



Bacteriology

Journal

ISSN 2153-0211



Academic
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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 100 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.

Key words: Mastitis, Milk-born, pathogens, milk, safety

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 (Ali, 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 (Bryan, 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 (Ali, 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 is 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 contaminated 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 is 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 countries, dairy production is an important part of the livestock production system in urban and pre-urban sectors of the country. 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 farmers dairy farming provides the opportunity for 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 demand for milk and its products (Haile *et al.*, 2010).

In Jimma town also market oriented small holder 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 and its products in the town is unsatisfactory due to many production constraints, mainly reproductive health problems that form 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 the fact 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 cause 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 udder of mammals. The main objective of this study was to evaluate the microbiological safety and to detect mastitis in the milk that is 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 $7^{\circ}41' \text{ N}$ latitude, $36^{\circ} 50' \text{ E}$ longitude;

the study area has an average altitude of 1,780 m above sea level. It lies in the climatic zone locally known as “Woynga Dada” (1,500-2,400 m 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 300°C and a mean annual minimum temperature of 140°C. The annual rainfall ranges from 1138-1690 mm. 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 7-9 pm using sterile containers maintaining sterile conditions. The microbial analysis was conducted within 1-3 h of collection and the samples were kept in the refrigerator at 4°C until microbial analysis was conducted.

Microbial analysis: From each sample 10 mL of milk was mixed with 90 mL of distilled water and homogenized in a flask for 5 min using shaker at 160 rpm. The homogenates were serially diluted from 10^{-1} - 10^{-5} and a volume of 0.1 mL aliquot of appropriate dilution was spread plated on pre-solidified plates and incubated at $30\pm 5^{\circ}\text{C}$. 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^{-1}) and later converted to log CFU mL^{-1} , by using the equation:

$$\text{CFU mL}^{-1} = \frac{\text{No. of colonies counted from plates (mean)}}{\text{Dilution factor} \times \text{volume plated}}$$

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 McCance, 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 0.1 g 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 5 mL nutrient broth. The inoculated cultures were incubated at 35°C for 24 h 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, 1984; Gregersen, 1978) endospore test; KOH-test, (Gregersen, 1978); catalase test, (MacFaddin, 1980); Coagulase test, (Cheesebrought, 2006) and IMViC tests (Cheesebrought, 2006).

DETECTION FOR MASTITIS

Detection of *Staphylococcus aureus*: For isolation purpose, 0.1 mL appropriate dilutions of the aliquot were spread-plated onto a sterile pre-solidified Blood Agar Plate (BAP) and incubated at

37°C for 48 h. 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 h 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 (NMC, 2004).

Detection of *Streptococcus agalactiae*: Zero point one milliliter appropriate dilutions of the aliquot was spread-plated on to a sterile per-solidified Blood Agra plate and incubated at 37°C for about 48 h. After incubation the suspected *streptococcus agalactiae* colonies were sub cultured on Nutrient Agra Plate (NAP) and incubated at 37°C for 24-48 h 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 (Keefe, 1997; NMC, 2004).

RESULTS

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 2). The isolates were identified, characterized and grouped in genus level based on John (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%).

In the present study, the mean count of Aerobic Mesophilic Bacteria (AMB) was the highest (3.23×10^7 CFU mL⁻¹) where as the lowest mean count was observed in Moulds (5.11×10^3 CFU mL⁻¹). The mean counts of Lactic Acid Bacteria (LAB) occupied the second rank that was (4.51×10^6 CFU mL⁻¹) following aerobic mesophilic bacteria counts and was followed by the mean count of yeasts (8.9×10^5 CFU mL⁻¹). The mean count of coliforms and aerobic bacterial spores occupied the forth and the fifth ranks (1.58×10^5 CFU mL and 1.81×10^4 CFU mL, respectively with a total mean count of 6.3×10^6 CFU mL, in general the present study indicates that all the mean counts of bacteria and fungi (yeasts and Moulds) were above the permissible level (Table 1).

The mean counts of Aerobic Mesophilic Bacteria (AMB) was the highest (7.5 log CFU mL⁻¹). Following aerobic mesophilic bacteria, the mean count of lactic acid bacteria (6.6 log CFU mL⁻¹) and yeasts (5.9 log CFU mL⁻¹) occupied the second and third ranks and those of coliforms (5.1 log CFU mL⁻¹) and aerobic bacterial spores (4.2 log CFU mL⁻¹) occupied the forth and the fifth stages, respectively (Fig. 1). Where AMB-Aerobic relsophilic Bacteria, LAB = Latic Acid Bacteria, coli-coliforms, ABS-aeroobic bacterial spores former.

