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RESEARCH ARTICLE

Factors contributed to the microbiology of traditional fermented camel milk (*Gariss*) produced in Al-Koma locality, North Darfur State, Sudan

Hafiz, I. I. Osman[1,2], Ibtisam, E. M. El Zubeir[2,3*]

[1] *Ministry of Production and Economic Resources, North Darfur State, Sudan, E-mail: hafizoma932@gmail.com*
[2] *Department of Dairy Production, Faculty of Animal Production, University of Khartoum, P. O. 321 Khartoum, Sudan.*
[3] *Institute for Studies & Promotion of Animal Exports, University of Khartoum, P.O. Box 321, Khartoum, Sudan, Email: Ibtisamelzubeir17@gmail.com, Ibtisam.elzubeir@uofk.edu. ORCID: 0000-0001-8173-7693*

***Corresponding author:** Ibtisam, E. M. El Zubeir, Ibtisamelzubeir17@gmail.com, Ibtisam.elzubeir@uofk.edu

ABSTRACT

It is meant in the present study to isolate and identify the lactic acid bacteria (LAB) and other contaminants associated with *Gariss*; traditional fermented camel milk; that produced and consumed by camel herders in the nomadic production systems in North Darfur State, Sudan. About 118 samples were collected during February 2018 from 4 different areas located at Al-Koma Locality (Sari; 30 samples, Om-Hageleeg; 30 samples, Om-Alhussain; 30 samples and Al-Koma; 28 samples). Rod shaped bacteria showed the isolation of *Lactobacillus* (Lb,) spp. (78.5%), *Bacillus* spp. (13.3%), *Propionibacterium* spp. (6.2%), *Bifidobacteria* spp. (1%), *Clostridium* spp. (0.5%) and *Bacteroides* spp. (0.5%). The result also illustrated that the LAB isolates from the 118 samples were identified as *Lactobacillus brevis* (67.3%), *Lb. acidophilus* (11.5%), *Lb. plantarum* (7.9%), *Lb. fermentum* (4.8%), *Lb. delbrueckii* (3.6%), *Lb. salivarius* (1.8%), *Lb. jensenii* (1.2%), *L. gasseri* (1.2%) and *Lb. casei* (0.6%). The spheric bacteria isolated were *Staphylococcus* spp. (32.4%), *Micrococcus* spp. (31.2%), *Streptococcus* spp. (26.8%) and *Enterococcus* spp. (9.6%). Moreover, the yeast (39%), Gram-positive bacteria (30.8%), Gram-negative bacteria (15.7%) and Gram-positive bacteria mixed with yeast were isolated from 14.5% of *Gariss* samples. The result showed significant ($P<0.01$) differences for microbial groups associated with *Gariss* collected from Al-Koma Locality. Also, the comparison of the different containers used for preparation of *Gariss* revealed significant ($P<0.01$) variation for the occurrence of microbial groups. The study concluded that the traditional containers; *Bokhsa* and *Siin;* used for fermenting *Gariss* in the field conditions contain various types of microorganisms. Their full identification and characterization should be done because of the possibility of isolating some of beneficial bacteria that might be of significant in the near future. Also, collaborative effort is needed to reduce the contamination of the product.

Keywords: isolation; identification; microbial group, lactic acid bacteria; *Gariss*; nomadic camel herders; Sudan

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1. Introduction

Traditionally, the camel milk is commonly consumed by most of the pastoral societies as fermented products as compared to the fresh milk[1-3]. The traditional fermented dairy products are known long ago in Sudan as they gained wide popularity and consumption among larger sector of the population[2,4]. In Sudan, the fermented camel milk (*Gariss*) is produced by semi-continuous fermentation process without adding starter cultures[2-3,5,-9]. The shepherds prepared *Gariss* in the fields during their driving the camels for grassing or pastures in far-away places[6,10]. The camel herders sustain their life by depending on *Gariss* only for several months[11,12].

The fermentation of milk including that from camels is a traditional ancestral processing method that is practiced worldwide. It involved transformation of lactose into lactic acid due to the action of natural dominated microflora (LAB and/ or yeasts) in the milk[13]. This fermentation has to be controlled by introduction of mesophilic LAB starter culture in order to improve this traditional spontaneous fermentation[1]. The biodiversity of microflora found in camel milk contribute to the production of diverse fermented products having specific flavor, taste and texture produced in the various regions of the world[13]. Camel milk and its fermented products in Sudan did not receive enough attention, although some studies have been conducted on the isolating and characterizing of the major microflora in camel fermented milk product[4,7,9,14-15]. However, most of these studies used limited numbers of samples; so more effort is needed for further studies in Sudan, especially where camels are concentrated. Hence in this study it is meant to isolate and identify some of dominant microorganisms found in *Gariss* produced and consumed in 4 different areas in North Darfur State, namely Al-lkoma Locality; and to compare some of the factors contributing to the variation of occurrence of the different types.

