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Assessment of Microbial Loads and Antibiotic Residues in Milk Supply in Khartoum State, Sudan

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Abstract: This study was conducted to evaluate the quality of milk supplied to consumers from 2 sources, which include farms and sales points in Khartoum State. Raw milk samples (72 samples) were collected from Khartoum North (36 samples) and Khartoum (36 samples). All milk samples were analyzed for total bacterial counts (TBC), coliform counts and psychrotrophic counts as well as measuring temperature and acidity. Moreover, antibiotic detection was performed using both Delvotest and Modified one plate test. The results indicated that the levels of TBC, coliform counts and psychrotrophic counts were ranged between $\log_{10}$ 8.279 to $\log_{10}$ 11.491 cfu mL^{-1}, $\log_{10}$ 3.845 to $\log_{10}$ 9.179 cfu mL^{-1} and 0 to $\log_{10}$ 1.690 cfu mL^{-1}. The acidity and temperature of milk samples were 0.11-0.3 and 13-30.7°C, respectively. In the present study, it was found that the mean level of TBC, coliform counts and acidity of milk samples from sales point's samples were found to be $\log_{10}$ 11.136±0.215 cfu mL^{-1}, $\log_{10}$ 8.802±0.254 cfu mL^{-1} and 0.219±0.048, respectively. These values were found to be higher than the values, which recorded for the milk samples that were collected from the farms ($\log_{10}$ 9.089±0.281 TBC, $\log_{10}$ 4.789±0.340 coliform, 0.158±0.032 acidity). However, psychrotrophic counts revealed higher mean value ($\log_{10}$ 0.227±0.523 cfu mL^{-1}) for farms samples compared to sales points samples ($\log_{10}$ 0.069±0.238). Antibiotic residues were detected in 28 (38.9%) of examined milk samples, 17 (23.6%) of these were in Khartoum North and 11 (15.3) were in Khartoum. On the other hand the milk samples from sales points had the highest percent of the antibiotic residues 20 (55.6%) compared to those detected in the farms 8 (22.2%). The 28 (38.9%) milk samples that showed positive results were confirmed for the presence of antibiotic residues after heat treatment and 26 (92.9%) of them remained positive.

Key words: Raw milk, microbiological quality, antibiotic residues, farms, sales points, Khartoum State, Sudan

INTRODUCTION

Milk and dairy products have become a major part of the human diet in many countries. Over many years, considerable attention has been paid to improve the yield, the compositional quality and the hygienic quality of milk (Harding, 1999). Raw milk quality is closely monitored to ensure processed product quality and safety; in addition, raw milk must meet other quality standards including freedom from drug residues, added water, sediment, contaminants and other abnormalities (Murphy and Boor, 2000). Also, hygiene of milk include prevention of contamination of milk by stable environment and milking equipment as well as controlling temperature and time in order to minimize the growth of pathogens (IDF, 1994). The presence of foodborne pathogens in milk is due to direct contact with contaminated source in the dairy farm environment and due to excretion from the udder of an infected animal (Oliver *et al*., 2005).

Ahmed (2004) studied the hygienic quality of raw milk offered for sale in Khartoum State, Sudan; she reported that raw milk might cause health hazards for humans if itconsumed without pasteurization or heat treatment.

Antimicrobial residues in milk cause series health problems for consumers in the form of antibiotic resistance or allergies (Shitandi and Sternesjö, 2003), as well as for the dairy industry, in the form of delays in bacteriological processes used to manufacture dairy products (Currie *et al*., 1998).

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The milk supply chain in Sudan is very weak and faced with so many constraints, of which the health hazards constitute an important issue. The aim of the present study was to evaluate the hygienic quality of milk supply in Khartoum State, detection of the presence of antibiotic residues in bulk milk using the Modified one plate test and Delvotest SP test and evaluate the effect of heat treatments on the presence of antibiotic residues in the examined milk samples.

