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Bacteriological Quality Assessment of Milk in Dairy Farms, Cafeterias and Wholesalers in Adigrat, Tigray, Ethiopia

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Abstract: Culturally consumption of raw milk is common in Ethiopia and it is sometimes associated with bacterial diseases due poor hygiene practice. Hence, this study was conducted from November 2011 to April 2012 with the objectives of assessing the bacteriological quality and safety of raw milk and determining the antimicrobial resistance pattern of the bacterial isolates in different settings of Adigrat town. A purposive sampling approach was used to collect a total of 60 milk samples. Standard plate count test, bacterial isolation and antimicrobial susceptibility pattern of isolated bacteria was determined. The mean value of bacterial load was found higher in cafeterias ($2.3x10^9$CFU/mL) followed by milk vending shops ($4.9x10^8$CFU/mL). Within dairy farms the mean values of bacterial load was highest in large scale farms. Among the milk samples collected, 45% of the farm settings, 60% of milk vending shops and 75% of cafeterias were graded as poor quality. *E. coli* and *S. aureus* were the common isolates. Antimicrobial susceptibility pattern showed that all the isolated bacteria were 100% susceptible to gentamicin and kanamycin. However, they were 100% resistant to penicillin G and amoxicillin. The quality of milk consumed in the study area was found inferior quality. Thus, awareness should be strengthened on hygienic methods of production, handling, transportation and distribution of milk among producers, milk vending shops and consumers in the town.

Key words: Adigrat · Antimicrobial Susceptibility · Bacterial Load · Cafeteria · Milk · Milk Vending Shop

INTRODUCTION

These days' food-borne diseases are becoming major public health concern worldwide particularly in the developing world. Among these food-borne health concerns, consumption of contaminated raw milk either from infected cows or due to poor hygiene during handling, transportation and processing are prior issue in developing countries. The long tradition of consumption of fresh and fermented raw milk products was subject to an important change in the late 19th century, as the developed countries began wide-scale pasteurization of milk to eliminate zoonotic bacterial pathogens such as *Mycobacterium bovis* [1]. Despite milk and milk products are considered as a complete diet in human nutrition, ensuring high hygienic quality of the products is very critical. As milk is a suitable substrate for microbial growth and development because of its fluid and semi-fluid nature and chemical composition [2, 3]. However, achieving this hygienic quality in the developing world and especially in the hot tropics is the major challenge.

Milk is synthesized in specialized cells of the mammary gland and virtually sterile when secreted into the alveoli of the udder. Beyond this stage of milk production, microbial contamination can generally occur from three main sources; within the udder, exterior of the udder and during surface milk handling and storage [4]. The major factors for contamination of milk and its products include the housing condition and management of dairy farms, level of hygiene in the dairy environment and hygienic standards of milk handling, transportation and storage conditions.

Although the counts can decrease to lower level after pasteurization, various bacterial pathogen isolates had been reported from raw milk samples taken from udder, milking bucket, storage container, at processing plant and milking environment [5]. In the developing

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world milk is subjected to more contamination during long distance transportation under high ambient temperature and without cold-chain facility and using materials which are not airtight [5]. Therefore, regular assessment of the bacteriological quality and safety of milk at all levels of value chain is important to safeguard the health of the community.

Wide spread reliance on antimicrobials in food animal production in the developing world has resulted in an increase in resistant strains of bacteria and then complicated treatment of infectious diseases in animals and humans [6-8]. In developing countries like Ethiopia there is no rule and regulation on the use of antimicrobials in food animals and because of this there is indiscriminate use of antibiotics in the country in the livestock industry.. Selection pressure through sustained use of antimicrobial agents at sub therapeutic concentrations in animal production systems/dairy cattle could result in the emergence, maintenance and horizontal transfer of antimicrobial resistance in commensal and pathogenic bacteria and favor genetic exchange of antimicrobial resistance determinants involving commensal bacteria [9, 10]. Antibiotic resistant strains of the bacteria currently pose a great public health hazards.

Tigray regional bureau of health has put different program initiatives in place to decrease possible food borne diseases. Regulatory enforcement and promotional activities are among the initiatives; however, lack of proper information flow on possible food contaminant sources and lack of integration with the livestock sector critically hinders the ability of the bureau to effectively regulate the situation. As a result studies in the region are indicating different bacterial pathogen isolates as in indication for poor quality of milk and other food sources [11, 12]. Even though, Adigrat town is one of the business centers in Tigray region, there is detailed assessment on the bacteriological quality and safety of milk produced and consumed in the town. Therefore, this study was initiated to assess the bacteriological quality and safety of raw milk produced and consumed in the town and to determine the antimicrobial resistance pattern of the bacterial isolates in different settings of town.

