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Original Article

Molecular Epidemiology Survey *of Staphylococcus aureus* Panton–Valentine Leukocidin-positive Isolated from Sanandaj, Iran

Abstract

Background: *Staphylococcus aureus* strains that are Panton–Valentine leukocidin (PVL) positive cause severe skin and soft tissue infections as well as necrotizing pneumonia. The presence of PVL gene is a marker for methicillin-resistant *S. aureus*; therefore, survey on prevalence and phylogenetic distribution of PVL is of great importance for public health. The aim of this research was molecular epidemiology survey of *S. aureus* PVL positive, isolated from two tertiary hospitals of Sanandaj. **Materials and Methods:** A total of 264 staphylococci isolates were collected from clinical specimens, hospital personnel and hospital environment of two tertiary hospitals of Sanandaj, in 2012 (Toohid and Besat). Bacterial cultures and biochemical tests were performed for *S. aureus* detection. Then, polymerase chain reaction (PCR) and repetitive sequence-based PCR (rep-PCR) were used for the determination of prevalence and molecular epidemiology of *S. aureus* PVL, respectively. Data were analyzed using the Fisher's exact test ($P < 0.05$). **Results:** From 264 staphylococci isolates, 88 (33.33%) were detected as *S. aureus*. Furthermore, 20 out of 88 (22.72%) strains of *S. aureus* were PVL positive according to PCR results. Rep-PCR showed six main clusters of *S. aureus* samples. PVL had similar clonality between different samples. No significant relationship was observed between PVL positive *S. aureus* and rep-PCR patterns ($P = 0.98$). **Conclusion:** These results showed that a clone of *S. aureus* PVL positive has spread between the community and hospital settings. Therefore, appropriate measures are required to prevent the spread of staphylococci and other bacteria in hospitals.

Keywords: *Hospitals, molecular epidemiology, Panton–Valentine leukocidin, Staphylococcus aureus*

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Introduction

Staphylococcus aureus is a Gram-positive bacterium that is associated with clinical syndromes such as skin and soft tissue infections (SSTIs), surgical infections, and invasive diseases. These bacteria produce different toxins and adhesion factors that are their virulence factors.[1,2] One of these virulence factors is Panton–Valentine leukocidin (PVL) that is composed of two components (*lukS*-PV and *luk*F-PV). PVL is encoded by the *luk*S-PV and *luk*F-PV genes. *S. aureus* PVL is a hetero-oligomeric pore-forming cytotoxin and has effect on monocytes, macrophages, and human polymorphonuclear leukocytes.[3,4] Strains of *S. aureus* which carry PVL gene often cause severe SSTIs, necrotizing pneumonia, and sepsis. Furthermore, the presence of PVL gene is a possible marker for community-associated methicillin-resistant *S. aureus* (CA-MRSA) strains.[5] Forasmuch as methicillin is the drug of choice for treatment of *S. aureus* infections, methicillin resistance in this bacterium, will complicate the treatment.[6] The risk of *S. aureus* infection is high in hospitals, and studies have shown that MRSA isolates with PVL-positive have emerged from hospitals and are increasing among patients.[2] On the other hand, polymerase chain reaction (PCR) is a rapid and versatile *in vitro* method for identification of bacteria, which amplifies target DNA sequences, present within a source of DNA. This method allows for selective amplification of a specific target DNA sequence.[7] In addition, repetitive extragenic palindromic (Rep)-PCR is a technique that is used to discriminate among bacterial species or strains of the same species. This technique includes the application of oligonucleotide primers based on families of short and highly conserved extragenic repetitive sequences.[8-10] Different studies have shown the prevalence of PVL toxin in *S. aureus* based on the molecular

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methods: Portillo *et al*. in Colombia by pulsed-field gel electrophoresis and multilocus sequence typing, showed that out of 270 CA-MRSA strains isolated from patients, 92% possessed the *luk*F-PV/*luk*S-PV genes.[11] In another study using the PCR method, Mine *et al*. in Japan reported that from 247 clinical samples with SSTI *S. aureus* infection, 6.2% were PVL-positive strains.[12] Elizur *et al*. in the USA showed by the rep-PCR that PVL-positive *S. aureus* transmitted between patients with cystic fibrosis *and* PVL isolated from the patients during their hospitalization and also their siblings were highly homologous.[13] Since the molecular epidemiology survey is importance in detecting and preventing the spread of bacteria, the aim of this study is determining the prevalence and molecular epidemiology of isolated *S. aureus* from samples that were obtained from two tertiary hospitals of Sanandaj, located in Kurdistan province, Iran.

