Iranian Journal of Microbiology
*Volume 15 Number 3 (June 2023) 408-413*

ORIGINAL ARTICLE

# The high cross-transmission in methicillin resistant *Staphylococcus aureus* between healthy and patient communities

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**Received:** May 2022**, Accepted:** January 2023

## ABSTRACT

**Background and Objectives:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the main causes of high mortality and morbidity in hospitals. This study was aimed to examine virulence factors, molecular typing, and the antibiotic resistance pattern of MRSA isolates in hemodialysis patients and healthy communities.
**Materials and Methods:** Total of 231 and 400 nasal samples were obtained from hemodialysis patients and healthy communities, respectively. Virulence factors profile was examined in two groups by PCR reaction. Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR) was used as a molecular typing approach.
**Results:** Overall, 35.49% (82/231) of hemodialysis patients were positive for *S. aureus*, and 47.56% (39/82) of isolates were positive for *mecA*. In a healthy community, 15% (60/400) of samples were positive for *S. aureus*, and 36.66% (22/60) were positive for *mecA*. The frequency of MDR was significantly higher in patients group (p-value < 0.00001). The frequency of *pvl* (p.value = 0.003932, P<0.05) and *tsst-1* (p.value = 0.003173, p < .05) were significantly higher in patients group. The highest frequency virulence factors in healthy individuals were related to *hla* (68.33%, 41/60), *hlb* (53.33%, 32/60), and *Acme/arcA* (46.66%) genes. Two groups were clustered by the ERIC-PCR method into 7 clusters and 2 single isolate with a 0.74 similarity index. Based on the results, each cluster was combination with healthy and patient isolates.
**Conclusion:** Our findings indicate a notable variation in the frequency of virulence factors between *S. aureus* isolates obtained from dialysis patients and the healthy community.

**Keywords:** Methicillin-resistant *Staphylococcus aureus*; Molecular typing; Virulence factors

## INTRODUCTION

*Staphylococcus. aureus*, as an opportunistic pathogen, is the cause of a wide range of infections such as suppurative disease, arthritis, osteomyelitis, infective endocarditis, necrotizing pneumonia, and toxic shock syndrome (TSS) (1) (2, 3). Some virulence factors produced by *S. aurus* play a key role in the escape of infection from the host immune system (3-6). The nasal carriage of *S. aureus* can be a reservoir for subsequent infections in individuals colonized with this pathogen (4, 5). The appearance of antibiotic-resistant *S. aureus* strains (methicillin-resistant *S. aureus* (MRSA) is now a serious threat in commu-

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nity and hospital settings. MRSA infections are one of the causes of the length of hospital stay and high morbidity and mortality, especially in people with HIV, cancer, diabetes, rheumatoid arthritis, and patients undergoing dialysis (6). *S. aureus* colonization is one of the main causes of endogenous infection in hemodialysis patients both time-to-first-bacteremia and the relapse of infection (7).

The increasing prevalence of antibiotic resistance is now a serious threat to public health (8).

Although MRSA infections were originally acquired only from hospital settings (HA-MRSA), Community-associated methicillin-resistant *S. aureus* (CA-MRSA) strains are found in the healthy community, but CA-MRSA infections can be the cause of infection in the community and hospital settings (9, 10).

A CA-MRSA strain is detected when the patient has no history of hospitalization, surgery, or the year before infection based on the US Centers for Disease Control and Prevention (CDC) guideline (11). The virulence factors variety is different between patients and healthy groups. The presence of virulence factors, such as the virulence-associated Panton-Valentine Leukocidin (PVL) gene in CA-MRSA, is responsible for leukocytosis and tissue necrosis (12). In this respect, Enterobacterial repetitive intergenic consensus sequence targeted PCR (ERIC-PCR) is a quick method for epidemiological typing for Gram-positive and -negative bacteria (13). ERIC sequences with 126 bp long are highly conserved central inverted repeats located in extragenic regions of the bacterial genome. These repeats are found in multiple copies in bacteria genomes (14, 15) which can be used for typing *S. aureus* isolates (16).