MATERIALS AND METHODS

Study Area: The study was conducted from November 2011 to April 2012 in Adigrat administrative town located in Eastern zone of Tigray National Regional State with a total human population of 57,588 (45.2, 54.8% male and female, respectively). Adigrat is situated at 115 Kms North East of Mekelle and 898 Kms North of Addis Ababa, capital city of Ethiopia. Geographically Adigrat is located at a latitude of 14° 16' North and 39° 27' East and with an altitude of 2497 meters above sea level (m.a.s.l). The mean annual rain fall ranges between 400 mm and 600 mm with a mean annual temperature range of 9.28 - 21.94°C. Agro climatically Adigrat is 80% middle land and 20% high land (BoFED, 2007, unpublished).

Study Methodology

Collection of Samples and Handling Procedures: A total of 60 (20 dairy farms, 20 milk vending shops and 20 cafeterias) study units were selected purposively on the voluntary basis to provide milk samples, accessibility to transportation facilities and level of consumers using the product. The dairy farms were categorized into three groups based on number of dairy cattle. Thus dairy farms having less than or equal to 5 dairy cattle were categorized as small scale, 5 to 10 dairy cattle as medium scale and greater than 10 dairy cattle as large scale dairy farms [13]. Accordingly, out of the 20 dairy farms included in this study, 4, 6 and10 were small, medium and large scale dairy farms, respectively.

15-20 mL of milk samples were collected from dairy farms, milk vending shops and cafeterias using sterile test tubes. Thus, a total of 60 pooled samples were collected. Each milk sample was labeled and placed in icebox and transported to Mekelle University College of Veterinary Medicine Microbiology Laboratory. The samples were kept in refrigerator at 4°C and culturing was conducted within 24 hours [14].

Bacterial Load Assessment in Milk Sample: Standard plate count (SPC) method was used in assessing the number of viable bacteria in milk and graded in to different categories according to its bacterial content. Serial tenfold dilution up to 10^7 dilutions was prepared for each sample using 0.85% sterile saline solution. Pour on plate method was used to make viable count. After incubation of 24-48 hours, plates from the different dilutions having bacterial colonies ranging from 30 – 300 were counted using the illuminated colony counter. The counts for each plate were expressed as colony forming unit of the suspension (CFU/mL) [15].

Bacteriological Examination of Milk Sample: Milk samples were graded as very good if the bacterial count did not exceed $2x10^5$CFU/mL, well if it was between $2x10^5$ and $1x10^6$ CFU/mL and fair if the count was between $1x10^6$ and $5x10^6$ CFU/mL of milk. While, samples showed bacterial count above $5x10^6$ CFU/mL were graded as poor quality [16].

RESULTS

Bacterial Load and Quality of Milk Samples: The present study indicated the presence of bacterial contaminants in milk samples collected from dairy farms, milk vending shops and cafeterias (Table 1). Highest mean bacterial count was observed in cafeterias followed by milk vending shops. In comparison within dairy farms, the highest mean bacterial count was recorded in large scale dairy farms.

Milk samples with a bacterial load ranging from $1x10^6$ to $5x10^6$CFU/mL were considered as poor quality but samples with bacterial load of less than $2x10^5$CFU/mL were graded as good quality (Table 2). Of the total milk collected 45% from the different farm settings, 60% from milk vending shops and 75% from cafeterias were graded of poor quality.

Bacteriological Examination: The milk samples with high bacterial load and graded as poor quality were further processed for bacteriological examination using different selective media and biochemical tests. Accordingly, different bacterial species with their respective prevalence rate were recorded (Table 3).

Antimicrobial Susceptibility Test: Antimicrobial susceptibility test was performed to all bacterial isolates (n = 29). All the isolated bacteria were found 100% susceptible to gentamicin and kanamycin. However, they were 100% resistant to penicillin G and amoxicillin. 70% of *Staphylococcus* species were intermediate and susceptible to polymyxin B and streptomycin, respectively. All *Streptococcus* species were resistant to polymyxin B and completely susceptible to streptomycin (Table 4).

