Prevalence of PVL-Containing MRSA Isolates Among Hospital Staff Nasal Carriers

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Abstract

Background: Staphylococcus aureus (S. aureus) carrying Panton-Valentine leukocidin (PVL) has become a serious global problem. Panton-Valentine leukocidin-positive Staphylococcus aureus can result in several infections, especially cutaneous ones. This study was conducted to determine the frequency of PVL-positive genes in methicillin-resistant Staphylococcus aureus (MRSA) among hospital staff nasal carriers.

Methods: Collectively, 270 nasal swabs were taken from the personnel of 5 university hospitals in Tehran, Iran. Then polymerase chain reaction (PCR) was used to detect the PVL gene.

Results: Among the samples taken, 72 (27%) S. aureus isolates were approved. Among the total isolates, there were 23 MRSA (32%) and 14 (19%) PVL gene-containing cases.

Conclusion: This study determined that a prevalence of strains exists among hospital staff members who are continuously in direct contact with patients. This may propose the significance of detecting the carriers and decolonizing them to reduce transmission of S. aureus in the hospital.

Keywords: methicillin-resistant Staphylococcus aureus, nasal carriers, Panton-Valentine leukocidin

Staphylococcus aureus (S. aureus) is one of the major human pathogens that can cause community and hospital-acquired (HA) infections. This bacterium is the most prevalent isolate taken from hospitalized patients. The global emergence of methicillin-resistant Staphylococcus aureus (MRSA) has turned into a serious public health problem. The bacterium is known as the most significant cause of nosocomial infections, which are resistant to different antibacterial classes. Antibiotic therapy has faced severe difficulties due to these strains.

The pathogenesis of S. aureus is caused by several virulence factors such as staphylococcal exoproteins. Among the exotoxins produced by S. aureus, some can selectively destroy phagocytes such as polymorphonuclear cells and monocytes. These exotoxins belong to the bi-component leukotoxin family, including S and F proteins. Panton-Valentine leukocidin (PVL) is a member of the staphylococcus leukotoxin family. It is a very important virulence factor in S. aureus, which is a pore-forming exotoxin, and its toxic effects result from the synergistic performance of 2 separate proteins (LUK S-PV and LUK F-PV).

Nasal nares are the best ecological niches for S. aureus, and S. aureus nasal carriers may transmit the pathogen among patients. It subsequently causes infections in susceptible hosts. The colonization of S. aureus in the nose is a cause of subsequent infections. Three principles prove that S. aureus is a very important risk factor for becoming infectious in the community and hospitals. First, the rate of infections related to the bacteria is much higher in the carriers. Second, studies comparing infecting isolates and nasal carriage isolates have shown that people are usually infected with the isolates they carry. Third, eradication of S. aureus in carriers following the administration of mupirocin has statistically decreased hospital infections in dialysis patients and those who have undergone surgeries.

Staphylococcus aureus strains containing the PVL gene have the potential to epidemiologically spread in the community. In fact, the PVL gene was first reported among community-acquired (CA) MRSA strains. For example, in 1 study, the PVL gene was diagnosed in 98% of CA-MRSA strains, which had caused nasal colonization.

Although it is said that CA-MRSA strains are more likely to produce PVL, some studies refer to the prevalence of PVL-containing S. aureus isolates from the community to the hospital. This is a remarkable risk to public health. Moreover, the analysis of MRSA isolates in Holland (2003) showed that 8% of nosocomial isolates carry locus for PVL. Therefore, the isolates do not only exist in communities but can also spread in hospitals. Consequently, early diagnosis and decolonizing carriers is inevitable and essential, since it can prevent person-to-person transmission of the isolates and, ultimately, fatal prevalence.

The design of this study was based on the importance of the aforementioned factors. These factors include person-to-person transmission, the fact that nasal carriers are a known risk factor for future infections, and the importance of PVL...
as a virulence factor in the bacteria. This study evaluated the prevalence of PVL-containing MRSA isolates among hospital staff nasal carriers who are directly and continuously in contact with patients.

Materials and Methods

Bacterial Isolates

In this research, 270 nasal swabs were taken from the personnel of Tehran University of Medical Sciences and Iran University of Medical Sciences hospitals (Children’s Medical Center, Shariati, Sina, Firoozgar, Hazrate Rasool) and were sent to the microbiology lab of the School of Public Health to confirm the diagnosis of S. aureus. A subculture was initially performed on blood agar. Catalase, coagulase, mannitol fermentation, and DNase tests were then applied.

DNA Extraction

DNA was isolated using a Bioneer kit (Bioneer, Daejeon, Korea) as recommended by the manufacturer, with the modification that 1.5 µl of lysostaphin (5 mM) was added to the bacterial suspension. Finally, the purified DNA was used for PCR.

PCR Assay

All the isolates were tested for the PVL gene using the PCR test in which a standard strain NCTC 13300 was used as a positive control, distilled water was used as negative control, and the coa gene was used as an internal control. The gene was amplified on an Eppendorf (Hamburg, Germany) thermocycler with the final volume of 50 µl containing 5 µl of 10x buffers, 1 U (0.5 µl) of Taq polymerase, 4 µl of the DNA template, 3 µl of MgCl₂ (10 mM), 1.5 µl of dNTP, 20 mMol of each primer (Luk PV-1 and Luk PV-2), and 32.5 µl of distilled water. DNA isolates were denatured for 5 minutes at 95°C, followed by 35 cycles of denaturing performed for 30 seconds at 92°C, with annealing at 55°C for 30 seconds, and extension at 72°C for 45 seconds. Finally, 10 minutes of final extension were performed at 72°C. Polymerase chain reaction (PCR) products were analyzed by electrophoresis through a 1.5% agarose gel.