MATERIALS AND METHODS

Sample collection: A total of seventy 2 bovine milk samples were collected during the period from July to September 2005. Thirty 6 samples were collected from the 6 big farms from Khartoum North and Khartoum and 36 of the samples were collected from six sales points from Khartoum North and Khartoum. The samples were collected into clean sterile bottles and cooled immediately at approximately 4-5°C in an ice bag until examinations were carried out. The samples were transported to the laboratory of the Department of Dairy Production, Faculty of Animal Production, University of Khartoum for analysis.

Examination of milk samples: The samples were examined for Total Bacterial Counts (TBC), coliform counts and psychrotrophic counts according to Harrigan and Mc Cance (1976). Plate count agar no. 298 (Biomark Laboratories) was used for enumeration of total bacterial counts and psychrotrophic counts. MacConkey's agar no. 779 (Biomark Laboratories) was used to determine coliform counts. Plates for enumeration of TBC and coliform incubated at 32°C for 48 h and 37°C for 24 h, respectively. Plates for enumeration of psychrotrophic counts were incubated at 7°C for ten days (Ballou *et al*., 1995). Developed colonies were counted using manual colony counter. The plate counting 25-250 colonies were selected as described by Houghtby *et al.* (1992). The number reciprocal of the dilution factor was recorded as cfu mL^{-1}. The acidity of the samples was determined according to Foley *et al.* (1974). Antibiotic residues were determined using Delvotest® SP- ampule Kit (202-Delvotest SP 100, test box, DSM Food Specialties, the Netherlands). The method was carried out according to the manufacture's instructions and Modified one plate test were used as described by Koenon-Dierick *et al.* (1995).

Effect of heating on antibiotic residues in milk: Positive and doubtful samples were kept refrigerated at 4°C and 24 h later were heated to 82°C for 10 minute; 0.1 cfu mL^{-1} of each sample were added to the ampule of the Delvotest SP test for confirmation (Molina *et al*., 2003).

Statistical analysis: The analysis was carried out using SPSS program (Statistical Package for Social Science). Positive and doubtful results by the Delvotest SP test were grouped as "positive plus doubtful" to treat these qualitative variables at 2 levels ("negative" and "positive plus doubtful"). Chi square was also used to determine the percentage of antibiotic residues. Analysis of Variance (ANOVA) was used to determine the effect of locations and source of milk samples on bacterial counts and physiochemical properties of the milk.

RESULTS

The hygienic quality of raw milk samples from Khartoum and Khartoum North (Khartoum State): The raw milk samples collected from Khartoum State showed that the average total bacterial count was $\log_{10}$ 10.112±1.060 cfu mL^{-1}. The milk samples collected from Khartoum North showed mean count of $\log_{10}$ 10.142±0.979 cfu mL^{-1}, with a maximum value of $\log_{10}$ 11.371 and minimum value of $\log_{10}$ 8.663 (Table 1). Similarly, the milk samples collected from Khartoum showed that the mean total bacterial count was $\log_{10}$ 10.083±1.149 and the maximum value was $\log_{10}$ 11.491 and the minimum was $\log_{10}$ 8.279 (Table 1). The milk samples collected from Khartoum State revealed non significant variation for total bacterial count ($p>0.05$).

The coliform counts of the milk collected from Khartoum State were found to be $\log_{10}$ 6.796±2.042, $\log_{10}$ 9.179 and $\log_{10}$ 3.845 cfu mL^{-1}, for mean, maximum and minimum values, respectively (Table 1). The samples collected from Khartoum North revealed coliform counts of $\log_{10}$ 6.801±2.045, $\log_{10}$ 9.090 and $\log_{10}$ 4.00 cfu mL^{-1}, respectively, while that collected from Khartoum showed colifrom counts of milk were $\log_{10}$ 6.791±2.068, $\log_{10}$ 9.179 and $\log_{10}$ 3.845 cfu mL^{-1}, respectively.

