Original Research Article

Rapid Detection of Panton-Valentine Leukocidin (PVL) Gene from *Staphylococcus aureus* Clinical Isolates

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A B S T R A C T

Infections caused by CA-MRSA have been associated with the presence of Panton-Valentine leukocidin (PVL) toxin which associated with increased disease severity, ranging from cutaneous infection requiring surgical drainage to severe chronic osteomyelitis and deadly necrotizing pneumonia. Panton-Valentine leukocidin (PVL) is a cytotoxin, one of the β-pore-forming toxins. PVL toxin targets cells of the human immune system like polymorphonuclear neutrophils (PMNs), monocytes, and macrophages forming pores on them that cause cytokine release and cell death by apoptosis or necrosis. The aim of this study is the rapid detection of PVL gene using real time PCR in *S. aureus* isolates recovered from community acquired *S. aureus* isolates from adult and pediatric infections, and to describe the prevalence of PVL gene positivity in community acquired. *S. aureus* strains. This study was conducted on one hundred and fifty clinical isolates of *S. aureus* isolates forming three groups (group one fifty community acquired isolates from adult infections, group two: fifty community acquired isolates from pediatric infections and group three: fifty hospital acquired isolates as a control group) isolated from different clinical specimens. All isolates were identified by conventional methods such as morphological identification, gram stain, and catalase test. Identification to species level by slide and tube coagulase, culture on DNase agar and mannitol salt agar. Then genotypic detection of PVL gene by real time polymerase chain reaction (PCR) was done. Regarding community acquired isolates from adults and pediatric infections (88%) were positive for presence of PVL gene by real time PCR while all the control isolates were PVL gene negative, of these community acquired *S. aureus* isolates (80%) were CA-MRSA while (20%) were CA-MSSA. In the present study regarding different types of infection (100%) of osteomyelitis were PVL gene positive but with no statistical significance. There was high statistical significance between skin and soft tissue infections and PVL gene positivity. There was also high statistical significance between skin soft tissue infection isolates and CA-MRSA. In conclusion, there is high percent of PVL gene positivity among CA-MRSA infections, so its rapid detection is recommended for early adjustment of antibiotic treatment.

Introduction

*Staphylococcus aureus* is a highly successful pathogen responsible for a variety of clinical problems ranging from folliculitis to endocarditis, osteomyelitis to pneumonia, and food intoxication to septic shock. A number of structural secreted virulence factors play a role in the pathogenesis of these various conditions and the evasion of
host defenses. One of these exoproteins, *Panton-Valentine leukocidin* (PVL), is produced by several community-acquired methicillin-resistant *S. aureus* and methicillin-sensitive *S. aureus* (CA-MRSA and CA-MSSA, respectively) clones that are spreading throughout the world (Mandell and Wunderink, 2012).

*Panton-Valentine leukocidin* (PVL) is a pore-forming toxin which is composed of two protein components (LukF and LukS) that very efficiently disrupt the cell membrane of neutrophils. PVL has been associated with chronic or recurrent skin and soft tissue infections and with necrotizing pneumonia, which also affect immune competent persons (Masiuk et al., 2010). The adjunctive use of antibiotics that suppress toxin production, such as clindamycin, linezolid, and rifampin, and intravenous immunoglobulin is advocated for the treatment of severe and invasive infections caused by PVL-producing strains (Badiou, 2010). The prevalence of the PVL genes was less common among MSSA isolates than among MRSA isolates from infections and colonization in several studies (Davied and Daum, 2010).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a well-known public health problem that emerged shortly after the introduction of methicillin, nafcillin, and oxacillin antibiotics (Mangliner et al., 2008). In 2000, the CDC created a case definition for a CA-MRSA infection as any MRSA infection diagnosed for an outpatient or within 48 hours of hospitalization if the patient lacks the health care-associated MRSA risk factors (Davied and Daum, 2012). CA-MRSA is known to cause predominantly skin and soft-tissue infections, but can also cause other severe community associated infections like myositis, pyomyositis, osteomyelitis and bacteremia (Rafee et al., 2012). These community-acquired MRSA (CA-MRSA) strains have epidemiological, clinical and microbiological features that distinguish them from the traditional hospital-based organisms Hospital Acquired MRSA (HA-MRSA) (Pantost and venditti, 2009).

