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2900 100th Street, Suite 309, Des Moines, IA 50322-3855, USA

Mohamed Ahmed,[1] Abraham Mikru,[2*] Million Adane,[3] and Henok Sileshi[4]

[1,4]Jigjiga University, P.O. Box 1020, Jigjiga, Somali, Ethiopia
[2*]Hawassa University, Dept. of Biology, P.O. Box 5, Hawassa, Ethiopia
[3]Ethiopian Biodiversity Institute, P.O. Box 30726, Addis Ababa, Ethiopia

Isolation of Lactic Acid Bacteria from Camel Milk and *in vitro* Assessment of Their Antagonism Against Selected Foodborne Pathogenic Bacteria

ABSTRACT

The use of lactic acid bacteria (LAB) as a protective culture offers a suitable alternative to chemical food preservatives. This study aimed to isolate LAB from raw and fermented camel milk and assess their antagonistic effects against selected bacterial pathogens (*Staphylococcus aureus, Escherichia coli,* and *Salmonella* Typhi). Twenty raw and 20 fermented camel milk samples were collected from Jigjiga, Ethiopia. Recommended microbiological protocols were followed to quantify, isolate, and identify representative LAB from the samples. Moreover, the antagonistic effect of the LAB isolates was assessed using the agar-well diffusion method. A total of 112 LAB isolates were classified into three genera: *Lactobacillus, Pediococcus,* and *Leuconostoc*. Of the 112 isolates, 94 (83.9%) showed antagonism against one or more of the test pathogens, with a mean diameter of the inhibition zone (MDIZ) ranging between 16.3 and 23.5 mm. More than 21% of the LAB isolates (18 of 83) that were antagonistic to *S. aureus* had an MDIZ ≥22 mm. However, only four and three LAB showed an MDIZ of > 22 mm against *E. coli* and *S.* Typhi, respectively. This study allowed the isolation of LAB from camel milk, with potential biotechnological applications as a protective culture. Further taxonomic identity confirmation of the promising LAB isolates and their performance as *in situ* protective cultures for food items is envisaged.

INTRODUCTION

Raw camel milk serves as food for many people worldwide and is commercially produced and sold in many countries *(39)*. The pastoral areas of Ethiopia are the main camel belts in the Horn of Africa, which includes the Afar and Somali regions, and the Borena and Kereyu areas of the Oromia region *(16)*. With an estimated 2.4 million heads, Ethiopia ranks third in the world in its camel livestock resource *(8)*. Of these, 458,760 are lactating with an annual milk production of 0.63 MMT *(24)*. Single-humped dromedary camels (*Camelus dromedarius*) are the predominant livestock in these locations. They produce more milk over a longer period than other animals under the same harsh conditions. Under similar arid conditions, camels yield an average of 4 litres of milk whereas cows yielded 1-2 litres of milk. However, the

*Author for correspondence: Phone: +251916867353; Email: mikruabraham@gmail.com

amount of milk produced per day depends on the lactation stage, type of feed, and management practices. It typically ranges from 0.5 to 8 liters, with a mean of 4.6±1.4 liters per day *(44)*. Therefore, they are the primary source of nutritious food and income for pastoralists in the region *(14)*.

Camel milk is traditionally consumed among the Somali pastoralists either fresh or in fermented form, known as "Sussa." The preparation of sussa is a small-scale artisanal practice among the Somalis whenever surplus camel milk is available. A clean wooden container is typically used to collect raw camel milk, which is then covered and left in a quiet place sheltered from dust for 24–48 h at room temperature (25–28°C). By this time, it turns sour and is suitable for consumption *(44)*. Although camel milk plays a significant role in the livelihood of pastoralists, its marketing potential has not been sufficiently assessed by the government and private sectors. The utilization of camel milk is largely governed by the needs of the community and other cultural and traditional limitations. However, selling camel milk among Ethiopian pastoralists is uncommon *(49)*. In additon, access to the market for camel milk is low for producers owing to remoteness from towns and roads. Being a highly perishable item, it is wasted because of the high ambient temperature and extended transportation time *(31)*. The value of annual milk and dairy product losses due to spoilage and lack of appropriate preservation technology across five African and Middle Eastern countries (Kenya, Tanzania, Uganda, Ethiopia, and Syria) is estimated to be over US$ 90 million *(32)*. Therefore, the need for locally available appropriate milk preservation technologies, in areas where modern pasteurization technology is not affordable, cannot be overemphasized.

Raw milk is endowed with natural antimicrobials, such as the lactoperoxidase system. However, the concentration of the components is not sufficient to thwart the growth of contaminating microorganisms. The system contains lactoperoxidase and small but sufficient concentrations of its substrate thiocyanate *(29)*. The key component that is specifically lacking in sufficient concentrations in raw milk is hydrogen peroxide *(25)*. Lactic acid bacteria (LAB), which naturally inhabit raw milk as the predominant flora, potentiate the antimicrobial effects of the lactoperoxidase system.

LAB are catalase-negative; thus, hydrogen peroxide, which is naturally produced under aerobic conditions, is not removed. This enhances the oxidation of thiocyanate to hypothiocyanate by the action of lactoperoxidase and hydrogen peroxide. Hypothiocyanate is a powerful oxidizing agent that reacts with the sulfhydryl groups of transport proteins in the bacterial membrane leading to their death. This is a very effective bactericidal agent, particularly in gram-negative bacteria, whereas lactic acid bacteria are relatively resistant *(3)*. LAB are generally regarded as safe because they are an integral part of starter cultures used in the production of traditional fermented foods. Through their metabolic activity, a complex system of competition for nutrients and binding sites, and the production of inhibitory bacteriocins, LAB exert antagonistic effects against a wide variety of human pathogens and food spoilage microorganisms *(4, 20)*. Therefore, it is highly desirable to develop and use selected and well-characterized LAB as a protective culture to ensure food safety and maintain the quality of raw milk and other non-dairy food items *(12, 23, 46)*.

The demand for organic food additives devoid of synthetic chemical preservatives is increasing among contemporary consumers. Various LAB species have been isolated and investigated worldwide to develop stater cultures and probiotics, including those in Ethiopia *(26, 33, 45)*. However, studies on the isolation of lactic acid bacteria for protective culture and food preservation purposes are lacking. Therefore, this study aimed to assess the diversity of LAB in raw and fermented camel milk and determine their potential biotechnological applications in controlling foodborne bacterial pathogens.

