Bacteriological quality assesement of raw cow's milk in and around Asossa, Ethiopia

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Abstract: A cross- sectional study was conducted in Asossa District of Benishangul Gumuz Regional State, Western Ethiopia from November 2015 to March 2016 to estimate the bacteriological quality of raw cow milk, antimicrobial susceptibility pattern, along with questionnaire survey to assess hygienic practices during milking, milk storage and transportation. The micro organisms were isolated from contaminated milk and their antimicrobial susceptibility was tested. The result revealed a high bacterial load in the raw cow milk, and an increased resistance of bacterial isolate to locally available anti bacterial agents. These results provide valuable information for the improvement of local dairy production and suggest the necessity of more effective control on the use of antibiotics.
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1. Introduction

Milk is one of the most common food sources in the human diet and is also a product that is directly available for consumption (Grimaud *et al*., 2009). Being a nutritious food, it is an excellent growth medium for bacteria, originating from contamination of the milk with environmental spoilage as well as pathogenic microorganisms during milking or milk handling process (Pospescu and Angel, 2009). This is especially true in developing countries where production of milk and various dairy products take place under rather unsanitary conditions and poor production practices (Zelalem and Faye, 2006).

Bacterial spoilage of raw milk depends upon various factors such as health of the animal, cleanliness of the housing area, the nature of feed, the water used at farm, the milk vessels / utensils for storage and essentially the hygiene of the milker / handler (Salman and Hamad, 2011).

The microbial load of milk is a major factor in determining its quality. It indicates the hygienic level exercised during milking, that is, cleanliness of the milking utensils, condition of storage, manner of transport as well as the cleanliness of the udder of the individual animal (Gandiya, 2001). Higher bacterial contents exist in developing countries where production of milk and various dairy products takes place under rather un sanitary conditions and poor production (Mogessie, 1990). This implies, high numbers of bacteria in raw milk usually indicate heavy contamination caused by handling, inadequate cooling or both. Mubarack *et al.,* (2010) and Lingathurai and Vellathurai (2010) have reported the presence of pathogenic bacteria to be a major threat to public health especially for those individuals who still consume raw milk.

The disease causing bacteria in the milk are Salmonella spp. *Mycobacterium bovis, Corynebacterium spp., Clostridium perfringens, Yersinia enterocolitica Coxiella burnetii, Brucella, Staphylococcus, Campylobacter jejuni Mycobacterium avium, Listeria spp., Escherichia coli* and coliforms. Many bacteria could get an easy access to milk and milk products such as *E. coli* and coliforms; they are often used as indicator organisms to confirm the bacterial contamination of milk. Most common pathogens that have been involved in milk borne diseases include Salmonella spp., *Staphylococcus aureus*, and *E. coli* (Vahedi *et al*., 2013). The quality and safety of raw milk can be evaluated by assessing hygiene indicator microorganisms. Total coliform, *E. coli* and *S. aureus* are used as hygienic parameters for milk production, as they indicate the conditions of raw milk obtaining and storage, and inadequate handling during the manufacturing process. These microorganisms are usually associated with food borne diseases and outbreaks, as recorded by official health organizations (Bouazza *et al*., 2012). The presence of these pathogenic bacteria in milk appeared as main public health concerns, especially for those people who still drink raw milk (Claeys *et al*., 2013).

Dominant of the people in the study area are agro pastoralists who kept large population of cattle to sustain their lives beside to this most of the people in the study area were having the habit of consuming raw cow milk as food source in addition to use of other products of milk like yogurt. Moreover, though there was study on milk quality in different parts of Ethiopia still there was little scientific study done in the study area about the hygienic condition of milk from production to consumption at different critical milk production points.

Therefore, the objective of this study were to determine the bacteriological load of raw cow's milk at different sampling points, isolate and identify the raw milk pathogens which have effect on human health and determine antimicrobial susceptibility pattern of the isolated bacteria from Asossa town and its surrounding.

2. Study Materials And Methdology

2.1 Study Area

The study was conducted in and around Asossa twon, which is located at 668Km North West of Addis Ababa. Asossa is the capital city of Benishangul-gumuz regional state located at 10° 04' north latitude and 34° 31' 59" east longitude. The altitude of the district ranges from 580-1500 meters above sea level and receives an annual rainfall of 900-1200mm with the mean minimum and maximum annual temperatures of 19°C and 34°C, respectively. The area has a sub humid climate with moderate hot temperature between daytime and night. The communities in the study area are relay predominantly on farming and cattle breeding.

