Journal of Medical Bacteriology

Virulence Genes Encoding for Panton Valentine Leucocidin and Toxic Shock Syndrome Toxin in Methicillin Resistant *Staphylococcus aureus*

Manisha Jain [1], Annapurna Parida [2], Vandana Rani [2], Rajni Gaind [2]*

1 Department of Microbiology, GB Pant Institute of Medical Education and Research, Delhi, India.

2 Department of Microbiology, Vardhman Mahavir Medical College and Safdarjung Hospital, Delhi, India

ARTICLE INFO

Article type:
Research Article

Article history:

Received	15	Jan	2025
Revised	12	Feb	2025
Accepted	22	Feb	2025
Published	05	May	2025

Keywords:
Methicillin resistance, Panton valentine leucocidin toxin, Toxic shock syndrome toxin.

*Corresponding Authors: Rajni Gaind: Department of Microbiology, Vardhman Mahavir Medical College and Safdarjung Hospital, Delhi, India. *Tel*: +91-11-26733027, *E-mail:* rgaind5@rediffmail.com

ABSTRACT

Background: The outcome of Staphylococcal infections ranging from mild skin infection to fatal necrotising pneumonia is determined by the co-presence of virulence factors such as enzymes, toxins (Panton valentine leucocidin & toxic shock syndrome toxin) and methicillin resistance. In this study the association of toxin genes encoding for PVL and TSST was seen with drug resistance.

Methods: *Staphylococcus aureus* isolates collected from clinical samples (pus, tracheal aspirate, wound swabs and blood) from inpatients of the tertiary care hospital. The isolates were analysed for presence of toxin genes and drug resistance. DNA extraction was done by boiling method and target genes were identified by PCR and gel electrophoresis. The data was analysed using Microsoft excel. Chi-square test was used for comparison of qualitative data. Unpaired t test was used for comparison of quantitative data. P value less than 0.05 was taken as significant.

Results: Methicillin resistance was seen in 54.8% (108/197) of the staphylococcal isolates. There was no significant difference in MRSA and MSSA distribution amongst hospitalized or outpatients. The PVL and TSST gene was present in 123 (62.4%) and 29 (14.7%) isolates respectively. The presence of both the PVL and TSST were significantly higher in MRSA ($P \leq 0.05$).

Conclusion: There was significantly higher association of virulence markers such as PVL and TSST in MRSA. The presence of toxins should be investigated from cases of non-resolving MRSA infections to initiate treatment effective against toxins for patient management.

- ***Please cite this paper as:*** Jain M, Parida A, Sharma V, Gaind R. Genes Encoding for Panton Valentine Leucocidin and Toxic Shock Syndrome Toxin in Methicillin Resistant *Staphylococcus aureus*. *J Med Bacteriol.* 2025; **13** (2): pp.23-30. DOI: 10.18502/jmb.v13i2.18653

Introduction

Staphylococcal infection ranges from small skin problems like folliculitis and carbuncle to life threatening conditions such as pneumonia, brain abscess and toxic shock syndrome (TSS) (1). The virulence of infections and thus the outcome of patients depends not only upon the host factors such as age, diabetes or presence of other comorbidities but also on the virulence factors of the microbe (1, 2). Given the large array of staphylococcal virulence determining factors and their distinct mechanisms of actions, the molecular basis of *S. aureus* pathogenicity is considered multifactorial.

PVL toxin (belongs to family of synergohymenotropic toxin) is produced by some strains of *S. aureus* with an increased ability to cause disease (3,4). Earlier studies had shown PVL association with MSSA and community acquired infection but newer studies are inconclusive as more and more PVL association with nosocomial infection is seen (5-7). PVL toxin producing *S. aureus* has been seen to be associated with skin and soft-tissue disease and is comparatively less common in colonisation and in other forms of invasive disease, such as pneumonia, musculoskeletal disease, and bacteraemia (8-10). Limited number of studies in the literature make it difficult to ascertain the role of PVL as a predictive marker of virulence or clinically severe disease beyond certainty.

