Assessment of safety and quality of fermented milk of camels, cows, and goats sold and consumed in five localities of Burkina Faso

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Abstract

Background and Aim: Fermented milk is food produced and consumed all over the world and plays an important role in human nutrition. This work aimed to evaluate the microbiological and physicochemical quality and mineral composition of fermented milk consumed in Burkina Faso.

Materials and Methods: A total of 114 samples of fermented milk from camels, goats, and cows were purchased in the market in five localities in Burkina Faso; Bobo Dioulasso, Djibo, Dori, Gorom-Gorom, and Sebba. Microbiological and physical parameters were monitored using standards methods.

Results: Microbiological analysis of fermented milks showed high average values of 7.60±1.50×10⁷ colony-forming unit per milliliter (CFU/ml), 5.72±3.60×10⁶ CFU/ml, 5.53±2.00×10⁵ CFU/ml, 1.97±0.18×10⁴ CFU/ml, 1.98±0.25×10³ CFU/ml, and 0.10±0.09×10¹ CFU/ml for total microbial flora, lactic acid bacteria, yeasts and molds, Staphylococcus aureus, total coliforms, and thermotolerant coliforms, respectively. None of the samples were contaminated by Salmonella or Shigella. The average values of pH, acidity, dry matter, ash, fats, proteins, and total carbohydrates content of samples were ranged, respectively: 3.830-4.137, 1.888-2.822%, 8.271-13.004%, 0.199-0.476%, 1.210-3.863%, 2.125-3.764%, and 3.080-5.428 % (w/w). Na/K and Ca/Mg ratio ranged from 0.104 to 0.909 and from 3.392 to 16.996, respectively. Total microbial flora, yeasts and molds, total coliforms, fats, calcium, potassium, iron, and zinc were significantly different.

Conclusion: This research contributed in the evaluation of the hygienic and nutritional qualities of local fermented milk. Results obtained in this study confirm the need to set up the training program on the sanitary condition to traditional maker’s to ensure the good fermented milk with high organoleptic and nutritional qualities.

Keywords: Burkina Faso, camel, cow, fermented milk, goat, sanitary quality.

Introduction

Milk is the natural product of the secretion of the mammary gland of a lactating female. It is an essential component of the diet of pastoral or agropastoral populations and also an important source of income in Sahelian countries. Milk plays an important role in bone growth, maintaining body integrity and health through its composition of minerals, fats, proteins, carbohydrates and vitamins [1]. Milk microbiota contains many bacteria, some are useful and necessary for her transformation to other products as lactic acid bacteria or molds used for the maturing of cheese and yeasts transforming sugars to alcohol [2-4].

According to composition, raw milk is an ideal medium for the growth of many microorganisms, unlike fermented milk, where there is a predominance of lactic acid bacteria with some contaminants as Bacillus, Staphylococcus, and Escherichia coli [5,6]. Cow milk is the most milk consumed in the world followed by that of goat, camel, buffalo, mare, and donkey. Taste of camel’s milk varies according to the pasture, is appreciated for its anti-infectious, anti-cancer, anti-diabetic, and reconstructive properties in convalescent patients [7,8]. In other countries as Central Asia, mare milk is used to replaces maternal milk for infants. In Africa and particularly in Burkina Faso, for ethnic and cultural reasons only, the milk of sheep, camel, goat, and cows is consumed. The use of unconventional milk (donkey and mare) is culturally important. Consumers of these products attribute to their medicinal and mystical properties during occult practices. Conventionally, the origin of the milk fermentation is correlated to the appearance of nomadic peoples (Fulani). Fermented milk is a traditional remedy used by the old medical sciences of agropastoral communities. In Burkina Faso, there is a greater diversity of dairy products in diets of the populations which include raw milk, fermented milk, pasteurized...
milk, yogurt, cheese, cream, butter, gappal, dèguè, and soap Fulani [9]. In the past, fermented dairy products as yogurt, fermented milks, and cheese have been recognized as foods with undeniable nutritional qualities [10-12]. Recently, a diversity of yogurt (yogurt with Moringa, pineapple, sweetened, and unsweetened) is sold by local producers in Burkina Faso. These foods are perishable and often contaminated by microorganisms, antibiotics, pesticides (insecticides), detergents, and disinfectants [13]. The hygienic quality of milk and dairy products is considered, as one of the major factors limiting their consumption. Other factors influencing the quality of these products include lack of knowledge in good hygiene practices, preservation conditions, and certain chemical additives used.

