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Prevalence of Panton-valentine gene in *Staphylococcus aureus* isolated from clinical samples and healthy carriers in Gorgan city, north of Iran

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***Aim. Staphylococcus aureus* (*S. aureus*) is a nosocomial and community acquired pathogen. *S. aureus* is a pathogen that causes several types of disease from skin infections to systemic diseases that is because of having several virulence factors such as enzymes, toxins, superantigens and Panton-Valentine leukocidin (*pvl*). *pvl* is a bi-component leukotoxin that destroy PMNs and monocytes and causes furunculosis, abscesses and necrotizing soft tissue infections in patients without any risk factors for such infections. The goal of this study was determine the prevalence of *pvl* gene in *S. aureus* isolated from patients and healthy carriers in Gorgan city, north of Iran.**

Methods. One hundred seventy isolates of *S. aureus*, 95 from patients and 75 healthy carriers, were collected during one year. After identification and purification, DNA extraction was done by phenol-chloroform method. Amplification of *pvl* gene was done by specific primer and polymerase chain reaction method.

Results. Among the 170 isolates of *S. aureus*, 20% contained *pvl* gene. The frequency of isolates contained *pvl* gene in MRSA and MSSA isolates were 21.6%, 19.3% , which was not statistically significant. The frequency of these genes was not related to age, sex and source of isolation from patients.

Conclusion. The frequency of *pvl* gene in this region were much higher than expected.

KEY WORDS: *Staphylococcus aureus* - Panton-Valentine leukocidin - Polymerase chain reaction.

Staphylococcus aureus (*S. aureus)* is a nosocomial and community acquired pathogen.[1]

S. aureus is a pathogen that causes several types of disease from skin infections to systemic diseases that is because of having several virulence factors such as enzymes, toxins , superantigens and Panton-Valentine leukocidin (*pvl*).[2] *pvl* is a bi-component leukotoxin that encode by *lukS-PV* and *lukF-PV* genes and transfer from temperate bacteriophages.[3]

pvl destroy PMNs and monocytes and causes furunculosis , abscesses and necrotizing soft tissue infections in patients without any risk factors for such infections [4] on the other hand *pvl* strain (meticillin-resistant or sensitive organisms) may be cause *Purpura fulminans* with a mortality of up to 60%.[4]

The goal of this study was determine the prevalence of *pvl* gene in *S. aureus* isolated from patients and healthy carriers in Gorgan city, north of Iran.

Materials and methods

Bacterial isolates

In this research we studied on 170 isolate of *S. aureus* (95 from patients and 75 healthy carriers) were collected during one year. Purification and confirm the diagnosis of *S. aureus* by biochemical tests were carried out as previously mentioned.[5-7]

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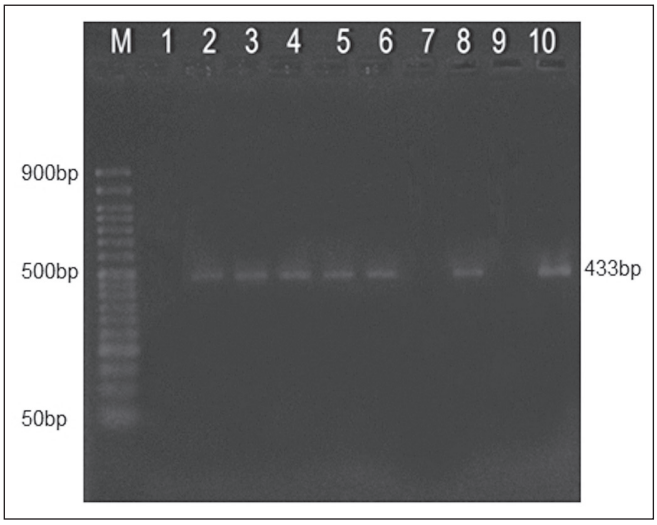

Figure 1.—Detection of the *pvl* gene by PCR (lanes 3-6,8,10). Lane 1, molecular weight marker; lanes 1 control- and 2 control +.