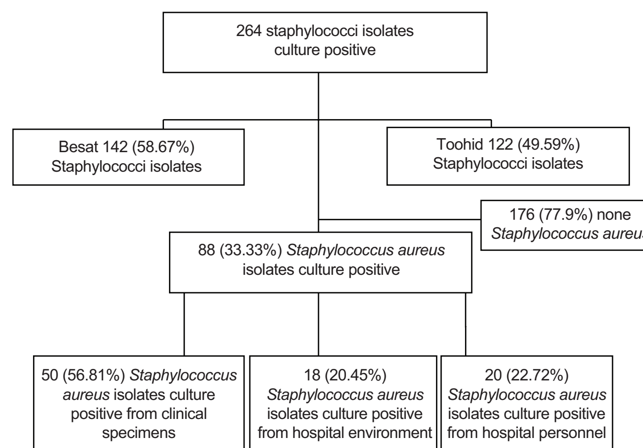
Materials and Methods

Samples

In this descriptive cross-sectional study, 264 staphylococci isolates were collected from April to March of 2012 from laboratories of two tertiary hospitals of Sanandaj city; Toohid and Besat, that are general government referral hospitals (122 isolates from Toohid and 142 isolates from Besat), Kurdistan province, Iran. 117 isolates were taken from clinical specimens including urine (60 samples), wound (12 samples), abscess (8 samples), blood (20 samples) and cerebrospinal fluid (CSF) (2 samples), respiratory tube (5 samples) and biopsy (1 sample). In this case, we did not directly contact with patient population, patient specimens that were collected previously were studied. However, our inclusion criteria including hospitalized patients in different clinical departments and in all age groups in both hospitals, that their culture samples were positive for staphylococci bacteria. Furthermore, 45 were collected from hospital personnel who were commuting between different wards of hospital, including (15 samples), nose swab (10 samples), and nasopharyngeal swab (20 samples). On other hand, 102 isolates were related to hospital environment and were taken from medical devices that used for patients' treatment in different wards. These devices including laryngoscope blades (30 samples), patient bed (35 samples), suction tank (17 samples), nebulizer (5 samples), and tourniquet (15 samples). All samples were taken from different wards including surgery (70 samples), pediatric (50 samples), internal (100 samples), and cardiac (44 samples) [Flow Chart 1].

Staphylococcus aureus detection

Samples were transferred to the laboratory of Microbiology Department of Faculty of Medicine of Kurdistan University of Medical Sciences, located in the city of Sanandaj, Iran. The above-mentioned isolates were diagnosed as *S. aureus* in the laboratory of microbiology by a standard



Flow Chart 1: *Staphylococcus aureus* isolates cultured at Toohid and Besat general government referral hospitals for the period April–March of 2012

microbiological procedure before the start of examinations as follows: At first, isolates were incubated at 37°C for 24 h on blood agar, then tube and slide coagulase, catalase, and DNase tests and manitol fermentation were performed on them. Also, the PCR was applied for thermonuclease (nuc) gene for *S. aureus* confirmation as a gold standard.[14]

DNA extraction

DNAs of *S. aureus* samples were extracted from bacterial colony using CinnaPure DNA extraction kit according to the manufacturer's instructions (SinaClon, Iran). Finally, the purified DNA was used for PCR.

