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MICROBIOLOGICAL QUALITY OF RAW MILK PRODUCED AND DISTRIBUTED IN KHARTOUM STATE, SUDAN

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ABSTRACT

This study was carried out to assess the microbiological quality of raw milk collected from distribution channels in Khartoum State, Sudan. A total of 150 samples were collected from small dairy farms, pick-up trucks and venders on donkeys' cart and transported to the laboratory at ≤4°C for total viable bacteria (TVB), *Staphylococcus aureus*, coliform bacteria and yeasts and moulds counts determination. The results showed that the number of TVB, coliform bacteria and *S. aureus* counts were higher in samples collected from Omdurman town (Log 9.29 ± 0.66, Log 7.11 ± 0.07 and Log 7.08 ± 0.54 cfu/ml, respectively), while the highest yeasts and moulds count was in samples collected from Khartoum North (Log 7.07 ± 0.60 cfu/ml). According to the distribution channels, the highest TVB, coliform bacteria, *S. aureus* and yeasts and moulds counts were in samples collected from pick-up trucks (Log 9.22 ± 0.64, Log 7.21 ± 0.25, Log 7.37 ± 0.57 and Log 7.17 ± 0.17 cfu/ml, respectively). In Omdurman town, the highest TVB count was in samples collected from pick-up trucks (Log 9.82 ± 0.16 cfu/ml), the highest coliform bacteria and *S. aureus* counts were in samples collected from venders on donkey cart (Log 7.24 ± 0.23 and Log 7.34 ± 0.23 cfu/ml, respectively), while the highest yeasts and moulds count was in samples collected from pick-up trucks and venders on donkey cart (Log 7.23 ± 0.19 and Log 7.23 ± 0.23 cfu/ml, respectively). In Khartoum North, the highest TVB, colifrom bacteria and *S. aureus* counts were in samples collected from pick-up trucks (Log 8.61 ± 0.46, Log 7.19 ± 0.28 and Log 7.52 ± 0.53 cfu/ml, respectively), while the highest yeasts and mould count was in samples collected from farms (Log 7.44 ± 0.30 cfu/ml). The result indicated poor bacteriological quality of raw milk samples from the three marketing distribution channels in two locations and there is an urgent need to implement good hygiene practices from farm through marketing channel to the consumer.

Keywords: raw milk, microbiological quality, marketing channels, Sudan.

INTRODUCTION

The safety of dairy products with respect to food-borne diseases is a great concern around the world, especially in developing countries where production of milk and various dairy products take place under rather unsanitary conditions and poor production practices (Ashenafi, 1990; Negash *et al*., 2012; Asaminew and Eyassu, 2011; Tola *et al*., 2007). To protect public health against milk borne infections, there are regulations that require proper hygiene handling of milk and its pasteurization, but in developing countries such regulations are not usually adhered, hence milk borne health risk is higher in these countries (Donkor *et al*., 2007).

Fresh milk drawn from a healthy cow normally contains low microbial load and generally contains less than 1000 cfu/ml of total bacteria, but the load may increase once it is stored at normal temperature (Salman and Hamad, 2011; Wallace, 2008). That means raw milk serves as an ideal medium for microbial growth such as *Lactobacillus*, *Lactococcus*, *Streptococcus* and yeasts and moulds. In addition, the detection of coliform bacteria or pathogenes in milk can be used as an indicator for udder infection (mastitis), contamination in milking utensils or water supply (Yuen *et al*., 2012).

Bacterial contamination of raw milk can originate from different sources including air, milking equipment, feed, soil, faeces and grass (Coorevits *et al*., 2008). Moreover, the number and types of micro-organisms in milk immediately after milking are affected by factors such as animal, equipment cleanliness, season, feed and animal health (Torkar and Teger, 2008). Oliver *et al*., (2005) reported that the presence of bacteria in milk can cause some reduction in the quality of raw milk and certain bacterial contaminants with their associated enzymes and toxins may even survive pasteurization and create health hazards.

