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RESEARCH ARTICLE

Assessment of PVL Gene Carriage in Methicillin-Resistant and Susceptible Staphylococcus aureus Isolates.

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Abstract: ***Background***: Various virulent factors are responsible for staphylococcal infections. The Panton-Valentine Leukocidin (PVL) gene encodes a cytotoxin associated with increased virulence. Understanding PVL gene prevalence in Methicillin-resistant S. aureus (MRSA) and methicillin-susceptible S. aureus (MSSA) is critical for guiding infection control and therapeutic strategies. ***Objective***: This study aimed to detect the prevalence of the PVL gene among clinical isolates of MRSA and MSSA. ***Methods***: Study was conducted on 100 non-duplicate S. aureus isolates collected from various clinical specimens. Isolates were classified as MRSA or MSSA using cefoxitin disk diffusion testing and confirmed by PCR amplification of the mecA gene. PVL gene detection was performed by PCR targeting the lukS-PV/lukF-PV genes. ***Results***: Out of all isolates, 42% were identified as MRSA and 58% as MSSA. The overall prevalence of the PVL gene was 32%. PVL was detected in 54.76% of MRSA and 15.51% of MSSA isolates. A statistically significant association was observed between PVL gene presence and methicillin resistance ($p < 0.05$). ***Conclusion***: The high prevalence of PVL gene observed in MRSA suggests a correlation between antimicrobial resistance and virulence potential.

Keywords: Panton-Valentine Leukocidin (PVL), Methicillin-resistant Staphylococcus aureus (MRSA), Methicillin-susceptible Staphylococcus aureus (MSSA), Virulence factors, Prevalence

INTRODUCTION

Staphylococcus aureus can cause infections ranging from simple skin and soft tissue infections to invasive diseases, such as bacteremia, endocarditis, and pneumonia [1].

Both community-associated (CA) and healthcare-associated (HA) strains pose serious clinical challenges. MRSA is responsible for approximately 100,000 deaths worldwide in 2019 [2], and continues to complicate treatment, leading to extended hospital stays and increased mortality as well as morbidity [3]. MSSA also still plays a major role in infections, often making it difficult to separate its virulence from resistance traits [1,3].

Panton–Valentine leukocidin (PVL) is a pore-forming toxin encoded by *lukS-PV* and *lukF-PV* [4,5]. PVL causing leukocyte lysis and intense inflammatory responses due to its pore forming nature [5]. Clinically, PVL-positive strains of *S. aureus* are often linked to recurrent skin abscesses and severe necrotizing pneumonia [4,6].

Existing literature indicates variable PVL prevalence among and MSSA and MRSA, depending on geography, strain lineages, and clinical source of isolates [6–8]. Latin American studies report diverse PVL carriage patterns, with documented isoform heterogeneity [7]; yet regional data particularly from the Indian subcontinent remains infrequent.

This study aims to determine the prevalence of the PVL gene among methicillin-resistant *S. aureus* and methicillin-susceptible *S. aureus* isolates and to evaluate any significant correlation with methicillin resistance.

MATERIALS AND METHODS

Study Design

This cross-sectional observational study was conducted between January and June 2023 to investigate the prevalence of the *Panton-Valentine Leukocidin* (PVL) gene among *Staphylococcus aureus* isolates, including methicillin-resistant (MRSA) and methicillin-susceptible (MSSA) strains.

Sample Collection

A total of 100 non-duplicate *S. aureus* isolates were collected from various clinical samples included pus, wound swabs, blood, and respiratory secretions at a tertiary care hospital.

Inclusion Criteria: All non-duplicate isolates phenotypically confirmed as *S. aureus*.

Exclusion Criteria: Duplicate isolates of *S. aureus* from the same patient and mixed cultures.

Bacterial Identification and Classification of MRSA/MSSA

Identification of *S. aureus* was done using Gram staining, inoculation on Blood Agar and Mannitol Salt Agar, Catalase test, Coagulase test, Phosphatase test.