MATERIALS AND METHODS

Study area

The study was conducted in Jigjiga City, the administrative center of the Somali Regional State of Ethiopia. It is located 610 KM southeast of the capital city, Addis Ababa, within the geographic coordinates 09°30′N-07°16′N latitude and 32°13′E-42°50′E longitude. The area has an average elevation of 1934 m above sea level, an average annual temperature of 25°C, and a mean annual rainfall of 598mm. According to the 2007 census, the district has a total population of 277,560, of which 149,292 were men and 128,268 were women. While 125,876 (45.35%) were urban inhabitants, a further 6,956 (2.51%) were pastoralists. The mean camel herd size among the pastoralists in Jigjiga has been estimated to be 20.4 and a standard error of 1.93 *(42)*. More than 90% of the population are Muslim (the sole consumers of camel milk), and 6.97% are Orthodox Christian. Taking the 2.5% annual population growth rate in Ethiopia, the current population of Jigjiga may be estimated to be 314,000.

Study design

A descriptive, and experimental study design was carried out by laboratory isolation and characterization of lactic acid bacteria (LAB) from raw and fermented camel milk samples from local breads of lactating camels in the area around Jigjiga City. The isolated LAB were phenotypically characterized and assessed for their antagonistic effects against selected foodborne pathogenic bacteria using *in vitro* experiments.

Sample size and sampling procedures

Purposive sampling was used by considering the availability of pastoralit households and their herd size in the suburbs of Jigjiga City. A total of 40 samples, consisting of 20 raw

and 20 fermented camel milk (Sussa) samples (250 ml per sample) were considered from random households who were willing to participate in the study after obtaining informed consent.

Sample collection

All samples were collected in sterile bottles following recommended aseptic procedure *(6)* and transported in an icebox to the Microbiology Laboratory of the Somali Regional State Diagnostics Laboratory and Research Center. Samples were analyzed within an hour of collection, and in case of delays, the samples were stored in a refrigerator at 4°C until analysis.

Milk sample and culture media preparation

Each sample of milk was mixed by repeated shaking and inverting the sample bottle manually for one-two minutes. After mixing each sample, a 10 mL aliquot was aseptically transferred with a sterile pipette into a bottle containing 90 ml of peptone water (1%) as a dilution blank. The mixture in the screw-capped bottle was mixed by manual shaking for about 1–3 minutes. From this bottle, further tenfold serial dilutions were prepared by transferring 1 ml aliquots into tubes containing 9 ml of sterile peptone water using a micropipette for up to 10^{-8}. All culture media used in this study were prepared according to the instructions of the manufacturers (Oxoid Limited, Basingstoke, England).

Enumeration and isolation of lactic acid bacteria (LAB)

LAB were enumerated and isolated using the spread-plate method on de Man, Rogosa, and Sharpe (MRS) agar (Oxoid). Using a micropipette, 0.1 ml aliquot of appropriate dilution of milk sample prepared as above was spread on MRS agar plates using a sterile bent glass rod and incubated at 32°C for 48 h in an anaerobic jar *(6)*. After incubation, the typical whitish colonies, with sizes between 0.4-2.5 mm, and convex elevation were counted. Plates with colonies between 25 and 250 were used to estimate the number of LAB per ml of the raw or fermented camel milk sample in colony forming units (CFU) per ml *(41)*.

For putative identification, colonies displaying the general characteristics of lactic acid bacteria were chosen from each plate for biochemical tests as described previously *(17, 47)*. Well-isolated, morphologically distinct colonies were selected from countable plates and purified by repeated streaking onto fresh MRS agar plates to obtain pure cultures. The isolated pure cultures were stored in 50% glycerol vials (a portion of a single colony emulsified in tubes containing a mixture of sterile 0.5 ml each of glycerol and MRS broth) at –20°C until further characterization.

Phenotypic characterization and putative identification of LAB

Each isolate was subjected to Gram staining and catalase test. Microscopic observation of Gram-stained smears for Gram reaction and endospore formation was conducted. The isolates that were Gram-positive, non-endospore formers, and catalase-negative isolates were putatively recognized as lactic acid bacteria.

Test for gas bubbles in glucose fermentation

To determine whether the LAB isolates were homofermentative or heterofermentative, the production of gas bubbles was checked. Tubes of modified MRS broth containing 1% glucose and inverted Durham's tubes were used to monitor gas (CO_2) generation from glucose. Separately, 50 µl of the LAB log phase culture was added into 9 ml MRS broth in separate tubes containing one percent glucose and inverted Durham tubes. The test tubes were incubated at 30°C for five days. Gas bubbles were checked and when they appeared in Durham's tubes over the course of five days indicating the production of CO_2, the isolate was recognized as heterofermentative LAB *(1, 22)*.

Determination of the antimicrobial activity of LAB preparation of the test foodborne pathogenic organisms

The test organisms (*Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* Typhi) were obtained in glycerol cryopreservation vials from the Somali Regional Diagnostics Laboratory and Research Center (SRDLRC). All are local isolates, *S. aureus* from raw cow's milk, *E. coli* and *S.* Typhi from raw beef samples in 2021. However, no published information exists about the strains. The inoculum of each test pathogen was prepared by taking a portion of the preserved culture with a wire loop into 5 mL nutrient broth tubes. The inoculated tubes were then incubated at 35°C for 24 h.

Screening for antagonistic activity of whole cell LAB isolates

The antagonistic activity of the LAB isolates was tested using the spot test method as described previously *(45)*. Briefly, plates of modified MRS agar were prepared and the bottom of the Petri dish was divided into grids of squares (ca. 4 cm^2) with a colored marker. Subsequently, 100 µL of 24 h broth culture of each test pathogen was spread on the surface of the pre-dried, modified MRS agar plates in duplicates using a sterile bent glass rod. Finally, 10 µL of the 48 h culture of each LAB isolate in modified MRS broth was spotted over the agar plates of the test pathogens in separate grids. All plates were incubated at 32°C for 48 h as indicated above. At the end of the incubation period, the plates were examined for zone inhibition around each LAB colony. LAB isolates that showed inhibitory activity against each test pathogen were selected for further studies using cell-free extracts.

