Study of virulence factors of Staphylococcus aureus from various clinical specimens in the tertiary care hospital.

1).Dr. Nilima Sanjay Naik
AssistantProfessor, Department of General Pathology and Microbiology,
Bharati vidyapeeth (Deemed to be university)
Dental College and hospital,
Sangli. Maharashtra
Mail ID-patilnilima@rocketmail.com

2).Dr.Sachin B Mangalekar, HOD &Professor, Department of Periodontology, Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, Wanlesswadi, Sangli-416414, Maharashtra
Email: drsachinbm@yahoo.com.

3. Dr. Sunil Tejaswi K L, Reader,
Department of Conservative and Endodontics, JSS DENTAL COLLEGE AND HOSPITAL, Mysore.
sunilkasipalli@gmail.com

4). Dr Rakesh Oswal
MDS Oral and MaxillofacialSurgeon, Professor and HOD,
Prakash Institute of Medical Science and Reaserch Health Centre,Islampur
drrgokol@yahoo.com

5)Dr. Shraddha Shetti
Associate Professor,
Department of Orthodontics and Dentofacial Orthopedics.
Bharati Vidyapeeth (Deemed to be University), Dental College and Hospital, Sangli. 416416.
Maharashtra.
Email:
shraddhashetti@gmail.com

6)Dr. Sangamesh G. Fulari
Associate Professor,
Department of Orthodontics and Dentofacial Orthopedics.
Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital. Sangli. 416416. Maharashtra
Email id : drsangameshfulari@gmail.com

Corresponding author : Dr.Sachin B Mangalekar, HOD &Professor, Department of Periodontology, Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, Wanlesswadi, Sangli-416414, Maharashtra Email: drsachinbm@yahoo.com

Aim and Objective:

This study aimed to identify the pathogenic gene in *Staphylococcus aureus*strain isolated from clinical specimens of burn patients in tertiary care hospital.**Material and Methods:** A total of 100 strains of *Staphylococcus aureus*were isolated and confirmed by using a standard biochemical and laboratory methodology in accordance with CLSI principles. All confirmed *S.aureus* were subjected to detection of *femB*, *mecA* and *PVL* markers by PCR.**Results:** Of the 100*S. aureus* strains, we found *mecA* gene in 30

eISSN 1303-5150

strains.Among these isolates, 10 strains did not possess the *femB* marker out of which 6 were *mecA* positive and 4 were *mecA* negative. *PVL* (lukS- lukF) markers were showed in 62 strains of *S. aureus*.**Conclusions:**Our study has shown a relatively high prevalence rate of *PVL* positivity in isolates fromtertiary care hospital. *PVL* carriage was associated with heterogeneous MRSA. We are concluded that the need of genetic studies on *S.aureus* infections in India and other parts of the world as well.
Abbreviations: *Staphylococcus aureus,PVL* (lukS- lukF), *fem B*, *mecA,* MRSA

DOI Number: 10.14704/NQ.2022.20.12.NQ77211 **NeuroQuantology2022;20(12): 2352-2360**

Introduction:

In the recent years, incidence of infection disease was increased, include infection diseases caused by *Staphylococcus aureus*. *Staphylococcus aureus* is an important pathogen responsible to cause a wide range of human infections such as minor skin infections, pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses including life threatening diseases [1].One major obstacle for the treatment of *S. aureus* infections is the development of antibiotic resistance in the isolates. This resistance phenomenon originated with penicillin, the first broad-spectrum antibiotic, which was discovered in the 1940s. Its adaptive power to antibiotics has resulted in the emergence of methicillin-resistant S. aureus (MRSA) in the beginning of the 1960s.Methicillin resistance is mediated by an acquired penicillin-binding protein, PBP2a, a peptidoglycan transpeptidase encoded by the mecA gene that has low affinity for beta-lactams. Thus, when the four native peptidoglycan synthetases (penicillin-binding proteins 1, 2, 3, and 4) are bound and inactivated by beta-lactams, PBP2a can still affect cell wall synthesis. *mecA* is harbored on the Staphylococcal chromosomal cassette mec (SCCmec), a genetic element that integrates site-specifically into the *S. aureus* chromosome [2].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.

Molecular characterization of SCCmec types of MRSA is very essential for studying the epidemiology of MRSA. The emergence of MRSA has increased and becoming a serious concern world-wide including India.

