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REVIEW ARTICLE

EVALUATION OF PANTON-VALENTINE LEUKOCIDIN GENE BY POLYMERASE CHAIN REACTION IN COMMUNITY-ACQUIRED METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

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ABSTRACT

Background- MRSA infections are become a growing problem in community as well as challenge for healthcare facilities. Community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) is a major global problem. **Aim-** to evaluate the presence of *PVL* toxin gene as a sole marker to validate the genotypic definition of the CA-MRSA isolates. **Material and methods**: 210 *S. aureus* strains were included in this study. 100 MRSA isolates identified by phenotypically using Cefoxitin disc diffusion test. Molecular detection of *mec-A* gene and *PVL* gene was done by PCR method. Results- We found that *PVL* toxin gene was present in 62/100 (62%) among all MRSA. Out of these 42/58 (72.41%) were CA-MRSA and 20/42 (42.61%) isolates were HA-MRSA. **Conclusion-** The present study suggested that the presence of the *PVL* toxin gene cannot be used as a reliable marker for the CA-MRSA infections.

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INTRODUCTION

Methicillin resistant *S. aureus* (MRSA) has been considered a major nosocomial pathogen in healthcare facilities but in the past decade, it has been observed emerging in the community also. The first case of community- associated MRSA (CA-MRSA) infection in the United States was reported in 1980[1]. CA-MRSA has been found to be composed of more varied clonal groups than hospital associated MRSA (HA-MRSA). It is usually contain a unique *SCCmec* type IV DNA element [2].

MRSA is mainly transmitted through skin-to-skin contact in community.[3] There is considerable concern that community-acquired MRSA would cause infections difficult to treat in the outpatient setting and would be increase the need for vancomycin therapy [4].

S. aureus expresses a variety of virulence factors, including Panton-Valentine-leukocidin (*PVL*). *PVL* is a two-component *S. aureus* pore-forming protein encoded by the *lukF-PV* and *lukS-PV* genes. The PVL genes have been identified as a stable marker of community-acquired methicillin-resistant *S.aureus* worldwide.[5]. It is a cytotoxin that causes leucocyte destruction and tissue necrosis, by forming pores in cellular membranes and it is mainly associated with primary skin infections, especially furuncles, and with other deep-seated infections including necrotizing community-acquired pneumonia, pyomyositis, ostiomylitis and septic arthritis. *PVL* is produced by fewer than 5% of the *S. aureus* strains [6, 7].

CA-MRSA strains not only increased into the hospital setting but also their association, mainly with the skin and soft-tissue infections in the recent years. Hence the screening of *PVL* gene among MRSA has gained importance[8].

Aim of our study is to assess the prevalence of CA-MRSA and HA-MRSA and to evaluate the presence of *PVL* toxin gene as a marker to validate the genotypic definition of the CA-MRSA isolates by PCR method.

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MATERIAL AND METHODS

For this study, we included all 210 *S. aureus* isolates from various clinical specimens which were isolated from inpatients and outpatients attending different departments at our institute Bharati Vidyapeeth university medical college and hospital, Sangli, from March 2011 to March 2012.

Case identification: Case identification was done according to criteria of CDC 2005. 'community acquired MRSA infection' in patients was considered if they had no contact with a healthcare facility within the preceding two years and if the sample was obtained within 72 hours after hospital admission.

Hospital-associated *S. aureus* isolates, as ones which were cultured from clinical specimens, were obtained at > 72 hours after the hospital admission of the patients or from patients with a history of hospitalization within six months of *S. aureus* isolation date[9].

Identification of *S. aureus*

S. aureus was identified by colony morphology, catalase test, tube coagulase test, mannitol fermentation and oxidation fermentation test. Methicillin resistance was also detected by disc diffusion method using cefoxitin disc (Hi-Media, Mumbai) on Muller-Hinton agar incubated at 37ºC for 24 hours.

Detection of MRSA

Phenotypic identification of MRSA

All isolates were subjected to detect MRSA phenotypically using Cefoxitin (30µg) disc-diffusion test (Hi-Media, India) following CLSI guidelines. The zone of inhibition was measured after 24 hrs incubation at 37°C and isolates were considered as susceptible at 22mm and resistant at 21mm zone of inhibition. ATCC 43300 and ATCC 25923 were included in every batch as standard strains for MRSA and MSSA respectively.

