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RESEARCH ARTICLE

BACTERIOLOGICAL QUALITY AND DETECTION OF BOVINE MASTITIS PATHOGENS OF MILK SOLD IN JIMMA TOWN

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ABSTRACT

Milk is a food that inherently favors the microbial growth. There are many sources of contamination of milk including the cows itself, the environment water, milking processes and equipments. The varied routes for the introduction of pathogens into milk preclude the production of milk that can be guaranteed to be safe for human consumption. The objective of this study was to evaluate the microbiological safety and to detect mastitis in milk that sold in Jimma town, south western Ethiopia. A total of 90 samples were collected from some randomly selected restaurants in the town. Standard methods were used for the enumeration of aerobic mesophilic bacteria, coliforms, aerobic bacterial spores, lactic acid bacteria, yeast and molds. Isolation and identification of the most important mastitis bacterial pathogens (*Staphylococcus aureus* and *Streptococcus agalactiae*) were also done. The data was analyzed by using SPSS version 15 and presented by using tables and graphs. The result of the study showed that the mean microbial counts were dominated by aerobic mesophilic bacteria (7.5 ± 0.8), lactic acid bacteria (6.6 ± 0.6), coliforms (5.9 ± 0.4), yeasts (5.1 ± 0.5) aerobic bacterial spores (4.2 ± 0.4) and moulds (3.7 ± 0.6). On the other hand, two most important mastitis pathogens were isolated and identified. Generally the microbial safety of milk sold in Jimma town was poor and it calls for special attention especially those concerned bodies inhabit in the town.

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INTRODUCTION

Milk which is a lacteal secretion of the mammary gland of mammals is a major component in human diet in all over the world and it has long been considered as a higher nutritious and valuable food that can be consumed by millions of people's daily in a variety of different products (Azhari *et al.*, 2010). Its nutrient composition makes it to serve as a good and ideal medium for the growth of many different kinds of microorganisms (Yigerm *et al.*, 2008), especially those pathogenic bacteria and therefore it can be considered one of the most perishable agricultural product because it can also easily be contaminated (Breyan, 1983). The safety of milk is considered very essential to the health and welfare of the people. Milk often contains microorganisms which causes all cases of dairy illness. Most of these microbes are continued to be of bacterial origin i.e. pathogens that have involved in communicable diseases associated with the consumption of Milk (Azhari *et al.*, 2010). Because of its specific production, it is impossible to avoid contamination of milk with microorganisms, and therefore, the microbial content of milk is the major feature in determining its quality i.e. the number and type of microorganisms in it immediately after its production (Ahmed, 2010). According to Lingaturia *et al.* (2011), microorganisms may gain entry into milk directly from dairy

cows experiencing clinical and sub-clinical mastitis which usually considered the most costly diseases of dairy cattle. *Sub-clinical mastitis* is considered the most economically important type of mastitis because of its long term effects on total milk yields. Production loss due to sub clinical mastitis was recently estimated to cost the dairy industry about 1 billion dollar annually, and additional costs of mastitis are due to the failure to receive quality premiums from milk purchasers. The other way that microbes get contaminate milk is from the farm environment particularly the water resource and utensils used for the storage of milk on the farm and during transportation of the product to the final receiver.

Fresh milk which drawn from a healthy cow normally contains a low microbial load (less than 100 ml^{-1}), but the load may significantly increase up to 100 times fold or more, once it is stored for some time at normal temperature. However keeping milk in clean containers at refrigerated temperature immediately after production process may delay the increase of initial microbial load and prevent the multiplication of microorganisms in milk between production at the farm and transportation to the required area (Ahmed, 2010). In Ethiopia, one of the most major developing country, dairy production is an important part of the livestock production system in urban and pre-urban sectors of the constitute of the agricultural production and market oriented small holder dairy farming is an emerging business which is becoming an important supplier of milk and milk products to the population. For small holder