The pathogenicity of *S. aureus* is related to a number of virulence factors that allow the organism to adhere, avoid the immune system and cause harmful effects to the host. The Pathogenicity of *S. aureus* depends on various bacterial surface components e.g., capsular polysaccharide and protein A and extracellular proteins like, coagulase, hemolysins, enterotoxins, toxic-shock syndrome toxin, exfoliatins, andvirulence factors, like *mec A* gene, Panton-Valentine-leukocidin (*PVL*) etc.However, the exact role of single virulence determinants for infection is difficult to establish.

PVL is a two-component *S. aureus* pore-forming protein encoded by the *lukF-PV* and*lukS-PV* genes. *PVL* is considered one of the important virulence factors of *S. aureus* responsible for destruction of white blood cells, necrosis and apoptosis. Panton Valentine leukocidin was named after Sir Philip Noel Panton and Francis Valentine who associated it with soft tissue infections in 1932. The first *PVL* positive MRSA was noticed in the late 1990s and these strains got scattered worldwide in recent years. These virulence genes play very important roles in bacterial pathogenesis and S*. aureus*could not be an exception to this. In addition to the limited treatment options, *S.aureus* strains acquire

eISSN 1303-5150

and express numerous virulence determinants that continue to increase its ability to cause a wide spectrum of human disease [3,4].

Data suggests these *PVL* producing *S. aureus* strains have high virulence. Limited data is available on the frequency of PVL positive *S. aureus* isolates from various other clinical infections.

FemB encodes enzyme in cross-linkingpeptidoglycan in *S. aureus*.*FemB* gene is used for spesies *S. aureus* confirmation [5]. Comprehensive literature search on data in India, suggests lack of data on the role of *PVL* in pathogenicity of *S. aureus* infections. Moreover, there is undue scarcity of data regarding presence of *pvl* gene *S. aureus* bacteraemia cases and its association with methicillin resistance[6].Having knowledge about the prevalence of MRSA and their virulence factors is useful for treatment and control of *S. aureus* infections

Therefore our study was aimed to determine the prevalence of *mec A, fem B and PVL* genes among *S. aureus* isolates from various clinical samples usingpolymerase chain reaction (PCR) in the tertiary care hospital.

MATERIAL AND METHODS

Source of sample: For this study, we included 100*S. aureus* isolates fromvarious clinical specimens which were isolated from inpatientsand outpatients attending different departments at our instituteBharati Vidyapeeth university medical college and hospital,Sangli.

Identification of *S. aureus*

S. aureus was identified by colony morphology, catalase test,tube coagulase test, mannitol fermentation test as per CLSI guideline.

Genotypic identification *of mecA* and *femB*

DNA was extracted from overnight cultures of *S.aureus* byCTAB-NaCl method [7]and was checked for quality andquantity using Nanodrop Spectrophotometer at 260/280 nm aswell as visually by horizontal gel electrophoresis in 1%agarose.1µl containing 60 ng of the extracted DNA was addedto 24µl of PCR amplification mix consisting of 16 µl of doubledistilled autoclaved water, 2.5µlof 10X Taq buffer (Tris with15 mM MgCl2),1µlof 2.5mM dNTP mix (Merck, India), 0.5 µlof3U Taq polymerase (Merck, India), and0.5mM of eachprimer given in the Table: 1. Amplifications were carried outusing thermal cycler with PCR conditions that consisted of 30cycles of denaturation at 94^0C for 45 s, annealing at 50^0C for45 s and extension at 72^0C for 1 min with a final extension at72^0C for 2 min. The PCR products were subjected to agarosegel electrophoresis using gel red dye (Biogenei, Bangalore) anddocumented[8].

PCR for the detection of *mecA* was carried out following themethod of Unal *et al*[9] and detection of *femB gene* was carriedout following the method of G. Jonas, *et al.* at I.C.M.R. Regional Medical ResearchCenter, Belgaum. Primer sequences used for *mecA* detection

were as follows,

eISSN 1303-5150

Table 1. Sequence of oligonucleotide primers for *mec A* and *fem B gene*

Target Gene	Primer name	Nucleotide sequence (5'-3')	Product length (base pairs)
Mec A gene	*Mec* A1	GTA GAA ATG ACT GAA CGT CCG ATA A	310
	Mec A2	CCA ATT CCA CAT TGT TTC GGT CTA A	
Fem B gene	*Fem* B1	TTA CAG AGT TAA CTG TTA CC	651
	Fem B2	ATA CAA ATC CAGCAC GCT CT	

Detection of PVL genes: 1 µl containing 60ng of extracted*S.aureus* DNA was added to24µl of PCR amplification mixconsisting of 18 µl of double distilled autoclaved water, 2.5µlof 10X Taq buffer (Tris with 15 mM MgCl2),1µl of 2.5 mMdNTP 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 PCRconditions that consisted of initial denaturation at 94^0C for 99sec followed by 10 cycles of denaturation at 94^0C for 60 s, annealing at 55^0C for 60 s and extension at 72^0C for 90 secfollowed by 25 cycles of denaturation at 94^0C for 60 s,annealing at50^0C for 60 s and extension at 72^0C for 90 s withfinal extension at 72^0C for 5 min.

