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The Study of Genetic Affinity of Methicillin Resistance Staphylococcus aureus Strains Isolated from Cream Pastries and Nasal Isolates at Shiraz Confectionaries

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ABSTRACT

Background: *Staphylococcus aureus (S. aureus)* is responsible for most cases of food poisoning all around the world. The carriers and manipulated foodstuffs are the main sources of bacteria transmission to ready-to-eat food. This study aims to determine the genetic affinity of methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from cream pastries and workers' nasal of Shiraz city confectioneries. **Methods:** 30 MRSA strains (7 nasal carriers, 23 food isolates) were selected from the bank of bacteria at Shiraz medical faculty. To determine the genetic affinity of the isolates, RAPD-PCR technique was performed using OLP6, OLP11, and OLP13 primers. RAPD-PCR patterns were analyzed using the software GelJ. **Results:** By using primer OLP6 only 5 RAPD-PCR patterns were produced from DNA ampliqons of creamy pastry isolates and were not enough to compare the genetic affinity of all the isolates. Based on 100% similarity, OLP13 primer produced 20 different patterns with some bands in the range of 1 to 11, and the OLP11 primer produced 22 patterns with some bands from 3 to 11 bands. At closely and possibly genetically related levels, the isolates are categorized into (13-15) and (1-5) clusters. In general, all the isolates are classified into human and food isolates. **Conclusions:** There was no genetic affinity of MRSA isolates regarding human and food samples; but, a high percentage of close genetic relationship between the isolates increases the possibility of bacteria transfer from humans to pastries and food poisoning.

Keywords: *Methicillin-resistant Staphylococcus aureus; Genetic affinity; Nasal carriers; Cream Pastry; RAPD-PCR.*

Introduction

Consuming the food stuff contaminated with pathogens and their toxins leads to hospitalization and economic losses (Marzano and Balzaretti, 2011). Food preparation and distribution might be accompanied by foodborne illnesses contaminated with various pathogens such as *Staphylococcus aureus (S. aureus)* (Lahou *et al.*, 2012, Lipcsei *et al.*, 2019).

S. aureus is non-motile, facultative anaerobic, Gram-positive cocci, mannitol utilization, thermo-

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stable nuclease, DNase, catalase and coagulase positive. The organism is able to grow in a wide range of temperatures, pH levels, and sodium chloride concentrations (up to 15%). *S. aureus* survives in a wide variety of foods especially those that require considerable handling while preparing. Humans and animals are the primary reservoirs of staphylococcus. This organism lives in nasal cavity, throat, hair, and on skin of healthy individuals (probably 20-30%). Moreover, *S. aureus* is one of the most common pathogens which produces staphylococcal enterotoxins (SEs) in food and results in foodborne poisoning (Argaw and Addis, 2015, Jay *et al.*, 2008). Studies showed that the handling of cream-filled pastries in the procedure of production potentially makes food incriminated in staphylococcal intoxication (Argudín *et al.*, 2010).

One of the most important challenges in medicine worldwide is antibiotic resistance, causing a silent global crisis. The prevalence of multi-drug resistance microorganisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) has been reported in developed and undeveloped countries. Since 1990, frequencies of MRSA strain occurrences have been reported and caused critical health problems all around the world. MRSA is not only resistant to beta-lactam antibiotics but also to various classes of antibiotics (Church and McKillip, 2021, Parthasarathy and Chougale, 2021). The *mecA* gene is responsible for resistance to methicillin which encodes the protein of PBP2a. PBP2a is responsible for a cross-link of peptidoglycans in the cell wall of bacteria. This protein does not interact with beta-lactams. Furthermore, MRSA imposes a lot of financial burden on health systems. MRSA carriers have no obvious signs or symptoms associated with this pathogen, and there is growing concern about the emergence of MRSA infection in patients. MRSA infection causes multiple problems such as hospitalization and death because of person-to-person transmission of infection which was reported by some researchers (Boucher and Corey, 2008, Hartman and Tomasz, 1984, Katayama *et al.*, 2000, Turner *et al.*, 2019).

