



Molecular Epidemiology of Panton-Valentine Leukocidin Harboring Hospital-Associated Methicillin-Resistant *Staphylococcus aureus* in Septicemic Children, Northeastern Iran, Bojnurd

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Received 2018 March 02; Revised 2019 January 12; Accepted 2019 January 16.

Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for an increasing number of serious hospital- and community-acquired infections in adults and children. Sepsis caused by *S. aureus* is one of the major health problems associated with treatment failure in adults; however, its clinical outcomes, the rate of treatment failure, and its molecular epidemiology are poorly understood.

Objectives: The objective of this study was to evaluate the molecular epidemiology of Panton-Valentine Leukocidin (PVL) harboring MRSA strains isolated from children's blood culture in Bojnurd.

Methods: Totally, 58 *S. aureus* strains were isolated from blood cultures in the major teaching hospital in Bojnurd. After the primary verification of Methicillin resistance by agar screening method, the isolated MRSA strains were confirmed with the detection of the *mecA* gene. *MecA*-positive strains evaluated for SCCmec, *agr*, and toxin profiles. Panton-valentine leukocidin- positive isolates were subjected to be evaluated for *spa* and sequence type (ST).

Results: Our data indicated 53.4% (31) of isolates were MRSA. Twelve (38.7%) of these isolates had PVL gene that 25% (3) of them had *tsst-1* gene and 58.3% (7) had *etb* gene. One (3.2%), 64.5% (20), and 32.2% (10) of these isolates belonged to SCCmec I, III, and IV, respectively. Predominant ST and *spa* types among PVL positive isolates were ST6 and t304, respectively.

Conclusions: We had an uncommon finding because PVL was routinely found in community-acquired MRSA, but in this study we found PVL harboring hospital-associated MRSA. A notable point about these isolates is that most of them belonged to Asian endemic clones.

Keywords: *Staphylococcus aureus*, Methicillin, Panton-Valentine Leukocidin, Sepsis, Child

1. Background

Bacterial bloodstream infections, called bacteremia, are life-threatening infections with a high mortality rate. These infections commonly appear with other serious infections such as urinary tract infections, endocarditis, and respiratory tract infections (1). Untreated bacteremia can progress to systemic inflammatory responses, sepsis, septic shock, and multiple organ dysfunction syndromes. A large number of Gram-negative and Gram-positive bacteria can lead to bacteremia. One of the

most important Gram-positive bacteria is *Staphylococcus aureus* (2). This bacterium is one of the major human pathogens that cause hospital- and community-acquired infections ranging from skin and soft-tissue mild infections to life-threatening diseases such as toxin-mediated diseases, pneumonia, and septicemia (3, 4).

Antimicrobial resistance has appeared soon after the first use of antibiotics to treat staphylococcal infections (5). Methicillin-resistant *Staphylococcus aureus* (MRSA) has been identified as a virulent bacterium in healthcare set-

tings since 1960, named hospital-associated MRSA (HA-MRSA) (6). Since 1990 the arrival of community-acquired MRSA (CA-MRSA) outside the hospitals has been increasingly reported and led to change in the epidemiology of MRSA (7). Community-acquired MRSA and HA-MRSA differentiated with genetic characteristics. Small *SCCmec* elements such as *SCCmec* IV and V presented in CA-MRSA instead of large elements such as *SCCmec* I, II, and III that are more common in HA-MRSA (8). Moreover, they express different patterns of specific virulence factors, including toxins and enzymes.

Phage harbored Panton-Valentine Leukocidin (PVL) is one of the most important toxins (8). This is a two-component toxin encoded by phage that can be inserted into the *S. aureus* genome (9). This toxin was introduced as one of the CA-MRSA markers, especially in skin and soft tissue infections (10). In Asia, the most prevalent MRSA clone is ST239 that is different from the USA and European clones (11-13). In previous works performed separately on *SCCmec* and sequence types in Iran, researchers have shown that the common types in HA-MRSA strains are *SCCmec* III and ST239 and in CA-MRSA are *SCCmec* IV and ST22, respectively (14-17).

2. Objectives

In this study we evaluated the molecular epidemiology of PVL-harboring MRSA strains isolated from children's blood culture in Bojnurd with multi-locus sequence typing (MLST), *spa* typing, *agr* typing, toxin profiling, and *SCCmec* typing methods.

