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Bacteriological Quality of Raw Camel Milk in Khartoum State, Sudan

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Abstract
The study was carried out in summer 2017 and winter 2018 in Khartoum State to investigate coliforms (total coliforms and Escherichia coli), Salmonella spp., Staphylo-coccus spp., yeasts, and molds in raw camel milk. Results indicated that coliform in the summer season, Staphylococcus aureus, yeasts and molds in summer and winter, Salmonella spp. were not detected in both seasons. Results revealed that Streptococcus spp and Staphylococcus spp. were the dominant bacteria in the raw camel milk.

Keywords*: Biological quality, raw camel milk, Staphylococcus spp., Streptococcus spp., Khartoum State, Sudan.*

I. Introduction

The estimated camel population in the world is around 22 million. Of this, 19.58 million are believed to be one-humped camels (*Camelus dromedarius*), while the remaining 2.42 million are two-humped bacterian camels (*Camelus bacterianus*). Camels live in the vast pastoral areas in Africa and Asia. The genus *camelus dromedarius* mainly live in the desert areas (arid), and the Bacterian camel (*Camelus bacterianus*) lives in the cooler areas. More than 60% of the dromedary camel population found in the four North East African countries viz. Somalia, Sudan, Ethiopia, and Kenya [1] Camels are very reliable milk producers during dry seasons and drought years when milk from cattle, sheep, and goats is scarce [2]. Numerous epidemiological reports proved that non-heat treated milk and raw-milk products represent one of the major factors responsible for illness caused by foodborne pathogens in pastoral communities [3]. Camel milk has been consumed for centuries by nomadic people for its nutritional value and medicinal properties. Pasteurized camel milk is currently produced and sold only in a few countries, including Saudi Arabia, United Arab Emirates, Kazakhstan, Mauritania, and Algeria [4].

Raw camel milk may contain microorganisms pathogenic for man. The contamination can generally occur from three main sources: within the udder, outside the udder, and from the surface of equipment used for milk handling and storage. Pathogenic bacteria may present in raw milk as a direct consequence of udder disease. The total number of the organism in milk as disease causative agent in relation to its evaluation for consumption is important. The notable disease-causing bacteria in milk are *Salmonella, Brucella, Staphylococcus, Listeria*, and coliforms. Coliforms are normal inhabitants of the large intestine, and their presence in milk could indicate faecal contamina-tion [5]

The quality of raw milk is a function of the animal's nutrition and health, chemical combination, and microbial activities. The two dominant factors of the quality are the time before delivery to the consumer and the condition of keeping the product. Microbial analysis of milk and milk products includes tests such as total bacterial count, yeasts and molds, and coliforms estimation. The high population of bacteria in aseptically drawn milk samples or the detection of harmful pathogenic microorganisms is evidence of unhygienic milk production conditions [6, 7]. The present study's objectives were to assess Sudanese raw camel milk's bacteriological quality obtained directly from the udder in the summer and winter seasons and to determine the dominant milk microflora.

II. Materials and Methods

A. Sampling

15 samples of raw camel milk were collected directly from the udder in five different areas from Khartoum State, Sudan (El-Sarha, El-Hatana, Soog Liby, Om-Dawn Ban, and Shambat). The ages of camels under the study were between 3 and 12 years. 15 samples were collected in May 2017 (summer), and 15 samples were collected in February 2018 (winter).

Raw camel milk samples (250ml) were collected in sterile screw bottles and kept in an iced box. All samples were transported to the Central Laboratory (Faculty of Agriculture, University of Khartoum, Sudan). The samples were analyzed for the total viable count and coliforms count, the pathogens *Salmonella* and *Staphylococcus aureus*, yeasts and molds, and types of microorganisms in raw camel milk.

B. Bacteriological analysis

The bacteriological tests considered for determining the bacterial load in raw camel milk samples were total bacterial count (TBC), coliforms count, the presence of the pathogens *Salmonella* and *Staphylococcus aureus*, yeasts and molds, and types of microorganisms in raw camel milk.

C. Total bacterial count

1ml of milk samples were diluted in 9ml of sterile peptone water and mixed thoroughly. After the preparation of serial dilutions, the volume (1ml) of appropriate dilutions was placed by the pour plate technique in duplicate. Standard plate count agar colonies were counted after plates were incubated at 37ºC for 48 hours[(8)].

