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ASSESSMENT OF MICROBIAL QUALITY OF FARM BUFFALO MILK

A. H. Soomro[1*], S. Raunaq[1], S. A. Sheikh[1], M. Khaskheli[2] and A. Talpur[2]

[1]Institute of Food Sciences and Technology, [2]Department of Animal Products Technology, Sindh Agriculture University, Tandojam, Pakistan

ABSTRACT

The microbial quality of farm buffalo milk was evaluated during this study. A total of 75 milk samples 25 each of fresh, bulk and transportation tank milk were screened for total viable count, coliform count, yeast and mould, and aerobic spore-former count. The results showed that the average TVC (5.51 log CFU/mL), coliform (5.45 log CFU/mL), yeast and mould (3.69 log CFU/mL) and spore former count (2.66 log CFU/mL) was significantly higher (P<0.05) in transportation tank milk samples, followed by bulk tank samples (5.29, 4.37, 3.60 and 2.59 log CFU/mL) and fresh milk samples (4.74, 3.51, 3.48 and 1.76 log CFU/mL). It was concluded from the findings of present investigation that average total viable count, coliform, yeast and mould, and aerobic spore former counts were higher in transportation tank milk as compared to fresh and bulk tank milk samples.

Keywords: microbiological quality, hygiene, buffalo milk

INTRODUCTION

Milk and its products are full of nutrients with moisture content and neutral pH. The milk even pasteurized or refrigerated easily favors the proliferation of microorganisms. Hence, the quality of the milk and its products are significantly influenced by the presence of microorganisms. At the time of milking a small number of bacteria are present in milk of healthy animals. However, it may be contaminated during milking operations, from the adjacent areas of the udder, utensils used during milking; the hands of milker and from soil (Bramley and McKinnon, 1990). Microorganisms which enter into milk or its products can easily multiply and cause spoilage of these products, which results to be unsafe due to potential health hazards. After milking the numbers and types of microorganisms in milk depends on animal health, quality of utensils used and season so the microorganisms in raw milk depend on the nature and extent of contamination which in turn varies with the conditions of milk production, and the subsequent storage conditions. However, certain strategies regarding feeding and housing may also have impact on the microbiological quality of milk and the rinsing water used for utensils may also increase the microbial population including pathogens

Corresponding author: aijaz68@hotmail.com

in raw milk (Bramley and McKinnon, 1990; Coorevits *et al*., 2008). During storage and transport the microbial quality of raw milk may change from predominant Gram-positive to Gram-negative bacteria and approximately made 90% of their population in stored cold raw milk (Martins *et al*., 2006). Among the milk microflora spore-forming microorganisms have ability to survive thermal treatments and later proliferate in final products (Abo-Elnaga *et al.,* 2002). In raw milk aerobic spore-forming bacteria (ASFB) belong to genus *Bacillus*. Their spores survive pasteurization and cause spoilage of raw, pasteurized and ultra-high temperature treated milk and dairy products (McGuiggan *et al.,* 2002; Coorevits *et al.,* 2008). Several workers have reported the presence of *Escherichia coli* (Soomro *et al*., 2002), *Listeria monocytogens* (Chandio *et al.*, 2007), lactic acid bacteria, coli forms, yeast, moulds, *Staphylococcus* spp. and *Clostridium* spp. in raw milk. Presence of these spoilage bacteria and pathogens in greater number may cause deterioration in the quality of buffalo milk, and may result in potential health hazard (Boycheva *et al*., 2002). As the buffalo milk is economically important in the country and from the food safety and quality point of view this study was conducted to assess the microbial quality of farm buffalo milk.

MATERIALS AND METHODS

Collection of milk samples

A total of 75 milk samples; 25 each from fresh milk, bulk tank milk and transportation tank milk were collected from dairy farms at different locations of Tandojam. The samples were aseptically collected in sterile bottles and transported under refrigeration to the Food Microbiology Laboratory, Institute of Food Sciences and Technology, Sindh Agriculture University Tandojam for microbial analysis.