Methicillin resistance is conferred by the mecA gene, which is located in the staphylococcal cassette chromosome mec (SCC mec). The SCC mec elements are different in HA-MRSA and CA-MRSA strains. HA-MRSA strains typically contain the larger type I, II, or III elements, whereas CA-MRSA strains contain the smaller type IV or V elements (Fritz et al., 2010). CA-MRSA strains differ from their HA-MRSA counterparts by usually being susceptible to clindamycin and other non-B-lactam antibiotics. They also typically carry the 2 genes encoding Panton-Valentine leukocidin (PVL) (Davied et al., 2008).

Therefore, detection of PVL gene helps in treatment and diagnosis of community acquired MRSA. One of the methods for detection of PVL gene is real time polymerase chain reaction (PCR). Real-time PCR is significantly faster than other detection methods. The combination of excellent sensitivity and specificity, low contamination risk, ease of performance and speed has made real-time PCR technology appealing to the clinical microbiology laboratory (Jukes et al., 2010). The aim of this study is the rapid detection of PVL gene using real time PCR in *S. aureus* isolates recovered from community acquired infections.
S. aureus isolates from adult and pediatric infections, and to describe the prevalence of PVL gene positivity in community acquired S. aureus strains.

**Materials and Methods**

This study was conducted on one hundred and fifty S. aureus clinical isolates that were collected from various clinical specimens, submitted for routine culture and susceptibility testing to the Main Microbiology Laboratory, Ain Shams University Hospitals, Cairo, Egypt. The isolates were collected as three groups (group one fifty community acquired isolates from adult infections, group two fifty community acquired isolates from pediatric infections and group three fifty hospital acquired isolates as control).

Isolates for adult and pediatric groups were collected as clinical isolates of S. aureus from specimens of out-patient clinic and recently hospitalized patients within 48 hours of hospitalization.

Isolates for Control group were collected as clinical isolates from hospitalized patients having one or more of the following health care-associated S. aureus risk factor: Hemodialysis, Surgery, Residence in a long-term care facility or hospitalization during the previous year, and Presence of an indwelling catheter or a percutaneous device at the time of culture, representing hospital acquired S. aureus infections.

Data was collected from patients about risk factors, antibiotics intake, associated medical diseases and relation to medical staff.

All isolates were identified using conventional methods as Gram stain and coagulase test, subculture on DNase agar, and mannitol salt agar. Antimicrobial susceptibility testing was carried out by the disk diffusion method according to CLSI guidelines using the interpretative criteria for cefoxitin, clindamycin, linezolid and rifampicin.

The isolates were stored in aliquots containing tryptic soya broth (Oxoid, UK.) at -70°C till used for Real-time PCR for detection of PVL gene.

**Polymerase chain reaction**

Real time Polymerase chain reaction (PCR) (Rotor gene 5plex) was performed for detection of PVL gene in the 150 isolates of adult, pediatric and control groups. Bacterial DNA was extracted using DNA extraction kit; QIA amp DNA Mini Kit supplied by Qiagen, USA. The reaction mixture included Quanti Tect SYBR Green PCR Master Mix (Qiagen, USA), Real time PCR was done using the following primers for PVL gene:

*PVL Forward: 5´ATG TCC TTT CAC TTT AAT TTC ATG AGT TTT 3´.

**PVL Reversed:5`CAT GCT ACT AGA AGA ACA ACA CAC TAT GG 3` (Shallcross, et al., 2010).

The PCR conditions were with initial denaturation step at 95°C for 10 min and 38 cycles of amplification consisting of: denaturation at 95°C for 15s., annealing at 60°C for 30s, extension at 72°C for 30s. Detection of the PCR amplified product was done by SYBR Green (Qiagen, USA).