3. Methods

3.1. Strains and Identification

We totally evaluated 58 strains of *S. aureus* isolated from blood cultures of children admitted to a university reference hospital in Bojnurd between September 2015 and April 2016. The ages of the patients were between 6 months to 12 years old. Thirty-five blood culture-positive patients (60.3%) were male and the 23 patients (39.7%) were female. All of these strains isolated from the patients (Table 1). Primary isolation of bacteria was performed using biphasic blood culture media (Pasteur Institute of Iran). Suspected positive blood cultures, sub-cultured to blood agar (Merck, Germany) and also MacConkey agar media (Merck, Germany). *Staphylococcus aureus* strains were characterized by laboratory microbiologic tests, including microscopic evaluation, DNase (Merck, Germany), coagulase (Razi Institute, Iran), mannitol fermentation (Merck, Germany), and catalase (Bahar Afshan, Iran). For classification of isolates

to community-acquired and hospital-acquired MRSA, we used criteria set by Clinical Laboratory Standards Institute (CLSI) and also *SCCmec* typing and toxin profiling methods (18).

3.2. Examination for Resistance to Methicillin

All isolates were screened for resistance to methicillin using cefoxitin disc (30 µg) (MAST DISKS™, UK) on mueller hinton agar media (Merck, Germany) completed with 4% NaCl. Minimum inhibitory concentration (MIC) was determined by E-test method (MAST DISKS™, UK).

3.3. Antimicrobial Susceptibility Test

Antibiotic susceptibility test was performed using minocycline, levofloxacin, ciprofloxacin, tetracycline, cotrimoxazole, gentamycin, clindamycin, and rifampicin antimicrobial disks using Kirby Bauer method (MAST DISKS™, UK) based on CLSI guidelines (18). All antibiotic disks used in this work were purchased from Mast, UK. *Staphylococcus aureus* ATCC 25923 used as the control.

3.4. DNA Extraction

The whole genome of *S. aureus* isolates was extracted using QIAamp® DNA blood mini kit (QIAGEN, Germany). As kit recommendations, lysostaphin (Sigma, Germany) was added at final concentration of 30 µg/mL to the lysis buffer.

3.5. PCR

PCR reaction was performed using TAKARA gradient PCR TP600 thermal cycler (TAKARA, Japan). We used Hot Star® 2X Master Mix (QIAGEN, Germany) for PCR reactions.

(I) Detection of the *mecA* gene. The presence of the *mecA* gene was evaluated by PCR as previously described (3).

(II) Multiplex PCR for the detection of toxin genes. The presence of the PVL, enterotoxin B and toxic shock syndrome toxin genes were evaluated by PCR as previously described (19).

(III) Typing of *SCCmec* and *agr*. *Agr* and *SCCmec* typing were performed as previously described (20).

3.6. Multi-Locus Sequence Typing

Multi-locus sequence typing was carried out by PCR and sequencing of the internal fragments of *yqi*, *tpi*, *glp*, *gmk*, *aro*, *pta*, and *arc* genes of *S. aureus* isolates on PVL-positive isolates as previously described (21).

3.7. Spa Typing

Spa typing was performed by PCR amplification of X region and sequence analysis of *spa* gene of the PVL-positive isolates as previously described (22).

Table 1. Phenotypic and Genotypic Characteristics of PVL-Positive MRSA Isolates

Strain	Gender/Age	Ward	Agr Type	SCCmec Type	Spa Type	Sequence Type	Virulence Genes	Antimicrobial Resistance
MRSA 1	M/4	Internal	I	III	t7688	ST97	<i>etb, tstI</i>	tet, gen
MRSA 2	M/1	Internal	I	IV	t304	ST6	<i>etb</i>	cip, gen
MRSA 3	M/3	Surgery	I	IV	t304	ST6	<i>tstI</i>	lev, cli, rif
MRSA 4	F/4	Internal	I	III	t037	ST239	-	lev, cli, rif
MRSA 5	F/3	Internal	I	IV	t304	ST6	<i>etb</i>	tet, cli, rif
MRSA 6	M/7	Internal	I	IV	t267	ST97	<i>etb</i>	cli, rif
MRSA 7	F/1	Surgery	I	IV	t304	ST6	<i>etb, tstI</i>	cot, cli
MRSA 8	F/6	Surgery	I	IV	t304	ST6	<i>etb</i>	gen, cli, rif
MRSA 9	M/3	Infectious	I	III	t037	ST239	<i>etb</i>	lev, cli
MRSA 10	M/1	Infectious	III	IV	t304	ST6	-	lev, cip, gen, cli, rif
MRSA 11	M/5	Internal	I	IV	t304	ST6	-	lev, cip, gen, cli, rif
MRSA 12	M/3	Surgery	I	IV	t189	ST239	-	-

Abbreviations: cip, ciprofloxacin; cli, clindamycin; cot, co-trimoxazole; *etb*, enterotoxin B; gen, gentamycin; lev, levofloxacin; rif, rifampicin; tet, tetracycline; *tstI*, toxic shock syndrome toxin 1.