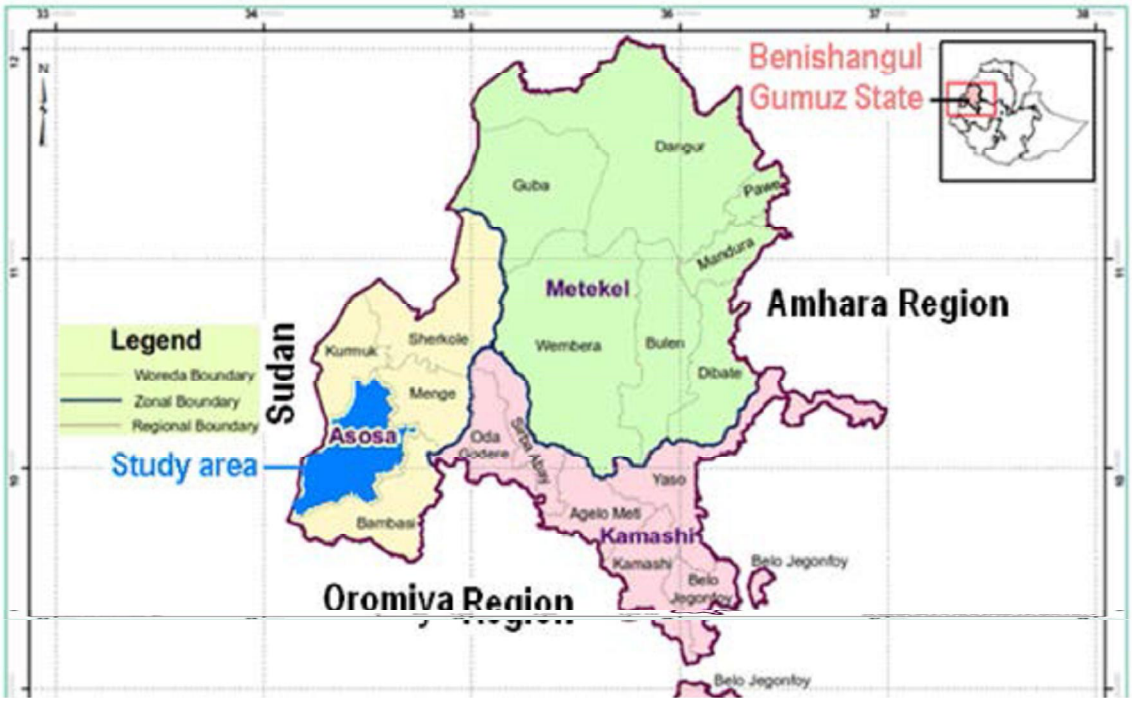

Figure 1: Source: (Disaster Risk management and Food Security Sector (DRMFSS, 2004 E.C): Administrative Map of Benishangul Gumuz.

2.2 Study Animals

The study animals were lactating dairy cows. The animals were managed under a semi-intensive management system.

2.3 Study Design and Sampling Technique

Cross-sectional study was conducted from November 2015 to March 2016 in dairy cows. Purposive sampling technique was used based on the accessibility, willingness of dairy animal and milk vending shop owners and only those owners who sold the milk were selected. Simple random sampling technique was also applied during the questionnaire survey.

2.4. Data collection

2.4.1. Questionnaire survey

A structured questionnaire was prepared to assess hygienic practices during milking, means of cleaning of the storage container, hygienic condition of transporting container to market and other related issues. A total of 132 individuals (100 from dairy farm and household and 32 from milk vending shop) were participated during the survey.

2.4.2. Milk Collection and Handling procedure

For the microbiological analysis a total of 100 samples of raw cow's milk was collected (34 milk sample from households, 34 milk sample from dairy farms, 6 milk samples from vending shops and 26 milk samples from cafeterias). 15-20 ml of milk samples were collected starting early in the morning from milk vending shops and cafterias, dairy farms and households (farmers) using sterile glass test tube.

The samples were properly labeled, kept in icebox and transported to the Asossa regional microbiology laboratory for bacteriological analysis

and samples were kept in refrigerator at +4 c^0 and culturing was done immediately.