Genotypic identification of MRSA

DNA was extracted from overnight cultures of *S.aureus* by CTAB-NaCl method[10] and was checked for quality and quantity using Nanodrop Spectrophotometer at 260/280 nm as well as visually by horizontal gel electrophoresis in 1% agarose.1µl containing 60 ng of the extracted DNA was added to 24µl of PCR amplification mix consisting of 16 µl of double distilled autoclaved water, 2.5µlof 10X Taq buffer (Tris with 15 mM MgCl2),1µlof 2.5mM dNTP mix (Merck, India), 0.5 µl of3U Taq polymerase (Merck, India), and0.5mM of each primer given in the Table: 1. Amplifications were carried out using thermal cycler with PCR conditions that consisted of 30 cycles of denaturation at 94^{0}C for 45 s, annealing at 50^{0}C for 45 s and extension at 72^{0}C for 1 min with a final extension at 72^{0}C for 2 min. The PCR products were subjected to agarose gel electrophoresis using gel red dye (Biogenei, Bangalore) and documented[11].

PCR for the detection of *mecA* was carried out following the method of Unal *et al*[12]. at I.C.M.R. Regional Medical Research Center, Belgaum. Primer sequences used for *mecA* detection were as follows,

Primer	Sequence	Amplicon size(bp)
mec A1	5'- GTA GAA ATG ACT GAA CGT CCG ATA A-3'	310(bp)
mec A2	5'- CCA ATT CCA CAT TGT TTC GGT CTA A-3'	

Detection of PVL genes: 1 µl containing 60ng of extracted *S.aureus* DNA was added to24µl of PCR amplification mix consisting of 18 µl of double distilled autoclaved water, 2.5µl of 10X Taq buffer (Tris with 15 mM MgCl2),1µl of 2.5 mM dNTP mix (Merck, India), 0.5 µl of 3U Taq polymerase (Merck, India) and 0.5mM of each primer.

Amplifications were carried out using thermal cycler with PCR conditions that consisted of initial denaturation at 94^{0}C for 99 sec followed by 10 cycles of denaturation at 94^{0}C for 60 s, annealing at 55^{0}C for 60 s and extension at 72^{0}C for 90 sec followed by 25 cycles of denaturation at 94^{0}C for 60 s, annealing at50^{0}C for 60 s and extension at 72^{0}C for 90 s with final extension at 72^{0}C for 5 min.

The PCR products were electrophoresed in 1% agarose gel and documented. PCR for the detection of *PVL gene* was carried out following the method of G. Lina, *et al.*[6] Primer sequences used for *PVL* detection were as follows,

Primer	Target gene	Sequence (5'-3')	Amplicon size(bp)
luk-PV-1	*luk –S*	ATC ATT AGG TAA AAT GTC TGG ACA TGA	433bp
luk-PV-2	*luk-F*	GCA TCA AGT GTA TTG GAT AGC AAA AGC	

RESULTS

210 *S. aureus* isolated from different samples included in this study. Among these 115(54.76%) recorded hospital associated *S. aureus* and 95(45.23%) stains were Community associated *S. aureus*. Out of these 100(47.61%) strains were MRSA and 110 (52.38%) were MSSA detected by cefoxitin disc diffusion test. Out of these 100 MRSA, 58 (58%) were CA-MRSA and 42 (42%) were HA-MRSA. These same 100 MRSA were confirmed for *mecA* gene by PCR. All 100 strains had *mecA* gene. Sensitivity and specificity of Cefoxitin disc diffusion test for detection of MRSA was 100%. We found that *PVL* toxin gene was present in 62/100 (62%) among all MRSA. Out of these 42/58 (72.41%) were CA-MRSA and 20/42 (42.61%) isolates were HA-MRSA and negative in 38/100(38%) isolates detected by PCR method shown in table No.1 and Fig 1.

Table 1 The percentage of *PVL* gene among cases of CA-MRSA and HAMRSA

Polymerase chain reaction for *PVL*	CAMRSA n=58	HAMRSA n=42	Total n=100
PVL positive isolates	42(72.41%)	20 (42.61%)	62 (62%)
PVL negative isolates	16(27.58%)	22(52.38%)	38(38%)

Abbreviations: MRSA- Methicillin-resistant Staphylococcus aureus, CA-MRSA- Community-associated MRSA, HA-MRSA- Hospital-associated MRSA, *PVL*: Panton-Valentine leukocidin.