The present study aimed to better understand the prevalence, molecular features, and antibiotic resistance pattern of CA-MRSA in healthy communities and HA-MRSA in hemodialysis patients in Tabriz, Iran.

## MATERIALS AND METHODS

This cross-sectional study was performed on the student community and hemodialysis patients in Tabriz, Iran. Ethical approval (Reference number: IR.TBZMED.REC.1398.989) for the study was obtained from the Tabriz University of Medical Sciences, Tabriz Iran ethics committee. The study was carried out from July 2017 to August 2018. In this study, two population including hemodialysis patients referred to the Imam Reza Hospital of Tabriz and healthy students (200 girls and 200 boys) from six different high schools in Tabriz city, Iran were studied. The sample size was determined based on Cochran formula (17). Individuals with previous antibiotic consumption and treated with decolonization drug were excluded from two populations.

**Bacteria identification.** The nasal, catheter and throat samples were obtained from 231 hemodialysis patients, and nasal samples were obtained from 400 students aged 16 to 17 years from high schools (mean age: 16.5) in Tabriz city from January 1, 2018, to March 1, 2018. The individuals were monitored for antibiotic consumption during the past three months, and individuals without previous antibiotic consumption were included in this study.

**Antimicrobial susceptibility testing.** The susceptibility pattern of isolates was determined to various antibiotics by Kirby Bauer disk diffusion testing based on the Clinical Laboratory Standards Institute (CLSI) guidelines as previously described (7, 15). Antibiotic disks used were as follows: oxacillin (1 μg), rifampicin (5 μg), clindamycin (2 μg), erythromycin (15 μg), linezolid (30 μg), cefazolin (30 μg), penicillin (6 μg), amoxicillin/clavulanic acid (20/10 μg), ampicillin (5 μg), cefoxitin (30 μg) (MAST, UK). *S. aureus* ATCC 33591 (methicillin-resistant) and *S. aureus* ATCC 25923 (methicillin-susceptible) were used as a positive control, and ultrapure water was as a negative control.

**The detection of virulence factors by PCR reaction.** The boiling approach was performed through TE buffer (10mM Tris, 1mM EDTA) to extract DNA from *S. aureus* isolates. Based on spectroscopy values at 260 nm and 260/280, the quantity and quality of DNA were evaluated. PCR reactions for *nucA* and *mecA* genes were performed to confirm *S. aureus* and MRSA isolates, respectively (18). Five virulence factors including *Arginine catabolic mobile element (ACME-arcA), Toxic shock syndrome toxin-1 (tsst-1), Alpha-hemolysin (hla), Beta-hemolysin (hlb),* and *Panton-Valentine leukocidin (pvl)* genes were selected due to key role of them in the increase of pathogenicity of *S. aureus.* ACME is an increasing factor of *S. aureus* colonization in the skin and mu-

cous membranes, *tsst-1* is mostly lead to toxic shock syndrome. *hla* is the cause of cell death and lysis, *Hlb* is mostly the main reason of lung and eye infections. *pvl* is known as the leading cause of leukocyte lysis and tissue necrosis (3, 19). The virulence factors frequency were examined by PCR reaction using specific primers (Table 1) in a 25 µL reaction for 30 cycles as follow: 94°C for 1 min, 49°C/53°C for 1 min, and 72°C for 1 min after an initial denaturation at 94°C for 4 min. The final extension was done at 72°C for 5 min. PCR products were detected by 1% agarose gel electrophoresis. For further validation, PCR products were sequenced.

**ERIC-PCR typing.** The genetic diversity of MRSA isolates was analyzed by ERIC PCR based on the number of copies of the ERIC sequence. ERIC – PCR reaction was performed based on specific primers (Table 1) after the initial denaturation at 95°C for 5 minutes and followed for 40 cycles (94°C for 1 minute, 50°C for 1 minute, and 72°C for 2 minutes). The final extension was done at 72°C for 10 minutes. A binary data matrix in NTSYS-pc 2.02 software package (20) was used for the analysis of ERIC patterns of the isolates. The dendrogram was made based on the Unweighted Pair Group Method using an arithmetic mean (UPGMA) algorithm in the NTSYS program.