2. Methodology

2.1. The study area and *Gariss* collection

One hundred and eighteen samples of *Gariss* were examined from 4 production sites in Al-Koma Locality that located in North Darfur State. Thirty samples were collected from Sari area (40 km from Al-Koma town) and 30 samples were collected from Om-Hageleeg (80 km from Al-Koma town). Also 30 samples were collected from Om-Alhussain area (40 km from Al-Koma town) and 28 samples were collected from Al-Koma town (80 km from El-Fasher town).

In this study, the samples of the examined *Gariss* were taken from camel producers in the nomadic production system during February 2018. The samples were collected in sterile containers with a capacity of 5 ml. After collection and labeling of the samples, the packing was done using an ice box container for transporting them to dairy microbiology laboratory at Department of Dairy Production, Faculty of Animal Production, University of Khartoum to conduct the analysis.

2.2. Microbiological examination

2.2.1. Preparation of media

The used media were in a dehydrated form, they kept in a hygroscopic environment in a cool, dark and dry place. They were prepared following the manufacturer's instructions and as outlined previously[16].

The culture media used include Plate count agar (HIMEDIA, M091), M17 broth (HIMEDIA, M1029), MRS broth (HIMEDIA, GM369), MacConkey agar 15% bile salts (HIMEDIA, M008) and Yeast extract agar (Scharlau 01-465). Cooked meat medium; R. C. medium; (HIMEDIA, M149), was used for preserving the isolates. Also, the OF, Arginine, peptone water (BIOMARK B035) and the bacteriological peptone (OXOID, 137) were used as test media.

2.2.2. Sterilization

The hot oven (170 ℃ for 2 hours) was used for sterilization of the glass wares including flasks, test tubes, Petri dishes, bottles and pipettes. Also, the autoclave was used for sterilizing the distilled water and culture media at 121 ℃ for 15 minutes (15 lbs pressure) except sugars that were sterilized at 115 ℃ for 5 minutes. Whereas, the benches, micro-pipettes and incubators were sterilized using alcohol solution (70%), while the loops, mouth of the bottles and slides were subjected to the direct flame as a method of sterilization[17].

2.2.3. Sub-culturing and purification of the bacterial isolates

All Gram positive suspected LAB were cultured in MRS and M17 agar and incubated for 24 hours at 37°C. Then the purification of the isolates was done by picking up part of a well typical isolated colony using sterile wire loop and streaked on the surface of a sterile Petri dishes, which containing a selective media for the appropriate organism. Sub-culturing for each organism was repeated until pure cultures; checked by Gram stain; were obtained[18]. The pure cultures were then streaked into the selective medium using bottles. The bottles were incubated at the optimum temperature for each organism. The Gram strain was done in order to ensure the purity of the streaked cultures. The bottles were stored at 4-8°C until they were used for the identification[18].

2.2.4. Identification of the isolates

Primary identification was performed by the examination of the cell morphology after staining of the isolate to be tested using Gram staining method that also showed the presence of spores, their shape and position[18]. The appearance of bacteria and their morphology were recorded[18]. Catalase test and oxidase test were also done[18]. Whereas, the motility test and oxidative-fermentative (OF) test were performed as was described earlier[16].

2.2.5. Secondary confirmatory biochemical tests used for *Lactobacilli* spp.

Purified colonies of *Lactobacillus* spp. were examined for growth at 15°C and 45℃[18], then subjected to arginine hydrolyses and sugars fermentation (arabinose galactose, lactose, maltose, mannitol, melezitose, melibiose and trehalose) test according to the previously outlined methods[16].

2.3. Statistical analysis

The obtained data were calculated on a percentage basis. Moreover, Chi square test was conducted to obtain the significant level at P<0.05 using SAS program[19].

3. Results

3.1. Primary testes used for identification of Gram-positive rod shape bacteria isolated from *Gariss* samples in Al-Koma Locality

The primary tests used for the identification of Gram-positive rod-shaped bacterial species (n= 210 isolates) were done as shown in **Table 1**.

Table 1. Primary tests used for identification of Gram-positive rod-shape bacteria isolated from *Gariss* samples collected from Al-Koma Locality, North Darfur State.

