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# Metagenomic Analysis of Bacterial Community Composition in *Dhanaan*: Ethiopian Traditional Fermented Camel Milk

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**Abstract**

This study was conducted to evaluate the safety and bacterial profile of *Dhanaan* (Ethiopian traditional fermented camel milk). The composition of the microbial community in *Dhanaan* samples was analysed by a metagenomic approach of 16S rRNA gene amplicon sequencing. Metagenomic profiling identified 87 different bacterial microorganisms (OTUs) in six samples analysed. Although the Dhanaan samples contained various lactic acid bacteria (LAB), they also all contained undesirable microorganisms in large proportions. The following LAB genera were identified: *Streptococcus, Lactococcus,* and *Weissella.* One *Streptococcus* species represented by OTU-1 (operational taxonomic unit) was found in all *Dhanaan* samples and the dominating species in four out of six samples. This common isolate was found to be closely related to *S. lutetiensis* and *S. infantarius*. Undesirable microorganisms from genera such as *Escherichia, Klebsiella, Enterobacter, Acinetobacter* and *Clostridium* were, however, also frequent, or even dominant in *Dhanaan* samples. Thus, this calls for a change in the *Dahnaan* manufacturing practice to an improved and safer production system. Starter cultures suitable for *Dhanaan* production might be developed from the *Streptococcus*, *Weissella*, and *Lactococcus* microorganisms identified in this study. However, further safety evaluation and technological characterization need to be conducted on strains defined by OTU-1, OTU-2, OTU-3, OTU-8, and OTU-35 before they can be used as food grade starter cultures.

**Keywords**: Metagenomic analysis; lactic acid bacteria; *Dhanaan*; OTU*;* fermented camel milk.

## Introduction

In many countries of dry zones of sub-Saharan Africa, camels (*Camelus dromedarius*) play a significant role in the lifestyle of pastoral communities owing to their adaptation to the hostile climatic conditions by providing milk, meat and transportation  More than half of the world's 28 million camel population are found in the East African countries of Somalia, Sudan, Ethiopia and Kenya (FAO STAT 2014). Nowadays, the camel is attracting an increased public interest since climate change is already influencing traditional cattle productivity, whereas the camel is capable of surviving in the harsh climatic conditions (Al haj and Al Kanhal 2010; Berhe *et al.* 2017; El-Agamy 2006).

Pastoralists' practice and recent scientific reports indicate that fermented and fresh camel milk have therapeutic properties against different diseases (Dubey, Lal, Mittal, & Kapur, 2015; Galil, Gader, & Alhaider, 2016; Mati *et al.* 2017; Mihic *et al.* 2016; Seifu, 2007). Fermented camel milk is a common traditional product in several parts of the world whereas products such as butter, cheese and yoghurt are not a traditional product from camel milk (Farah *et al.* 2007). Fermented camel milk has different names in different parts of the world; *Shubat* in Turkey, Kazakhstan and Turkmenistan, *Suusac/susa* in Kenya and Somalia, *Gariss* in Sudan, *Dhanaan* and *Ititu* in Ethiopia (Farah, Mollet, Younan, & Dahir, 2007; Seifu, 2007; Shori, 2012). *Dhanaan* is common in the pastoral areas of Eastern part of Ethiopia mainly in Somali and Oromia regions and *Ititu* is produced in the Kereyu area of Oromia region in the Eastern part of the country (Seifu, 2007; Seifu, Abraham, Kurtu, & Yilma, 2012).