2.4.3. Bacterial load assessment of raw milk sample

Milk quality control is an essential component of any milk processing industry whether small, medium or large scale. The high nutritive value of milk makes it an ideal medium for the rapid multiplication of bacteria, particularly under unhygienic storage conditions and at ambient temperatures (Marshall, 1992). There is no single test done at the processing plant, which can determine the hygienic quality of milk but commonly, methylene blue reduction test, total plate counting, lactometer test, PH test and so on is done to assess quality of milk (McKenzie, 2009).

2.4.3.1. Methylene blue reduction test

The test is an indirect method to assess the bacterial count of the milk. It gives indication about the sanitary and keeping quality of milk and helps in grading the raw milk samples (Benson, 2002). Methylene blue reduction test has been employed to check for the overall microbial load and quality control of milk and other liquid foods (Impert *et al*., 2002). It is assumed that, the greater the number of microorganisms, the more the oxygen demand and lesser the oxygen concentration in the medium resulting in the faster disappearance of the color. This fact has been used as a broad indicative test of a microbial load representing microbial quality of milk (Nandy and Venkatesh, 2010).

10 ml of raw cow's milk with 10 ml sterile pipette were added aseptically in to sterile test tube and then 1ml of methylene blue reagent were added with sterile pipette to the solution and the test tube containing the solution were closed carefully with the rubber stopper without contaminating it. Then solutions were mixed by inverting the tube two times and place the tube in a water bath maintained at 37°C. The tubes were observed after 30 minutes of incubation and an hourly interval for decolonization (IDF, 1990). Methylene blue reduction test result was judged based on the discoloration time where samples with discoloration time of less than 2 hour, 2-6 hour, 6-8 hours and more than 8 hours were judged as poor, fair, good and very good respectively (Bilal *et al.,* 2011).

2.4.3.1 Standard plate count test

Standard plate count test is test which is useful in assessing the number of total viable bacterial in the raw milk based on which the milk can be graded in to different categories according to bacterial content in the milk. Tenfold serial dilution up to 10^6 was prepared for each sample using 9ml of 0.85% sterile saline water. Pour on plate method was used to prepared viable count by adding 1ml of diluted sample in to petridish then adding 15-20ml of sterilized molten standard plate count agar in to petridish with gentle rotation to mix the solution and allow the agar to solidify for 5 minutes. After incubation for 24-48 hours plate with different dilution having bacterial colony ranging from 30 to 300 were selected and counted using colony counter and the count for each plate were expressed as colony forming unit of the suspension (kebede, 2005).

Table 1: bacteriological standards of raw milk as prescribed by bureaus of indian standards (BIS) (IS-1479, PART-3-1997)

Grade	Standard plate count per ml (10^5)
Very good	<2
Good	2-10
Fair	10-50
Poor	>50

Source: (Sherikar *et al*., 2004)

2.4.4. Isolation and identification of bacteria

Isolation and identification of bacteria was done by plating the milk samples on both general and selective media as indicated in table 2. Firstly, all the samples were cultured on to the nutrient agar (Oxiod) for bacterial growth characterization. Secondly, the different biochemical tests were conducted such as gram staining, catalase test, KOH test and oxidase test. Again the colony was cultured on to MacConkey agar (Himedia) to isolate Gram negative lactose fermenting (coliforms) and non- lactose fermenting microorganisms. Lactose fermenting bacteria was pink in color whereas non lactose fermented remains colorless. The bacterial isolated from MacConkey agar were sub-cultured on eosin methylene blue (EMB) agar (Himedia). Lactose fermenters such as Escherichia coli was small and have a metallic sheen The lactose non-fermenting Gram negative non-coliform (colourless) isolates were also sub- cultured and confirmed on selective media. Salmonella Shigella (SS) agar (Himedia) and XLD agar (Himedia) was used for the isolation of Salmonella and Shigella species. Salmonella colonies showed as black appearance on salmonella shigella agar and pale to pink colony having blackening center on the XLD agar were confirmed. On the other hand, those gram positive bacteria were sub cultured on to Manitol salt agar (Himedia) which is used for isolation of staphylococcus species based on their ability to utilize manitol sugar and Edward base medium which is used for isolation of streptococcal species directly from the general medium and on manitol salt agar bacteria colonies was having small, sized, pale, pink and yellowish color was observed. Further isolation and identification was done by conducting secondary biochemical tests such as indole test, motility test, citrate utilization test, methylene red vogues proskaeure test, carbohydrate utilization test (glucose,

lactose, sucrose and maltose), oxidation fermentation test, and triple sugar iron test and finally identification was made to its genus and species level based up on biochemical characteristics (Quinn *et al*., 2002).