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farmers during provides the opportunity to effective use of land, labor and feed resources that generates regular incomes (Wubete, 2004; Tolosa et al., 2010). Milk and milk products are economically important farm commodities and dairy farming is an investment option for many peoples. Currently the trend of rapidly increasing human population together with growing urbanization creates even greater markets and increased of demand for milk and its products (Haile et al., 2010). In Jimma town also market oriented small beholder dairy farming is at its immature stage to supply the milk demand to the ever-growing population in the town (Tolosa et al., 2010). The microbiological safety of milk its products in the town is unsatisfactory due to many production constrains, mainly reproductive health problems that forms the bottleneck in the production process and productivity in the livestock sub-sectors. Therefore, it is justifiable to generate scientific information on the safety of dairy products in the town (Gashaw et al., 2011). In the previous years several researches were done in Jimma town to assess the microbiological safety of milk due to that milk and milk products are being the major sources of food and nutrients for the inhabiting population of the town. Despite the work of researchers on assessing the microbiological safety of milk in the town, no current research was done that investigates and detects the microbial pathogens that causes mastitis (an inflammatory disease of the udder in mammals) that pose a great deal of destructions and spoilage in milk and its products. Therefore, to fill this gap the study was conducted to investigate the microbiological safety, in special reference to detect pathogenic microbes that cause mastitis in the under of mammals. The main objective of this study was to evaluate the microbiological safety and to detect mastitis in the milk that sold in Jimma town, southwestern Ethiopia.

MATERIALS AND METHODS

Study Area Description

The study was conducted in Jimma University, at Jimma town, which is located south west of Addis Ababa. The town is located 70°41' N latitude, 360° 50' E longitude; the study area has an average altitude of 1,780m above sea level. It lies in the climatic zone locally known as "Woynga Dada" (1,500-2,400m above sea level) which is considered ideal for agriculture as well as human settlement. The town is generally characterized by warm weather with a mean annual maximum temperature of 30°C and a mean annual minimum temperature of 14°C. The annual rainfall ranges from 1138-1690mm. The maximum precipitation occurs during the three months period (June, July and August), with minimum rainfall occurring in December and January. From a climatic point of view, abundant rainfall makes this area one of the best watered of Ethiopian highland areas, conducive for agricultural production (Alemu et al., 2011).

Sample Size and Sampling technique

A total of 100 samples were collected from thirty restaurants found in Jimma town from March to April 2013. The samples were purchased and collected in the time between 7 pm to 9 pm using sterile containers maintaining sterile conditions. The microbial analysis was conducted within one to three hours of collection and the samples were kept in the refrigerator at 4°C until microbial analysis was conducted.

Microbial analysis

From each sample 10ml of milk was mixed with 90 ml of distilled water and homogenized in a flask for five minutes using shaker at 160 rpm. The homogenates were serially diluted from 10⁻¹ to 10⁻⁵ and a volume of 0.1 ml aliquot of appropriate dilution was spread plated on pre-solidified plates and incubated at 30±5°C. The colonies were counted from plate containing microbial colonies between 30 and 300. The counted colonies were expressed in colony forming units per milliliter (CUF/ml) and later converted to log CFUml⁻¹, by using the formula.

$$\text{CFUml}^{-1} = \frac{\text{number of colonies counted from plates (mean)}}{\text{Dilution factor} \times \text{volume plated}}$$

Microbial enumeration and Identification

From the appropriate serial dilution total Aerobic Mesophilic counts (TAMC) on plate count agar (PCA) (Weil et al., 2006), aerobic bacteria spore count on (PCA) (Acco et al, 2003); Count of Enterobacteriaceae on violate red bile glucose agar (VRBA) (Weil et al., 2006); counts of lactic acid bacteria on Mann rogosa Sharpe (MRS) agar media, (Harrigan and maccnce, 1976); Count of coliform on violate red bile agar (VRBA) (Weil et al., 2006); and Counts of yeast and molds on potato dextrose agar supplemented with 0.1g chloramphenicol (Spencer et al., 2007) were done with appropriate temperature.

