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ORIGINAL RESEARCH

Extended-Spectrum Beta-Lactamase Producing *Escherichia coli* in Raw Cow Milk At Selling Points and Determinants of Contamination in and Around Chencha, Southern Ethiopia

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Introduction: Bacterial foodborne infections are a major public health concern globally, and the emergence of antimicrobial-resistant bacteria in food worsens the associated problems which are alarming issues. The current study aimed to isolate *E. coli*, determine antimicrobial resistance patterns, estimate the prevalence of extended-spectrum beta-lactamase-producing *E. coli*, and determinants of raw cow milk contamination at selling points in and around Chencha, Southern Ethiopia from January 2021 to April, 2022.

Methods: A total of 384 milk samples were collected randomly using a cross-sectional study and processed in the laboratory for evaluation of microbial load, bacterial isolation, and antimicrobial susceptibility patterns. The determinants were assessed through a prepared questionnaire, and descriptive statistics and multivariable logistic regression analysis were performed using SPSS version 21.

Results: Out of 384 milk samples analyzed for microbial loads, 5.3±1.68 log and 2.17±1.51 log CFU/mL respectively were the mean values of total viable count and total coliform count. A total of 118 (30.7%) samples were contaminated with *E. coli* of about 11.8% extended-spectrum beta-lactamase producers. Notably, 91 (77.1%) of isolates were multiple drug-resistant isolates. The multivariable logistic regression showed that variables of educational status of milk handlers, hand washing activities, nose picking habit of milk handlers, milk container cleaning activity, milk container type, physical abnormal milk checking status, and attended training on hygienic food handling practices type were significantly associated with the milk contamination in the study area.

Conclusion: The results implied that the raw milk samples served in the study area contained bacterial pathogens and a higher microbial load than recommended standards. Our data also confirmed the presence of multiple drug resistant and extended-spectrum beta-lactamase producing *E. coli*. Therefore, a close follow-up and training of milk handlers is needed. Besides, consumers should be made aware of the risks of consuming raw milk.

Keywords: antimicrobial susceptibility patterns, associated factors, Gamo zone, milk borne *Escherichia coli*

Introduction

Nowadays, one of the significant factors escalating the threat caused by bacterial pathogens from foods of animal origin is an increasing resistance to multiple antimicrobial agents.[1] Multi-drug resistant bacteria associated with food animals, environments, and food handlers have resulted in their dissemination via the food chain.[2]

Escherichia coli (E. coli) is an important food-borne pathogen that causes hemorrhagic colitis and the hemolytic-uremic syndrome in humans and may cause serious morbidity and large outbreaks worldwide.[1,2] The global emergence and spread of extended-spectrum beta-lactamase-producing *E. coli* (ESBLPEC) have been a critical public health issue in which the therapeutic options associated with these strains are fairly limited, so the rapid identification of this resistance and its determinants can help to eliminate the infection effectively.[1,3]

Overall, the problem is more alarming in developing countries like Ethiopia, where there is a huge burden of infectious diseases, a lack of surveillance networks, laboratory incapacity, and inappropriate diagnostics.[4] Studies

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conducted in different parts of Ethiopia have shown the poor sanitary conditions of food catering establishments and the presence of pathogens.[4,5] However, no studies were observed regarding ESBLPEC and associated factors of isolates. In the present study area context, the consumption of raw milk is traditional and consumer demand is increasing. As a result, it is essential to have the knowledge of prevalence of ESBLPEC and associated factors of milk contamination. Therefore, this paper aimed to isolate *E. coli*, determine antimicrobial resistance patterns, and estimates the prevalence of ESBLPEC and associated risk factors of milk contamination in and around Chencha of Gamo zone, Southern Ethiopia.

Materials and Methods

Description of Study Area

The study was conducted in and around Chencha, Gamo Zone, Southern Ethiopia over one year (January 2021-April 2022). Chencha (Chencha town and Chencha Zuria woreda) is one of the administrative towns and woredas in the Gamo zone located 37 kilometers north of Arba Minch town. Agro-ecologically, it is highlands and known for its potential of cow milk. It is traditional for people in the area to consume raw milk (CSA, 2008).

Population

Source population

Cow milk at the selling points.

Study Population

Raw milk ready for consumption.

Study Design

A cross-sectional study design was conducted in January 2021-April 2022 to investigate *E. coli* isolates, estimate the prevalence of extended-spectrum beta-lactamase-producing *E. coli*, and assess associated risk factors of milk contamination in Chencha town, Gamo Zone, Southern Ethiopia.