Preparation of cell-free crude extract of LAB

Each LAB isolate was grown overnight at 37°C first in a 100 ml capacity conical flask containing 50 ml MRS broth supplemented with 1% glucose. The overnight growth culture was filtered through a Millipore filter with a pore diameter of 0.22 µm. The cell-free culture filtrate from each LAB isolate was collected as a crude extract separately and aseptically to test its antimicrobial activity against the test foodborne pathogens.

Antagonistic effect of cell-free extract against the pathogens

The test pathogens were grown overnight (24 h) in nutrient broth, and 10 µL of the growth medium was mixed with molten modified MRS soft agar (0.7% agar) at approximately 48°C. The mixture was then overlaid onto the surface of the modified MRS agar plates. After solidification, wells were made on overlaid modified MRS agar plates with a sterile 7 mm diameter cork-borer. The floors of the wells were sealed with a drop of modified MRS soft agar (0.7% agar). Wells on duplicate plates were filled with 50 µL cell-free filtrate from each LAB isolate. A well filled with sterile distilled water served as the negative control. For the positive control, the culture filtrate of a known LAB (Local Isolate of LAB from tomato and obtained from the lab) with antagonistic activity against each test pathogen was included. In all cases, the filtrate in the wells was allowed to diffuse for 4 h at 40°C and was incubated overnight at 37°C *(45)*. At the end of the incubation period, the antimicrobial activity was checked by noting the inhibition zone around the well and measuring it using a caliper in mm.

Data analysis

LAB were enumerated in duplicate plates from the appropriate dilution of samples following the standard plate count method *(30)*. Final values were transformed into $\log_{10}$ colony-forming units (CFU) per mL. The average counts of LAB between the raw and fermented milk samples were compared using an independent *t*-test, and *P*-value < 0.05 was considered statistically significant. The relative effectiveness of the LAB isolates against each test pathogen was categorized and presented in frequency tables.

RESULT AND DISCUSSION

Enumeration of presumptive LAB in the milk samples

LAB were encountered in all 40 camel milk samples analyzed. Accordingly, the average LAB count of the raw camel milk samples was 5.52 $\log_{10}$ CFU/ml (log unit) and ranged from 4.77–6.18 log units *(Table 1)*. On the other hand, the average LAB count of the fermented camel milk samples (7.4 log units) was higher than that of the raw milk samples and ranged between 6 – 7.96 log units. The observed difference in the average LAB counts between the raw and fermented milk samples was statistically significant ($P<0.05$). Of the 20 raw milk samples, 8 (40%) showed LAB counts < 5 log units, 10 (50%) had LAB counts between 5 and 6 log units, and two (10%) had a count > 6 log units *(Table 1)*. In contrast, 12 of the 20 (60%) fermented camel milk samples showed LAB counts ≥ 7 log units, while the remaining 8 (40%) samples showed counts between 6 and 7 log units *(Table 1)*.

The mean LAB count of the raw camel milk samples (5.52 log units) in the present study was much higher than that of Zhao et al. *(51)* who reported an average LAB count of 6.02×10^3 CFU/ mL (equivalent to 3.78 log unit) from 15 raw camel milk samples in Mongolia. The results of this study indicate a higher LAB count in fermented camel milk than in raw camel milk samples. In agreement with this study, Taye et al. *(43)* reported that *Lactobacillus* species were more abundant in fermented cow's milk (8.36 $\log_{10}$ CFU/ml) samples than in raw cow's milk (7.67 $\log_{10}$ CFU/ ml) samples. Similarly the count of *Lactococcus* species was higher in fermented cow's milk (9.44 $\log_{10}$ CFU/ml) samples than in raw cow's milk samples (7.05 $\log_{10}$ CFU/ ml). A similar finding was reported by Khalil and Anwar, *(27)* based on a study of fresh cow's milk and commercial yogurt samples in Bangladesh.

The higher LAB count in the fermented camel milk was apparently the result of the multiplication of the indigenous LAB that entered the raw milk during the storage period under favorable extrinsic conditions. With the accumulation of inhibitory factors from the multiplication of LAB and the rising acidity of fermented milk, suppression of other contaminating microbes would further enhance the multiplication of LAB in the absence of competition *(4)*.

Isolation and putative identification of LAB from the milk samples

A total of 166 distinct presumptive LAB colonies were isolated from countable MRS agar plates, consisting of 98 isolates from fermented camel milk and 68 isolates from raw camel milk. Further phenotypic characterization based on the score of key features confirmed 112 of the 166 isolates were LAB *(Table 2)*. Therefore, a total of 112 LAB isolates, consisting of 35 from raw and 77 from fermented camel milk samples were isolated and putatively identified.

Phenotypic characterization based on key morphological, physiological, and biochemical features allowed the putative identification of the 112 LAB isolates into three genera *(Table 3)*. Accordingly, the most frequently encountered LAB isolates were those related to the genera *Lactobacillus* (80 or 71.4%), *Pediococcus* (17 or 15.2%), and *Leuconostoc* (15 or 13.4%). The majority of the isolates related to the genus *Lactobacillus* (62 of 80 or 77.5%) were recovered from fermented milk samples, of which 49 (43.8%) were homofermentative and 13 (11.6%) were heterofermentative *(Table 3)*. The remaining 18 isolates related to the genus *Lactobacillus* were obtained from the raw milk samples.

TABLE 1. Enumeration of presumptive lactic acid bacteria (LAB) in samples of raw and fermented camel milk collected in the suburbs of Jigjiga city, Somali Region of Ethiopia

Raw milk sample	Number of LAB in $\log_{10}$ CFU/ml	Fermented milk sample	Number of LAB in $\log_{10}$ CFU/ml
Rcm1	4.83	Fcm1	7.92
Rcm2	4.89	Fcm2	6.94
Rcm3	5.81	Fcm3	6.95
Rcm4	4.77	Fcm4	6.99
Rcm5	5.83	Fcm5	7.94
Rcm6	4.84	Fcm6	7.96
Rcm7	4.919	Fcm7	6.00
Rcm8	5.88	Fcm8	6.00
Rcm9	5.83	Fcm9	7.93
Rcm10	4.90	Fcm10	6.98
Rcm11	5.94	Fcm11	7.88
Rcm12	6.81	Fcm12	7.95
Rcm13	5.88	Fcm13	7.94
Rcm14	5.00	Fcm14	7.89
Rcm15	4.99	Fcm15	7.00
Rcm16	5.81	Fcm16	6.97
Rcm17	5.85	Fcm17	7.93
Rcm18	6.78	Fcm18	6.96
Rcm19	4.91	Fcm19	7.89
Rcm20	5.88	Fcm20	7.90
Minimum	4.77		6.00
Maximum	6.81		7.96
Average	5.52		7.40
StadDev	0.64		0.66

LAB = Lactic acid bacteria, Rcm = Raw milk sample, Fcm = Fermented milk sample

TABLE 2. The number of LAB isolated from raw and fermented camel milk in Jigjiga city, Somali, Ethiopia

LAB Isolates	Raw milk	Fermented milk	Total
Total presumptive LAB isolates	68	98	166
Confirmed LAB isolates	35	77	112

Moreover, 17 (15.2%) isolates related to *Pediococcus* species were isolated from raw milk samples.