**Statistical analysis.** Statistical analysis was carried out by the SPSS software. The variations in the prevalence of virulence factors and antibiotic resistance were compared by Chi-square test ($p<0.05$). Each test was performed at least three times. Data were presented as mean ± standard deviation (SD) or 95% confidence interval (CI).

## RESULTS

**Antimicrobial susceptibility.** Overall, 35.49% (82/231) of hemodialysis patients and 15% (60/400) healthy individuals were positive for *S. aureus*. Antibiotic resistance profile to 11 antibiotics was determined on 82 *S. aureus* isolates from hemodialysis patients and 60 *S. aureus* isolates from the healthy community by the disc diffusion agar. In both groups, the highest antibiotic resistance was observed against ampicillin, and the highest sensitivity was to linezolid antibiotic. The frequency of MRSA isolates in patients, and healthy individuals based on resistance to cefoxitin was equal to 68.04% ± 1.0% and 18.34% ± 1.6%, respectively. Details of the results of the disc diffusion test are shown in Fig. 1. Based on results, 31.66% ± 2.5% (19/60) of *S. aureus* isolates in the healthy group were multi-drug resistant (MDR) based on resistance to 3 or more classes (7) while 70.73% ± 2.0% (58/82) of *S. aureus* isolates were MDR in patients group. The frequency rate of MDR was significantly higher in patients group (p-value < 0.00001).

**Detection of MRSA isolates and virulence factors by PCR reaction.** Identification of all *S. aureus* isolates were confirmed based on the presence *nucA* gene. In the healthy group, 36.66% ± 1.7% (22/60) of

**Table 1.** The sequence of primers used for PCR

| Primer | Primer sequence (5´→3´) | Size (bp) | Ref |
|---|---|---|---|
| *mecA* | F: 5 ′ - AGAAATGACTGAACGTCC -3 ′ | 305 | (7) |
| | R: 5 ′ - ATTCCACATTGTTTCGGTC - ′ 3 | | |
| *hla* | F: 5 ′ - GTACAGTTGCAACTACCT -3 ′ | 253 | (19) |
| | R: 5′- CTTTCCAGCCTACTTTTTTATCAGT -3 ′ | | |
| *hlb* | F: 5 ′ - GTGCACTTACTGACAATAGTGC -3 ′ | 313 | (19) |
| | R: 5′- GTTGATGAGTAGCTACCTTCAGT -3 ′ | | |
| *Acme-arcA* | F: 5′- CTAGGTGCATAAATGTACGTG -3′ | 577 | (26) |
| | R: 5′- CCAGAAGTACGCGAGAAC -3′ | | |
| *tsst-1* | F: 5 ′ -ACAAGCGCTATTTTTATTTCAG-3 ′ | 271 | (3) |
| | R: 5′-CCCATCCCCAACCACTTTT-3 ′ | | |
| *pvl* | F: 5′- AGGTAAAATGTCTGGACATG-3′ | 427 | (27) |
| | R: 5′-GCATCAACTGTATTGGATAGC-3′ | | |
| ERIC | F: 5′- ATGTAAGCTCCTGGGGATTC-3′ | Variables | (15) |
| | R: 5′- AAGTAAGTGACTGGGGTGAG-3′ | | |

the isolates were positive for *mecA*, while 47.56% ± 1.3% (39/82) of the isolates in the patient's group were positive for *mecA*. The most frequency virulence factors in healthy individuals were related to *hla* (68.33% ± 1.2%, 41/60), *hlb* (53.33% ± 1.3%, 32/60), and *Acme/arcA* (46.66% ± 1.6%) while in patients group, highest frequency of virulence factors were related to *hla* (47.56% ± 1.0%, 39/82), *Acme/arcA* (45.12% ± 1.4%, 37/82) and *pvl* (39.02% ± 1.8%, 32/82) (Fig. 2). The frequency of *pvl* (p.value = 0.003932, P < 0.05) and *tsst-1* (p.value = 0.003173, p < .05) were significantly higher in patients group.