Seifu (2007) reported that pastoralists in Shinile and Jigjiga area of Eastern Ethiopia prefer to produce *Dhanaan* to other dairy products for its perceived high nutritional value, high demand by urban dwellers, and preference of consumers for its taste and flavour as well as long shelf life. Pastoralists in eastern Ethiopia indicated that the storage stability of *Dhanaan* is long and it can stay for a couples of months when accompanied with continuous back slopping, i.e., inoculating a new batch of milk with a small amounts of a previously fermented *Dhanaan* (Seifu, 2007). Production of *Dhanaan* in Eastern Ethiopia is a long tradition and made by placing unpasteurized camel milk in a smoked container, wrapping the container with a piece of cloth and keeping it at ambient temperature (25°C-35°C) over extended period of time. *Dhanaan* has a white opaque colour, sour taste and thin consistency. It has similar appearance like cow milk *Ergo* (Ethiopian traditional fermented milk) but has very thin gel (Biratu and Seifu, 2016). However, it shows syneresis on storage. The pastoralists in Eastern Ethiopia claim that *Dhanaan* is a safe product for human consumption and has therapeutic properties. They believe that pasteurization destroys the medicinal properties of the product.

On the other hand, spontaneously fermented African camel dairy products are reported to be sources of contaminating microbes of public health concern (Biratu & Seifu, 2016; Jans ,Bugnard, Njage, Lacroix, & Meile, 2012). No information is available concerning the microbial profile of Dhanaan. However, similar products from other African regions have been analysed. Abdelgadir *et al.* (2008) analysed the microbial profile of Sudanese fermented camel milk, Gariss, and found the

dominating LAB to be *Streptococcus infantarius, Lactobacillus fermentum, Lactobacillus helveticus,* and *Enterococcus faecium*. Jans *et al.* (2017) also reported that African fermented dairy products are dominated by technologically important microorganisms such as *Streptococcus infantarius subsp. infantarius* (Sii), *Lactococcus lactis, Lactobacillus spp*. and yeasts. The authors recommend the evaluation and use of the *Streptococcus infantarius* variants as a potential culture for future application to enhance food safety and security of the African dairy products. Safe and high quality fermented dairy products should preferably contain a microbial community dominated by lactic acid bacteria (LAB) belonging to species with a history of safe use whereas spoilage microorganisms and pathogens must be reduced to an insignificant level. LAB belonging to the genera *Lactobacillus* and *Lactococcus* are generally considered safe whereas Gram-negatives belonging to the phylum Proteobacteria are generally considered undesirable and potentially unsafe. Several LAB species of other genera than *Lactococcus* and *Lactobacillus* can contribute to quality and safety. With this study we have analysed the microbial quality of Ethiopian Dhanaan and we tried to identify LAB of potential use as starter cultures in order to develop safe practise for the production of Dhanaan.

## Materials and Methods

### Sample collection and DNA extraction

Six *Dhanaan* samples were collected from different market selling points (1 from Bombas, 2 from Gende sherka and 3 from Babile) areas in Somali and Oromia regional states of Eastern Ethiopia. *Dhanaan* in traditional containers is commonly sold to the customers in the areas around the streets. The samples were collected in sterilized containers and transported under ice box to Haramaya University Dairy laboratory. Samples arrived at the university lab within one hour of collection and were stored at 4 °C until DNA extraction.

Total genomic microbial DNA from the fermented milk samples were extracted using milk bacterial DNA isolation kit (Product #21550, Norgen Biotek) according to the manufacturers´ protocol. Extraction of Chromosomal DNA was done at Haramaya University Dairy laboratory, Ethiopia. The DNA concentration was measured using Qubit dsDNA assay (Qubit 3.0 Fluorometer, Q32851, Invitrogen). The extracted DNA samples were stored at -20 °C until transported to the Technical University of Denmark for metagenomic sequencing.

### PCR amplification and Library preparation of the V3 region of the 16S rRNA gene

PCR amplification of the V3 region of 16S rRNA genes from microbial community DNA of *Dhanaan* samples was done as described by Laursen *et al.* (2016). The primers used were forward primer (PBU [primer bacterial universal] 5`-A-adaptor-TCAG-barcode-CCTACGGGAGGCAGCAG-3´) and reverse primer (PBR [primer bacterial reverse] 5´-trp1-

adaptor- ATTACCGCGGCTGCTGG-3´). Both primers include sequencing adaptors. The PCR product was purified and processed for ion torrent sequencing as described in Fugl et al. (2017).

