

## Incidence of *Escherichia coli* in Raw Cow's Milk in Khartoum State

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**Abstract:** The present study was undertaken to estimate the incidence of opportunistic pathogen, *E. coli*, in raw cow's milk in Khartoum State (Khartoum, Khartoum North and Omdurman). Hundred raw milk samples were randomly collected from different localities/sources of Khartoum State and were inoculated on the relevant bacteriological media. Confirmation was performed using a series of biochemical tests. The results revealed that 63% of the samples were *E. coli* positive. The highest numbers of milk samples contaminated with *E. coli* were obtained from Khartoum north vending shops and Khartoum milk vendors. From twenty samples 9, 10 and 12 contaminated samples were detected from Khartoum, Khartoum North and Omdurman farms respectively with contamination with Coliforms ranging between  $3.86 \pm 0.1$  and  $4.18 \pm 0.01$  and *E. coli* between  $3.53 \pm 0.1$  and  $3.93 \pm 0.01$ . Although, fresh cow milk samples collected from milk vendors and milk vending shops were contaminated with almost the same load of Coliform bacteria  $4.11$  and  $4.01 \log_{10}$  cfu/mL, but the count of *E. coli* ranged from  $3.54$ - $3.90 \log_{10}$  cfu/mL. The highest mean value of coliform was found in milk from Khartoum farms with  $15.0 \times 10^4$  cfu/mL, while the lowest mean count of  $3.857 \pm 0.02 \log_{10}$  cfu/mL was detected in milk obtained from the Omdurman farms.

**Key words:** Raw milk, human diet, pathogenic bacteria, coliform bacteria, *E. coli*, public health

### INTRODUCTION

Raw or processed milk is a well-known good medium that supports the growth of several microbes with resultant spoilage of the product or infections/intoxications in consumers (Murinda *et al.*, 2004; Oliver *et al.*, 2005). Microbes may gain entry into raw milk directly from dairy cows experiencing sub clinical or clinical mastitis (Rodojcic-Prodaova and Necev, 1991), from the farm environment particularly the water source (Eberhart, 1977) and utensils used for the storage of milk on farm or during transportation (Freedman, 1977). Markets and consumers for raw milk and their products have existed in many parts of the world. Raw unpasteurized milk is consumed directly by a large number of people in rural areas and indirectly by a much larger segment of the population via consumption of several types of cheeses. Among the main reasons that people may believe that the raw milk and their products have advantages or value over the pasteurized one. Being a highly nutritious medium, therefore many bacteria including spoilage and pathogenic bacteria can grow and propagate in it. Generally, bacteria in the milk can occur through colonization of the teat canal or an infected udder (clinical and subclinical mastitis) or gets contaminated at various stages be it from the animal, milker (manual as well as automated), extraneous dirt or unclean process water (Banwart, 1989; Gruetzmacher and Bradley, 1999; Hayes *et al.*, 2001; Philips and Griffiths, 1990; Rohde, 1985; Stewart, 1978). Mastitis, milk quality and

dairy food safety are all much interrelated. Collectively, the presence of food-borne pathogens in unpasteurized raw milk either directly or indirectly increases the risk of ingestion and transmission of food-borne pathogens and ingestion of potentially harmful toxins. Many microorganisms can get access to milk and products, among these are *E. coli*. Coliforms and *E. coli* are often used as marker organisms. Recovery and counting of *E. coli* is used as reliable indicator of fecal contamination and indicates a possible presence of enteropathogenic and/or toxigenic microorganisms which constitute a public health hazard. *E. coli* is one of the main inhabitants of the intestinal tract of most mammalian species, including humans and birds. Most *E. coli* are harmless, but some are known to be pathogenic bacteria, causing severe intestinal and extra intestinal diseases in man (Kaper *et al.*, 2004). The output of dairy and dairy products from Sudan is increasing day by day in their internal market. Considering its economic potential, extensive and intensive exploitation of cow milk can both contribute to the nutrient requirements of the Sudanese public and increase the income of farmers. In view of the growing public awareness about food safety and quality, knowledge of the microbial and chemical composition of milk is of great significance for further development of its hygienic processing into high quality consumer products. Until now, information on such aspects is scant and scattered. Thus, the objective of this study was to investigate the occurrence of the opportunistic pathogen *E. coli* in cow's milk in Khartoum State, Sudan.