Biochemical analysis

After enumeration of aerobic mesophilic bacteria, 10 to 15 colonies with distinct morphological such as color, size and shape were picked from countable plates and aseptically transferred into a tube containing 5ml nutrient both. The inoculated cultures were incubated at 35°C for 24 hrs cultures were purified by plating and preserved on slants at 4°C. Finally the obtained organisms were characterized based on John (2012) bacterial classification manual with gram staining, (Gram, 1884, Gregerson, 1987); endospore test; KOH-test, (Gregerson, 1987); catalase test, (Mac Faddin, 1980); Coagulase test, (cheese brought 2006), and IMViC tests (cheese brought 2006).

Detection for mastitis

Detection of *Staphylococcus aureus*

For isolation purpose, 0.1ml appropriate dilutions of the aliquot were spread- plated onto a sterile per-solidified Blood Agar Plate (BAP) and incubated at 37°C for 48 hrs. presence of *Staphylococcus aureus* on the basis of their morphological aspects of BAP and then set b- cultured on nutrient Agar plate and incubated at 37°C for 24- 48 hrs to get a pure culture. The pure isolates on the nutrient agar plate (NAP) were preserved and maintained for biochemical test differentiation and characterization. Final identification of *S. aureus* was done based on gram- staining, catalase tests, sugar fermentation and coagulase test (National Mastitis Council, 2004).

Detection of *streptococcus agalactiae*

0.1 ml appropriate dilutions of the aliquot was spread- plated on to a sterile per- solidified Blood Agra plate and incubated

at 37⁰C for about 48 hrs. After incubation the suspected *Streptococcus agalactiae* colonies were sub cultured on nutrient Agra plate (NAP) and incubated at 37⁰C for 24-48 hrs to get a pure culture. The pure isolates on the NAP were preserved and maintained for biochemical test differentiation and characterization. The final identification of *Streptococcus aralactiae* was done based on gram- staining, catalase tests and growth (production) on 5 ml nutrient broth containing 6.5% Nacl solution (Wilson, 1997; National Mastitis Council, 2004).

RESULT

Microbial counts

From the total of 100 samples analyzed 118 bacterial strains of aerobic mesophilic bacteria, 105 bacterial strains of lactic acid bacteria and 84 bacterial strains of coliforms with a total number of 307 bacterial strains were isolated (Table 3). The isolates were identified, characterized and grouped in genus level based on Johns (2012) bacterial classification systematic. From these isolates gram-negative bacteria (52.8%) were dominated the gram-positive bacteria (47.2%). The predominant bacterial group was found to be *Bacillus spp.* (33.9%) followed by *Lactobacillus spp.* (32.4%), *Escherichia spp.* (30.9%) and *Lactococcus spp.* (27.6%).

mean counts of bacteria and fungi (yeasts and Moulds) were above the permissible level (Table 2). The mean counts of aerobic mesophilic bacteria (AMB) was the highest (7.5 log CFU/ml).

Table 3. Microbial strains and their frequency distribution

Micro organisms	Bacterial spp	Total number	(%) frequency
Aerobic Mesophilic Bacteria	Bacillus	40	33.9
	Aeromonas	32	27.1
	pseudomonas	29	24.6
	Acinitobactor	17	14.4
	Total	118	100%
Lactic Acid Bacteria	Lactobacillus	34	32.4
	Lactococcus	29	27.6
	Enterococcus	23	21.9
	Aerococcus	19	18.1
	Total	105	100%
Coliforms	Escherichia	26	30.9
	Citrobactor	22	26.2
	Enterobactor	19	22.6
	Klebsiella	17	20.3
	Total	84	100%

Following aerobic mesophilic bacteria, the mean count of lactic acid bacteria (6.6 long CFU/ml) and yeasts (5.9 log CFU/ml) occupied the second and third ranks and those of

Table 2. Mean Microbial counts (CFU/ml and LogCFU/ml) of milk in Jimma town, south western Ethiopia