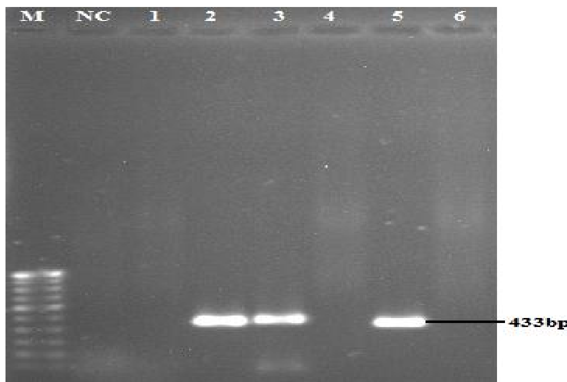

Fig. 1 Agarose gel electrophoresis showing the PCR patterns of, 433-bp amplified genes of *PVL*. Lane M: 100bp DNA ladder, Lane NC: *PVL* negative control, Lanes 1 to 6: Clinical isolates CA-MRSA.

DISCUSSION

MRSA infections are become rising problem and challenge for healthcare institutions. The data on the prevalence of MRSA among the Indian isolates of *S. aureus* is variable in the different studies. In our study we observed 47.61% MRSA is correlates with study of Loveena Oberoi *et al*. They reported 47.42% prevalence of MRSA[13].

Community-acquired MRSA is also new rising pathogen. The clinical presentation of infection by *S. aureus* might depend on a variety of factors not yet totally understood[14].

The Pathogenicity of *S. aureus* depends on various bacterial surface components (e.g., capsular polysaccharide and protein A) and extracellular proteins (e.g., coagulase, hemolysins, enterotoxins, toxic-shock syndrome toxin, exfoliatins, and Panton-Valentine leukocidin (*PVL*). However, the exact role of single virulence determinants for infection is difficult to establish.

PVL is a toxic substance produced by some strains of *S. aureus* which is associated with an increased ability to cause disease[15]. In 1932, Panton and Valentine described *PVL* as a virulence factor belonging to the family of synergohymenotropic toxins[16].

Epidemiological data and clinical data have also suggested that the high virulence possible of CA-MRSA is associated with the bearing of the *PVL* toxin gene that produces a series of chemicals contributing to the invasiveness of MRSA strains[17,18]. Due to association of *PVL* gene mainly in skin and soft tissue infection has gained importance in the detection of the *PVL* toxin in the MRSA population[8].

Changing epidemiology of CA-MRSA can become serious problem for the clinicians in the near future[19]. However, the screening for the *PVL* toxin among CA-MRSA was started recently[20].

We recorded 58% *S.aureus* strains were CA-MRSA and 42% were HA-MRSA, our result correlates with study of Hoda Kholeif *et al*. they recorded 63.04 % CA-MRSA and less than study of Sahar M Ali *et al* they reported the prevalence of *PVL* gene was 46.8 % and 12.8% among CA- MRSA and HA-MRSA isolates, respectively[21,22].

The prevalence of CA-MRSA infection varies by geographic area. Different investigators found that community-associated MRSA is up to 70% in other areas [23, 24].

We found that *PVL* gene in 72.41% CA-MRSA and 42.61% in HA-MRSA correlates with study by Ben Jomaa-Jemili *et al.* in their study they found 79% prevalence of *PVL* gene in CA-MRSA and and 51% HA-MRSA[25]. While Larsen *et al*, and Davis *et al* found that 69.4% and 54% of CA-MRSA were positive for *PVL* gene respectively. It is somewhat less than our observations[26, 27]. Prevalence of *PVL* gene in MRSA is variable around the world. its might be explained by the strong association between *PVL* gene carriage with certain *mec A* gene subtype IV & V and the distinct geographical distribution of *mec A* subtypes as proposed by various recent studies[3].

In this study *PVL* toxin gene was detected in CA-MRSA as well as HA-MRSA, irrespective of their types. Our findings agreed with few other studies which said that the presence of the *PVL* toxin could not be used as a sole marker for CA-MRSA[21, 22]. On the contrasting, several other studies have reported *PVL* toxin in 100% CA-MRSA, whereas not reported *PVL* from HA-MRSA thus they concluded that *PVL* toxin gene was a reliable marker of the CA-MRSA infections[20, 28]. Our result also suggested that, the prevalence of the *PVL* toxin varied in different geographical regions to region.

CONCLUSION

We are concluded that the prevalence of the *PVL* toxin among the *MRSA* is increased not only in CA-MRSA but also in HA-MRSA irrespective of their types. Our study also suggested that the presence of the *PVL* toxin was no longer a reliable marker for the CA-MRSA infections. Furthermore genetic studies are required to find out a reliable marker for recognition of CA-MRSA.

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