Tests	Type of bacteria					
	Lactobacillus **spp.**	***Bacteroides*** **spp.**	***Bifidobacteria*** **spp.**	***Bacillus*** **spp.**	***Propinonibacterium*** **spp.**	***Clostridium*** **spp.**
Shape	R	R	R	R	R	R
Spore	-	-	-	-	+	-
Gram-stain	+	+	+	+	+	+
Motility	-	-	-	-	-	-
Growth in air	+	+	+	+	+	+
Catalase	-	-	+	-	-	-
Oxidase	-	+	-	-	-	-
Glucose	+	+	+	-	+	+
OF	F	F/O	F	F	F	F

3.2. Microbial findings in *Gariss* samples produced and consumed in Al-Koma Locality

Gariss produced in Al-Koma Locality. (**Table 2**) revealed significant (P<0.01) variation in its microbial content. The occurrence of Gram-negative bacteria in *Gariss* samples obtained from Sari area was 4%, Gram-positive bacteria was 30.6%, yeasts mixed with Gram-positive bacteria were 17.4% and yeast alone was 27.4%. *Gariss* samples from Om-Hageleeg showed the Gram-negative bacteria of 44%, the Gram-positive bacteria of 22.4%, Gram-positive bacteria mixed with yeasts were 8.7% and yeasts alone was 14.5%. For *Gariss* samples collected from the area of Om-Alhussain, Gram-negative bacteria revealed 20%, Gram-positive bacteria was 26.5%, yeasts mixed with Gram-positive bacteria were 30.4%, while yeasts alone was 22.6% (**Table 2**). *Gariss* samples collected in Al-Koma area showed the occurrence of Gram-negative bacteria in 32%, Gram-positive bacteria in 20.4%, yeasts mixed with Gram-positive bacteria in 43.5% and yeasts alone in 35.3% of the examined samples (**Table 2**).

Table 2. Microbial content of *Gariss* samples collected from camel herders in Al-Koma Locality, North Darfur State.

Microbial group	Areas				Total	Chi square	Significant level
	Sari	Om-Hageleeg	Om-Alhussain	Al-Koma			
Gram-negative bacteria	1(4%)	11(44%)	5(20%)	8(32%)	**25(15.7%)**		
Gram-positive bacteria	15 (30.6%)	11(22.4%)	13(26.5%)	10(20.4%)	**49(30.8%)**		
Yeasts and Gram-positive bacteria	4(17.4%)	2(8.7%)	7(30.4%)	10(43.5%)	**23(14.5%)**	**19.26**	**0.023****
Yeast	17(27.4%)	9(14.5%)	14(22.6%)	22(35.3%)	**62(39%)**		
Total	**37(23.3%)**	**33(20.8%)**	**39(24.5%)**	**50(31.4%)**	**159(100%)**		

****=** Significant at P<0.01

3.3. The effect of the containers used in keeping *Gariss* on the occurrence of bacteria and yeasts in Al-Koma Locality

Comparison of the occurrence of different microbial groups in *Gariss* prepared using the different containers revealed significant (P<0.01) variation (Table 3). The bacteria in the samples collected from plastic containers revealed the presence of Gram-negative bacteria (89.7%) and the Gram-positive bacteria (73.7%). Also, the highest occurrences of Gram-positive bacteria mixed with yeasts were found in *Gariss* samples kept into plastic containers (90%). The occurrence of yeast alone was detected in 78% of the *Gariss* samples that prepared using plastic containers. However, *Gariss* samples obtained from *Siin* showed that the Gram-negative bacteria, Gram-positive bacteria, Gram-positive bacteria mixed with yeasts, and yeasts alone were 2.6%, 21.1%, 10%, and 22%, respectively (Table 3). Moreover, *Gariss* samples that collected from *Bokhsa* revealed the presence of Gram-positive bacteria (5.3%) only, while, those obtained from stainless steel containers showed the presence of Gram-negative bacteria only (7.7%) as shown in **Table 3**.

Table 3. Comparison of occurrence of different group of bacteria and yeasts in *Gariss* samples kept in different containers in Al-Koma Locality, North Darfur State.

Microbial group	Type of Containers				Total	Chi square	Significant level
	Plastic	*Siin*	*Bokhsa*	Stainless Steel			
Gram-negative bacteria	35(89.7%)	1(2.6%)	0(0.0%)	3(7.7%)	**39(28.5%)**		
Gram-positive bacteria	14(73.7%)	4(21.1%)	1(5.3%)	0(0.0%)	**19(13.9%)**		
Yeasts and Gram-positive bacteria	18(90%)	2(10%)	0(0.0%)	0(0.0%)	**20(14.6%)**	**21.39**	**0.011****
Yeasts	46(78%)	13(22%)	0(0.0%)	0(0.0%)	**59(43.1%)**		
Total	**113(82.5%)**	**20(14.6%)**	**1(0.7%)**	**3(2.2%)**	**137(100%)**		

**= Significant at P<0.01

3.4. Isolation and identification of Gram-positive cocci from *Gariss* samples collected in Al-Koma Locality

Nine primary tests were done on 176 bacterial isolates of Gram-positive cocci (**Table 4**). This was done by examining the culture and its produced pigmentation under the microscope using the oily lens. The general proportion of the cocci isolated from *Gariss* samples include *Staphylococcus* spp. (32.4%), *Micrococcus* spp. (31.2%), *Streptococcus* spp. (26.8%) and *Enterococcus* spp. (9.6%) as shown in **Table 4**.

