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Research Article

# Microbiological and Anti-Microbial Quality of Raw Milk Produced in Dairy Farms in Khartoum and Gezira States, Sudan

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## Abstract

A stratified random study was conducted between 2019 and 2023 across 99 dairy farms in all localities of Khartoum and Gezira States. The primary aim of the study was to detect and enumerate coliform bacteria in raw milk, assess awareness regarding antibiotic residues in raw milk, and evaluate farm cleanliness. A pre-structured questionnaire was utilized to collect data on the area, farm hygiene practices, drug usage, and methods of disposing of antibiotic-contaminated milk. Bacterial isolates were identified using biochemical reactions and sugar fermentation tests. The study revealed that 74 (74.7%) of the investigated farms used antibiotics and antiparasitic. Alarmingly, 96 (97.0%) farms sold milk from cows treated with antibiotics for human consumption. Additionally, 69 (69.7%) dairy workers were unaware of the harmful effects of chemical residues in milk on human health. The average total plate count (TPC) in milk was $2.157 \times 10^5$ cfu/ml across the three localities of Khartoum State and $8.45 \times 10^5$ cfu/ml in Gezira State. The average most probable number (MPN) for coliforms counts in Khartoum, Bahri, Omdurman and Gezira were 236.49 ± 55.15, 146.00 ± 29.29, 259.23 ± 51.39, and 175.57 ± 14.96, respectively. The prevalence of E. coli in milk samples varied significantly between regions. Positive samples in Khartoum State were 9 (64.3%), 17(80.9%), and 10 (90.9%) in Khartoum, Bahri and Omdurman, respectively, whereas it was found 25 (47.1%) in Gezira State. There was a significant association between the area and the presence of E. coli in milk with p- value .007). The study also highlighted a significant association between regions and the frequency of manure removal with P ≥± 0.05. A chi-square test further showed an insignificant relationship between the area and the TPC in milk. Moreover, a high degree of association was observed between coliform counts and manure disposal practices P ≥± 0.05. In Conclusion, the findings indicate that both coliform counts and TPC in milk from the investigated farms exceeded the internationally accepted limits. This underscores the urgent need for improved farm hygiene practices, proper sanitation of equipment, and strict adherence to national milk safety standards.

**Keywords:** TPC; Coliforms; Anti-Microbial Residues; Dairy Farms

## Introduction

Coliform bacteria are a broad group of rod-shaped, Gram-negative bacteria that are commonly found in the environment, particularly in the intestines of warm-blooded animals, including humans. Coliforms are used as indicator organisms for fecal contamination in water, milk, and other substances [1], which can pose a significant health risk due to the possibility of exposure to harmful pathogens. Coliform tests are also used to monitor the safety of food products, especially those that may have come into contact with contaminated water or have been handled unsanitarily [2].

Milk is virtually sterile when it is synthesized in a healthy cow's udder, and may become contaminated with bacteria during or after

milking. Some disease-causing organisms (pathogens) can be shed through cow feces and may contaminate the outside of the udder and teats, the farm environment, water, bedding and the milking equipment [3,4]. Although optimal growth conditions for bacteria are different for different organisms, milk contains important nutritional components, and therefore, it is also an ideal medium for the growth of many different bacteria [5]. Microbes may enter raw milk directly from dairy cows experiencing sub clinical or clinical mastitis from the farm environment particularly the water source [6].

Raw milk can be contaminated with residue of anti-microbial agents which are used to treat a variety of diseases or added intentionally to increase the usable life of the product. It was reported that 80% of conventional dairy herds use antibiotics for treatment of mastitis which was the first disease and remains the most common reason for administration of antibiotics in cattle [7,8]. Milk supplies containing higher concentrations of antibiotics are not acceptable, because of their allergenic properties and inhibitory effect on culturing processes of fermented milk products. The residues maybe also responsible for the development of resistant strains of bacteria [9].