TSST-1 is encoded by the gene tst, which is carried on mobile genetic elements (MGE) named staphylococcal pathogenicity islands (Sa PAIs) that lie within the *S. aureus* chromosome (3, 11). Colonization or infection by TSST-1 superantigen producing *S. aureus* strains have adverse effect on the clinical outcome of the patients. Previous studies have shown prevalence of tsst-1 gene in *S. aureus* to be 20% (3). Toxin-suppressing antibiotics, such as clindamycin, linezolid, and rifampin, are recommended for treating such patients (12).

The methicillin resistant isolates are generally resistant to the multiple antimicrobial drugs thus limiting the therapeutic options. Moreover, toxin producing MRSA isolates are emerging throughout the world (5, 6, 13). In cases of co-presence of multiple virulence factors the treatment regimen needs to be regulated.

The present study thus planned to evaluate the association of PVL and TSST gene in the methicillin sensitive and resistant *S. aureus* isolates.

Materials and Methods

The study was conducted in the Department of Microbiology, VMMC & Safdarjung Hospital. It is a prospective observational study. The study was approved by institutional ethical committee vide letter no. S. No IEC/VMMC/SJH/Project/ December/2018-18 dated 31.01.2019. All clinical isolates of *Staphylococcus aureus* isolated from samples like pus, tissue, tracheal aspirate, BAL and blood were included in this study. For better analysis samples without completed TRF (test requisition forms) were rejected. Samples were processed as per standard microbiological techniques. Briefly they were streaked on blood agar and MacConkey agar and incubated for 24 hours at 37 ºC. White or golden yellow coloured, pin head colonies with or without haemolysis on blood agar were stained with gram stain. Gram positive, cluster forming and catalase producing cocci were further analysed by furazolidone sensitivity, Coagulase test, mannitol fermentation and Deoxyribonuclease (DNase) production.

Staphylococcus aureus Screening

Isolates phenotypically identified as *Staphylococcus aureus* by coagulase test, anaerobic fermentation of mannitol and DNase production were included in this study.

In test tubes containing sterile normal saline 3-5 colonies were inoculated to make suspension. One

ml of this suspension was transferred to Eppendorf's tubes. DNA was extracted by boiling at 95 °C. Nanodrop spectrophotometer (Nanodrop 2000c ThermoScientific) was used for DNA quantification. Extracted DNA were preserved at -80 ºC for future reference.

Molecular detection of target genes

Target gene identification was done through polymerase chain reaction (PCR) followed by gel electrophoresis. DNA amplification was performed in a Master cycler® Pro PCR system (Eppendorf, Hamburg, Germany).

The Master Mix of 25µl reaction volume were prepared using template DNA, 1X PCR buffer, forward and reverse primers, 200 µM of each deoxy nucleoside triphosphate(dNTPs), 1.5mM MgCl2 and 1U Taq DNA Polymerase. The amplification was done in Master cycler® Pro PCR system (Eppendorf Master cycler EPS thermo-module, Hamburg, Germany) using following conditions (initial elongation at 94 °C for 5 min; followed by 30 cycles of 94 °C for 45 seconds, 56 °C for 45 seconds and 72 °C for 1min; and a final extension step at 72 °C for 5 min).

The designed primers used were PVL-F (forward primer) GCTGCACAAAACTTCTTGGAATAT of 85 base pairs and PVL-R (reverse primer) AGGACACCAATAAATTCTGGATTG of 85 base pairs for target gene pvl. For tst H primer used were ACCCCTGTTCCCTTATCATC (forward primer) and TTTTCAGTATTTGTAACGCC (backward primer).

After target amplification gel electrophoresis was done with 100 base-pair molecular size standard ladder (Thermofischer Scientific, Massachusetts, USA) in 1.5% weight/volume(w/v) agarose gel prepared in 0.5X Tris-borate ethylene diamine tetra acetic acid buffer (Sigma-Aldrich Pvt Ltd., India) and containing 0.04 µg/ml of ethidium bromide (Sigma-Aldrich Pvt Ltd., India). With help of UV illuminator and Gel DocTM (Bio-Rad, Hercules, California, USA) the amplicon bands were photographed.