Table 2: Growth on selective media and biochemical characteristics

Tests	**Bacterial types**			
	Staphylococcus auerus	Other *staphylococcus* species	*E.coli*	*Salmonella* species
Catalase	+	+	+	+
Oxidase	-	-	-	-
KOH	-	-	+	+
Hemolysis	+	-	-	-
Manitol salt agar	+	+	-	-
EMB agar	-	-	+	-
XLD	-	-	-	+
Citrate utilization	-	-	-	-
O-F	Fermentative	Fermentative	Fermentative	Fermentative
Motility	-	-	+	+
Indole	-	-	+	-
MR	+	+	+	+
VP	-	-	-	-
TSI gas	-	-	+	-
TSI sugar slant	+	+	+	-
TSI sugar butt	+	+	+	+
TSI H2S	-	-	-	+
Glucose	+	+	+	+
Maltose	+	+	+	+
Lactose	+	+	+	-
Sucrose	+	+	+	-

KOH= Potassium hydroxide, EMB=eosin methylene blue, XLD=xylose lysine desoxychocolate agar, MR=methyl red, VP = voges proskaeure, TSI=triple sugar iron (Source: Quinn *et al*., 2002).

2.4.5. Antibiotic susceptibility test

An antimicrobial susceptibility test by disc diffusion method has been used with antibiotic discs (oxiod). Antibiotic susceptibility tests were performed on all individual pure isolate as *S. auerus* (38), *E.coli* (6) and other *Staphylococcus* species (8) and again those bacteria in mixed infection were further sub cultured to purify where (24) *Staphylococcus auerus*, (28) salmonella species, (48) *E.coli* and (16) other *Staphylococcus* species were isolated and thus a total of (62) *S. auerus*, (54) *E.coli*, (28) *salmonella* spp and (24) other *Staphyloccus* species were included to determine their antibiotic susceptibility profiles. Fresh cultures were prepared by inoculating nutrient broth (oxiod) with the isolated bacteria and incubated for at least 2 to 8 hours. A sample of 1ml from each isolate suspension was spread plated on Mueller Hinton agar (oxiod). Five different antibiotic discs were used for both gram positive and gram negative bacterial isolates as indicated in table 3a and 3b. Antibiotic discs were gently pressed on to the inoculated Mueller Hinton agar to ensure intimate contact with the surface and the plates were incubated aerobically at 37 °C for 24 hours. Then based on the inhibition zone, diameter for antimicrobial agent the bacterial isolates were classified as resistant, intermediate or susceptible and interpreted according to zone size interpretation chart (CLSI, 2014).

Table 3a: Staphylococcus susceptibility pattern

Antimicrobial agent	**Disc potency**	**S***	**I***	**R***
Penicillin	10 unit	29	-	28
Cloxacillin	5µg	22	-	21
chloramphenicol	30 µg	18	13-17	12
Tetracycline	30 µg	19	15-18	14
Vancomycin	30 µg	12	10-11	9

*S=Susceptible, I=Intermediate, R=Resistant

Table 3b: Enterobacteriacae susceptibility pattern

Antimicrobial agent	Disc potency	S*	I*	R*
Gentamycin	10 µg	15	13-14	12
Streptomycin	25 µg	15	12-14	11
Chloramphenicol	30 µg	18	13-17	12
Tetracycline	30 µg	15	12-14	11
Sulphonamide	300 µg	17	13-16	12

2.5. Data Analysis

A data base was developed to store qualitative and quantitative data from the cross sectional study using Microsoft Excel 2007 spread sheet. STATA version 11 was used to compute descriptive statistics of variables collected during the study. Overall bacterial load was calculated using descriptive statistics of the sample through frequencies and cross tabulations. Bacterial isolates and antimicrobial susceptibility test were described by frequency and percentage, comparison of bacterial isolates and antimicrobial susceptibilities were performed and the proportion of bacterial resistant to each antibiotic was calculated. P-value <0.05 was reported as statistically significant.

2.6. Data Quality Assurance and Quality Control

Regular monitoring of field and laboratory works was conducted and quality of field data collection and transportation was assured and checked for completeness consistency at the site of data collection. The overall study was checked by the advisor for its validity and successfully completeness of the study. Preservatives and other chemicals were tested against predetermined specifications to ensure consistent product quality.