PCR-based typing methods are useful techniques to survey *S. aureus* of various origins and to monitor their spread. RAPD-PCR is a rapid and easy method based on PCR technique, and despite some typing methods (the same AFLP, RFLP, PFGE,..) it does not require the genetic content of the organism. One or more primers (1 to 13 nucleotides) are used to produce randomly amplified ampliqons of DNA templates (Theresa *et al.*, 2021, Williams *et al.*, 1990). RAPD-PCR technique can identify genetic variation and establish strain-specific fingerprinting by short synthetic oligonucleotide primers with a random sequence of about 9-10 bases in length (Olive and Bean, 1999).

Several studies certified RAPD-PCR method as an easy, economically beneficial and reliable technique with high sensitivity to study genetic affinity and find the origin of MRSA strains. Researchers emphasize that an experimental-based program is necessary to perform RAPD-PCR technique correctly and accurately. This technique is applicable to epidemiological studies (Aycicek *et al.*, 2005, Miao *et al.*, 2018, Mobasherizadeh *et al.*, 2016, Reinoso *et al.*, 2004).

It is necessary to identify the source of contamination and pathogen before spreading diseases or the occurrence of an epidemic situation. Molecular typing or fingerprinting methods have been used to perform epidemiological studies and subsequently prevent the spread of the disease. The level of genetic similarity of pathogenic strains between species is a key point to indicate and control the origin of isolates and prevent infection spreading.

This study aims to identify the level of genetic affinity of MRSA strains isolated from cream pastries samples and workers' nasal of Shiraz confectionaries, south of Iran.

Materials and Methods

Identification of MRSA strains

A cross-sectional study was conducted and cream pastries and nasal swabs were collected from Shiraz confectionaries and biochemical tests (Gram staining, Catalase, DNase, Mannitol salt agar, Baired parker agar and Coagulase) were

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performed. Then, MRSA strains were identified by PCR technique using special primers (*femA* and *mecA* gens) and disc diffusion method (Cefoxitin).

DNA extraction

30 MRSA strains isolated from cream pastries and nasal of workers at Shiraz confectionaries were selected (bank of bacteria strains, Shiraz medical faculty). The DNA of MRSA strains was extracted using a gram-positive DNA Kit (MBST, Tehran, Iran) as described by the manufacturer and stored at -20 °C**.**

RAPD-PCR

RAPD-PCR was performed with oligonucleotide primers OLP6, OLP11, and OLP13 (**Table 1**) according to Zare *et al.*'s method (Zare *et al.*, 2019).

Table 1. The Use of RAPD primers and their sequences this study.

Target genes	Oligonucleotide sequence(5'-3')	Tm
OLP6	5'-GAGGGAAGAG -3'	36^{OC}
OLP11	5'- ACGATGAGCC -3'	36^{OC}
OLP13	5'- ACCGCCTGCT -3'	36^{OC}

APD-PCR was carried out in a total volume of 25 µl containing 2.75 µl of oligonucleotides primers (3 µM), 1.75 µl dNTPs (200 µM of each deoxynucleoside triphosphate), 2 µl of MgCl2 (3.5 mM), 0.5 µl of Taq DNA polymerase (2.5 U), 2 µl of 10X PCR buffer, 12.5 µl of sterile distilled water, and 3µl of DNA template (Cinna Gen, Iran).

Amplification of DNA fragments was performed in MJ Mini Thermal Cycler (BIO-RAD, USA) with initial denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 1 minute, annealing at 36 °C for 90 seconds and extension at 72 °C for 2 min, with a final extension of 7 min at 72 °C.

1.5% agarose gel containing 0.5 µg/ml DNA safe stain (Cinna Gen, Iran) was prepared and placed into electrophoresis chamber containing TAE 0.5 x (Iranian novin gen company). RAPD-PCR products loaded in wells of agarose gel then turned electrophoresis system on. Electrical power was adjusted to 80 volts for 60 to 90 minutes in 0.5x TAE buffer. After that, DNA fragments in gels were visualized under UV light by Gel Doc (UVitec, UK). 100 base pairs and 10 kilo base pairs DNA ladders were also used as DNA fragment size markers in all runs.

Analysis of RAPD-PCR patterns

RAPD-PCR patterns obtained by each primer was analyzed using GelJ software. Cluster analyses was performed and dendrograms were created based on unweighted pair-group method with averaging (UPGMA). A dice similarity coefficient was conducted to estimate the percentage of genetic affinity. To classify the results of phylogenic trees, the similarities of isolates were classified into three categories: indistinguishable differences between patterns (100% genetically similar), closely related strains or clones (85-99% similarity and 3 band differences), possibly related (≥65% similarity) according to recommendations of Tenover (Tenover *et al.*, 1995).

