Open Access ORIGINAL ARTICLE

Association of PVL Gene with SCC*mec* Typing in MRSA (both community associated and hospital-acquired) from a Tertiary Care Hospital, Lahore

Rai Gul Fraz[1], Farhan Rasheed[1], Muhammad Waseem[2], Iffat Javed[2], Amina Asif[3], Iqra Jamil[4]

[1] Department of Pathology, Allama Iqbal Medical College, Lahore; [2] Department of Pathology, Amna Inayat Medical College, Sheikhupura; [3] Department of Pathology, Postgraduate Medical Institute, Lahore; [4]Department of Microbiology, University of Central Punjab, Lahore

ABSTRACT

Introduction: Antibiotic resistance is a major problem in staphylococcal infections. Resistance against Methicillin in *S. aureus* is due to the transfer of mobile cassettes of specific genes in *S. aureus* called Staphylococcal Cassette Chromosome *mec* (SCC*mec*). 13 types of SCC*mec* have been identified till now. Various clinical syndromes are associated with the presence of the PVL gene. Therefore, the present study was designed to check the prevalence of PVL gene and SCC*mec* types in CA-MRSA and HA-MRSA.

Materials and methods: This cross-sectional descriptive study was done in LGH/PGMI, Lahore. MRSA isolates were phenotypically confirmed by Cefoxitin (30) disc on Mueller-Hinton agar. DNA extraction was done by using a Genomic DNA purification kit. Detection of PVL gene and SCC*mec* types was done with PCR using specific primers.

Results: Out of 89 isolates, the maximum isolates were from pus swabs (45%), followed by wound swabs (15.5%). It was found that 55 (61.8%) were HA-MRSA isolates, while 34 (38.2%) were CA-MRSA isolates. PVL gene was found in 37% of isolates. Among PVL-positive samples, 36.4% were HA-MRSA, while 63.6% were CA-MRSA. The most common SCC*mec* in HA-MRSA was type III (69%), while in CA-MRSA, SCC*mec* type IVa was predominant (35.4%).

Conclusion: The present study provides insight into the prevalence of the PVL gene and different SCC*mec* types in CA-MRSA and HA-MRSA. The most common SCC*mec* type is linked with HA-MRSA.

Authors' Contribution:
[1,2]Conception; Literature research; manuscript design and drafting; [2,3] Critical analysis and manuscript review; [1,4]Data analysis; Manuscript Editing.

Correspondence:
Iqra Jameel
Email: Iqrajameel@hotmail.com

Article info:
Received: March 20, 2023
Accepted: August18, 2023

Cite this article.Fraz RG, Rasheed F, Waseem M, Javed I, Asif A, Jamil I. Association of PVL gene with SCCmec typing in MRSA (both community associated and hospital-acquired) from a Tertiary Care Hospital, Lahore.J Islamabad Med Dental Coll. 2023; 12(3):158-163.
DOI: https://doi.org/10.35787/jimdc.v12i3.963

Funding Source: Nil
Conflict of interest:Nil

Introduction

Staphylococcus aureus, a ubiquitous pathogen, is a Gram-positive coccus, forming grape-like clusters, non-sporing, non-motile, catalase-positive facultative anaerobe, having a diameter of 0.5-1.5 µm. Antibiotic resistance is a major problem in staphylococcal infections, which may lead to an increase in the length of hospital stay and treatment failure.[1] Infections caused by

community-associated and hospital-acquired Methicillin-resistant *Staphylococcus aureus* (MRSA) have turned out to be a serious problem across the world.[2]

MRSA isolates are frequently resistant to several non-β-lactam antibiotics, including macrolides, lincosamides, aminoglycosides, and tetracyclines.[3]Resistance against Methicillin in *S. aureus* is due to the transfer of mobile cassettes of specific genes in *S. aureus* called Staphylococcal Cassette Chromosome *mec* (SCC*mec*), and coagulase-negative *staphylococci* were the origin which transfer SCC*mec* cassette to *S. aureus* ST250. SCC*mec* consists of 3 components: (i) *mec* gene complex, (ii) Ccr (cassette chromosome recombinase) gene complex and (iii) J regions.[4] The *mec*A and *mec*C genes in the SCC*mec* cassette are responsible for methicillin resistance. There are 13 types of SCC*mec*, and all are integrated at the attB site of *the S. aureus* genome, possessing significant characteristics.[5] For SCC*mec* typing, conventional PCR techniques are more widely used; however, advanced techniques include whole genome sequencing and the use of bioinformatics tools for subsequent data analysis.