In Sudan milk is produced mostly in non-organized way and usually it is being supplied to the consumers as raw milk from the urban and rural areas either directly from farms, by milk vendors or from the groceries. The distribution of milk to the consumers is completely in poor hygienic conditions [10]. However, about 82.7% of consumers prefer to purchase fresh raw milk [11]. Most of the farmers were not aware of antimicrobial resistance (78.7%) as well as and 56% of raw milk users [12]. One of the requirements of production of high- quality milk is maintaining the bacteria count level of microorganisms in the raw milk.

The objectives of this study were to detect and enumerate the coliform bacteria in raw milk, in dairy farms in Khartoum and Gezira States, and to evaluate the awareness of farmers to the antimicrobial resistance.

## Material and Methods

Stratified random sampling technique was used to select 99 dairy farms in Khartoum and Gezira States. A pre-structured questionnaire was prepared to obtain data regarding the area, hygienic statues of the farm, antimicrobial awareness and drugs used. Two hundred and twenty-seven of milk samples were collected from different areas in Khartoum and Gezira States. All the farms in the two States almost followed the same pattern in cow milking. No milking machines were used in all farms. Milk was milked in small buckets from every cow and collected either in plastic or Aluminum container which was put under shade. Different animal breeds were found in every farm. Holstein-Friesian cows, cross breeds, and local breeds all were kept together. Samples were collected in sterile containers, preserved under cold conditions till transferred to the lab for analysis.

### Preparation of serial dilutions

Ten ml from each sample of raw milk were transferred to 90 mL sterile normal saline 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 normal saline, ($10^{-2}$) and so on ($10^{-3}$, $10^{-4}$, $10^{-5}$ …) as described by [13].

### Total plate count (TPC)

TPC test was performed using plate count agar and serial dilutions according to [14]. In duplicate plates with 30-200 colonies per plate, all colonies were counted and the dilution was recorded to calculate the total number of colonies. If plate exceeded 200 colonies, the count was recorded as uncountable. If the count is less than 30 colonies the actual number on lowest dilution was recorded [15].

### Total coliforms count (TCC)

TCC presumptive test was done according to [12,16]. The Lauryl tryptose broth was used as the media for the presumptive test for total Coliforms count. Peptone water was used as a diluent; this result in a dilution of $10^1$, $10^2$, $10^3$, $10^4$ and $10^5$. A Durham tube was inserted into each Lauryl Tryptose tube. 1 ml of each dilution was pipetted into 3 Lauryl Tryptose tubes. All tubes were incubated at 35 to 37°C for 48 h and then examined for gas formation in the Durham tubes.

### Confirmed test for coliforms

Each positive (gassing) Lauryl Tryptose tube was gently agitated and a loopful of suspension was transferred to tube of "brilliant green" bile broth. All the tubes were incubated at 35 to 37°C; any gas formation in Durham's tubes with slight turbidity in the media was regarded as positive confirmed test. Results were interpreted using the MPN tables based on combination of confirmed gassing of Lauryl Tryptose broth tubes for three consecutive dilutions [16].

### Confirmed test for *E. coli*

According to [16], Levine Eosin methylene blue (L-EMB) agar was used. The plates were streaked with a loopful of suspension from confirmed positive brilliant green bile broth culture. Plates were incubated at 35°C for 18 to 24 h. Discrete dark centered nucleated colonies with or without metallic sheen were regarded as a positive test.

### Biochemical tests

According to [17] different biochemical and sugar tests were performed to identify other coliforms.

### Data analysis

SPSS ver. 20 was used for descriptive (the mean standard error of the mean) and analytical statistics (ANOVA and chi square test).