Type of Microorganisms	Microbial mean count (CFU/ml)	Microbial mean count (LogCFU/ml)	Microbial mean count (Log CFU/ml ± SD)
Aerobic Mesophilic bacteria	3.23x 10 ⁷	7.5	7.5 ± 0.8
Lactic acid bacteria	4.5x10 ⁶	6.6	6.6 ± 0.6
Coliforms	8.9 x10 ⁵	5.9	5.9 ± 0.4
Yeasts	1.58x10 ⁵	5.1	5.1 ± 0.5
Aerobic bacterial spores	1.81x10 ⁴	4.2	4.2 ± 0.4
Moulds	5.11x10 ³	3.7	3.7 ± 0.6

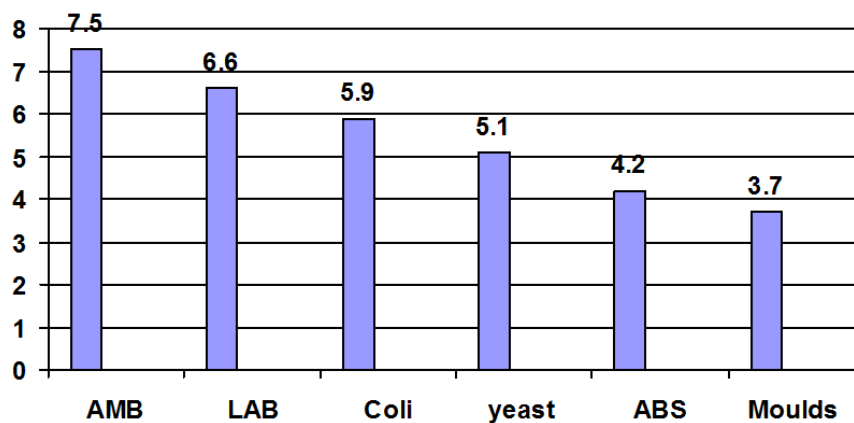


Figure 1. Mean microbial counts of milk samples collected form Jimma town, southern Ethiopia

In the present study, the mean count of aerobic mesophilic bacteria (AMB) was the highest (3.23x10⁷ CFU/ml) where as the lowest mean count was observed in Moulds (5.11x10³ CFU/ml). The mean counts of lactic acid bacteria (LAB) occupied the second rank that was (4.51x10⁶ CFU/ml) following aerobic mesophilic bacteria counts and was followed by the mean count of yeasts (8.9 x10⁵ CFU/ml). The mean count of coliforms and aerobic bacterial spores occupied the fourth and the fifth ranks (1.58x10⁵CFU/ml and 1.81x10⁴ CFU/ml respectively with a total mean count of 6.3x10⁶ CFU/ml. in general the present study indicates that all the

coliforms (15.1 log CFU/ml) and aerobic bacterial spores (4.2 log CFU/ml) occupied the fourth and the fifth stages respectively (Figure 1) Where AMB-Aerobic mesophilic Bacteria, LAB= Lactic Acid Bacteria, coli-coliforms, ABS-aerobic bacterial spores former.

Biochemical identification of Microorganisms

The isolated and purified microorganisms through repeated sub-culturing on nutrient agar media were subjected to different biochemical tests and identified based on john's bacterial identification systematic in the genus level and

presented in their frequency distribution. The table below shows that the predominant micro flora of milk in the present study was *Bacillus spp.* (33.9%) followed by *Lactobacillus* (32.4%) and *Escherichia spp.* (30.0%) where as the lowest bacterial strains found from the present study was *Acinetobacter spp.* (14.4%) followed by *Aerococcus spp.* (18.1%).