Polymerase chain reaction assay

The following primers were used for the PCR assay; 5′-GTGGTCCATCAACAGGAGGT-3′ and reverse; 5′-CCTCCACCTGATAAGCCGTT-3′, 968 bp (SinaClon, Iran) (*luk*S-PV-*lukF*-PV). *S. aureus* strains NCTC 13300 was used as positive control for PVL gene and distilled water as negative control. DNA amplification was performed on an Eppendorf thermocycler in a final volume of 25 µL containing; 2 µL of each primer, 3 µL of template DNA, 5.5 µL distilled water, and 12.5 µL master mix. Amplification parameters were performed in initial denaturation at 95°C for 5 min at 1 cycle, followed by 35 cycles of denaturation at 95°C for 45 s, annealing at 57°C for 45 s, and extension at 72°C for 1 min, with final extension at 72°C for 10 min at 1 cycle.[14,15]

Repetitive-polymerase chain reaction assay

The rep-PCR in this study was performed using forward; rep 1R-I, 5'-IIIICGICG ICATCIGGC-3' and rep 2-I, 5'-ICGICTTATCIGGCCTAC-3'. 25 µl volumes containing 1 µl template DNA, 1 µl of each primer, 12.5 µl master mix and 9.5 µl distilled water. The rep-PCR amplification conditions for rep primer were the following: Initial denaturation at 94°C for 2 min, and 35 cycles of denaturation at 92°C for 1 min, annealing at 40°C for 1 min, extension at 65°C for 8 min and final extension at 65°C for 8 min.[14]

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Composed matrix of 0 and 1

After electrophoresis, the presence or absence of bands were determined using Jaccard similarity with the numbers 0 and 1 for the isolates, respectively. Detection of bands and dendrogram was performed by averaged similarity of the matrix with the use of the algorithm of the Unweighted Pair-Group Method in the SAHN program of the NTSYSpc version 2.02 (Rohlf FJ, 1999, Numerical Taxonomy and Multivariate Analysis System, Setauket, New York, USA, Exeter Software). Joining the nearest neighbor clustering method was used to detect the relationships among the analogous groups.[14]

Statistical analysis

Data were obtained from this study were analyzed using SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, Illinois, USA). For this purpose, descriptive statistics were used to determine the frequency and percentage, we used Chi-square and Fisher's exact test to compare qualitative variables ($P < 0.05$).

Results

Staphylococcus aureus detection results

Out of 264 staphylococci isolates, 88 (33.33%) were detected as *S. aureus* strains. Fifty from 117 (42.73%), 20 from 45 (44.44%), and 18 from 102 (17/64%) staphylococci isolates that were isolated from clinical specimens, hospital personnel, and hospital environment were detected as *S. aureus*, respectively [Flow Chart 1].

Polymerase chain reaction assay results

Of 88 strains of *S. aureus* that were examined for PVL gene by the PCR method (PCR products were confirmed using the positive control *S. aureus* strains NCTC 13300 [*luk*S-PV-*luk*F-PV]), 20 (22.72%) were PVL-positive. Results showed that the isolated PVL-positive strains were related to hospital personnel (5 samples; 5.68%), environmental samples (5 samples; 5.68%), and clinical specimens (10 samples; 11.36%), respectively [Figure 1].

Repetitive-polymerase chain reaction assay results

The rep-PCR profiles grouped 88 samples into 6 main clusters (C1–C6) with 77 patterns. 25% of similarity was observed between groups [Figure 2]. Nineteen patterns were PVL positive. PVL had a similar clonality among clinical specimens, hospital personnel and the environment. All the results from the rep-PCR are presented in Table 1. No significant relationship was observed between PVL positive *S. aureus* and rep-PCR patterns ($P = 0.98$).

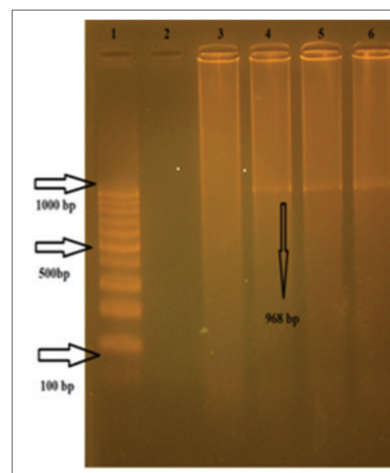

Figure 1: Gel electrophoresis of the polymerase chain reaction products from Panton–Valentine leukocidin gene; Line 1: Marker, molecular weight (100–1000 bp), Line 2: Positive control, Line 3: Negative control, Line 4–6: Positive examples of Panton–Valentine leukocidin genes (bond length = 968 bp)