## Results

A total of 227 milk samples were collected from 99 dairy farms as shown in table 1.

| Area | Milk | Number of farms |
|---|---|---|
| Khartoum | 37 | 14 |
| Bahri | 76 | 21 |
| Omdurman | 36 | 11 |
| Gezira State | 76 | 53 |
| Total number | 227 | 99 |

**Table 1:** Distribution of the total number of samples.

### Farms cleanliness and manure removal

Some farmers used to clean their farms daily (14,1%) (n = 14), while two-thirds used to clean their farms weekly (65.7%) (n = 67), others once a month (18.2%) (n = 18) (Figure 1). The result also highlighted a significant association between regions and the frequency of manure removal P ≥± 0.05.

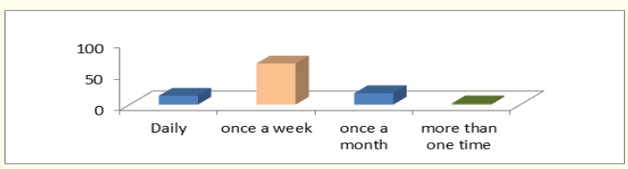


**Figure 1:** Farm cleanliness and manure removal.

### Milk usage following antibiotic therapy

Only 1 (17.1%) of farmers in Khartoum locality withdraw milk from antibiotic-treated cows, while 2 (3.8%) feed this milk to calves. Most farmers 13 (92.9%) mix the treated milk with milk from untreated cows (Table 2). Statistically there was no significant differences between areas and milk use following antibiotic therapy with P ≤ 0.05.

| Area | | Milk after treatment with antibiotics and medicines | | | Total | Sig. |
|---|---|---|---|---|---|---|
| | | Withdrawn | Sold | Given to calves | | |
| Area | KH | 1 (7.1%) | 13 (92.9%) | 0 | 14 | |
| | BA | 0 | 21(100) | 0 | 21 | |
| | OM | 0 | 11 (100%) | 0 | 11 | .248 |
| | GZ | 0 | 51 (96.2%) | 2 (3.8%) | 53 | |
| Total | | 1 | 96 | 2 | 99 | |

**Table 2:** Association between areas and usage of milk after antibiotic therapy.

### Awareness of the antibiotic residues in milk

In Khartoum State, 10 (71.4%), 6 (28.5%), and 0 (0.0%) of respondents from Khartoum, Bahri, and Omdurman localities, respectively, were aware of the residual effects of antibiotics in milk. In Gezira State, only 14 (26.4%) of respondents demonstrated similar awareness. The variation in awareness levels between localities was statistically significant (P ≤ 0.05) (Table 3).

| | | Knowledge | | Total | Sig. |
|---|---|---|---|---|---|
| | | Yes | No | | |
| Area | KH | 10(71.4%) | 4(28.6%) | 14 | |
| | BA | 6(28.5%) | 15(71.4%) | 21 | 0.001 |
| | OM | 0 | 11(100%) | 11 | |
| | GZ | 14(26.4%) | 39(73.6%) | 53 | |
| Total | | 30 | 69 | 99 | |

**Table 3:** Association between areas and awareness of the antibiotic residues in milk.

### TPC and MPN in milk samples in the investigated areas

The average TPC across the two states was $5.46 \times 10^5 \pm 143 \times 10^3$ and the average TPC in Khartoum, Bahri, Omdurman and Gezira were $1.79 \times 10^5 \pm 26 \times 10^3$, $3{,}18 \times 10^5 \pm 15 \times 10^3$, $1.50 \times 10^5 \pm 10 \times 10^3$ and $8.45 \times 10^5 \pm 260 \times 10^3$, respectively. Similarly, the average total coliform count across the four areas was 187.21 ± 14.19. The mean coliform counts in Khartoum, Bahri, Omdurman and Gezira were 236.49 ± 55.15, 146.00 ± 29.29, 259.23 ± 51.39, and 175.57 ± 14.96, respectively. The differences in TPC and coliform counts between the areas were not statistically significant. (P ≤ 0.05) (Table 4; Figure 2).

