INTERNATIONAL JOURNAL OF CURRENT ADVANCED RESEARCH
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 12; Issue 06(B); June 2023; Page No. 2171-2174
DOI: http://dx.doi.org/10.24327/ijcar.2023.2174.1476

Research Article

ASSOCIATION OF PANTON VALENTINE LEUKOCIDIN (PVL) GENES WITH METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA): TERTIARY CARE HOSPITAL BASED STUDY IN MANIPUR, INDIA

K Reena Devi, S Damrolien*, Ksh Mamta Devi, Kh Sulochana Devi and Th Nabakumar Singh

Department of Microbiology RIMS, Imphal, Manipur, India 795004

A R T I C L E I N F O

Article History:
Received 06th March, 2023
Received in revised form 14th April, 2023
Accepted 23rd May, 2023
Published online 28th June, 2023

Key words:
PV leukocidin, HA-MRSA, CA-MRSA

A B S T R A C T

Background and objectives: Panton-Valentine leukocidin (LukS-PV and lukF- PV) is a cytotoxin that causes leukocyte destruction and tissue necrosis. PVL-carrying *Staphylococcus aureus* strains are more virulent and highly transmissible strains than PVL-negative *Staphylococcus aureus.* To date, PVL has become the most essential and significant virulence factor of community-acquired (CA) S.aureus. This study was conducted to determine the prevalence of Panton-Valentine leukocidin genes in identified CA-MRSA and HA-MRSA isolated from various clinical samples in a tertiary care hospital.

Methods: A prospective cross-sectional study was conducted in a tertiary care hospital at RIMS, Imphal. The study was conducted over a period of three years, from October 2018 to September 2021. The isolates were obtained from various clinical samples such as blood, pus, wound swabs, aspirate, urine, and sputum. PCR amplification of Mec A and PVL genes was performed to check the prevalence among the identified CA-MRSA and HA-MRSA.

Results: 348 Methicillin-resistant Staphylococcus aureus samples were analyzed for Mec A and PVL genes. All 348 samples show positive for Mec A (100%).306 (87.93%) were positive for PVL genes. All 348 MRSA was further identified as HA-MRSA 124 (35.63%) and CA-MRSA 224(64.36%). The PVL-positive rate was high in CA-MRSA 201(89.73%) compared to HA-MRSA 105(84.67%).

Conclusion: Antimicrobial resistance is a major global health concern and of MRSA is a serious threat. PVL gene with added virulence further worsens the clinical outcome among infected patients. Hence the knowledge of its prevalence adds an insight among the infection control practitioners to adhere to effective prevention control.

INTRODUCTION

Methicillin resistant Staphylococcus aureus (MRSA) is endemic in India and is a dangerous pathogen for hospital-acquired infection. MRSA can cause mild to severe infectious diseases, such as pyogenic skin and soft tissue infection, food poisoning, suppurative pneumonia, pyogenic endocarditis, osteomyelitis, and otitis media. Hospital-acquired MRSA infections significantly burden morbidities, mortalities, and healthcare resources. Continued isolation and characterization of this fatal organism are crucial for the proper prevention and control[1].

Because of the elaboration of several different virulence factors, Staphylococcus aureus, has become a threat to our lives. Panton-Valentine leukocidin (PVL) is one of the most important virulence factors of S. aureus. This beta pore-forming cytotoxin is associated with tissue necrosis and also disrupts leukocyte membranes[2]. PVL-carrying Staphylococcus aureus strains are more virulent and highly transmissible[3].In recent times, there have been an overall increase in the prevalence of PVL-positive Staphylococcus aureus worldwide. Variable prevalence rates have been reported from different countries, i.e., 12.8% in china[4], 30% in Germany[5]45.3% in Japan, and 97% in USA[6]. To date, PVL has become the most important significant virulence factor of community-acquired Staphylococcus aureus. The prevalence of PVL gene among HA-MRSA and CA-MRSA has not been adequately reported in India. This study was undertaken to investigate the prevalence of PVL among HA-MRSA and CA-MRSA in this region of India.

MATERIALS & METHODS

A total of 348 isolates were obtained from various clinical samples such as blood, pus, wound swabs, aspirate, urine, and sputum Department of Microbiology of RIMS hospital during the period of 3 years from October 2018 to September 2021. The ethical clearance was taken from the institutional ethics committee, RIMS.

Corresponding author:* **S Damrolien
Department of Microbiology RIMS, Imphal, Manipur, India 795004

Confirmation and storage of Staphylococcus Aureus Isolates

All these isolates were confirmed as MRSA by using standard techniques[7]. The isolates were inoculated into the semi-solid nutrient agar and stored at -20°C until further study.