(6.4 log CFU/ml) in milk from Egypt and this mean count of coliforms is much higher than the acceptable standard mean of count given by United States to be < 2 log CFU/ml. The occurrence of coliforms in milk may therefore be considered as a real indicator of the fecal contamination with the possibility existing associated pathogens and also the public

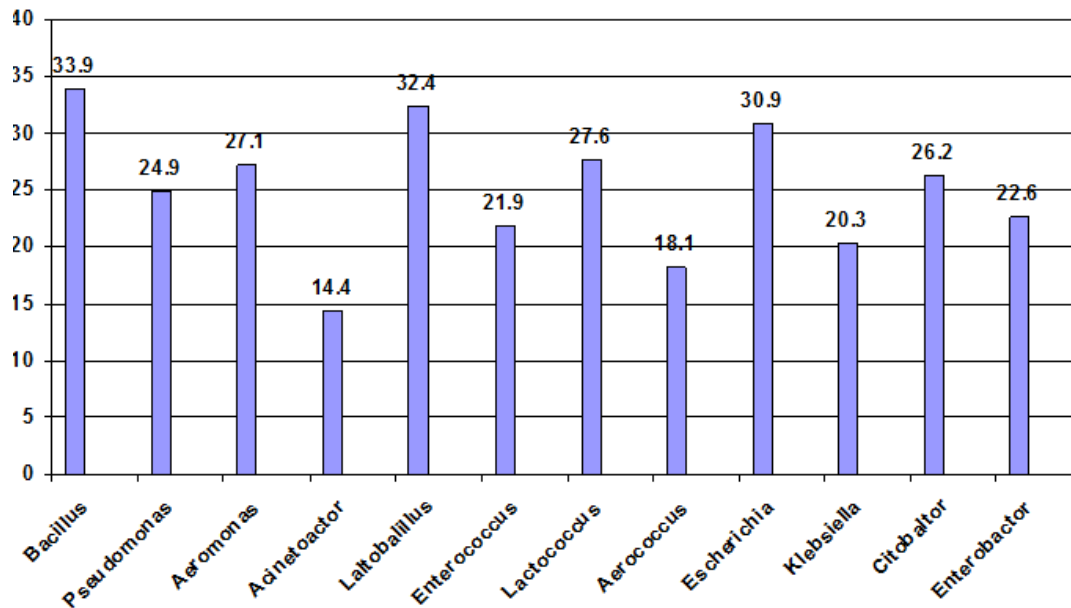


Figure 2. Microbial strains and their frequency distribution, Jimma town, south western Ethiopia

DISCUSSIONS

Dairy farming is a growing sector in many developing countries like Ethiopia. It assures food security for low-income urban and semi-urban population and provides a livelihood for along number of peoples who would otherwise be unable to establish other businesses (Aseffa, 2010). In contrast to this potential benefit, the quality of milks is often poor and lacks the appreciation for safe consumption, and as a result milk is perceived to be one of the major public health risk (WHO, 1996). Furthermore there was significant report of health problems that have been associated with milk and its products as reported by (Mossel, 1982) from Netherlands. In the present study, the microbiological safety of milk that sold in Jimma town was determined by the enumeration of aerobic mesophilic bacteria (7.5 log CFU/ml), Yeasts (5.1 log CFU/ml), Lactic acid bacteria (6.6 log CFU/ml), coliforms (5.9 log CFU/ml). Aerobic bacteria spores (4.2 log CFU/ml) and Moulds (3.7 log CFU/ml). The mean value of aerobic mesophilic bacteria count (7.5 log CFU/ml) in milk samples collected from Jimma town was in agreement with the findings of Mutukumira *et al.*, (2001) who found the mean count of (7.4 log CFU/ml) in milk samples collected from Khartoum state. However, according to the United States maximum standard limit of the mean value of aerobic mesophilic bacterial count (2.9 log CFU/ml-5.7 log CFU/ml) the recorded mean of these bacterial groups in the present study is above the acceptable level and so it is unsatisfactory level. This could be originated from lack of hygienic practices during preparation procedures. The mean count of coliforms in the present study is in line with Mossel (1922) who reported mean count of coliforms (5.8 log CFU/ml) in milk from Khartoum state. In contrast to the present study, Majer *et al.*, (1973) reported higher number

health hazards of *E. coli* has been emphasized by many authors because these bacterial groups have been implicated human cases of gastroenteritis, epidemic diarrhea in infants, sporadic diarrhea in adults as well as in cases of food poisoning (Mossel, 1982). The aerobic bacterial spore (ABS) count (4.2 log CFU/ml) of the this study is somewhat lower as compared to findings reported by Ahn *et al.*, (2007) who found a mean count (6.4 log CFU/ml) in milk from United States. According to Cornell University dairy food science the maximum standard limit of aerobic bacterial spore count in milk should be (4.3 log CFU/ml) but the present study mean count is above the maximum standard level and is thus unsatisfactory. The higher counts of aerobic bacterial spores in the present study could be due to the high temperature resistant nature of bacterial spores in the milk.

