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Research Article

Detection of Panton – Valentine Leukocidin Toxin by Polymerase Chain Reaction in Methicillin Resistant *Staphylococcal aureus* Isolates

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Abstract

Introduction: *Staphylococcus aureus* is a major pathogen causing a diversity of life–threatening systemic infections. The MRSA is known to have high probability of PVL toxin gene. The frequency of PVL-producing MRSA in various clinical samples were detected by PCR.

Methods: 218 MRSA isolates from heterogeneous clinical samples received in microbiology laboratory were processed to detect mecA and PVL gene by PCR.

Results: In the present study, out of 218 isolates, 192 were mecA positive by PCR. Of the 192 mecA positive isolates, 104 (54.16%) samples were positive for PVL toxin.

Conclusion: The PCR system used in the study is specific for the mec A and PVL. This may lead to specific therapeutic approaches targeting PVL in severe PVL-related staphylococcal syndromes.

Keywords: MRSA; Mec A; PVL Toxin; PCR

Introduction

Staphylococcus aureus is a major pathogen causing a diversity of life–threatening systemic infections [1]. Despite the introduction of active antimicrobial agent, it remains as a major cause of hospital and community acquired infections [2], leading to high morbidity and mortality [1]. The reports from India suggest there is increasing incidence of MRSA throught [3-5].

The MRSA is known to have high probability of PVL toxin gene [6]. The PVL toxin is a binary toxin comprising two proteins "LukF-PV", "LukS-PV" [6,7]. PVL exhibits cytolytic activity on leukocytic cells when the two components function in combination [8,9]. PVL targets the cells of the human immune system, such as polymorphonuclear neutrophils (PMNs), monocytes, and macrophages [10-12]. The mechanism of action of PVL toxin is depicted in figure 1.

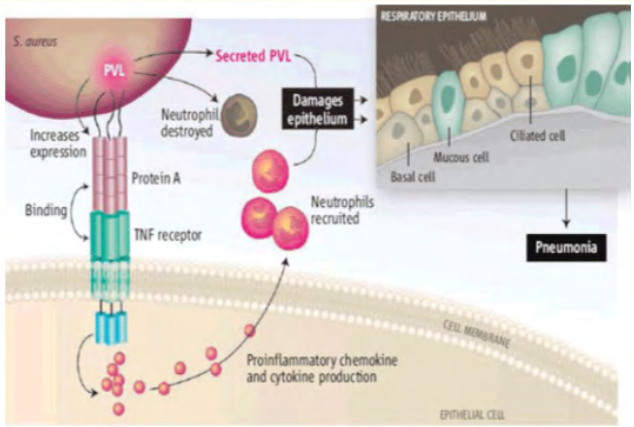


Figure 1: Mechanism of PVL toxin in respiratory Epithelium.

PVL-producing strains can be detected by PCR, ELISA and matrix-assisted laser desorption ionization-time of flight mass spectrometry method[6]. PVL is detected in clinical practice and treatment regimens may be adjusted. The adjunctive use of antibiotics that suppress toxin production, such as clindamycin, linezolid, and rifampin, intravenous immunoglobulin, surgical evacuation and drainage of necrotic lesions is advocated for the treatment of infections caused by PVL-producing strains.

The importance of PVL as a potential virulence factor led to investigate the frequency of PVL-producing *S. aureus* in various clinical samples.

Aim

To detect the Panton –Valentine Leukocidin toxin by PCR in Methicillin resistant Staphylococcal isolates.

Objectives

The main objectives of the present study are:

- To isolate and identify Methicillin resistant *Staphylococcus aureus* (MRSA) from clinical samples.
- To detect mecA gene by PCR in MRSA isolates.
- To detect PVL toxin by PCR in MRSA isolates.

Methodology

The study was conducted from November 2022 to May 2022 at Sri Lalithambigai Medical College and Hospital, Chennai. Institutional ethical committee clearance was obtained. A total of 218 MRSA isolates from heterogeneous clinical samples received in microbiology laboratory were stored in nutrient agar vials at -20°C and processed to detect mecA and PVL gene.

