PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
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Pakistan Journal of Nutrition 9 (7): 651-653, 2010
ISSN 1680-5194

Microbiological Safety of Raw Milk in Khartoum State, Sudan: 2- Khartoum-North City

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Abstract: Sixteen random samples of raw cow's milk were collected from Khartoum North in Sudan. Samples were analyzed for microbiological properties included total plate count (TPC), total coliforms (TC), fecal coliforms (FC), *Staphylococcus aureus*, Salmonella, lactic acid bacteria (LAB), spore forming bacteria (SFB) and yeast. The results showed higher counts for all the microorganisms studied. Average of TPC, TC, FC, S. *aureus*, SFB, LAB and Yeast were 9.88 x 10^6, 5.43 x 10^4, 1.56 x 10^4, 1.2 x 10^6, 1.23 x 10^2, 7 x 10^4 and 9.63 x 10^5 cfu/mL, respectively. The microbial profiles found had non-conformance to the standards. Based on the exceedingly high microbial counts found in this study, it could be concluded that this milk type poses a serious health risk in the study areas.

Key words: Cow milk, pathogenic and indicator bacteria, human food

INTRODUCTION

Cow's milk has long been considered a highly nutritious and valuable human food and is consumed by millions daily in a variety of different products. Its nutrient composition makes it an ideal medium for bacterial growth and therefore it can be considered one of the most perishable agricultural products because it can so very easily be contaminated (Bryan, 1983, Bramley and McKinnon, 1990; Heeschen, 1993). Raw Milk (RM) often contains microorganisms which may cause food borne diseases (Adesiyun *et al*., 1995; Steele *et al*., 1997; Headrick *et al*., 1998).

Because of the specific production it is impossible to avoid contamination of milk with micro-organisms therefore the microbial content of milk is a major feature in determining its quality (Rogelj, 2003). He stated that the number and types of microorganisms in milk immediately after milking are affected by factors such as animal and equipment cleanliness, season, feed and animal health. Bacterial contamination of raw milk can originate from different sources: air, milking equipment, feed, soil, faeces and grass (Coorevits *et al*., 2008). He also stated that it is hypothesized that differences in feeding and housing strategies of cows may influence the microbial quality of milk. Rinsing water for milking machine and milking equipment washing also involve some of the reasons for the presence of a higher number of micro-organisms including pathogens in raw milk (Bramley and McKinnon, 1990).

The main objectives of this study were to investigate the microbial quality of cow milk and detect the pathogenic bacteria and enumerate the bacteria that may cause changes in raw cow milk in Khartoum North, Sudan and the distribution of those bacteria.

MATERIALS AND METHODS

Microbiological analysis: Samples of cow milk were obtained from Khartoum north district. Milking was done manually twice a day at 7.00am and 5.00pm. A total of 16 samples of raw cow milk were collected at four locations. At each location, samples of approximately 500 ml were taken aseptically from the bulk milk container into sterile glass bottles. The milk was collected within 15 min of milking at ambient temperatures and was analyzed immediately after arrival at the laboratory (Microbiology Laboratory, Food Research Centre, Khartoum North). All methods of analysis were carried out according to Harrigan and MacCance (1976), unless otherwise indicated.

Sample treatment: Representative 10 ml were aseptically mixed with 90 ml distilled water and homogenized by shaking. Subsequent decimal dilutions were prepared with the same diluents and in all cases duplicate-counting plates were prepared of appropriate dilutions.

Total count of mesophilic aerobic bacteria (TC): was enumerated according to Harrigan and MacCance (1976) in pour plates of plate count agar (Oxoid), after incubation at 37°C for 2 days.

Lactic acid bacteria (LAB): was enumerated according to Harrigan and MacCance (1976). Appropriate dilutions were plated on De Man, Rogosa and Sharpe medium (MRS, Merck, Germany), after incubation at 37°C for 3 days.

***Staphylococcus aureus*:** *Staphylococcus aureus* was performed on Baird-Parker Agar (Oxoid). The plates were incubated at 37°C for 48 h.