The chain of people involved in dairy production in such under-developed countries extends from milk production farms (farmers, farm workers and veterinarians) and transportation to milk market or small vendors to reach the final consumers either through milk venders or from shops (Salman and Hamad, 2011). In Sudan, the raw milk distributed for consumption is not subjected to proper quality control measures which are needed (Mohamed and El Zubeir, 2007). In Khartoum, 95% of milk is distributed as raw milk to the consumers (Salman and Hamad, 2011). Moreover, most of milk producers in Khartoum State are unaware of the effect of animal health and environmental conditions on producing safe milk due to absence of full certification of employees, absence of technical and staff, retardation of milk production and processing system and lack of training and extension programs (Abdalla and Elhagaz, 2011).

This study was carried out to investigate the occurrence and load of microorganisms in raw milk produced and distributed in Khartoum State through different marketing channels.

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MATERIALS AND METHODS

Milk sampling

The raw milk samples were collected from Omdurman and Khartoum North towns of Khartoum State, Sudan. A total of 150 samples were randomly collected of which 75 samples were collected from Omdurman and 75 samples from Khartoum North. In each town 25 samples were collected from each of traditional farms (locally known as *zariba*), pick-up trucks and venders on donkey carts. The samples were collected in dry clean sterile glass bottles (25 ml), preserved in ice box at ≤4°C and transported to the laboratory for microbiological examination.

Preparation of serial dilutions, culturing and enumeration of microorganisms

Milk sample (11 ml) was aseptically transferred to 99 ml sterile peptone water solution and thoroughly mixed to make 10^{-1}. From the previous dilution, 1 ml was transferred to another sterile test tube containing 9 ml sterile peptone water to make 10^{-2}. This procedure was repeated to make ten dilutions (10^{-1}-10^{-9}). Using a sterile tip, 1 ml from each dilution was carefully transferred into sterile Petri plates followed by adding 10-15 ml of the desired medium. The plates were then covered and mixed by gentle agitation and incubated at the proper temperature.

For enumeration of total viable bacteria, the standard methods agar medium was used, and the plates were incubated at 32°C for 48 hr (Houghtby *et al*., 1992). MacConkey agar medium was used for the enumeration of coliform bacteria, and the plates were incubated at 32°C for 48 hr (Christen *et al*., 1992). *S. aureus* was determined using mannitol salt agar medium, and the plates were incubated at 37°C for 48 hr (Abdalla and Hussein, 2010). The number of yellow colonies in un-crowded plates was counted. Yeast extract medium was used for the enumeration of yeasts and moulds, and the plates were incubated at 25°C for 5 days (Frank *et al*., 1992). For all microorganisms tested the number of colonies in each dilution was multiplied by the reciprocal of the dilution and recorded as colony forming units (cfu/ml).

Purification and identification of organisms

Cultures were examined with naked eyes for growth and colony morphology. Purification and identification of microorganisms was carried out according to Barrow and Felthman (1993).

Statistical analysis

The statistical analysis was performed using Statistical Analysis Systems (SAS, ver. 9). General Linear Models were used for the determination of the effect of location and marketing channel on the microbiological quality of raw milk. Means were separated by LSD test at $P \leq 0.05$.

RESULTS AND DISCUSSIONS

Microbiological quality of raw milk collected from Omdurman and Khartoum North towns