This work aimed to evaluate the microbiological, physicochemical, and nutritional qualities of fermented milk produced and consumed in Burkina Faso.

Materials and Methods

Ethical approval

Ethical approval does not apply to this type of study. Samples of fermented milk were purchased from the vendors and analyzed in our laboratory.

Sampling

A total of 114 fermented milk samples (camel, cow, and goat) produced by the traditional method, purchased from the markets and streets of five cities in Burkina Faso, were collected aseptically from local producers and transported to the laboratory at 4-5°C using icebox for the different analysis. Figure-1 and Table-1 presented, respectively, sampling sites and samples coding.

Microbiological analysis

Microbiological analyses of fermented milk were performed according to standard methods described in the manual of microbiological analysis. The bacterial populations in fermented milk were enumerated after prepared stock solution and decimal dilutions according to standard microbial methods. 10 ml of the sample were added to 90 ml of sterile buffered peptone water, and serial dilutions were monitored with this suspension. All tests were done in duplicate. The results were expressed as colony-forming unit per milliliter (CFU/ml). Total microbial flora was enumerated on plate count agar after incubation at 30°C during 24-48 h. Lactic acid bacteria were enumerated on plates of Man, Rogosa, and Sharpe agar, after incubation at 37°C for 24-48 h anaerobically (anaerobic jars with Anaerocult A). Yeasts and molds were enumerated on Sabouraud CAF agar with chloramphenicol, after incubation at 25°C for 3-5 days. Total coliforms and thermotolerant coliforms were counted on eosin methylene blue agar at 37°C and 44°C for 24-48 h. Staphylococcus aureus were counted on Baird-Parker agar supplemented with tellurium egg yolk and incubated at 37°C for 24-48 h, the black brilliant or dark gray colonies surrounded a clear halo were selected and tested for the confirmation (Gram, catalase, and coagulate tests). The research of Salmonella or Shigella spp. was carried by pre-enrichment with buffered peptone water followed by enrichment in Rappaport-Vassiliadis broth and isolation on Salmonella-Shigella agar for 24 h at 37°C after each part.

Physicochemical analysis

The samples were mixed and analyzed in duplicate for the determination of different parameters physicochemical. The pH was determined using a digital pH meter (WATERPROOF-PC5). Titratable acidity, dry matter, ash, fats, and protein contents were determined according to AOAC [14]. Total carbohydrates were calculated according to this formula: Total carbohydrate = Total solids - (Fat + Protein + Ash) [15].

Minerals determination

For the determination of mineral elements, the ash was dissolved in 100 ml of concentrated HNO₃.
Table-1: Coding of samples.

| Fermented milk | Bobo Dioulasso | Djibo | Dori | Gorom-Gorom | Sebba |
|----------------|---------------|-------|------|-------------|-------|
| Camel          | -             | CaJ   | CaD  | CaG         | CaS   |
| Cow            | CoB           | CoJ   | CoD  | CoG         | CoS   |
| Goat           | -             | GoJ   | GoD  | GoG         | GoS   |

Table-2: Characteristics of the calibration curves of minerals.

| Mineral | Standard concentration (mg/L) | Standard solution | Standard gas | Dependence | Correlation coefficient |
|---------|-------------------------------|-------------------|--------------|------------|-------------------------|
| Ca²⁺    | 0.0551                        | 422.70            | HNO₃         | y=0.07993×c | 0.9985                  |
| Fe²⁺    | 0.1904                        | 248.20            | HNO₃         | y=0.02311×c | 0.9985                  |
| K⁺      | 0.1497                        | 766.50            | HNO₃         | y=0.039×c   | 0.9776                  |
| Mg²⁺    | 0.0098                        | 285.20            | HNO₃         | y=0.45101×c | 0.9895                  |
| Na⁺     | 0.0477                        | 589.00            | HNO₃         | y=0.09215×c | 0.9899                  |
| Zn²⁺    | 0.0499                        | 213.90            | HNO₃         | y=0.08810×c | 0.9889                  |

*λ*=Flame photometer reading, *c*=Concentration in mg/L, *y*=Wavelength, Ca²⁺=Calcium, K⁺=Potassium, Na⁺=Sodium, Mg²⁺=Magnesium, Fe²⁺=Iron, Zn²⁺=Zinc