Table 1: Summary of bacterial count from milk samples

Source of sample	No of Sample	Average count	Minimum count	Maximum count	Mean/Std deviation	*p-value*
Dairy farm						
SS	4	2.4×10^7	1.49×10^7	3.05×10^7	7.36/0.139	
MS	6	5.6×10^7	5×10^7	6.07×10^7	7.74/0.03	< 0.001
LS	10	8.4×10^7	7.4×10^7	9.9×10^7	7.92/0.05	
Total	20	6.37×10^7	1.49×10^7	9.9×10^7	7.75/0.23	
Milk shops	20	4.59×10^8	1.05×10^7	8.9×10^8	8.14/0.42	
Cafeterias	20	2.34×10^9	1×10^8	9.95×10^9	9.18/0.40	< 0.001

SS = Small Scale, MS = Medium Scale, LS = Large Scale

Table 2: Quality of milk samples tested on the basis of bacterial load

		Milk Quality grade	
Site of collection	Sample size	Good quality	Poor quality
Dairy farm			
SS	4	3 (75%)	1 (25%)
MS	6	4 (66.6%)	2 (33.3%)
LS	10	4 (40%)	6 (60%)
Total	20	11 (55%)	9 (45%)
Milk vending shops	20	8 (40%)	12 (60%)
Cafeterias	20	5 (25%)	15 (75%)
Total	60	24 (40%)	36 (60%)

Table 3: Frequency of bacterial species isolated from milk samples

Bacterial species Isolated	Dairy farm	Milk vending shops	Cafeterias	Total Samples Positive no. (%)
S. aureus	1	2	3	6 (20.0)
Staphylococcus species other than *S. aureus*	1	1	2	4 (13.3)
Streptococcus species	1	1	2	4 (13.3)
E. coli	1	1	4	6 (20.0)
Other *coliform bacteria*	1	1	2	4 (13.3)
Non coliform bacteria	1	1	3	5 (16.6)
Total	6	7	16	29

Table 4: Antimicrobial resistance profiles of *Staphylococcus* and *Streptococcus* species

Bacterial	*Staphylococcus* species (n=10)			*Streptococcus* species (n=4)		
Antimicrobial pattern	R	I	S	R	I	S
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
Amoxicillin	10 (100)	0	0	4 (100)	0	0
Tetracycline	1 (10)	6 (60)	3 (30)	0	1 (25)	3 (75)
Streptomycin	1 (10)	2 (20)	7 (70)	0	0	4 (100)
Polymyxin B	0	7 (70)	3 (30)	4 (100)	0	0
Pencillin G	10 (100)	0	0	4 (100)	0	0
Kanamycin	0	0	10 (100)	0	0	4 (100)
Gentamicin	0	0	10 (100)	0	0	4 (100)

R = resistance, I = intermediate, S = susceptible

Table 5: Antimicrobial resistance profiles of bacteria under *Enterobacteriaceae* family

Bacterial	*E. coli*(n=6)			Other coliform (n=4)			Non- coliform(n=5)		
Antimicrobial pattern	R	I	S	R	I	S	R	I	S
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
Amoxicillin	6 (100)	0	0	4 (100)	0	0	5 (100)	0	0
Tetracycline	0	2 (33.3)	4 (66.6)	2 (50)	1 (25)	1 (25)	0	2 (40)	3 (60)
Streptomycin	1 (16)	2 (33.3)	3 (50)	0	1 (25)	3 (75)	0	2 (40)	3 (60)
Polymyxin B	1 (16)	4 (66.6)	1 (16)	3 (75)	1 (25)	0	3 (60)	2 (40)	0
Pencillin G	6 (100)	0	0	4 (100)	0	0	5 (100)	0	0
Kanamycin	0	0	6 (100)	0	0	4 (100)	0	0	5 (100)
Gentamicin	0	0	6 (100)	0	0	4 (100)	0	0	5 (100)

66.6% *E. coli* showed intermediate resistance to polymyxin B and susceptible to tetracycline. 75% of other coliform bacteria were resistant to polymyxin B and susceptible to streptomycin. 60% of the non-coliform bacteria were resistant to polymyxin B (Table 5).

DISCUSSION

Cow's milk may be contaminated from different sources and at different processes. The results obtained from the present study on the bacteriological quality of raw milk also justify the contamination of milk with bacterial organisms at different stages of the value chain. The results of the current study indicate that the situation is critical and needs real improvement. According to the information of the dairy farm owners, most of their workers are not aware of the hygienic handling of milk and they are not trained in the processing and handling of milk aseptically.