3.8. Nucleotide Sequencing

QIAquick® Gel Extraction Kit (QIAGEN- Germany) was used for PCR product purification. The PCR products were sequenced in both directions with an ABI 3730XL DNA analyzer.

4. Results

Thirty-one of 58 analyzed *S. aureus* isolates (53%) were MRSA. Of these 12 were PVL-positive and various antimicrobial resistance patterns were found to rifampicin, clindamycin, gentamycin, co-trimoxazole, tetracycline, ciprofloxacin, and levofloxacin in these isolates. All of these isolates were susceptible to vancomycin and minocycline. The patients' data and complete antibiogram results of these isolates are listed in Table 1. Twenty-five percent and 75% of MRSA isolates belonged to SCCmec III and IV, respectively. Also, 91.7% and 8.3% belonged to *agr* I and III, respectively. Evaluation of *tsst-1* and *etb* genes led to the finding of 3/12 (25%) *tsst-1* and 7/12 (58.3%) *etb* gene harboring isolates. We had three PVL-positive SCCmec III isolates. Most of our PVL-positive isolates belonged to ST6 (Table 2). *Spa* typing revealed that the most prevalent *spa* type among PVL-harboring isolates was 304 (Table 2). Regarding MLST and *spa* typing results, we can infer that the most prevalent types in our isolates are Asian endemic types.

5. Discussion

The present study describes genetic characteristics of PVL harboring HA-MRSA strains isolated from blood cul-

tures of children admitted to teaching hospital in Bojnourd. We found 25% (3/12) of PVL gene harboring HA-MRSA in children are less than five years old. Khosravi et al. reported that the prevalence of PVL gene harboring MRSA in burn patients in Ahvaz-Iran was 7.23% and PVL gene harboring MSSA was 33.3% (23). In another study in Shiraz-Iran, Hoseini Alfatemi et al. reported that the prevalence of PVL gene between MRSA isolates was 5.47% (24). Panton-Valentine Leukocidin is commonly found in SCCmec IV MRSA strains that are often classified as community-acquired. Momtaz and Hafezi reported 40.9% PVL gene harboring isolates in clinical samples that most of them belonged to SCCmec type V (25). A similar result was reported by Dormanesh et al. They reported 63.5% PVL-positive MRSA that most of them belonged to SCCmec type V (26).

Surprisingly in this study, we found the PVL gene in SCCmec type III MRSA often classified as HA-MRSA. This is in concordance with some previous findings that proved the sole existence of PVL is not a decisive clue for characterization of an MRSA as CA-MRSA (27-31). Havaei et al. reported that the prevalence of PVL harboring isolates in five Hospitals of Tehran was 24.2%. Of these, 61.8% was HA-MRSA (32). Rasigade et al. expressed three hypotheses about the relationship between SCCmec elements and PVL gene acquisition. First, the subset of *S. aureus* lineages that are prone to the stable integration of SCCmec IV (for example ST8/USA300 or ST80) is also prone to the integration of the PVL gene (33). Second, there is no direct interaction between the PVL gene and SCCmec integration. Third, the PVL-harboring MSSA lineages pre-exist

Table 2. The Prevalence of Various *Spa* and Sequence Types Among PVL-Positive MRSA Isolates

	<i>Spa</i> Types					Sequence Types		
	t037	t7688	t304	t267	t189	ST239	ST6	ST97
Percent	16.7	8.3	58.3	8.3	8.3	25	58.3	16.7

to PVL positive MRSA (33). Based on the last hypothesis, if PVL-harboring MSSA were prone to accept any *SCCmec* element, they would likely integrate the most prevalent *SCCmec* element in the environment. In our isolates similar to other Asian countries, the most prevalent HA-MRSA clone is ST239-*SCCmec* III (11, 12, 34). Prevalence of PVL-harboring *SCCmec* III MRSA is uncommon but interestingly we found PVL-positive *SCCmec* III MRSA in our region.

Song et al. reported the variable presence of the PVL gene in various *SCCmec* types. About 10% of their *SCCmec* I, 7.7% of *SCCmec* III, and 16.3% of *SCCmec* IV isolates were PVL-positive (13). All of *SCCmec* II isolates were negative for PVL; however, in our study the presence of PVL in various *SCCmec* types was different and we just found PVL gene among *SCCmec* III and IV isolates (Tables 1 and 2). Most of our PVL-positive *SCCmec* III isolates belonged to major Asian endemic clone (CC8/ST239). Perhaps the reason for this is that PVL encoding phages have been recently integrated into dominant Asian MRSA clones. This would suggest the previously mentioned hypothesis, PVL phage insertion into pre-existing MRSA lineages, rather than the accepted model in which *SCCmec* inserted itself into PVL-positive MSSA strains (33).