The mean counts of lactic acid bacteria (LAB) in the present study was (6.6 log CFU/ml). This is in line with the microbiological studies made on milk samples in Nigeria (Cheniguen *et al.*, 2007), whose counts were 6.4 log CFU/ml. However, according to the South Africa maximum standard level of lactic acid bacterial count in milk (5 log CFU/ml), the mean count of the present study is above the permissible level and is unsatisfactory. In the present study the mean count of yeasts and those of Moulds were 5.1 log CFU/ml and 3.7 log CFU/ml respectively. Yeasts are not commonly the cause of defect in dairy farming unless they ferment lactose found in milk. In this case they can grow rapidly and produce a characteristic yeasty or fruity flavor and obvious gas (Davis and Wilby, 1990). Yeasts also produce toxic effects against undesired microorganisms in the intestinal tract (Jacobsen and Naruijvo, 1996). Most importantly the total microbial counts are used for estimate viable microbial population in milk and

reflect the hygienic practices used in the production and handling of milk (Moughtby *et al.*, 1994). On the other hand, pathogenic bacteria could also present in milk as a direct consequence of udder diseases in dairy hared mammals; this disease is commonly called mastitis which is an inflammation in the mammary tissue of milk producing mammals that is often caused by bacterial infection. Among the most important mastics causing bacterial pathogens *Staphylococcus aureus* and *Streptococcus agalactiae* occupies the first and second rank respectively. These bacterial pathogens cause a contagious mastitis that is transferred from one individual cow to another during infection (Sinell, 1973). In the present study, these two most important mastitis causing pathogenic bacteria species were isolated and identified according to the National mastitis council, (2004) mastitis detection procedure. The presence of mastitis in milk shows the high level of poisoning of the milk that emanated from the cows udder itself which have been infected with these bacterial pathogens. Mastitis pathogens in milk pose a higher threat to public heath if milk is not pasteurized properly. On the other hand the improper use of antibiotics to eliminate mastitis pathogens is another public heath concern. The careless therapy with antibiotic against mastitis can read to this residue in milk but very little information is available concerning the effect of mastitis treatment in cows on human health and welfare (Galal *et al.*, 2007). Effective programs for mastitis control that promote diary food safety are based on identifying the pathogen present, developing effective tools to control mastitis pathogens and observing practices that reduce the risk antibiotic contamination of milk. Controlling mastitis is very critical because the condition has significant implications such as financial loss of diary farmers, adverse effect on cow's welfare and potential effects on public health (Sinell, 1973).

Conclusion

Generally the evaluation of microbial safety of milk sold in Jimma town revealed the high microbial counts. Hence it is necessary to minimize microbial contamination which can be achieved through health animal and milkieer and hygienic practices followed in diary farming. High microbial counts and the accuracy of pathogens are likely to affect the keeping quality and safety of milk as well as products derived from it. The achievement of hygiene in dairy farm directly influences the production's economic result and health safety perspectives in humans. It is therefore critically important to ensure high quality raw milk produced from healthy animals under hygienic conditions and that control measures are applied to protect human health. On the other hand the delivery of milk safety programs by veterinarians is an important over all component of a diary production medicine program. Preventing mastitis and improving milk quality is virtually important role that contributes to improved animal well- being, enhanced farm profitability and better assurances that food is being produced in a safe and sustainable way. Diary veterinarians should seek out involvement in education programs that focus on research based methods and advancements in mastitis control. Milk quality programs must continue to advance with changes in pathogens changes in milking equipment and low housing systems and as associated expectations evolve.

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