Case definition: **HA-MRSA** was defined as one cultured from a clinical specimen obtained ≥72hrs after a patient's hospital admission or whose sources of isolation were associated with risk factors for HA-MRSA infection (e.g. recent hospitalization, recent surgery, residence in a long-term care facility, drug use)[8] within one year of MRSA isolation date. **CA- MRSA** isolate was defined as one cultured < 72 hrs of a patient's hospital admission or whose sources of isolation were not associated with risk factors for HA-MRSA infection[9].

PCR for detection of mec-A and pvl genes

The primer pairs for mec-A and pvl genes were taken from the published sequence by Oliveria *et al*[10] and McClure *et al*[11], respectively. Primers were blasted and commercially obtained from Eurofins, Bangalore, India. PCR was performed by using Multiplex PCR kit (Qiagen, Hilden, Germany) with slight modification of final reaction volume of 25 µl (12.5 µl mastermix, 2.5 µl primer mix, 3µl of DNA template and 7µl of RNase-free water). Thermocycling conditions and visualization of products were done as per the manufacturer's instructions. Reference strains ATCC 43300 and 25923 were used as positive and negative controls for mec-A gene, respectively and ATCC 43300 was used as negative control for pvl gene.

Thermocycling conditions and visualization of products were done as per manufacturer's instructions.

Antibiotic susceptibility testing of MRSA isolates

Kirby-Bauer disc diffusion method[12] and by automated method Vitek 2 compact system (Biomerieux, France) using ASTP-628 in accordance with the manufacturer's instructions and CLSI guidelines[13].

RESULTS

All 348 Methicillin resistant Staphylococcus aureus were screen with cefoxitin disc and showed amplifications with Mec-A genes.

Table1 Distribution of MRSA cases according to age group

Age Group	Frequency	Percent
1 - 15 yr	65	18.7
16 - 25 yr	37	10.6
26 - 45 yr	107	30.7
46 - 75 yr	120	34.5
Above75 yr	19	5.5
Total	348	100.0

From the table-1, it may be observed that in the sample of 348 MRSA cases, highest number of them belong to the age range of 46 - 75 years with 34.5% which is followed by the age range of 26 - 45 years with 30.7%, and lowest 5.5% pertains to the age range of 75 years and above.

Among 348 isolates, 124 (35.63%) met the definition of HA-MRSA and 224 (64.36%) CA-MRSA.

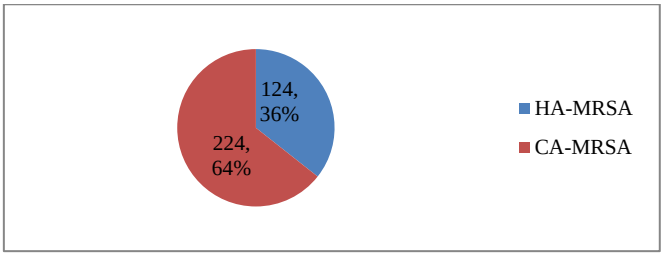

Fig 1 Type of MRSA

Table 2 MRSA cases according to panton- valentine leukocydin (PVL)

Parameters		Phanto valentine leukocydin (PVL)			χ^2 -value	df	P -value
		Positive	Negative	Total			
Infection type	Community Acquired Infection	192(89.7%)	22(10.3%)	214(100.0%)	1.675	1	.196
	Health Care Associated Infection	114(85.1%)	20(14.9%)	134(100.0%)			
	Total	306(87.9%)	42(12.1%)	348(100.0%)			

χ^2 –value; df: degree of freedom; P-value: probability due to chance factor

In order to test the toxin difference between the infection types, the table-3 is introduced by using χ^2–test as statistical tool. It is found that percentage of positive panton-valentine leukocydin is visibly higher than that of percentage of negative panton- valentine leukocydin. This is found true in both community acquired infection and health care associated infection MRSA cases. Nevertheless, the insignificant test value (P=0.196) indicates that there is no significant variation of the pattern of panton-valentine leukocydin between community acquired infection and health care associated infection MRSA cases.