The results of the microbiological examination of the raw milk samples from Omdurman and Khartoum are presented in Table-1. The average values of total viable bacteria count were 9.29 ± 0.66 and 8.23 ± 0.76 $\log_{10}$ cfu/ml for Omdurman and Khartoum North, respectively. The total viable bacteria count of milk collected from Omdurman was significantly higher ($P<0.001$) than that of Khartoum North. The findings are consistent with those of Abdalla and Elhagaz (2011) who reported TVB count of Log_{10} 8.93 cfu/ml for raw milk samples collected from cows without application of hygienic practices, and Log_{10} 8.13 cfu/ml when sanitary hygienic practices were applied prior to milking. TVB count in this study is higher than that reported by Franciosi *et al*., (2009) and Tasci (2011). The higher TVB count in Omdurman could be attributed to the fact that most dairy farms in this area are around the city; therefore, venders transport milk to collection centers and/or directly to consumers without cooling. As a consequence for the lack of cooling facilities, contamination of milk is highly expected. These results are in agreement with those of Ali *et al*., (2010) and Hussain (2001). In another study, El Zubeir *et al*., (2007) found that the milk collected from Khartoum North and Omdurman has relatively high viable bacterial count and concluded that unsanitary conditions in the farms associated with mishandling of milk and lack of cooling during transportation could be the reason for this high bacterial load. Moreover, under tropical conditions like Sudan, the high temperature could enhance the growth and multiplication of bacteria in raw milk (Barakat, 1995), and the source of contamination of raw milk is manifold and starting from the milking procedure (Hempen *et al*., 2004). Unsanitary conditions with hand milking are common in the study area, for that as expected high numbers of microorganisms in milk are obtained from different sources. The milkers and handlers of milk determine the quality of milk because they contribute directly a significant number of bacteria from their hands and clothes (Gupta, 2008), in addition to flies falling into the milk are also another source of contamination. Under high ambient temperature and lack of cooling facilities, contaminated raw milk represents a very good medium for bacterial growth and multiplication (Hempen *et al*., 2004). The count of *S. aureus* bacteria was $\log_{10}$ 7.08 ± .54 cfu/ml in samples collected from Omdurman, and $\log_{10}$ 6.91 ± .78 cfu/ml in milk collected from Khartoum North. These results are much higher than the findings of Abdalla and Elhagaz (2011), Al-Tahiri (2005), Ali *et al*., (2010) and Torkar and Teger (2008). Contamination of raw milk after handling under non hygienic conditions and mastitis considered as another source of *S. aureus* (Fook *et al*., 2004). *S. aureus* is a ubiquitous organism that occurs in the mucous membranes and skin of most warm blooded animals including human beings, also is widely recognized as a major causative agent of clinical and subclinical mastitis in dairy cattle (Burnie *et al*., 2000). The high

count of *S. aureus* in both towns might be due to contamination by humans since up to 60% of humans are nasal carriers of *S. aureus* and 5-20% are carrying the organism as part of their normal skin flora (Asperger, 1994).

The mean value of coliform bacteria count in milk collected from Omdurman (Log_{10}7.11 ±. 07 cfu/ml) was higher than those from Khartoum North (log_{10} 6.61 ± 0.74 cfu/ml). High significant variation (P<0.001) in coliform bacteria count between the two towns was found. These results are in agreement with El Zubeir *et al*., (2007) who found that the mean coliform bacteria count was 7.98×10^5 cfu/ml in Omdurman and 6.86×10^6 cfu/ml in Khartoum North. However, the findings are higher than those of Abdalla and Elhagaz (2011), Al-Tahiri (2005), Ali *et al*., (2010), Franciosi *et al*., (2009), Tasci (2011) and Torkar and Teger (2008). The high coliform bacteria count might be due to poor hygiene during production of milk, fecal contamination and unclean equipments. Prejit *et al*., (2007) reported that coliform organism in milk can get entry through environmental and fecal contamination and this organism becomes a major source of milk contamination because it can rapidly build up and multiply in moist and milk residues on the surface of equipment.

The mean value of yeast and moulds count in milk from Khartoum North and Omdurman log_{10} 7.07 ± .60 and 7.06 ± .37 cfu/ml, respectively. This result is in agreement with that reported by Ali *et al*., (2010) and Tasci (2011) and higher than that reported by A-Tahiri (2005).

Table-1. Microbiological quality of raw milk from Omdurman and Khartoum North towns.