In the present study, the PVL-positive isolates were evaluated for the presence of toxin genes, including *tst 1* and *etb*. We observed *tst 1* and *etb* genes in PVL-harboring strains and it can be an indicator of high pathogenicity of these isolates. The emergence of ST239/*SCCmec* III MRSA strains carrying *tst 1*, in addition to PVL could be an alarming sign because these strains belong to major hospital-acquired clones. Actually, toxins encoded by these genes are associated with pathogenicity of *S. aureus*. The genetic evaluation showed that a mainland genetic base of CA-MRSA strain does not relate to HA-MRSA, indicating that CA-MRSA does not appear from residential HA-MRSA (35). One of the most prevalent MRSA genetic backgrounds in Asia is ST239 and *spa* type t037 (36). The majority of our PVL-positive HA-MRSA isolates belong to ST239 and *spa* type t037. Only one ST239 isolate had *SCCmec* IV (CA-MRSA). Moreover, we didn't have CA-MRSA strain with *spa* type t037. Regarding these facts and also the high-prevalence rate of *spa* type t037 in HA-MRSA, it is possible that there is a different genetic background of HA- and CA-MRSA in our isolates.

5.1. Conclusions

In conclusion, for the first, time we have shown PVL is not restricted to *SCCmec* IV CA-MRSA isolates in Bojnurd, Iran contrary to the previous hypothesis. It seems that PVL carrying phages were integrated into pre-existing endemic MRSA strains; therefore, a previously accepted hypothesis about markers of CA-MRSA strains could be speculated. Our findings and some previous studies suggest that the PVL gene could not be an appropriate marker for CA-MRSA (9, 10, 27, 37-39). Altogether, these findings are alarming with respect to the genetic background of these HA-MRSA strains (ST239/*SCCmec* III), since it is possible that these highly virulent strains spread to other hospitals in various geographical regions.

Footnotes

Authors' Contribution: Reza Besharat: laboratory analysis; Majid Ghafouri: study design; Saghar Safamanesh: laboratory analysis and English editing; Mahsa Khosrojerdi: sample collection and scientific advice; Kiarash Ghazvini: scientific advice; Sara Nojumi: sample collection; Toktam Memariani: laboratory analysis; Hosein Lashkardoost: statistical analysis; Amir Azimian: study design, laboratory analysis and write manuscript.

Conflict of Interests: All authors declare no conflicts regarding this manuscript.

Ethical Considerations: This study was approved by Ethics Committee of North Khorasan University of Medical Sciences for using patient samples with number 92P713.

Financial Disclosure: All authors of this manuscript have not any relevant financial interests or financial conflicts.

Funding/Support: This work was supported by North Khorasan University of Medical Sciences (grant number: 92P713).

References

- Fazzeli H, Arabestani MR, Esfahani BN, Khorvash F, Pourshafie MR, Moghim S, et al. A new multiplex polymerase chain reaction assay for the identification a panel of bacteria involved in bacteremia. *Adv Biomed Res.* 2013;2:7. doi: 10.4103/2277-9175.107972. [PubMed: 23930252]. [PubMed Central: PMC3732891].