Table 4. Primary tests used for the identification of Gram-positive cocci isolated from *Gariss* samples collected from Al-Koma Locality, North Darfur State.

Suspected bacterial species	Tests									No of isolates
	Shape	Spore	Gram stain	Motility	Growth in air	Catalase	Oxidase	Glucose	OF test	
***Staphylococcus* spp.**	S	-	+	-	+	-	-	+	F	**57(32.4%)**
***Micrococcus* spp.**	S	-	+	-	+	-	+	+	F	**55(31.2%)**
***Streptococcus* spp.**	S	-	+	-	+	+	-	+	F	**47(26.8%)**
***Enterococcus* spp.**	S	-	+	-	+	+	+	+	O/F	**17(9.6%)**
Total										**176(100%)**

3.5. Types of rod-shaped bacteria isolated from *Gariss* samples collected from Al-Koma Locality

Table 1 and **Table 5** showed the general proportion of the rod shape bacteria isolated from *Gariss* samples. Among the isolates, *Lactobacillus* spp. (78.5%), *Bacillus* spp. (13.3%), *Propionibacterium* spp. (6.2%), *Bifidobacteria* spp. (1.0%), *Clostridium* spp. (0.5%) and *Bacteroides* spp. (0.5%) were found (**Table 5**).

Table 5. Types of rod-shaped bacteria isolated from *Gariss* samples collected from Al-Koma Locality, North Darfur State.

Types of bacteria	Frequencies	Percentage
***Lactobacillus* spp.**	165	78.5%
***Bacillus* spp.**	28	13.3%
***Propionibacterium* spp.**	13	6.25%
***Bifidobacteria* spp.**	2	1.0`%
***Bacteroides* spp.**	1	0.5%
***Clostridium* spp.**	1	0.5%
Total	**210**	**100**

3.6. Types of *Lactobacillus* spp. identified from *Gariss* produced and consumed in Al-Koma Locality

The result of the primary and secondary tests conducted for the identification of the isolated bacteria (**Table 1** and **Table 6**) revealed the presence of 210 species that suspected to be *Lactobacillus* spp. In the current result (**Table 7**), the predominant isolates (118) were identified as *Lb. brevis* (67.4%) followed by *Lb. acidophilus* (11.5%) compared to *Lb. plantarum* (7.9%), *Lb. fermentum* (4.8%), *Lb. delbruckii* (3.6%), *Lb. salivarius* (1.8%), *Lb. gasseri* (1.2%), *Lb. jensenii* (1.2%) and *Lb. casi* (0.6%).

Table 6. Secondary testes used for identification of *Lactobacillus spp*. isolated from *Gariss* samples collected from Al-Koma Locality, North Darfur State.

Tests	*L. brevis*	*L. acidophilus*	*L. plantarum*	*L. fermentum*	*L. delbrueckii*	*L. salivarius*	*L. gasseri*	*L. jensenii*	*L. casei*
Growth at 15°C	-	-	-	-	-	+	+	+	-
Growth at 45°C	+	+	+	+	+	-	-	-	+
Arginine	+	-	-	+	+/-	-	-	+	-
Sugar fermentation									
Arabinose	-	-	-	-	-	-	+	+	+
Galactose	+	+	+	+	+	+	+	+	+
Lactose	+	-	-	+	+	+	+	+	+
Maltose	+	+	+	+	-	+	+	+	+
Manitol	+	+	-	+	-	+	+	-	-
Melezitose	-	-	-	-	-	+	-	-	-
Melebiose	-	-	-	+	+	-	+	+	+
Trehalose	-	+	-	+	+	+	+	-	+

Table 7. Comparison of occurrence of *Lactobacillus spp*. identified from *Gariss* samples collected from Al-Koma Locality.

Bacteria	Area				Total	Chi square	Significant level
	Sari	Om-hageleeg	Om-Alhussain	Al-Koma			
L. brevis	29(26.1%)	33(29.7%)	16(14.4%)	33(29.7%)	**111(67.4%)**	**28.24**	**0.250^{NS}**
L. acidophilus	2(10.5%)	3(18.8%)	6(31.6%)	8(42.1%)	**19(11.5%)**		
L. plantarum	5(38.5%)	1(7.7%)	6(46.2%)	1(7.7%)	**13(7.9%)**		
L. fermentum	2(25%)	3(37.5%)	1(12.5%)	2(25%)	**8(4.8%)**		
L. delbrueckii	1(16.7%)	0(0.0%)	2(33.3%)	3(50%)	**6(3.6%)**		
L. salivarius	1(33.3%)	1(33.3%)	1(33.3%)	0(0.0%)	**3(1.8%)**		
L. gasseri	0(0.0%)	0(0.0%)	1(50%)	1(50%)	**2(1.2%)**		
L. jensenii	1(50%)	0(0.0%)	1(50%)	0(0.0%)	**2(1.2%)**		
L. casei	0(0.0)	0 (0.0)	0(0.0)	1(100)	**1(0.6%)**		
Total	**41(24.8%)**	**41(24.8%)**	**34(20.6%)**	**49(29.7%)**	**165(100%)**		