The psychrotrophic counts of the milk samples collected from Khartoum State were found as $\log_{10}$ 0.148±0.412 cfu mL^{-1}, $\log_{10}$ 1.690 cfu mL^{-1} for mean and maximum values, respectively, while the minimum was reported as 0 (Table 1). The psychrotrophic mean counts of milk were $\log_{10}$ 0.069±0.238 cfu mL^{-1} and 0.227±0.523 cfu mL^{-1} for the milk samples collected from Khartoum North and Khartoum, respectively (Table 1).

Comparison of some hygienic quality measurements of raw milk samples from farms and sales points in Khartoum State: The raw milk samples collected from different farms (Table 2) showed that the mean total bacterial count was found to be $\log_{10}$ 9.089±0.281 cfu mL^{-1}. The milk samples collected from farms of Khartoum North showed mean count of $\log_{10}$ 9.200±0.242 cfu mL^{-1}, while the maximum value was $\log_{10}$

Table 1: Comparison of some quality measurement of raw milk samples from Khartoum and Khartoum North

Measurement		Khartoum North	Khartoum	Total
Log total bacterial counts (cfu mL^{-1})	M±Std	10.142±0.979	10.083±1.149	10.112±1.060
	Max	11.371	11.491	
	Min	8.663	8.279	
Log coliform counts (cfu mL^{-1})	M±Std	6.801±2.045	6.791±2.068	6.796±2.042
	Max	9.090	9.179	
	Min	4.00	3.845	
Log psychrotrophic counts (cfu mL^{-1})	M±Std	0.069±0.238	0.227±0.523	0.148±0.412
	Max	1.041	1.690	
	Min	0	0	
Acidity	M±Std	0.189±0.049	0.188±0.054	0.189±0.514
	Max	0.30	0.29	
	Min	0.11	0.12	
Temperature (°C)	M±Std	28.40±3.22	27.70±4.88	28.05±4.12
	Max	30.70	30.50	
	Min	17.00	13.00	

In this and the following tables: M = Mean, Std = Standard deviation, Min = Minimum, Max = Maximum

Table 2: Comparison of the hygienic quality of raw milk samples from farms and sales points of Khartoum State

		Farms			Sales points		
Item		KN	K	Sub total	KN	K	Sub total
Log TBC (cfu mL^{-1})	M+Std	9.200±0.242	8.977±0.280	9.089±0.281	11.083±0.192	11.189±0.229	11.136±0.215
	Max	9.616	9.352	9.615	11.371	11.491	11.491
	Min	8.663	8.279	8.279	10.813	10.643	10.643
Log coliform counts (cfu mL^{-1})	M+Std	4.807±0.395	4.771±0.284	4.789±0.340	8.794±0.205	8.810±0.301	8.802±0.254
	Max	5.377	5.093	5.377	9.089	9.180	9.180
	Min	4.00	3.845	3.845	8.341	8.114	8.114
Log Psych.C (cfu mL^{-1})	M+Std	0	0.455±0.674	0.227±0.523	0.138±0.327	0	0.069±0.238
	Max	0	1.690	1.690	1.041	0	1.041
	Min	0	0	0	0	0	0
Acidity	M+Std	0.157±0.026	0.158±0.038	0.158±0.032	0.220±0.047	0.218±0.051	0.219±0.048
	Max	0.21	0.25	0.25	0.30	0.29	0.30
	Min	0.11	0.13	0.11	0.16	0.12	0.12
Temp.	M+Std	29.27±0.63	25.46±6.19	27.36±4.74	27.52±4.39	29.93±0.34	28.73±3.30
	Max	30.1	30.2	30.2	30.7	30.5	30.7
	Min	28	13	13	17	29	17

KN = Khartoum North, K = Khartoum, TBC = Total bacterial count, Psych = Psychrotrophic, Temp = Temperature

9.616 and the minimum value was $\log_{10}$ 8.663 cfu mL^{-1} (Table 2). In Khartoum, the milk samples collected from the farms showed that the mean total bacterial count was $\log_{10}$ 8.977±0.280 cfu mL^{-1}, the maximum value was $\log_{10}$ 9.352 and the minimum value was $\log_{10}$ 8.279 (Table 2).