| | | Mean TPC | SEM | Sig. | Mean Coliform | Std. Error | Sig. |
|---|---|---|---|---|---|---|---|
| MPN | KH | $1.79 \times 10^5 \pm 26 \times 10^3$ | $26 \times 10^3$ | | 236.49 ± 55.15 | 55.15 | |
| | BA | $3{,}18 \times 10^5 \pm 15 \times 10^3$ | $15 \times 10^3$ | 0.079 | 146.00 ± 29.29 | 29.29 | |
| | OM | $1.50 \times 10^5 \pm 10 \times 10^3$ | $10 \times 10^3$ | | 259.23 ± 51.39 | 51.39 | .164 |
| | GZ | $8.45 \times 10^5 \pm 260 \times 10^3$ | $260 \times 10^3$ | | 175.57 ± 14.96 | 14.96 | |
| | Av. | $5.46 \times 10^5$ | $143 \times 10^3$ | | 187.21 ± 14.19. | 14.19 | |

**Table 4:** Coliform count (MPN) and TPC in milk samples in the investigated areas.

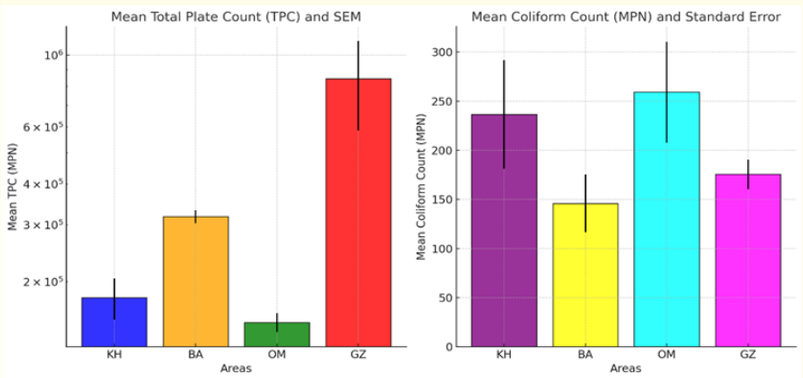


**Figure 2:** Mean TPC and Coliform.

## Association between areas and *E. coli* in milk samples

The percentage of positive milk samples for *E. coli* in milk was 21 (64.3%) in Khartoum locality area, 39 (80.9%) in Bahri locality area, 22(90.9%) in Omdurman locality area and 58 (47.1%) in Gazira State areas. There was significant association between the areas and the presence of *E. coli* in milk (p- value .007) (Table 5).

| | | ***E. coli* in milk** | | **Total** | **Sig.** |
|---|---|---|---|---|---|
| | | **Positive** | **Negative** | | |
| Area | KH | 21 (64.3%) | 11 | 32 | |
| | BA | 39 (80.9%) | 9 | 48 | |
| | OM | 22 (90.9%) | 3 | 25 | 0.007 |
| | GZ | 58 (47.1%) | 64 | 122 | |
| Total | | 140 | 87 | 227 | |

**Table 5:** Association between areas and *E. coli* in milk samples.

## Discussion

This study aimed to detect and enumerate the coliform bacteria in raw milk and to evaluate the awareness of farmers to the issue of antimicrobial resistance in dairy farms in Khartoum and Gezira States, In this study the frequency of manure removal from the farms reflects the degree of the cleanliness of the farm. The findings highlighted a significant association between regions and the frequency of manure removal with P ≥ 0.05. Better frequency of manure removal was reported by [18] who found that half (50%) of the dairy farmers used to remove their cows' manure weekly, one-quarter (25%) used to remove it daily, and one-quarter (25%) used to remove it monthly.

In the present study, according to the frequency of farm cleaning, and the general conditions of the farm environment in terms of available space for animals, absence of insects, drying of the floors and the cleanliness of feeding and drinking troughs 14.1% of the farms were classified as "Good", 55.6% were in "Fair" reasonable condition while 30.3% were in "Poor" conditions. This finding was comparable to [19] who showed that 53% of the farm owners adopted traditional type of animal keeping where the fences were from acacia trees and the general environment is poor. Similar findings also were reported by [20] who investigated dairy farm in Bahri locality and scored low values (between 40.7% and 75.2%). This low classification of dairy farms in term of general hygiene also supported the findings of [21] who stated that there is no rigid system of inspection and that dairy farms are not complying with sanitary standards.