Microbial analysis of milk

The quarter strength Ringer's solution was prepared for serial dilutions.

Enumeration of total viable count

The enumeration of total viable counts (TVC) was determined (IDF, 1991). The milk samples (1 ml) from 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and/ or 10^{-6} dilutions were used in triplicate and transferred into Petri dishes and sterile warm (45 $\pm 1^{o}$C) plate count agar medium (15ml) was mixed with inoculums and at 30°C plates were incubated for 24-48 h.

Enumeration of coliform count

The coliform count was performed as reported by British Standard Institute (BSI, 1993). The test samples (1 ml) of 10^{-1}, 10^{-2}, 10^{-3} and/ or 10^{-4} dilutions were transferred into sterile Petri dishes in triplicates and warm (45 $\pm 1^{o}$C) MacConkey agar medium (15ml) was poured and mixed with inoculums and incubation was carried out at 37^{o}C for 24-48 hours.

Enumeration of yeast and mould counts

Yeasts and moulds were examined as described by IDF, (1990). The milk sample (1 ml) of 10^{-1}, 10^{-2}, 10^{-3} and/ or 10^{-4} dilutions were transferred into sterile

Petri dishes in triplicates and potato dextrose agar (15ml) at a temperature of 45 ±1^{o}C was used and mixed with the inoculum and incubated at 25^{o}C for 72 hours.

Enumeration of aerobic spore-former counts

Aerobic spore former counts were enumerated as reported by Marshall (1993). The milk sample (1 ml) was mixed in Ringer's dilution (9 ml) and heated (80^{o}C for 10min) to eliminate the vegetative cells, then kept into freezer for 10min. Heat treated sample (1 ml) of 10^{-1}, 10^{-2}, 10^{-3} and/ or 10^{-4} dilutions were pipetted into triplicates Petri plates and warm (45 ±1^{o}C) sterile nutrient agar medium (15 ml) was mixed with inoculums and incubated at 55^{o}C for 48 h. The blank plates without samples were used as control in all the counts. All the results were expressed as CFU/ml.

Statistical analysis

The data obtained was analyzed statistically (Origin Version 5.1).The significant differences between means were calculated by ANOVA using Duncan's multiple-range test at $P< 0.05$.

RESULTS

Total viable count

The results presented in Figure 1 revealed that mean total viable count of fresh, bulk tank and transportation tank milk was 4.74, 5.29 and 5.51 log CFU/mL, respectively. It was observed that the total viable count was higher in transportation tank milk, whereas, statistically significant differences ($P< 0.05$) in the total viable count of fresh, bulk and transportation tank milk samples were shown in the analysis of variance.

Coliform count

The mean coliform count of fresh, bulk tank and transportation tank milk was 3.51, 4.37 and 5.45 log CFU/mL, respectively (Figure 2). The results indicated that the coliform count was significantly higher ($P< 0.05$) in transportation tank milk as compared to bulk tank and fresh milk samples.

Yeast and mould count

The average yeast and mould count of fresh, bulk tank and transportation tank milk was 3.48 3.60 and 3.69 log CFU/mL, respectively (Figure 3). The transportation tank milk had slightly higher count. A non- significant difference ($P< 0.05$) among the three sample sources was observed.

Aerobic spore-former count

The average aerobic spore former count of fresh, bulk and transportation tank milk was 1.76, 2.59 and 2.66 log CFU/mL, respectively (Figure 4). The higher count was observed in transportation tank milk samples. There was highly significant difference ($P< 0.05$) in the aerobic spore former count among the sources.