Table 1: Mean Microbial counts (CFU and log CFU mL⁻¹) of milk in Jimma town, south western Ethiopia

Type of microorganisms	Microbial mean count (CFU mL ⁻¹)	Microbial mean count (Log CFU mL ⁻¹)	Microbial mean count (Log CFU mL ⁻¹ ± SD)
Aerobic mesophilic bacteria	3.23×10^7	7.5	7.5±0.8
Lactic acid bacteria	4.5×10^6	6.6	6.6±0.6
Coliforms	8.9×10^5	5.9	5.9±0.4
Yeasts	1.58×10^5	5.1	5.1±0.5
Aerobic bacterial spores	1.81×10^4	4.2	4.2±0.4
Moulds	5.11×10^3	3.7	3.7±0.6

Table 2: Microbial strains and their frequency distribution

Micro organisms	Bacterial spp.	Total No.	Frequency (%)
Aerobic mesophilic bacteria	<i>Bacillus</i>	40	33.9
	<i>Aeromonas</i>	32	27.1
	<i>Pseudomonas</i>	29	24.6
	<i>Acinitobactor</i>	17	14.4
	Total	118	100.0
Lactic acid bacteria	<i>Lactobacillus</i>	34	32.4
	<i>Lactococcus</i>	29	27.6
	<i>Enterococcus</i>	23	21.9
	<i>Aerococcus</i>	19	18.1
	Total	105	100.0
Coliforms	<i>Escherichia</i>	26	30.9
	<i>Citrobactor</i>	22	26.2
	<i>Enterobactor</i>	19	22.6
	<i>Klebsiella</i>	17	20.3
	Total	84	100.0

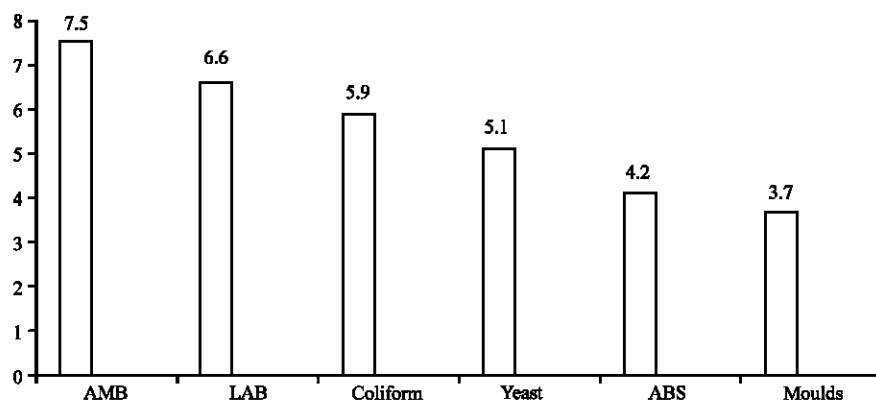


Fig. 1: Mean microbial counts of milk samples collected from Jimma town, southern Ethiopia

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%) whereas the lowest bacterial strains found from the present study was *Acinitobactor* spp. (14.4%) followed by *Aerococcus* spp. (18.1%).

From the present study, the predominant bacterial strain found was *Bacillus* spp. (33.9%) followed by *Lactobacillus* spp. (32.4%), *Escherichia* spp. (30.9%) and *Lactococcus* spp. (20.6%) (Fig. 2) from the total isolates, (52.6%) of the bacterial strains were found to be gram-negative and the rest (48.2%) were gram-positive.

DISCUSSION

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 a large number of people who would otherwise be unable to establish other businesses (Aseffa, 2010). In

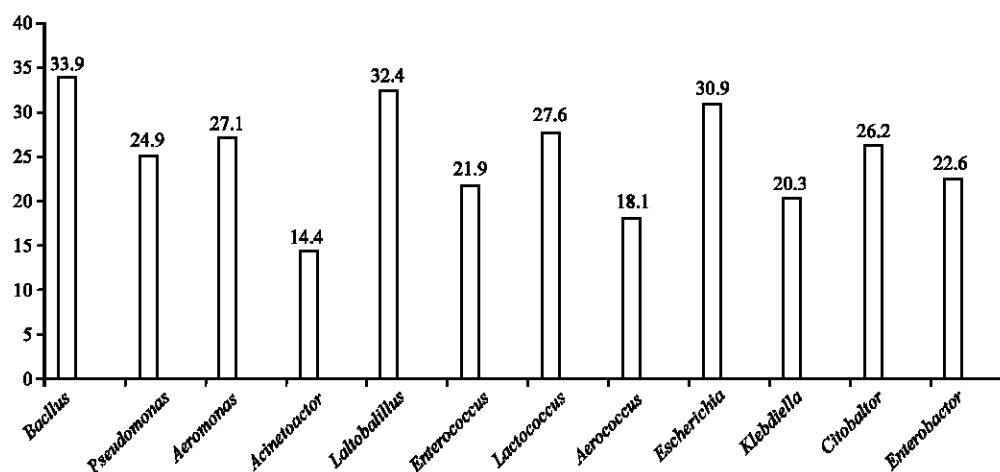


Fig. 2: Microbial strains and their frequency distribution, Jimma town, south western Ethiopia