Many other virulence factors, such as Panton-Valentine leucocidin (PVL), which is encoded by lukF-PV and lukS-PV, are responsible for *S. aureus's* widespread infection rate. PVL encoded a pore-forming toxin that attacks white blood cells such as monocytes and neutrophils. It is associated with various clinical diseases, mainly skin infections and necrotizing pneumonia.[6] PVL was considered to be associated with five predominant CA-MRSA strains epidemiologically.[7] Among these, the USA300 clone is the major epidemic CA-MRSA strain globally, which is extremely pathogenic and causes intractable infections of the skin.

Hence, the current study was designed to check the prevalence of PVl and different SCC*mec* types in clinical and Community-acquired isolates of Methicillin-resistant *Staphylococcus aureus.*

Methodology

The present study was done in the Microbiology laboratory of PGMI/LGH and the Immunology Laboratory of UHS, Lahore, over one year from 01-09-2021 to 01-09-2022. It was a cross-sectional descriptive study design, and a non-probability consecutive sampling technique was used to collect the samples. The sample size was calculated according to the formula.

$$n = \frac{z^2_{1-\frac{\alpha}{2}}\, p(1-p)}{d^2}$$

Level of confidence = 1-α= 95%

Anticipated population proportion margin of error = (P) = 36.1%

Margin of Error = 10%

Sample size = 89

All types of clinical samples that yielded MRSA were included in the present study. Samples from both genders were included. While *S.aureus* strains sensitive to Methicillin were excluded. Repetitive samples from the same patient were also excluded. Samples were collected and subcultured on blood and MacConkey agar. Confirmation of *S.aureus* was done by gram staining and different biochemical tests. Methicillin resistance was confirmed by 30 µg cefoxitin discs on Mueller-Hinton agar plates according to CLSI guidelines 2021. A bacterial suspension of 0.5 McFarland standard of turbidity was used.

DNA extraction of confirmed MRSA isolates was done by using a genomic DNA purification kit (ThermoFisher Scientific, Germany) according to the manufacturer's guidelines. Primers used for the detection of different SCC*mec* types and PVL genes were designed by using LaserGene bioinformatics software. Primers were reconstituted with TE buffer. The working primer was prepared in a separate vial by adding 10µl of the stock primer in 90µl of the TE buffer 8. The set of primers used for

PCR amplification is given in Table I. (Supplementary Material)

PCR amplification of SCC*mec* types and PVL gene was performed in a thermal cycler. Reaction mix of 20 µl was prepared as 10x PCR buffer (2.0 µl), dNTPs (0.5 µl), $MgCl_2$ (1.2 µl), Forward and Reverse primer (2.0 µl) each, Template DNA (7.0 µl) Taq DNA polymerase (0.4 µl), ddH_2O (4.9 µl). The PCR cycle was run at 95℃ for 4 min, followed by 30 cycles of 94℃ for 45 sec, 52℃ for 1 min, and 72℃ for 1 min, and a final extension at 72℃ for 10 min. All amplified products were run on 1.2% stained agarose gel along with a DNA ladder. (Sigma-Aldrich, UK.). Visualization of bands at specific molecular target sizes was confirmed in the Gel documentation system by UV illuminator. (BionexusInc, USA).[11] Results were analyzed by using Microsoft Excel 2010 through frequencies and percentages

Results

Among 89 confirmed isolates of MRSA, 45% were collected from pus swabs, followed by wound swabs (15%) and blood (12%). In contrast, 28% of isolates were collected from different specimens, including Fluids/aspirates, CSF, sputum, CVP tip, and urine. Among positive samples, it was found that 55 (61.8%) were HA-MRSA isolates, while 34 (38.2%) were CA-MRSA isolates. Among total samples, 37% (n=33) isolates were found to be positive for the PVL gene, among which 12 (36.4%) were HA-MRSA and 21 (63.6%) were CA-MRSA.