**ERIC PCR genotyping.** Based on the ERIC-PCR gel analysis exhibited 1 to 12 bands ranging from 100 to 900 bp.

Dendrogram data generated from the computer-designed analysis indicated a high genetic dissimilarity among healthy and patient's groups (Fig. 3).

The genetic dissimilarity MRSA isolates based on PCR results for *mecA* positive isolates (22 isolates from healthy community and 39 isolates from patients) were clustered into 7 clusters and 2 single isolates with similarity index:0.74. Based on results, in each cluster a combination with the healthy community (H) and patient's isolates were observed. ERIC-type A was identified as the predominant type comprised of 39 isolates (63.93% ± 1.0%). The other clusters were including 2 to 4 isolates.

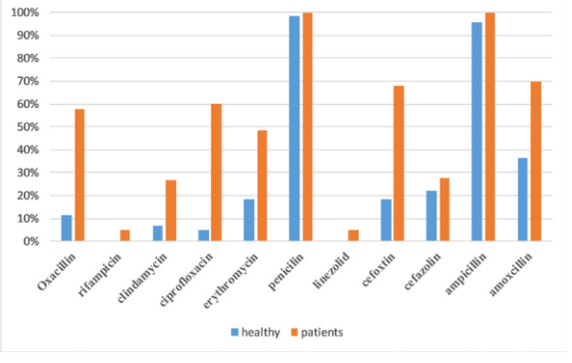


**Fig. 1.** Results of antibiotic susceptibility test of *S. aureus* isolates from hemodialysis patients and healthy community.

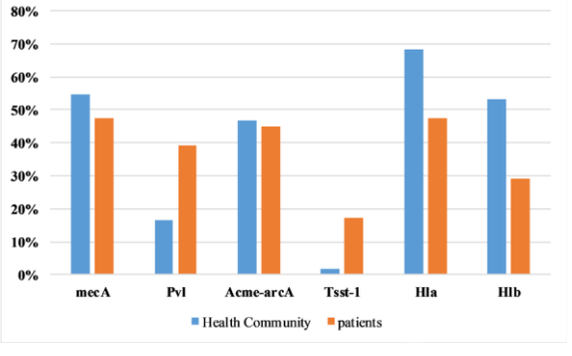


**Fig. 2.** Virulence factors profile of *S. aureus* isolates obtained from hemodialysis patients and healthy community.

## DISCUSSION

The frequency of virulence factors is the main cause of *S. aureus* pathogenicity in both HA-MRSA and CA-MRSA. In this study, 68.04% of clinical isolates obtained from hemodialysis patients were MRSA based on resistance to cefoxitin. The frequency of MRSA was more than the recent study in Tabriz, in which 40.8% of clinical isolates were identified as MRSA (21). Based on a systematic review and meta-analysis, MRSA prevalence was almost 43% in Iran from 2000 to 2016 (22). In a study conducted in the north of Iran, 36.9% of hemodialysis patients were *S. aureus* carriage, which 74.2% of them were MRSA (23).

In this study, 18.34% of isolates obtained from a healthy community were MRSA which was less than the MRSA rate in students (4.5%) from central IRAN (24).

The highest frequency virulence factors in healthy individuals were related to *hla* (68.33%, 41/60), *hlb* (53.33%, 32/60), and *Acme/arcA* (46.66%) genes while *hla* (47.56%, 39/82), *Acme/arcA* (45.12%,37/82) and *pvl* (39.02%,32/82) were most frequency in patients.

In the healthy group, 16.66% of the isolates were positive for the *pvl* gene; 11.66% (7/60) of the isolates were positive for both *pvl* and *ACME-arcA* genes. A significant correlation was observed between the presence of the *ACME-arcA* gene and resistance to methicillin ($p<0.05$) in the two groups. At the same time, 90% (9/10) of the *pvl*-positive isolates were sensitive to methicillin ($p<0.05$). In the patients' group, 12.37% were positive for both *hla* and *hlb* genes, while 53.33% of the isolates were positive for both genes in the healthy group.