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 *et al.* (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 ($7.5 \log \text{CFU mL}^{-1}$), Yeasts ($5.1 \log \text{CFU mL}^{-1}$), Lactic acid bacteria ($6.6 \log \text{CFU mL}^{-1}$), coliforms ($5.9 \log \text{CFU mL}^{-1}$). Aerobic bacteria spores ($4.2 \log \text{CFU mL}^{-1}$) and Moulds ($3.7 \log \text{CFU mL}^{-1}$). The mean value of aerobic mesophilic bacteria count ($7.5 \log \text{CFU mL}^{-1}$) in milk samples collected from Jimma town was in agreement with the findings of Hayes *et al.* (2001) who found the mean count of ($7.4 \log \text{CFU mL}^{-1}$) in milk samples collected from Khartoum state. However, according to the united state maximum standard limit of the mean value of aerobic mesophilic bacterial count ($2.9 \log \text{CFU mL}^{-1}$ – $5.7 \log \text{CFU mL}^{-1}$) 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 confirms in the present study is in line with Mossel *et al.* (1982) who reported mean count of coliforms ($5.8 \log \text{CFU mL}^{-1}$) in milk from Khartoum state. In contrast to the present study, Majer *et al.* (1973) reported higher number ($6.4 \log \text{CFU mL}^{-1}$) 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 $<2 \log \text{CFU mL}^{-1}$. The occurrence of coliforms in milk may there fore be considered as a real indicator of the fecal contamination with the possibility existing associated pathogens and also the public 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 *et al.*, 1982).

The Aerobic Bacterial Spore (ABS) count ($4.2 \log \text{CFU mL}^{-1}$) of the this study is somewhat lower as compared to findings reported by Ahn and Youssef (2007) who found a mean count ($6.4 \log \text{CFU mL}^{-1}$) in milk from united state. According to Corneal University dairy food science the maximum standard limit of aerobic bacterial spore count in milk should be

(4.3 log CFU mL⁻¹) 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 Bacterial (LAB) in the present study was (6.6 log CFU mL⁻¹). This is inline with the microbiological studies made on milk samples in Nigeria (Cheriguene *et al.*, 2007), whose counts were 6.4 log CFU mL⁻¹. However, according to the South Africa maximum standard level of lactic acid bacterial count in milk (5 log CFU mL⁻¹), 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⁻¹ and 3.7 log CFU mL⁻¹, 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 (Acco *et al.*, 2003).

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 NMC (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 milkier and hygienic practices followed in dairy 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 dairy 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. Dairy 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.