Table II: Frequency of different SCC*mec* types in MRSA isolates

SCC *mec* type	Percentage	
	HA-MRSA n=55 (%)	CA MRSA n=34 (%)
III	38 (69)	4 (11.8)
IVa	6 (10.9)	12 (35.3)
IVc	3 (5.5)	6 (17.6)
IVe	0 (0)	6 (17.6)
IVb	2 (3.6)	3 (8.9)
II	3 (5.5)	0 (0)
V	1 (1.8)	1 (2.9)
I	2 (3.7)	0 (0)
NT	0 (0)	2 (5.9)

Association of PVL with different SCC*mec* types showed that among 47 isolates of SCC*mec* types I, II and III, 9(19.1%) were PVL positive, while 38 (80.9%) isolates were PVL negative. Among 40 isolates of IV subtypes and V, 33 (82.5%) were PVL-positive, while 7 (17.5%) were PVL-negative.

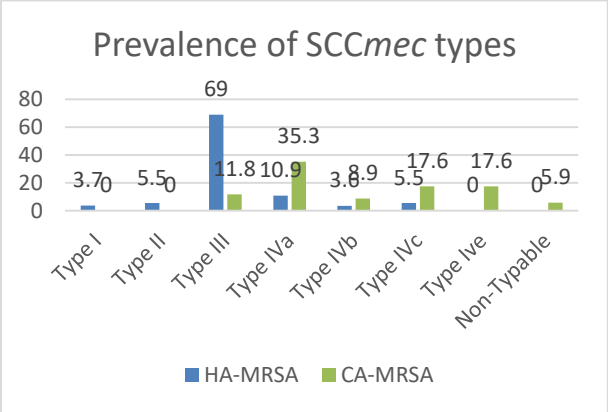

Figure 1: SCC*mec* types in HA-MRSA and CA-MRSA

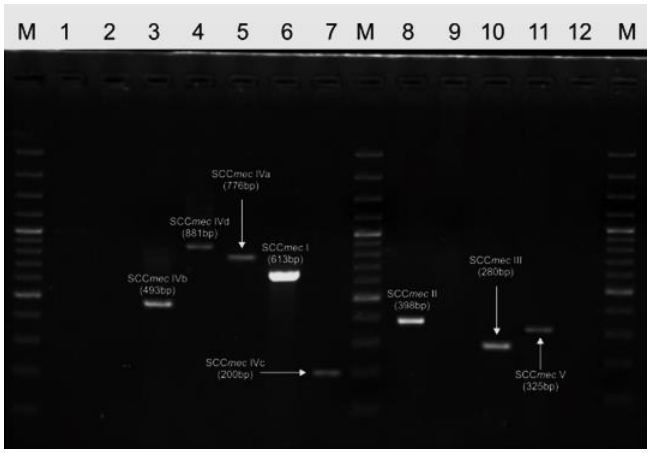

Figure 2: Multiplex PCR identifies SCC *mec* types and subtypes. Lane 1 is negative control. Lanes 3, 4, 5, 6, 7, 8, 10 and 11 represent SCC *mec* type IVb, IVd, IVa, I, IVc, II, III and type V, respectively. Lane

12 is empty. M is the 100bp DNA ladder (Thermo Scientific).

Discussion

Methicillin-resistant *S. aureus* is one of the most communal organisms linked with nosocomial infections and has been recognized as a major problem for infection control.[12] Acquiring resistance against commonly used antibiotics is mainly because of the presence of the PVL gene and different SCC*mec* types, which can be found in both HA-MRSA and CA-MRSA.