The PCR products were electrophoresed in 1% agarose gel anddocumented. PCR for the detection of *PVL gene* was carriedout following the method of Lina, *et al* .[10]. Primer sequencesused 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	

eISSN 1303-5150

Result:

In this study total 100confirmed isolates of*S.aureus* from various clinical specimens like Pus, wound Swab, blood, urine, fluids etc were subjected to detection of *fem B, Mec A* and *PVL* gene markers by polymerase chain reaction.Among these isolates we found *mecA* gene in 30 (30%) strains of *S. aureus* these strains were considered as MRSA and 60 were negative for *mecA*gene were considered as Methicillin sensitive Staphylococcus aureus (MSSA) as PCR is gold standard method for detection of MRSA.

Among the 100 isolates, 10 (10 %) did not possess the *femB* marker out of which 6 were *mecA* positive and 4 were *mecA* negative (Table No.1). PCR for the *PVL* marker*lukS- lukF-PV* revealed that 26out of 30 *mecA* positive isolates harbored this gene while it was 36 out of 70 (48.8%) in *mecA* negative. Out of 100 *S. aureus* 62 strains showed *lukS- lukF* markers (Table No.2).

Table 1. Result of association of *MecA*, with *FemB* markers among *S.aureus*

	Mec A +ve MRSA(n=30)	*Mec A –ve* MSSA (n=70)
femB +ve	24	66
femB -ve	06	04
Total	30	70

Table 2. Result of association of MecA and lukS- lukF markers among *S.aureus*

	Mec A +ve MRSA(n=30)	*Mec A –ve* MSSA(n=70)
PVL +ve	26	36
PVL -ve	04	34
Total	30	70

Discussion:

The presence of virulence genes in the *S. aureus* either the *mec A* gene or *PVL* gene in the increases their ability to resist antibiotics through altered gene products. The involvement of carriers is another important factor which spreads the infections in hospitals. Several studies have reported the use of *mecA* as marker for MRSA detection and *fem B*genes for recognition of *S. aureus* species.From the statistical analysis, the prevalence of MRSA was calculated as 30%. The study by Tacconelli E *et al.*, [11] showed similar results. In a study from north India the prevalence of MRSA was 46% [12]. It is higher than this study.

PVL-positive *S. aureus*, as a rapidly emerging worldwideand become the problem to global

eISSN 1303-5150

public health. Even though heightened surveillance and improved case recognition might have played a role in increased reporting of such strains, the rapid growth of *PVL*-carrying *S. aureus* nonetheless should not be undermined. *PVL* positive CA-MRSA infections are common in parts of Europe and USA. The first *PVL* positive MRSA was noticed in the late 1990s and these strains got scattered worldwide in recent years. Data suggests these PVL producing MRSA strains have high virulence.Comparatively, countries in western and central Africa boast a higher prevalence rate at 57%, raising valid concerns for the spread of virulent *PVL*-positive MRSA strains [13].

Similarly, data from across the world a varied picture for the prevalence of PVL-positive *S. aureus*: 30% in Germany [14], 11.6% in Singapore [15], 12.8% in China [16) and a striking 97% in the United States [17].Limited datais available on the frequency of *PVL* positive *S. aureus* isolates from various other clinical infections.

Comprehensive literature search on data in Northern India, suggests lack of data on the role of *PVL* in pathogenicity of *S. aureus* infections. However, the worldwide scheme of *PVL* among MRSA isolates varies. A lower prevalence of *PVL* has been reported from other parts of world 5% in France and 14.3% in Bangladesh [18,19] reflecting the significant variation in prevalence of *PVL* among geographical areas and communities. H. Kaur et al. [20], from India, have reported overall 62.85% prevalence of PVL among MRSA andthis is correlate to present study, we found 62% but is much higher than most reports worldwide.