Studies from many countries have indicated that LAB are dominant in milk, particularly camel milk *(35, 50)*. In agreement with this study, *Lactobacillus* species and *Leuconostoc* species were among the dominant LAB reported by Elgadi et al. *(13)* studies on milk samples from camels, cows, goats, and ewes in Khartoum state, Sudan.

In the present study, *Lactobacillus* (71.43%) was the dominant genus isolated from the raw and fermented camel

TABLE 3. Phenotypic characterization and putative identification of LAB isolates from raw and fermented camel milk in Jigjiga, Somali Region of Ethiopia

Number of LAB (%)	Sample Source	Cell shape	Cellular arrange	Gram stain	Catal	Endo-spore	Gas (CO_2)	Mode of Fermentation	Putative identity
49 (43.8)	FM	Bacilli	Single	+	-	-	-	Homo	*Lactobacillus*1
15 (13.4)	FM	Cocci	Chains	+	-	-	+	Hetro	*Leuconostoc*
13 (11.6)	FM	Bacilli	Chains	+	-	-	+	Hetro	*Lactobacillus*2
17 (15.2)	RM	Cocci	Pairs	+	-	-	-	Homo	*Pediococcus*
18 (16.1)	RM	Bacilli	Single	+	-	-	+	Hetro	*Lactobacillus*3

Catal = Catalase test, FM = Fermented milk, RM = Raw milk, Homo = Homofermentative, Hetro = Hetrofermentative.
+ = Positive or present, - = Negative or absent

milk samples. This is in agreement with a previous finding by Seifu et al. *(40)* who reported that of the 146 LAB isolated from fermented camel milk (Ititu), 58% were *Lactobacillus*, 25% *Lactococcus*, and 17% *Enterococcus*. Rahman et al. *(37)*, also reported the identification of 48 LAB isolates where *Lactobacillus* and *Enterococcus* were predominant. Likewise, Hawaz et al. *(21)* also reported the predominance of *Lactobacillus* species, accounting for more than 70% of the LAB species isolated from camel milk samples in Babile, eastern Ethiopia.

In contrast with the present study, Hassaine et al. *(18)* reported that the majority of LAB isolated from raw camel milk in Algeria were cocci consisting of *Enterococcus* (30.43%) and *Lactococcus* (21.74%), whereas 47.83% of the isolates belonged to the genus *Lactobacillus*. Similarly, Akhmetsadykova et al. *(2)*, reported that most of the isolated LAB from raw and fermented camel milk samples in Kazakhstan were cocci (70% of 118 isolates) belonging to *Enterococcus*, *Leuconocstoc* and *Lactococcus*. This finding contrasts with the observations of the present study. The predominance of cocci rather than Lactobacilli in camel milk has also been reported in previous studies including studies on fermented camel milk in Sudan *(8)*, camel milk from Moroco *(28)* and fermented camel milk samples from Xinjiang, China *(38)*. Based on a study of raw camel milk, Figuiri et al. *(15)* isolated and identified five *Lactococcus lactis*, one *Lactobacillus pentosus*, two *Lactobacillus plantarum*, one *Lactobacillus brevis*, and one *Pediococcus pentosaceus*.

The variations in the dominant LAB genera isolated from raw and fermented camel milk among the different studies may be due to some factors including differences in the environment or methods used in the identifications. The exact ecological cues that select for specific genera or species of LAB are not clear, but one may speculate several biotic and abiotic factors, including gross geographic location, climate, the species of camel, type of feed, manner of husbandry, milking environment, and utensils. Attributing specific LAB genera in milk to the type of source animal is premature and requires further substantiation with a controlled study.

Primary screening for antagonistic activity of the LAB isolates

Initial screening for antagonistic activity against selected foodborne pathogenic bacteria was performed based on whole-cell spot tests on all 112 putatively identified LAB isolates. Of these, 94 (83.93%) showed antagonistic action against one or more of the tested pathogens *(Fig. 1)*. The most frequently observed antagonistic activity was against *S. aureus* (83 of 94 or 88.3%), followed by *S.* Typhi (81 of 94 or 86.2%) and *E. coli* (75 of 94 or 79.8%). LAB isolates with antagonistic activity against multiple test pathogens (broad spectrum) were also observed including against *S. aureus* + *E. coli* (13.8%), against *S. aureus* + *S.* Typhi (20.2%), against *E. coli* + *S.* Typhi (11.7%) and against all three pathogens (54%).

In a similar study, Hawaz et al. *(21)* reported the antagonistic effect of 21 LAB isolates against both gram-positive and gram-negative test bacterial pathogens isolated from 30 raw camel milk samples from Babile, Eastern Ethiopia. In agreement with the present study, the majority of their isolates belonged to the genus *Lactobacillus*, but unlike the present study, all 21 isolates were reported to show varying degrees of broad-spectrum antagonistic action against test strains of *S. aureus*, *E. coli*, *S.* Tphi, and *Pseudomonas aeruginosa*.