Also, 11.66% of the *pvl*-positive isolates in the healthy group were positive for the *ACME-arcA* gene, consistent with previous research (25). In contrast, in the patients' group, 13.40% of the isolates were positive for both virulence factors, and 4.12%

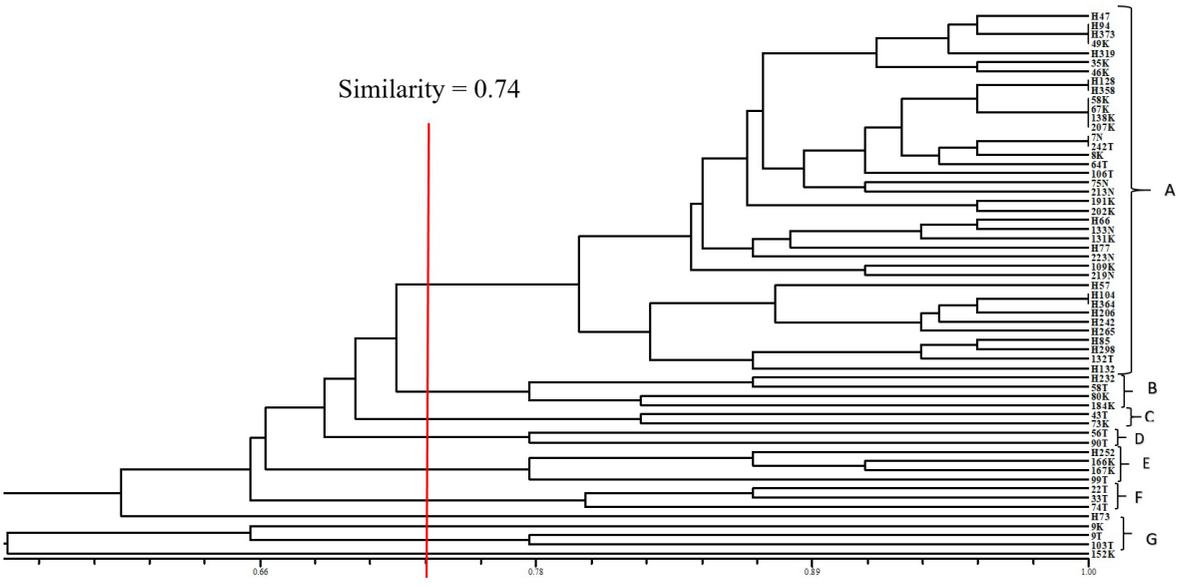


**Fig. 3.** Dendrogram of ERIC-PCR analysis for *S. aureus* isolates obtained from hemodialysis patients and healthy community. H: isolates obtained from nasal of healthy community, K: isolates obtained from catheter, T: isolates obtained from throat of dialysis patients, and N: isolates obtained from nasal of dialysis patients

carried *pvl* and *tsst-1.* Only 1.03% of the isolates in this group were positive for four genes, including *pvl*, *tsst-1*, *ACME-arcA*, and *mecA.* Based on results, there is a different profile of virulence factors in patient and healthy groups.

This study shows a significant relationship between the presence of the *ACME-arcA* gene and the frequency of *mecA*-positive strains in a healthy population. There is no relationship between the presence of *pvl* and *mecA* so that 14.29% *pvl* positive isolates were MRSA in the healthy group. In the patient group, 15.67% of the isolates were positive for *ACME-arcA* and *mecA.* Two groups were clustered by the ERIC-PCR method into 7 clusters and 2 single isolates with a 0.74 similarity index. Based on the results, each of the clusters were including both healthy and patient isolates.

## CONCLUSION

Our findings indicate a notable variation in the frequency of virulence factors between *S. aureus* isolates obtained from dialysis patients and the healthy community. The genetic relatedness of the isolates from dialysis patients and healthy group was high, indicating cross-transmission within patient and healthy communities.