### DNA sequencing of 16S rRNA gene libraries

Sequencing of the prepared libraries was performed (Fugl *et al.* 2017) using the Ion Personal Genome Machine (PGM) with an Ion 318 chip kit (Thermo Fisher Scientific) and processed as described in Fugl *et al.* (2017). Briefly: sequences were analysed in CLC Genomic Workbench (version 8.5 CLC bio, Qiagen, Aarhus, Denmark). Reads were quality controlled, demultiplexed, and trimmed. Reads below 125 bp and above 180 bp were discarded. Operational taxonomic unit (OTU) clustering, chimera filtering, mapping of reads to OTUs, and generation of OTU table was done according to the UPARSE pipeline (Edgar 2013). The nucleotide sequences of the V3 regions of the OTUs identified was deposited at GenBank (Benson *et al.* 2017) with submission number SUB5459365 and accession numbers ranging from MK789774-MK789846. Taxonomy was assigned using the ribosomal database project classifier (Wang *et al.* 2007) with confidence threshold of 0.5 and the Greengenes database v.13.8 (DeSantis *et al.* 2006). Taxonomic assignments were checked by BLASTN against the 16S ribosomal RNA sequences database at NCBI (national centre for biotechnology information). The relative abundance of a given taxon was calculated by considering the total number of reads in the sample.

## Results

The composition of the bacterial communities of six *Dhanaan* samples was analysed by sequencing DNA molecules generated by PCR amplification of the V3 region of the 16S rRNA genes using universal primers. Between 20,000 and 40,000 16S rRNA V3 amplicon sequences were obtained from each sample. The sequences were assigned to a total of 87 different OTUs. Table 1 shows the relative abundance of the OTUs contributing with more than 2% in each *Dhanaan* sample. Table 2 lists the possible taxonomy (species assignment) of the 16 OTUs of Table 1 contributing with more than 2% of the total bacterial 16S amplicon sequences in any of the *Dhanaan* samples. Table 3 summarizes the Key findings regarding quality and safety of the analysed Dhanaan samples. The taxonomy of the OTUs was investigated by applying a BLASTN analysis on the V3 16S rRNA sequence against the NCBI non-redundant 16S sequence database. The sequences determined for 73 of the 87 OTUs have been deposited at GenBank; 14 sequences ranging in length from 135 to 145 did not fulfil the minimum requirement of 150 set by GenBank. The results of the microbial community analysis indicate that all Dhanaan samples analysed contain microorganisms unsuitable for human consumption. One of the Dhanaan samples, sample number 6, had a microorganism belonging to the genus *Escherichia* as the most abundant OTU. The lowest amount of Gram-negatives was detected in sample 3 in which an *Escherichia* amounted to 3.9% of the microbial community.

## Discussion

Microbial communities of the *Dhanaan* samples were found to be complex ecosystems with organisms belonging to the phylum Firmicutes and Proteobacteria. We found 16 OTUs to exceed 2 % in relative amount in any of the six Dhanaan samples. Ten of the 16 OTUs belong to the phylum Proteobacteria and six belong to Firmicutes.

Eight of the ten OTUs of Proteobacteria are from the family Enterobacteriaceae, representing genera like *Escherichia, Klebsiella, Salmonella, Tatumella, Enterobacter*, and *Hafnia*. The Enterobacteriaceae are indicators of poor hygiene (Quigley *et al.* 2013). Pathogens are common among the Enterobacteriaceae and the frequency and high level in the *Dhanaan* samples raise a serious health concern. Proteobacteria were also in one sample represented by two OTUs belonging to the genus *Acinetobacter*. *Acinetobacter* has not been reported to be a beneficial food culture (Bourdichon *et al.* 2012) and the presence of *Acinetobacter* is indicative of contamination from the environment probably soil (Quigley *et al.* 2013; Al Atrouni *et al.* 2016). In the sample containing *Acinetobacter*, the dominating organism belongs to the genus *Escherichia*, indicating also poor hygienic practices of the pastoralists.