Discussion

CA-MRSA that carries PVL has become a global problem in the field of health.[16] The PCR method in this study showed that out of 88 strains of *S. aureus*, 22.7% were PVL carriers. Abimanyu *et al*. in India in 2013 using Triplex PCR for the detection of PVL MRSA in 100 clinical isolates of *Staphylococcus* spp.[17] reported that 10 out of 25 (40%) MRSA isolates were positive for PVL gene, which was higher than the results of this study. *S. aureus* gained resistance to antibiotics and spread within the hospital environment, so the prevalence rate of resistant *S. aureus* is relatively high.[15] Also, differences in rates of prevalence are possibly due to different geographical areas and the type of assay used to diagnose the gene, as well as hygiene in hospitals.[18,19] In another study, Ohadian Moghadam *et al*. in Iran by the PCR assay, showed that out of 56 isolated *S. aureus* that were collected from the abscess and wound of patients in hospitals of the city of Isfahan, 14.3% were PVL positive.[18] In the present study as well, clinical specimens such as urine, wound, abscess, blood, and CSF were examined, and PVL positive *S. aureus* were higher in urine and blood compared to other clinical specimens. *S. aureus* that harbors PVL gene is the major cause of SSTIs (such as furuncle and abscess).[1] On the other hand, *S. aureus* causes urine and blood infections, and different studies have reported the prevalence of PVL positive *S. aureus* to be high in blood and urine isolates.[20] Rastegar Lari *et al*. in Iran reported the prevalence of PVL among hospital staff by nasal carriers and using the PCR assay.[21] In Rastegar Lari's study, among 270 nasal swabs, 14 (19%) had PVL gene. Results in this study showed that 5.18% and 4.20% PVL positive isolates were related to personnel nasal and environmental samples, respectively. Bacterial genes are mobile among isolates and spread among different environments and people. Therefore, it is

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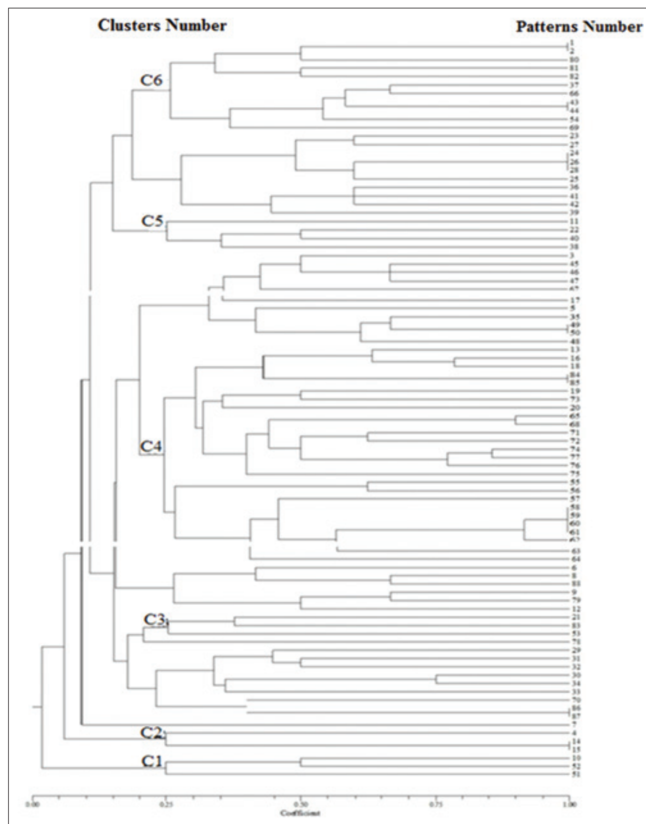