DISCUSSION

Milk is an important food and is recognized as a complete diet due to its essential nutrients. Inappropriately, due to lack of cold chain transport system, the quality of milk at consumer level is barely maintained and this also results in the presence of spoilage agents and food borne pathogens (Adesiyun *et al*., 1998). The level of contamination mainly depends on the conditions in which milk is produced which include animal housing, the milk harvesting, equipment, local trading, storage and transport. Therefore, the hygienicity of a product used for human intake could not be maintained due to these factors. Even the transport of milk at 4°C could not be able to check the growth of some microorganisms and produce defects if they exceed the recommended levels set by Food and Agriculture Organization (FAO, 1998). Total viable counts are the estimation of viable bacterial population in milk and reflect the hygienic processes adopted during production and handling of the milk (Houghtby *et al*., 1994). The findings of present study are in accord with the results of Lee *et al*. (1983) they observed that raw milk obtained from Seoul, Korea had TVC ranged from 4×10^6 to 2.7×10^7CFU/mL. However, findings in present investigation suggested that the total viable count in fresh and bulk milk samples were within permissible limits. These results are further in line with those of Supino *et al*. (2004) who reported that total bacterial count in raw milk was 5.23 Log CFU/mL. Similarly, Mesfine *et al.* (2015) observed 6.67 Log_{10} CFU/mL. Furthermore, the total viable count of transportation tank milk is greater than the fresh and bulk milk. This might be due to the poor hygienic conditions (Saeed *et al*., 2009) and lack of the cooling facilities (Murphy and Boor, 2000) and duration of milk storage at the farms before marketing. Moreover, Gran *et al.* (2002) mentioned that unhygienic practices during milking process affect the microbiological quality of raw milk. Moreover, ineffective sanitizing routine of post milking process that leaving herds' manure in contact with udder may also add in prevalent bacterial population of raw milk.

Delay in the supply of raw milk to milk collection centers or market together without bringing down the temperature at 4-5°C could also be detrimental to the raw milk quality, which may allow the speedy proliferation of bacterial load within short period of time. Furthermore, the use of unhygienic conditions and /or improper storage at farm may contribute in bacterial proliferation. The present investigation is in accord with the results of Lingathurai *et al.* (2009) that the milk is easily contaminated after milking and during transportation to the milk collecting center or markets due to external contamination or increased temperature.

The incidence of coliforms may act as an indication of the sanitary conditions and generally confirms the fecal contamination during production, handling and storage of milk. Hence, poor hygienic conditions at the farm, low quality water used in utensil washing and unhygienic milking practices during storage may cause increase in the coliform count (Fook *et al*., 2004). Whereas, lower coliform counts may occur due to proper cleaning and handling system and due to hygienic practices applied at farm that result in production of high quality raw milk. Likewise, coliform count ranged between 2.50 - 2.61 Log CFU/mL as observed by Khan *et al*. (2008). Whereas, Mosu *et al.* (2013) have observed lower count in raw milk samples from collected from Ethiopia. However, Fekadu

(1994) found higher coliform counts than Desmasures *et al.* (1997), they reported that 84% fresh cow milk samples had <100 CFU/mL coliform counts. The surface of the moist or under washed milking equipments may contain coliforms which can enter into milk and contaminate the raw milk in the countries where sanitation is unwarranted and there is absence of the cold chain system. Apart from food safety and public health issues, the coliforms also produce off flavors and ultimately decrease the shelf life of dairy products.

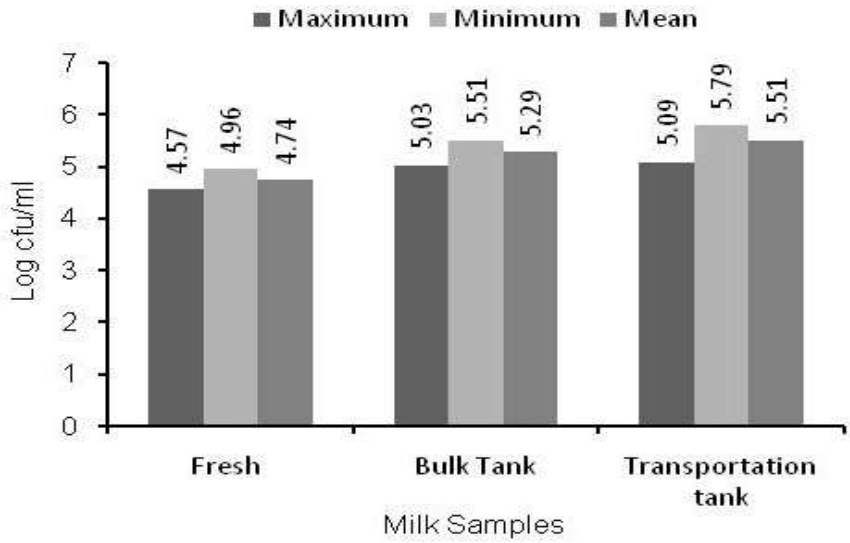

Figure 1. Total viable count (log Cfu/mL) of fresh, bulk tank and transportation tank milk samples