Sampling Technique

A total of 385 milk samples were collected. Before the milk sample collection, the checklists and questionnaire were filled and finally, the milk samples were collected by simple random sampling technique.

Sample Size Determination

The milk sample size was determined using a single population proportion formula with the following assumptions:

$$N = \frac{(Z\alpha/2)^2 p_{exp}(1-p_{exp})}{d^2}$$

Where N is number of required sample; p_{exp} is expected prevalence; $Z_{\alpha/2}$ is Z value at 95% confidence interval and d is the margin of error which is 5%.

The sample size was calculated according to the formula given by thru field (2018) considering an expected prevalence of 50% with a 95% confidence level and 5% margin of error. Accordingly, the minimum sample size (n) was found to be 384.[6]

Eligibility Criteria

Inclusion Criteria

Raw cow milk at selling points included in the study.

Exclusion Criteria

Milk under refrigerated and boiled were excluded due to low bacterial load.[4]

Sample Collection Methods

Checklist/Questionnaire

Before milk sample collection, observation of milk handlers regarding basic hygiene practices such as the use of hair cap, clean overcoat, and nose touching habits were done based on the prepared checklist. A structured questionnaire was used to collect information about educational status, attended training, medical check-ups, hand washing practices, milk container washing practices, and the type of milk containers used as indicated in Annex I.

Milk Sample

The milk samples were collected with the most careful attention to avoid extraneous contamination while drawing and handling. The samples were collected in sterile test tubes and immediately transported in an icebox to the microbiology laboratory, department of Biological Science, Arba Minch University for bacteriological analysis. The samples were analyzed within 2–4 hrs.[7]

Laboratory Analysis

Microbial Load Analysis

A 25mL of milk sample was homogenized in 225mL buffered peptone water using a homogenizer. The final homogenate gave a 1:10 dilution. Serial dilution up to 10^{-6} was performed to evaluate total viable count (TVC) and total coliform count (TCC).[8]

Total viable count

0.1mL from each serial dilution was spread onto plate count agar (PCA) (Himedia) and then incubated at 37° C for 24h. After incubation, distinct colonies ranging 30–300 on PCA were counted and calculated according to ISO7218:2007 using the following formula,[9] then expressed in colony forming unit per milliliter (CFU/mL). Finally, it was grouped based on the criteria set by hazard analysis and risk assessment in the management of food safety and quality for bacteriological limit standards for human consumption.[10]

$$\frac{CFU}{ml} = \frac{\sum C}{V \times 1.1 \times d}$$

Where: $\sum C$ is the sum of the colonies on the two Petri dishes counted from two successive dilutions; V is the volume of inoculum placed in each Petri dish which was 0.1 (in milliliters) and d is the first dilution retained.

Total coliform count

From each serial dilution, 0.1mL aliquot was spread onto McConkey agar with crystalline violet (Himedia) and incubated at 37° C for 24hr. The colonies on media ranging from 30 to 300 were counted, and results were calculated according to ISO7218:2007 using the above-cited formula.[9] Then it was categorized according to the criteria set by hazard analysis and risk assessment in the management of food safety and quality for bacteriological limit standards for human consumption.[10]

Bacterial Isolation

Isolation of *Escherichia coli*

Suspected pure colonies from Maconkey agar of total colony count determined were sub-cultured on nutrient agar. Then pure cultures of bacterial isolates were subsequently subjected to biochemical tests (triple sugar iron test, methyl red Voges–Proskauer test, citrate, motility, gas formation, and hydrogen sulfide production and indole reactions). Morphological and physiological characteristics of isolated bacteria were determined by adopting standard laboratory methods as shown in Annex II. Corresponding American-type culture collection strains were utilized as reference standards to validate the biochemical identification.[11]

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility test was performed by the Kirby Bauer disc diffusion method as per clinical Laboratory Standards Institute (CLSI) guidelines on Muller Hinton agar (Oxoid, Basingstoke, England). Three to five

morphologically identical and fresh bacterial colonies were suspended on distilled sterile water suspension to make turbidity equivalent to 0.5 MacFarland standards according to CLSI. Using the sterile cotton swab, the inoculum was seeded on Mueller–Hinton agar at the center and evenly spread onto the medium. The antimicrobial discs were applied on the surface of the medium after 15 minutes of inoculation. Afterward, plates were incubated at 35°C for 24hr and then diameters of the zones of inhibition around the discs were measured to the nearest millimeter using a ruler and categorized as sensitive, intermediate, and resistant according to the standardized table described in Clinical and Laboratory Standards Institute.[12]