The *Dhanaan* samples contained six OTUs belonging to the phylum Firmicutes. One of these belongs to the genus *Clostridium,* most likely to *Clostridium tyrobutyricum.* Clostridia are generally unwanted in fermented products as some clostridia are pathogenic and no clostridia have been reported to be beneficial food culture (Bourdichon *et al.* 2012). *Clostridium tyrobutyricum* is a common environmental contaminant in milk able to cause spoilage of cheese, commonly known as late blowing. The other five OTUs are lactic acid bacteria (LAB) belonging to the genera *Lactococcus, Streptococcus,* and *Weissella.*

The LAB of the *Dhanaan* samples might represent a potential source of starter cultures needed for a safer and improved procedure for *Dhanaan* manufacturing. Three of the five "lactic OTUs" belong to the genus *Streptococcus*. OTU-1 is the most abundant in four of the *Dhanaan* samples and the second most abundant in sample 1. Based on the 16S sequence, OTU-1 belongs to the *Streptococcus equinus* complex also containing *Streptococcus lutetiensis* and *Streptococcus infantarius.* Strains belonging to this group have frequently been isolated from fermented dairy products in Africa (Abdelgadir *et al.* 2008; Jans *et al.* 2012). However, *Streptococcus lutetiensis* and *Streptococcus infantarius* have not yet been recognized as beneficial food cultures (Bourdichon *et al.* 2012). The dominating strain in sample 1 (defined by OTU-35) with 60% of all reads is closely related to *Streptococcus sanguinis* and *Streptococcus cameli*. *Streptococcus cameli* is a new species recently isolated from Morocan raw camel milk (Kadri *et al.* 2015) and this species does not yet have a history of use in fermented foods. The third OTU belonging to the genus *Streptococcus,* OTU-8, was found as a non-dominating component in two of the *Dhanaan* samples. The taxonomy placed this microorganism close to *Streptococcus salivarius* and *Streptococcus thermophilus*. *Streptococcus thermophilus* is commonly used as a starter culture for the manufacture of yoghurt (Bourdichon *et al.* 2012). The fourth "lactic OTU", OTU-2, was found in every *Dhanaan* sample in relative amounts between 2 and 28 %. The strain defining OUT-2 is closely related to *Weissella*

*cibaria* and *Weissella confusa*. These two species have previously been found in fermented foods and they are listed in the inventory of microorganisms with beneficial use (Björkroth *et al.* 2002; Bourdichon *et al.* 2012). However, *Weissella* is not commonly used in starter cultures (Fessard and Remize 2017). *Weissella confusa* and *cibaria* are hetero-fermentative lactic acid bacteria and *Weissella* strains could therefore contribute with important flavour notes to the *Dhanaan* products and they might be interesting candidates to develop into *Dhanaan* starter cultures. The fifth "lactic OTU", OTU-3, was identified as *Lactococcus lactis*. *Lactococcus lactis* is commonly used in starter cultures for the manufacture of cheese and fermented milk and is one of the species on the European QPS list (Bourdichon *et al.* 2012; Ricci *et al.* 2017). We can therefore conclude that this component of the *Dhanaan* microbiota is a beneficial component, which could also be a candidate for a *Dhanaan* starter culture.

Generally, the *Dhanaan* samples were dominated by pathogenic bacteria which have major public concern. The high content of Gram-negatives in all six Dhanaan samples analysed demonstrates that the current production practice is unsafe and needs improvement. Therefore, the pastoralists' practice of preparation of *Dhanaan* needs to be modified and the health issue should be considered. The pastoralists' perception towards the resistance of heat treatment of camel milk should be given a due attention in transforming the manufacturing practices to safe production. The practice of heat treatment accompanied with good manufacturing practices to avoid recontamination of the products should be implemented to produce safe and quality product. Strains of the LAB found in the *Dhanaan* samples that might serve as sources of starter cultures include the genus *Streptococcus, Weissella*, and *Lactococcus* which would be suitable for improved safety of *Dhanaan* products.