Secondary screening of LAB isolates for antagonism against test pathogens

All 94 lactic acid bacteria (LAB) isolates that showed positive antagonistic effects against one or more of the test pathogens in the whole-cell spot tests were retested using cell-free culture filtrate by the agar-well diffusion method. All showed antagonistic effect against the respective

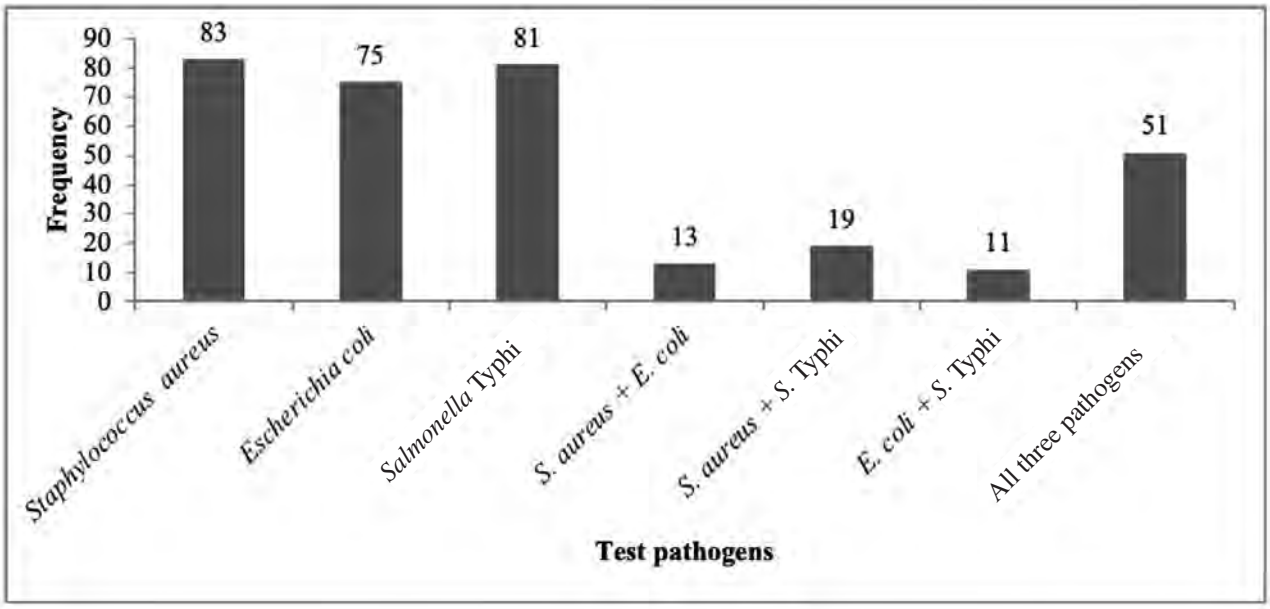

FIGURE 1. The frequency distribution of lactic acid bacteria (LAB) isolates (n = 94) from raw and fermented camel milk samples with the spectrum of antagonistic effect against three bacterial pathogens in Jigjiga.

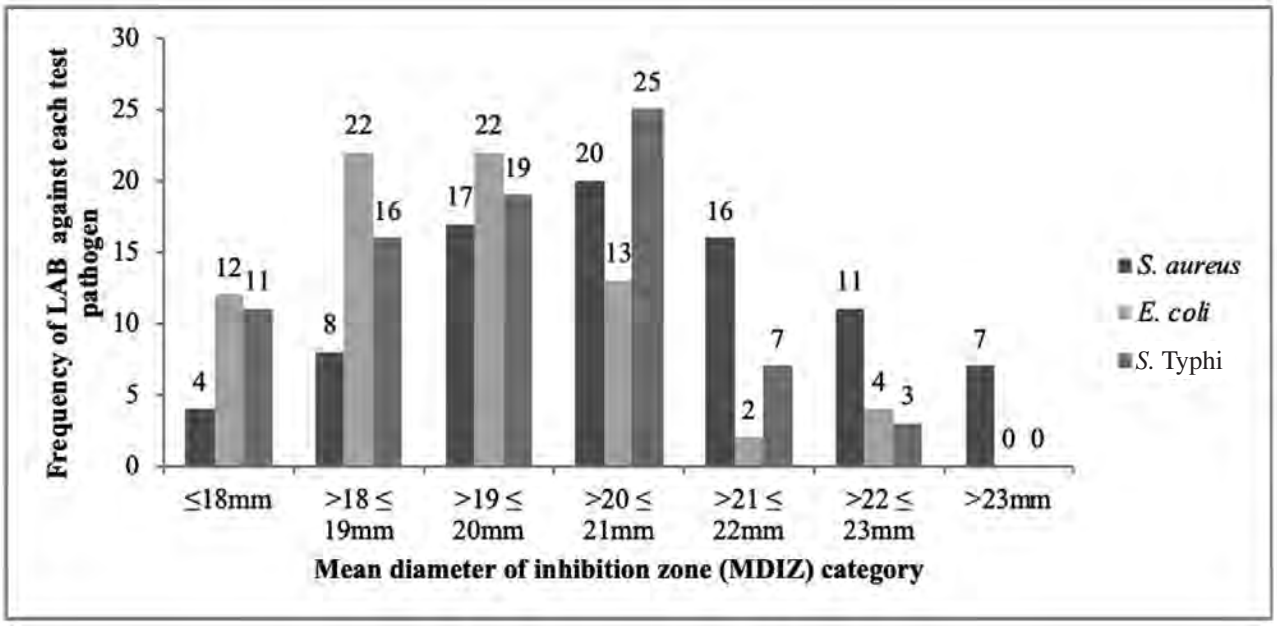

FIGURE 2. The frequency distribution of LAB isolates from raw camel milk by the magnitude of their antagonistic effect against selected foodborne pathogenic bacteria, 2022, Jigjiga, Somali, Ethiopia.

test pathogens with a mean diameter of inhibition zone (MDIZ) ranging between 16.3 mm against *E. coli* to 23.5 mm against *S. aureus* *(see Appendix Table 1)*. Accordingly, 83 LAB isolates showed antagonistic activity against *S. aureus*, with MDIZ ranging from 17.5 mm (HeLbRCM99) - 23.5 mm (HFLbFCM41). Of the 83 LAB isolates that showed antagonistic activity against *S. aureus*, 54 (65.1%) demonstrated an MDIZ greater than 20 mm *(Fig. 2)*. More than 21% of the LAB isolates (18 of 83 isolates) that were antagonistic to *S. aureus* with spot tests demonstrated an MDIZ of ≥22 mm with the agar well diffusion test using cell-free culture filtrate. All 18 isolates were derived from fermented camel milk samples, consisting of 15 isolates related to homofermentative *Lactobacillus* species and

TABLE 4. Frequency distribution of antagonistic LAB genera isolated from fermented camel milk samples against three pathogenic bacteria by mean diameter of inhibition zone (MDIZ) category

