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# Microbiological Safety of Raw Milk in Khartoum State, Sudan: 1-Khartoum and Omdurman Cities

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**Abstract:** Twenty four random samples of raw cow milk were collected from Khartoum and Omdurman Cities. Samples were analyzed for microbiological population, included Total Plate Count (TPC), Total Coliforms (TC), *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, Lactic Acid Bacteria (LAB) and spore forming bacteria. Microbiological enumeration revealed for the counts of total mesophilic aerobic bacteria, 5.86 log cfu/ml; lactic acid bacteria, 4.47 log cfu/ml; coliforms 2.76 log cfu/ml; *E. coli* 1.63 log cfu/ml; *Staphylococcus aureus*, 1.92 log cfu/ml and 2.38 log cfu/ml Spore forming bacteria. The microbial profiles found had non-conformance to the Standard. 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:** Milk, human diet, pathogenic bacteria,

## INTRODUCTION

Milk is a major component in human diet all over the world, but it also serves as a good medium for growth of many microorganisms, especially pathogenic bacteria. Thus, the quality of milk is considered essential to the health and welfare of a community. Also, all cases of dairy illness continued to be of bacterial origin, pathogens that have involved in communicable diseases associated with the consumption of milk include *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Campylobacter*, *Yersinia* pathogenic *Escherichia coli* and *Clostridium botulinum* (Adesiyun et al., 1995; Hahn, 1996).

The detection of coliform bacteria and pathogens in milk indicates a possible contamination of bacteria either from the udder, milk utensils or water supply used (Olson and Mocquot, 1980; Bonfoh et al., 2003).

Fresh milk drawn from a healthy cow normally contains a low microbial load (less than 1000 ml$^{-1}$, but the loads may increase up to 100 fold or more once it is stored for some time at normal temperatures (Richter et al., 1992). However, keeping milk in clean containers at refrigerated temperatures immediately after milking process may delay the increase of initial microbial load and prevent the multiplication of microorganisms in milk between milking at the farm and transportation to the processing plant (Adesiyun, 1994; Bonfoh et al., 2003). Contamination of mastitis milk with fresh milk may be one of the reasons for the high microbial load of bulk milk (Jeffery and Wilson, 1987).

In Sudan milk is considered one of the oldest kind of food and so many people depend on its products. Cow's milk is predominant, but some people depend on goat milk where the goats are kept by the families and camel milk by nomadic people.

The aim of this research project was to evaluate the quality of milk available to the consumers, comparing two different regions. The samples of milk were evaluated to determine the bacteriological quality.

## MATERIALS AND METHODS

### Microbiological analysis

**Collection of samples:** Twenty four samples of cow milk were collected randomly from different regions of Khartoum and Omdurman. Milking was done manually twice a day at 7.00am and 5.00pm.

The samples collected aseptically in sterile containers and transported to the microbiology laboratory, Food Research Centre, Khartoum North, where the analysis was done.

Sterilization, serial dilution and preparation of the media were done according to Harrigan and MacCance (1976).

**Total Bacterial Count (TBC):** Total viable count was carried out using the pour plate method described by Harrigan and MacCance (1976). Appropriate dilution (10$^{-1}$ up to 10$^{-6}$) of the samples was plated on Standard Plate Count Agar (Oxoid). The plates were incubated at 37$^{\circ}$C for 48 h.

**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$^{\circ}$C for 10 min to destroy vegetative cells. The tube was cooled and 1 ml from this dilution was aseptically transferred into sterile Petri dish. To each plate melted Starch Milk Agar (SMA) was added. The plate's inoculums were mixed with the medium and

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allowed to solidify. The plates were incubated at 37°C for 2 days.

**Enumeration of total coliforms:** Standard multiple tube fermentation technique described by multiple tube system was used for samples examination, which is described into three tests:

**Presumptive test:** MacConkey Broth (Oxoid) was used. 1 ml was added to MacConkey broth culture medium with a Durham's tube. All tubes were incubated at 37°C for 24 h. For positive tubes, numbers of bacteria were looked out from statistically calculated, MPN prepared tables used for Most Probable Number (Andrews, 1992).

**Confirmatory test:**
- The medium used for this test was Brilliant Green Bile (BGB) Broth a selective medium for *E. coli* (Oxoid). The tubes were inoculated with positive tubes (MacConkey Broth), incubated at 44°C for 48 h. After incubation, tubes were examined for gas production and colour changing from green to yellow.
- A loopfull of suspension from positive BGB broth tubes were streaked on Eosin Methylene Blue (EMB) agar (Oxoid). The plates were incubated at 37°C for 24 h (Harrigan and MacCance, 1976).

**Enumeration of *Staphylococcus aureus*:** Enumeration of *Staphylococcus aureus* was performed on Mannitol Salt Agar (Oxoid). The plates were incubated at 37°C for 48 h. yellow colonies were counted and checked for gram and catalase reactions. Also, the isolated colonies were checked for their coagulation on rabbit plasma. Catalase positive Gram negative colonies were spread cultured on Trypticase Soya Agar slants for further characterization (Harrigan and MacCance, 1976).

***Salmonella*:** Detection of *Salmonella* was carried out according to Harrigan and MacCance (1976). Thus, 25 ml of the sample were added to 250 ml of sterile nutrient broth (Oxoid) and incubated for 24 h at 37°C. 2 tubes of Selenite cystein broth (Oxoid) were inoculated with 1 ml from the nutrient broth and incubated for 24 h at 37°C. Positive tubes were streaked on Bismuth Sulfite agar (Oxoid) and incubated at 37°C for 24 h. The pure colonies were then subjected to the confirmatory tests.