Parameter (log_{10} cfu/ml)	**Location** (mean±SD)		
	Omdurman	**Khartoum North**	**SL**
Total viable bacteria count	9.29 ± 0.66[a]	8.23 ± 0.76[b]	***
Coliform bacteria count	7.11± 0.07[a]	6.61 ± 0.74[b]	***
S. aureus count	7.08 ± 0.54[a]	6.91 ± 0.78[a]	NS
Yeasts and moulds count	7.06 ± 0.37[a]	7.07 ± 0.60[a]	NS

Means in the same row bearing similar superscripts are not significantly different (P>0.05)
*** = P<0.001
NS= Not significant
SL = Significance level
SD = Standard deviation

Microbiological quality of raw milk collected from different marketing channels

The results of mean values of the microbiological quality of milk samples collected from different marketing channels are summarized in Table-2. Milk on pickup trucks was highly contaminated with TVB, coliform bacteria, *S. aureus* and yeasts and moulds (Log_{10} 9.22 ± 0.64, 7.21 ± 0.25, 7.37 ± 0.57 and 7.17 ± 0.17 cfu/ml, respectively). These results are in disagreement with the findings of Karthikeyan and Pandiyan (2013) who reported high TVB in venders' milk due to the long interval between milking and consumption and the prevalence of favorable temperature for bacterial multiplication. In this study the high TVB might be due to poor hygienic practices in farm during handling of milk. Milkers and workers wearing dirty clothes and shoes during milking cows and selling this contaminated milk directly to consumers or to venders for transportation and distribute milk to the household might contribute to this poor milk quality. Hussain (2001) found that milk collected from venders had the highest TVB (6.18 ± 0.96 cfu/ml) followed by farms (6.08 ± 0.49 cfu/ml) and groceries (6.02 ± 0.89 cfu/ml). The high TVB might be due to the inadequate sanitary conditions during milking as well as unhygienic collection and transportation conditions. Moreover, venders transporting milk in containers without cooling have a negative effect on the microbiological quality of milk (Ali *et al*., 2010). Omer and Abdelgadir (2014) reported that no system of milk cooling is applied in Khartoum State. Fook *et al*., (2004) reported that the highest TVB recorded in milk samples from farm might be due to poor hygienic practices in farm during milking process and ineffective sanitizing routine on post milking process such as leaving herds manure in contact with cow's udder.

The high coliform count in this study might be due to the traditional means of production and handling as well as transportation and irregular marketing of the product. These results are in agreement with Hussain (2001) who reported that duration by venders takes 2-3 hours without cooling for transporting milk on donkeys from the farms and/or collection centers to consumer. During this time milk containers are opened frequently, accordingly milk is subjected to contamination. Presence of high coliform bacterial count in milk is suggestive of unsanitary conditions of practices during production, processing, distribution and storage.

The results of high *S. aureus* in vender's milk are higher than those reported by Ali *et al*., (2010) who found the average *S. aurues* to be 1.2×10^6 cfu/ml. The higher contamination was probably originated from cow's udder. Andreoletti *et al*., (2009) mentioned that *S. aurues* is presented within the farm environment and carried by approximately half the human population, and added that

absence of cooling during transportation is another factor that might increase *S. aurus* in milk. If milk is not refrigerated, the enterotoxigenic of *S. aureus* strains can grow and produce enterotoxin (O'Ferrall-Berndt, 2003). Rysanek *et al*., (2007) reported that post milking teat disinfection is especially effective against the contagious pathogens. The high count of *S. aureus* and coliform bacteria is due to poor personal hygiene practices (Yuen *et al*., 2012). Abdalla and Elhagaz (2011) reported that TVB, coliform and *S. aurues* count showed high number when hygiene practices were not applied. Moreover, fresh raw milk collected from retailers were heavily contaminated which might be due to infected udders, unhygienic milking procedures or equipment and inferior microbiological quality of water used for cleaning utensils. Lack of knowledge about clean milk production and lack of potable water for cleaning purposes were some of the factors that contributed to the poor hygienic quality of milk from farms or at collection centers in Khartoum State (Ali *et al*., 2010).

Yeasts and moulds count was in milk from pickup trucks (Log_{10} 7.17 ± 0.17 cfu/ml), followed by milk samples collected from farms and venders on donkey cart (Log_{10} 7.13 ± 0.49 and 6.96 ± 0.68 cfu/ml. respectively). The high count in this study might be due to poor hygiene of equipment during handling and processing of milk, and indicates unsanitary conditions of handling and contamination from environment (Prejit *et al*., 2007).