According to some other reports, the prevalence of *PVL* positive *S. aureus* is less than 5% in France and 35% in Cape Verde Islands [21,22]. D Souza et al. reported 64% *PVL* positive MRSA from Mumbai [23], which is quite high, compared to other countries and in our study 62% *PVL* positive *S.aureus* was seen. The high prevalence of PVL may also be due to misuse of antibiotics in the country causing selective pressure for development of resistant strains along with the virulence factor i.e. *PVL*.The high percentage of *PVL* producing *S.aureus* is interesting and can be due to the carriage of large parts of *SCC mec* including the *luk-F and luk-S* genes to other resistant strains of *S.aureus* by various ways of recombination. *PVL* has been detected only occasionally in strains of *S. aureus* from Western Europe (5%) [24].

Kobayashi et al. reported that though *femB* genes is detectable only in *S. aureus,* an absence of *femB* gene does not mean that the isolate is not *S. aureus.* They showed up to 3% of *S.aureus* isolates in their study were negative for *femB*. In our study, we found 10% *femB* negative isolates out of which, 6 were *mecA* positive, and 4 were *mecA* negative. Therefore we conclude that *femB* negative *S.aureus* may be present in much higher numbers in clinical specimens than previously reported. This is correlates with the studies of Kobayashi et al [25].

Conclusion:

Conclusions: In recent years, increased resistance has been a serious threat.Our study has shown a relatively high prevalence rate of *PVL*positivity in isolates from tertiary care hospital. As *PVL* is a marker of pathogenicity and *PVL* positive strains are more virulent, these cases need to be monitored and adequately treated. We are concluded that the appropriate clinical and public health measures such as screening should be undertaken for *PVL* positive *S. aureus* infections and there is further need of genetic studies on *S.aureus* infections in India and world as well.

eISSN 1303-5150

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgement: Authors thank to contribution of staff of I.C.M.R. Regional Medical Research Center, Belgaum, Department of Microbiology for their immense help and support and

References:-

1. Ki V, Rotstein C. Bacterial skin and soft tissue infections in adults: A review of their epidemiology, pathogenesis, diagnosis, treatment and site of care. Can J Infect Dis Med Microbiol 2008;19(2):173-184.
2. Nilima R. Patil, M.V. Ghorpade et al, Association of virulence factor (panton–valentine leukocidin) with mecagene in staphylococcus aureus isolates in tertiary care hospital, asian j pharm clin res, vol 11, issue 2, 2018, 113-116
3. Diep BA, Chambers HF, Graber CJ, Szumowski JD, Miller LG, Han LL, et al. Emergence of multidrug-resistant, community-associated, methicillin-resistant Staphylococcus aureus clone USA300 in men who have sex with men. Ann Intern Med 2008;148:249-57.6.
4. Gilbert M, MacDonald J, Gregson D, Siushansian J, Zhang 6. K, Elsayed S, et al. Outbreak in alberta of community-acquired (USA300) methicillin-resistant Staphylococcus aureus in people with a history of drug use, homelessness or incarceration. CMAJ 2006;175:149-54
5. Towner K J et al Development and evaluation of a PCR-based immunoassay for the rapid detection of methicillin-resistant Staphylococcus aureus *J. Med. Microbiol,* 1998;**47** pp 607–613.
6. Rani JaiswalAditi GargPiyush TripathiVimala Venkatesh,Epidemiology of Panton Valentine Leukocidin in clinical Staphylococcus aureus isolates - A prospective study at a tertiary care centre in North India. Clinical Epidemiology and Global Health 15 (2022) 101006
7. Ausubel FM, Brent R, Kingston R, Moore DE, Seidman, *et al*. Protocols in Molecular Biology, 4th edn. John Wiley and Sons Inc (1999). New York.
8. Jonas D, Grundmann H, Hartung D, Daschner FD, Towner KJ. Evaluation of the mecA-fem B Duplex Polymerase Chain Reaction for Detection of Methicillin Resistant Staphylococcus aureus. Eur J Clin Microbiol Infect Dis 1999; 18: 643-47.
9. Unal S, Werner K, Degirolami P, Barsanti F, Eliopoulos G. Comparison of tests for detection of methicillin resistant Staphylococcus aureus in a clinical microbiology laboratory. Antimicrob Agents Chemother(1994); 38:345-47.
10. Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, *et al*. Involvement of Panton- Valentine leukocidin-producing Staphylococcus aureus in primary skin infections and pneumonia. Clin Infect Dis (1999);29(5):1128-32.
11. Tacconelli E, Venkataraman L, De Girolami PC, D Agata EM. Methicillin-resistant *Staphylococcus aureus* bacteraemia diagnosed at hospital admission: distinguishing between community-acquired versus healthcare-associated strains. *J Antimicrob Chemother*. 2004;53(3):474-79