LAB with MDIZ	Frequency and % of LAB with MDIZ against the indicated pathogen					
Category	***S. aureus***	**%**	***E. coli***	**%**	**S. Typhi**	**%**
Homofermentative *Lactobacillus* species (n = 47)						
≤18mm	0	0	6	15	5	12.5
>18 ≤ 19mm	0	0	14	35	10	25
>19 ≤ 20mm	5	11.63	15	37.5	11	27.5
>20 ≤ 21mm	13	30.23	5	12.5	11	27.5
>21 ≤ 22mm	10	23.26	0	0	3	7.5
>22 ≤ 23mm	12	27.9	0	0	0	0
>23mm	3	6.98	0	0	0	0
Total	43	100	40	100	40	100
Isolates related to *Leuconostoc* species (n = 11)						
≤18mm	0	0	0	0	0	0
>18 ≤ 19mm	2	20	5	62.5	0	0
>19 ≤ 20mm	5	50	1	12.5	1	10
>20 ≤ 21mm	2	20	2	25	6	60
>21 ≤ 22mm	1	10	0	0	2	20
>22 ≤ 23mm	0	0	0	0	1	10
>23mm	0	0	0	0	0	0
Total	10	100	8	100	10	100
Heterofermentative *Lactobacillus* species, (n = 8)						
<18mm	0	0	0	0	1	14.29
>18 ≤ 19mm	0	0	0	0	1	14.29
>19 ≤ 20mm	0	0	0	0	3	42.86
>20 ≤ 21mm	0	0	2	40	2	28.57
>21 ≤ 22mm	4	57.14	0	0	0	0
>22 ≤ 23mm	1	14.29	3	60	0	0
>23mm	2	28.57	0	0	0	0
Total	**7**	**100**	**5**	**100**	**7**	**100**

MDIZ = Mean diameter of inhibition zone, LAB = Lactic acid bacteria, *E. coli* = *Escherichia coli, S. aureus* = *Staphylococcus aureus,* S. Typhi = *Salmonella* Typhi

three isolates related to heterofermentative *Lactobacillus* species *(Table 4)*. The exact inhibitory principles and their concentrations in the culture filtrates were not defined but an MDIZ ≥22 mm is equivalent to the susceptibility breakpoint for 30 µg of cefoxitin standard discs against *S. aureus (48)*.

With regard to *E. coli*, a total of 75 LAB isolates showed antagonistic effects with MDIZ ranging between 16.3 mm (HeLbRCM106) and 22.6 mm by HeLbFCM75 *(Appendix Table 1)*. More than a quarter of (19 of 75 or 25.3%) LAB isolates that showed antagonistic effects on *E. coli* with spot tests demonstrated MDIZ greater than 20 mm with the agar well diffusion test *(Table 5)*. Only four (5.34%) of the LAB isolates with an antagonistic effect with spot test against *E. coli* showed MDIZ greater than 22 mm by the agar well diffusion test method. This MDIZ is slightly greater than the sensitivity breakpoint for most standard beta-lactam

TABLE 5. Frequency distribution of antagonistic LAB genera isolated from raw camel milk samples against three pathogenic bacteria by MDIZ category

LAB with MDIZ	Frequency and % of LAB with MDIZ against the indicated pathogen					
Category	***S. aureus***	**%**	***E. coli***	**%**	***S.* Typhi**	**%**
Isolates related *Pediococcus* species ($n = 13$)						
≤18mm	1	10	1	10	0	0
>18 ≤ 19mm	4	40	2	20	2	18.2
>19 ≤ 20mm	0	0	4	40	2	18.2
>20 ≤ 21mm	5	50	2	20	4	36.4
>21 ≤ 22mm	0	0	1	10	1	9.1
>22 ≤ 23mm	0	0	0	0	2	18.2
>23mm	0	0	0	0	0	0
Total	**10**	**100**	**10**	**100**	**11**	**100**
Heterofermentative *Lactobacillus* species ($n = 15$)						
≤18mm	3	23.1	5	41.7	5	38.5
>18 ≤ 19mm	2	15.4	1	8.3	3	23.1
>19 ≤ 20mm	7	53.9	2	16.7	2	15.4
>20 ≤ 21mm	0	0	2	16.7	2	15.4
>21 ≤ 22mm	1	7.7	1	8.3	1	7.7
>22 ≤ 23mm	0	0	1	8.3	0	0
>23mm	0	0	0	0	0	0
Total	**13**	**100**	**12**	**100**	**13**	**100**

MDIZ = Mean diameter of inhibition zone, LAB = Lactic acid bacteria, *E. coli* = *Escherichia coli*, *S. aureus* = *Staphylococcus aureus*, *S.* Typhi = *Salmonella* Typhi

antibiotic discs against *Enterobacteriaceae (45)*. All four lactic acid bacteria that showed MDIZ >22 mm against *E. coli* were related to Heterofermentative *Lactobacillus* species, consisting of three derived from fermented camel milk samples *(Table 5)* and one from raw camel milk samples *(Table 6)*.

Likewise, 81 (86.2%) of the LAB isolates that showed antagonistic activity against *Salmonella* Typhi with spot test demonstrated MDIZ ranging from 16.6 mm by HeLbRCM100 to 22.8 mm by LeuFCM57 with agar well diffusion method *(Appendix Table 1)*. The LAB isolates that showed MDIZ > 22 mm against *S.* Typhi were only 3 (3.7%), consisting of one isolate related to *Leuconostoc* species derived from fermented camel milk samples *(Table 4)* and two isolates related to *Pediococcus* species derived from raw camel milk samples *(Table 5)*. The genera *Lactobacillus* and *Pediococcus* are LAB that are frequently used on a large scale in the production and preservation of many foods or as probiotics for humans and animals *(50)*. In one study, Hathout and Aly *(19)* demonstrated the shelf-life extension of a traditional beverage, Talbina (mix of barley flour and milk), by more than a week using two *Lactobacillus* species as bio-preservatives.

The majority of LAB that demonstrated antagonistic effects against the test pathogens with a mean diameter of inhibition zone ≥20 mm were derived from the fermented camel milk sample. All the homofermentative and heterofermentative *Lactobacillus* species from fermented camel milk and the LAB isolates from raw camel milk (*Pediococcus* species and heterofermentative *Lactobacillus* species) demonstrated higher efficacy or MDIZ against *S. aureus* than against *E. coli* and *Salmonella* Typhi. This suggests that they have higher antimicrobial activity against gram-positive than gram-negative bacteria.