## Conclusion

Metagenomic profiling showed classical and non-classical species of LAB under the genera *Streptococcus*, *Lactococcus*, and *Weissella* as well as unsafe microorganisms like *Klebsiella*, *Enterobacter*, *Acinetobacter*, and *Clostridium* were found in the *Dhanaan* samples. OTU-1 (operational taxonomic unit) was the dominating streptococcus unit in four out of six samples. This common isolate was found to be closely related to *S. lutetiensis* and *S. infantarius*. The presence of significant amount of potentially pathogenic microorganisms in the *Dhanaan* samples indicates the need for the transformation of the traditional practices of *Dhanaan* manufacturing to an improved and safer production system. LAB species under OTU-1, OTU-2, OTU-3, OTU-8, and OTU-35 might be technologically important lactic acid bacteria for the fermentation of camel dairy products. However, further safety evaluation and characterization need to be conducted on such species before being considered as food grade bacteria.

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### Conflict of interest

The authors declare that there is no conflict of interest

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Table 1: Relative abundance of OTUs present in amounts larger than 2% of the total bacterial communities in the *Dhanaan* samples

| **Sample 1** | | **Sample 2** | | **Sample 3** | | **Sample 4** | | **Sample 5** | | **Sample 6** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Total reads=34118 | | Total reads=32342 | | Total reads=37173 | | Total reads=27749 | | Total reads=20591 | | Total reads=28276 | |
| OTU | % | OTU | % | OTU | % | OTU | % | OTU | % | OTU | % |
| OTU-35-g.*Streptococcus* | 60.1 | OTU-1-g.*Streptococcus* | 32.4 | OTU-1-g.*Streptococcus* | 39.9 | OTU-1-g.*Streptococcus* | 44.7 | OTU-1-g.*Streptococcus* | 23.9 | OTU-4-g.*Escherichia* | 38.7 |
| OTU-1-g.*Streptococcus* | 14.9 | OTU-6-g.*Clostridium* | 21.2 | OTU-3-g.*Lactococcus* | 33.3 | OTU-7-g.*Klebsiella* | 18.5 | OTU-4-g.*Escherichia* | 20.5 | OTU-2-g.*Weisella* | 27.9 |
| OTU-9-f. *Ent* | 9.1 | OTU-2-g.*Weissella* | 18.2 | OTU-2-g.*Weissella* | 14.8 | OTU-5-g.*Klebsiella* | 16.5 | OTU-7-g.*Klebsiella* | 18.9 | OTU-10-f.*Ent.* | 7.1 |
| OTU-7-g. *Klebsiella* | 3.1 | OTU-8-g.*Streptococcus* | 6.3 | OTU-4-g. *Escherichia* | 3.9 | OTU-4-g.*Escherichia* | 6.0 | OTU-5-g.*Klebsiella* | 18.7 | OTU-8-g.*Streptococcus* | 5.5 |
| OTU-2-g. *Weissella* | 2.3 | OTU-7-g.*Klebsiella* | 3.1 | | | OTU-48-f. *Ent* | 5.0 | OTU-2-g.*Weissella* | 5.0 | OTU-1-g.*Streptococcus* | 5.1 |
| OTU-48-f. *Ent* | 2.2 | OTU-4-g. *Escherichia* | 2.9 | | | OTU-2-g.*Weissella* | 3.7 | OTU-3-g.*Lactococcus* | 5.0 | OTU-11-g. *Acinetobacter* | 4.2 |
| | | OTU-9-f. *Ent* | 2.8 | | | OTU-3-g.*Lactococcus* | 3.1 | OTU-48-f.*Ent* | 4.4 | OTU-51-g. *Acinetobacter* | 3.8 |
| | | OTU-27-f. *Ent* | 2.3 | | | | | | | OTU-40-f.*Ent* | 3.5 |
| | | OTU-35-g.*Streptococcus* | 2.3 | | | | | | | | |
| | | OTU-5-g.*Klebsiella* | 2.0 | | | | | | | | |
| Others | 8.4 | Others | 6.5 | Others | 8.1 | Others | 2.5 | Others | 3.7 | Others | 4.2 |