3. Result

3.1. Questionnaire Survey

3.1.1. Information on housing condition, animal health and hygienic status of milk collecting materials

A total of 100 from house hold and dairy farm owners were interviewed for the hygienic practice during milk collection to distribution periods. As indicated in Table 3, from the households and dairy farm owners, majority (95%) of the respondents kept their animals in non-concrete type of housing system, most (50%) of the respondent clean their barn twice per week 78% of respondents had the habit to wash the udder and teat of their animals before milking. Moreover, 43% of the individual were used cold water with detergent (omo / soap) to wash the udder of animals where 47% of the respondents use common cloth to dry the teat. However, none of the respondents were practice teat dipping and milk quality tests, on the other side, 68% of the respondent vaccinate and dewormed their animals.

Table 4: Questionnaire survey on milk hygienic during milking practice

Variable	Frequency	Percentage
Bedding condition		
Concrete	5	5
None-concrete/soil	95	95
Barn cleaning frequency		
Once/week	30	30
Twice/week	50	50
More than two/week	20	20
Udder and teat washing before milking		
Yes	77	77
No	23	23
Udder washing material		
Cold water	15	15
Cold water and detergent	43	43
Warm water	9	9
Warm water and detergent	11	11
No washing	22	22
Udder and teat drying		
Common cloth	47	47
Individual cloth	31	31
No drying	22	22
Teat dipping		
Yes	0	0
No	100	100
Means of disease prevention		
Vaccination and deworming	68	68
Vaccination	32	32
Milk quality test		
Yes	0	0
No	100	100
Sanitizing milking equipment		
Yes	100	100
No	0	0
Source of water for equipments		
Pipelines	15	15
Wells	75	75
Others	10	10
Use of local plants for fumigation		
Yes	46	46
No	54	54
Hand wash before milking		
Yes	83	83
No	17	17
Hand wash b/n cows		
Yes	30	30
No	70	70
Milking procedure used		
Hand	100	100
Machine		
Milking frequency per day		
Once	0	0
Twice	100	100

3.1.2. Information on milk storage and transporting

A total of 32 respondents from milk vending shops (n = 6) and cafeteria (n=26) were participated during the study period. Most (43.75%) of the respondent collected their milk from individual households. Table 4 summarizes milk storage and transporting practices.

Table 5: Milk storage and transporting practices

Variables		Frequency	Percentage
Source of milk	Dairy farms	10	31.25
	Milk selling cooperatives	8	25
	Households	14	43.75
Material for collection of milk	Plastic	28	87.5
	Metallic	4	12.5
Equipment washing material	Cold water and detergent	14	43.75
	Warm water and detergent	18	56.25
Time of milk collection	Early morning	26	81.25
	Afternoon		
	Both	6	18.75
Storage material of milk	Plastic	26	81.25
	Metallic	6	18.75
Duration of milk stayed in shop	One day	100	100
	Two day		
	More		
Other product of milk sold at shop	Yogurt	2	6.25
	Pasteurized milk	24	75
	Both	6	18.75
Any cooling system used	Yes	24	75
	No	8	25

3.2. Microbial Load Assessment of Raw Cow's Milk

3.2.1. Methylene blue reduction test

Majorities (48%) of the sample were graded as poor and 18 of them were graded as fair depending on the methylene blue reduction test result interpretation standard. Table 6 summarizes methylene blue reduction test result.

Table 6: Methylene blue reduction test result

Discoloration time (dt)	Judgment	Frequency	Percentage
Less than 2hr	Poor	48	48
2-6hr	Fair	18	18
6-8hr	Good	27	27
Greater than 8hr	Very good	7	7

3.2.2. Standard plate count test

majority (45%) of the milk sample collected from the different points in the study area were graded as poor and 21% of milk samples were graded as fair based on their microbial loads. Similarly, the rates of mixed infection were higher in dairy farms and lower in vending shops and cafeterias with bacterial load ranging from 7.08log 10 to 7.41 log10. There was statistically significant (p = 0.009) on the bacterial load observations among the three source of milk samples. Table 6a and b summarize the total bacterial count of raw milk.