Inclusion criteria

All consecutive MRSA isolates from clinical samples.

Exclusion criteria

- Clinical samples with MSSA isolates
- MRSA isolates isolated from the same patient.

Mec A and PVL gene detection by PCR

In the study the mecA positive MRSA isolates were detected and in those isolates PVL gene was identified. The PCR reagents i.e.; sterile water, assay buffer, dNTP mix, Template DNA, Forward primer, Reverse primer and Taq DNA polymerase were mixed.

The primers used in this PCR are for mecA:

- F primer: 5′CTGGTGAAGTTGTAATCTG-3′
- R primer: 3′ATCGATGGTAAAGGTTGGC-5′

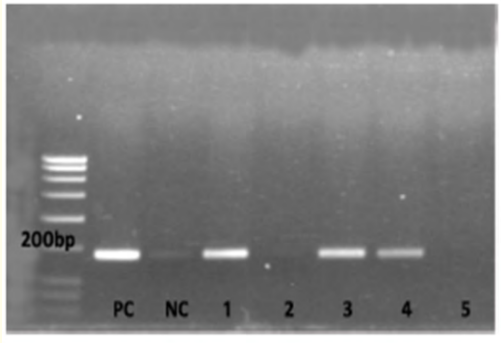
The primers used in this PCR are for PVL are

- F primer: 5′ ATCATTAGGTAAAATGTCTGGACATGATC -3′
- R primer: 3′ GCATCAAGCTGTATTGGATAGCAAAAGC-5′

Initial denaturation was done at 94°C for 1 minute and denaturation cycle is continued for 30 cycles each for 30 seconds. The primary annealing of the template and the primers takes place at 48°C – 54°C for 30 seconds in each cycle.

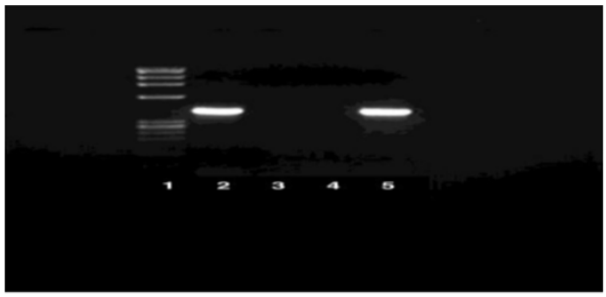
The extension step by Taq polymerase was done at 72°C for 1 minute. The bases are coupled to the primer at the 3′ side. The final extension was done for 5 -10 minutes.

Following PCR amplification, using Bromothymol blue as tracking dye, it was visualised under UV transilluminator. The PCR of mecA and PVL in figure 2 and figure 3 respectively.



M: Molecular ladder ;
PC: Positive Control;
NC: Negative Control ;
1, 3, 4: Positive clinical isolate ;
2, 5: Negative clinical isolates.

Figure 2: mecA gene in PCR.



1- Molecular wt ladder ;

2,5 - Positive clinical isolates;

3,4- Negative clinical isolates

.

Figure 3: PVL gene in PCR.

Results

The present study was carried out at the Department of Microbiology, V.M.K.V Medical College and VMH. Majority of the patients were of age 41-50 years. The age distribution is shown in table 1.

Age Group (Years)	No. of Cases
<10	23
11-20	21
21-30	14
31-40	33
41-50	57
51-60	33
61-70	19
>71	18
Total	218

Table 1: Age Distribution.

Out of 218 patients, 137 (62.8%) patients were males and 81 (37.2%) were females.

Out of the total 218 samples, 143 samples were pus, 24 urine samples, 16 sputum samples, 16 blood samples and others - 19 samples depicted in chart 1.