eISSN 1303-5150

12. Arora S, Devi P, Arora U, Devi B. Prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) in a tertiary care hospital in northern India. *J Lab Physicians.* 2010;2:78–81.
13. S.Breurec, C. Fall, R. Pouillot et al., "Epidemiology of methicillin-susceptible *Staphylococcus aureus* lineages in five major African towns: high prevalence of Panton-Valentine leukocidin genes," *Clinical Microbiology and Infection*, vol. 17, no. 4, pp. 633–639, 2011.
14. S. Monecke, P. Slickers, M. J. Ellington, A. M. Kearns, and R. Ehricht, "High diversity of Panton-Valentine leukocidin-positive, methicillin-susceptible isolates of *Staphylococcus aureus* and implications for the evolution of community-associated methicillin-resistant *S. aureus*," *Clinical Microbiology and Infection*, vol. 13, no. 12, pp. 1157–1164, 2007.
15. A. S. Rossney, A. C. Shore, P. M. Morgan, M. M. Fitzgibbon, B. O'Connell, and D. C. Coleman, "The emergence and importation of diverse genotypes of methicillin-resistant *Staphylococcus aureus* (MRSA) harboring the panton-valentine leukocidin gene (*pvl*) reveal that *pvl* is a poor marker for community-acquired MRSA strains in Ireland," *Journal of Clinical Microbiology,* 2007, vol. 45, no. 8, pp. 2554–2563.
16. F. Yu, Z. Chen, C. Liu et al., "Prevalence of *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes among isolates from hospitalised patients in China," *Clinical Microbiology and Infection*, 2008vol. 14, no. 4, pp. 381–384.
17. Z. C. Karahan, A. Tekeli, R. Adaleti, E. Koyuncu, I. Dolapci, and O. A. Akan, "Investigation of Panton-Valentine leukocidin genes and SCCmec types in clinical *Staphylococcus aureus* isolates from Turkey," *Microbial Drug Resistance*, 2008,vol. 14, no. 3, pp. 203–210.
18. Holmes A., Ganner M., McGuane S., Pitt T. L., Cookson B. D., Kearns A. M. *Staphylococcus aureus* isolates carrying Panton-Valentine leucocidin genes in England and Wales: frequency, characterization, and association with clinical disease. *Journal of Clinical Microbiology.* 2005;43(5):2384–2390. doi: 10.1128/jcm.43.5.2384-2390.2005.
19. Afroz S., Kobayashi N., Nagashima S., Alam M. M., Hossain A. B. M. B., Rahman M. A. Genetic characterization of Staphylococcus aureus isolates carrying Panton Valentine Leukocidin genes in Bangladesh. *Japanese Journal of Infectious Diseases.* 2008;61:393–396.
20. Kaur H., Purwar S., Saini A., et al. Status of methicillin resistant Staphylococcus aureus infections and evaluation of PVL producing strains in Belgaum, South India. *Journal of Krishna Institute of Medical Sciences University.* 2012;1(2):43–51.
21. Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, Vandenesch F, Etienne J. Involvement of Panton-Valentine leukocidin producing Staphylococcus aureus in primary skin infections and pneumonia. Clin Infect Dis 1999; 29: 1128-32
22. Aires-de-Souza M, Conceicao T, Lencastre HD. Unusually high Prevalence of Nosocomial Panton Valentine Leukocidin-Positive Staphylococcus aureus Isolates in Cape Verde Islands. J Clin Microbiol 2006; 44: 3790-93.

23. D Souza ND, Rodrigues C, Mehta A. Molecular Characterization of Methicillin Resistant Staphylococcus aureus with Emergence of Epidemic Clones of Sequence type (ST) 22 and ST 772 in Mumbai, India. J Clin microbial 2010; 48: 1806-11.2360
24. Pre´vost G, Couppie´ P, Pre´vost P, et al. Epidemiological data on *Staphylococcus aureus* strains producing synergohymenotropic toxins. J Med Microbiol 1995;42:237–45.
25. Kobayashi N, Wu H, Kojima K, Taniguchi K, Urasawa S, Uehara N, Omizu Y, Kishi Y, Yagihashi A, Kurokawa I. Detection of mecA, femA and femB genes in clinical strains of Staphylococci using polymerase chain reaction. Epidemiol Infect 1994; 11: 259-66.

eISSN 1303-5150