In the present study, we determined the prevalence of the PVL gene and different SCC*mec* types in Community-associated and hospital-acquired MRSA isolates. In our study, the maximum number of MRSA isolates were isolated from pus samples (45%), followed by wound swabs (28%) and blood (12%). In accordance with our results, Maham Akram, in 2022, also reported 36.2% isolation of MRSA from pus samples 12. Various other studies also reported the maximum number of MRSA isolations from pus and wound swabs.[13,14]

In the present study, among 89 MRSA isolates, 55 (61.8%) were HA-MRSA, while 34 (38.2%) were CA-MRSA isolates. A study conducted in China in 2022 also reported similar results in which community-associated MRSA (CA-MRSA) infections accounted for 24% of the total isolates. Similar results were reported from China in 2013, in which maximum numbers of MRSA isolates were found to be Hospital-acquired. [15]

Panton-Valentine leucocidin is generally used as an indicator for Community-associated MRSA, accountable for skin and soft tissue infections 16. In our study, the PVL gene was present in 37% (n=33) isolates, among which 12 (36.4%) were HA-MRSA and 21 (63.6%) were CA-MRSA. A study conducted in 2022 reported a 35% prevalence of the PVL gene in MRSA isolates, which was in accordance with our results.[18] However, a study conducted in India reported a 63% prevalence of the PVl gene in MRSA isolates, which indicates a higher prevalence than our study.[19] Various reports from different countries reported lower pervasiveness of the PVL gene among isolates of MRSA (5% in France, 4.9% in the UK, 14.3% in Bangladesh)[18], demonstrating how the prevalence of PVL differs significantly between different geographic areas and populations.

Investigation of SCC*mec* types improves understanding of the epidemiology of MRSA and detection of relatedness among different strains. 98% of our isolates were typeable for SCC*mec* by PCR. Various studies around the world work on SCC*mec* typing by PCR to identify the most prevalent SCC*mec* type in their region and reported variations in typeability that were wholly less than 100%. Various researchers reported similar results to our findings of type ability (98% in Denmark, 97.4% in Portugal, 96.4% in Palestine).[19]

The high frequency of SCC*mec* type III (69%), followed by SCC*mec* type IVa (10.9%) and type II and IVc (5.5%) each in our study, was in accordance with the results of the study conducted in Alexandria, which reported 57% prevalence of SCC*mec* type III.[20] Various other studies across the world also reported similar results.[21,22] Conversely, studies conducted in Saudi Arabia and Kuwait reported SCC*mec* type IV as the most prevalent type.[23,24] The discrepancy in the SCC*mec* types distribution from different geographic regions can be attributed to limited proficiencies of the conventional PCR detection method and differences in the specificity and sensitivity of primers used.

In CA-MRSA, the most prevalent type was found to be type IVa (35.3%), followed by type IVc and IVe (17.6%) each in our study, which was in accordance with the study conducted in Japan in 2022 in which SCC*mec* type IV accounted for 44% of total samples in community-associated MRSA.[25]In our study, the presence of SCC*mec* type I, II and III in CA-MRSA demonstrated the spread of HA-MRSA in the Community, which was already reported in the

previous studies conducted in China, the Philippines, Korea and Veitnam.[17]

Conclusion

The present study provides insight into the prevalence of the PVL gene and different SCC*mec* types in community-associated and hospital-acquired MRSA isolates. The most common SCC*mec* type linked with HA-MRSA was type III, while type IV was more prevalent in CA-MRSA. Increased association of the PVL gene with HA-MRSA is of major concern. More knowledge about the characterization of SCC*mec* types and transfer of PVL gene from CA-MRSA to HA-MRSA will help in better understanding for monitoring and managing MRSA infections.