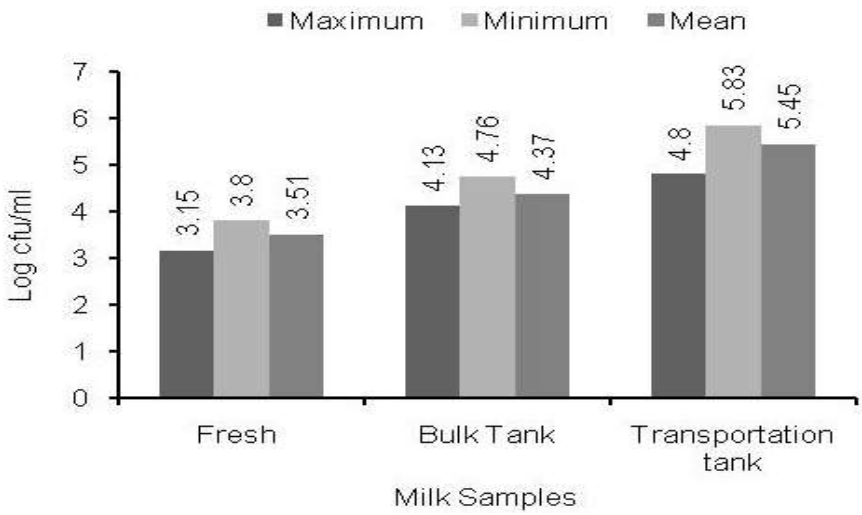

Figure 2. Coliform count (log Cfu/mL) of fresh, bulk tank and transportation tank milk samples

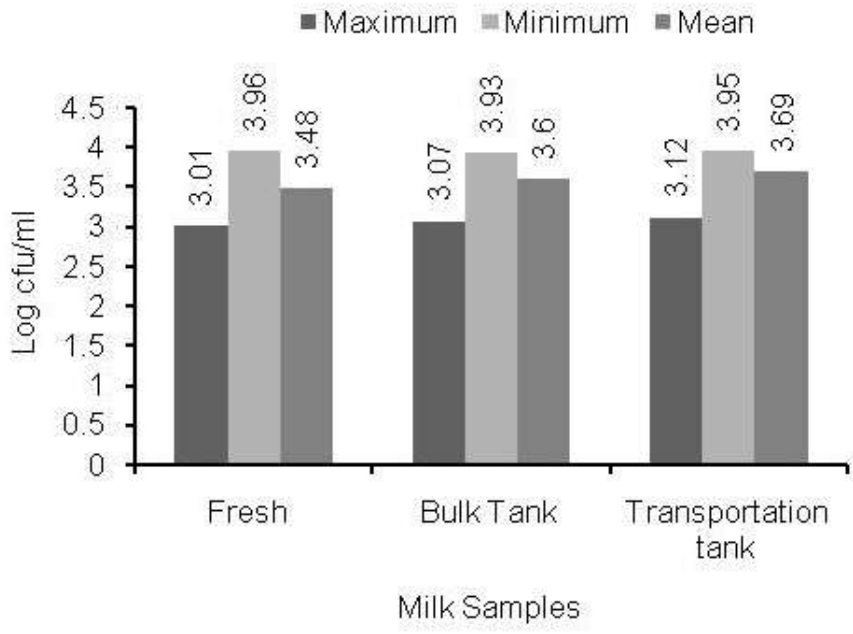

Figure 3. Yeast and mould count (log Cfu/mL) of fresh, bulk tank and transportation tank milk samples