Primers used for coa gene as an internal control were as follows:

**Coa 1** 5'-CGA GAC CAA GAT TCA ATA AG-3'; and **Coa 2** 5'-AAA GAA AAC CAC TCA CAT CAGT-3' with relevant produced product of 900 bp.

Also, primers used for the PVL gene were as follows:

**Luk PV-1** 5'-ATCATTAGTAAAAATGTCTGCACAT-GATCCA-3'; and **Luk PV-2** 5'-GCATCAASTGTATTGGATAGCCA-AAAGC-3', with relevant produced product of 433 bp.

Methicillin Susceptibility Testing

Susceptibility to antibiotics was determined by the agar disc diffusion method using Muller Hinton agar medium containing 2% NaCl, oxacillin disc (MAST Diagnostics, Merseyside, U.K.). All plates were incubated at 35°C overnight.

Results

Among the 270 samples taken from the personnel of the hospitals under study, approximately 72 (27%) S. aureus isolates were approved. Among the total isolates there were 23 MRSA (32%) and 14 (19%) PVL gene-containing cases.

Discussion

In the present study, 270 nasal swab samples were taken from the personnel of the referenced hospitals, and 72 (27%) cases were recognized as nasal carriers of S. aureus. A PCR assay was used for diagnosing the PVL gene in S. aureus isolates. The prevalence of MRSA, the PVL gene among the studied S. aureus isolates, and MRSA-PVL isolates were 32%, 19%, and 10%, respectively. In a study conducted in Germany in 2007, the prevalence of MRSA isolates among hospital carriers of the isolates (health care workers [HCWs]) was reported at 11.3%, among which 9.1% of the isolates were PVL(+) MRSA and 2.2% PVL(-) MRSA. In another study carried out in southeast Queensland, Australia (2008), the prevalence of MRSA-PVL was 0.7%, and in Germany (2006), the rate was 9.6% and 5.8% among residents and personnel of a nursing home in Germany. A study performed in Holland/German border-area hospitals revealed prevalence rates of MRSA isolates in nasal swab samples at 6.5% and 1.4% in Germany and Holland, respectively. The prevalence rate and MRSA-PVL positive genes of the nasal swab samples was 13.2% among children in northern Taiwan. The prevalence of MRSA is highest in Asian countries such as China and Taiwan. It also seems that infections resulting from CA-MRSA are important phenomena in Asian countries.

The global emergence of MRSA is a significant challenge for public health. Recently, new variants with grave characteristics have been found. These variants are known as CA-MRSA because they are primarily found in communities and among people without the risk factors of infection. Community-acquired MRSA can cause soft-tissue infections and deep dermal infections and usually carry the PVL gene. In fact, 2 groups of MRSA bacteria have already been identified: CA-MRSA and HA-MRSA, which is worldwide and leads to hospital infections, particularly in health centers. In 1993, MRSA isolates were reported in a small population in Australia for the first time. Since then, infections with similar isolates were reported throughout the country, and the isolates had multiple antibiotic resistances. Infection due to MRSA bacteria in the community has been proven and is known as CA-MRSA infection. Also, S. aureus isolates are diagnosed in high-risk groups, including school children, residents of nursing homes, drug abusers, barracks personnel, and people in close contact with persons infected with this isolate. Successful MRSA strains have acquired several virulence factors.

One of these factors is PVL, which can cause necrosis and accelerate apoptosis and destruction of polymorphonuclear and mononuclear human cells. Presently, MRSA-PVL positive isolates are increasingly reported in health care systems. Preventing *staphylococcus* infections and decreasing the prevalence of MRSA are essential. The relationship between nasal carriers of S. aureus isolates and infections by detecting strains with equal genotypes has been proven. The genetic variety of the strains reflects clinical representations; it is noteworthy that the presence of PVL is proven
in most MRSA isolates. In 1 research study, infection spread because PVL MRSA was positive in a population of a region in southeastern Germany. In the mentioned study, the prevalence of infection resulting from positive PVL MRSA was studied for a 9-month period and involved 83 subjects and personnel. The potentiality of the isolates for causing a larger prevalence was proven. Therefore, the presence of carriers for MRSA and PVL-containing MRSA, particularly in hospitals, is significant for different reasons. These isolates can cause pandemics, are transmittable to patients, and can have dreadful consequences. The other issue is that the transmission quality of such isolates causes infection in carriers. Our findings show that among hospital staff who come in close contact with hospitalized patients (our study population included nurses, physicians, operating room and anesthesiology technicians), there were S. aureus nasal carriers, among whom PVL-containing MRSA were diagnosed. This has raised concerns because healthy carriers, who do not show any symptoms or signs of severe disease, can still cause epidemics, increase the incidence of severe disease among patients, and raise mortality rates by transmitting the strains. As we know, HA-MRSA are strains with multiple resistances to antibiotics. In case the strains contain virulence genes like PVL, their risks and the consequent concerns would increase. 

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