The milk samples collected from sales points in Khartoum State revealed mean total bacterial count of $\log_{10}$ 11.136±0.215 cfu mL^{-1} (Table 2). The milk samples collected from sales points in Khartoum North and Khartoum showed mean total bacterial counts of $\log_{10}$ 11.083±0.192 and $\log_{10}$ 11.189±0.229 cfu mL^{-1}, respectively. However, the maximum and the minimum values for the milk samples collected from sales points in Khartoum North and Khartoum were found as $\log_{10}$ 11.371 and $\log_{10}$ 11.491 cfu mL^{-1} and $\log_{10}$ 10.813 and $\log_{10}$ 10.643 cfu mL^{-1}, respectively (Table 2). Moreover the milk samples collected from the different sampling points showed highly significant variations for total bacterial counts ($p<0.05$). The colifrom count was estimated as $\log_{10}$ 4.789±.340 cfu mL^{-1} for the milk samples collected from different farms of Khartoum State (Table 2). The farms that located in Khartoum North showed that the mean, the maximum and the minimum values of the coliform counts of $\log_{10}$ 4.807±0.395, $\log_{10}$ 5.377 and $\log_{10}$ 4.00 cfu mL^{-1}, respectively (Table 2). Similarly the coliform counts of the milk samples collected from the farms of Khartoum were found to be $\log_{10}$ 4.771±0.284, $\log_{10}$ 5.093 $\log_{10}$ and 3.845 cfu mL^{-1}, respectively (Table 2).

The milk samples collected from the sales points of Khartoum State revealed mean coliform count of $\log_{10}$ 8.802±0.254 cfu mL^{-1} (Table 2). The milk samples collected from Khartoum North and Khartoum sales points showed mean coliform counts of $\log_{10}$ 8.794±0.205 and 8.810±0.301 cfu mL^{-1}, respectively as shown in Table 2. The milk samples showed highly significant differences ($p<0.05$) for coliform counts from different sampling points, while non significant differences ($p>0.05$) due to variations of cities. Psychrotrophic bacteria was detected only in the milk samples collected from some farms that located in Khartoum and sales points of Khartoum North with mean count of $\log_{10}$ 0.455±0.674 and $\log_{10}$ 0.138±0.327 cfu mL^{-1}, respectively (Table 2).

The average acidity of milk samples that collected from Khartoum Sate was found to be 0.189±0.514, the maximum was 0.30 and the minimum was 0.11. The mean values for acidity were 0.189±0.049 and 0.188±0.054 for milk samples collected from Khartoum North and Khartoum, respectively (Table 1). The mean value of acidity of the milk samples collected from the farms and the sales points of Khartoum North were 0.157±0.026 and 0.220±0.047, respectively (Table 2). Similarly, the milk samples collected from the farms and sales points in Khartoum revealed 0.158±0.038 and 0.218±0.051, respectively (Table 2). There were highly significant differences ($p<0.05$) in the values of acidity for the milk samples collected from the different sampling points, while non significant differences ($p>0.05$) were obtained for acidity of the milk samples collected from the different cities.

The mean temperature of the milk samples collected from Khartoum State was 28.05±4.12°C, the minimum was 13.00°C and the maximum was 30.7°C. The mean values of the temperature of the milk samples collected from Khartoum North and Khartoum were estimated as 28.4±3.22 and 27.7±4.88°C, respectively (Table 1). The mean value of temperature of milk samples collected from the farms and the sales points of Khartoum North were 29.27±0.63 and 27.52±4.39°C, respectively (Table 2). However, the temperature of the milk samples collected from Khartoum farms and sales points were recorded as 25.46±6.19°C and 29.93±0.34°C, respectively (Table 2). There were highly significant differences ($p<0.05$) in the temperature of the milk samples collected from different sampling points, while non significant differences ($p>0.05$) were found for the milk samples collected from the different cities.