Table 7a: Standard plate count test result

Cfu/ml (10^5)	Judgment	Frequency	Percentage
Greater than $5x10^6$	Poor	45	45
$1x10^6$-$5x10^6$	Fair	21	21
$2x10^5$-$1x100^6$	Good	30	30
Less than $2x10^5$	Excellent	4	4

Table 7b: Mean ±SE of standard plate count test

Source	N	Mean	Dilution (10^{-5})	mean ± SE	p-value
Dairy farm	34	121.23	$1.21x10^{7}$	7.08±0.128	0.009
Households	34	197.85	$1.978x10^{7}$	7.29±0.134	
Shops and cafeterias	32	265	$2.65x10^{7}$	7.42±0.140	

*N = No. of samples, SE = Standard error

3.2.3. Isolation and identification of the microorganisms

Bacterial isolation and identification was done with commonly available material in the study area. *S. auerus*, other *staphylococcus* species, *E.coli* and *salmonella* species were identified by both primary and secondary biochemical tests. Out of 100 milk sample collected at different sources, majority of isolate were *S. auerus* (38%) and other staphylococcus species (8%) followed by *E.coli* (6%).

Moreover, 28% of milk sample from milk vending shops and cafeteria were contaminated with mixed infection/bacteria (two or more of the isolated bacteria) in the study area table 8, showed milk sample collected from dairy farm, households and milk vending shops were positive for *staphylococcus auerus* each consisting of 19%,15% and 4% respectively. The difference in bacterial species among the three source of specimens were statistically significant (p-value=0.00).

Table 8: Isolated bacterial species from different source of milk samples

Species	Source of milk			Total
	Dairy farm	Households	Shops	
Staph. auerus	19 (19%)	15(15%)	4(4%)	38 (38%)
E.coli	2 (2%)	4(4%)	0(0%)	6 (6%)
Other Staph. Spp	5 (5%)	3(3%)	0(0%)	8 (8%)
Mixed bacteria	8(8%)	12(12%)	28(28%)	48 (48%)
Total	34(34%)	34 (34%)	32 (32%)	100(100%)

3.2.4. Antimicrobial susceptibility test

In this study, five different antibiotic discs were used against each bacterial isolates of gram positive (*staphylococcus auerus* and other *staphylococcus* species) (Table 9) and gram negative bacteria (*E.coli* and *salmonella* species) (Figure 2). *Staphylococcus auerus* were 100% susceptible to penicillin, intermediate (90.3%) to vancomycin and (93.5%) resistant to tetracycline. 75% and 58.3% of the other staph staphylococcus species were intermediate to tetracycline and vancomycin, respectively. On the other hand, all of the *E.coli* that were isolated from the samples during the study period were resistant to tetracycline and 92.59% of these bacterial were also were resistant to sulphonamide.

Table 9: Antimicrobial susceptibility test result of staphylococcus species

Antibiotic agent	Disc potency	N	Staphylococcus auerus			N	Other staphylococcus		
			S	I	R		S	I	R
Penicillin	10 unit	62	100%	0%	0%	24	100%	0%	0%
Cloxacillin	5µg	62	90.3%	0%	9.7%	24	91.67%	0%	8.33%
Chloramphenicol	30µg	62	80.6%	0%	19.4%	24	95.8%	0%	4.2%
Tetracycline	30µg	62	6.5%	0%	93.5%	24	75%	0%	25%
Vancomycin	30µg	62	9.7%	90.3%	0%	24	58.3%	47.7%	0%