Positive control for pvl gene was control strain of *Staphylococcus aureus* USA300. Reference strains for tst H gene used were control strain of *Staphylococcus aureus* Mu50.

Statistical analyses

The data was entered in excel sheet and comparison between two parameters was done by using Chi square ($\chi 2$) test and p value was calculated for each parameter. P value less than 0.05 was taken as statistically significant.

Results

Over a period of 3 months 197 consecutive *Staphylococcus aureus* were included in this study. Age of the patients ranged from two months to 86 years with median age of 25 years. A total of 23 samples were from paediatric age group. Out of 197 patients, 119(60%) were male and 78(40%) were female. A total of 152(77%) *S. aureus* were isolated from pus samples, 23(11.6%) from wound swabs and 11(10.7%) were from blood. Another 11 isolates were from other clinical samples such as tissue, high vaginal swabs and tracheal aspirates. Patients from OPD (outpatient department) were 100(50%) and 97(50%) were inpatients among these five were under treatment in intensive care units (ICU) and 92 patients were in different wards (like burns, general surgery, orthopaedics, obstetrics and others). Methicillin resistance screening test by using Cefoxitin disk was positive in 108 isolates (54.8%) and 89 (46%) were identified as MSSA (methicillin sensitive *Staphylococcus aureus*). The distribution of MRSA and MSSA in OPD and inpatients is shown in table 1.

Inducible clindamycin resistance was detected in 23 of the isolates of which 16 (69%) were MRSA and 7(31%) were MSSA, Table 2. Clindamycin

was not significantly associated with methicillin resistance, p value >0.05(0.1306).

Table 1. Prevalence of MRSA and MSSA in outpatient and inpatient departments.

Count of Location type	MRSA screening test		
Location type	MRSA	MSSA	Grand Total
ICU	3	2	5
WARDS	54	38	92
OPD	51	49	100
Grand Total	108	89	197

PVL gene was detected in 62.4% (123/197) of all *Staphylococcus aureus*. PVL genes was present in 76 (61.8%) MRSA and 47 (38.2%) MSSA isolates and the association with methicillin resistance was found to be significant, p value <0.05(0.011), Table 3.

Table 2. Methicillin resistance relation to inducible clindamycin resistance

	Inducible Clindamycin resistance		
	Present	Absent	Total Number(n)
MRSA	16	92	108
MSSA	7	82	89
Total	23	174	197

PVL gene was found in 64% (n=64) of *S. aureus* isolated from OPD samples which is similar to PVL positive inpatient isolates, 60% (n=59) and the difference was not statistically significant, p value = 0.05(0.055), Table 4.

The tst gene was present in 14.7% of all staphylococcal isolates.

Table 3. Prevalence of PVL in MRSA vs MSSA.

	PVL positive	PVL negative	Total Number(n)
MRSA	76	32	108
MSSA	47	42	89
Total	123	74	197

The test gene was present significantly in MRSA (24%, n=26) as compared to only 3% of MSSA isolates, p value <0.05(0.0001), Table 5.

Table 4. Prevalence of PVL gene in different location type.

Location type	PVL positive	PVL negative	Total Number(n)
ICU	2	3	5
WARDS	57	35	92
OPD	64	36	100
Total	123	74	197

When trying to analyse the correlation between the presence of PVL and TSST it was observed that there was no correlation between the two genes. The presence of one gene was independent to the presence of another gene, p value > 0.05(0.106), Table 6.

Table 5. Prevalence of TSST in MRSA vs MSSA

	TSST Positive	TSST Negative	Total Number(n)
MRSA	26	82	108
MSSA	3	86	89
total	29	168	197

Table 6. Correlation of PVL and TSST.