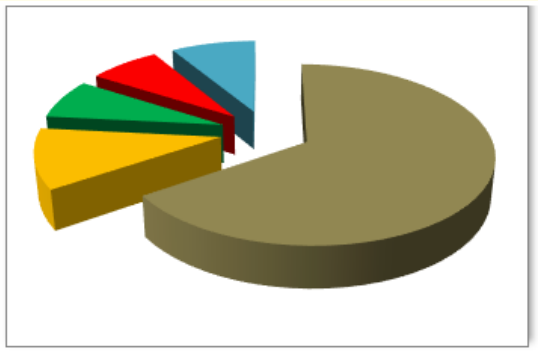


Chart 1: Distribution of samples.

In the present study, out of 218 isolates, 192 were mecA positive by PCR. Of the 192 mecA positive isolates, 104 (54.16%) samples were positive for PVL toxin. It is shown in chart 2.

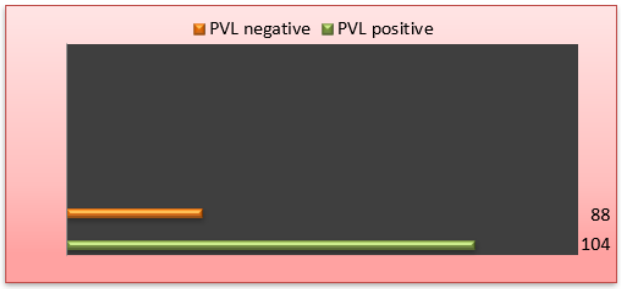


Chart 2: PVL genes detected by PCR.

Discussion

218 consecutive clinical samples in which MRSA isolated were taken.

Out of 218 cases, majority patients were between 41-50 years of age. This may be due to Diabetes, Hypertension, waning immunity and hormonal abnormalities as reported by Prajna., *et al.* [13].

In the present study male to female ratio was 2:1. The increased rate of infection among males is that they are more prone for injuries

leading to fractures due to outdoor occupation and smoking which is the predisposing factor for gangrene. A similar observation has been made by Siddiq., *et al.* who has reported a male to female ratio of 2.6:1 [14] and Prajna., *et al*. who has reported 6:4 [13].

In the study pus samples were high in proportion (66.6%) of which furncles were high. A similar observation was seen by Prajna., *et al*. (87%) [13], Siddiq., *et al*. (62%) [14], Tandel., *et al*. (76.1%) [15], Horieh sadari., *et al*. (63%) [16], Hare Krishna Tiwari., *et al*. (76%) [17].

In the present study mecA gene was detected in 192 isolates. All Cefoxitin resistant genes were mecA positive. The prevalence of PVL toxin was 54.16%. This is consistent with Nadija., *et al*. [18], Harleen Kaur., *et al*. [19] and Souza., *et al*. [20], Gillet., *et al*. [21] and Cedric Badiou., *et al*. [6].

The PVL genes were not detected in *S. aureus* strains in those causing urinary tract infections and in blood infections. This was similar to studies of Gillet., *et al*. [21] and Souza., *et al*. [20].

Conclusion

In this study, PVL genes were more frequent in strains causing disease by direct invasion and tissue destruction (primary skin infections) than in strains causing secondary infections (infective endocarditis, urinary tract infection, TSS, or enterocolitis). Studies suggest that PVL positive *S. aureus* strains are associated with skin infection [22-24], bone and joint infections, and pneumonia.

The PCR system used in the study is specific for the mec A and PVL. Since previous methods for PVL detection are somewhat cumbersome, requiring detection of the toxin by immunodiffusion with rabbit antibodies, this method would be useful for routine testing. The early diagnosis of PVL-positive *S. aureus* infections by this method will allow physicians to rapidly identify PVL-associated diseases.

In conclusion, PVL appears to be a possible virulence factor associated with necrotic lesions of the skin and subcutaneous tissues (e.g., furuncles) and also pneumonia; This may lead to specific therapeutic approaches targeting PVL in severe PVL-related staphylococcal syndromes.

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