Detection of Extended-Spectrum Beta-Lactamases Producing Escherichia Coli

A phenotypic confirmatory test of *E. coli* for extended-spectrum beta-lactamase production was performed on standard disc diffusion technique using indicator drugs. Briefly, the *E. coli* organism isolated was spread onto a Mueller–Hinton agar (oxoid, England) by preparing a suspension turbidity equivalent to 0.5 McFarland standards. Then, two indicator antimicrobial discs, ceftazidime(30 g) and amoxicillin (20 g)/clavulanic acid (10 g) were placed 15 mm center to center on media. Then incubated for 24 hrs an enhanced zone of inhibition in between the discs was considered a positive test.[12,13]

Data Quality Control

Data quality was ensured from data collection up to final laboratory identification by following the prepared standard operating procedure (SOP). The performances of the prepared media were checked by inoculating control strains *E. coli* ATCC 25922 which was obtained from the Ethiopian Public Health Institute. Culture media was prepared according to the manufacturer's instruction, and the sterility was checked by incubating 5% of the prepared media at 37 °C for 24 hours and observing bacterial growth. Those batches of the media that show the growth were discarded and re-prepared.

Data Analysis

Data were collected, entered, cleaned, and analyzed using SPSS version 21 software according to the study objectives. Estimation of proportions was used to summarize the prevalence and antimicrobial susceptibility patterns of *E. coli and* ESBLPEC at 95% CI and 5% margin of error. The descriptive summaries were presented with text and tables. Multivariable logistic regression analysis was made to obtain odds ratio and confidence intervals for statistically associated variables.

Result and Discussion

Socio-Demographic Characters and Hygienic Practices of Milk Handlers at Milk Selling Points

In this study, 384 study participants were included with a 100% response rate. The majority of the participants (65.7%) were females and about 54% of workers had the educational level of elementary school. In contrast, the study conducted in the other part of Ethiopia showed that more than 50% of milk handlers were males.[14] The present finding also showed that about 42% and 45% of the participants were respectively washing milk containers and their hands with water only. This was in agreement with the previous studies as mentioned above. It was observed that more than 80% of workers did not wear clean gowns and hair cups. According to the participants' responses, it was found that only 8.6% of them had received training on hygienic food handling practices as indicated in Table 1. Similarly, Bereda et al reported that the untrained food handlers working on food services were vehicles of disease-causing microorganisms.[15] It is plausible that poor hygiene adds to the microbial contamination of milk as well and the lack of continuous training and low education level contributed to the lack of food hygiene knowledge concerning several key aspects of safe food production.[16] The discrepancy in food hygiene practice level in this study might be due to variations in the study tool used, time of the study, and variation in socio-demographic and socioeconomic status.[17]

Table 1 Socio-Demographic Characteristics and Hygienic Practices

Variables	Category	Frequency(n)	Percentage (%)
Sex	Male	132	34.3
	Female	252	65.7
Educational level	Illiterate	55	14.3
	1–8	208	54.3
	9–12	65	17.1
	College and above	56	14.3
Attended training on food hygiene	Yes	33	8.6
	No	351	91.4
Using hair cap	Yes	55	14.3
	No	329	85.7
Using a gown/apron	Yes	77	20
	No	307	80
Health Check per year	Yes	22	5.7
	No	362	94.3
Nose-picking habit while working on milk	Yes	165	42.9
	No	219	57.1
Cleaning milk container	Detergent and water	222	57.9
	water only	162	42.1
Hand washing	Detergent and water	211	55
	water only	173	45
Abnormal milk Check	Yes	242	62.9
	No	142	37.1
Milk container	Plastic Jerkan	263	68.6
	Aluminum coated Milk tank	121	31.4

Microbial Load Evaluation

In the current study, a total of 384 milk samples were analyzed for microbial load and all the samples were found positive for aerobic mesophilic microorganisms. About 60 (15.7%) samples had exceeded the microbial load limit standards for human consumption concerning TVC. It was shown that the overall mean value of TVC 5.3log with a standard deviation of ±1.68; and the mean TVC of the current result was failed to comply with the standard set for raw milk intended for direct human consumption (<5log CFU/mL).[18] Similarly, higher mean TVC (6.36–9.82 log CFU/mL) values were reported in different parts of Ethiopia.[15–19] The higher count in milk could be attributed to the substandard hygienic conditions practiced during production and subsequent handling.[20]