	TSST Positive	TSST Negative	Total Number(n)
PVL Positive	22	101	123
PVL Negative	7	67	74
Total	29	168	197

Discussion

Staphylococcus aureus is present as a part of normal microbial flora at sites like the nares, axilla, vagina, pharynx, and skin in nearly one third of adult population (1). The most common infections caused by *S. aureus* are abscesses of skin and soft tissues that can spread locally (carbuncle, cellulitis, impetigo bullosa, or wound infection) or become invasive (1,2). The progression of staphylococcal infection depends manifold on the virulence factors of the organism such as enzymes, toxins and drug resistance.

Of the 197 samples that yielded *S. aureus* in culture, 118(60%) were from male and 79 (40%) from female patients but the difference was not statistically significant. The findings are similar to studies having reported statistically insignificant difference in the prevalence of *S. aureus* in male and females. In this study *S. aureus* was identified mainly from pus samples (n=152, 77%) collected from different sites. Though the prevalence of *S. aureus* is higher than the previous reports from same parts of India (61-64%), but it supports the fact that SSTI are the most common infections caused by *S. aureus* (14).

Methicillin resistance has been one of the most studied virulence determinants to understand the prognosis of any staphylococcal infection. Genes that are responsible for methicillin resistance also contributes for resistance to other antibiotics so identifying MRSA is an important step to regulate treatment.

MRSA prevalence in our study was 54%. This is same as the national prevalence but higher than the global average of 41.2% (2009) (15). No significant difference was found between the prevalence of MRSA in hospital acquired infections (53%, n=57) and community acquired infections (47%, n=51). It could be because patients coming to these tertiary care centers were mostly referred and already exposed to antibiotics so a clear distinction of community and hospital acquired infection was not possible. This supports the fact that methicillin resistance has gradually spread into communities.

In the present study all isolates were penicillin resistant. CA MRSA strains are less resistant than HA MRSA and most patients can be treated with aminoglycosides, fluoroquinolones, erythromycin or clindamycin (15).

With increased MRSA prevalence use of clindamycin as a treatment option has increased. MRSA strains that are susceptible to clindamycin in disk diffusion test, but resistant to erythromycin, can become resistant to clindamycin due to the presence of erythromycin ribosomal methylase (*erm*) genes. This can lead to clindamycin treatment failure in presence of any macrolide. Previous studies had found the prevalence of inducible clindamycin resistance to be 43%-78% irrespective of age and methicillin susceptibility status (16). In this study 23 of 197 (11%) were found to show inducible clindamycin resistance by Kirby bauer disk diffusion method. 70% of these cases were associated with MRSA isolates. Further genetic study needs to be done to see the erm gene presence in the population.

Besides clindamycin other drugs having good susceptibility were cotrimoxazole (96%) followed by gentamycin (77%). There was no discernible difference in resistance to these drugs for MRSA or MSSA isolates. In a previous study done from same part of India resistance to gentamycin was not there in comparison to 18 % in our study (16,17). In case of methicillin resistance other treatment options such as gentamycin and

cotrimoxazole are being increasingly used. Increased resistance for gentamycin can be attributed to these antibiotic selective practices.

Vancomycin has been often used as a last resort drug for the treatment of MRSA infections. In the present study also all the *Staphylococcus* isolates were sensitive to vancomycin and linezolid. Thus, these drugs can still be used for severe life-threatening MRSA infection however the use should be regulated as resistance has started to develop against these agents also.

