JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2015. Vol. **9**(2), p. 1001-1005

Prevalence of *hlg* and *pvl* Genes in Methicillin Resistant *Staphylococcus aureus*(MRSA) Isolated from Health Care Staff in Mofid Children Hospital,Tehran, Iran

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(Received: 09 February 2015; accepted: 19 April 2015)

Methicillin Resistance *Staphylococcus aureus*(MRSA) is a type of *Staphylococci* that is resistant to the antibiotics such as methicillin,cloxacillin,dicloxacillin,naficillin and cephalosporins. The*hlg*and *pvl* are two important genes that confer virulence traits in MRSA strains.Gamma-hemolysin is toxicfor human erythrocytes, whereas Panton-Valentine Leukocidin (PVL) is toxic for human and rabbit macrophages and polymorphonuclear(PMNs)cells. In present study, we sought to examine the prevalence of MRSA strains and detect the genes of *hlg* and *pvl* in health care staff. The descriptive studywas conductedfromJanuarytoDecember 2014. In this survey,*two hundred twenty-nine*nose specimens were taken from the health carestaff of Mofid Children Hospital, Tehran.The isolates were identified as *S.aureus*based on biochemical and phenotypical tests.To determine the profile of antibiotic resistance of *S.aureus*isolates,the disk diffusion method (Kirby-Bauer) was used according to 2013 CLSI guidelines.MRSA strains were ascertained by resistance to oxacillin and cefoxitin. The PCR assayswere used for detection of *hlg* and*pvl*genes.PCR product was sequenced and thedata analyzed using SPSS software (version 19).Health care staff included 200 (87.33%) female and 29 (12.66%) male. Out of 229 samples, 27(12%) isolates were positive for *S.aureus*of which 21 (77.7%) were MRSA and 6 (22.3%) were MSSA (Methicillin Sensitive *S. aureus*).The results of PCR showed that 18 (85.71%) of MRSA isolates harbored *hlg*genebut the isolates were negative for the presence of*pvl* gene.In conclusion, gamma-hemolysinappears to be a more possible virulence factor than Panton-Valentine Leukocidin in MRSA isolates.

Key words: Methicillin Resistance *Staphylococcus aureus,hlg*gene, *pvl*gene, medical staff.

*S.aureus*is an important agent of infections inhuman. These infections can become threat the human life due to the ability to produce virulence factorsincluding toxins and adhesion factors. Panton-Valentine Leukocidin (PVL) is a virulence factor which producedby *S. aureus* strains often associated to causing necrotizing pneumonia. The leukocidinhas been detected among (MRSA) strains. This toxin is produced in fewer than five percent of *S.aureus* isolates[1,2].*S.aureus*, the most virulent *Staphylococcus* strains, is also the most pathogen isolated from hospitalized patients.*S. aureus* causes a wide spectrum of diseases such as skin infection and pneumonia [3].MRSA strains are resistant to certain drugs and antibiotics such as methicillin,cloxacillin,dicloxacillin,naficillin and cephalosporins.One of the most important reasonsfor increasing of MRSA strains is overuse of antibiotics and powerful drugs than essential for less serious infections.Methicillin was

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introduced to overcome infectious caused by beta-lactamase-producing *S.aureus* but resistant strains promptly spread in healthcare facilities and in the community. Furthermore,in a survey was reported that approximately 1-2% of people carry MRSA isolates on their skin or nose[4,5].The*hlg*and *pvl*are two important virulent genes in MRSA strains. Thegamma-hemolysin has toxic properties for human erythrocytes, whereas PVL is leukotoxic for human and rabbitmacrophages and polymorphonuclearcells (PMNs).For this reason, the genes have important role in infections caused by MRSA[6].The purposes of the present study were to examine the prevalence of MRSA strains in health care staff and to detect*hlg* and *pvl* genes in MRSAisolates.

METHODS

Sample collection

The descriptive studywas conducted from January to December 2014. In this survey, 229 specimens were taken from the health care workers thereby that a sterile moistened swab was inserted into each nostril to approximately 1 cm depth,and rotated five times.The samples were cultured on sheep blood agar and incubated at 37°C.

Isolates Identification

All *Staphylococcus*isolates were inoculated on to mannitol salt agar medium and incubated at 37°Cfor overnight. Then the isolates were identified as *S.aureus*based on biochemical and phenotypical tests.Resistance tooxacillin and cefoxitin was performed using Disk diffusion methodon all *S. aureus* isolatesaccording to 2013CLSI guidelines.