Figure 2: Dendrogram analysis of genomic similarity *Staphylococcus aureus* strains (C; clusters C1–C6 with 19 patterns)

possible that a different mechanism of gene transfer, such as horizontal gene transfer, be the reason for transfer of these genes between serotypes. In addition, genetic changes cause bacteria transmission in different areas.[21-24] In the present study, the rep-PCR was used to detect relationships between bacteria species or strains of the same species. Also, the predominate cluster was H4 with 32 patterns. Church *et al*. in Canada by the rep-PCR in 54 MRSA isolates detected seven groups. In this study, the rep-PCR showed seven main clusters among 88 samples of *S. aureus*.[25] Studies have shown that the rep-PCR is a partial, good, rapid and reliable method for bacteria identifying, outbreak screening, molecular typing, genomic fingerprinting, and proving relationships within and among bacteria strains, which can be used in epidemiological studies of microbial infections and infection control.[9,10,25-27] Studies have shown that rep-PCR is a partial good, rapid, and reliable method in screening during outbreaks, molecular typing, genomic fingerprinting and confirming the connection between bacteria and different types of bacteria species. This method can be used in epidemiological studies related to bacterial infections, as well as infection control. Also, Abdel-Haq *et al*. in the USA in 2009, using the PCR reported that from 93 patients with community-onset MRSA, 87.1% carried PVL gene; while the rep-PCR in Abdel-Haq's study showed different patterns of isolates with PVL. Gene expression in *S. aureus* is controlled by regulator genes; therefore, the distribution of

Table 1: Distribution of Panton-Valentine leukocidin-positive *Staphylococcus aureus* strains in our study

Patterns number of PVL-positive *S. aureus*	Number of PVL-positive *S. aureus* strains	Number of PVL-positive *S. aureus* strains in each hospital		Number of PVL-positive *S. aureus strains* in samples				Number of PVL-positive *S. aureus* strains in hospital wards			
		T	B	U	BL	N/S	E	S	P	I	C
p1	2	2	-	2	-	-	-	2	-	-	-
p2	1	-	1	-	-	1	-	-	-	-	1
p3	1	-	1	-	-	1	-	-	-	1	-
p5	1	-	1	1	-	-	-	1	-	-	-
p8	1	-	1	-	-	-	1	1	-	-	-
p12	1	1	-	-	1	-	-	1	-	-	-
p14	1	-	1	1	-	-	-	1	-	-	-
p15	1	1	-	-	1	-	-	-	-	1	-
p19	1	-	1	-	1	-	-	-	-	-	1
p20	1	1	-	1	-	-	-	1	-	-	-
p27	1	1	-	1	-	-	-	1	-	-	-
p35	1	-	1	-	-	1	-	1	-	-	-
p37	1	1	-	-	-	1	-	-	-	1	-
p40	1	-	1	-	-	-	1	-	1	-	-
p45	1	1	-	-	-	1	-	-	1	-	-
p47	1	-	1	-	-	-	1	-	-	-	1
p50	1	-	1	-	-	-	1	-	-	1	-
p72	1	-	1	-	1	-	-	-	-	-	1
p73	1	-	1	-	1	-	-	-	-	-	1
Total (19)	20	8	12	6	5	5	4	9	2	4	5

T: Toohid, B: Beasat, U: Urine samples, BL: Blood samples, N/S: Nasopharynx swab samples, E: Environment samples, S: Surgery ward, P: Pediatric ward, I: Internal ward, C: Cardiac ward, *S. aureus*: *Staphylococcus aureus*, PVL: Panton-Valentine leukocidin

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types of *S. aureus* with similar genes in strains, according to different origins and sources is different.[28] In this study, there was not any relationship between the rep-PCR patterns and the *S. aureus* PVL positive samples ($P > 0.05$). The aim of molecular typing via rep-PCR in this research was the detection of *S. aureus* PVL positive in different clones. Twenty PVL positive samples were in H1, H2, H5 and H6 clusters which represent a common origin. In the end, strengths of this study were; large numbers of samples, different sources of samples, valid method and diagnostic criteria, functionality, and ease of implementation, as well as its low cost. But, the weaknesses of this study can be limitations in sampling and number of samples due to the lack of time and financial resources. In addition, possible contamination in laboratories could cause false results in bacteria detection, in both phenotypic and molecular methods. In the PCR, determining qualitative results is only possible postreaction, and in this method, it is possible for some of target sequences to not become amplified. In addition, the proliferation of large DNA sequences is not possible in this method, and it is likely to proliferate sequences other than the target sequence, which will lead to false results. PCR reaction is inhibited by the presence of certain chemicals such as ethanol, phenol, and isopropanol. Therefore, the occurrence of these problems in the reaction should be prevented. It is hoped that the results of this research, would be applied for infection control in different wards of hospitals.