Table-3: Microbiological parameters of different fermented milk samples.

| Samples | TMF×10⁹ | LAB×10⁹ | Y&M×10⁹ | *S. aureus*×10³ | TC×10³ | TTC×10³ | SS |
|---------|---------|---------|---------|-----------------|--------|---------|-----|
| CaD (n=6) | 0.39±0.27 | 0.43±0.34 | 5.44±3.00 | 0.18±0.06 | 0.13±0.06 | 0.06±0.03 | Nd |
| CaG (n=10) | 4.08±1.80 | 2.53±2.37 | 0.34±2.70 | 0.21±0.05 | 0.06±0.04 | 0.02±0.11 | Nd |
| CaS (n=4) | 0.41±0.10 | 3.10±2.90 | 5.53±2.00 | 1.97±1.18 | 0.17±0.07 | 0.08±0.04 | Nd |
| CaJ (n=4) | 4.60±1.39 | 4.08±3.60 | 0.47±0.28 | 1.23±0.39 | 0.08±0.06 | 0.02±0.00 | Nd |
| CoB (n=10) | 3.76±3.20 | 2.37±1.30 | 0.63±0.30 | 0.62±0.53 | 0.17±0.12 | 0.03±0.04 | Nd |
| CoD (n=10) | 0.50±0.32 | 0.52±0.27 | 0.47±0.26 | 0.54±0.52 | 0.21±0.17 | 0.04±0.01 | Nd |
| CoG (n=10) | 0.55±0.26 | 4.37±4.00 | 4.25±3.50 | 0.86±0.64 | 0.26±0.12 | 0.04±0.07 | Nd |
| CoS (n=10) | 3.38±0.89 | 5.20±2.70 | 3.82±1.00 | 1.03±0.30 | 1.31±0.28 | 0.08±0.07 | Nd |
| CoJ (n=10) | 7.60±1.50 | 3.33±1.38 | 0.41±0.26 | 0.81±0.64 | 0.16±0.12 | 0.04±0.03 | Nd |
| GoD (n=10) | 4.99±2.70 | 5.57±3.20 | 4.18±3.00 | 0.87±0.84 | 0.16±0.09 | 0.03±0.03 | Nd |
| GoG (n=10) | 0.67±0.22 | 3.09±1.10 | 4.81±2.96 | 1.54±0.13 | 1.98±0.38 | 0.06±0.04 | Nd |
| GoS (n=10) | 4.05±2.80 | 5.72±3.60 | 0.33±0.09 | 0.82±0.70 | 0.57±0.25 | 0.07±0.06 | Nd |
| GoJ (n=10) | 0.33±0.13 | 0.53±0.28 | 3.27±2.95 | 1.13±1.02 | 0.43±0.50 | 0.10±0.12 | Nd |
| P-value | 0.0000**** | 0.052 (NS) | 0.016* | 0.084 (NS) | 0.0000**** | 0.405 (NS) | --- |

Values bearing different letters in a column are significantly different (p<0.05), NS=Not significant, TMF=Total microbial flora, LAB=Lactic acid bacteria, Y&M: Yeasts and molds, S. aureus=Staphylococcus aureus, TC=Total coliforms, TTC=Thermotolerant coliforms, SS=Salmoneilla or Shigella, Nd=Not detected

at 0.5 M. The composition in Ca²⁺, potassium (K⁺), sodium (Na⁺²), magnesium (Mg²⁺), iron (Fe²⁺), and zinc (Zn²⁺) was determined by Fast Sequential Atomic Absorption Spectrometer AA240FS according to AOAC [14]. Table-2 presented the characteristics of analytical curves.

Statistical analysis

The data were analyzed using analysis of variance by program XLSTAT 2017 and modeled using R software, version 3.4.2 (R Foundation for Statistical Computing, Austria).

The results were expressed as average ± standard deviation. The difference between the means was calculated using least significant difference Fisher’s test, and p<0.05 was considered statistically significant.