TABLE I.—*Characteristics of patients and healthy carriers of Staphylococcus aureus pvl -producing gene*

Property	number of isolates	number (%) of isolates *pvl* gene	P-value
Age group			
— <20	40	10 (25%)	0.6
— 20-45	85	16 (18.8%)	
— >45	45	8 (17.8%)	
Sex			
— Man	83	13 (15.7%)	0.1
— Woman	87	21 (24.1%)	
Type of infection			
— Urine	29	8 (27.6%)	0.2
— Wound	23	7 (30.4%)	
— Blood	22	3 (13.6%)	
— Others	21	2 (9.5%)	
— Healthy	75	14 (18.7%)	0.4
— Carrier			
— Patient	95	20 (21.1%)	
Methicillin resistance			
— MRSA	51	11 (21.6%)	0.4
— MSSA	11	23 (19.3%)	

DNA extraction

DNA was extracted using phenol-chloroform method that used lyzozyme, sarcosyl, proteinase K, RNAse, phenol and chloroform without using lysostaphin and boiling.[8] The purified DNA was assessed by electrophoresis and stored at -20 °C.

pvl gene detection

Polymerase chain reaction was carried out for all the isolates (Eppendorf Thermocycler, Hamburg, Germany) for the *pvl* gene in a final volume of 50 µl, in which a standard strain USA300 was used as a positive control for *pvl* gene detection and distilled water was used as negative control. The primer sequences for the *pvl* genes were as follows: for luk-PV-1, 5´-TCATTAGGTAAAATGTCTGGACATGATCCA-3´ for luk-PV-2,5´-GCATCAASTGTATTGGATAGCAAAAGC-3´.[9]

PCR matrix was containing 1 U of Taq polymerase(GENET BIO), 5 µL of the DNA template, 4 µL $MgCl_2$(GENET BIO), 1 µL of dNTP(GENET BIO) and 1 µL of each primer. DNA isolates were denatured for 5 minutes at 94 °C, followed by 30 cycles of denaturing performed for 30 seconds at 94 °C, with annealing at 56 °C for 30 seconds, and extension at 72 °C for 60 seconds. Finally, 5 minutes of final extension were performed at 72 °C. PCR products were analyzed by electrophoresis through a 1.5% agarose gel with ethidium bromide.

Methicillin susceptibility testing

Susceptibility to methicillin was determined by the agar disc diffusion method using Muller Hinton agar medium containing 2% NaCl, oxacillin disc (MAST Diagnostics, Merseyside, UK). All plates were incubated at 35 °C overnight. Molecular detection of this gene was performed.[6]

Statistical analysis

The data entered in SPSS ver 16 and χ^2 test were used for statistical analysis. A P-value of less than 0.05 was considered to be statistically significant.

Results

Among the 170 *S. aureus isolates*, 34(20%) cases were carry *pvl* gene. The distribution of these isolate among person with age less than 20 years (25%) and patients (21.1%) was higher than other groups. The frequency *pvl* gene among MRSA isolates (21.6%) was more than MSSA, but there was not any significant difference between them (P>0.05).

Discussion

Pvl strain is associated with skin and soft tissue infections such as furuncles and abscesses, subcutane-

ous tissue and also necrotic suppurative lesions,[9] It has also been isolated from necrotizing pneumonia patients without known risk factors that are necessary for such infection (community-acquired *S. aureus*).[10]

On the other hand, an association between *pvl* strain and severity has been reported in lung and bone infection. Although, this is not important for skin and soft tissue infection; but the site of infection may be has an important role in infections that causes by *pvl* strain.[11]

Our study demonstrated that the frequency of *pvl* isolates were 20%. This prevalence is similar to some other studies performed in various region in Iran such as Tehran [7, 12] as well as some of countries like Spanish, Nigeria and Detroit.[13-15] It is interesting to note that the prevalence of *pvl* in this study were higher than other studies in neighbor country [16-18] and lower than African [19, 20] and American countries.[21-23]

Different epidemiological factors such as type of strain (USA300), resistance to methicillin, CA-MRSA (community-associated MRSA) or HA-MRSA (hospital-associated MRSA), condition of patient and type of infectious have role in variability of prevalence of *pvl*.