Routinely species identification and susceptibility testing are done to guide the clinicians however to characterize *Staphylococcus aureus* study of virulence determinants is important. Presence of toxins like PVL and TSST has been seen to be associated with poor prognostic outcomes of *S. aureus* infections. Necrotizing-pneumonia caused by PVL positive *S. aureus* is associated with a mortality rate of up to 75% (8,10). No widespread prevalence studies of PVL and TSST genes are present for our population. In this study 62% of all *S. aureus* isolates were found to carry PVL gene. The prevalence of PVL from blood samples was only 50%. This discrepancy might be due to the very small number of blood samples taken for this study. Previous Indian studies had also shown a higher prevalence of 62-64% in comparison to a very low prevalence seen internationally (5-35%) (6, 9, 10, 18-20). The authors believe the difference in the prevalence of PVL could be because of selection bias of patients as most of the patients coming to a tertiary care set up are already exposed to antibiotics and unintentionally only the ones with non-responding or severe infection gets included in the study group. The prevalence of PVL gene in MRSA strains was higher at 70% as compared to 52% in MSSA. This is somewhat similar to previous studies having seen higher PVL prevalence of approximately 85.1% in MRSA and of 48.8% in MSSA (10,18,19,20). There is no statistical significance between PVL positivity in isolates from OPD setting to hospitalized patients in this study in contrast to previous studies showing significant association of PVL gene with CA-MRSA and recommending screening of CA-MRSA isolates for PVL gene (3,4). Our finding contradicts the previous belief that PVL is more prevalent in community acquired Staphylococcal infections. This can be considered as a result of gradual increase in hospitalization where patients normal microbial flora gets replaced with prevalent hospital strains. On discharge patients carry these new strains back to the community.

Toxic shock syndrome is a multisystem disease with rapid onset fever, hypotension, erythematous rash, and mucosal hyperemia, followed by desquamation and multiorgan involvement (21). TSS toxin was considered to be associated with tampon use but recent studies had shown increased incidence amongst non-tampon users (22). In our study 14.7% *S. aureus* isolates carried the tst gene. This is similar to the global prevalence rate which is less than 20% (3,13, 22). There was significant difference in the association of tst gene to MRSA (24%, n=24) and MSSA isolates (3%, n=5). This differs from previous study where MRSA association (0.2%) with tst is less than MSSA (16%) (3,23). In a previous study 44% of all *Staphylococcus aureus* from blood sample and around 72% of MRSA were reported to carry the tst gene (24). In the present study also 30% (n=3) of the isolates from blood carried the tst gene in comparison to 14% (n=19) of the isolates obtained from pus samples. Screening for tst gene in all *S. aureus* isolate provides no extra benefit in treatment of patients but if done only from suspected cases of bacteremia it can help for early decision making to add toxin neutralizers in severe life-threatening conditions.

Staphylococcus aureus is associated with multiple virulence factors as seen in the present study. The PVL gene was seen in 70% and the tst gene was present in 24% of MRSA isolates. Both the genes were present simultaneously in 11 % of all the *Staphylococcus aureus* isolates but surprisingly in 86% of MRSA isolates. In case of

non-responding patients and in severe life-threatening infections authors recommend the molecular characterization of *Staphylococcus aureus* isolates as there is co-existence of multiple virulence factors.

Conclusion

Results of this research could help the clinicians in better management of patients by regulating the therapeutic regimen as per the presence of toxins in the isolate. Further outcome studies can be planned to look for the clinical significance of such multiple virulence factors.

Acknowledgements

None.

Funding Information

No funding was received for this study.

Ethics approval and consent to participate

The study was approved by institutes ethical committee.

Conflict of interest

The authors have no conflict of interest to declare.

References

1. Tong SY, Davis JS, Eichenberger E, et al. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev* 2015; 28(3):603-61.
2. Archer GL. *Staphylococcus aureus* : A Well–Armed Pathogen. *Clin Infect Dis* 1998; **26**: 1179-81.
3. Dinges MM, Orwin PM, Schlievert PM. Exotoxins of *Staphylococcus aureus*. *Clin Microbiol Rev* 2000; **13**:16-34.
4. Panton PN, Valentine FCO. Staphylococcal Toxin. *Lancet* 1932; **219**: 506-8.
5. Vandenesch F, Naimi T, Enright MC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying panton-valentine leukocidin genes: Worldwide emergence. *Emerg Infect Dis* 2003;9: 978-84.
6. Patil NR, Ghorpade MV, Roy S, et al. Evaluation of panton-valentine leukocidin gene by polymerase chain reaction in community-acquired methicillin resistant *Staphylococcus aureus*. *Int J Recent Sci Res* 2016; **7**:8132-5.
7. Shallcross LJ, Fragaszy E, Johnson AM, et al. The role of the Panton-Valentine leucocidin toxin in staphylococcal disease: A systematic review and meta-analysis. *Lancet Infect Dis* 2013; **13**:43-54.
8. Gillet Y, Issartel B, Vanhems P, et al. Association between *Staphylococcus aureus* strains carrying gene for panton-valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. *Lancet* 2002; **359**:753-9.
9. Lina G, Piémont Y, Godail-Gamot F, et al. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis* 1999; **29**:1128-32.
10. Holmes A, Ganner M, McGuane S, et al. *Staphylococcus aureus* isolates carrying panton-valentine leucocidin genes in England and Wales: Frequency, characterization, and association with clinical disease. *J Clin Microbiol* 2005; **43**:2384-90.
11. Becker K, Friedrich AW, Lubritz G, et al. Prevalence of genes encoding pyrogenic toxin superantigens and exfoliative toxins among strains of *Staphylococcus aureus* isolated from blood and nasal specimens. *J Clin Microbiol* 2003; **41**:1434-39.
12. Hodille E, Rose W, Diep BA, et al. The role of antibiotics in modulating virulence in