* N = no of isolates

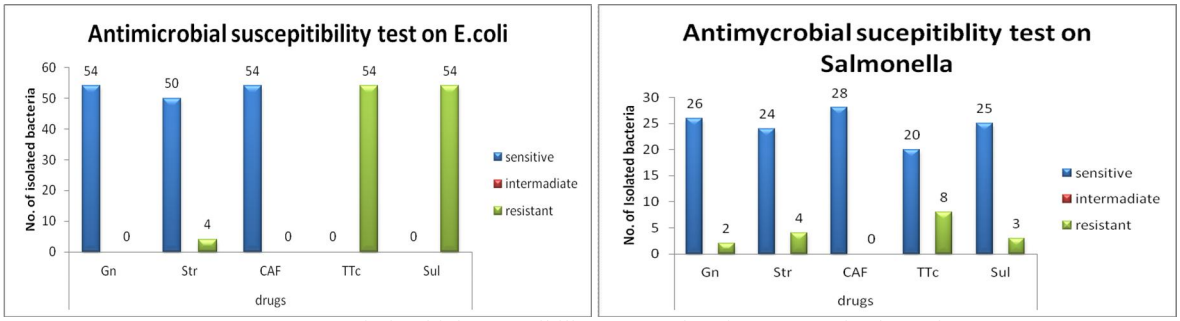

Figure 2: Antimicrobial susceptibility test results of gram negative bacteria

4. Discussion

Barn hygiene is important in maintaining the living environment of the animal. The current study is comparable to the study conducted by Abebe *et al*. (2012) in Ezha district of the Gurage zone, Southern Ethiopia who reported 11.7%, 39% and 47% cleaned the barn once per week, twice and three times per week, respectively. On the other side, the current study revealed that less frequency of cleaning their barns comparing to the reports by Meles *et al*. (2015) and zelalem (2012) as 75% and 87%, respectively who had the practices of cleaning their barns daily. This difference may be raised because of the respondents in the study area were kept their animal in open air or in their home vicinity which is difficult to clean the area regularly except those of dairy farm holders.

Most of dairy animal owners had the habit of washing the udder of cows before milking. Similar results were reported from North western Ethiopian highlands Yitaye *et al*. (2009), Alehegne, (2004) from debre ziet and Haile *et al*. (2012) from Hawasa. However, some reports by Meles *et al*. (2015) and Abebe *et al*. (2012) indicated that less habit of washing the udder of cows before milking. Most of the respondents used common close (towel) to dry the udder and teat of animals. This result is in line with report by Haile *et al*. (2012) from Hawassa. But, better udder drying practice than report presented by Tsegaye and Gebreegzher (2015) from Wolaita Zone, Southern Ethiopia.

Equipment used for milking, processing and storage determine the quality of milk and milk products. Accordingly to this study 87.5 % of the respondents were use plastic jars and 12.5 % of the respondents used metallic/ aluminum materials. Comparable figure 100 was reported by Abebe *et al*. (2012) where all of the households in the study area use plastic material and this study was greater comparable to study reported by Meles *et al*. (2015) where over 60 % of the responds were use plastic materials. To wash milkier hand, udder of their cow and equipments for storage and transportation of milk about 43.75 and 56.25 of the respondents were cold water and warm water with detergent soap/ omo, respectively. This is because most of the cows were kept in non-concrete type of house where there was no litter and to remove the contamination from the surface since they consider that most of the contamination was result from the environment. This is comparable with Yitaye *et al*. (2009) in north western, Ethiopian highlands and the reports of (Haile *et al*., 2012) from Hawassa, southern, Ethiopia. Majority (75%) of the respondent had deep well water for different purpose though the quality of water is not well known. This study is greater than study reported by Meles *et al*., 2015) but, disagree with the work done by Abebe *et al*. (2012) as majority of the respondent had access to river water.

In the current study, most of raw cow's milk shows better discoloration time which indicates low bacterial load. The result is not in line with study conducted by Worku *et al.* (2012) which had short discoloration time with poor grade. The difference could be due to the difference in hygienic practices such as using detergents to clean the material and the udder, care of animals and following of hygienic condition during milk production. The shorter time required for the disappearance of the blue colour is indicative of a higher microbial load (Bongard *et al*., 1995; Marker *et al*., 1997). This may be due to poor milk handling practices during milking, poor animal health services, and use of poor potable water which were linked to markedly high total bacterial count (Nandy *et al*., 2007).

The microbial content of milk indicates the hygienic levels during milking that include cleanliness of the milking utensils, proper storage and transport as well as the wholesomeness of the udder of the individual cow (Spreer, 1998). Standard plate count (SPC) is one of the most commonly used microbial quality tests for milk and milk products. The overall mean bacterial count of cow's milk in the study area was from 7.08 log10 (1.21x10 7) to 7.41 log10 cfu/ml (2.65x107) from different milk collection points and the result indicated high load of bacteria were obtained from milk vending shops and cafeterias.

The total aerobic bacterial count of this study was comparable figure with the study conducted by Beyene (1994) in Southern, Ethiopia that he got average aerobic bacterial count of 7.7log cfu/ml, Tola (2002) in Eastern, Wollega that he got average aerobic bacterial count of 7.4log10, Tassew and Seifu (2011) at Bahir Dar Zuria with the overall mean of 7.58log10*cfu*/ml, Worku *et al.* (2012) who reported bacterial count from 7.36 -7.88 log10 *cfu*/ml of raw cows' milk in Borana, Ethiopia and Mosu *et al.* (2013) at selected dairy farms in Debre Zeit town that he got average aerobic bacterial count of 7.07log cfu/ml. Moreover, this study was in line with study by Endale *et al.* (2013) where the overall mean bacterial count of cow's milk in mekelle was 7.39log10 cfu/ml at different points. However, the bacterial count obtained from current result was higher than that of work done by Ashenafi and Beyene (1994) reported as 6.32log10 cfu/ ml, Ombui *et al.* (1995) reported as 5log10 cfu/ml and Bonfoh *et al.* (2003) reported as 7 log10 cfu/ml). This is because of microbial load has highly associated with the hygienic condition practiced during harvesting to distribution process since the source of milk contamination is most of the time from the

external environment than within the udder of the animals.