Results

The average densities of various microorganisms determined are summarized in Table-3. This result showed a significant load of total microbial flora with high variations of 0.33-7.60×10⁹ CFU/ml. Lactic acid bacteria were found at 0.43-5.72×10⁹ CFU/ml. Yeasts and molds count ranged from 0.33 to 5.53×10³ CFU/ml, showing an increasing trend during fermentation. According to the results of this study, total coliform densities were 0.06 at 1.98×10³ CFU/ml, and thermotolerant coliform densities ranged from 0.02 to 0.10×10³ CFU/ml. *S. aureus* densities were in the range of 0.18-1.97×10⁵ CFU/ml in traditional fermented milk and yogurt sold at acidic pH (>3). Table-3 reveals that *Salmonella* and *Shigella* were absent in all analyzed samples. After analyzing the distribution of centers gravity classes on principal factorial plane (Figure-2), we can be noted some closeness between the types of milk and variables. The variables and samples are visualized in the factorial plane formed on dimensions 1 and 2 (71% of variance explained, Figure-2). According to dimension 1, CoS and GoS were highly contaminated with total coliforms, *S. aureus*, and lactic acid bacteria, unlike CaD and CoD which were less...
Figure-2: Principal component analysis distribution of fermented milk samples and ellipse of inertia different species on the factorial plane according to microbiological parameter.

Figure-3: Principal component analysis distribution of fermented milk samples and ellipse of inertia different species on the factorial plane according to physicochemical parameters (a), ascending hierarchical clusters according to physicochemical parameters of fermented milk from different species (b).
Table-4: Physicochemical profile of different fermented milk samples.

| Samples | pH | Acidity | Total carbohydrate (%) | Dry matter (%) | Protein (%) | Ash (%) | Fats (%) |
|---------|----|---------|------------------------|----------------|-------------|---------|----------|
| CaG (n=6) | 3.94±0.05 | 2.56±0.17 | 7.45±0.10 | 3.60±0.21 | 2.36±0.17 | 3.60±1.17 | 2.08±0.17 |
| CaJ (n=4) | 3.98±0.10 | 2.59±0.21 | 7.69±0.20 | 3.90±0.15 | 2.39±0.12 | 3.76±1.05 | 2.27±0.10 |
| CoD (n=4) | 3.90±0.15 | 2.60±0.21 | 7.83±0.20 | 4.00±0.15 | 2.40±0.12 | 3.86±1.05 | 2.38±0.10 |
| GoD (n=6) | 3.90±0.15 | 2.61±0.21 | 7.86±0.20 | 4.00±0.15 | 2.41±0.12 | 3.86±1.05 | 2.39±0.10 |

Values bearing different letters in a column are significantly different (p<0.05). NS = Not significant. Dry matter = Total solids

contaminated. The dimension 2 reveals that CaJ, CaS, CoJ, GoD, GoJ, and GoG were highly contaminated with yeasts and molds and thermotolerant coliforms, but they were weakly contaminated by total microbial flora, while CaG, CoB, and CoG were contaminated with total microbial flora but weakly contaminated by yeasts and molds and thermotolerant coliforms.