Results

Randomly amplified arbitrary DNA of isolates resulted in RAPD-PCR products. These products electrophoresed in agarose gel and presented RAPD-PCR patterns. Patterns obtained by primer OLP6 did not result in producing the considerable number of DNA bands, and application of this primer just produced some patterns for 5 isolates of cream pastries samples. Therefore, the results of this primer were not acceptable to compare the genetic affinity of all isolates. The analysis of RAPD-PCR patterns by GelJ software (gel analyzer) performed to obtain phylogenic trees (**Figures 1** and **2**).

The patterns of PCR products using primer OLP11 revealed that RAPD-PCR patterns of 8 food isolates collected from one or different confectionaries were genetically similar (100%) which was not distinguishable by GelJ software. The patterns produced by primer OLP13 identified that 8 isolates of food samples from different confectionaries were genetically similar (100%) to each other (**Table 2**).

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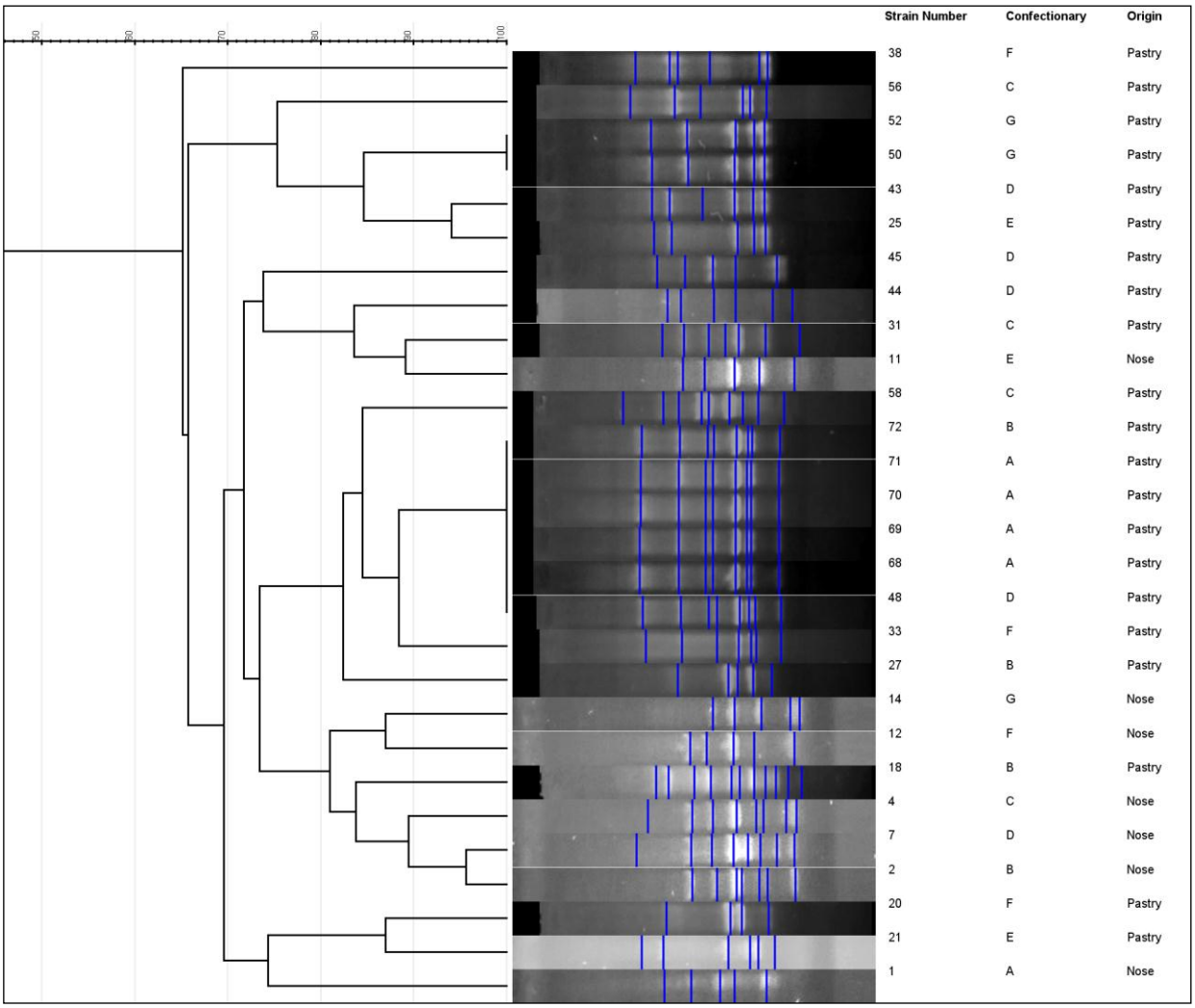