Table 1: Microbiological parameters of raw cow milk samples collected from different sources in Khartoum North

Region	No. samples	TB	TC	FC	Staph	SFB	LAB	Yeasts
		cfu/ml						
Kh. North (Morning)	8	9.88×10^6	5.43×10^4	1.56×10^4	1.20×10^6	1.23×10^2	7×10^4	9.63×10^5
Kh. North (Night)	8	9.42×10^6	5.11×10^4	1.23×10^4	0	1.12×10^2	6.4×10^4	4.3×10^5

TB: Total Bacterial Count; TC: Total Coliforms; FC: Fecal Coliforms; SFB: Spore Forming Bacteria; Staph: *Staphylococcus aureus*, LAB: Lactic Acid Bacteria

Enumeration of total coli forms: Presumptive test was done using MacConkey broth (Oxoid) and tubes were incubated at 37°C, examined for gas production and growth after 24 h. A confirmation test was done using BGB broth for total coliform and EMB agar (Oxoid) for *E.coli* and incubated at 37°C for 18-24 h. Two typical colonies from each EMB plate were picked and transferred to plate count agar slants for morphological and biochemical tests (Harrigan and MacCance, 1976).

Enumeration of total spore forming bacteria: The colony count method to determine the total spore forming bacteria was followed as described by Harrigan and MacCance (1976). A test tube of suitable dilution is heated in water bath at 80°C for 10 min to destroy vegetative cells. The tube is cooled and 1 ml from this dilution was aseptically transferred into sterile Petri dishes. To each plate melted Starch Milk Agar (SMA) was added. The plate's inoculums were mixed with the medium and allowed to solidify. The plates were incubated at 37°C for 2 days.

Yeast: Yeasts were enumerated by surface plating on malt extract agar (Oxoid) with 0.01% chloramphenicol as bacterial inhibitor and incubated aerobically at 25°C for 2-3 days (Harrigan and MacCance, 1976).

RESULTS AND DISCUSSION

Hygiene quality was determined by the enumeration of total bacterial, total coliforms, faecal coliforms and *Staphylococcus* sp. The result (Table 1) indicated high contamination of milk samples: TBC (6.5×10^4 cfu/mL to 2.17×10^7 cfu/mL and an average 1×10^7 cfu/mL, TC: 3.6 $\times 10^3$ cfu/ml to 15.25×10^4 cfu/mL with an average of 5.93 $\times 10^4$ cfu/mL, FC: 3.3×10^3 cfu/ml to 2.8×10^4 cfu/mL with an average 1.56×10^4 cfu/mL.

The rates of *S. aureus* found in the examined milk samples are very variable "1.26×10^5 to 4.3×10^6 germs/mL with an average of 1.2×10^6 *S. aureus*/mL. This higher contamination was probably originated from cow's udder. This result is higher than those found by Hamama (1989); Fook *et al.* (2004) and the cows milk with normal food. The contamination of the milk by *S. aureus* is often original but can also occur after handling draft in non-hygienic conditions. *Staphylococcus aureus* is a poor competitor and is readily outgrown by lactic acid-producing microorganisms, so its growth is limited in raw milk (Holsinger *et al.*, 1997; Asperger, 1994). Raw milk may contain microorganisms pathogenic to man and their source may lie either within or out side the udder. Pathogenic bacteria may present in raw milk as a direct consequence of udder disease. Among the organisms commonly producing mastitis are *Staphylococcus aureus* and *Escherichia coli* and all are pathogenic (Sinell, 1973). Contamination of raw milk by pathogenic bacteria from source external to the udder may be caused by *salmonellae* strains, which produce many out breaks of *enteritis* (Robinson *et al.*, 1979).

The average values of coliform counts/ml of milk samples collected from Khartoum north was 5.43×10^4 cfu/ml this result is in agreement with the finding of Mutukumira *et al.* (1996), who found the coliform bacteria 3.2×10^2 to 2.3×10^5. Saitanu *et al.* (1996) examined and found that the total coliform count of <1000 cfu/ml.

Total bacterial counts or total aerobic colony counts are used to estimate viable bacterial populations in milk and reflect the hygienic practices used in the production and handling of the milk (Houghtby *et al.*, 1994). The results of this investigation are in agreement with the finding of Lee *et al.* (1983) conducted an experiment in Seoul (Korea) and found that the bacterial count in raw milk ranged from 4×10^6 to 2.7×10^7 cfu/ml.

Yeasts are not commonly the cause of defect in dairy products unless they ferment lactose. In this case, they can grow rapidly and produce a characteristic yeasty or fruity flavor and obvious gas (Davis and Wilbey, 1990). They also produce metabolites, e.g. short-chain fatty acids and other compounds, with known toxic effects against undesired micro-organisms in the intestinal tract (Jacobsen and Narvhus, 1996).