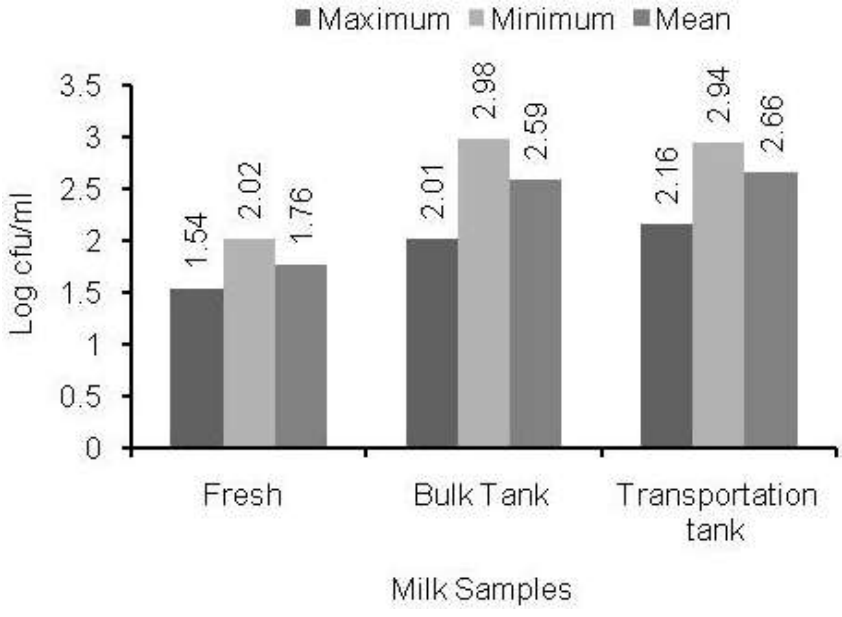

Figure 4. Aerobic spore-former count (log Cfu/mL) of fresh, bulk tank and transportation tank milk samples

Usually, the presence of yeast in raw milk is in low numbers (Fleet, 1990) this may probably due to the presence of psychrotrophic bacteria in milk which utilizes growth substrates of milk or due to excretion of bacterial metabolic end products which may result in inhibition of yeast in milk (Viljoen, 2001). Torkar and Teger (2008) determined 2.0 log CFU/mL yeast and mould counts. Entry of these organisms might be due to contamination through unclean udder surface and teat area. The use of unhygienic utensils used in milking and transport may also contribute to the poor hygienic quality (Parekh and Subhash, 2008). Yeasts may cause defects in dairy products by fermenting lactose and that result in gas production with characteristic yeasty or fruity flavor (Davis, 2002). In this study the increased yeasts and mould count may be due to the cross contamination of milk at the farm. The increased yeast and mould count is undesirable as some of the moulds may produce toxins and may act as pathogens (Guarino *et al.,* 1996). The spore-forming micro-organisms survive pasteurization and multiply in the final products (Foltys and Kirchnerova, 2006). The aerobic spore-former bacteria generally originate from the manure, forages and soil that may contaminate the milk. The numbers of aerobic spore-former bacteria found in this study were generally higher than the findings of Boor *et al*. (1998). Similarly, Barbano *et al*. (2006) also reported low count of spores in raw milk. The udder and teat sanitation before milking could reduce the raw milk microbial load and proper hygienic conditions applied during storage and processing may also decrease the microbial contamination of the end product.

CONCLUSION

Average total viable count, coliform count, yeast and mould count and aerobic spore former count were found higher in transportation tank milk as compared to bulk tank and fresh milk samples. Appropriate sanitary measures should be applied from the point of production to consumption. More attention should be focused on low temperature transportation of milk from collection to selling points.

REFERENCES

Abo-Elnaga, H. I., F. Z. Hegazi and I. G. Abo-Elnaga. 2002. Spore-forming rods surviving oiling the raw milk and implicated in later spoilage of the product. Arch. Lebensm. Hyg., 53: 86-89.