**Results and Discussion**

Regarding detection of PVL gene by PCR, PVL gene was positive in 42 (84%) of community acquired isolates in adult group, 46 (92%) in pediatric group while all the
hospital acquired isolates (control group) were PVL gene negative, with a highly statistical significance between the two groups (p value < 0.001) (table 1).

Out of 50 community acquired S. aureus isolates 46 (92%) in adult group and 34 (68%) in pediatric group were CA-MRSA. While 4 (8%) in adult group and 16 (32%) in pediatric group were CA-MSSA. Whereas in the control group out of 50 hospital acquired isolates 47 (94%) were HA-MRSA and 3 (6%) were HA-MSSA.

Out of 46 CA-MRSA 38 (76%) were PVL gene positive in adult group while out of 34 CA-MRSA 31 (91%) were PVL gene positive in pediatric group. There is higher percentage of PVL gene positivity among cases of CA-MRSA but there was no statistical significance between CA-MRSA and CA-MSSA as regards PVL positivity (P value >0.05).

In the adult group, Out of 50 S. aureus isolates for each adult and pediatric groups 14 (28%), 20 (40%) were from patients taking antibiotics, 4 (8%) from patients with history of contact to relative medical staff, and 13 (26%), 14 (28%) of patients having associated medical diseases in adults and pediatric groups respectively. As regard PVL gene positivity there was no statistically significant association with any of these risk factors in either of the groups (p >0.05).

In the adult group, The fifty S. aureus isolates were collected from different types of infection, out of 50 isolates 28 (56%) were from skin and soft tissue followed by pneumonia 9 (18%) followed by UTI 6 (12%) then bacteremia 5 (10%) and osteomyelitis 2 (4%). There were 23 (46%), 7 (14%), 6 (12%), 4(8%), 2(4%) PVL gene positive S. aureus isolates respectively. It was found that 100% of osteomyelitis were PVL gene positive. On comparing the results of PVL gene according to different types of infections, there is high statistically significant association between soft tissue infections, UTI and PVL gene presence (P < 0.01) and there is a border line significance for pneumonia (P value =0.05).There was also high statistical significance between CA-MRSA and skin and soft tissue infection cases. While in the pediatric group, out of 50 CA S. aureus isolates 22 (44%) were from skin and soft tissue followed by osteomyelitis 12 (24%), and the lowest percentage was from bacteremia 5 (10%) and UTI 5 (10%) then pneumonia 3 (6%) and other infections 3(6%).It was found that 100% of isolates from these infections were PVL gene positive. On comparing the results of PVL gene positivity according to different type of infections, there was no statistical significance (P >0.05).In the control group, out of 50 HA S. aureus isolates 23(46%) were from skin and soft tissue followed by bacteremia 13 (26%), and the lowest
percentage was from pneumonia 12(24%) and UTI 0 (0%) then osteomyelitis 0 (0%) and other infections 2(4%).

In the adult group, out of 50 S. aureus isolates 20 (40%), 19 (38%), 17(34%) were sensitive to clindamycin, linezolid and rifampicin respectively, whereas 30 (60%), 31 (62%), 33(66%) were resistance to clindamycin, linezolid and rifampicin respectively. For pediatric group, out of 50 S. aureus isolates 34 (85%), 26 (52%) were sensitive to clindamycin, and rifampicin respectively, whereas 16 (15%), 24 (48%) were resistance to clindamycin, and rifampicin respectively. All S. aureus isolates from pediatric group were sensitive to Linezolid antibiotic. There was no statistical significance between antimicrobial susceptibility and PVL gene positivity. While in the control group, out of 50 S. aureus isolates 21 (42%), 36 (72%), 9(18%) were sensitive to clindamycin, linezolid and rifampicin respectively, whereas 29 (58%), 14 (28%), 41(82%) were resistance to clindamycin, linezolid and rifampicin respectively. Only Linezolid had statistically higher sensitivity in the hospital acquired control group 36 (72%) than the community acquired adult group 19 (38%) (p<0.01). The percentage of PVL gene positivity is higher among resistant strains of S. aureus to Clindamycin (46%), Linezolid (46.8%) and Rifampicin (54%). A statistically significant association was found between PVL gene positivity and Linezolid resistance (p<0.05) only.