REFERENCES

1. Cornejo-Juárez P, Vilar-Compte D, Pérez-Jiménez C, Ñamendys-Silva SA, Sandoval-Hernández S, Volkow-Fernández P. The impact of hospital-acquired infections with multidrug-resistant bacteria in an oncology intensive care unit. International Journal of Infectious Diseases. 2015 Feb 1;31:31-4. https://doi.org/10.1016/j.ijid.2014.12.022
2. Vaidya P, Pawar G, Krishnamurthy N. Community-acquired MRSA infections—Three recent cases and an overview of CA MRSA infections. Pediatric Infectious Disease. 2015 Jan 1;7(1):8-12. https://doi.org/10.1016/j.pid.2014.12.005
3. Sharma P, Dahiya S, Manral N, Kumari B, Kumar S, Pandey S, et al. Changing trends of culture-positive typhoid fever and antimicrobial susceptibility in a tertiary care North Indian Hospital over the last decade. Indian Journal of Medical Microbiology. 2018 Jan 1;36(1):70-6. https://doi.org/10.4103/ijmm.IJMM_17_412.
4. Akram J, Khan AS, Khan HA, Gilani SA, Akram SJ, Ahmad FJ, et al. Extensively drug-resistant (XDR) typhoid: evolution, prevention, and its management. BioMed Research International. 2020 May 2, 2020. https://doi.org/10.1155/2020/6432580
5. Grema HA, Geidam YA, Gadzama GB, Ameh JA, Suleiman A. Methicillin resistant Staphylococcus aureus (MRSA): a review. Adv Anim Vet Sci. 2015;3(2):79-98. | http://dx.doi.org/10.14737/journal.aavs/2015/3.2.79.98
6. Radhakrishnan A, Als D, Mintz ED, Crump JA, Stanaway J, Breiman RF, et al. Introductory article on global burden and epidemiology of typhoid fever. The American journal of tropical medicine and hygiene. 2018 Sep;99(3 Suppl):4. 10.4269/ajtmh.18-0032
7. Diep BA, Otto M. The role of virulence determinants in community-associated MRSA pathogenesis. Trends in microbiology. 2008 Aug 1;16(8):361-9. https://doi.org/10.1016/j.tim.2008.05.002
8. Sajid S, Rahman SU, Mohsin Gilani M, Sindhu ZU, Ali MB, Hedfi A, et al. Molecular characterization and demographic study on infectious bursal disease virus in Faisalabad district. Plos one. 2021 Aug 16;16(8):e0254605. https://doi.org/10.1371/journal.pone.0254605
9. Stanaway JD, Reiner RC, Blacker BF, Goldberg EM, Khalil IA, Troeger CE, Andrews JR, Bhutta ZA, Crump JA, Im J, Marks F. The global burden of typhoid and paratyphoid fevers: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet Infectious Diseases. 2019 Apr 1;19(4):369-81.https://doi.org/10.1016/S1473-3099(18)30685-6
10. Marchello CS, Hong CY, Crump JA. Global typhoid fever incidence: a systematic review and meta-analysis. Clinical Infectious Diseases. 2019 Mar 7;68(Supplement_2):S105-16. https://doi.org/10.1093/cid/ciy1094
11. Sajid S, ur Rahman S, Mohsin M. IgY: A Key Isotype and Promising Antibody for the Immunoprophylaxis Therapy of Infectious Bursal Disease Virus Infections. https://doi.org/10.48022/mbl.2205.05013
12. Akram M, Haider MH, Riaz S. Molecular detection of mecA gene in Methicillin-resistant Staphylococcus aureus isolated from clinical specimens: A descriptive case series study from Pakistan. In2022 19th International Bhurban Conference on Applied Sciences and Technology (IBCAST) 2022 Aug 16 (pp. 375-380). IEEE. 10.1109/IBCAST54850.2022.9990562
13. Gittens-St Hilaire MV, Chase E, Alleyne D. Prevalence, molecular characteristics and antimicrobial susceptibility patterns of MRSA in hospitalized and nonhospitalized patients in Barbados. New Microbes and New Infections. 2020 May 1;35:100659. https://doi.org/10.1016/j.nmni.2020.100659
14. Gustave K. Colonization and Infection with MRSA and CRKP and Its Result in an Increased Mortality Rate Within the Intensive Care and High-