## MATERIALS AND METHODS

All the samples were collected in sterilized container at random from different localities of Khartoum State, and were brought in ice box to the Food microbiology and Biotechnology Laboratory, Food Research Centre, Khartoum North, for the isolation of *E. coli*.

**Microbiological methods:** The study was carried out in the Department of Food Microbiology, Food Research Centre, Khartoum North, during the period from March to September 2009.

**Preparation of serial dilutions:** Ten ml from each sample of raw sorghum flour and fermented dough were transferred to 90 mL sterile peptone water (0.1%) and thoroughly mixed to give 1:10 dilution 'first dilution'; serial dilutions were prepared by transferring one ml from first dilution ( $10^{-1}$ ) to 9 ml peptone water, ( $10^{-2}$ ) and so on ( $10^{-3}$ ,  $10^{-4}$ , ...) as described by Harrigan and McCance (1976).

**Coliform bacteria, *E. coli*:** The coliform test was done according to Harrigan (1998) by plating one ml sample onto MacConkey agar media. The plates were incubated at 37°C for 48 h and the counts were presented as colony forming unites per gram (cfu/g). Plates showing positive coliform were subjected to the confirmatory test using Brilliant green bile lactose broth in test tubes with inverted Durham tubes and incubated at 44°C for 48 h. Each positive tube was subcultured into E.C. broth medium and then incubated at 44.5°C for 24 h. Tubes showing gas productions were considered *E. coli* positive.

All the samples positive for *E. coli* contamination were confirmed using Gram's staining, cultural and biochemical examinations. For the isolation and identification of *E. coli*, the enriched sample was cultured on selective medium Levine Eosin Methylene Blue (EMB) Agar and incubated at 37°C for 24 h (Harrigan and MacCance, 1976). Morphologically typical colonies (at least 4/plate) producing metallic sheen were taken into nutrient broth for further identification. Biochemical tests were performed to confirm *E. coli* using Gram staining, Catalase test, Indole, Methyl red, Voges- Proskauer test, Nitrate reduction, Urease production, Simmon's citrate agar, and various sugar fermentation tests (Table 1).

**Statistical analysis:** Bacterial load and mean counts of coliform, *E. coli* were statistically analyzed by one way Analysis of Variance. Significant differences between treatments were determined using Turkey's multiple range test at  $p = 0.05$

## RESULTS AND DISCUSSION

The results of the present study are summarized in the Table 2 and 3. According to these results the highest

Table 1: Biochemical characterization of *E. coli*

Biochemical test	Reaction
Lactose fermentation	+ve
Catalase	+ve
Simmon's Citrate	-ve
Indole Production	+ve
Nitrate Reduction	+ve
Methyl Red	+ve
Voges- Proskauer	-ve
Urease	-ve
<b>Acid from Sugar</b>	
(a) Glucose	+ve
(b) Mannitol	+ve
(c) Lactose	+ve
(d) Salicin	+ve
(e) Sucrose	+ve

number of *E. coli* contaminated samples was recorded in Khartoum milk vendors and Khartoum North vending shops samples (16 out of 20 samples), followed by raw milk samples obtained from Omdurman (12 out of 20 samples). The least number of contaminated samples was detected in raw milk samples obtained from Khartoum farms (9 out of 20 samples).

Within columns, mean±SD followed by the same letter do not differ significantly using Turkey's test,  $p \leq 0.05$ .

From Table 2 it is shown that all tested samples were found to be contaminated with Coliform bacteria and heavily loaded with *E. coli*. Although high numbers of samples were contaminated, the least contamination with Coliforms and specifically *E. coli* was detected in samples collected from Omdurman farms, 3.86 and 3.53  $\log_{10}$  cfu/mL for Coliforms and *E. coli* respectively. The highest contamination with *E. coli* was shown in samples of Fresh cow milk collected from Khartoum farms and Khartoum milk vendors, 3.93 and 3.90  $\log_{10}$  cfu/mL respectively. Samples from Khartoum North farms and vending shops were found to have the same load of Coliforms and *E. coli*. Significant difference ( $p \leq 0.05$ ) in Coliforms load was found between Khartoum farms, Khartoum North farms and Khartoum vending shops and Omdurman farms but there is no significant difference between the load of Khartoum farms and Khartoum milk vendors. Coliform bacteria can be carried into milk duct of the cow during milking by suction of the milking machine and then flushed out during subsequent milking without causing clinical symptoms of infection. Previous studies provided evidence that *Escherichia coli* are frequently occurring organism in milk. The methods of production, transportation, handling and sale of milk are entirely unhygienic. The results of the present study showed that 63 out of 100 milk samples were contaminated with *E. coli*. High incidence of *E. coli* was found in different types of milk by many researchers (Naqvi, 1972; Martin *et al.*, 1986; Hanjra and Khan, 1989; Ahmed and Sallam, 1991; Sharma and Joshi, 1992; Adesiyun, 1994). Contamination of milk and milk products, with pathogenic bacteria is largely due to processing, handling, and unhygienic conditions. *E. coli*