Figure 1. phylogenic tree of *S. aureus* strains isolated from nasal and cream pastries samples of Shiraz confectionaries using RAPD-PCR and primer OLP11.

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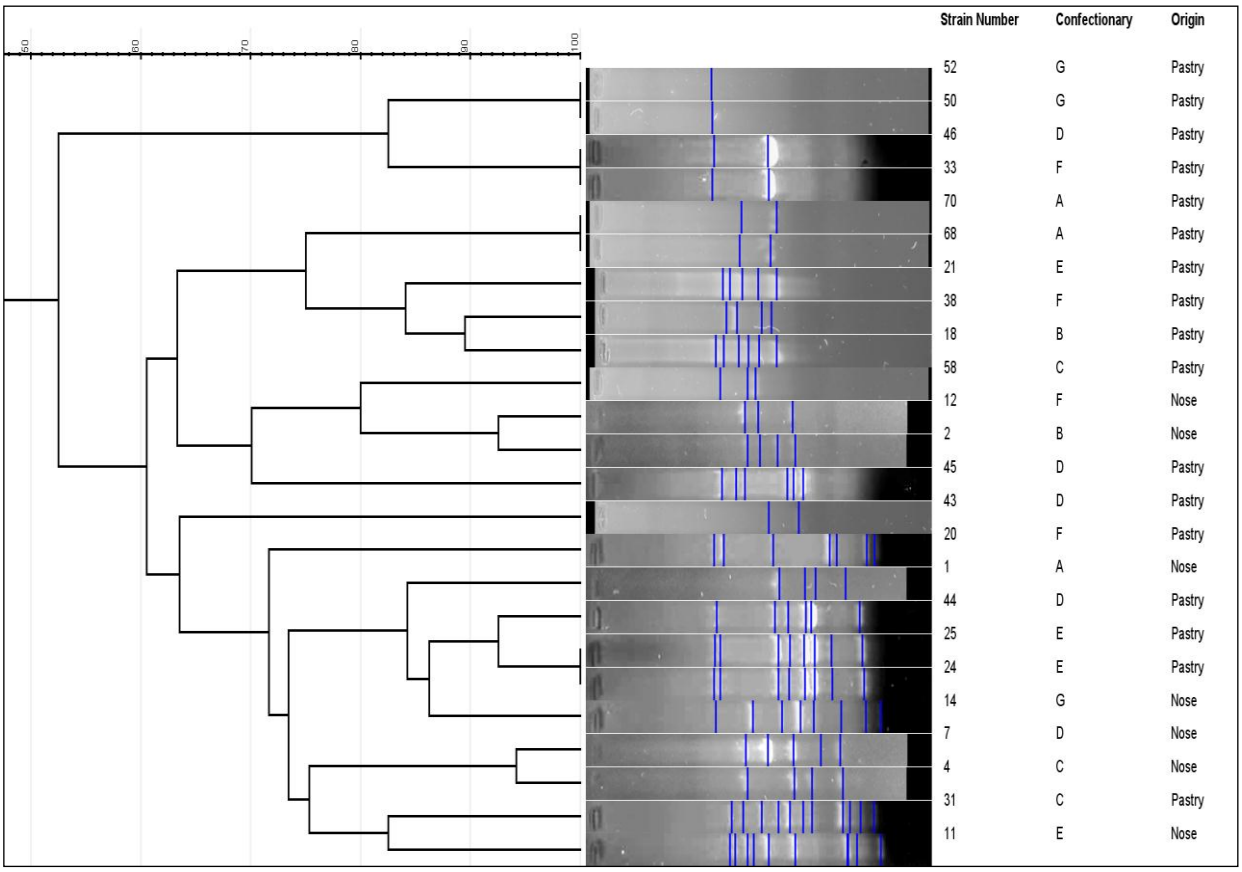

Figure 2. phylogenic tree of *S. aureus* strains isolated from nasal and cream pastries samples of Shiraz confectionaries using RAPD-PCR and primer OLP13.