In agreement with this study, Musiy et al. *(34)* found that *Lactobacillus* fermentum from raw sheep milk Bryndza had greater antimicrobial activity against *S. aureus* and *Listeria monocytogenes* than against *E. coli*. This may be due to variations in the bacterial cell wall structure. The cell wall of Gram-positive bacteria is mostly peptidoglycan *(10)*, which

TABLE 6. Selected LAB isolates from raw and fermented camel milk samples that showed a broad spectrum of antagonism against two or more of three test pathogens with a MDIZ ≥ 20 mm in Jigjiga, Somali region of Ethiopia

S. aureus + *E. coli*	*S. aureus* + *S.* Typhi	*E. coli* + *S.* Typhi	All three pathogens
HFLbFCM24	HFLbFCM15	PcRCM80	HFLbFCM4
HeLbFCM68	LeuFCM59	PcRCM85	HFLbFCM21
	HeLbFCM65		HFLbFCM33
	PcRCM87		HFLbFCM37
			HeLbFCM69
			HeLbFCM77
			PcRCM84
			HeLbRCM96

HFLbFCM = Homofermentative *Lactobacillus* species from fermented camel milk, LeuFCM = *Leuconostoc* spp. from fermented camel milk, HeLbFCM = Heterofermentative *Lactobacillus* from fermented camel milk samples, PcRCM = *Pedicoccus* species from raw camel milk, HeLbRCM = Heterofermentative *Lactobacillus* species from raw camel milk, *E. coli* = *Escherichia coli, S. aureus* = *Staphylococcus aureus, S.* Typhi = *Salmonella* Typhi

can be the target of bacteriocins. In contrast, the cell wall of Gram-negative bacteria is mainly lipopolysaccharide, which makes them resistant to many antimicrobials, especially those with a large molecular size and unable to penetrate the cell wall to reach their inhibitory sites *(11)*. However, the finding of this study is in contrast with that of Ohenhen et al. *(36)*, who observed the highest and lowest zone of inhibitions by *Lactobacillus plantarum* for *E. coli* and *S. aureus*, respectively.

The majority of lactic acid bacteria isolates (38 of 51) that showed a broad spectrum of antagonistic activity against all three test pathogens were derived from fermented camel milk samples *(see Appendix Table 1)*. These consisted of 29 isolates related to homofermentative *Lactobacillus*, six isolates related to *Leuconostoc* species, and three related to heterofermentative *Lactobacillus* species. Eight of the LAB isolates with antagonistic activity against all three test pathogens demonstrated MDIZ ≥20 mm which consisted of four homofermentative *Lactobacillus* species and two heterofermentative *Lactobacillus* species derived from fermented camel milk samples. The remaining two isolates, one related to *Pediococcus* species and the other to heterofermentative *Lactobacillus* species, came from raw camel milk samples *(Table 6)*.

Two LAB isolates from fermented camel milk samples, consisting of the homofermentative *Lactobacillus* species (HFLbFCM24) and heterofermentative *Lactobacillus* species (HeLbFCM68), showed a broad spectrum of antagonism against *S. aureus* and *E. coli* with an MDIZ of ≥20 mm *(Table 6)*. Similarly, two *Pediococcus* species (PcRCM80 and PcRCM85) showed antagonism against *E. coli* and *S.* Typhi with MDIZ ≥20 mm. Four LAB isolates showed a broad spectrum of antagonism against *S. aureus* and *S.* Typhi with MDIZ ≥ 20 mm *(Table 6)*. These consisted a homofermentative *Lactobacillus* species from fermented camel milk samples (HFLbFCM15), *Leuconostoc* species from fermented camel milk sample (LeuFCM59), a heterofermentative *Lactobacillus* species from fermented camel milk samples (HeLbFCM65), and *Pediococcus* species from a raw camel milk sample (PcRCM87).

Hawaz et al. *(21)* reported the study of 30 raw camel milk samples from farming households in Babile, eastern Ethiopia. Twenty-one LAB isolates demonstrated a broad spectrum of antagonism against four test bacterial pathogens with an MDIZ ranging from 1 to 12 mm. In the present study, 51 isolates demonstrated a broad spectrum of antagonism against three test pathogenic bacteria with MDIZ > 16 mm. *E. coli*, *S. aureus* and *S.* Typhi were used as test pathogens in both studies. However, although strain differences in the sensitivity of the test pathogens used in the two studies may exist, the LAB isolates in the present study demonstrated a superior antagonistic effect. Based on the study of raw camel milk samples from southern Algeria, Bentoura et al. *(9)* reported the antagonistic effect of four *Lactobacillus* species isolates against six test bacterial pathogens with MDIZ ranging from 2 to16 mm.

CONCLUSION

This study aimed to assess the LAB load of raw and fermented camel milk samples and isolate representative LAB strains with antagonistic activity against selected foodborne bacterial pathogens (*S. aureus*, *E. coli*, and *S.* Typhi). Despite the limitations of the methods for taxonomic

identification of the LAB isolates and lack of determination of the active principles of the antagonistic activities, the following conclusions were drawn. The average LAB count of the fermented camel milk samples was higher than that of raw camel milk samples. Phenotypic methods allowed the putative identification of 112 LAB isolates as *Lactobacillus* species, *Leuconostoc* species, and *Pediococcus* species. Of the 112 LAB isolates, 94 (83.9%) were antagonistic to one or more of the test pathogens, with an MDIZ ranging between 16.3–23.5 mm. Sixteen LAB isolates showed a broad spectrum of antagonistic activity, with an MDIZ >20mm. This study demonstrated that camel milk is a good source of LAB, with potential biotechnological applications as a protective or starter culture in the food industry. Further studies should be conducted on the full systematic identity of the selected LAB isolates that showed a broad spectrum of antagonistic activity and their application as protective cultures in situ for fermented and non-fermented food items.