2. Holubar M, Meng L, Deresinski S. Bacteremia due to methicillin-resistant *Staphylococcus aureus*: New therapeutic approaches. *Infect Dis Clin North Am*. 2016;**30**(2):491-507. doi: [10.1016/j.idc.2016.02.009](https://doi.org/10.1016/j.idc.2016.02.009). [PubMed: [27208769](https://pubmed.ncbi.nlm.nih.gov/27208769/)].
3. Pishva E, Havaei SA, Arsalani F, Narimani T, Azimian A, Akbari M. Detection of methicillin-resistance gene in *Staphylococcus epidermidis* strains isolated from patients in Al-Zahra Hospital using polymerase chain reaction and minimum inhibitory concentration methods. *Adv Biomed Res*. 2013;**2**:23. doi: [10.4103/2277-9175.108008](https://doi.org/10.4103/2277-9175.108008). [PubMed: [23977651](https://pubmed.ncbi.nlm.nih.gov/23977651/)]. [PubMed Central: [PMC3748670](https://pubmed.ncbi.nlm.nih.gov/PMC3748670/)].
4. Peerayeh Najar S, Azimian A, Behzadian Nejad Q, Kashi M. Prevalence of agr specificity groups among *Staphylococcus aureus* isolates from university hospitals in Tehran. *Lab Med*. 2009;**40**(1):27-9. doi: [10.1309/lmgb9gb82wkdanwf](https://doi.org/10.1309/lmgb9gb82wkdanwf).
5. Havaei SA, Azimian A, Fazeli H, Naderi M, Ghazvini K, Samiee SM, et al. Isolation of Asian endemic and livestock associated clones of methicillin resistant *Staphylococcus aureus* from ocular samples in North-eastern Iran. *Iran J Microbiol*. 2013;**5**(3):227-32. [PubMed: [24475328](https://pubmed.ncbi.nlm.nih.gov/24475328/)]. [PubMed Central: [PMC3895559](https://pubmed.ncbi.nlm.nih.gov/PMC3895559/)].
6. Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J, et al. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. *JAMA*. 2003;**290**(22):2976-84. doi: [10.1001/jama.290.22.2976](https://doi.org/10.1001/jama.290.22.2976). [PubMed: [14665659](https://pubmed.ncbi.nlm.nih.gov/14665659/)].
7. Brennan GI, Shore AC, Corcoran S, Tecklenborg S, Coleman DC, O'Connell B. Emergence of hospital- and community-associated panton-valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* genotype ST772-MRSA-V in Ireland and detailed investigation of an ST772-MRSA-V cluster in a neonatal intensive care unit. *J Clin Microbiol*. 2012;**50**(3):841-7. doi: [10.1128/JCM.06354-11](https://doi.org/10.1128/JCM.06354-11). [PubMed: [22189119](https://pubmed.ncbi.nlm.nih.gov/22189119/)]. [PubMed Central: [PMC3295151](https://pubmed.ncbi.nlm.nih.gov/PMC3295151/)].
8. Skov RL, Jensen KS. Community-associated methicillin-resistant *Staphylococcus aureus* as a cause of hospital-acquired infections. *J Hosp Infect*. 2009;**73**(4):364-70. doi: [10.1016/j.jhin.2009.07.004](https://doi.org/10.1016/j.jhin.2009.07.004). [PubMed: [19786313](https://pubmed.ncbi.nlm.nih.gov/19786313/)].
9. Shrestha B, Singh W, Raj VS, Pokhrel BM, Mohapatra TM. High prevalence of panton-valentine leukocidin (PVL) genes in nosocomial-acquired *Staphylococcus aureus* isolated from tertiary care hospitals in Nepal. *Biomed Res Int*. 2014;**2014**:790350. doi: [10.1155/2014/790350](https://doi.org/10.1155/2014/790350). [PubMed: [25045702](https://pubmed.ncbi.nlm.nih.gov/25045702/)]. [PubMed Central: [PMC4087282](https://pubmed.ncbi.nlm.nih.gov/PMC4087282/)].