OTU, operational taxonomic unit; *Ent*, *Enterobacteriaceae*; g and f indicate identification at genus and family level, respectively. Sample 1 (Bombas); Sample 2 and 3 (Gende sherka); Sample 4, 5 and 6 (Babile)

Table 2: Taxonomy of abundant OTUs found in *Dhanaan* samples

| OTU | [a]Accession # | Genus | species | Accession # | % Identity |
|---|---|---|---|---|---|
| OTU-1-g.*Streptococcus* | MK789774 | *Streptococcus* | *alactolyticus* | NR_041781 | 100 |
| | | | *danieliae* | NR_117375 | 100 |
| | | | *equinus* | NR_113594, NR_114642, NR_042052 | 100 |
| | | | *lutetiensis* | NR_115719, NR_037096, NR_042051 | 100 |
| | | | *macedonicus* | NR_037002 | 100 |
| | | | *pasteurianus* | NR_043660 | 100 |
| | | | *porcorum* | NR_108477 | 99 |
| | | | *infantarius* | NR_104991 | 99 |
| OTU-2-g.*Weissella* | MK789775 | *Weissella* | *cibaria* | NR_036924 | 100 |
| | | | *confusa* | NR_113258, NR_040816 | 100 |
| OTU-3-g.*Lactococcus* | MK789776 | *Lactococcus* | *lactis* | NR_116443, NR_113958, NR_040956, NR_113925, NR_040954, NR_113960, NR_040955 | 100 |
| OTU-4-g.*Escherichia* | MK789777 | *Brenneria* | *alni* | NR_116340 | 100 |
| | | *Escherichia* | *albertii* | NR_025569 | 100 |
| | | | *coli* | NR_114042, NR_112558 | 100 |
| | | | *fergusonii* | NR_074902, NR_114079, NR_027549 | 100 |
| | | | *vulneris* | NR_119109 | 100 |
| | | *Shigella* | *dysenteriae* | NR_026332 | 100 |
| | | | *flexneri* | NR_026331 | 100 |
| | | | *sonnei* | NR_104826 | 100 |

| | | | | | |
|---|---|---|---|---|---|
| OTU-5-g.*Klebsiella* | MK789778 | *Klebsiella* | *pneumoniae* | NR_041750 | 100 |
| OTU-6-g.*Clostridium* | nd | *Clostridium* | *acidisoli* | NR_028898 | 100 |
| | | | *tyrobutyricum* | NR_044718 | 100 |
| OTU-7-g.*Klebsiella* | MK789779 | *Klebsiella* | *variicola* | NR_025635 | 100 |
| | | *Pectobacterium* | *carotovorum* | NR_125539 | 100 |
| OTU-8-g.*Streptococcus* | MK789780 | *Streptococcus* | *salivarius* | NR_042776 | 100 |
| OTU-9-f. *Enterobacteriaceae* | | | *thermophilus* | NR_042778 | 99 |
| | | | *vestibularis* | NR_042777 | 99 |
| | MK789781 | *Cronobacter* | *sakazakii* | NR_118087, NR_115942, NR_044076 | 100 |
| | | *Enterobacter* | *cloacae* | NR_118011, NR_102794, NR_117679, NR_113615 | 100 |
| | | | *ludwigii* | NR_042349 | 100 |
| | | | *oryzendophyticus* | NR_125586 | 100 |