## ACKNOWLEDGEMENTS

This study was supported by the Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

## REFERENCES

1. Budzyńska A, Skowron K, Kaczmarek A, Wietlicka-Piszcz M, Gospodarek-Komkowska E. Virulence factor Genes and antimicrobial susceptibility of *Staphylococcus aureus* strains isolated from blood and chronic wounds. *Toxins (Basel)* 2021; 13: 491.
2. Lindsay JA, Holden MT. *Staphylococcus aureus*: superbug, super genome? *Trends Microbiol* 2004; 12: 378-385.
3. Zeinalpour Ahrabi S, Rahbarnia L, Dehnad A, Naghili B, Ghaffari Agdam MH, Nazari A. Incidence of Oxacillin-susceptible *mecA*-positive *Staphylococcus aureus* (OS-MRSA) isolates and TSST-1 virulence factor among high school students in Tabriz, Northwest of Iran. *Arch Clin Infect Dis* 2019;14(4): e85341.
4. Magro G, Biffani S, Minozzi G, Ehricht R, Monecke S, Luini M, et al. Virulence genes of *S. aureus* from dairy cow mastitis and contagiousness risk. *Toxins (Basel)* 2017; 9: 195.
5. Rahbarnia L, Farajnia S, Naghili B, Ahmadzadeh V, Veisi K, Baghban R, et al. Current trends in targeted

therapy for drug-resistant infections. *Appl Microbiol Biotechnol* 2019; 103: 8301-8314.