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APPENDIX

TABLE 1. Distribution of LAB genera isolated from raw and fermented camel milk samples with their mean diameter of inhibition zone (MDIZ) in mm against three test bacterial pathogens based on agar-well diffusion test of cell-free culture filterate in Jigjiga

No.	Homofermentative *Lactobacillus* spp.	*S. aureus* MDIZ in mm	*E. coli* MDIZ in mm	*S.* Typhi MDIZ in mm
1	HFLbFCM1	23	18	-
2	HFLbFCM2	19.75	-	19.9
3	HFLbFCM3	-	19	21.75
4	HFLbFCM4	22.8	20.7	20.5
5	HFLbFCM5	22	18	19
6	HFLbFCM6	23	-	19.75
7	HFLbFCM7	21	19.45	20
8	HFLbFCM8	20.4	18.5	-
9	HFLbFCM9	22.85	19	21.4
10	HFLbFCM10	20.8	-	18.7
11	HFLbFCM11	19.9	20.5	16.9
12	HFLbFCM12	21.5	18.5	19.1
13	HFLbFCM13	22.6	18.5	20.5
14	HFLbFCM14	22	16.5	21.7
15	HFLbFCM15	22.4	-	20.35
16	HFLbFCM16	23.5	19.2	19
17	HFLbFCM17	-	19.4	18.5
18	HFLbFCM18	22.5	19.5	-
19	HFLbFCM19	20.5	19.6	18.35
20	HFLbFCM20	21.5	19.1	19.75
21	HFLbFCM21	20.7	20.5	21
22	HFLbFCM22	20.2	18.5	19.8
23	HFLbFCM23	23.1	18	18.7
24	HFLbFCM24	20.1	20.5	-
25	HFLbFCM25	19.5	19.5	-
26	HFLbFCM26	21.9	18.5	20.55
27	HFLbFCM27	21	19.55	19.3
28	HFLbFCM28	22	-	19.3
29	HFLbFCM29	20.3	18.3	-
30	HFLbFCM30	22.2	19	19.55
31	HFLbFCM31	21.5	20	18.95
32	HFLbFCM32	22	18.5	17.9
33	HFLbFCM33	20.5	20.5	20.5
34	HFLbFCM34	20	17.5	-
35	HFLbFCM35	23.05	19.5	19
36	HFLbFCM36	22.5	19.5	20.7

Continued on the next page.

TABLE 1. Distribution of LAB genera isolated from raw and fermented camel milk samples with their MDIZ in mm against three test bacterial pathogens based on agar-well diffusion test of cell-free culture filterate in Jigjiga (cont.)

No.	Homofermentative *Lactobacillus* spp.	*S. aureus* MDIZ in mm	*E. coli* MDIZ in mm	*S.* Typhi MDIZ in mm
37	HFLbFCM37	21.95	20	20.55
38	HFLbFCM38	22.5	19.8	17
39	HFLbFCM39	-	16.5	19.4
40	HFLbFCM40	20.5	18.5	17.3
41	HFLbFCM41	22.65	-	18.5
42	HFLbFCM42	21.4	18.1	19.4
43	HFLbFCM43	22.1	19.2	20.6
44	HFLbFCM44	-	19.5	16.9
45	HFLbFCM45	21	-	18.3
46	HFLbFCM46	20.6	18.5	20.9
47	HFLbFCM47	19.25	18.6	20.75
Leuconostoc spp.				
48	LeuFCM51	19.05	20.1	20.4
49	LeuFCM53	18.5	-	21.2
50	LeuFCM55	19.2	-	20.75
51	LeuFCM56	-	19	20.25
52	LeuFCM57	19.2	19.5	22.8
53	LeuFCM58	20	18.75	20
54	LeuFCM59	21	-	21
55	LeuFCM60	19.65	18.3	-
56	LeuFCM61	19	20.5	20.9
57	LeuFCM62	21.75	18.95	20.9
58	LeuFCM63	20.65	18.5	21.8
Heterofermentative *Lactobacillus* spp.				
59	HeLbFCM65	22.15	-	20.3
60	HeLbFCM66	21.5	-	18
61	HeLbFCM68	21.4	22.15	-
62	HeLbFCM69	21.7	22.5	20
63	HeLbFCM72	-	20.35	18.35
64	HeLbFCM74	23.2	-	19.2
65	HeLbFCM75	23.8	22.6	19.9
66	HeLbFCM77	21.65	20.9	20.75
Pediococcus spp.				
67	PcRCM78	18.7	-	20.5
68	PcRCM79	19	18.4	19.6
69	PcRCM80	-	20.5	21.9
70	PcRCM82	20.25	19.9	-

Continued on the next page.

TABLE 1. Distribution of LAB genera isolated from raw and fermented camel milk samples with their MDIZ in mm against three test bacterial pathogens based on agar-well diffusion test of cell-free culture filterate in Jigjiga (cont.)

No.	Homofermentative *Lactobacillus* spp.	*S. aureus* MDIZ in mm	*E. coli* MDIZ in mm	*S.* Typhi MDIZ in mm
71	PcRCM84	20.5	21.75	22.5
72	PcRCM85	-	20.4	20.7
73	PcRCM86	19	19.5	18.8
74	PcRCM87	20.4	-	22.3
75	PcRCM89	20.15	19	-
76	PcRCM91	18	-	19.6
77	PcRCM92	20.5	19.75	20.5
78	PcRCM93	-	19.5	20.6
79	PcRCM94	18.5	17.3	18.5
Heterofermentative *Lactobacillus* spp.				
80	HeLbRCM96	21.5	21	21.5
81	HeLbRCM97	18.4	17.5	19
82	HeLbRCM98	19.85	17.8	18.7
83	HeLbRCM99	17.5	-	19
84	HeLbRCM100	19.5	20	16.6
85	HeLbRCM101	19.85	19.1	-
86	HeLbRCM102	-	18.4	19.4
87	HeLbRCM104	19.5	18	18
88	HeLbRCM105	17.8	17.3	16.95
89	HeLbRCM106		16.3	17.8
90	HeLbRCM107	17.9	-	18
91	HeLbRCM108	19.25	21.6	19.2
92	HeLbRCM109	19.6	-	20.5
93	HeLbRCM110	19.5	22.5	-
94	HeLbRCM111	18.8	20.3	20.4

MDIZ = Mean diameter of inhibition zone
HFLbFCM = Homofermentative *Lactobacillus* species from fermented camel milk
LeuFCM = *Leuconostoc* spp. from fermented camel milks
HeLbFCM = Heterofermentative *Lactobacillus* from fermented camel milk samples
PcRCM = *Pedicoccus* species from raw camel milk
HeLbRCM = Heterofermentative *Lactobacillus* species from raw camel milk
E. coli = *Escherichia coli, S. aureus* = *Staphylococcus aureus*, *S.* Typhi = *Salmonella* Typhi