In this study 69 (69.7%) of the investigated farmers were not know the harmful effect of chemical residues in milk on human health. In addition, 96 (97.00%) of the investigated dairy farms sold milk from cows treated with antibiotics for human consumption. It also supports the finding of [22] who stated that the78.7% of the antibiotic users and 56% of the milk consumers lack knowledge about the emerging threat of antimicrobial resistance in bacteria and/or antibiotic residues in animal products. This observation also was coincided with the findings of [12,23]. The results were in agreement with [10] who showed that antibiotic treated cows were milked together with the healthy ones and 85% of the farmers lacked the necessary information about antibiotic residues in milk.

This study revealed that the highest TPC was observed in Gezira, ($8.45 \times 10^5 \pm 26 \times 10^3$) followed by Bahri, Khartoum, and Omdurman. The difference in TPC count was statistically insignificant. Furthermore, the highest SEM, in Gezira samples indicating greater variability or uncertainty in TPC measurements compared to other localities. The average TPC in Gezira State was above the limit internationally accepted ($5 \times 10^5$ CFU/ml). Higher counts were obtained by [20] who obtained TPC $13.8 \times 10^5 \pm 6 \times 10^4$cfu in milk tank samples in Bahri area. Higher result also was obtained by [24] who revealed high contaminated TPC level in milk ($2.55 \times 10^7$cfu/

ml). [10] reported that more than half of the samples (55.3%) had a count of less than or equal $9 \times 10^5$ cfu/ml.

The average Coliform count in this study was 187.21 ± 14.19CFU. The highest mean coliform count is found in Omdurman (259.23 ± 51.39) while the lowest is in Bahri (146.00 ± 29.29). These differences were statistically insignificant. Standard errors are relatively smaller, with Gezira (GZ) having the lowest error, indicating more consistent measurements. The variation in coliform counts may reflect differences in milk handling practices, storage conditions, or water quality used during production and processing [1]. reported coliform counts ranging between 190 and 258 CFU in Khartoum state, which is comparable to the current findings. The higher coliform counts may be due to milking cows inside the barns which can predispose milk to fecal contamination.

The current study revealed that Omdurman exhibits the highest percentage of positive E. coli samples (90.9%), suggesting a potential issue in milk hygiene practices in this locality. Gezira showed the lowest positivity rate (47.1%), but it also has the largest total sample size, which may influence the observed trends. The P = 0.007 indicates that the differences in E. coli presence among the areas were statistically significant. This highlights regional disparities in milk contamination levels [1], observed a lower E. coli positive sample (32%) in Khartoum state.

## Conclusion

This study found significant variations in the prevalence of *E. coli* across the studied regions. Furthermore, a significant association was observed between the presence of *E. coli* and the areas, as well as a strong link between coliform counts and manure disposal practices. Notably, the average TPC and Coliform count exceeded acceptable limits in both Khartoum and Gezira States. Most of the investigated farms used to sell milk from cows treated with antibiotics for human consumption without any regards to withdrawal period. The awareness of dairy workers regarding the harmful effect of chemical residues in milk on human health was found low. Unsanitary conditions of farms and accumulation of manure within the barns was found in most of the farms.

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## Competing Interest

The authors declare that they have no competing interests.

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## Authors' Contributions

This work was carried out in collaboration between authors. Author (1) collected the research data, conducted laboratory analysis, and contribute to drafting the initial manuscript. Author (2) conceptualized the initial idea, performed supervision over the research, drafted the initial manuscript and carried out correspondence duties. Author (3) contributed to checklist preparation, and edited, reviewed the final manuscript. All authors read and approved the final manuscript.

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