Antibiotic residues of the milk samples collected from different cities in Khartoum State: The results obtained by both microbiological assays; The Delvotest-SP test and Modified one plate test revealed the same results as recorded in Table 3. The present result showed that 28 (38.9%) of the milk samples collected from Khartoum State were found to be positive for antibiotic residues tests. Moreover, 17 (47.2%) of the positive samples were detected in the milk samples collected from Khartoum North, while 11 (30.6%) were found in the milk samples collected from Khartoum. The raw milk collected from the different farms in Khartoum State (Table 3) revealed that the total milk samples showing positive detection of antibiotic residues were 8 (22.2%). These were reported as 6 (33.3%) and 2 (11.1%) in Khartoum North and Khartoum, respectively (Table 3). On the other hand, the milk samples collected from the sales points showed 20 (55.6%) positive samples for antibiotic residues (Table 3). These positive samples were estimated as 11 (61.1%) and 9 (50%) for the milk samples collected from Khartoum North and Khartoum, respectively (Table 3).

Table 3: Frequencies of antibiotic residues in raw milk samples from farms and sales points of Khartoum State using two screening methods

Source		Sales points	Farms	Total
Delvo- test	Khartoum	9 (50%)	2 (11.1%)	11 (15.3%)
	Khartoum North	11 (61.1%)	6 (33.3%)	17 (23.6%)
	Average	20 (55.6%)	8 (22.2%)	28 (38.9%)
Modified one plate test	Khartoum	9 (50%)	2 (11.1%)	11 (15.3%)
	Khartoum North	11 (61.1%)	6 (33.3%)	17 (23.6%)
	Average	20 (55.6%)	8 (22.2%)	28 (38.9%)

Effect of heat treatment on antibiotic residue: Most of the milk samples that showed positive results (28; 38.9%) were confirmed for the presence of antibiotic residues after heat treatment. Following heat treatment (26; 92.9%) of the total positive samples remained positive. The 2 milk samples that were negative after boiling the milk were among the samples collected from the collection pints in Khartoum North.

DISCUSSION

In the present study, the fresh raw milk samples collected from Khartoum North and Khartoum in Khartoum state were heavily contaminated by bacteria (Table 1 and 2). This result supported Mohamed and El Zubeir (2007) who found high average of bacterial counts in the milk samples collected from Khartoum State. Also, it was in agreement with Jayarao *et al.* (2004) who reported that the herd size and farm management practices had considerable influence on somatic cell and bacterial counts in bulk tank milk. Possible reasons for the high counts could be due to infected udders of the cows, unhygienic milking procedures or equipment and inferior microbiological quality of water used for cleaning utensils as well as the milk storage conditions (Godefay and Molla, 2000). However, improvement in milk hygiene reduces the bacterial content of raw milk (Jung, 2002). In addition to the lack or inefficient of cooling facilities was an important factor of the high count as reported previously by Ahmed (2004).

The total bacterial counts and the coliform counts were found to be significantly affected by the sampling point. This might be due to the traditional methods of distribution and transportation of milk (Elmagli *et al.*, 2006). Since the vendors take hours to transport raw milk form farms or sales points to the consumers in cans without cooling, which supported Godefay and Molla (2000), Bonfoh *et al.* (2003) and Fudle Elseed (2005). In addition, containers are opened frequently that milk might subjected to contamination.

It is probable that the low count of psychotrophic bacteria obtained during the present study was due to high temperature and unavailability of cooling during transportation and storage of milk. This result is in agreement with Chye *et al.* (2004) who reported that the psychrotrophic counts in the tropical countries were lower than the count for milk produced in the temperate countries. The psychrotrophic count was considered lower than the count of milk produced in temperate countries, which could reach as high as 10^6 cfu mL^{-1} (Reinheimer *et al.*, 1990).