In the case of TCC, the contamination rate was found to be 65.7% with an overall mean of 2.17 log and 15.7% of total samples having total coliform bacterial load limit above the recommended level for human consumption. The mean values of TCC observed in the current study were slightly higher than both the recommended values set for raw milk intended for direct human consumption (<2log CFU/mL)[18] and the American Public Health Service for grade A milk which was <2log;[21] however much lower when compared with previous studies conducted in West Shewa zone of Oromia, Ethiopia[22] and Wolaita.[17] The high coliform counts are associated with the level of hygiene and are an indication of fecal contamination from animals, humans, and/or the environment.[23]

Altogether, it was extrapolated as 44(11.4), 258(67.2), 38(10), and 44(11.4), respectively, were found to be satisfactory, marginal, unsatisfactory, and potentially toxic for human consumption at raw state according to criteria set by hazard analysis and risk assessment in the management of food safety and quality for a bacteriological limit standard for human consumption standards[10] (as shown in Table 2). In contrast, previous studies in Nepal showed that 25%, 30%, and 45% of total evaluated milk samples respectively were found satisfactory, fairly satisfactory, and poor quality for human consumption.[24] Generally, the high counts of bacteria observed in the present study were attributed to a lack of awareness related to milk hygiene, low level of educational status, absence of training on clean milk production, poor transportation

Table 2 Bacteriological Contamination and Load of Raw Cow Milk Collected from Arba Minch and Chencha Town, Gamo Zone

Counts	Total Tested Sample	Growth Status		Microbial Load (log10 CFU\mL)			Acceptability of the Examined Milk Based on Microbiological Load			
		No growth No (%)	Positive sample No (%)	Min	Max	Mean ± SD CFU\mL	Satisfactory No (%)	Marginal No (%)	Unsatisfactory No (%)	Potential risk No (%)
TVC	384	84	300	0.00	8.52	5.3±1.68	148(38.6)	176(45.8)	60(15.7)	
TCC	384	132(34.3)	252(65.7)	0.00	6.23	2.17±1.51	143(37.2)	181(47.1)	60(15.7)	
Overall extrapolation							125(32.5)	177(46)	82(21.3)	

conditions, poor hygiene of milking utensils and milker's hands as well as lack of good hygiene in and around milking environments, milk processing and handling at selling points.[25]

Isolation of Escherichia coli from Milk

Based on colony morphology, gram stain, and biochemical characteristics; the percentage of bacterial isolates retrieved from the milk samples is depicted in Table 3 and the representative images of main findings indicated in Annex III. Accordingly, a total of 118 *E. coli* isolates were detected. As an indicator of hygiene and sanitary quality, the presence of *E. coli* suggests that consumers are at a greater risk of suffering food-related diseases due to these pathogens.[26]

In the current study, the isolation rate of *E. coli* was found 30.7% with a p-value <0.05. The overall isolation rate of *E. coli* in the study was slightly higher than the results of a previous study conducted in Tigray, Ethiopia (25%),[27] however, it was lower than that of a study in Bishoftu, Ethiopia (42%)[28] and Iran (69%).[29] The possible reason for the differences in the isolation rates could be due to fluctuations in handling practices, post-handling of the milk, and general hygiene standards maintained at various stages of the milk processing chain. Besides, the sample size, design of the study, methodology used, as well as geographical location might have contributed to fluctuations in the isolation rate of bacterial isolates.[18,30] Detection of *E. coli* in milk often reflect fecal origins; however, coliforms of environmental origin have been also reported in milk.[20]

Factors Associated with Escherichia coli Contamination at Milk Selling Point

In the present study, a total of eighteen risk factors in the first step were analyzed using univariate analysis, and the eleven variables (sex of milk handlers, educational status, attended training on hygienic food handling practices, using clean gown/overcoat, wearing of clean head/hair cover, health check per year, nose picking habit during working period, cleaning milk container, hand washing activity, milk container type and abnormal milk checking physically) were significant at p-values <0.25. Then, these variables were entered into the multivariate analysis. The findings revealed that the milk contamination by *E. coli* was significantly associated with variables of educational status of milk handlers, hand washing activities, nose picking habit of milk handlers, milk container cleaning activity, milk container type, physical abnormal milk checking status, and attended training on hygienic food handling practices with p-values <0.05.