4. Discussion

In this study (**Table 1** and **2**), Gram-positive bacteria (30.8%), Gram-negative bacteria (15.7%), mixture of yeast and Gram-positive bacteria (14.5%) and yeast alone (39%) were found in the *Gariss* examined from Al-Koma Locality. Similarly, a previous report showed that traditional *Gariss* had lactic acid bacteria and yeasts[15]. Also, a combination of LAB and yeasts are involved during the production of traditional fermented camel milk '*Suusac*' in Kenya[20]. Moreover, *Geotrichum penicillatum*. *Candida krusei* and *Rhodotorula mucilaginosa* the were the identified yeasts[20]. The yeast counts was slightly lower in *Gariss* samples collected from women herders in the transhumance system of camel husbandry (log 6.99±0.13) compared to those live as nomads (log 7.02±0.3)[7]. The reason could be because of their prevalence in the milk, as high counts of yeast and mould was reported in the milk of camels[21-22]. This high occurrence of yeast is because yeasts are commonly associated with traditional fermented dairy products[23]. Similarly, the presence of yeasts showed a high number (log 8 cfu per gram) associated with the indigenous fermented milks in Africa, which have potential contribution together with the wide range of LAB to the characteristics of the product[24]. On the other hand, the microbial profile of *Gariss* was significantly influenced by the management system and the preparation conditions[7].

The highest occurrence of Gram-positive and Gram-negative bacteria was reported for the *Gariss* samples collected from plastic containers (**Table 3**). Furthermore, the bacterial load in *Gariss* was influenced by the herders' seasonal movement, in addition to additives added to the products and the containers used for its production[9]. However, the presence of Gram-positive bacteria was only found in the *Gariss* samples collected from *Bokhsa*. This might be because *Bokhsa* is a woody container that has pores, which help in suppressing the activities of the starter culture and hence preserving its viability compared to other used containers[9]. Moreover, the gourds together with environmental factors might help in providing the necessary selective forces that help in the evolution of the unique LAB strains[20]. A previous reported showed the highest means for the total bacterial counts were estimated for *Gariss* obtained from the plastic (log 7.31±0.04) and *Siin* (log 7.30±0.04) containers[7]. Meanwhile, lower mean (log 6.94±0.08) was found in *Gariss* prepared using *Bokhsa.* However, significantly high means counts were obtained for the *Lactobacillus* spp. and *Streptococcus* spp. (log 7.48±0.13 and 7.36±0.13, respectively) in *Gariss* collected from *Bokhsa* compared to those kept into other containers. Meanwhile, stainless steel containers revealed lower mean counts of *Lactobacillus* spp. and *Streptococcus* spp. (log 6.54±0.18 and 6.54±0.19, respectively) in *Gariss*[9]. The reason could be attributed to the fact that stainless containers have the ability to absorb heat faster than any other containers[9] .

The samples of *Gariss* made in *Bokhsa* and *Siin* showed the lowest occurrence for Gram-negative bacteria (0% and 2.6%, respectively) compared to other used containers as shown in **Table 3**. The reason could be because the materials from which *Bokhsa* and *Siin* are made enable cleaning and washing more easily beside their ability for keeping the product cool for more time, unlike to the plastic and stainless steel that characterized by losing the heat quickly[9]. However, unlike the present study, the growth of coliform bacteria

was not detected in *Gariss* that was obtained from Butana[7]. The range of the coliform reported in *Gariss* was log 3.2 to 3.5 cfu/ ml[4]. Also, low coliform numbers were found in *Suusac*[20].

Variation was found when comparing the occurrence of Gram-negative bacteria and yeast in *Gariss* kept in all used containers in the current study areas (**Table 3**). However, the variation of the used containers revealed non significant values for the yeast count in the examined samples of *Gariss*[8-9]. The data showed higher prevalence of Gram-negative bacteria in *Gariss* samples prepared using plastic containers (89.7%) could be attributed to unhygienic milk production[1-2,8-9,22,26]. Moreover, lacking of udder wash and disinfectant within camel herders' communities is another reason specially when the containers made from plastic is used for preparation and storing of the products. This because it is difficult to clean its bottom and that commonly the water used is of low microbiological quality[9].