Adesiyun, A. A. L., Webb and S. Rahman. 1998. Microbiological quality of raw cow milk at collection centres in Trinidad. J. Food Prot., 58 (4): 139-146.

Barbano, D. M, Y. Ma and M. V. Santos. 2006. Influence of raw milk quality on fluid milk shelf life. J. Dairy Sci., 89: 15-19.

Boor, K. J, D.P. Brown, S. C. Myrphy, S. M. Kozlowski and D. K. Bandler. 1998. Microbiological and chemical quality of raw milk New York State. J. Dairy Sci., 81: 1743-1748.

Boycheva, S., T. Dimitrov, M. Tsankova and T. Iliev. 2002. Investigation on microflora of buffalo milk. Bulg. J. Agric. Sci., 8: 279-282.

Bramley, A. J. and C. H. McKinnon. 1990. The microbiology of raw milk. *In*: Dairy Microbiology, I, (Ed. Robinson, R.K.). London, New York, Elsevier. Applied Sci., p 171.

BSI. 1993. Determination of enterobacteriaceae, *In:* microbiological examination

of food and animal feeding stuffs. BSI 5763, British Standards Institution, London, U.K.

Chandio, T. H., A. H. Soomro, M. B. Bhutto, P. Dewani and G. Shah. 2007. Occurrence of *Listeria monocytogenes* in bovine milk in Hyderabad, Pakistan. Ann. Microbiol., 57 (3): 341-344.

Coorevits, A., V. D. Jonghe, J. Vandroemme, R. Reekmans, J. Heyrman, W. Messens, P. D. Vos and M. Heyndrick. 2008. Comparative analysis of the diversity of aerobic spore-forming bacteria in raw milk from organic and conventional dairy farms. J. Syst. Appl. Microbiol., 31: 126-140.

Davis, J. G. 2002. Microbiology of cream and dairy desserts. *In:* Dairy Microbiology (Ed: Robinson, R.K.) London and New York. Applied Science Publishers, 41-108.

Desmasures, N., F. Bazin and M. Gueguen, 1997. Microbiological composition of raw milk from selected farms in the Camembert region of Normandy. J. Appl. Microbiol., 83: 53-58.

FAO, 1998. Microflore du lait. Le lait et les produitslaitiersdans la nutrition humaine. Collection FAO: Alimentation et nutrition n° 28. ISBN 92-5-20534-6. Catalogageavant publication de la Bibliothèque David Lubin FAO, Rome.

Fekadu, B. 1994. Present situation and future aspects of milk production, milk handling and processing of dairy product in Southern Ethiopia. Food production strategies and limitations. The case of Aneno, Bulbula and Dongora in Southern Ethiopia, Ph.D. Thesis, Dept. Food. Sci. Agri. Uni. Norway.

Fleet, G. H. 1990. Yeast in dairy products. J. Appl. Bacteriol., 68:199-211.

Foltys, V. and K. Kirchnerova. 2006. Mesophilic and psychrotropic aerobic sporulating microorganisms in raw cow's milk. Central Euro. J. Biol., 1 (4): 545-560.

Fook, Y. C., A. Aminah and K. A. Mohammad. 2004. Bacteriological quality and safety of raw milk in Malaysia. Food Microbiol., 21:535-541.

Gran, H. M., A. N. Mutukumira, A. Wetlesen and J. A. Narvhus. 2002. Smallholder dairy processing in Zimbabwe: hygienic practices during milking and the microbiological quality of the milk at the farm and on delivery. Food Control, 13: 41-47.

Guarino, A., G. Fusco, A. Merola, M. Romano and D. Fenezia. 1996. Microbiological and chemical requirements of buffalo milk production in some farms in Southern Italy. Recent Research Developments in Buffalo Production: proceedings of the Second Asian Buffalo Association Congress, Momongan, V. Ducusin, R. J. T. Maala, C. P. Bondoc, O. L. Del Barrio, A.N. (Eds.). College, Laguna (Philippines): PSAS Foundation Inc., ISBN 971-91760-0-8. p. 487.