Table 2. Genetic affinity levels of MRSA strains isolated from workers' nasal and cream pastries samples of Shiraz confectionaries based on analysis of phylogenic trees (RAPD-PCR patterns using primers *OLP11* and *OLP13*).

Indistinguishable (100% similarity)	Closely related (85-99% similarity)	Possibly related (65-84% similarity)
OLP11		
2 food isolates	2 human isolates (96%)	
6 food isolates	2 food isolates (95%)	
	3 food isolates (89%)	
	1 human isolate and 1 food isolate (88.5%)	All isolates
	7 food isolates (88%)	
	4 food isolates (85%)	
OLP13		
2 food isolates	2 human isolates (94%)	4 food isolates (83%)
2 food isolates	2 human isolates (92%)	5 food isolates (75%)
2 food isolates	3 food isolates (92%)	5 human isolates and 5 food isolates (72%)
2 food isolates	2 food isolates (90%)	2 human isolates and 2 food isolates (71%)
	1 human isolate and 3 food isolates (86%)	

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In the present study, the genetic affinity of nasal and cream pastries of MRSA isolates were evaluated utilizing OLP6, OLP11, and OLP13 primers in RAPD-PCR technique.

Using primers *OLP13* and *OLP11* produced RAPD-PCR patterns from 1-11 and 4-11 bands respectively. Analysis of RAPD-PCR patterns produced by *OLP13* and *OLP11* oligonucleotides, based on a100 percent similarity, classified the isolates into 20 and 22 clusters respectively. The analysis based on closely genetically related levels indicated that the phylogenic trees of OLP11 classified isolates into 13 clusters, but phylogenic trees of OLP13 separated the isolates into 15 different clusters. At the level of possibly genetically related, 1 and 5 clusters were performed by primers OLP11 and OLP13 respectively.

Total DNA fragments amplified by RAPD-PCR technique primers were 296 bands that included 113 and 183 bands obtained by primers OLP13 and OLP11 respectively. The results showed that the highest number and variety of DNA fragments were produced by primer OLP11.

Discussion

RAPD-PCR patterns of nasal carriers and cream pastries isolates were not quite similar (100%); but, some *S. aureus* strains isolated from workers' nasal were not only possibly or closely genetically related to the isolates of cream pastries produced by the same workers but also these patterns were possibly or closely genetically related to other isolates of cream pastries samples produced in other confectionaries. One of the most common reasons was that the workers were working at several confectionaries in Shiraz city. The other reason was that some confectionaries were not able to balance the supply and demand process; therefore; they had to find another source for retaining their consumers. These reasons and some factors the same knowledge of workers about food hygiene and their hygiene practices resulted in cross-contamination of ready-to-eat cream pastries with *S. aureus* in confectionaries. One study identified that total scores of workers' hygiene practices in Shiraz confectionaries were not acceptable in preventing food contamination while preparing and packaging the cream pastries as ready-to-eat products, because there was a significant relation between hygiene practices and contamination of products observed (Masihi Nezhad *et al.*, 2022).

RAPD profiles of *S. aureus* strains isolated from workers and mastitis cases in the study by Pereira *et al*. (Pereira *et al.*, 2002) were different, and no similarity was observed; however, 2 human strains and 1 cow strain had a familiar genetic profile. The strains were divided into 5-9 clusters.

Rinoso *et al*. studied genetic affinity of *S. aureus* strains isolated from patient and healthy carriers, cows, and milk samples using three primers OLP6, OLP11, and OLP13 by RAPD-PCR. The isolates were classified into 11 clusters. No considerable genetic relation was found according to the origins of isolates (Reinoso *et al.*, 2004).