Table-4 presents the average values of physicochemical parameters of fermented milk collected from different localities. A significant decrease in pH from 3.830 to 4.137 and a significant increase in acidity from 1.888 to 2.822 were found for fermented milk samples. The dry matters and ashes ranged, respectively, from 8.271 to 13.004 and from 1.994 to 4.761. Dry matter contents varied from 8.271% to 13.004% and ash values were significantly different between the samples collected from 0.199% to 0.476%. The biochemical composition of fermented milk samples varied as follows: Fats (1.210-3.863%), proteins (2.125-3.764%), and total carbohydrates (3.080-5.428%). Analyzing the distribution of center gravity classes on principal factorial plane (Figure-3a), it reveals a homogeneity of the groups of milk from different animal species according to the physicochemical parameters. Dimension 1 indicated that CaG, CaD, GoG, and GoD contain high rate of fats, ash, dry matter, and less rate of total carbohydrate, contrary to CaJ, GoS, CoS, and CoG who are rich total carbohydrate and poor in fats, ash, and dry matter. Dimension 2 reveals a high acidity, low pH, and low rate of protein samples content for the following CaS, CoD, CoJ, and CoB while GoJ is rich in protein and has high pH. The Ascending Hierarchical Clustering (AHC) led to a dendrogram which regrouped three major clusters according to their physicochemical parameters from different species (Figure-3b). The first cluster included the following samples CaG, GoG, CoB, CaD, and GoD. The second cluster included the fermented milk samples CaS, CoD, CoJ, and GoJ, and the third cluster contained the samples CoG, CoS, CaJ, and GoS. Table-5 shows that the mean values of major elements (Ca²⁺, Na⁺, and K⁺) in fermented milk were 855.430 (CaJ), 424.296 (CaG), and 1427.383 (CoG) while the mean values of some minor elements (Fe²⁺, Zn²⁺, and Mg²⁺) were 4.421 (CoJ), 7.450 (CoD), and 104.941 (CoD), respectively. The Na⁺/K⁺ and Na⁺/K⁺ ratios obtained for the different fermented milk were ranged 0.104-0.909 and 3.392-6.464, respectively. Analyzing the distribution of centers gravity classes on the main factorial plane (Figure-4a), we can observe that camel milk with cow milk was close but goat milk deviated by its composition. The result of principal component analysis performed on the minerals concentration of different fermented milk samples showed that the first two axes explained 69.0% of the variation observed (Figure-4a). Therefore, only the first two axes were used to describe the relationship between mineral concentration and species samples. Dimension 1 shows that CoS, CoB, CaG, and CoG were poor in K⁺, but
CaS and CaJ were highly rich in K+. Dimension 2 reveals that CaD was rich in Zn$^{2+}$ and Na$^{2+}$ and poor in Fe$^{2+}$, Ca$^{2+}$, and Mg$^{2+}$, while CoJ, CoD, GoD, GoS, GoJ, and GoG were rich in Fe$^{2+}$, Ca$^{2+}$, and Mg$^{2+}$ but poor in Zn$^{2+}$ and Na$^{2+}$. The AHC led to dendrogram which regroups three major clusters according to their minerals concentration (Figure-4b). The first cluster included the fermented milk samples from goat. The second cluster included the fermented milk samples from camel and cow (Bobo and Sebba). The third cluster contained the fermented cow’s milk samples from the remaining localities (Djibo, Dori, and Gorom-Gorom).

**Discussion**

The quality and safety of fermented foods are decisive factors for producers and consumers. Microbial densities obtain during this study were higher than those reported by Bonfoh et al. [16], Koussou et al. [17], Katinan et al. [18], and De et al. [19]. Total microbial flora in samples was high according to the criteria presented in Codex Standard for Fermented Milks which lays down the minimum sum of microorganisms 10$^7$ CFU/ml. The presence of high densities of microorganisms in these samples could be due to poor handling, inadequate heat treatment, and environmental conditions during the preparations and sale. As for the lactic acid bacteria, these results did not reflect poor quality of milk but more contributing to the improvement of its sanitary quality, because these microorganisms produce antibacterial substances and particular organic acids [20-24]. Yeasts are responsible for producing alcohol during the fermentation of milk. These results were similar to those reported by Savadogo et al. [25], Al-Tahiri [26], and Serhan and Mattar [27]. The assessment of hygienic quality detected the presence of thermotolerant coliforms and *S. aureus* in fermented milk. Several
Table-5: Concentrations in mineral of different samples (mg/Kg).

| Samples       | Ca/D (n=4) | CaG (n=3) | CaS (n=3) | CoD (n=4) | CoG (n=3) | CoS (n=5) | GoJ (n=3) |
|---------------|------------|------------|------------|------------|------------|------------|------------|
|               | 267.594±0.015 | 161.165±0.020 | 2.829±0.006 | 1083.825±0.001 | 1002.359±0.000 | 5.216±0.424 | 0.158      |
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|               | 267.594±0.015 | 161.165±0.020 | 2.829±0.006 | 1083.825±0.001 | 1002.359±0.000 | 5.216±0.424 | 0.158      |
|               | 267.594±0.015 | 161.165±0.020 | 2.829±0.006 | 1083.825±0.001 | 1002.359±0.000 | 5.216±0.424 | 0.158      |
|               | 267.594±0.015 | 161.165±0.020 | 2.829±0.006 | 1083.825±0.001 | 1002.359±0.000 | 5.216±0.424 | 0.158      |
|               | 267.594±0.015 | 161.165±0.020 | 2.829±0.006 | 1083.825±0.001 | 1002.359±0.000 | 5.216±0.424 | 0.158      |