Houghtby, G. A., L. J. Maturin and E. K. Koenig. 1994. The microbiological count methods. *In:* Marshall R.T. (Ed.). Standard methods for the examination of dairy products. American Public Health Association, Washington, DC., pp: 213-246.

IDF. 1990. Enumeration of yeast and molds in milk and milk products. Yeast and molds at 25°C. *In:* International Dairy Federation, Brussels, Belgium.

IDF. 1991. Enumeration of microorganism in milk and milk products. Colony counts at 30°C. *In:* International Dairy Federation, Brussels, Belgium.

Khan, M. T. G., M. A. Zinnah, M. P. Siddique, M. H. A. Rashid, M. A. Islam and K. A. Choudhury. 2008. Physical and microbial qualities of raw milk collected from Bangladesh Agricultural University dairy farm and the surrounding villages. J. Vet. Med., 6 (2): 217-221.

Lee, J. T., S. Y. Park, I. K. Korea and H. U. Kin. 1983. Comparative analysis of quality of raw milk in Korea. Korean J. Dairy Sci., 5: 22-28.

Lingathurai, S., P. Vellathurai, S. Vendan and A. A. P. Anand. 2009. A comparative study on the microbiological and chemical composition of cow milk from different locations in Madurai, Tamil Nadu. Ind. J. Sci. Technol., 2 (2): 51. 54.

Marshall, R. T. 1993. Tests for groups of microorganisms of dairy products. *In:* Standard methods for the examination of dairy products, American Public Health Association, Washington, USA. pp. 271-286.

Martins, M. L., C. L.O. Pinto, R. B. Rocha, E. F. Araujo and M. C. D. Vanetti. 2006. Genetic diversity of Gram-negative proteolytic, psychrotrophic bacteria isolated from refrigerated raw milk. Int. J. Food Microbiol., 111: 144-148.

McGuiggan, J. T. M., D. R. McCleery, A. Hannan and A. Gilmour. 2002. Aerobic spore forming bacteria in bulk raw milk: factors affecting the number of psychrophilic, mesophilic and thermophilic bacillus spores. Int. J. Dairy Technol., 55: 100-107.

Mesfine, S., T. Feyera and O. Muhammad. 2015. Microbiological quality of raw cow's milk from four dairy farms in Dire Dawn city, Eastern Ethiopia. World J. Dairy Food Sci., 10 (1): 9-14.

Mosu, S., M. Mergersa, Y. Muhie, D. Gebremedin and S. Keskes. 2013. Bacteriological quality of bovine raw milk at selected dairy farms in Debre Zeit town, Ethiopia. Comp. J. Food Sci. Technol. Res., 1 (1): 1-8.

Murphy, S. C. and K. J. Boor. 2000. Trouble-shooting sources and causes of high bacterial count in raw milk. Dairy Food. Environ. Sanit., 20 (8): 606-611.

Parekh, T. S and R. Subhash. 2008. Molecular and bacteriological examination of milk from different milk animals with special reference to coliforms. Curr. Res. Bacteriol., 1 (2): 56-63.

Saeed, A. E. A, E. M. Zubeir and O. A. O. Owni. 2009. Antimicrobial resistance of bacteria associated with raw milk contaminated by chemical preservative. World J. Dairy Food Sci., 4 (1): 65-69.

Soomro, A. H., M. A. Arain, M. Khaskheli and B. Bhutto. 2002. Isolation of *Escherichia coli* from raw milk and milk products in relation to public health sold under market conditions at Tandojam, Pakistan. Pak. J. Nutr., 1 (3):151-152.

Supino, M. T., M. Gallo, G. Capo, C. Morena, G. Durante and G. Galiero. 2004. Buffalo milk produced in the province of Salerno: Evaluation of sanitary and product parameters. Bubalus Bubalis, 10: 22-26.

Torkar, K. G. and S. G. Teger. 2008. The microbiological quality of raw milk after introducing the two day's milk collecting system. Acta Agric. Slov., 92: 61-74.

Viljoen, B. C. 2001. The interaction between yeast and bacteria in dairy environments. Int. J. Food Microbiol., 69: 37-44.

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