Staphylococcus aureus causes a range of infections, from mild skin infections to septicemia. Shortly after the introduction of methicillin in the early 1960s, methicillin-resistant S. aureus (MRSA) emerged and now causes serious nosocomial infections worldwide. Despite its spread in hospitals and nursing homes, MRSA has not disseminated in the wider community until recently. Such infections not acquired at a healthcare setting or institution is considered community associated MRSA (CA-MRSA). The discovery of Panton-Valentine leukocidin (PVL), encoded by lukF-PV and lukS-PV genes, was reported in the 1930s, and PVL has been associated with 1.6% of S. aureus strains in England and Wales in 2002. MRSA strains producing PVL toxin have been associated with an increase in severe skin infections and a new syndrome of community-associated necrotizing pneumonia in children. The PVL genes are carried on the phage SLT, but PVL has not been identified with epidemic or endemic hospital-acquired MRSA strains (Rayan et al., 2005). The present study aimed to detect the PVL gene in community acquired S. aureus as one of the virulence factors of community acquired S. aureus and also in hospital acquired S. aureus (control) using real-time PCR as one of the rapid and accurate methods.

This study was conducted on one hundred and fifty S. aureus clinical isolates that were collected from various clinical specimens. The isolates were collected as three groups (group one fifty community acquired isolates from adult infections, group two fifty community acquired isolates from pediatric infections and group three fifty hospital acquired isolates as control). All isolates were identified using conventional methods as Gram stain and catalase. Then, the isolates were identified to the species level by slide and tube coagulase test, subculture on DNAse agar, and mannitol salt agar. Antimicrobial susceptibility testing was carried out by the disk diffusion method according to CLSI guidelines using the interpretative criteria for cefoxitin, clindamycin, linezolid and rifampicin. Real-time PCR was made for detection of PVL gene.
In the current study, PVL gene was positive in 42 (84%) of community acquired isolates in adult group, 46 (92%) in pediatric group while all the hospital acquired isolates (control group) were PVL gene negative. Similarly Baran et al., 2010 conducted a study on thirty strains that were phenotypically identified as MRSA and after assessing the risk factors, 28 (93.3%) of them were classified as health-care associated (HCA) and 2 (6.7%) of them as community acquired (CA). Panton-Valentin leucocidin (PVL) gene positivity was detected in only CA-MRSA isolates (2/2; 100%). Panton-Valentine Leukocidin (PVL) genes have been identified as a stable marker of CA-MRSA strains worldwide (Morrison et al., 2006).

On the other hand, WD et al., 2010 conducted a study on 1104 S. aureus isolates community acquired and hospital acquired. 31.8% (351) were community-acquired S. aureus. Panton-Valentine Leukocidin (PVL)-positive community-acquired S. aureus strains were 35.3% only. Also Gülmez et al., 2012 conducted a study to detect SCCmec types and PVL gene in S.aureus strains isolated from various infections. They found that Ten out of 446 (2.2%) were PVL positive strains that were all methicillin susceptible S.aureus (MSSA). Muttaiyah et al., 2010 stated that in Auckland, PVL-positive MSSA was strongly associated with the diagnosis of SSTI; 48% of all MSSA isolates associated with SSTI were lukSF-PV positive. The reported incidence of PVL-positive MSSA SSTI in the literature ranges from 6.8% in a teaching hospital in Michigan to 93% in a New York prison endemic with the PVL-positive USA300 MSSA strain (Muttaiyah et al., 2010).