The sampling point affected the values of acidity significantly during present study, the mean value agreed with Mohamed and El Zubeir (2007). This could be due to the high numbers of bacterial count and high atmospheric temperature (Table 1 and 2), which supported Ahmed (2004) and Mohamed and El Zubeir (2007).

The average mean value of the temperature of milk samples was 28.05±4.12°C. This might be due to the higher temperature in Sudan. Also, this might be due to the lack of cooling facilities of milk. The above results are in agreement with Mohamed and El Zubeir (2007) who reported that the milk samples had high temperature due to the higher atmospheric temperature. Moreover, she concluded that the handling temperature of the milk might facilitate the growth and multiplication of pathogenic and non pathogenic bacteria during production, transportation and distribution, which might lead to spoilage of milk and change the product to unsafe food.

The screening of milk samples by the Delvotest SP test and Modified one plate test showed that 38.9% of the milk samples positive for antibiotic residues. The present result is higher Barakat (1995) as he found 8.7% of the milk samples from Khartoum State were contaminated by antibiotic. Similarly, El Sherbini and El sayed (1993) in Egypt found 7.8% of the examined samples were positive. Jones and Seymour (1992) reported that the use of excessive of approved drugs or prolonged drug clearance and multiple dosing as the source of antibiotic in milk. On the other hand, Shitandi and Sternesjö (2003) reported that the small-scale producers in Kenya, with fewer resources and lacking relevant knowledge, might not follow recommended treatment regimes when using antibiotics, as manifested in the higher prevalence of antimicrobial drug residues in their milk. The results of antibiotic detection in raw milk samples from farms and sales points showed 22.2 and 55.6% positive results, respectively. The high incidence of antibiotic residues in the milk samples from the sales points was in agreement with Taj Elsir (2001) who reported that the high percentage of antibiotic residue in raw milk collected from vendors might be due to the use of antibiotics as preservatives to increase the shelf life of the raw milk. Irwin *et al.* (2003) suggested that minimizing level of disease occurrence is a way to minimize the use of antibiotics, which achieved by maintaining sanitation and hygiene.

The milk samples that showed positive results for antibiotic residues were confirmed for the presence of inhibitors after heat treatment. Following heat treatment, 26 (92.9% of the total contaminated samples) remained positive. Several studies have reported that false-positive results might be due to the high levels of natural inhibitors in mastitic milk and colostrums, which can cause false-positive result in the microbial growth inhibition assay (Kang and Kondo, 2001; Yamaki *et al.*, 2004; Kang *et al.*, 2005). The false-positive result in Delvotest assay was found to correlate with an increase in lactoferrin and lysozyme concentrations (Kang and Kondo, 2001). Few of the samples 2 (7.1%) examined during the present study could be classified as false positive. Similar finding was reported by Kang *et al.* (2005) who found that 3 samples exhibited positive results from 24 positive samples subjected to heat treatment. In addition, this variation might be due to the different antibiotics, which contributed the milk, since Abdulrhman (2001) reported the absence of the effect of heating on tylosin, while very slight influence on oxytetrcycline and penicillin were observed. This reduction might also be related to the presence of thermosensitive anti-microbial compounds, which might contaminate the milk (Yamaki *et al.*, 2004). Moreover, Kang *et al.* (2005) stated that the heat treatment of milk is a fast, simple and inexpensive method to remove false-positive results.

The results obtained during the present study clearly indicated that the microbial quality of raw milk produced in Khartoum State is low. Moreover, this study presented that lack of hygienic measures at milk production chains might be the reasons of the high bacterial counts and occurrence of antibiotic residues. Both are likely to affect the keeping quality and the safety of raw milk as well as products derived from it. Non significant differences were observed in the measurement of the milk quality between the areas studied, although the milk samples from the sales points was highly contaminated than those from farms. The higher level of incidences of antibiotic residues in milk samples collected from sales points might be due to the adulteration by addition of antibiotic to the milk in order to prolong its shelf life.

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