In this study most *pvl*-positive isolates were observed in wound (30.4%) specimens confirming some of the results.[12, 24, 25] Also, 27.6% of urine specimens were *pvl* positive that could have a participating role in virulence, therefore, we suggest to survey gene expression.

It is noteworthy that, *pvl* gene has been detected in MRSA strains more than MSSA strain confirming the observations of other studies.[20, 24, 26]

Conclusions

In conclusion, *pvl* was much frequent in strains isolated from patients, women, age group less than 20, than in strains isolated from carriers, men and other age groups, but there was no evident relationship between this gene and other characteristics, statistically (P-value >0.05).

Finally, we suggest detection of type strain of this region for monitoring and control of this organism to reduce distribution of staphylococcal infections and also determine gene expression in clinical isolates to clarify the role of this proteins in phathogenesis.

References

1. Fey PD, Said-Salim B, Rupp ME, Hinrichs SH, Boxrud DJ, Davis CC *et al.* Comparative molecular analysis of community- or hospital-acquired methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 2003;47:196-203.
2. Rastegar Lari AA, Ohadian Moghadam S, Abdossamadi Z, Asghari B. Prevalence of PVL-containing MRSA isolates among hospital staff nasal carriers LABMEDICINE 2011;42:283-6.
3. Boakes E, Kearns AM, Ganner M, Perry C, Hill RL, Ellington MJ. Distinct bacteriophages encoding Panton-Valentine leukocidin (PVL) among international methicillin-resistant Staphylococcus aureus clones harboring PVL. J Clin Microbiol 2011;49:684-92.
4. Cheung AS, Aboltins CA, Daffy JR, Stanley PA. Necrotising pneumonia due to Panton-Valentine leukocidin-positive methicillin-sensitive Staphylococcus aureus. Med J Aust 2008;188:373.
5. Rahimi-Alang S, Cheraghali F, Yazarlou S, Amini A, Shakeri F, Ghaemi EA. Frequency of methicillin resistant Staphylococcus aureus in health care. Zahedan Journal of Research in Medical Sciences 2011;13:17-22.
6. Vaez H, MoradI AV, Ghaemi EA. Evaluation of methicillin resistance Staphylococcus aureus isolated from patients in Golestan province-north of Iran. African Journal of Microbiology Research 2011;5:432-6.
7. Rastegar Lari AA, Ohadian Moghadam S, Abdossamadi Z, Asghari B. Prevalence of PVL-Containing MRSA Isolates Among Hospital Staff Nasal Carriers. LABMEDICINE 2011;42.
8. Shakeri F, Golalipour M, Rahimi Alang S, Ghaemi EA. Spa Diversity among MRSA andMSSA Strains of Staphylococcus aureus in North of Iran. Hindawi Publishing Corporation. Int J Microbiol 2010;2010. pii: 351397.
9. 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;29:1128-32.
10. Zhu YH, Liu PQ, Weng XG, Zhuge ZY, Zhang R, Ma JL *et al.* Short communication: Pheromonicin-SA affects mRNA expression of toll-like receptors, cytokines, and lactoferrin by Staphylococcus aureus-infected bovine mammary epithelial cells. J Dairy Sci. 2012;95:759-64.
11. Ritz N, Curtis N. The Role of Panton-Valentine leukocidin in Staphylococcus aureus musculoskeletal infections in children. Pediatr Infect Dis J 2012;31:514-8.
12. Havaei SA OMS, Pourmand MR, Faghri J. Prevalence of genes encoding bi-component leukocidins among clinical isolates of methicillin-resistant Staphylococcus aureus. Iranian J Publ Health 2010;39:8-14.
13. Casado-Verrier B, Gomez-Fernandez C, Pano-Pardo JR, Gomez-Gil R, Mingorance-Cruz J, Moreno-Alonso de Celada R *et al.* [Community-acquired methicillin resistant Staphylococcus aureus skin and soft tissue infections in Madrid: prevalence study.]. Enferm Infecc Microbiol Clin 2012;30:300-6.
14. Esan CO FO, Lin J, Shittu AO. Characterization of Staphylococcus aureus isolates obtained from health care institutions in Ekiti and Ondo States, South-Western Nigeria. AJMR 2009;3:962-8.
15. Haque NZ, Arshad S, Peyrani P, Ford KD, Perri MB, Jacobsen G *et al.* Analysis of pathogen and host factors related to clinical outcomes in patients with hospital-acquired pneumonia due to methicillin-resistant Staphylococcus aureus. J Clin Microbiol. 2012;50:1640-4.
16. Alp E, Klaassen CH, Doganay M, Altoparlak U, Aydin K, Engin A *et al.* MRSA genotypes in Turkey: persistence over 10 years of a single clone of ST239. J Infect 2009;58:433-8.
17. Nam HM, Lee AL, Jung SC, Kim MN, Jang GC, Wee SH *et al.* Antimicrobial susceptibility of Staphylococcus aureus and characterization of methicillin-resistant Staphylococcus aureus isolated from bovine mastitis in Korea. Foodborne Pathog Dis 2011;8:231-8.