Dependency Units in Barbados. Infection Control & Hospital Epidemiology. 2020 Oct;41(S1):s173-4. https://doi.org/10.1017/ice.2020.703
15. Guerrier G, Erneau M, Mathurin M, Ouabo A, Murakami C, Rull M, et al. Surgical-site infection following cesarean section in Haiti: incidence and associated pathogens. 10.7727/wimj.2015.419
16. Brown PD. Multiple-locus VNTR Analyses of Methicillin-resistant Staphylococcus aureus from Jamaica. Infectious Diseases: Research and Treatment. 2015 Jan;8:IDRT-S31084. doi:10.4137/IDRT.S31084.
17. Chuang YY, Huang YC. Molecular epidemiology of community-associated meticillin-resistant Staphylococcus aureus in Asia. The Lancet infectious diseases. 2013 Aug 1;13(8):698-708. https://doi.org/10.1016/S1473-3099(13)70136-1
18. Mostafa N, Salah M, Elashrey A. Incomplete Hemolytic MRSA Strains Associated with Hemolysin and Panton-Valentine Leucocidin Virulence Genes as a Cause of Blood Stream Infections. Egyptian Journal of Medical Microbiology. 2022 Oct 1;31(4):141-9. 10.21608/EJMM.2022.264208
19. Steele AD, Hay Burgess DC, Diaz Z, Carey ME, Zaidi AK. Challenges and opportunities for typhoid fever control: a call for coordinated action. Clinical Infectious Diseases. 2016 Mar 15;62(suppl_1):S4-8.https://doi.org/10.1093/cid/civ976
20. Alseqely M, Newton-Foot M, Khalil A, El-Nakeeb M, Whitelaw A, Abouelfetouh A. Association between fluoroquinolone resistance and MRSA genotype in Alexandria, Egypt. Scientific reports. 2021 Feb 19;11(1):1-9. https://doi.org/10.1038/s41598-021-83578-2
21. Wang WY, Chiueh TS, Sun JR, Tsao SM, Lu JJ. Molecular typing and phenotype characterization of methicillin-resistant Staphylococcus aureus isolates from blood in Taiwan. PLoS One. 2012 Jan 23;7(1):e30394. https://doi.org/10.1371/journal.pone.0030394
22. Hashemizadeh Z, Hadi N, Mohebi S, Kalantar-Neyestanaki D, Bazargani A. Characterization of SCCmec, spa types and Multi-Drug Resistant of methicillin-resistant Staphylococcus aureus isolates among inpatients and outpatients in a referral hospital in Shiraz, Iran. BMC research notes. 2019 Dec;12(1):1-6. https://doi.org/10.1186/s13104-019-4627-z
23. Alkharsah KR, Rehman S, Alkhamis F, Alnimr A, Diab A, Al-Ali AK. Comparative and molecular analysis of MRSA isolates from infection sites and carrier colonization sites. Annals of clinical microbiology and antimicrobials. 2018 Dec;17:1-1.https://doi.org/10.1186/s12941-018-0260-2
24. Alfouzan W, Udo EE, Modhaffer A, Alosaimi A. Molecular characterization of methicillin-resistant Staphylococcus aureus in a tertiary care hospital in Kuwait. Scientific reports. 2019 Dec 6;9(1):1-8. https://doi.org/10.1038/s41598-019-54794-8
25. Yamaguchi T, Nakamura I, Sato T, Ono D, Sato A, Sonoda S, et al. Changes in the genotypic characteristics of community-acquired methicillin-resistant Staphylococcus aureus collected in 244 medical facilities in Japan between 2010 and 2018: A Nationwide surveillance. Microbiology Spectrum. 2022 Aug 31;10(4):e02272-21.https://doi.org/10.1128/spectrum.02272-21