10. Shashindran N, Nagasundaram N, Thappa DM, Sistla S. Can panton valentine leukocidin gene and clindamycin susceptibility serve as predictors of community origin of MRSA from skin and soft tissue infections? *J Clin Diagn Res*. 2016;**10**(1):DC01-4. doi: [10.7860/JCDR/2016/14531.7036](https://doi.org/10.7860/JCDR/2016/14531.7036). [PubMed: [26894063](https://pubmed.ncbi.nlm.nih.gov/26894063/)]. [PubMed Central: [PMC4740590](https://pubmed.ncbi.nlm.nih.gov/PMC4740590/)].
11. Havaei SA, Vidovic S, Tahmineh N, Mohammad K, Mohsen K, Starnino S, et al. Epidemic methicillin-susceptible *Staphylococcus aureus* lineages are the main cause of infections at an Iranian university hospital. *J Clin Microbiol*. 2011;**49**(11):3990-3. doi: [10.1128/JCM.05445-11](https://doi.org/10.1128/JCM.05445-11). [PubMed: [21940478](https://pubmed.ncbi.nlm.nih.gov/21940478/)]. [PubMed Central: [PMC3209102](https://pubmed.ncbi.nlm.nih.gov/PMC3209102/)].
12. Ghaznavi-Rad E, Nor Shamsudin M, Sekawi Z, Khoon LY, Aziz MN, Hamat RA, et al. Predominance and emergence of clones of hospital-acquired methicillin-resistant *Staphylococcus aureus* in Malaysia. *J Clin Microbiol*. 2010;**48**(3):867-72. doi: [10.1128/JCM.01112-09](https://doi.org/10.1128/JCM.01112-09). [PubMed: [20089756](https://pubmed.ncbi.nlm.nih.gov/20089756/)]. [PubMed Central: [PMC2832418](https://pubmed.ncbi.nlm.nih.gov/PMC2832418/)].
13. Song JH, Hsueh PR, Chung DR, Ko KS, Kang CI, Peck KR, et al. Spread of methicillin-resistant *Staphylococcus aureus* between the community and the hospitals in Asian countries: An ANSORP study. *J Antimicrob Chemother*. 2011;**66**(5):1061-9. doi: [10.1093/jac/dkr024](https://doi.org/10.1093/jac/dkr024). [PubMed: [21393157](https://pubmed.ncbi.nlm.nih.gov/21393157/)].
14. Japoni A, Jamalidoust M, Farshad S, Ziyaeyan M, Alborzi A, Japoni S, et al. Characterization of SCCmec types and antibacterial susceptibility patterns of methicillin-resistant *Staphylococcus aureus* in Southern Iran. *Jpn J Infect Dis*. 2011;**64**(1):28-33. [PubMed: [21266752](https://pubmed.ncbi.nlm.nih.gov/21266752/)].
15. Fatholahzadeh B, Emameini M, Gilbert G, Udo E, Aligholi M, Modarressi MH, et al. Staphylococcal cassette chromosome mec (SCCmec) analysis and antimicrobial susceptibility patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in Tehran, Iran. *Microb Drug Resist*. 2008;**14**(3):217-20. doi: [10.1089/mdr.2008.0822](https://doi.org/10.1089/mdr.2008.0822). [PubMed: [18694326](https://pubmed.ncbi.nlm.nih.gov/18694326/)].
16. Fatholahzadeh B, Emameini M, Aligholi M, Gilbert G, Taherikalani M, Jonaidi N, et al. Molecular characterization of methicillin-resistant *Staphylococcus aureus* clones from a teaching hospital in Tehran. *Jpn J Infect Dis*. 2009;**62**(4):309-11. [PubMed: [19628913](https://pubmed.ncbi.nlm.nih.gov/19628913/)].
17. Japoni-Nejad A, Rezazadeh M, Kazemian H, Fardmousavi N, van Belkum A, Ghaznavi-Rad E. Molecular characterization of the first community-acquired methicillin-resistant *Staphylococcus aureus* strains from Central Iran. *Int J Infect Dis*. 2013;**17**(11):e949-54. doi: [10.1016/j.ijid.2013.03.023](https://doi.org/10.1016/j.ijid.2013.03.023). [PubMed: [23706379](https://pubmed.ncbi.nlm.nih.gov/23706379/)].
18. Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial disk susceptibility tests*. 11th ed. Approved Standard; 2010.
19. Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, et al. Involvement of panton-valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis*. 1999;**29**(5):1128-32. doi: [10.1086/313461](https://doi.org/10.1086/313461). [PubMed: [10524952](https://pubmed.ncbi.nlm.nih.gov/10524952/)].