All confirmed *S. aureus* strains were tested for methicillin resistance by the Kirby-Bauer disc diffusion method using cefoxitin disc (30 µg) . Zone diameters ≤19 mm was reported as methicillin-resistant and ≥22 mm was considered as methicillin sensitive [9].

Detection of the *mecA* gene done by PCR, using the following primers:
Forward: 5'-AAA ATC GAT GGT AAA GGT TGG C-3'
Reverse: 5'-AGT TCT GCA GTA CCG GAT TTG C-3'
Expected product: 533 bp.
Detection of PVL (*lukS and lukF*) Gene done by conventional PCR using the following primers [9].
Forward: 5'-ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A-3'
Reverse: 5'-GCA TCA AST GTA TTG GAT AGC AAA AGC-3'
Product size: 433 bp.

Statistical Analysis

Statistical analysis was performed using SPSS v26.0. Proportions were compared using the chi-square (χ^2) test to assess the association between methicillin resistance and PVL gene carriage. p-value < 0.05 was considered statistically significant.

RESULTS:

Prevalence of MRSA

Out of the 100 *S. aureus* isolates, 42% were confirmed as MRSA by cefoxitin screening. These same isolates were confirmed for *mecA* gene by PCR. All strains had *mecA* gene. Among all *S. aureus,* 58% were classified as MSSA.

Prevalence of PVL Gene: The overall prevalence of the PVL gene was 32%. Among PVL-positive isolates: **54.76%** were MRSA and **15.51%** were MSSA

This indicates a higher prevalence of PVL gene in MRSA strains.

Statistical Association

The association between methicillin resistance and PVL gene carriage was statistically significant.

Table 1. Distribution of PVL Gene Among MRSA and MSSA

Isolate Type	PVL Positive	PVL Negative	Total	Prevalence of PVL
MRSA	23	19	42	54.76%
MSSA	9	49	58	15.51%
Total	32	68	100	32%

DISCUSSION:

Knowledge regarding the prevalence of MRSA and its virulence factors is useful for treatment as well as control of community and hospital acquired *S. aureus* infections. PVL-positive MRSA is often associated with community-acquired infections that exhibit more aggressive clinical manifestations.

This study demonstrates a 32% overall prevalence of the *Panton-Valentine Leukocidin (PVL)* gene among *Staphylococcus aureus* isolates, with a significantly higher frequency in MRSA strains **(54.76%)** compared to MSSA (**15.51%).** These findings suggesting a strong association between methicillin resistance and PVL carriage. Previous studies had reported the prevalence of *pvl* gene was from 2% - 35% (11, 12).

Our findings are correlates with studies from Southeast Asia and parts of the Middle East, where prevalence of *PVL* in MRSA isolates ranged between 40% and 80% [13] and less than a UK-based study by Shallcross et al. [14] reported a much lower PVL prevalence of 2.1%.

Some Indian studies found similar results, emphasized that PVL was more detected in MRSA than MSSA across multiple regions. Our findings are correlates with study of Thakar et al, Ghosh et al. Rathore et al, Singh et al. Kumar et al [15,16,17,18,19]. they reported 47.2% ,34.1% ,50.2%,45.7%,51.4%respectively.

Some African studies reported, prevalence of *PVL* ranging from 17% - 74% depending on the region and strain type [20]. Study in the United States also reported higher prevalence of PVL gene in MRSA [21]. Several studies have reported variable PVL gene prevalence, influenced by regional factors, infection type, and patient demographics.

CONCLUSION:

The high prevalence of the PVL gene in MRSA isolates indicates a concerning overlap of virulence and antimicrobial resistance in local *S. aureus* strains. This significant association highlights the rising burden of PVL-positive MRSA in India and underscores the need for routine screening, surveillance, and focused infection control. Monitoring these strains is crucial for guiding appropriate therapeutic interventions.

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