According to the current result, the use of water only to wash milk containers and hands respectively had 2.5 and 2 times higher likelihood of milk contamination with *E. coli* than that of with both detergent and water. This result was in agreement with the previous study conducted in central Ethiopia that showed the use of water without detergents for washing milk containers increased the risk of milk contamination with milk-borne pathogens by three

Table 3 Prevalence of Bacterial Isolates from the Raw Cow Milk Samples in the Study Area

Number of samples	*E. coli* n (%)	X^2	p-value
384	118(30.7)	2.659	0.013

times.[31] This shows that the use of some disinfectant products before handling and milk storage in containers can have beneficial effects on reducing the levels of pathogens.[32] Our findings also revealed that the odds of milk contamination at the selling points with trained handlers on hygienic food handling practices reduces the risk by 80% and this result was supported by a previous study from Addis Ababa, Ethiopia, that showed the odds of milk from trained handlers reduced the bacterial contamination by 3.5 times higher.[33] Similarly, reports from another region of Ethiopia showed that the absence of training, low level of education, and poor hygienic practices of workers in food areas as very important risk conditions for contamination of raw milk.[31]

In the current study, it was observed that the odds of milk contamination with *E. coli* in plastic jerkan are 2.7 times higher likelihood than that of the aluminum-coated container. Similarly, Donkor et al showed that the use of plastic jerkan milk containers was found to be a potential risk factor associated with bacterial contamination.[34] According to the present result, the odds of milk contamination at selling points that did not check the abnormal milk physically tend to have a two times higher likelihood of contamination than that of doing a check of abnormal milk physically. On the other hand, the variables including sex of respondent, wearing of hair cup, wearing a gown, and health check per year were not associated statistically at a 95% confidence interval. The overall association between the variables of interest and the contamination of *E. coli* in milk is indicated in Table 4.

Antimicrobial Susceptibility Patterns of Isolates

Antimicrobial susceptibility patterns observed in the current study revealed that the majority of isolates of *E. coli* were found to be 100% susceptible to ciprofloxacin and co-trimoxazole followed by gentamicin (97.7%), Augmentin (88.4%) and Ceftazidime (88.4%) as shown in Table 5. A similar trend of sensitivity of ciprofloxacin and gentamicin was observed in a study done in another part of Ethiopia.[2] In contrast to our findings, the highest resistance to ciprofloxacin in

Table 4 Multivariate Logistic Regression Analysis of the Risk Factors of Milk Contamination with E. Coli

Variables		OR	CI at 95%	p-value
Sex of respondents	Male	1.1	0.75–12.09	0.62
	Female	Ref	Ref	Ref
Educational status	Illiterate	3	2.01–8.07	0.02
	1–8	3	1.00–16.40	0.00
	9-12	2	1.04–23.89	0.00
	College and above	Ref	Ref	Ref
Attended training on food hygiene	Yes	0.2	0.04–0.69	0.04
	No	Ref	Ref	Ref
Using hair cap	No	1.3	0.52–2.81	0.2
	Yes	Ref	Ref	Ref
Using a gown/apron	No	1.3	1.12–2.84	0.09
	Yes	Ref	Ref	Ref
Health Check per year	Yes	0.6	0.09–2.81	0.06
	No	Ref	Ref	Ref
Nose-picking habit while working on milk	Yes	1.4	1.04–2.25	0.04
	No	Ref	Ref	Ref
Cleaning milk container	water only	2.5	1.21–14.07	0.00
	Detergent and water	Ref	Ref	Ref
Hand washing	only water	2	1.62–23.98	0.02
	Detergent and water	Ref	Ref	Ref
Abnormal milk Check	No	2	1.55–6.75	0.00
	Yes	Ref	Ref	Ref
Milk container	Plastic Jerkan	2.7	1.12–6.67	0.02
	Aluminum coated Milk tank	Ref	Ref	Ref

Abbreviation: *Ref: reference point.*

Table 5 Antimicrobial Susceptibility Patterns of Bacterial Isolates from Raw Cow Milk in the Study Area