20. Boye K, Bartels MD, Andersen IS, Moller JA, Westh H. A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCCmec types I-V. *Clin Microbiol Infect*. 2007;**13**(7):725-7. doi: [10.1111/j.1469-0691.2007.01720.x](https://doi.org/10.1111/j.1469-0691.2007.01720.x). [PubMed: [17403127](https://pubmed.ncbi.nlm.nih.gov/17403127/)].
21. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol*. 2000;**38**(3):1008-15. [PubMed: [10698988](https://pubmed.ncbi.nlm.nih.gov/10698988/)]. [PubMed Central: [PMC86325](https://pubmed.ncbi.nlm.nih.gov/PMC86325/)].
22. Harmsen D, Claus H, Witte W, Rothganger J, Claus H, Turnwald D, et al. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. *J Clin Microbiol*. 2003;**41**(12):5442-8. [PubMed: [14662923](https://pubmed.ncbi.nlm.nih.gov/14662923/)]. [PubMed Central: [PMC309029](https://pubmed.ncbi.nlm.nih.gov/PMC309029/)].
23. Khosravi AD, Hoveizavi H, Farshadzadeh Z. The prevalence of genes encoding leukocidins in *Staphylococcus aureus* strains resistant and sensitive to methicillin isolated from burn patients in Taleghani Hospital, Ahvaz, Iran. *Burns*. 2012;**38**(2):247-51. doi: [10.1016/j.burns.2011.08.002](https://doi.org/10.1016/j.burns.2011.08.002). [PubMed: [21924558](https://pubmed.ncbi.nlm.nih.gov/21924558/)].
24. Hoseini Alfatemi SM, Motamedifar M, Hadi N, Sedigh Ebrahim Saraie H. Analysis of virulence genes among methicillin resistant *Staphylococcus aureus* (MRSA) strains. *Jundishapur J Microbiol*. 2014;**7**(6):e10741. doi: [10.5812/jjm.10741](https://doi.org/10.5812/jjm.10741). [PubMed: [25371805](https://pubmed.ncbi.nlm.nih.gov/25371805/)]. [PubMed Central: [PMC4217665](https://pubmed.ncbi.nlm.nih.gov/PMC4217665/)].
25. Momtaz H, Hafezi L. Methicillin-resistant *Staphylococcus aureus* isolated from Iranian hospitals: Virulence factors and antibiotic resistance properties. *Bosn J Basic Med Sci*. 2014;**14**(4):219-26. doi: [10.17305/bjbm.2014.4.34](https://doi.org/10.17305/bjbm.2014.4.34). [PubMed: [25428674](https://pubmed.ncbi.nlm.nih.gov/25428674/)]. [PubMed Central: [PMC4333963](https://pubmed.ncbi.nlm.nih.gov/PMC4333963/)].
26. Dormanesh B, Siroosbakhsh S, Khodaverdi Darian E, Afsharkhas L. Methicillin-resistant *Staphylococcus aureus* Isolated from various types of hospital infections in pediatrics: Pantone-valentine leukocidin, Staphylococcal chromosomal cassette mec SCCmec phenotypes and antibiotic resistance properties. *Jundishapur J Microbiol*. 2015;**8**(11):e11341. doi: [10.5812/jjm.11341](https://doi.org/10.5812/jjm.11341). [PubMed: [26862375](https://pubmed.ncbi.nlm.nih.gov/26862375/)]. [PubMed Central: [PMC4741056](https://pubmed.ncbi.nlm.nih.gov/PMC4741056/)].
27. Brust T, da Costa TM, Amorim JC, Asensi MD, Fernandes O, Aguiar-Alves F. Hospital-associated methicillin-resistant *Staphylococcus aureus* carrying the PVL gene outbreak in a public hospital in Rio de Janeiro, Brazil. *Braz J Microbiol*. 2013;**44**(3):865-8. [PubMed: [24516453](https://pubmed.ncbi.nlm.nih.gov/24516453/)]. [PubMed Central: [PMC3910202](https://pubmed.ncbi.nlm.nih.gov/PMC3910202/)].
28. Rossney AS, Shore AC, Morgan PM, Fitzgibbon MM, O'Connell B, Coleman DC. The emergence and importation of diverse genotypes of methicillin-resistant *Staphylococcus aureus* (MRSA) harboring the