Nikbakht *et al.* reported that phylogenic tree revealed RAPD-PCR profiles of *S. aureus* which were isolated from the personnel and patients at 2 hospitals in Iran were not similar and distinguishable. The isolates made 43 profiles and were clustered into 18 categories at 50 percent of similarity (Nikbakht *et al.*, 2008).

Idil, N. and I.S. Bilkay performed RAPD-PCR to determine the locality of MRSA isolated from the different wards of hospitals in Turkey. The size of DNA bands was in the range of 200 to 1500 base pairs and the analysis of the phylogenic trees classified the isolates into 2 groups and 3 clusters. Researchers reported that RAPD profiles of *S. aureus* isolated from the same ward were genetically similar (Idil and Bilkay, 2014).

Hasan Aycicek *et al*. confirmed that RAPD-PCR technique was a successful method for finding the origin of MRSA strains isolated from several wards of Turkish hospitals. RAPD patterns analysis divided the strains into 2 groups and 3 clusters. The results demonstrated that the strains isolated from the same wards produced indistinguishable genetic profiles, and RAPD was a suitable method to find the origin of the organism

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(Aycicek *et al.*, 2005).

Mobasherizadeh *et al* analyzed the patterns while using GE6, OLP6, OLP13, and 1254 primers in RAPD-PCR method to determine variations in the genetics of MRSA isolated from 410 healthy children. The phylogenic trees showed 5 groups with different patterns (Mobasherizadeh *et al.*, 2016).

To find the genetic affinity of *S. aureus* isolated from several sources, Hakimi *et al* implemented AP-7 primer and RAPD-PCR method. 34 patterns and 6 clusters were made, and the analysis of the phylogenic trees resulted in the transmission of strains between several hosts because 5 clusters of isolates originated from the same source, and 4 clusters originated from several resources (Hakimi Alni *et al.*, 2017).

Bannon *et al*. used RAPD-PCRE method to survey the genetic affinity of *S. aureus* strains isolated from patients referred to 2 hospitals in Iraq using special primers. Then, RAPD profiles of 19 isolates were analyzed. The isolates remained in 1 main group and 19 subgroups, which suggested it was related to the compatibility power of S. aureus with several host cells.

According to the overall analysis of phylogenic trees, the authors classified all the isolates into two categories human and food ones (Banoon *et al.*, 2019). A study regarding the genetic relation of *S. aureus* detected in foodstuff, human infections, coin, and mastitis samples collected by Zare *et al*. showed that isolates did not originate from common resources, and there was no relation between patterns and resources. OLP13 produced more DNA bands than other primers, and the size of DNA fragments was between 250 -1500 (b.p). The results revealed an extended genetic variety of *S. aureus* strains (Zare *et al.*, 2019).

The experiments resulted in producing oligonucleotides band size in the range of 250 to 6500 base pairs; this revealed the sensitivity of RAPD-PCR technique and genetic variety among nasal and cream pastries isolates of Shiraz confectionaries.

In RAPD-PCR technique, the primers are randomly attached to DNA strand and the results cannot be repeated. The technique is experiment-based, so the concentration of master mix materials is crucial to obtain better results. On the other hand, some primers do not produce enough RAPD patterns from PCR ampliqons for comparing genetic affinity of all the isolates. Unsuitable primer and unbalanced master mix material resulted in loss or failure of RAPD pattern and to made phylogenic tree and consequently disrupts analysis of genetic affinity.

In general, by using more primers and a proper design of PCR method, the researchers can overcome the main problems of the method because it is a suitable and low-cost method for DNA fingerprinting of organisms.

Conclusion

The combination of phylogenic trees did not confirm the genetic affinity of nasal carriers and cream pastries isolate because MRSA strains are classified into two categories of human and food isolates. Analysis showed that some human and food isolates were possibly or closely related to each other genetically. Analysis of phylogenic trees supports this phenomenon that human and food isolates may probably originate from a common source; the transmission of the *S. aureus* strains from workers' nasal to cram pastry products in the confectionaries of Shiraz city is possible.

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Conflict of interests

Authors declared no conflict of interest.

Authors' contributions

Motamedi Far M, Boniadian M, and Masihi Nezhad AA designed the research; Masihi Nezhad AA conducted the research; Naziri Z and Masihi Nezhad AA analyzed data; and Masihi Nezhad AA wrote the paper. Boniadian M had primary responsibility for final content. All the authors read and approved the final manuscript.

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