Table 2 continued

| | | | | | |
|---|---|---|---|---|---|
| | | *Leclercia* | *adecarboxylata* | NR_114154, NR_104933, NR_117405 | 100 |
| | | *Salmonella* | *enterica* | NR_044372, NR_044371, NR_119108, NR_074910, NR_074799, NR_104709, NR_116126, NR_044373 | 100 |
| OTU-10-f. *Enterobacteriaceae* | MK789782 | *Tatumella* | *morbirosei* | NR_116282 | 99 |
| | | *Buttiauxella* | *agrestis* | NR_041968 | 99 |
| | | | *ferragutiae* | NR_025329 | 99 |
| | | | *gaviniae* | NR_025330 | 99 |

| | | | | | |
|---|---|---|---|---|---|
| | | | *noackiae* | NR_036919 | 99 |
| | | *Kluyvera* | *intermedia* | NR_114153, NR_028802, NR_112007 | 99 |
| OTU-11-g. *Acinetobacter* | MK789783 | *Acinetobacter* | *albensis* | NR_145641 | 99 |
| OTU-27-f. *Enterobacteriaceae* | MK789797 | *Enterobacter* | *hormaechei* | NR_042154 | 99 |
| | | | *tabaci* | NR_146667 | 99 |
| | | | *mori* | NR_116430 | 99 |
| | | *Shimwellia* | *blattae* | NR_074908, NR_116602, NR_116478 | 99 |
| | | *Cronobacter* | *malonaticus* | NR_044060 | 99 |
| OTU-35-g.*Streptococcus* | MK789805 | *Streptococcus* | *cameli* | NR_134817 | 99 |
| | | | *sanguinis* | NR_113260, NR_024841, NR_111994 | 99 |
| OTU-40-f. *Enterobacteriaceae* | MK789808 | *Hafnia* | *alvei* | NR_044729, NR_112985 | 99 |
| | | *Obesumbacterium* | *proteus* | NR_025334 | 99 |
| | | *Buttiauxella* | *warmboldiae* | NR_028893 | 99 |
| | | | *izardii* | NR_025331 | 99 |
| | | | *brennerae* | NR_025328 | 99 |
| | | *Lelliottia* | *nimipressuralis* | NR_044976 | 99 |
| | | | *amnigena* | NR_024642 | 99 |
| OTU-48-f. *Enterobacteriaceae* | MK789815 | *Enterobacter* | *cloacae* | NR_118568 | 99 |
| | | | *xiangfangensis* | NR_126208 | 99 |
| | | | *asburiae* | NR_024640 | 99 |
| | | *Erwinia* | *billingiae* | NR_104932 | 99 |
| | | *Klebsiella* | *alba* | NR_132596 | 99 |
| OTU-51-g. *Acinetobacter* | MK789818 | *Acinetobacter* | *pittii* | NR_117621, NR_117930, NR_116774 | 100 |
| | | | *calcoaceticus* | NR_042387 | 100 |

OTU, operational taxonomic unit; g and f indicate identification at genus and family level, respectively

[a]**Accession #** are for deposits in Genbank.

Nd; not deposited.

Table 3: Key findings regarding quality and safety of Dhanaan samples analysed

| **Positively affecting Dhanaan quality** | **Negatively affecting safety of Dhanaan** |
|---|---|
| All six samples contain LAB in which OTU-1 (operational taxonomic unit) was the dominating streptococcus unit in four out of six samples. This common isolate was found to be closely related to *S. lutetiensis* and *S. infantarius* | All six samples contain Gram negative microorganisms |
| Fraction of LAB range from 50 to 90% of the microbiota | Fraction of Gram negatives range from 4 to 60% of the microbiota |
| LAB could be a source of safe starter cultures specific for camel milk fermentation | In one Dhanaan sample, Escherichia is the most frequent microorganism |
| | Safety of the LAB present in Dhanaan needs to be investigated further |