.9 and 17.04% respectively. *E. coli* is a poor contagious bacterium, and the highest prevalence of other pathogens of mastitis particularly *Staphylococcus*, *Streptococcus* and *Corynebacterium* lead to rise in somatic cell count of milk which limits the multiplication of the coliforms has also shown that *Pseudomonas* is intermittently shed from the udder and does not frequently appear in milk.

In our study from 384 fresh raw milk samples, 8.07% milk samples were known to be contaminated with *E. coli*. This was in agreement with the studies by [30-32] who reported 8.75%, 10.3% and 11.2% from Malaysia, Ghana and Hawassa in Southern Ethiopia respectively. The current finding was lower as compared to [33] who found 21% from India and [34] who reported quarter prevalence of 17.9%. The recent study was relatively higher as compared to the report by [35] who found 3.88% prevalence of *E. coli* from raw milk of cows in Iraq. The differences in the prevalence of *E. coli* in our study and other researchers could be attributed to variations in the study area, sample size, methodology we used and season.

In this study, the prevalence of clinical and subclinical mastitis in the study area was 1.04% and 7.03% respectively. This was in agreement with [36] who reported the prevalence of clinical and subclinical mastitis 8.3% and 1.8% respectively, lactating cows from smallholder dairy farms in Sellalle area, Central Ethiopia. The difference in the recent study from past studies might be attributed to differences in environmental conditions, management differences in the production system (intensive, semi-intensive, and extensive), ecology, hygienic practices and methodological differences among these studies.

In the present study, the occurrence of *E. coli* has been found significantly associated with different parity numbers of (x^2=7.7706; P=0.021) which is in agreement with reported by [37,20,38,39]. On the other hand, the present finding revealed that there was no association between different groups of age, lactation stage, breed and body condition with the occurrence of *E. coli* and our results agree with that of [32] in cows in Hawassa, who reported no association of the prevalence of mastitis with age, lactation stage, body condition and history of mastitis.

In this study the highest percentages of *E. coli* isolates were isolated from cows with eight and above parity number (20%) when compared to those of parity number greater than or equal to three and less than or equal to seven (moderate) (11.11%) and less than three parity number (few) (4.32%). This finding was in agreement with [40] who reported 31.5% from cows with age group from seven to ten years. 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 [40].

A high prevalence of *E. coli* was detected in right hind (9.57%) and left-fore quarter (8.24%) followed by left hind (8.16%) and right-fore quarter (6.31%). This finding is in agreement with that of [25] who reported a high prevalence in hind quarter, in contrast, [41,42] reported a high prevalence in four quarters. The high prevalence in hind quarters might presumably be associated with increased chance of hind quarters being soiled with urine and faces or by the tail leading to poor udder management [43].

A total of 31 *E. coli* isolates were tested against 10 antimicrobials based on CLSI guidelines and all *E. coli* isolates were found to be 100% susceptible to gentamicin Ciprofloxacin, Menoperem, Ceftriaxone and Cefotaxime followed by Tetracycline (87.1%), Amoxicillin + cluvanate acid (87.09%), sulphamethoxazole-trimethoprim (83.87%) and Ampicillin (70.96%). All isolated *E. coli* were found to be 22.58% resistant to Ampicillin followed by Trimethoprim + sulphamethoxazole (16.12%), Tetracycline (12.9%), Amoxicillin + clavulanate acid (9.67%) and Cefoxitin (6.45%). Relatively similar findings have been reported by [44] All *E. coli* isolates were 100% susceptible to gentamicin, 15% resistant to sulphamethoxazole-trimethoprim from Burkinafaso. The result of this study was almost comparable with the work of [45,46] who reported the susceptibility against *E. coli* were 100% for Ciprofloxacin and Gentamycin and resistance against Tetracycline (86.88%from Ethiopia and Pakistan respectively. Our result was relatively less than [47] who reported sulphamethoxazole-trimthoprim (76%) was susceptible to *E. coli* from Mekelle, Ethiopia. Therefore, in this study gentamycin, Ciprofloxacin, Meropenem, Ceftriaxone and Cefotaxime were found to be the most effective drugs against *E. coli* infection in the study area.

Conclusion and Recommendations

- This study clearly indicates that fresh raw cow's milk was found to be highly contaminated with the *E. coli* organisms. Since many people still drink fresh 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, has significant association with occurrence of *E. coli*; whereas there was no significant difference among different age, breed, lactation stage and body condition. Based on the antimicrobial susceptibility pattern, *E. coli* isolates were found to be highly susceptible to gentamicin, ciprofloxacin, meropenem, ceftriaxone and cefotaxime whereas resistant to ampicillin, trimethoprim+sulphamethoxazole, tetracycline, amoxicillin and clavulanate acid. Based on the above remarks, the following recommendations need to be considered:

- To ensure the quality of raw milk, everyone engaged in milk and dairy production chain should be trained for hygienic practices.

- In order to protect consumers from zoonotic AMR, food safety management programs should be implemented and highly considered.

- Awareness should be given to the community at risk, whole sellers and distributers.

- Consistent teat dips should be applied after milking.

Declarations Ethics Approval and Consent to Participate

Animal Health Institute (AHI) Research Ethics Review Committee (WUREC) approved this research before actual data

collection. A consent sheet was prepared in English and attached to the tool on a separate page regarding the purpose, description, anticipated benefits, and other relevant aspects of the study, and signed informed consent was taken from all respondents prior to data collection for animal owners of above 18 years of age. Thus, the authors declare that all methods were performed in accordance with the relevant guidelines and regulations.

Availability of Materials and Data

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

All authors contributed to the manuscript to the final submission. Conceptualization, Data curation, analysis, and writing the original draft were performed by Shubisa Abera, Investigation, methodology, validation, and supervision were majorly done by Mekonnen Addis while visualization, reviewing, and editing was done by Shubisa Abera. Finally, all authors read and approved the final manuscript submission.

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