**Lactic Acid Bacteria (LAB):** Numbers of LAB were determined on selective media MRS agar. Appropriate dilutions were plated on MRS agar and incubated anaerobically using the anaerobic jars and the BBL, Gas Pak, anaerobic system envelopes (Becton, Dickinson, Cockeysville, USA) at 37°C for 48 h. Enumeration of Lactic acid bacteria was determined using MRS medium incubated at 30°C for 48 h. After

incubation, colonies were enumerated, recorded as colony forming units (cfu) per milliliter of the products (Harrigan and MacCance, 1976).

**Statistical analysis:** All microbial counts were converted to the base -10 logarithm of the number of colony forming units per ml of raw cow milk samples (log cfu/ml) and from the means and their standard deviations were calculated. Data were analyzed using Analysis of Variance (ANOVA) through the General Linear Models (GLM) procedure of the statistical analysis system software (SPSS version-11.5, 2003). Least significant differences were used to separate means at $p < 0.05$.

## RESULTS AND DISCUSSION

The microflora of raw cow milk is presented in Table 1. Difference among milks from different regions of cow raw milk were studied by analysis of variance (Table 1) of the two regions. The highest ($p < 0.05$) average loads of TBC, LAB and coliforms were observed with respect to the average counts of *E. coli*.

Raw milk contained an average TBC of 5.86 log cfu/ml. It is a high count of TBC and should be due to inadequate sanitary condition during milking, collection and transport. Raw milk in Italy (Supino *et al.*, 2004) had total bacterial counts of 5.23 log cfu/ml, which is of the same order of our data. LAB constituted a major part of the microflora with an average 4.47 log cfu/ml. Boycheva *et al.* (2002) observed that LAB and psychrotrophs predominated in Bulgarian buffalo milk.

The average load of *S. aureus* was 1.92 log cfu/ml. Fook *et al.* (2004) reported considerably higher levels of *S. aureus* in other cow milk, with 35% of samples having 4.2 log cfu/ml. Since S. aureus is potentially hazardous at $>10^4$ cfu/ml (Han *et al.*, 2005), all cow milk samples were within an acceptable level.

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 growth is limited in raw milk (Holsinger *et al.*, 1997; Asperger, 1994).

On the other hand *Salmonella* was not detected in any samples that collected from Khartoum or Omdurman. The presence of *Salmonella* as pathogenic microorganism is a health problem and this in accord with the finding of Gazzar *et al.* (1992) who reported that *Salmonella* spp., become a major concern for the dairy industry due to out breaks of illness.

The average levels of coliform bacteria and *E. coli* were 2.76 and 1.63 log cfu/ml respectively. These counts were higher than those reported by Desmasures *et al.* (1997), who reported that 84% of samples of French cow milk had coliform counts <100 cfu/ml and 80% had *E. coli* counts ≤10 cfu/ml. *E. coli* may be considered an

Table 1: Microbiological loads of cow milk (log cfu/ml) in Khartoum district

| Region | TBC | SFB | LAB | Coliforms | *E. coli* | *S. aureus* |
|---|---|---|---|---|---|---|
| Khartoum (morning) [n = 6] | 5.17±0.46[a] | 3.08±0.11[a] | 4.53±0.64[a] | 2.41±0.21[a] | 1.63±0.13[a] | 2.15±0.53[a] |
| Khartoum (night) [n = 6] | 6.40±0.31[b] | 2.44±0.21[b] | 5.01±0.31[b] | 3.36±0.08[a] | 1.65±0.41[b] | 2.34±0.47[a] |
| Omdurman (morning) [n = 6] | 5.45±0.34[b] | 1.61±0.26[b] | 3.66±0.20[ab] | 2.21±0.29[a] | 1.46±0.17[b] | 1.40±0.72[b] |
| Omdurman (night) [n = 6] | 6.37±0.14[b] | 2.35±0.41[a] | 4.61±0.61[ab] | 3.09±0.11[a] | 1.89±0.09[b] | 1.81±0.17[c] |
| Average | 5.86±0.31 | 2.38±0.27 | 4.47±0.44 | 2.76±0.18 | 1.63±0.20 | 1.92±0.47 |

*Means±SD; [abc]Means bearing different superscripts in the same column differ significantly (p<0.05); TBC: Total Bacterial Count;
SFB: Spore Forming Bacteria; LAB: Lactic Acid Bacteria

indicator microorganism of faecal contamination and other enteric pathogens. The presences of large numbers of coliform bacteria are suggestive of unsanitary conditions or practices during production, processing, distribution or storage (Thomas *et al.*, 1971).

Pathogenic bacteria may also be present in raw milk as a direct consequence of clinical or subclinical mastitis (Giesecke *et al.*, 1994). Among the organisms commonly producing mastitis, *Streptococcus aureus* and *E. coli* are pathogenic for man (Bramley and McKinnon, 1990).

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).

Generally, fresh raw milks collected from retailers were heavily contaminated. Possible reasons for the counts could be due to infected udders of the cows, unhygienic milking procedures or equipment and/or inferior microbiological quality of water used for cleaning utensils and animals, as well as the milk storage conditions. The milking process, especially the equipment associated with it, introduces the greatest proportion of microorganisms in raw milk (Olson and Mocquot, 1980; Cousin, 1982).

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