Conclusion

These results showed the presence of PVL in *S. aureus* isolates with different origins and spread of this bacterium that contains PVL. Using phenotypic tests for isolates cannot distinguish the isolates containing PVL; therefore, molecular methods are used for detection of *S. aureus* isolates that have PVL. On the other hand, PVL is one of the prominent virulence factors of this bacterium and by early diagnosis of this disease; it is possible to prevent its transfer and fatal prevalence. Despite the prevalence of *S. aureus*, detection of its reservoir and finding appropriate methods for preventing its spread must be considered.

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Conflicts of interest

There are no conflicts of interest.

References

1. Baba-Moussa L, Sina H, Scheftel JM, Moreau B, Sainte-Marie D, Kotchoni SO, *et al.* Staphylococcal Panton-Valentine leucocidin as a major virulence factor associated to furuncles. PLoS One 2011;6:e25716.
2. Chadwick SG, Prasad A, Smith WL, Mordechai E, Adelson ME, Gygax SE. Detection of epidemic USA300 community-associated methicillin-resistant *Staphylococcus aureus* strains by use of a single allele-specific PCR assay targeting a novel polymorphism of *Staphylococcus aureus* pbp3. J Clin Microbiol 2013;51:2541-50.
3. Brown ML, O'Hara FP, Close NM, Mera RM, Miller LA, Suaya JA, *et al.* Prevalence and sequence variation of Panton-Valentine leukocidin in methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains in the United States. J Clin Microbiol 2012;50:86-90.
4. Crémieux AC, Dumitrescu O, Lina G, Vallee C, Côté JF, Muffat-Joly M, *et al.* Panton-Valentine leukocidin enhances the severity of community-associated methicillin-resistant *Staphylococcus aureus* rabbit osteomyelitis. PLoS One 2009;4:e7204.
5. AlFouzan W, Al-Haddad A, Udo E, Mathew B, Dhar R. Frequency and clinical association of Panton-Valentine leukocidin-positive *Staphylococcus aureus* isolates: A study from Kuwait. Med Princ Pract 2013;22:245-9.
6. Chen Y, Chatterjee SS, Porcella SF, Yu YS, Otto M. Complete genome sequence of a Pantón-Valentine leukocidin-negative community-associated methicillin-resistant *Staphylococcus aureus* strain of sequence type 72 from Korea. PLoS One 2013;8:e72803.
7. Hu X, Zhang Y, Zhou X, Xu B, Yang M, Wang M, *et al.* Simultaneously typing nine serotypes of enteroviruses associated with hand, foot, and mouth disease by a GeXP analyzer-based multiplex reverse transcription-PCR assay. J Clin Microbiol 2012;50:288-93.
8. Caburlotto G, Lleo MM, Gennari M, Balboa S, Romalde JL. The use of multiple typing methods allows a more accurate molecular characterization of Vibrio parahaemolyticus strains isolated from the Italian Adriatic Sea. FEMS Microbiol Ecol 2011;77:611-22.
9. Rao BM, Surendran PK. Genetic heterogeneity of non-O1 and non-O139 Vibrio cholerae isolates from shrimp aquaculture system: A comparison of RS-, REP- and ERIC-PCR fingerprinting approaches. Lett Appl Microbiol 2010;51:65-74.
10. Mancuso M, Avendaño-Herrera R, Zaccone R, Toranzo AE, Magariños B. Evaluation of different DNA-based fingerprinting methods for typing *Photobacterium damselae* ssp. *piscicida*. Biol Res 2007;40:85-92.
11. Portillo BC, Moreno JE, Yomayusa N, Alvarez CA, Cardozo BE, Pérez JA, *et al.* Molecular epidemiology and characterization of virulence genes of community-acquired and hospital-acquired methicillin-resistant *Staphylococcus aureus* isolates in Colombia. Int J Infect Dis 2013;17:e744-9.
12. Mine Y, Nakasone I, Yamamoto Y, Utani A, Yamane N, Uezato H, *et al.* Dissemination of Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* in Okinawa, Japan. J Dermatol 2013;40:34-8.
13. Elizur A, Orscheln RC, Ferkol TW, Dunne WM Jr., Storch GA, Cannon CL. Transmission of Panton-Valentine leukocidin-positive *Staphylococcus aureus* between patients with cystic fibrosis. J Pediatr 2007;151:90-2.
14. Ramazanzadeh R, Narenji H. Molecular epidemiology of the *Staphylococcus aureus* by Rep-PCR method in Sanandaj hospitals. Life Sci J 2013;10:117-21.