Table-2. Microbiological quality of raw milk from different marketing channels in Khartoum State.

Parameter (Log_{10} cfu/ml)	**Marketing channels** (mean ± SD)			**SL**
	Farm	**Pick-up trucks**	**Venders on donkey cart**	
Total viable bacteria count	9.06 ± 0.64[a]	9.22 ± 0.64[a]	8.82 ± 0.84[b]	***
Coliform bacteria count	6.72 ± 0.54[b]	7.21 ± 0.25[a]	6.65 ± 0.89[c]	***
S. aureus count	6.77 ± 0.45[c]	7.37 ± 0.57[a]	6.86 ± 0.81[b]	***
Yeasts and moulds count	7.13 ± 0.49[b]	7.17 ± 0.17[a]	6.96 ± 0.68[c]	*

Means in the same row bearing similar superscripts are not significantly different (P>0.05)
*** = P<0.001
* = P<0.05
SL = Significance level
SD = Standard deviation

The microbiological quality of milk samples collected from different distribution channels in Omdurman and Khartoum North is presented in Table-3. In milk samples collected from Omdurman, the highest TVB was in milk collected from pick-up trucks (Log_{10} 9.82 ± 0.16 cfu/ml). The higher mean count of coliform, *S. aureus* bacteria and yeasts and moulds were found in milk collected from venders on donkeys' cart (log_{10} 7.24 ± 0.23, 7.34 ± 0.43 and 7.26 ± 0.23 cfu/ml respectively). These results are in agreement with Hussain (2001) who found that in Omdurman the highest coliform bacteria count was in milk collected from venders, while the lowest count was in milk collected from farms. In Khartoum North the higher TVB, coliform and *S. aureus* count was in milk collected from pick-up trucks (Log_{10} 8.61 ± 0.19, 7.19 ± 0.28 and 7.52 ± 0.53 cfu /ml, respectively). The outbreak of staphylococcal poisoning in Khartoum North was due to milk purchased from vendors (Suliman and Mohamed 2010). Milk is very easily contaminated when collected unhygienically and handled carelessly leading to quick spoilage (Sherwani *et al*., 2013). Hussain (2001) reported that in Khartoum North, the lowest TVB and coliform bacteria were found in farms, while the highest count were found in venders. Hence, distribution channels in Khartoum State are considered a major source of contamination. These results are in agreement with Suliman and Mohamed (2010) who reported that most of the raw milk sold in Khartoum State is of low hygienic quality due to the traditional means of milking and distribution using donkeys or cars (vendors).

Table-3. Microbiological quality of raw milk collected from different marketing channels in Omdurman and Khartoum North towns (mean ± SD).

Parameter (Log_{10} cfu/ml)	**Omdurman town**			**Khartoum North town**		
	Farm	**pick-up trucks**	**Venders on donkey cart**	**Farm**	**pick-up trucks**	**Venders on donkey cart**
Total viable bacteria count	9.54 ± 0.40	9.82 ± 0.16	8.52 ± 0.43	8.58 ± 0.46	8.61± 0.19	7.51± 0.84
Coliform bacteria count	6.85 ± 0.69	7.12 ± 0.22	7.24 ± 0.23	6.85 ± 0.27	7.19 ± 0.28	6.05 ± 0.93
S. aureus count	6.61 ± 0.43	7.30 ± 0.43	7.34 ± 0.43	7.00 ± 0.47	7.52 ± 0.53	6.49 ± 0.78
Yeasts and moulds count	6.82 ± 0.44	7.23 ± 0.19	7.23 ± 0.23	7.44 ± 0.30	7.11 ± 0.12	6.64 ± 0.82
SD = Standard deviation						

CONCLUSIONS

The current study concluded that the microbiological quality of raw milk obtained from Omdurman and Khartoum North was very low. Raw milk samples distributed through venders on donkey cart and pick-up trucks had a high bacterial load in Omdurman and Khartoum North. The introduction of proper hygiene practices from milk producers through the distribution channels to consumers should be applied and suitable facilities for transporting milk should be used.

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