Antimicrobial Susceptibility testing

To determine the antimicrobial sensitivity patterns of MSSA and MRSA strains, disk diffusion method (Kirby-Bauer) was used.Theantibiotcs which used in our study including penicillinG (10units), cefpodoxime (10µg), oxacillin (1µg), vancomycin (30µg), linezolide(30µg), clindamycin (2µg), ciprofloxacin (5µg), rifampicin (5µg), teicoplanine (30µg), cefepime (15µg), erythromycin (15µg), cefotaxim (30µg), azithromycine (15µg), ceftazidim (30µg), aztreonam (30µg), minocycline (30µg), doxycycline (30µg), trimethoprime-sulfametoxazole (25µg) and ceftriaxone(30µg) (Mast, UK).Zone diameters were measured after incubation at 37°Caccording 2013CLSI guidelines.*S.aureus* ATCC 29213was used as control.

DNA Extraction and Identification of *hlg* and *pvl*genes using PCR

DNA was extracted by Accuprep genomic DNA extraction kit (Cat.No.K.3032,Bioneer,Korea).The concentration ofextracted DNA was measured and then stored at -20°C.The detection of *hlg* and *pvl*was performed in MRSA isolatesusing specific primersand PCR assay (Table 1). The PCR reaction mixture contained 13 µl ofPCR mix, 1µl each primer, 8 µl of distilled water, and2µl of DNA template for adjusting to a final volome of 25µl. The clinical MRSA strains harbored *hlg* and *pvl* genes were used as positive control.The PCR products were analyzedon 1.2% agarose gel and stained with ethidium bromide(0.5µg/ml) and viewed by UV transilluminator.The presence of 937bp and 433bp fragments were positive for *hlg*and*pvl*genesrespectively. The PCR product of *hlg* gene was sequenced.

Statistical analysis

Demographics data was analyzed using Statistical Package for Social Sciences (SPSS) software (version 19).

RESULTS

In current study, 229 health care workersaged 23-49 years old from 16 different hospital wards including Infectious, Gastrointestinal, Pediatric Intensive Care Unit, Neonatal Intensive Care Unit,Surgery,Dialysis, Emergency, Laboratory, Radiology were studied.Two hundred (87.33%) were female and 29 (12.66%) were male.The results of antibacterial sensitivity testing of staphylococci isolated are showed in Table 2.Twenty- seven (12%) isolateswere *S. aureus*; 21(77.7%) isolates were MRSA and 6 (22.3%) were MSSA.The results of PCR of *hlg*and*pvl*genes showed that 18(85.71%) of MRSA isolates were positivefor the presence of *hlg* and all MRSA isolates were negativefor the presence of *pvl*.The *hlg* gene was documented in GenBank with accession number KM116014.

DISCUSSION

There is increasing recognition that MRSA is the most virulent *Staphylococcal* strain which resistant to methicillin. MRSA and Vancomycin Resistant *Staphylococcus aureus* (VRSA) are known worldwide as risk factors for infectious disease in skin, respiratory and soft tissue. Iran has known as one of the countries that has low prevalence of MRSA among countries of world. The worldwide increasing of MRSA is a remarkable challenge in public health [7,8,9]. Our results indicatethat 21health care workers harbored MRSA strains in their noses. The rate of *hlg*gene in isolatedMRSA was 18(85.71%) compared with the rate of *pvl*gene with a prevalence of 0%. The findings of the study suggest that the *hlg*gene may be one of the commonvirulent genes in the MRSA isolated from medical staff.According to the results of one study from Iran in 2013, the prevalence of MRSA isolates among health care workers was

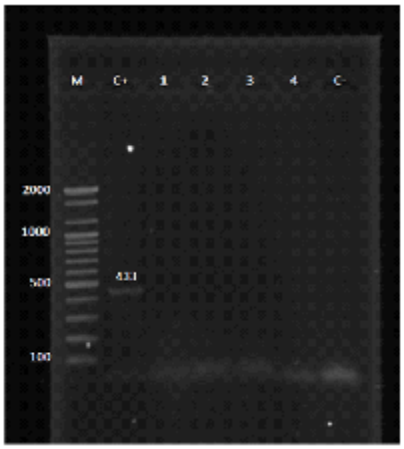

Fig. 1. Detection of the *pvl*gene. Lane 1, molecular weight marker100bp; C+: Control positive; Lanes 1-4 MRSA isolates; C- : Control negative