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15. 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;38:247-51.
16. Adler A, Givon-Lavi N, Moses AE, Block C, Dagan R. Carriage of community-associated methicillin-resistant *Staphylococcus aureus* in a cohort of infants in Southern Israel: Risk factors and molecular features. J Clin Microbiol 2010;48:531-8.
17. Abimanyu N, Krishnan A, Murugesan S, Subramanian GK, Gurumurthy S, Krishnan P. Use of triplex PCR for rapid detection of PVL and differentiation of MRSA from methicillin resistant coagulase negative staphylococci. J Clin Diagn Res 2013;7:215-8.
18. Ohadian Moghadam S, Havaei SA, Pourmand MR. Prevalence of methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin gene in cutaneous infections in the city of Isfahan. J Med Bacteriol 2012;1:9-16.
19. Cummings KL, Anderson DJ, Kaye KS. Hand hygiene noncompliance and the cost of hospital-acquired methicillin-resistant *Staphylococcus aureus* infection. Infect Control Hosp Epidemiol 2010;31:357-64.
20. 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;39:8-14.
21. Rastegar Lari A, Pourmand M, Ohadian Moghadam S, Abdossamadi Z, Ebrahimzadeh Namvar A, Asghari B. Prevalence of PVL-containing MRSA isolates among hospital staff nasal carriers. Lab Med 2011;42:283-6.
22. Sharma A, Chaturvedi AN. Prevalence of virulence genes (ctxA, stn, OmpW and tcpA) among non-O1 Vibrio cholerae isolated from fresh water environment. Int J Hyg Environ Health 2006;209:521-6.
23. Goel AK, Jiang SC. Genetic determinants of virulence, antibiogram and altered biotype among the Vibrio cholerae O1 isolates from different cholera outbreaks in India. Infect Genet Evol 2010;10:815-9.
24. Waturangi DE, Joanito I, Yogi Y, Thomas S. Use of REP- and ERIC-PCR to reveal genetic heterogeneity of Vibrio cholerae from edible ice in Jakarta, Indonesia. Gut Pathog 2012;4:2.
25. Church DL, Chow BL, Lloyd T, Gregson DB. Comparison of automated repetitive-sequence-based polymerase chain reaction and spa typing versus pulsed-field gel electrophoresis for molecular typing of methicillin-resistant *Staphylococcus aureus*. Diagn Microbiol Infect Dis 2011;69:30-7.
26. Güler I, Kiliç H, Atalay MA, Perçin D, Erçal BD. Genotyping of nosocomial methicillin-resistant *Staphylococcus aureus* strains isolated from clinical specimens by rep-PCR. Mikrobiyol Bul 2011;45:581-91.
27. Mohajeri P, Farahani A, Feizabadi MM, Davoodabadi A, Noroozi B. The prevalence of ESBL isolates of *Acinetobacter baumannii* using pulsed-field gel electrophoresis. Zahedan J Res Med Sci 2014;16:20-3.
28. Abdel-Haq N, Al-Tatari H, Chearskul P, Salimnia H, Asmar BI, Fairfax MR, *et al.* Methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitalized children: Correlation of molecular analysis with clinical presentation and antibiotic susceptibility testing (ABST) results. Eur J Clin Microbiol Infect Dis 2009;28:547-51.