6. Goudarzi M, Navidinia M, Beiranvand E, Goudarzi H. Phenotypic and molecular characterization of methicillin-resistant *Staphylococcus aureus* clones carrying the Panton-Valentine leukocidin genes disseminating in Iranian hospitals. *Microb Drug Resist* 2018; 24: 1543-1551.
7. Ghavghani FR, Rahbarnia L, Naghili B, Dehnad A, Bazmani A, Varshochi M, et al. Nasal and extra nasal MRSA colonization in hemodialysis patients of north-west of Iran. *BMC Res Notes* 2019; 12: 260.
8. Rahbarnia L, Farajnia S, Khaneshi H, Farajnia H, Naghili B, Tanomand A. Detection of blaOXA-23 and $bla_{NDM-1}$ carbapenemase among clinical isolates of *A. baumannii* in Tabriz, north-west of Iran. *Gene Rep* 2020; 18: 100555.
9. Nakaminami H, Takadama S, Ito A, Hasegawa M, Jono C, Noguchi M, et al. Characterization of *SCCmec* type IV methicillin-resistant *Staphylococcus aureus* clones increased in Japanese hospitals. *J Med Microbiol* 2018; 67: 769-774.
10. David MZ, Cadilla A, Boyle-Vavra S, Daum RS. Replacement of HA-MRSA by CA-MRSA infections at an academic medical center in the midwestern United States, 2004-5 to 2008. *PLoS One* 2014; 9(4): e92760.
11. Buck JM, Como-Sabetti K, Harriman KH, Danila RN, Boxrud DJ, Glennen A, et al. Community-associated methicillin-resistant *Staphylococcus aureus*, Minnesota, 2000–2003. *Emerg Infect Dis* 2005; 11: 1532-1538.
12. Ahmad NI, Yean Yean C, Foo PC, Mohamad Safiee AW, Hassan SA. Prevalence and association of Panton-Valentine Leukocidin gene with the risk of sepsis in patients infected with methicillin resistant *Staphylococcus aureus. J Infect Public Health* 2020; 13: 1508-1512.
13. Sivakumar M, Dubal ZB, Kumar A, Bhilegaonkar K, Vinodh Kumar OR, Kumar S, et al. Virulent methicillin resistant *Staphylococcus aureus* (MRSA) in street vended foods. *J Food Sci Technol* 2019; 56: 1116-1126.
14. Wilson LA, Sharp PM. Enterobacterial repetitive intergenic consensus (ERIC) sequences in *Escherichia coli*: Evolution and implications for ERIC-PCR. *Mol Biol Evol* 2006; 23: 1156-1168.
15. Lotfi F, Shojaie M, Rahbarnia L, Dehnad A, Naghili B, Lotfi H. Molecular characterization and genetic diversity of multidrug-and extensively drug-resistant *A. baumannii* clinical isolates. *Gene Rep* 2022; 26: 101455.
16. Hsu B-M, Chen J-S, Lin I-C, Hsu G-J, Koner S, Hussain B, et al. Molecular and anti-microbial resistance (AMR) profiling of methicillin-resistant *Staphylococcus aureus* (MRSA) from hospital and long-term care facilities (LTCF) environment. *Antibiotics (Basel)* 2021; 10: 748.
17. Uakarn C, Chaokromthong K, Sintao N. Sample size estimation using Yamane and Cochran and Krejcie and Morgan and green formulas and Cohen statistical power analysis by G* Power and comparisions. *Apheit Int J* 2021; 10: 76-86.
18. Appiah VA, Pesewu GA, Kotey FCN, Boakye AN, Duodu S, Tette EMA, et al. *Staphylococcus aureus* nasal colonization among children with sickle cell disease at the Children's Hospital, Accra: prevalence, risk factors, and antibiotic resistance. *Pathogens* 2020; 9: 329.
19. Dehnad A, Agdam MHG, Rahbarnia L, Naghili B, Saffarian P. Detection of hemolysine genes in methicillin-resistant *S. aureus* isolates obtained from a healthy population in north-west of Iran. *Gene Rep* 2020; 21: 100874.
20. Rohlf F. NTSYS-PC: Numerical taxonomy and multivariate analysis system. Version 2.1. Exeter Software, Appl Biostat, New York. 1998.
21. Ahmadishoar S, Pour NK, Sadeghi J, Nahaei MR, Kheirkhah B. Molecular epidemiology of clinical isolates of methicillin-resistant *Staphylococcus aureus* by multilocus sequence typing in northwestern (Tabriz) and southern (Kerman) of Iran: The emergence of MRSA ST4848-*SCCmec* III. *Gene Rep* 2021; 24: 101212.
22. Dadashi M, Nasiri MJ, Fallah F, Owlia P, Hajikhani B, Emaneini M, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) in Iran: a systematic review and meta-analysis. *J Glob Antimicrob Resist* 2018; 12: 96-103.
23. Ghasemian R, Najafi N, Makhlough A, Khademloo M. Frequency of nasal carriage of *Staphylococcus aureus* and its antimicrobial resistance pattern in patients on hemodialysis. *Iran J Kidney Dis* 2010; 4: 218-222.
24. den Heijer CD, van Bijnen EM, Paget WJ, Pringle M, Goossens H, Bruggeman CA, et al. Prevalence and resistance of commensal *Staphylococcus aureus*, including meticillin-resistant *S. aureus*, in nine European countries: a cross-sectional study. *Lancet Infect Dis* 2013; 13: 409-415.
25. Motamedi H, Rahmat Abadi SS, Moosavian SM, Torabi M. The Association of Panton-valentine leukocidin and *mecA* genes in methicillin-resistant *Staphylococcus aureus* isolates from patients referred to educational hospitals in Ahvaz, Iran. *Jundishapur J Microbiol* 2015; 8(8): e22021.
26. Sabat AJ, Ilczyszyn WM, van Rijen M, Akkerboom V, Sinha B, Kluytmans J, et al. Genome-wide analysis reveals two novel mosaic regions containing an ACME with an identical DNA sequence in the MRSA ST398-t011 and MSSA ST8-t008 isolates. *J Antimicrob Chemother* 2015; 70: 1298-1302.
27. Hoppe P-A, Hanitsch LG, Leistner R, Niebank M, Bührer C, von Bernuth H, et al. Periorbital infections and conjunctivitis due to Panton-Valentine Leukocidin (PVL) positive *Staphylococcus aureus* in children. *BMC Infect Dis* 2018; 18: 371.