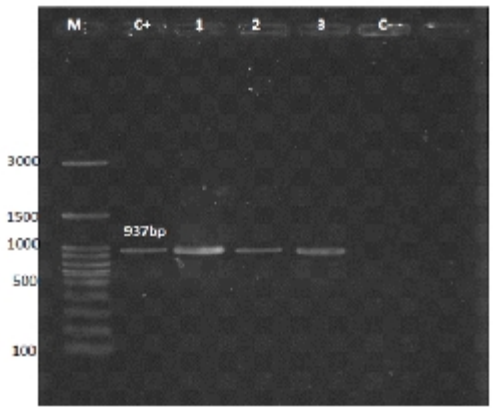

Fig. 2. Detection of the *hlg*gene. Lane 1, molecular weight marker100bp; C+: Control positive; Lanes 1-3 MRSA isolates; C- : Control negative

Table1. Specific primers for *pvl*and *hlg* genes

Name	Sequences (5' -> 3')
luk-PV-1	ATCATTAGGTAAAATGTCTGGA CATGATCCA
luk-PV-2	GCATCAASTGTATTGGATAGCAAAAGC
hlg-1	GCC AATCCGTTATTAGAAAATGC
hlg-2	CCATAGACGTAGCAACGGAT

Table 2. Amplification protocol for detection of *hlg* and *pvl*genes

Cycle	Time	Temprature(°C)
1	4 Min	94
30	45 Sec45 Sec1 Min	945572
1	5Min	72

Table 3. Antibiotic resistant pattern of *Staphylococci*isolated from health care providers

Antibiotics	21MRSA/ Resistant N(%)	6MSSA/ Resistant N(%)
Linezolid	0	0
Azithromycine	10(47.62%)	0
Erythromycin	11(52.38%)	0
Clindamycin	11(52.38%)	0
Penicillin	20(95.24%)	5(83.33%)
Trimethoprime/ Sulfametoxazol	4(19.4%)	0
Doxycycline	6(28.57%)	0
Minocycline	3(14.28%)	0
Teicoplanine	6(28.57%)	2(33.33%)
Rifampicin	5(23.8%)	0
Cefpodoxime	12(57.14%)	2(33.33%)
Ceftazidim	17(80.95%)	4(66.67%)
Cefotaxim	4(19.4%)	2(33.33%)
Ceftriaxone	12(57.14%)	2(33.33%)
Vancomycin	0	0

17.5%(10).Researchers also reported the rate of MRSA isolated from patients in Germany (6.5%), Iran (3.2%),USA (3.4%) and French (6.6%) [11,12,13,14,15,16].One research conducted by Prevost .et al. from western Europe showed that PVL toxin in strains of *S. aureus* is less than 5%.This result is close with our study[17]. According to our findings, PVL genewasnot foundin MRSA strains.In other study that was performed by Lina et al, fromof 172 clinical samples, 64 yielded positive PVL amplification,whereas the gamma-hemolysin genes were isolated in all samples.MRSA strains associated with several syndromes harbored the PVL genes[2].Our results are in disagreement with those ofby Prevost, in that, they indicated that gamma-hemolysin is produced by 19% of *S. aureus* strains and PVL was isolated in <5% of *S. aureus* clinical samples[17].For treatment of infections caused by MRSA that are resistant to several antibiotics, Linezolid is used[18]. In this study there was no resistance documented against linezolid. Methicillin Resistance *S.aureus* isolates showed variable resistance to azteronam, ceftriaxone, penicillin, cefpodoxim and erythromycin. Resistance to penicillin similar to other study [19,20].

CONCLUSION

The*hlg*geneappears to be a possible common virulence factor inMRSA strains.On the basis of ourresults, 27 medical staff harbored this bacterium which could spread in wards of hospital and cause infection in hospitalized patients.We recommended that thehealth care workerswere evaluated and monitoredfor the presence of MRSA strains in their noses.If they had MRSA, they must be treated by property drugs.Each medical center must implement a periodic program of surveillance and controlof antibiotic resistance of *S. aureus* strains.

ACKNOWLEDGEMENTS

We would like to thank personnel of Pediatric Infectious Research Center,Mofid Children Hospital, Tehran, for their assistances in this project.

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