In the current study, different bacterial isolate were detected from milk sample collected from different sources with higher prevalence of microbial contamination in the form of mixed bacterial infection (*S. auerus*, other staphylococcus species, *E.coli* and salmonella species). Similar species of microorganism were isolated by Merhawit *et al.* (*2014)* from Adigrat, Tigray, Ethiopia. *S. aureus, E. coli* and non-coliform bacteria like *Salmonella* and *Shigella* are some of the main bacterial pathogens associated with food-borne infections. Similar bacterial contaminants have been reported by other investigators in food, water and environmental samples (Haftu *et al*., 2012 and Haileselassie *et al*., 2012).

In the present study, *S. auerus* was the dominant bacteria isolated from the sample.

The study is similar with reports by Workineh *et al.* (2002) and Dego and Tareke (2003) from Addis Ababa and Southern, Ethiopia, respectively. In addition, this study was in line with researches done by Bitaw *et al.* (2010), Endale *et al.* (2013), Tesfaye *et al*. (2013) and Vadehi *et al.* (2013).

However, this study was comparatively higher than study reported by Amistu *et al*. (2015) from samples collected from different critical points in Oromia regional state to retail centers at Addis Ababa. This is because udder has a lot of micro flora that can capable of contaminating the milk besides, the environmental contaminants of the milk that result from hygienic practice followed during production system.

The antibiotic susceptibility test conducted in the current study revealed that, all of staphylococcus species isolated form milk sample were fully (100) susceptible to penicillin and followed by cloxacillin and chloramphenicol. All of the *E. coli* isolated was susceptible to gentamycin, chloramphenicol and streptomycin but resistant to tetracycline and sulphonamide. Moreover, all of the salmonella species isolated during study were susceptible to all drugs.

This study was in contrarily to Mueena *et al*., (2014) who reported that all of *S. auerus* isolates were found 100% resistant to Penicillin and Amoxicillin and Begum *et al*. (2007) revealed that *S. aureus* was 82.86% resistant to Penicillin-G. The difference could be raised from the strains of *staphylococcus*. This is may be because of, regular use of the drug in treating of animals that may result in development of resistance. This is carried on plasmids and transposons which can pass from one staphylococcal species to another (Werckenthin *et al*., 2001). However the study is in line with the study of Mueena *et al*. (2014) where *S. auerus* was sensitive to Cloxacillin (100%), and Abebe *et al*. (2013) showed the resistance of *S. aureus* to tetracycline (73.2%), in milk samples taken from dairy cows around Addis Ababa. This may be resulted from continuous use of tetracyline in animal treatment which may lead to development of resistant strains. Besides, majority of the *E. coli* and salmonella species isolated in the study area was susceptible to chloramphenicol, gentamycin and streptomycin. This study was similar with the study conducted by Singh (2011) who reported Chloramphenicol and Gentamicin as the best antimicrobial drugs against *E. coli* and Salmonella species. In addition, Rashed *et al*. (2011) reported Antibiotic resistance pattern of *E. coli* isolated from raw milk exhibited 100% resistance against Tetracycline.

5. Conclusion

The present finding indicated that, the bacteriological load obtained from different source of milk producers were higher which was mainly associated with the hygienic practice during collection, storage and distribution. Heavy contamination of milk sample with mixed bacterial isolate was encountered from milk vending shops and cafeterias. In addition, antimicrobial sensitivity test result showed that some isolated staphylococcus species were susceptible to penicillin. In the same way, *E.coli* and *salmonella* species isolate were susceptible to chloramphenicol. However, *S. auerus* and *E.coli* were resistant to tetracycline. In general, the higher bacterial load in the raw cow milk, the type of bacterial isolate and the increase resistance of bacterial isolate to locally available antibacterial agent have been observed. Therefore, proper strategies or corrective measures have to be implemented and designed in dairy production on milk handling and misuse of drugs in the study area.

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