REFERENCES

- Acco, M., F.S. Ferreira, J.A.P. Henriques and E.C. Tondo, 2003. Identification of multiple strains of *Staphylococcus aureus* colonizing nasal mucosa of food handlers. *Food Microbiol.*, 20: 489-493.
- Ahmed, K., 2010. Challenge testing of microbiological safety of raw milk cheeses. *Afr. J. Microbiol. Res.*, 6: 3366-3379.
- Ahn, J. and A. Youssef, 2007. Selective inactivation of aerobic bacterial spores by pressure assisted thermal processing. *Inter. J. Food Microbiol.*, 113: 321-329.
- Alemu, A., W. Tsegaye, L. Golassa and G. Abebe, 2011. Urban malaria and associated risk factors in Jimma town, South-West Ethiopia. *Malar J.*, Vol. 10. 10.1186/1475-2875-10-173
- Ali, A.A., 2010. Microbiological safety of raw milk in Khartoum State Sudan: 2- Khartoum-North city. *Pak. J. Nutr.*, 9: 615-630.
- Aseffa, A., 2010. The microbiological safety of pasteurized and raw milk from milk processing plants in and around Addis Ababa. Addis Ababa, Ethiopia.
- Bryan, F.L., 1983. Epidemiology of milk-borne diseases. *J. Food Prot.*, 46: 637-649.
- Cheesebrought, A., 2006. Laboratory Manual. Central Food Research Institute Press, Budapest, Hungary.
- Cheriguene, A., F. Chougrani, A. Bekada, M. El Soda and A. Bensoltane, 2007. Enumeration and identification of lactic microflora in Algeria goats' Milk. *African J. Biotechnol.*, 6: 1854-1861.
- Davis, M. and K. Wilby, 1990. Prevalence of subclinical mastitis in dairy cattle. *J. Anim. Plant Sci.*, 21: 477-480.
- Galal, K., G. Sender and K.A. Korwin-Kossa, 2007. Public Health Hazards Due to Mastitis in Dairy Cows. Soult Valley University Press, Qenal, Egypt.
- Gashaw, A., F. Worku and S. Flulugeta, 2011. Assessment of smallholder dairy production system and their reproductive health problems in Jimma Town. South Western Ethiopia, Jimma University, College of Agriculture and Veterinary Medicine, Jimma, Ethiopia.
- Gram, H.C., 1984. Über die isolierte färbung der schizomyceten in schnitt- und trockenpräparaten. *Fortschr. Mediz.*, 2: 185-189, (In German).
- Gregersen, T., 1978. Rapid method for distinction of gram-negative from gram-positive bacteria. *Eur. J. Applied Microbiol. Biotechnol.*, 5: 123-127.
- Haile, R., G. Wells and R. Swanson, 2010. An outbreak of enterotoxigenic *E. coli* food born disease traced to imported French cheese. *J. Dairy Sci.*, 83: 264-274.
- Harrigan, W.F. and M.E. McCance, 1976. Laboratory Methods in Food and Dairy Microbiology. Academic Press, London, pp: 17-68.
- Hayes, M.C., R.D. Ralyea, S.C. Murphy, N.R. Carey, J.M. Scarlett and K.J. Boor, 2001. Identification and characterization of elevated Microbial counts in bulk tank raw milk. *J. Dairy Sci.*, 84: 292-298.

- Jacobspn, S. and R. Naruijvo, 1996. *Staphylococcus aureus* in the Significance of Pathogenic Microorganism in Raw Milk. International Dairy Federation (IDF), Brussels, Belgium.
- John, L., 2012. An introduction to bacterial identification. <http://www.splammo.net/bact102>.
- Keefe, G.P., 1997. *Streptococcus agalactiae* mastitis: A review. Can. Vet. J., 38: 429-437.
- Lingaturia, A., M. Arian, M. Khaskheli and B. Bhutto, 2011. Isolation of *E. coli* from raw milk and milk products in relation to public health. Pak. J. Nutr., 1: 151-152.
- MacFaddin, J.F., 1980. Biochemical Tests for Identification of Medical Bacteria. 2nd Edn., Williams and Wilkins, USA.
- Majer, T.H., E. Hofer and A. Tibana, 1973. Incidence of listeria monocytogens in cheese produced in rio de janeiro Brazil. J. Food Prot., 61: 354-356.
- Mossel, D.R., R. Ryoba and E. Karimuribo, 1982. Prevalence of clinical and sub clinical mastitis and quality of milk on small holder diary farms in Tanzania. J. South Africa Vet. Assoc. Moroporo, Tanzania, 80: 163-168.
- NMC, 2004. Microbiological Procedures for the Diagnosis of Bovine Udder Infection and Determining Milk Quality. National Mastitis Council Inc., Arlington, VA.
- Sinell, L., 1973. Contagious and environmental mastitis. Global organiziaon for mastitis control and milk quality, England.
- Spencer, K., F.T. Jon and A.L. Spencer, 2007. Food Microbiology Protocols. Homana Press, New Jersey, India.
- Tolosa, T., M. Refera, Y. Deneke, A. Gashaw, K. Supre and S. Devligher, 2010. Milk production and marketing system in Jimma Town. South Western Ethiopia, Jimma, Ethiopia.
- WHO, 1996. Milk and Milk Products. Tota MC Graw Publishing Company, Bombay, New Dehali.
- Weil, Q., S. Hwang and J. Chen, 2006. Microbiological quality of ready- to eat food products in Southern Jaiwan. J. Food Drug Analy., 14: 68-73.
- Wubete, A., 2004. Bacteriological quality of bovine milk in small holder dairy farms in Debre-Zeit, Ethiopia. M.Sc. Thesis, Faculty of Veterinary Medicine, Addis Ababa University.
- Yigerm, S., F. Beyene, A. Tegene and B. Gebremedehin, 2008. Diary Production, Processing and Marketing System of Shaslemene- Dilla Area, South Ethiopia. International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia.