Infect Epidemiol Med. 2016 Summer; Volume 2, Issue 3: 14-19 DOI:10.18869/modares.iem.2.3.14

Original Article

Microbial Safety of Masske: A Traditional Butter from South of Khorasan, Genetic Similarity of Pathogenic Bacteria Indicators

Fariba Ghiamati Yazdi[1], Masoud Yavarmanesh[2*], Morteza Khomeiri[1], Morteza Mahdavi[3]

[1]Department of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, IR Iran
[2]Department of Food Science and Technology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, IR Iran
[3]Department of Animal Sciences, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, IR Iran

**Corresponding author:* Masoud Yavarmanesh, Department of Food Science and Technology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, IR Iran. Tel.: +985118795620,E-mail: yavarmanesh@um.ac.ir

Submitted: April 21, 2014; **Revised:** July 08, 2014; **Accepted:** Aug 02, 2014

Background: Masske is a traditional Iranian butter made from yoghurt. The first aim of this study was to isolate and identify the nonlactic pathogenic microflora by culture and molecular methods of identification, and the second purpose was to identify genetic similarity of the isolated bacteria in Masske.
Materials and Methods: In order to detect pathogenic dominant indicator microorganisms, a number of 150 bacterial isolates from three Masske samples, which may comprise the repetitive isolates and could grow on appropriate media for *Staphylococci* and *E.coli*, were classified into 8 groups according to their phenotypic characterization followed by chemical tests. Then 2approximately similar isolates from each group were chosen (total 18 isolates; we selected 3 isolates from 2 groups of eight), and the sequencing of 16S rRNA gene was done for subsequent analysis.
Results: Among 18 bacterial isolates, *Staphylococcus hominis* was the most frequently isolated species during the manufacture of Masske as the presence of this bacterium was confirmed in 14 out of 18 samples. Also, the presence of *Staphylococcus epidermidis* and *Escherichia coli* was identical across the samples (for each one, 2 out of 18).
Conclusion: Our results based on cultural and molecular methods suggest making some improvements to the hygiene of Masske manufacture due to the high population of minor pathogens.

Keywords: Butter, Microbial safety, Pathogenic bacteria, Genetic similarity

[DOR: 20.1001.1.25884107.2016.2.3.7.1]

1. Background

Traditional dairy products comprise a huge recognized resources of phenotypic and genetic microbial variety that may have a lot of probable biotechnological applications (1-5). Among all dairy products, different types of fermented milk products have a prominent role across the globe. The nature of the milk product is based on its type, the pretreatment , and the fermentation conditions and next processing. They largely comprise of lactic acid bacteria (LAB); however, staphylococci, coliforms, yeasts, and moulds can also exist. These traditional foods have persisted over decades in different countries (6-8).

The foods poisoned by staphylococci (SFP) are the most important causes of gastroenteritis in the world (9). Symptoms of SFP have a rapid onset (from 2 to 6 hours) of abdominal cramps, nausea, and vomiting, sometimes followed by diarrhea (10, 11). Milk and milk products are the best vehicles for staphylococcal food poisoning (12-17). They are frequently responsible for SFP, (10). These products have high potency to be contaminated by a variety of microorganisms because of their high nutritive value and complex chemical composition. Among *Staphylococci*, coagulase-negative ones have been considered as minor pathogens. These kinds of pathogens were considered to be minor based on the fact that the minor pathogens are not reported quantitatively from each laboratory (18). In addition to the pathogenic *Staphylococcus aureus*, potentially pathogenic coagulase-negative *Staphylococci* (CoNS) have also been identified as the carriers of genes for persistence beyond macrolides (19, 20).

The CoNS have become the most common bacterial pathogens isolated from milk samples in many countries causing bovine intramammary infections (21-23). They are opportunists and adhere to the metal devices to produce a protective biofilm. The ability to produce biofilm enables CoNS to persist on milking equipment as well as on the milker’s hands, which serves as a major source of staphylococcal spread (24, 25). CoNS have traditionally been considered to be normal skin microflora, which as opportunistic bacteria can cause mastitis.

Masske is a dairy product with an attractive appearance, an integrated texture, an acceptable sour aroma, a light yellow color, which is semisolid at room temperature (26-28). Its flavor serves as a important role for its acceptance (28). Masske is made by fermentation of milk fat. It is produced in the special provinces of Iran: Mazandaran and Khorasan provinces, respectively, as well as in Turkey, Afghanistan, and Balkans Peninsula. It is always produced in a traditional hand work from the past generations. It can be made from the mixture of unpasteurized ewe or goat milk or only one type of milk (29). The main steps of Masske production are presented in Figure 1. The Fermentation process is completed by both natural microflora and little inoculums, during several days, and the phase changes are fulfilled by tuluming. The Shallaghi and Shiraz are two milk fat byproducts with the lower fat contents that are used to provide drink buttery yoghurt. Due to the lack of using special heating operation to produce this product, it is probable that pathogenic bacteria could remain in it, so its safety should be checked before use.

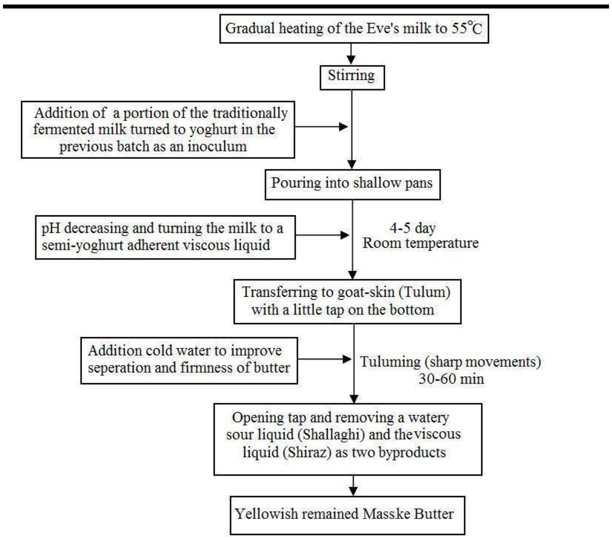


Figure1. Flow scheme of the manufacturing process of Masske butter

2. Objectives

The main goal of this study was to identify the predominant species and the genetic similarity of remaining pathogenic bacteria using classical cultural and molecular techniques to provide a phylogenetic tree and genetic similarities matrix.

3. Material and methods

3.1. Sampling of Masske

Three types of Masske designated as BB (butter of Borjuk), BBO (butter of Band Ozbak), and BR (butter of Riab) were come together from local markets in different areas of Gonabad (a southern city of Khorasan province). The samples were intentionally collected from regions where Masske has been traditionally produced in households for centuries. All samples were provided from sheep's milk. BB, BBO and BR were collected from the farms located in the south-western, south-eastern and eastern regions of Gonabad. Samples were collected from the batches of Masske that had been produced independently in different times of the year. The collected samples were put in sterile bags at 4 °C, transferred to the laboratory and the further analyses were performed within the following 24 hr.

3.2. Enumeration and isolation of microbial population

Masske samples, (25 g each), were homogenized in 225 mL of steriled and cooled 2% (w/v) tri-sodium citrate dihydrate solution (pH=7.5) and preheated to 40 °C. Ten-fold serial dilution and surface culturing were performed according to the method designed by Karl-Heinz and Van-Hoorde (30, 31).

3.3. Total plate counts

Total microbial counts were examined on plate count agar (Merck, Darmstadt, Germany), by use of the pour-plate technique. Microbial community were enumerated after 24-48 hr of incubation at 30°C (1, 7).

3.4. Coliforms

Coliforms were cultured on Violet Red Bile Lactose Agar (VRBLA) (Merck, Darmstadt, Germany), by the pour-plate method. Microbial community were counted after 24-48 hr of incubation at 30 °C (1, 7). For detection and isolation of *E. coli* specifically, the MPN method was performed by inoculation of 1 mL aliquots from the first three dilutions into 3 Lauryl Sulfate broth (LS) (Merck, Darmstadt, Germany) with inverted Durham tubes in them and incubated at 35 °C. The tubes were examined after 24 hr for gas production. For each gassing LS tube from the Presumptive test, we did the same for EC broth with Durham tubes and incubated EC tubes for 24 hr at 44.5 °C and examined them for gas production. We removed a loop full of EC broth from gas-positive tubes then carried out the same test in Tripton Water medium (Merck, Darmstadt, Germany) and incubated the tubes for 24 hr at 44.5 °C. Observation of purple ring is the indicator for doing further streak culturing on EMB agar for 24 hr at 35◦C (7, 32).

3.5. Staphylococci

Dilutions were grown on Baird–Parker agar (B–PA; Merck) supplemented with egg yolk tellurite solution (Merckaccording to the method previously fulfilled by other researchers (1, 7, and 32).

3.6. Phenotypic characterization of isolates

We isolated 150 bacterial isolates from three Masske samples. Fifteen isolates had been grown on VRBA and EMB agar and also made turbid with gas in LSB and EC media tubes. We classified these 15 isolates into two groups according to the shapes of their colonies (all these isolates were indole/ methyl red positive). The rest of the grown isolates on BPA were classified into six different groups according to their colony appearance, catalase test, resistance to 10 and 15% NaCl, and temperature resistance of 15 and 45 ºC.

3.7. Molecular identification of microbial isolates

Eighty pured colonies from EMB and BP agar media were stored frozen at −80 °C until the analyses were done. Cultures were recovered in the mentioned media with 100µl milliQ water Steriled pearls were added to the 0.5 ml microtubes and the tubes were mixed vortex for 5 mins, the microbial suspensions were transferred to the new tubes and heated for 20min at 70 °C. The tubes were centrifuged for 10 min at 13,000 g and 50 µl of supernatants (cell free extracts) were transferred into new tubes for resuspending the pellets to 100µl milliQ water Finally 50 µl of upper liquid parts of supernatants was used as DNA template for PCR analysis. The rest of the DNA isolates which didn't get a sharp band with the above method, were extracted by DNA kit (Roche, Germany) based on the manufacturer's protocol (1, 24, and 31).

3.8. Amplification of 16S rRNA Genes

PCR amplifications were carried out in a 25 µl volume using a Thermal Lab cycler (Sensequest, Germany) according to the method developed in other studies (31, 33-35). Amplicons were sequenced by Macrogen Advancing Thought Genome Co., Korea. Homology comparisons were performed using the Basic Local Alignment Search Tool (BLAST), available online at the National Centre for Biotechnology Information (NCBI) homepage (http://www.ncbi.nlm.nih.gov) (36).

3.9. Bioinformatic analysis

Alignment was performed through the ClustalW algorithm with its default parametersIn the Next step the MEGA software version 5 was and the phylogenetic tree was

[DOR: 20.1001.1.25884107.2016.2.3.7.1]

calculated based on the Neighbour-joining procedure with the Maximum Composite Likelihood model (37, 38 and 39). The validity of the tree was also verified based on the random sampling (Bootstrap) method with a thousand repetitions (40).

3.10. Statistical analysis

Standard deviation and average comparisons of log.cfug^{-1} counted colonies on four media of PCA, BPA, VRBA, and EMB were conducted by MINITAB software version 16 and analyzed based on Turkey test at the level of confidence of 95%.

4. Result

4.1. Enumeration of bacteria

According to Figure 2, the numbers of hygienic-indicator Populations were considerable in the all of the Masske samples, reaching their highest levels at sample assigned by BBO for total counts (7.6 log.cfug^{-1}), sample assigned by BB for coliforms (5.6 log.cfug^{-1}) and for *E.coli* (3.3 log.cfug^{-1}), and sample assigned by BR for staphylococci (6.5 log.cfug^{-1}). The results were in agreement with Alegria and colleagues (2009) about the total aerobic counts and coliforms of the curd and 3 day and 7 day ripened cheese for coliforms and Staphylococci respectively (1). Our results were also in agreement with Sengül's study (2006), in five of the fifteen samples (41). The 16s rRNA sequencing showed the genus, species, and strains of the 18 isolates susceptible for being *Staphylococci* and *Escherichia*. As we expected, all the 18 isolates belonged to these two genera. The results indicated that 14 isolates belonged to *Staphylococcus hominis*, 2 to *Staphylococcus epidermidis*, and 2 to *E. coli* (Table1). The results also indicated that two *E. coli* isolates were obtained from sheep's milk and not from butter; it may be because of the process of pH reduction in Masske production that kills some gram-negative bacteria such as *E. coli*. On the other hand, raw milk is a good rich nutrient resource for them.

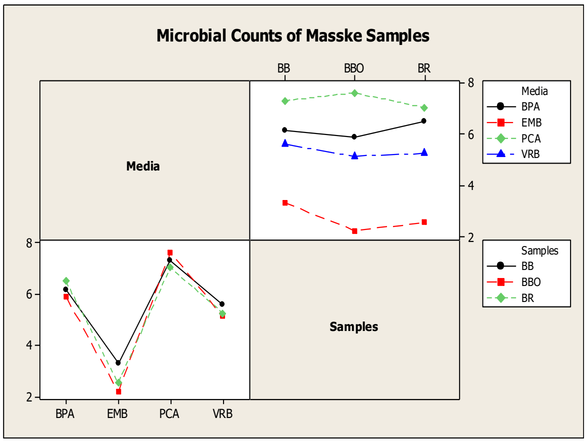


Figure2. Intractionpragh of microbial counts of Masske samples.

Table1. Majority of microorganisms identified from Masske samples isolated in three varied culture media.

isolate No.	isolate Name	Name in matrix	Name of bacteria in 16s rRNA sequencing	%iIdentity	Accession No.
1	B176	GIV1	*Staphylococcus hominis* strain *77 (BC26)*	99	(KF254627.1)
2	B60	GIV2	*Staphylococcus hominis* strain *77 (BC26)*	99	(KF254627.1)
3	B27	GII1	*Staphylococcus hominis* strain *HACF7*	99	(KC581939.1)
4	B95	GIV3	*Staphylococcus hominis* strain *77 (BC26)*	99	(KF254627.1)
5	B14	GII2	*Staphylococcus hominis* strain *HACF7*	99	(KC581939.1)
6	B213	GI1	*Staphylococcus hominis* strain *A8DI*	99	(KC898305.1)
7	B189	GIV4	*Staphylococcus hominis* strain *77 (BC26)*	100	(KF254627.1)
8	B216	GIII1	*Staphylococcus hominis* strain *AW14*	100	(JX281775.1)
9	B224	GIV5	*Staphylococcus hominis* strain *77 (BC26)*	100	(KF254627.1)
10	B212	GII3	*Staphylococcus hominis* strain *HACF7*	99	(KC581939.1)
11	B61	GIII2	*Staphylococcus hominis* strain *AW14*	98	(JX281775.1)
12	B16	GIV6	*Staphylococcus hominis* strain *77 (BC26)*	99	(KF254627.1)
13	B231	GIV7	*Staphylococcus hominis* strain *77 (BC26)*	98	(KF254627.1)
14	B64	GIV8	*Staphylococcus hominis* strain *77 (BC26)*	100	(KF254627.1)
15	B117	S.e(R)	*Staphylococcus epidermidis* strain *WIF14*	94	(HM480310.1)
16	M97	S.e1	*Staphylococcus epidermidis* strain *258 (P37A)*	100	(KF254632.1)
17	M66	E1	*Escherichia coli* strain *KVP104*	98	(JX290084.1)
18	M247	E2	*Escherichia coli* strain *BAB-538*	100	(KF535120.1)

4.2. Identification of isolates

We isolated 150 bacterial isolates from three Masske samples. Fifteen isolates had been grown on VRBA and EMB agar and also made turbid with gas in LSB and EC media tubes. We classified these 15 isolates to two groups according to the shapes of their colonies (all these isolates were indole and methyl red positive). The rest of the isolates on BPA were classified into six different groups according to their colony appearance, catalase test, resistance to 10 and 15% NaCl, and temperature resistance of 15 and 45 ºC.

Group I colonies were nonpigmented, flat (gray or grayish white), smooth, mucoid or slimy, which could grow strongly in the presence of 10% NaCl and 45 ºC but not 15 ºC.

Group II Colonies were black, opaque, and 1mm diameter, which could grow only in 45 ºC.

Group III colonies were yellowish-orange, watery, and bigger than group II, which could grow weakly in the presence of 10% NaCl, however, in 45 ºC they were noticeable. No growth was observed in 15 ºC and 15% NaCl. Group IV colonies were catalase negative and were omitted from other tests. Group V colonies were the same as Group III but couldn't grow at any percentage of NaCl and temperatures of

[DOR: 20.1001.1.25884107.2016.2.3.7.1]

[DOR: 20.1001.1.25884107.2016.2.3.7.1]

15 and 45 ºC. Group VI colonies were black but grew weakly in the presence of 10% NaCl and 45 ºC.

4.3. Cladograms

According to genetic similarity that was seen throughout the cladogram, *S. hominis* were classified into four groups and the other three species to only one group. According to Figure 2, the four groups of Staphylococci had varied similarity (45-100%). We selected a reference (marked with "R" letter) species for each group for the best comparison. As we see in Figure 2 and 3, we had four critical points where bootstraps had arisen up to 99-100% because of the importance of these points. We saw that the genetic distances from strains started from these points, so the groups were classified according to them. These genetic distances showed that two *E. coli* strains had more differences between themselves, rather than the Staphylococci. Moreover, the cladogram marked three points where the genetic differences had increased between four groups of *Staphylococci* (14-16, 36, 42).

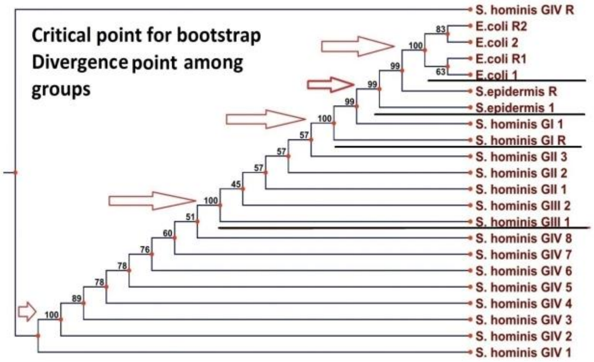


Figure3. Genetic dendrogram of *Staphylococci* and *Escherichia* isolated from Masske based on bootstrap

4.4. Similarity-distance matrix

The matrix shows that (the numbers above main diameter represent genetic distances and the numbers below talk the number of nucleotide coverage) among *Staphylococci* groups, GI, GII, and GIII differences were minute and in the range of 0 to 20%, and the nucleotide coverage (nc) reached up to 670-673 (Figure 4). For *S. epidermidis*, there was no difference and nc was 673. On the other hand, genetic distance between the first three groups of *S.hominis* and the fourth group (GIV) was high (72%) and nc was reduced to 359-360. Unexpectedly, the difference between the first three groups (GI, GII and GIII) and *E. coli* was 68%, which was less than the difference between the first three groups and the fourth group of *S.hominis* (GIV) (72%). Maybe it is because of the different origin of *S.hominis GIV*. Finally, *S.hominis GIV* and *E.coli* had the most distance (186%) and the lowest nc (269).The results also show that the most correlations belonged to strains of *E.coli* (855-850). These results are comparabe to the results obtained from Hebremedhin et al., (2008) (43).

The results also show that the Staphylococci in Group IV have a little Cognation relationship with the other Staphylococci strains indicators in dairy products and plant resources; however, according to the reference strain that was obtained from feces (36). This Cognation relationship is very close to strains isolated from animal feces. So it is important to make a solution to eliminate the present pathogens without affecting on the nutritive values of product.

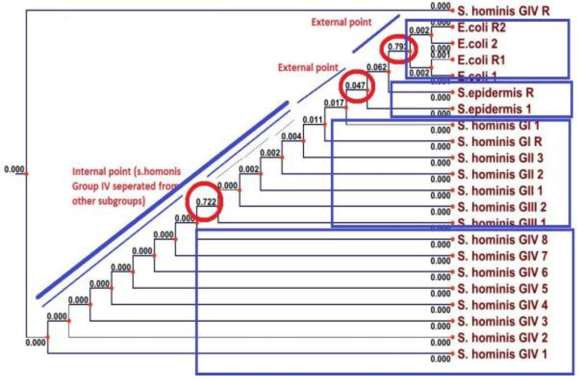


Figure4. Genetic dendrogram of *Staphylococci* and *Escherichia* isolated from Masske based on genetic distances.

5. Discussion

In the present study, the variety of dominant species of pathogenic indicators in analyzed Masske samples was recognized through classical culturing isolation and DNA based techniques. This study supplied a phylogenetic tree for the assessment of genetic diversity and the relationships between those species. The present data illustrated that *S.hominis* was the predominant isolate recovered from the examined Masske samples. The predominance of *S.hominis* among the CoNS was also recorded earlier in sheep's dairy product (24, 25).

In order to assess intense changes in the microbial community and the microbial genetic biodiversity during the process of making Masske, we should attention a main factor that influences the microbial quality and quantity during this process.

The conditions provided for microbial growth and the major compounds exist in every sample during Masske production together, make some of the microbial species abundant; raw milk is a good source of gram negative bacteria (GNB) such as *E. coli* and coliforms that prefer neutral pH of environment to survive and grow. During Masske production, when the milk is turned to yoghurt, the pH decreases vigorously, so most of these bacteria die. Only the microbial flora that can tolerate this pH decrease can survive. Finally, when the yoghurt is turned to Masske, pH increases slightly, and these surviving microorganisms can grow. On the other hand, the microbial diversity of goat skin and also the human skin that forms the product may influence the Masske final microflora. This microbial population is originated from the normal resident flora of udder and teat skin too (44).

The results indicate that although the numbers of Staphylococci were remarkable, strains of pathogenic *Staphylococci* such as *Staphylococcus aureus* were never detected in the BPA counting plates and also in sequencing. The pathogenic microorganisms were *E. coli*, *S. hominis*, and *S. epidermidis*. The high rate of *S.hominis* may be due to the localization of this microorganism inside or/and outside of the udder and also in human skin flora, which have been entered from hands skin during hand-formation process of Masske. Many studies in different regions worldwide have investigated the occurrence of CoNS. The prevalence of intramammary infections with CoNS was reported in Finland (50% of the positive for bacterial growth) (45). In a similar study that was done in Norway, the occurrence of CoNS was 16% (46), while in Germany CoNS were isolated from 9% of the dairy samples in a total of 80 dairy herds (47). In two dairy herds in Canada, CoNS were the most common bacteria (51%) causing intramammary infection (IMI)(48). Although these species of Staphylococci don’t have the pathogenicity capacity as *S. aureus*, lately the Centers for Disease Control and Prevention's National Nosocomial infection surveillance system has reported that *S. epidermidis* is involved in 33.5% of blood system infection diseases with the hospital originality which are called nosocomial blood stream infection. So the importance of coagulase-negative staphylococci (CoNS) has increased and they have become the predominant pathogens isolated from subclinical infections in several countries (47-49) and categorizes in the groups of pathogens responsible for diverse nosocomial infections. Most of the strains have troublous resistance to many types of antibiotics such as penicillin, methicillin, tetracycline, , and so on, which makes the infections caused by these bacteria very difficult to treat (50).

6. Conclusion

It could be concluded that CoNS are important minor pathogens in Masske traditional butter. Such species can cause subclinical and nosocomial infections by its consumption. This reflects the environmental hazard such as udder health, raw milk microbial load, sanitary conditions of dairy farms and the people who work there. Further investigations should be carried out on the epidemiology of CoNS-causing disease. More reliable identification methods would be beneficial for the classification of CoNS species isolates based on virulent genes not only 16S rRNA.

Conflict of Interests

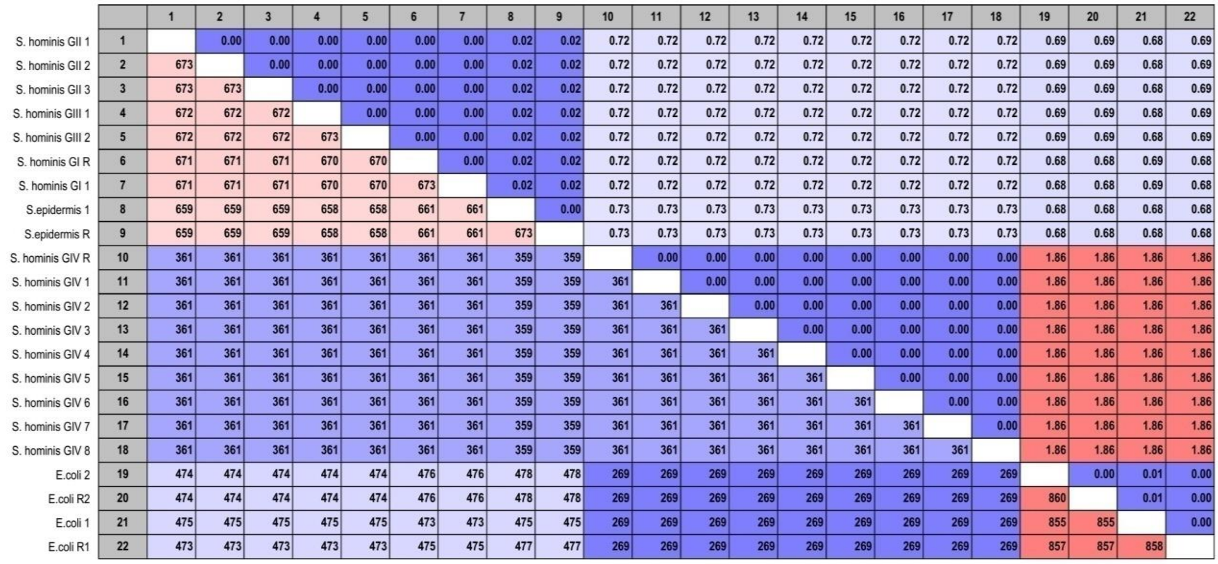
The authors declare they have no conflict of interests.

Acknowledgements

This research was funded by Ferdowsi University of Mashhad (FUM)..

Authors’ Contribution

Conceived and designed the experiments: Masoud Yavarmanesh, Morteza Khomeiri and Fariba Ghiamati Yazdi. Performed the experiments: Fariba Ghiamati Yazdi. Analyzed the data: Fariba Ghiamati Yazdi, Masoud Yavarmanesh, Morteza Khomeiri and Morteza Mahdavi. Contributed reagents/material/ analysis tools: Fariba Ghiamati Yazdi and Morteza Mahdavi. Wrote the paper: Fariba Ghiamati Yazdi and Morteza Mahdavi. Paper revision: Masoud Yavarmanesh, Morteza Khomeiri, Fariba Ghiamati Yazdi and Morteza Mahdavi.



		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
S. hominis GII 1	1		0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.69	0.69	0.68	0.69
S. hominis GII 2	2	673		0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.69	0.69	0.68	0.69
S. hominis GII 3	3	673	673		0.00	0.00	0.00	0.00	0.02	0.02	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.69	0.69	0.68	0.69
S. hominis GIII 1	4	672	672	672		0.00	0.00	0.00	0.02	0.02	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.69	0.69	0.68	0.69
S. hominis GIII 2	5	672	672	672	673		0.00	0.00	0.02	0.02	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.69	0.69	0.68	0.69
S. hominis GI R	6	671	671	671	670	670		0.00	0.02	0.02	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.68	0.68	0.69	0.68
S. hominis GI 1	7	671	671	671	670	670	673		0.02	0.02	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.72	0.68	0.68	0.69	0.68
S.epidermis 1	8	659	659	659	658	658	661	661		0.00	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.68	0.68	0.68	0.68
S.epidermis R	9	659	659	659	658	658	661	661	673		0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.68	0.68	0.68	0.68
S. hominis GIV R	10	361	361	361	361	361	361	361	359	359		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.86	1.86	1.86	1.86
S. hominis GIV 1	11	361	361	361	361	361	361	361	359	359	361		0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.86	1.86	1.86	1.86
S. hominis GIV 2	12	361	361	361	361	361	361	361	359	359	361	361		0.00	0.00	0.00	0.00	0.00	0.00	1.86	1.86	1.86	1.86
S. hominis GIV 3	13	361	361	361	361	361	361	361	359	359	361	361	361		0.00	0.00	0.00	0.00	0.00	1.86	1.86	1.86	1.86
S. hominis GIV 4	14	361	361	361	361	361	361	361	359	359	361	361	361	361		0.00	0.00	0.00	0.00	1.86	1.86	1.86	1.86
S. hominis GIV 5	15	361	361	361	361	361	361	361	359	359	361	361	361	361	361		0.00	0.00	0.00	1.86	1.86	1.86	1.86
S. hominis GIV 6	16	361	361	361	361	361	361	361	359	359	361	361	361	361	361	361		0.00	0.00	1.86	1.86	1.86	1.86
S. hominis GIV 7	17	361	361	361	361	361	361	361	359	359	361	361	361	361	361	361	361		0.00	1.86	1.86	1.86	1.86
S. hominis GIV 8	18	361	361	361	361	361	361	361	359	359	361	361	361	361	361	361	361	361		1.86	1.86	1.86	1.86
E.coli 2	19	474	474	474	474	474	476	476	478	478	269	269	269	269	269	269	269	269	269		0.00	0.01	0.00
E.coli R2	20	474	474	474	474	474	476	476	478	478	269	269	269	269	269	269	269	269	269	860		0.01	0.00
E.coli 1	21	475	475	475	475	475	473	473	475	475	269	269	269	269	269	269	269	269	269	855	855		0.00
E.coli R1	22	473	473	473	473	473	475	475	477	477	269	269	269	269	269	269	269	269	269	857	857	858	

Figure 5. Similarity-distance Matrix of *Staphylococci* and *Escherichia* isolated from Masske based on genetic distances.

Funding/Support

This study was kindly supported by a grant from Gorgan University of Agricultural Sciences and Natural Resources.

References

1. AlegríaA, Álvarez-Martín P, Sacristán N, Fernández E, Delgado S, Mayo B. Diversity and evolution of the microbial populations during manufacture and ripening of Casín, a traditional Spanish, and starter-free cheese made from cow's milk. Int J Food Microbiol.2009; 136(1): 44-51.
2. Palys T, Nakamura LK, Cohan FM. Discovery and classification of ecological diversity in the bacterial world: the role of DNA sequence data. Int J Syst Bacteriol. 1997; 47(4):1145-56.
3. Topisirovic L, Kojic M, Fira D, Golic N, Strahinic I, Lozo J. Potential of lactic acid bacteria isolated from specific natural niches in food production and preservation. Int J Food Microbiol. 2006; 112(3):230-5.
4. VanHylckama VJE, Rademaker JL, Bachmann H, Molenaar D, Kelly WJ, Siezenm RJ. Natural diversity and adaptive responses of *Lactococcuslactis*. Curr Opin Biotechnol. 2006; 17(2):183-90.
5. Wouters JTM, Ayad EHE, Hugenholtz J, Smit G. Microbes from raw milk for fermented dairy products. Int Dairy J. 2002; 12(2):91-109.
6. Stanley G. Cheeses. In: BJB Wood. Microbiology of Fermented Foods. 2nd ed. England: Blackie Academic & Professional, Ltd; 1998.p. 263-307.
7. Harrigan WF. Laboratory Methods in Food Microbiology. 3rd ed. England: Academic Press, Ltd; 1998.
8. Zamfir M, Vancanneyt M, Makras L, Vaningelgem F, Lefebvre K, Pot B, et al. Biodiversity of lactic acid bacteria in Romanian dairy products. Syst Appl Microbiol. 2006; 29(6):487-95.
9. Jablonski LM, Bohach GA. *Staphylococcus aureus*.In: Michael PD, Robert LB. Food microbiology: fundamentals and frontiers.1st ed. USA: ASM Press, Ltd; 1999.p. 353-75.
10. Jorgensen HJ, Mork T, Caugant DA, Kearns A, Rorvik LM. Genetic Variation among *Staphylococcus aureus* strains from Norwegian Bulk Milk. Appl environ Microbiol. 2005; 71(12):8352-61.
11. Le Loir Y, Baron F, Guatier M. *Staphylococcus aureus* and food poisoning. Genetics and Molecular Research.2003; 2(1):63-76.
12. Foschino R, Invernizzi A, Barucco R, StradiottoK. Microbial composition, including the incidence of pathogens, of goat milk from the Bergamo region of Italy during a lactation year. J Dairy Res. 2002; 69(2):213-25.

13. Scallan E, Hoekstra RM, Angulo FJ. Foodborne illness acquired in the United States major pathogens. Emerg Infect Dis. 2011; 17(1):7-15.
14. Lima CG, Loiko MR, Casarin LS, Tondo EC. Assessing the epidemiological data of *Staphylococcus aureus* food poisoning occurred in the State of Rio Grande do Sul, Southern Brazil. Braz J Microbiol. 2013; 44(3):759-63.
15. BalabanN, Rasooly A. Staphylococcal enterotoxins. Int J Food Microbiol.2000; 61(1):1-10.
16. Kadariya J, Smith TC, Thapaliya D. *Staphylococcus aureus* and *Staphylococcal* Food-Borne Disease: An Ongoing Challenge in Public Health. Biomed Res Int. 2014; 2014: 827965.
17. ArgudínMÁ, Mendoza MC, Rodicio MR. Food poisoning and *Staphylococcus aureus* enterotoxins. Toxins. 2010; 2(7):1751-73.
18. Dingwell RT, Leslie KE, Schukken YH, Sargeant JM, Timms LL. Evaluation of the California mastitis test to detect an intramammary infection with a major pathogen in early lactation dairy cows. Can Vet J. 2003; 44(5):413–416.
19. Schlegelova J, Vlkova H, Babak V, Holasova M, Jaglic Z, Stosova T, et al. Resistance to erythromycin of Staphylococcus spp. isolates from the food chain. Vet Med. 2008; 53(6):307-14.
20. Luthje P, Schwarz S. Antimicrobial resistance of coagulase-negative staphylococci from bovine subclinical mastitis with particular reference to macrolide lincosamide resistance phenotypes and genotypes. J Antimicrob Chemother. 2006; 57(5):966-9.
21. Pyorala S, Taponen S. Coagulase-negative staphylococci emerging mastitis pathogens. Vet Microbiol. 2009; 134(1-2):3–8.
22. Otto MR. Veterinary medicine: a textbook of the diseases of cattle, sheep, pigs, goats and horses. 9th ed. W.B. Saunders Company Ltd; 2000.
23. Simojoki H, Orro T, Taponen S, Pyorala S. Host response in bovine mastitis experimentally induced with Staphylococcus chromogenes. Vet Microbiol. 2009; 134(1-2):95–9.
24. Pilipčincová I, Bhide M, Dudriková E, Trávniček M. Genotypic characterization of coagulase-negative staphylococci isolated from sheep milk in Slovakia. Acta Vet Brno. 2010; 79(2): 269–75.
25. El-Jakee JK, Aref NE, Gomaa A, El-Hariri MD, Galal HM, Omar SA, et al. Emerging of coagulase negative staphylococci as a cause of mastitis in dairy animals: An environmental hazard. Int J Vet Sci Med. 2013; 1(2):74-8.
26. Gonfa A, Foster H A, Holzapfel WH. Field survey and literature review on traditional fermented milk products in Ethiopia. Int J Food Microbiol. 2001; 68(3):173-86.
27. Ongol MP, Asano K. Main microorganisms involved in the fermentation of Ugandan ghee. Int J Food Microbiol. 2009; 133(3):286-91.
28. Sserunjogi ML, Abrahamsen RK, Narvhus J. A review paper: current knowledge of ghee and related products. Int Dairy J. 1998; 8(8):677-88.
29. Jokovic N, Nikolic M, Begovic J, Jovcic B, Savic D, Topisirovic L. A survey of the lactic acid bacteria isolated fromSerbian artisanal dairy product kajmak. Int J Food Microbiol. 2008; 127(3):305-11.
30. Karl-Heinz S. Gram-positive cocci. In: Vos P, Garrity G, Jones D, Krieg NR, Ludwig W,Rainey FA, et al. Bergey’s Manual of Systematic Bacteriology. USA: Springer Dordrecht Heidelberg London New York, Ltd; 2009.
31. Van Hoorde K, Verstraete T, Vandamme P, Huys G. Diversity of lactic acid bacteria in two Flemish artisan raw milk Gouda-type cheeses. Food Microbiol. 2008; 25(7):929-35.
32. Schleifer KH, Bell JA. Genus *Staphylococcus.* In: Fred AR, Karl-Heinz S. Bergey's Manual of Systematic Bacteriology.1st ed. USA: Williams and Wilkins,Ltd; 2005. p. 392-433.
33. Walter J, Tannock GW, Tilsala-Timisjarvi A, Rodtong S, Loach DM, Munro K, Alatossava T. Detection and identification of gastrointestinal Lactobacillus species by using denaturing gradient gel electrophoresis and species-specific PCR primers. Appl Environ Microbiol. 2000; 66(1):273-303.
34. Gala E, Landi S, Solieri L, Nocetti M, Pulvirenti A, Giudici P. Diversity of lactic acid bacteria population in ripened ParmigianoReggiano cheese. Int J Food Microbiol. 2008; 125(3):347-51.
35. Stackebrandt E, Goodfellow M. 16S/23S rRNA sequencing. In: Lane DJ. Nucleic Acid Techniques in Bacterial Systematics. 1sted. John Wiley and Sons, Ltd; 1991. p. 115-75.
36. Blast. Basic Local Alignment Search Tool, National Center for Biotechnology Information, National Library of Medicine (NLM), National Institutes of Health (NIH). USA. 2011; http://www.ncbi.nlm.nih.gov/BLAST.
37. Tamura K. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol Biol Evol. 2011; 28(10):2731-9.
38. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987; 4(4):406-25.
39. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci USA. 2004; 101(30):11030-5.
40. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. Evolution. 1985; 39(4):783-91.
41. Sengül M. Microbiological characterization of civil cheese, a traditional Turkish cheese: microbiological quality, isolation and identification of its indigenous *Lactobacilli*. World J Microbiol Biotechnol. 2006; 22(6):613-8.
42. Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. J Mol Biol. 2000; 17(4):540-52.
43. Hebremedhin BG, Layer F, König W, König B. Genetic Classification and Distinguishing of Staphylococcus Species Based on Different Partial gap, 16S rRNA, *hsp*60, *rpo*B, *sod*A, and *tuf* Gene Sequences. J Clin Microbiol. 2008; 46(3):1019-25.
44. Richard KR.The microbiology of raw milk. In: James VC. Dairy Microbiology Handbook. 3rd ed. USA: Wiley and Sons, Ltd; 2002. p. 39-85.
45. Pitkala A, Haveri M, Pyorala S, Myllys V, Honkanen-Buzalski T. Bovine mastitis in Finland 2001 – prevalence, distribution of bacteria and antimicrobial resistance. J Dairy Sci. 2004; 87(8):2433-41.
46. Osteras O, Solverod L, Reksen O. Milk culture results in a large Norwegian survey effect of season, parity, days in milk, resistance and clustering. J Dairy Sci. 2006; 89(3):101-23.
47. Tenhagen BA, Koster G, Wallmann J, Heuwieser W. Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. J Dairy Sci. 2006; 89(7):2542-51.
48. Lim GH, Leslie KE, Kelton DF, Duffield TF, Timms LL, Dingwell RT. Adherence and efficacy of an external teat sealant to prevent new intramammary infections in the dry period J Dairy Sci. 2007; 90(3): 1289-300.
49. Koivula M, Mantysaari EA, Pitkala A, Pyorala S. Distribution of bacteria and seasonal and regional effects in a new database for mastitis pathogens in Finland. Acta Agr Scand A-An. 2007; 57(2):89-96.
50. Rasheed MU. *Staphylococcus epidermidis*: A commensal emerging as a pathogen with increasing clinical significance especially in nosocomial infections. Int J Microbiol. 2007; 3(2):1453-64.

How to cite this article: Ghiamati Yazdi F, Yavarmanesh M, Khomeiri M, Mahdavi M. Microbial Safety of Masske: A Traditional Butter from South of Khorasan, Genetic Similarity of Pathogenic Bacteria Indicators. Infection, Epidemiology and Medicine. 2016; 2(3): 14-19.

[DOR: 20.1001.1.25884107.2016.2.3.7.1]