Table 2: Summarizes the over all percentages of *E. coli* contamination in milk samples

Source/locality	Total number of analyzed samples	Number of <i>E. coli</i> positive samples	Percentage (%) of <i>E. coli</i> positive
Khartoum farms	20	9	45
Khartoum North farms	20	10	50
Omdurman farms	20	12	60
Khartoum vending shops	20	16	80
Khartoum North vending shops	20	16	80
Total	100	63	

Table 3: Mean counts of coliform, *E. coli* in raw cow milk sample collected from local dairy farms in Khartoum State.

Region	Coliform (log <sub>10</sub> cfu/mL)	<i>E. coli</i> (log <sub>10</sub> cfu/mL)
Khartoum farms (n = 20)	4.18±0.01 <sup>a</sup>	3.93±0.01 <sup>c</sup>
Khartoum North farms (n = 20)	4.10±0.1 <sup>b</sup>	3.57±0.1 <sup>e</sup>
Omdurman farms (n = 20)	3.86±0.02 <sup>d</sup>	3.53±0.1 <sup>e</sup>
Khartoum milk venders (n = 20)	4.11±0.01 <sup>a</sup>	3.90±0.03 <sup>c</sup>
Khartoum North vending shops (n = 20)	4.10±0.2 <sup>b</sup>	3.54±0.2 <sup>e</sup>

is one of the bacteria that exist in the normal microflora of the intestinal tract of humans and warm blooded animals. *E. coli* is, furthermore, a known causative agent of diarrhea and other food-borne related illnesses through the ingestion of contaminated foodstuffs. Pathogenic members of the coliform group as well as the Enterobacteriaceae family are represented by genera such as *Salmonella* and *Shigella* and are found in the intestines of humans and animals (Collins *et al.*, 1995; Hayes *et al.*, 2001; Le Minor, 1984; Rowe and Gross, 1984). Most strains of *E. coli* are nonpathogenic (Stender *et al.*, 2001). However some strains differ from Commensal in that they express virulence factors molecules directly involved in pathogenesis thereby causing disease (Schroeder *et al.*, 2004). *E. coli* frequently contaminates food and it is a good indicator of fecal pollution (Dilielo, 1982; Soomro *et al.*, 2002; Benkerroum *et al.*, 2004). Presence of *E. coli* in milk products indicates the presence of enteropathogenic microorganisms, which constitute a public health hazard. Enteropathogenic *E. coli* can cause severe diarrhoea and vomiting in infants, and young children. The incidence of the species of *E. coli* itself in milk and milk products, as a possible cause of food born disease, is not significant if *E. coli* is normally a ubiquitous organism (Hahn, 1996), yet the pathogenic strains if present could be harmful to consumers.

### CONCLUSION AND RECOMMENDATION

The results obtained in this study concluded that raw Cow's milk available to consumers in Khartoum state (March-September 2009) was highly contaminated with the opportunistic pathogen *E.coli*. High and strict preventive measures like regular washing and sterilization of dairy equipment, utensils, milker's hands, and animal udders, pasteurization of milk before distribution to consumers and eradication of diseased animals from the herd are highly recommended. In this respect pasteurization and immediate cooling to 5°C of milk could be more effective.

The magnitude of the problem of bacterial contamination deserves more elaborative studies from the point of production of milk and milk products to the point of consumption and at all intermediary levels. To identify potential milk safety hazards and to ensure production of safe and high quality product, food safety management programme, the Hazard Analysis Critical Control Point (HACCP), should be implemented and highly considered.

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