The Gram-negative bacteria in *Gariss* samples revealed 28.5% of the total isolates in the examined samples (**Table 3**). The current study assumed that most of the isolated Gram-negative bacteria might belong to coliform group of bacteria (Not shown data). Coliforms are associated with raw camel milk[22,26]. Moreover, *E. coli* was detected in raw milk obtained from the udder of the camels[26]. Furthermore, *E. coli, Enterobacter aerogenes* and *Klebsiella* spp. (40%, 30% and 30%, respectively) were identified in *Gariss* collected from Al Gadarif State in Sudan[8-9]. Some microorganisms from undesirable genera including *Escherichia, Enterobacter, Klebsiella* and *Acinetobacter* were frequently dominant in *Dhanaan* samples; fermented camel milk; from Ethiopia[27]. The *Escherichia coli* was also isolated from all examined *Suusac* samples in Kenya, while *Shigella* spp. and *Klebsiella* spp. were found in 88.1% and 77.4% of the examined samples, respectively[28]. Moreover, the means $\log_{10}$ counts for *E. coli*, *Shigella* spp., and *Klebsiella* spp. revealed 3.135, 2.784 and 3.138, respectively[28]. The reason could be due to the multiplication of coliforms bacteria during the natural fermentation, resulted in problems in the final products as the numbers of lactic acid bacteria revealed very low at the beginning of the fermentation[24]. The occurrence of coliforms in the product is due to the poor hygiene practices, which might create potential risks to the health of the consumers[22,26,28,29-30]. The risk is high when the local producers are consuming raw milk[9,29-31]. As a tradition, most of the fermented products made from camel milk are fermented naturally without any heat treatment and with no added starter cultures[1,3,5-10]. Moreover, during their movement season, the sandy winds are vey common and that most of the herders opened and closed the containers for storing *Gariss* frequently without paying attention to its cleaning and/ or renewable[9]. This regardless of the fact that camel milk has inhibitory effect against most of the pathogenic bacteria due to its high content of the several protective proteins that are associated with camel milk, including vitamin C, immunoglobulins, lactoperoxidase, lactoferrin and lysozymes[32,]. Thus, food-borne diseases might associated with poorly produced camel milk, while the naturally occurred antimicrobial agents provide limited protection against some of the specific pathogens such as *S. aureus*[33].

Staphylococcus spp. were predominated in the examined *Gariss* samples (32.4%) as shown in Table 4. Similarly, low occurrence of *S. aureus* (29%) was found in *Suusac*[34]. However, higher prevalence rate was reported when isolating *Staphylococcus aureus* (63.09%) from the samples analyzed in Kenya, which revealed

a mean count of log 2.784[28]. The significant of *S. aureus* is associated with its involvement in food-borne diseases causing gastroenteritis when consuming the contaminated food[35]. The isolation and description of *S. aureus* were shown in raw milk obtained from camel in India[36], Saudi Arabia[37], Egypt[38], Morocco[29,39], Ethiopia[40], Kenya[34,41] and Sudan[26][33,42]. The presence of staphylococci in camel samples in the marketing chain indicated significant contamination and/or microbial build up due to handling of milk at ambient temperatures[34].

The presence of *Micrococcus* spp. (31.2%) in the samples examined in the current study (**Table 4**), supported those reported for the *Micrococcus* found in *Suusac*[34]. The Micrococcus genus can play a positive role during the ripening of cheeses because of its high proteolytic and lipolytic activities[43].

The data of this study (**Table 4**), revealed the occurrence of *Streptococcus* spp. in *Gariss* samples was 26.8%. In a similar study, about 18% of *Streptococcus* spp. were isolated from Egyptian traditional camel milk[44]. The counts of *Streptococcus* spp. in *Gariss* produced by camel herders in the transhumance and nomadic production system in Butana area, Eastern Sudan revealed log 6.47±0.35 and 6.85±0.33, respectively[7,9,10]. Moreover, the increasing loads for *Streptococcus* spp. in *Gariss*, to which some spices were added suggesting their action in elimination of bacteria contaminated the product, hence the chance for the growth of *Streptococcus* spp. is increased[8,9]. On the other hand, the *S. infantarius* subsp. *Infantarius* was one of the dominant LABs in the *Gariss* samples examined[15]. Similarly, *Streptococcus* species was found in all *Dhanaan* samples and that *S. lutetiensis* and *S. infantarius* appeared as the commonly isolated bacteria[27]. Both organisms sre not yet considered among the starter culture of benefit in food[45]. The high occurrence of *Streptococcus infantarius* subsp. *Infantarius* in Kenya, Somalia, Sudan, Mali and Côte d'Ivoire has strongly indication of its pivotal role in the African traditional dairy fermentations potentially parallel to that of typical western dairy species *S. thermophilus*[46]. The putative health risks might arise upon consuming high amounts of food contain the live cells of *Streptococcus infantarius* subsp. *infantarius*[46].