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Semaan et al. [35], Cesbron-Lavau et al. [36], and Ayyash et al. [34] obtained similar values from traditional dairy products (milk and darfieh cheese), but other compositions are reported in review by Clark and Mora-Garcia [37]. Numerous studies reported that fermented milk and yogurt contain some nutritive components such as peptides and fatty acids, which are produced during fermentation. These components were known to modulate the immune system [38]. Yadav and Shukla [39] reported that fermented milk consumption could prevent the effect of ulcerative colitis. Camel milk differs from other milk (bovine) in its composition and protein content and structure, and, therefore, is expected to possess functional and bioactive properties different from bovine milk. Camel milk has an excellent reputation as nutritious food, with most of its therapeutic value related to its biological properties such as antioxidant activity [7].

The fermented milk samples were a rich source of Ca\(^{2+}\) and Mg\(^{2+}\), which are produced during fermentation. These compounds were known to modulate the immune system [38]. Numerous studies reported that fermented milk could prevent the effect of ulcerative colitis. Camel milk differs from other milk (bovine) in its composition and protein content and structure, and, therefore, is expected to possess functional and bioactive properties different from bovine milk. Camel milk has an excellent reputation as nutritious food, with most of its therapeutic value related to its biological properties such as antioxidant activity [7].

Table-5 showed the average contents of minerals in various traditional fermented milks of Burkina Faso. The results revealed that camel fermented milk contains the highest concentration in Na\(^{+}\) and Ca\(^{2+}\). High concentrations of Ca\(^{2+}\), Mg\(^{2+}\), and Zn\(^{2+}\) were reported by Navarro-Alarcón et al. [39] in commercial fermented milk of goat and cow, but Wang et al. [32] reported high concentrations to K\(^+\) (1724 mg/Kg) and Ca\(^{2+}\) (1409 mg/Kg) in fermented goat milk. The concentration of Ca\(^{2+}\), K\(^+\), and Mg\(^{2+}\) in fermented milks could be due to the activity of the lactic bacteria during fermentation process [40,41]. The high rate of Ca\(^{2+}\) in milk of Djibo, Dori, and Gorom-Gorom is due to the high presence of limestone (CaCO\(_3\)) in the water of these cities: In general, the minerals concentration in fermented milk depends on the species, its individual characteristics, feeding method, rearing area, nature of metal of the material containing milk, degree of food contamination and drinking water, lactation stage, and health condition of female. The Na\(^{+}/K^+\) ratio in the body helps to control blood pressure; fermented milk is a food source having impact in lowering blood pressure [42]. The Na\(^{+}/K^+\) ratios (0.104-0.909) were obtained for the different fermented milk samples, and this low Na\(^{+}/K^+\) ratio can help to control blood pressure. The Ca\(^{2+}/Mg^{2+}\) ratio (3.392-6.464) for food was within the recommended value higher at 1.00 [42]. The fermented milk samples were a rich source of Ca\(^{2+}\) and Mg\(^{2+}\). K\(^+\) and Ca\(^{2+}\) are the most important elements for bone growth, development, metabolism, and health maintenance.

**Conclusion**

The traditional fermented milks and dairy products are important sources of functional nutrients. The fermented milks sold and consumed in Burkina Faso showed high variability in microbiological, physico-chemical quality, and the minerals concentration. This study revealed that traditional fermented milk is a very important source of nutrients and functional food due to compounds in fats, proteins, carbohydrate, and low Na\(^{+}/K^+\) ratio. The presence of lactic acid bacteria and yeast improves the organoleptic qualities of fermented milk and brings beneficial effects to consumers, while the presence of certain bacteria such as coliforms and *S. aureus* is a risk for the milk quality and the health of consumers. This work has important implications for the commercialization of fermented milk based on camel and goat milk. The sanitary practices followed by producers during handling, storage, and processing are generally poor. Based on the overall evaluation of the results, training to the local producers on good hygiene practices is necessary to improve sanitary quality of fermented milk sold in Burkina Faso.

**Authors’ Contributions**

HC collected the samples and wrote the original draft of the manuscript. HC, JUM, and SMD analyzed the samples, NSS, AS, and FT organized the data, helped in writing, and review of the manuscript. CZ, YT, and AlyS supervised the study, validated the results from analysis, and review of the manuscript. All authors have read and approved the final version.

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**Competing Interests**

The authors declare that they have no competing interests.

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