In the current study out of 100 S. aureus isolates 89 (89%) are MRSA, followed by MSSA which form 11 (11%) of the total study isolates. In a similar study done in Sudan by Kheder et al., 2012, out of 35 strains of S. aureus isolated from surgical samples, 25 (71.4%) were found to be MRSA. Levels reported elsewhere differed substantially ranging from 22% - 65%. However, there are evidences that the prevalence of MRSA has increased worldwide (Kheder et al., 2012). On the other hand a study made by Egyir et al., 2014 in Ghana on 903 staphylococci collected, 308 (34%) were identified as S. aureus and 595 (66%) as coagulase negative staphylococci. MRSA and MSSA isolates formed nearly (3%), (97%) respectively. The prevalence of MRSA (3%) was lower than those reported in other African countries such as Nigeria (20%), Algeria (45%) and in a multicenter study (15%) involving five major African towns. Egyir et al., 2014 attributed the low MRSA frequency reported in their study to the low consumption of antimicrobial agents such as fluoroquinolones and third generation cephalosporins in Ghana, because they are expensive and are usually prescribed for acute infections. Usage of the aforementioned antimicrobial agents has been shown to correlate with an increase in MRSA prevalence (Egyir et al., 2014). In another western country WD et al., 2010 found that CA-MRSA accounted for 4% (14) of S. aureus isolates.

Also Shallcross et al., 2010 conducted a study on 390 isolates in England, they found that Methicillin-resistant S. aureus with PVL was rare (0.8% of all isolates) but PVL with methicillin-sensitive S. aureus was common (9.0% of all specimens). Also Gülmez et al., 2012 found MRSA to be 97 (34%) out of 285 S. aureus isolates. The observed MRSA prevalence among clinical isolates in Ghana is similar to those reported in European countries with low MRSA prevalence, such as the Scandinavian countries and The Netherlands (Egyir et al., 2014).
Table 1 Comparison between case group and control group regarding PVL gene presence

<table>
<thead>
<tr>
<th>Staphylococcus aureus isolates</th>
<th>Community acquired group</th>
<th>Hospital acquired Control group</th>
<th>$X^2$</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVL positive</td>
<td>88(88%)</td>
<td>0(0%)</td>
<td>72.414</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PVL negative</td>
<td>12(12%)</td>
<td>50(100%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the current study, there is high statistically significant association between soft tissue infections, UTI and PVL gene presence ($P < 0.01$) and there is a border line significance for pneumonia ($P = 0.05$). Similarly, WD et al., 2010 found that Panton-Valentine Leukocidin (PVL)-positive community-acquired S. aureus strains were more commonly associated with skin abscesses than other SSTIs (29.4% versus 5.9%, $P < 0.01$). Also Shallcross et al., 2010 found that 9.7% of clinical isolates and 20.8% of skin and soft tissue specimens contained the genes for PVL. Also Gülmez et al., 2012 isolated 7 PVL positive strains out of 446 (1.5%) causing skin and soft tissue infections. They stated that CA-MRSA strains might harbour Panton-Valentine leukocidin (PVL), an important virulence factor in skin and soft tissue infections. They added that Strains carrying PVL has the ability to penetrate undamaged skin and cause more severe infections.

In conclusion there is a high percent of PVL gene positivity among CA-MRSA infections, so its rapid detection is recommended for early adjustment of antibiotic treatment. As the adjunctive use of antibiotics that suppress toxin production, such as clindamycin, linezolid, and rifampin, and intravenous immunoglobulin is advocated for the treatment of severe and invasive infections caused by PVL-producing strains. More studies are required for implementing cheaper methods for detection of PVL toxin directly from specimens such as specific enzyme-linked immunosorbent assay (ELISA) or immunochromatographic test (ICT) methods, a step for routine work up for PVL toxin detection in S. aureus clinical isolates.

References


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