D. Coliforms count

Total and fecal coliforms enumerations were carried out on VRBA (Violet Red Bile Agar) medium. 9ml of samples were diluted in 90ml of sterile peptone water, and decimal dilutions up to 10^{-6} were prepared. Duplicate dilutions from each dilution were mixed with (VRBA) medium. The plates were incubated at 30ºC for 24 hours and at 44ºC for 24 hours for total and faecal coliforms, respectively. Colonies were counted[(8)].

E. Detection of Salmonella

25ml of milk samples were pre-enriched in 225ml of sterile buffered peptone water at 37ºC for 24 hours. Then 10ml of the pre-enriched sample was incubated in Selenite Cystine Broth at 42ºC for 24 hours. About 0.1ml of the selective enrichment was then streaked onto Xylose Lysine Desoxycholate Agar plates. The plates were incubated at 37ºC for 24 hours. The cells were observed under the microscope [9].

E. Detection of Staphylococcus aureus

9ml samples were aseptically transferred into 90ml of sterile peptone water and mixed thoroughly; the decimal dilutions up to 10^{-6} were prepared. 0.1ml from each dilution was transferred into the surface of the Baird Parker Agar medium. The plates were incubated at 37ºC for 24 hours, then colonies were confirmed by the coagulate test [10].

F. Yeasts and molds enumeration

The surface spreading technique was used to enumerate yeasts and molds [8]. From suitable dilutions, 0.1ml was spread onto malt extract agar medium containing 0.1g chloramphincol to spress bacterial growth. Plates were incubated at 28ºC for 5 days. Colonies were counted using the colony counter (Quebec Colony Counter). The results were expressed as cfu/ml for each sample.

G. Bacterial isolation and identification

The isolation and identification of the bacteria are, according to Barrow and Feltham (1993) [11]. The purified isolates of bacteria were identified to the criteria: cultural characteristic of isolates, shape, color, odor, elevation, margin, consistency, growth, and size of colonies. The colonial characteristic on the different and selective media and hemolysis of blood agar, Gram's stain, reaction, Motility, Aerobic growth, and Biochemical tests.

III. Results and Discussion

Results obtained by enumeration of the different microbial flora of raw camel milk samples were shown in Tables (1, 2) in summer and winter, respectively. Generally, the counts in summer samples were higher than those in winter. The total viable bacterial count ranged from 4.2×10^2 to 5.4×10^4 cfu/ml in summer and winter, respectively. It is worth mentioning that there are currently no microbiological standards concerning camel milk. Therefore, standard European Union (EU) microbiological limits (TBC $\leq 10^2$ CFU/ml) for acceptable cow milk [12]. TBC is a good indicator for monitoring the sanitary conditions practiced during the production and handling of raw milk. High total bacterial counts in raw milk mainly reflect the poor hygienic condition under which the milk was handled, including the storage temperature and poor health of milking animals [13]. Another source of milk contamination hands of milkers and udders before milking. This shows that the practice of hygienic practices was inappropriate. The water used for washing may not be clean. Water used for washing at the milking level could be one factor since there was a shortage of water in the study areas.

Table (I): Microbial loads of raw camel milk produced in Khartoum State, Sudan (summer)

Sample No.	Total viable bacterial count (cfu/ml)	Coliforms count (cfu/ml)		Salmonella count (cfu/ml)	Staphylococcus count (cfu/ml)	Yeasts and molds count (cfu/ml)
		Total coliform	*E. coli*			
1	3.5×10^3	0	0	0	0	2.3×10^2
2	6.0×10^3	0	0	0	4.0×10^2	0
3	8.3×10^3	18	0	0	0	2.0×10^2
4	7.5×10^2	14	0	0	0	0
5	8.6×10^2	0	0	0	0	0
6	5.1×10^3	0	0	0	2.0×10^2	0
7	7.1×10^3	0	0	0	0	0
8	6.6×10^3	0	0	0	3.6×10^2	0
9	5.4×10^4	0	0	0	0	0
10	5.3×10^3	0	0	0	0	0
11	4.1×10^3	4	0	0	3.3×10^2	0
12	2.4×10^3	5	0	0	0	0
13	4.1×10^4	0	0	0	0	0
14	4.7×10^4	6	0	0	3.0×10^2	0
15	4.1×10^2	0	0	0	0	0

cfu: Colony-forming unit

Table (II): Microbial loads of raw camel milk produced in Khartoum State, Sudan (winter)