Staphylococcus aureus. *Clin Microbiol Rev* 2017; **30**(4):887-917.
13. Fitzgerald JR, Sturdevant DE, Mackie SM, et al. Evolutionary genomics of *Staphylococcus aureus*: Insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic. *PNAS* 2001; **98**: 8821-6.
14. Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, India. Methicillin resistant *Staphylococcus aureus* (MRSA) in India: prevalence & susceptibility pattern. *Indian J Med Res* 2013 ; **137**(2):363-9.
15. Lakhundi S, Zhang K. Methicillin-Resistant *Staphylococcus aureus*: Molecular Characterization, Evolution, and Epidemiology. *Clin Microbiol Rev* 2018; **31**(4):e00020-18.
16. Siberry GK, Tekle T, Carroll K, et al. Failure of Clindamycin treatment of methicillin-resistant *Staphylococcus aureus* expressing inducible clindamycin resistance in vitro. *Clin Infect Dis* 2003; **37**:1257-60.
17. Mohanty S, Kapil A, Dhawan B, et al. Bacteriological and antimicrobial susceptibility profile of soft tissue infections from Northern India. *Indian J Med Sci* 2004; **58**(1):10-15.
18. Moussa I, Shibl AM. Molecular characterization of methicillin-resistant *Staphylococcus aureus* recovered from outpatient clinics in Riyadh, Saudi Arabia. *Saudi Med J* 2009; **30**(5):611-7.
19. Afroz S, Kobayashi N, Nagashima S, et al. Genetic characterization of *Staphylococcus aureus* isolates carrying Panton-Valentine leukocidin genes in Bangladesh. *Jpn J Infect Dis* 2008, **61**:393-6.
20. Aires-de-Sousa M, Conceição T, De Lencastre H. Unusually high prevalence of nosocomial panton-valentine leukocidin-positive *Staphylococcus aureus* isolates in cape verde islands. *J Clin Microbiol* 2006; **44**:3790-3.
21. Todd J, Fishaut M, Kapral F, et al. Toxic-shock syndrome associated with phage-group-i staphylococci. *Lancet* 1978; **312**:1116-8.
22. El-Ghodban A, Ghenghesh KS, Márialigeti K, et al. PCR detection of toxic shock syndrome toxin of *Staphylococcus aureus* from Tripoli, Libya. *J Med Microbiol* 2006; **55**:179-82.
23. Schlebusch S, Schooneveldt JM, Huygens F, et al. Prevalence of *Staphylococcus aureus* strains in an Australian cohort, 1989-2003: Evidence for the low prevalence of the toxic shock toxin and Panton-Valentine leukocidin genes. *Eur J Clin Microbiol Infect Dis* 2009, **28**:1183-9.
24. Peck KR, Baek JY, Song JH, et al. Comparison of genotypes and enterotoxin genes between *Staphylococcus aureus* isolates from blood and nasal colonizers in a Korean hospital. *J Korean Med Sci* 2009; **24**(4):585-91.