The occurrence of *Enterococcus* spp. revealed 9.6% (Table 4). Similarly, the microflora of *Gariss* samples examined include 10% of *Enterococcus* spp. (10%)[4]. *Enterococcus* spp. were found in both *Gariss* and *Shubat* and they might help in the development of aroma during fermentation process[47]. Moreover, the *Enterococcus* spp. present in the fermented camel milk were differentiated into *Enterococcus faecalis, Enterococcus faecium* and *Enterococcus durans*[48]. Also, *E. faecium* was found in 55.6% of the investigated *Gariss* samples in Sudan[15]. However, among LAB, the genus *Enterococcus* is of particular interest because it is found particularly in the intestine of humans and animals and in many food products, including milk[49]. However, limited studies were carried out on the isolation of bacteriocin-producing strains from raw camel milk, which presents specific biological characteristics and beneficial traits[50].

The data in **Table 5** revealed the isolation of 9 species from the *Gariss* samples belonging to *Lactobacillus* (78.5%). Similarly, *Lactobacillus* spp. had been isolated from fermented camel milk (*Gariss*)[4,7-9,14-15]. Moreover, the mean counts of *Lactobacilli* spp. revealed log 6.83±0.33 and 6.55±0.32 in the

Gariss produced by camel herders in the transhumance and nomadic camel production systems, respectively[7].

The isolated bacteria (**Table 5**), including *Propionibacterium* spp. (6.25%), *Bifidobacteria* spp. (1.0`%), *Bacteroides* spp. (0.5%) and *Bacillus* spp. (13.3%) supported the report stating that *Lactobacillus*, *Propionibacterium*, *Bifidobacterium* and *Bacillus* are the bacterial genera that are found in the fermented dairy products[51] The different sensory properties are found in the traditional and fermented dairy products because of the diversity of their microbiological content[51]. *Gariss* has *Bifidobacterium lactis*, which has favorable role in lowering the cholesterol level in both plasma and liver[52]. On the other hands, 8 potential probiotics *Bifidobacteria* spp. were isolated from camel milk and that Bifidobacteria showed variation regarding their survival in the gastrointestinal conditions[53].

In the present study; unfortunately; one isolate belonging to *Clostridium* spp. (0.5%) was found in *Gariss* samples (Table 1 and 5). The *Clostridium* spp. were found in samples collected from fermented camel milk in Ethiopia[27]. This organism might be spoilage or pathogenic, which supported the previous report that the camel herders do not heated their milk before processing or consumption[31]. Milk contamination might occurred from the external surfaces of animals as well as during milking, transportation, storage and processing[54]. This because milk has high nutritional value and its neutral pH and high-water activity enable the proliferation of many microorganisms[54].

Some variations were found in the types and frequencies of the isolated fermented Lactobacilli associated with the samples in the present (Table 6 and 7) and previous reports. This because the *Lactobacillus* spp. showed wide range and diverse habitats with some application in the industrial and medical fields[55]. This diversity together with some of the recent findings regarding the characters of LAB lead to the important of recalcifying them into new genus[55.56]. Accordingly, the *Lactobacillus brevis* was renamed as *Levilactobacillus* (*Lev.*) *brevis*, *Lactobacillus plantarum* as *Lactiplantibacillus* (*Lpb.*) *plantarum*, *Lactobacillus fermentum* as *Limosilactobacillus* (*Lim.*) *fermentum*, *Lactobacillus salivarius* to *Ligilactobacillus* (*Lig.*) *salivarius* and *Lactobacillus casi* to *Lacticaseibacillus* (*Leb.*) *casi*[55.56].

The *L. brevis* (67.3%) is the most predominant bacterial species among the 9 Lactobacilli isolated from *Gariss* samples followed by *Lactobacillus acidophilus* (11.5%) that showed the highest percentage in all localities (Table 6 and 7). *Lact. brevis* (16.7%) and *Lact. acidophilus* (4.16%) were previously isolated from *Gariss* samples obtained from Gezira State[7]. Moreover, the *L. brevis* was similarly isolated from *Gariss* reported in 20% of the samples investigated[8,9]. *Lactobacillus acidophilus* has probiotic properties[55,57]. Thus, their presence on the traditional *Gariss* (**Table 6** and **7**) suggested probiotic feature of this product. This is especially because the milk from camel reported to have probiotic characteristics[58]. Moreover, the unique fermented dairy products made from camels and their diverse microflora depend on the methods of production technology and the ecological localities in which they are produced[9,59,60]

The low occurrence of *Lb. plantarum* (7.9%) and *Lb. fermentum* (4.8%) as shown in **Table 6** and **7** are unlike the results showed that 8 (33.3%) of the *Gariss* samples containing *Lb. planturum* and 3 (12.5%) *Lb.*

fermentum[7]. Previously, 7 strains of *Lb. fermentum* and 3 strains of *Lb. plantarum* from *Gariss* samples were isolated[4]. Also, the occurrence of *Lb. plantarum* in 16% of the examined *Suusac*, samples were reported in Kenya[20]. Recently *Lb. plantarum* has been moved to the genus Lactiplantibacillus due to its association with both milk and plants and it is also dominant in the fermented vegetable, olive and meats[56]. The low frequencies of *Lb. plantarum* in *Gariss* samples examined during the present study might be due to the less additives used in the area of the current study. The high occurrences of *Lb. plantarum* in *Suusac* and *Gariss* was probably due to the addition of onion pulp, and seeds of fenugreek and black cumin during the fermentation[10]. Moreover, the health-effectiveness of some *Lb. plantarum* against the cancer cell is reported[58].