- panton-valentine leukocidin gene (pvl) reveal that pvl is a poor marker for community-acquired MRSA strains in Ireland. *J Clin Microbiol.* 2007;**45**(8):2554–63. doi: [10.1128/JCM.00245-07](https://doi.org/10.1128/JCM.00245-07). [PubMed: [17581935](https://pubmed.ncbi.nlm.nih.gov/17581935/)]. [PubMed Central: [PMC1951240](https://pubmed.ncbi.nlm.nih.gov/PMC1951240/)].
29. Said-Salim B, Mathema B, Braughton K, Davis S, Sinsimer D, Eisner W, et al. Differential distribution and expression of panton-valentine leucocidin among community-acquired methicillin-resistant *Staphylococcus aureus* strains. *J Clin Microbiol.* 2005;**43**(7):3373–9. doi: [10.1128/JCM.43.7.3373-3379.2005](https://doi.org/10.1128/JCM.43.7.3373-3379.2005). [PubMed: [16000462](https://pubmed.ncbi.nlm.nih.gov/16000462/)]. [PubMed Central: [PMC169154](https://pubmed.ncbi.nlm.nih.gov/PMC169154/)].
 30. Diep BA, Carleton HA, Chang RF, Sensabaugh GF, Perdreaux-Remington F. Roles of 34 virulence genes in the evolution of hospital- and community-associated strains of methicillin-resistant *Staphylococcus aureus*. *J Infect Dis.* 2006;**193**(11):1495–503. doi: [10.1086/503777](https://doi.org/10.1086/503777). [PubMed: [16652276](https://pubmed.ncbi.nlm.nih.gov/16652276/)].
 31. Hu Q, Cheng H, Yuan W, Zeng F, Shang W, Tang D, et al. Panton-valentine leukocidin (PVL)-positive health care-associated methicillin-resistant *Staphylococcus aureus* isolates are associated with skin and soft tissue infections and colonized mainly by infective PVL-encoding bacteriophages. *J Clin Microbiol.* 2015;**53**(1):67–72. doi: [10.1128/JCM.01722-14](https://doi.org/10.1128/JCM.01722-14). [PubMed: [25339405](https://pubmed.ncbi.nlm.nih.gov/25339405/)]. [PubMed Central: [PMC4290966](https://pubmed.ncbi.nlm.nih.gov/PMC4290966/)].
 32. Havaei S, Moghadam SO, Pourmand M, Faghri J. Prevalence of genes encoding bi-component leukocidins among clinical isolates of methicillin resistant *Staphylococcus aureus*. *Iran J Public Health.* 2010;**39**(1):8–14. [PubMed: [23112984](https://pubmed.ncbi.nlm.nih.gov/23112984/)]. [PubMed Central: [PMC3468970](https://pubmed.ncbi.nlm.nih.gov/PMC3468970/)].
 33. Rasigade JP, Laurent F, Lina G, Meugnier H, Bes M, Vandenesch F, et al. Global distribution and evolution of panton-valentine leukocidin-positive methicillin-susceptible *Staphylococcus aureus*, 1981-2007. *J Infect Dis.* 2010;**201**(10):1589–97. doi: [10.1086/652008](https://doi.org/10.1086/652008). [PubMed: [20367458](https://pubmed.ncbi.nlm.nih.gov/20367458/)].
 34. Havaei SA, Azimian A, Fazeli H, Naderi M, Ghazvini K, Samiee SM, et al. Genetic characterization of methicillin resistant and sensitive, vancomycin intermediate *Staphylococcus aureus* strains isolated from different Iranian hospitals. *ISRN Microbiol.* 2012;**2012**:215275. doi: [10.5402/2012/215275](https://doi.org/10.5402/2012/215275). [PubMed: [23762750](https://pubmed.ncbi.nlm.nih.gov/23762750/)]. [PubMed Central: [PMC3664199](https://pubmed.ncbi.nlm.nih.gov/PMC3664199/)].
 35. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying panton-valentine leukocidin genes: Worldwide emergence. *Emerg Infect Dis.* 2003;**9**(8):978–84. doi: [10.3201/eid0908.030089](https://doi.org/10.3201/eid0908.030089). [PubMed: [12967497](https://pubmed.ncbi.nlm.nih.gov/12967497/)]. [PubMed Central: [PMC3020611](https://pubmed.ncbi.nlm.nih.gov/PMC3020611/)].
 36. Goudarzi M, Bahramian M, Satarzadeh Tabrizi M, Udo EE, Figueiredo AM, Fazeli M, et al. Genetic diversity of methicillin resistant *Staphylococcus aureus* strains isolated from burn patients in Iran: ST239-SCCmec III/t037 emerges as the major clone. *Microb Pathog.* 2017;**105**:1–7. doi: [10.1016/j.micpath.2017.02.004](https://doi.org/10.1016/j.micpath.2017.02.004). [PubMed: [28179118](https://pubmed.ncbi.nlm.nih.gov/28179118/)].
 37. Jung J, Song EH, Park SY, Lee SR, Park SJ, Sung H, et al. Emergence of panton-valentine leukocidin-positive ST8-methicillin-resistant *Staphylococcus aureus* (USA300 clone) in Korea causing healthcare-associated and hospital-acquired bacteraemia. *Eur J Clin Microbiol Infect Dis.* 2016;**35**(8):1323–9. doi: [10.1007/s10096-016-2668-y](https://doi.org/10.1007/s10096-016-2668-y). [PubMed: [27209287](https://pubmed.ncbi.nlm.nih.gov/27209287/)].
 38. Sun DD, Ma XX, Hu J, Tian Y, Pang L, Shang H, et al. Epidemiological and molecular characterization of community and hospital acquired *Staphylococcus aureus* strains prevailing in Shenyang, Northeastern China. *Braz J Infect Dis.* 2013;**17**(6):682–90. doi: [10.1016/j.bjid.2013.02.007](https://doi.org/10.1016/j.bjid.2013.02.007). [PubMed: [23916451](https://pubmed.ncbi.nlm.nih.gov/23916451/)].
 39. Lima DF, Brazao NB, Folescu TW, Neves FP, Ferreira AG, Santos EA, et al. Panton-valentine leukocidin (PVL) gene carriage among *Staphylococcus aureus* strains with SCCmec types I, III, IV, and V recovered from cystic fibrosis pediatric patients in Brazil. *Diagn Microbiol Infect Dis.* 2014;**78**(1):59–62. doi: [10.1016/j.diagmicrobio.2013.10.004](https://doi.org/10.1016/j.diagmicrobio.2013.10.004). [PubMed: [24211217](https://pubmed.ncbi.nlm.nih.gov/24211217/)].