Conclusion: The microbiological quality was only marginally acceptable with respect to the total bacteria count. Nevertheless, the presence of pathogenic and indicator bacteria, such as *E. coli*, coliforms and *S. aureus* indicate that to the growth of these organisms may lead to a hazard against public health. Therefore practice and regulations, such as on-site pasteurization and implementation of HACCP following established standards, should be introduced to facilitate the production of cow milk of high quality and safety.

REFERENCES

Adesiyun, A.A., L. Webb and S. Rahman, 1995. Microbiological quality of raw cow milk at collection centers in Trinidad, J. Food Prod., 58: 448.

Asperger, H., 1994. *Staphylococcus aureus*. In: Monograph on the significance of pathogenic microorganisms in raw milk, International Dairy Federation, Brussels, pp: 24-42.

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 Science, pp: 171.

Bryan, F.L., 1983. Epidemiology of milk-borne diseases. J. Food Prot., 46: 637-649.

Coorevits, A., V. De Jonghe, J. Vandroemme, R. Reekmans, J. Heyrman, W. Messens, P. De Vos and M. Heyndrickx, 2008. Comparative analysis of the diversity of aerobic-spore-forming bacteria in raw milk from organic and conventional dairy farms system. Appl. Microbiol., in press.

Davis, J.G. and R.A. Wilbey, 1990. Microbiology of Cream and Dairy Desserts. In: Dairy Microbiology (Ed.: Robinson, R.K.). London and New Jersey, Applied Science Publishers, pp: 41-108.

De Man, J.D., M.A. Rogosa and M.E. Sharpe, 1960. Medium for the cultivation of lactobacilli. J. Appl. Bacteriol., 23: 130-135.

Fook, Y.C., A. Aminah and K.A. Mohd, 2004. Bacteriological quality and safety of raw milk in Malaysia. Food Microbiol., 21: 535-554.

Hamama, A., 1989. Thesis of Ph.D. Minnesota University USA, Handbook of food microbiology. Ministry for health, Morocco.

Harrigan, W.F. and M.E. MacCance, 1976. Laboratory Methods in Food and Dairy Microbiology, Academic Press, London.

Headrick, M.L., S. Korangy, N.H. Bean F.J. Angulo, S.F. Altekruse, M.E. Potter and K.C. Klontz, 1998. The epidemiology of raw milk associated food borne disease out breaks reported in the United States, 1973 through 1992. Am. J. Public Health, 88: 1219-1221.

Heeschen, W.H., 1993. Introduction In: Monograph on the significance of the pathogenic microorganisms in raw milk, International Dairy Federation Brussels, pp: 8-11.

Holsinger, V.H., K.T. Rajkowski and J.R. Stabel, 1997. Milk pasteurization and safety: A brief history and update. Rev. Sci. Tech. Off. Int. Epiz., 16: 441-451.

Houghtby, G.A., L.J. Maturin and E.K. Koenig, 1994. 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.

Jacobsen, N. and J. Narvhus, 1996. Yeasts and their possible beneficial and negative effects on the quality of dairy products. Int. Dairy J., 6: 755-768.

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

Mutukumira, A.N., S.B. Feresu, J.A. Narbhus and R.K. Abrahamsen, 1996. Chemical and Microbiological Quality of raw milk produced by small holder farmers in Zimbabwe. J. Food Prot., 59: 984-987.

Robinson, D.A., W.J. Edgar, G.L. Gibson, A.A. Matcheit and A.A. Robertson, 1979. Campylobacter enteritis associated with consumption of unpasteurized milk L. (1979). Br. Medical J., 1: 1171.

Rogelj Mleko, I., 2003. In: Mikrobiologija zivil zivalskega izvora (Eds.: Bem, Z., Adamic, J., Zlender, B., Smole Mozina, S., Gašperlin, L.). Ljubljana, Biotehniška fakulteta, Oddelek za zivilstvo, pp: 515-538.

Sinell, H.J., 1973. Food Infections from Animals. In: The Microbiological Safety of Foods. Hobbs, B.C. and J.H.B. Christian (Eds.). Academic Press, London and New York.

Steele, M.L., W.B. Mcnab, C. Poppe, M.W. Graffiths, S. Chen, S.A. Degrandis, L.C. Fruhner, C.A. Larkin, J.A. Lynch and J.A. Odumeru, 1997. Survey of Ontario bulk tank milk for food borne pathogens. J. Food Prot., 60: 1341-1346.

Saitanu, I.A., K.R. Chuanchuen, S. Nuanuarsuwan, C. Koowatananukul and V. Rugkhaw, 1996. Microbiological quality of raw cow milk. Thai J. Vet. Med., 26: 193-214.