Sample No.	Total viable bacterial count (cfu/ml)	Coliforms count (cfu/ml)		Salmonella count (cfu/ml)	Staphylococcus count (cfu/ml)	Yeasts and molds count (cfu/ml)
		Total coliform	*E. coli*			
1	6.0×10^3	0	0	0	0	5.8×10^2
2	3.4×10^3	0	0	0	4.0×10^2	0
3	4.0×10^3	0	0	0	0	3.0×10^2
4	3.8×10^2	0	0	0	0	3.3×10^2
5	5.8×10^2	0	0	0	0	3.5×10^2
6	7.5×10^3	0	0	0	2.0×10^2	2.1×10^2
7	6.6×10^3	0	0	0	0	0
8	3.4×10^3	0	0	0	3.6×10^2	0
9	4.8×10^4	0	0	0	0	0
10	2.9×10^3	0	0	0	0	0
11	3.0×10^3	0	0	0	3.3×10^2	0
12	4.3×10^3	0	0	0	0	0
13	3.6×10^4	0	0	0	0	0
14	4.4×10^4	0	0	0	3.0×10^2	3.0×10^2
15	4.0×10^2	0	0	0	0	0

cfu: Colony-forming unit

Also, milking in the study areas full of dust and dung and without shade could negatively impact the quality of the milk produced in terms of pathogenic microorganisms. The microbiological quality of raw milk should be of major concern to the producers, the processors, and the general public because bacteria in milk can degrade milk components, decrease shelf life and acceptability of processed products and cause illnesses in human beings[14].

The value of coliforms counts observed in the present study (Table 2) was much lower when compared with the recommended values given by the American Public Health Association[15] and EU [12] (<100 CFU/ml). High numbers of coliforms in milk indicate that the milk has been contaminated with faecal materials. It is an index of the hygienic standard used in the production of milk. This could be attributed to insufficient pre-milking udder preparation, poor handwashing practice of milker. When present in any food, coliforms signal the possibility of enteric pathogens and unhygienic conditions under which the food was produced and handled [16].

Staphylococcus aureus ranged from 2.5×10^2 to 5.0×10^2 cfu/ml and from 2.0×10^2 to 4.0×10^2 cfu/ml in summer and winter respectively (Tables 2,3). This result agrees with Asfour and Anwer (2015), who reported that nearly 70% of camel milk samples are contaminated with *Staphylococcus aureus*. This could be due to poor hygienic practices and the presence of subclinical mastitis. Also, Asfour and Anwer (2015) reported that *S. aureus* was detected in 3.33% of camel milk samples tested in Egypt. On the other hand, Semereab and Molla (2001) reported higher rates (31.5%) of isolation *S. aureus* from camel milk in Ethiopia's far region.

Yeasts and molds ranged from 0 to 2.3×10^2 cfu/ml and from 2.1×10^2 to 5.8×10^2 cfu/ml in summer and winter, respectively (Tables 1, 2). The yeasts and molds content in Moroccan camel's milk was high, with an average raised to 4.6 log CFU/ml [20]. The high counts of yeasts and molds in milk are rather uncommon since the natural milk pH favors bacteria dominance[21].

The type of bacteria isolated from contaminated raw camel milk samples under the present study include *Staphylococcus* spp. (24.3%), *Strepto-coccus* spp. (21.6%), *Micrococcus* spp. (18.9%), *Bacillus* spp. (16.2%), coliforms (8.1%) and *Pseudomonas* spp. (2.7%). This result revealed that *Staphylococcus* spp. and *Streptococcus* spp. are the dominant bacteria isolated in raw camel samples.

This result contrasts with Abdullah and Sabry (2009) results, who reported that *E. coli* was isolated from 33 (66%) of the 50 raw camel milk and product samples tested. Also, Soomro *et al.* (2002), Chye *et al.* (2004), and Aly and Galal (2002) reported that *E. coli* was found to be the highest percentage of isolates from raw camel milk.

Conclusion

The present study showed that the raw camel milk samples collected during the summer were highly contaminated because of higher ambient temperatures. The hygienic level of milk was affected by various characteristics and practices of milkers like washing hands and udder and teat dip application. The presence of coliform bacteria indicates the poor hygienic condition in which milk is produced and marketed and could be pathogenic.

The major isolates were *Staphylococcus* spp. and *Streptococcus* spp. Therefore, in Khartoum State, Sudan, strict hygienic control measures to improve hygienic conditions of milk from production to consumers should be implemented. The work on the determination of camel milk standards in Sudan should be initiated.

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