Compared to the three study targets, the highest mean value of microbial load (2.34×10^9 CFU/mL) was recorded from cafeteria followed by milk vending shops (4.59×10^8 CFU/mL). This could be due to high exposure to dusts and pathogenic organisms from the environment, cleanliness of transporting materials and handlers. These values exceed the WHO standard limit for food products and water [17] and the mean value showed a significant difference between the different settings/target groups ($p = 0.001$). World Health Organization highlighted in its report that food prepared in large quantities are liable to contamination. The need for milk products is forcing the dairy producer to increase their production capacity. Though the dairy establishments offer good economic opportunities, milk supplied to the consumers should also get proper handling and preservation in order to safeguard public health. The present finding is in line with the report of Wubete [5] on the bacteriological quality of milk from dairy operations. The main reasons associated with the higher level of bacterial contamination of the milk in these settings could be due to the lack of knowledge on proper handling of milk, less hygienic conditions in the environment, poor interior quality of material used for milk transportation and storage, lack of proper transportation facilities (cooling system and time) and water supplied for washing utensils.

With regard to the different farming systems visited during the study period, the highest mean of total viable count of microbial load was observed in large scale dairy farms (8.4×10^7CFU/mL) followed by medium scale dairy

farms ($5.6x10^7$ CFU/mL) and the mean difference is significant ($p = 0.001$). Furthermore, according to standards of raw milk quality [16] of the TAPC, 25, 33.3 and 60% of the raw milk samples collected from small scale, medium scale and large scale dairy farms respectively indicating a poor grade quality. This might be due to poor husbandry management, poor hygienic conditions and environmental factor that facilitate easy access of the organisms due to close contact. Additionally, environmental contamination of the milk supply at farm level due to general hygienic failure such as degree of cleanliness of facilities and animals, milking equipment and, conditions of milkers are among the major factors that contribute to the deterioration of milk quality. The probable reason for higher load of bacteria in large scale farms could be due to involvement of many personnel in the milking and handling process of milk, very close contact of animals due to limited space and bulk collection of milk i.e. if one cow is infected, it can contaminate the whole bulk milk.

With regard to isolation of bacterial pathogens from the three sampling farms, the highest bacterial isolates were recovered from cafeterias and milk vending shops. The marketing condition which is unhygienic practicing of the employee milk sellers could lead to recontamination of the milk and resulted in higher load and isolates of bacteria [3]. The findings of the present study are in agreement with other observations regarding the contamination of milk and other ready to eat food stuffs [11, 18-20].

The bacteriological quality and safety of milk is not only affected by the bacterial counts, but also the type and strain of the bacteria are very important. The bacterial contaminants isolated during the present study include *S. aureus,* non *S. aureus* species*, Streptococcus* species*, E. coli,* other coliform bacteria and non-coliform bacteria. *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 [3, 11, 12, 19-23]. *E. coli,* other coliform and non-coliform bacteria were the most frequently isolates in the present study and this is also supported by other findings [3, 20].

The antimicrobial susceptibility test carried out in this study indicated that all isolated bacteria were 100% susceptible to gentamicin and kanamycin. This could be due to the fact that less application of these antibiotics in the study area. The resistance (100%) of the isolated microorganisms to penicillin G and amoxicillin reported is also in agreement with the higher reported resistance of bacterial isolates to amoxicillin/ampicillin [11, 12]. The resistance of *S. aureus* to penicillin and amoxicillin may be attributed to the production of beta-lactamase, an enzyme that inactivates penicillin and closely related antibiotics. Moreover, the probable reasons for the development of resistance by these organisms are associated with prolonged and indiscriminate usage of these antimicrobials in the study area.

CONCLUSION

The results obtained in this study showed that milk available to the consumer in Adigrat town has low quality considering the different stepwise contamination of the milk. The quality of milk produced and channeled starting from the different dairy farm settings in the study area was substandard. The major factor that contributed to poor quality of milk in the study area is due to less hygienic standard, due to lack of awareness and carelessness of employee and absence of strict sanitation control measures. Therefore, in order to improve the quality and safety of milk produced and distributed to the consumers in the study area awareness creation programs should be initiated to dairy industries, milk distributors, restaurants and consumers. Furthermore, establishment of operation standards and strict enforcement of regulatory measures on hygienic standards are of critical importance. To reduce the widespread of antimicrobial resistance it is important to implement systemic application of an *in vitro* antibiotic susceptibility test prior to the use of antibiotics in farm animals and also developing and monitoring standards on the use of antibiotics in farm animals.

Competing Interests: We declare that we do not have competing interest on all activities pertaining this research work.

Authors' Contribution: Merhawit Reda, Habtamu Taddele, Berihun Afera and Abrha Bsrat conceived the investigation; MR generated the idea, performed the field and laboratory experiments, analyzed the data and prepared the paper. HT, BA and AB facilitate and participated in the investigation and supervision of all activities, read and improving of the manuscript.

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