18. Yamaguchi T, Nakamura I, Chiba K, Matsumoto T. Epidemiological and microbiological analysis of community-associated methicillin-resistant Staphylococcus aureus strains isolated from a Japanese hospital. Jpn J Infect Dis 2012;65:175-8.
19. Breurec S, Fall C, Pouillot R, Boisier P, Brisse S, Diene-Sarr F *et al.* Epidemiology of methicillin-susceptible Staphylococcus aureus lineages in five major African towns: high prevalence of Panton-Valentine leukocidin genes. Clin Microbiol Infect 2011;17:633-9.
20. Jimenez JN, Ocampo AM, Vanegas JM, Rodriguez EA, Garces CG, Patino LA *et al.* Characterisation of virulence genes in methicillin susceptible and resistant Staphylococcus aureus isolates from a paediatric population in a university hospital of Medellin, Colombia. Mem Inst Oswaldo Cruz 2011;106:980-5.
21. McCullough AC, Seifried M, Zhao X, Haase J, Kabat WJ, Yogev R *et al.* Higher incidence of perineal community acquired MRSA infections among toddlers. BMC Pediatrics 2011;11:96.
22. Sola C, Paganini H, Egea AL, Moyano AJ, Garnero A, Kevric I *et al.* Spread of epidemic MRSA-ST5-IV clone encoding PVL as a major cause of community onset staphylococcal infections in Argentinean children. PLoS One 2012;7:e30487.
23. Miller LG, Eells SJ, Taylor AR, David MZ, Ortiz N, Zychowski D *et al.* Staphylococcus aureus colonization among household contacts of patients with skin infections: risk factors, strain discordance, and complex ecology. Clin Infect Dis 2012;54:1523-35.
24. 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(1):86.
25. Diep BA, Sensabaugh GF, Somboonna N, Carleton HA, Perdreau-Remington F. Widespread skin and soft-tissue infections due to two methicillin-resistant Staphylococcus aureus strains harboring the genes for Panton-Valentine leucocidin. J Clin Microbiol 2004;42:2080-4.
26. Vourli S, Vagiakou H, Ganteris G, Orfanidou M, Polemis M, Vatopoulos A *et al.* High rates of community-acquired, Panton-Valentine leukocidin (PVL)- positive methicillin-resistant S. aureus (MRSA) infections in adult outpatients in Greece. Euro Surveill. 2009;14.

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