Antimicrobials	*E. coli*		
	S	I	R
Ampicillin	25(21)	5(4.6)	88(74.4)
Cefoxitin	38(32.5)	14(11.7)	66(55.8)
Gentamicin	115(97.7)	3(2.3)	0
Tetracycline	82(69.8)	8(7)	27(23.2)
Ciprofloxacin	118(100)	0	0
Cotrimoxazole	118(100)	0	0
Chloramphenicol	80(67.7)	18(15.2)	20(16.9)
Augmentin	104(88.4)	3(2.5)	11(9.3)
Ceftazidime	104(88.4)	0	14(11.8)

Sudan[35] and co-trimoxazole (50%) in central Ethiopia[36] were observed. The present study showed that a higher level of resistance of *E. coli* against ampicillin (74.4%) followed by cefoxitin (55.8%) was observed. Similarly, the highest resistance of ampicillin was reported from studies conducted in central Ethiopia (68.7%).[36] This might be attributed to the continuous usage of penicillin derivatives in food animals.[37]

Multiple Drug Resistant Isolates

In the present study, the pathogens that were resistant to three or more antimicrobial classes were considered as MDR according to CLSI, 2021. It was found that more than 77% of isolates were MDR indicated in Table 6. The percentage of MDR isolates of *E. coli* observed in our finding was much higher than that detected in previous studies of Mekelle, Ethiopia (28.24%),[2] Indonesia (9.1%)[38] and Nepal (28%).[39] According to our results, the observed patterns reflect the use of these antimicrobials in the study area, and it shows that *E. coli* has been exposed to these drugs. Another possible reason for the observed pattern is the availability and price of these drugs. It was noticed that these drugs are widely available from agro-vet distributors as well as human pharmacies and can be purchased easily without any prescription from an authorized facility.[40,41]

Prevalence of Extended Spectrum Beta-Lactamase Producing Escherichia Coli

On the other hand, increasing resistance to third-generation cephalosporin has become a cause for concern about Enterobacteriaceae and in recent years, extended-spectrum beta-lactamases producing Enterobacteriaceae isolates have shifted from the hospital to the community, and the environment.[35] The results of the current study further revealed that 14(11.8%) of *E. coli* isolates were found to be extended-spectrum beta-lactamase enzyme producers which is an alarming issue and this finding was higher than the previous studies conducted in Indonesia (1.7%).[38] Even though the third-

Table 6 MDR Bacterial Isolates from Raw Cow Milk in the Study Area

MDR	*E. coli*
R3	41(34.7)
R4	30(25.4)
R5 and above	20(16.9)
Sub-total	91(77.1%)

Note: R= number of antimicrobials disc in which bacterial isolate resistant.

generation cephalosporin is not used in food animals in the study area, it was widely used in humans which can contribute to resistant pathogens when working on food.[23]

Conclusion and Recommendation

The overall results of the current study provided that some of the raw milk served in the study area contained higher bacterial loads than recommended standards, indicating poor management practices. In addition, *E. coli* was detected with a high percentage of MDR which poses serious risk factors for the health of consumers. The detection of ESBLPEC in our findings can pose a risk of transmission of resistant strains to humans and environments. Our findings also determined the educational status of milk handlers, hand washing activities, milk container cleaning activity, milk container type, physical abnormal milk checking status, and attended training on hygienic food handling practices were statically predisposing factors of *E. coli* contamination of milk at the selling point. Therefore, it needs improvement of microbiological quality and safety in the study area in the future. And also further investigation at molecular level should be required for confirmation.

Based on this finding, the following recommendations are forwarded;

- The concerned government bodies should coordinately act to prevent the microbial contamination of milk.
- Programmed monitoring and inspection of milk for proper hygiene, handling, and sanitary practices should be prioritized as an immediate intervention by professionally qualified food safety officers.
- Effective surveillance programs at a multidisciplinary level at the molecular-level investigation should be done to better understand and minimize the emergence of resistant strains of bacteria associated with milk.
- Further research on the producer's level should be required to establish control approaches to milk contaminations.

Data Sharing Statement

All the relevant data are within the manuscript.

Ethical Consideration

The verbal informed consent process was approved by the Institutional Review Board of college of Medicine and Health Science, Arba Minch University (Ref. No IRB/174/12/17/03/2020). The research ethics was taken into consideration; the research did not expose client's information to protect the privacy and confidentiality of the owners in order to avoid jeopardizes the owners businesses, and the guidelines outlined in the declaration of Helsinki were followed.

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Disclosure

The author declares no competing interests in this work.

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