Some of the isolated Lactobacilli (**Table 6** and **7**) supported those isolated by Ashmaig who found *Lactobacillus brevis*, *Lb. plantarum*, *Lb. fermentum* and *Lb. gasseri* in addition to other species in *Garris*[14]. Also, high rate of *Lactobacillus fermentum* (26.67%), followed by *Lactobacillus acidophilus* (20%) and *Lactobacillus plantarum* (20%) compared to *Lactobacillus brevis* (13.33), *Lactobacillus casei* (13.33%) and *Lactobacillus delbreckii* (6.67%) was found in *Gariss* used by camel herders in the nomadic production system in Al Gadarif State, Eastern Sudan[8,9]. On the other hand, the *Lb. delburekii* and *Lb. acidophilus* were isolated in addition to *Lb. bulgaricus* from traditional *Rayeb* milk, which is Egyptian fermented camel milk[44]. The isolated *Lb. delbrueckii* (**Table 6** and **7**) might contribute to both acid and flavor production as well as probiotic properties[53].

The 3 isolates of *Lb. salivarius* (1.8%) were found in *Gariss* samples (**Table 6** and **7**) supported the presence of this organism in the fermented camel milk in Kenya[20]. Similarly, *t*he presence of *Lactobacillus salivarius* (1%) was reported in *Chal*, a traditional fermented camel milk in Iran[61]. Moreover, the isolation of *Lb. acidophilus* (11.5%) and *Lb. casei* (0.6%) as shown in **Table 6** and **7** were in accord to the result reported on the increase of using strains of *Lb. acidophilus* and *Lb. casei* in the manufacture of probiotic yoghurts[62].

In the present study, 2 isolates for each of *Lb. gasseri* (1.2%) and *Lb. jensenii* (1.2%) were identified (**Table 6** and **7**). Similarly, the *Lb. gasseri* was isolated from *Garris* samples[14]. However, *Lb. gasseri* is one of the Lactobacilli that have been found in kefir grains[53].

The *Lactobacillus jensenii* (ATCC 25258) was among the 6 strains that exhibiting the highest radical scavenging activity during the fermentation of milk[63]. On the other hand, both strains of *Lactobacillus gasseri* and *jensenii* were isolated from the vaginas of healthy premenopausal women[64,65]. Moreover, the antimicrobial properties and stability of the biomolecules that produced by *Lactobacillus jensenii* and *Lactobacillus gasseri* (P_{6A} and $P_{65,}$ respectively) suggest their potential antimicrobial activity against the gastrointestinal, urogenital tracts and skin infectious pathogens[[66].

Some of the beneficial lactic acid bacteria that were found in the fermented camel milk should receive more attention. Hence, this study supported the conclusion stating that the beneficial LAB found in camel's milk have potentiality as new, natural and functional sources for the dairy technology explored[14]. This

because of the important roles of lactic acid fermentation process in the preservation of food, improving of organoleptic properties and increasing its acceptability and yield[10].

5. Conclusion

Wide variations and diversity were found for the lactic and non-lactic acid bacteria in *Gariss* obtained from nomadic production system in North Darfur State by camel herders. The locations as well as the containers used for preparing *Gariss* revealed significant variations on the occurrence of the different microbial group. *Bokhsa* and *Siin* are found as the best containers to prepare and store fermented camel milk. Moreover, different types of beneficial bacteria were isolated from *Gariss* in addition to other types of pathogenic or spoilage bacteria that should be identified is order to prevent and control their presence in such important food. Hence, the present study recommends provision of extension and essential services including veterinary supervision to improve camels' wealth in the country. This is beside capacity buildings targeting the best and accurate methods of processing and preservation of camel milk and products. Also, it is important to highlight the use of the pure or mixed selected types of fermentative bacteria and yeasts for the utilization of camel dairy products in pastoral and urban areas.

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Author contribution

Hafiz Osman: Study design, Methodology, Investigation, Data analysis, Writing – original draft.

Ibtisam El Zubeir: Study design, Investigation, Supervision, Funding acquisition, Writing – reviewing and editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data for this research will be available upon request.

Ethics approval

Not applicable.

Consent to participate

Both authors consent to participate.

Consent for publication

The authors consent for publication of this mauscript.

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