DISCUSSION

In the present study, very high rates of resistance, particularly penicillin, ciprofloxacin, erythromycin, and trimethoprim/sulfamethoxazole were observed in both cases. However, a study reported by kazakova *et al*[14] reported a community-associated MRSA (CA-MRSA) clone isolated from US football prayer with skin abscess. The strains were susceptible to most antimicrobial agents except β-lactams and macrolides. The first PVL-positive MRSA was noticed in the late 1990s and these strains got scattered worldwide in recent years[16]. The role of PVL is boosting the virulence of S.aureus, and their pathogenicity is being deliberated. Panton-Valentine leukocidin raises the pathogenicity of *S. aureus* by necrosis, quickening apoptosis, and damage of polymorphonuclear and mononuclear cells, thereby contributing to mortality and morbidity[16]. PVL is generally used as a marker for community-acquired MRSA, liable for deep dermal infections including soft tissue[17.18]. However, in this present study, it may not be a marker for CA-MRSA since the rate of PVL production in HA-MRSA is also high. However, the worldwide scheme of PVL among MRSA isolates varies. A lower prevalence of PVL has been reported in other parts of world (5% in France, 4.9% in UK, 8.1% in Saudi Arabia, and 14.3% in Bangladesh)[19,.20,.21,.22] reflecting the significant variation in the prevalence of PVL among geographical areas and communities. Kaur *et al*.[23], from India, have reported an overall 62.85% prevalence of PVL among MRSA and MSSA (MRSA: 85.1% and MSSA: 48.8%), which delineates a higher

prevalence of PVL among MRSA which is similar to our findings.

The majority of the isolates were reported from pus (35.82%) in HA-MRSA and (69.15 %) in CA-MRSA. Our study collaborates with a similar study conducted in South India, which isolated 80 % of cases from pus[24].In our study, PVL gene is predominantly present in CA-MRSA (90.65%) compared to HA-MRSA (85.07 %).At prevalence rate of 35.17% in HA-MRSA and 34.11% in CA-MRSA for the age group (46 – 75) table 1. The PVL positive isolate fails to show any age-related predilection. Previous studies have shown a strong predisposition of PVL-positive *S. aureus* isolates for younger and previously healthy patients[25,26]. In a 2008 Australian study by Munckhof *et al*., the authors observed a steady decline in PVL occurrence with increasing age, which they ascribed to the age-associated strengthening of immunity and to the natural penchant of children and young adults to acquire PVL-positive *S. aureus* from skin contamination during playful and contact sports[26]. This increased likelihood of PVL-positive *S. aureus* to infect younger age groups, as seen in earlier studies, may be attributed to the fact that a majority of PVL-carrying *S. aureus* isolates from these studies were community-acquired.

As with age, PVL prevalence also exhibited no association with infection site or hospital of origin. Although the highest numbers of PVL-carrying *S. aureus* isolates were recovered from pus (35.82%), blood (25.37%) in HA-MRSA and pus (69.15%), and urine (15.42%) in CA-MRSA, the prevalence rate difference between the sites did not reach statistical significance. Along similar lines, the 85.07% carriage rate of PVL in HAMRSA was not statistically significantly different from the 90.65% prevalence rate of PVL in CA-MRSA. On the basis of vastly different occurrences of the PVL-positive organisms in different parts of the world, it appears that the PVL carriage depends largely on the geographical location and the organisms that are endemic in a particular locality.

CONCLUSION

In conclusion, PVL may no longer be a reliable marker for CA-MRSA isolates; rather, all MRSA may be an important reservoir of PVL toxin. The current study reflects the elevated level of multi-drug resistant strains in the community. The presence of pvl among multi-drug resistant MRSA may be a fatal and challenging condition. Hence the knowledge of its prevalence adds an insight among the infection control practitioners to adhere to effective prevention protocol.

References

1. Algammal AM, Hetta HF, Elkelish A, Alkhalifah DHH, Hozzein WN, Batiha GE, El Nahhas N, Mabrok MA (2020) Methicillin-Resistant Staphylococcus aureus (MRSA): one health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. Infect Drug Resist 13: 3255-3265
2. B. Shrestha, W. Singh, V. S. Raj, B. M. Pokhrel, and T. M. Mohapatra, "High Prevalence of Panton-Valentine leukocidin (PVL) genes in nosocomial-acquired staphylococcus aureus isolated from tertiary care hospitals in Nepal," BioMed Research International, vol. 2014, Article ID 790350, 7 pages, 2014.
3. J. Kaneko and Y. Kamio, "Bacterial two-component and heteroheptameric pore-forming cytolytic toxins: structures, poreforming mechanism, and organization of the genes," *Bioscience, Biotechnology, and Biochemistr*, vol. 68, no. 5, pp. 981–1003 2004.
4. 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*, vol. 14, no. 4, pp. 381–384, 2008.
5. 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.
6. D. J. Skiest, K. Brown, T. W. Cooper, H. Hoffman-Roberts, H. R. Mussa, and A. C. Elliott, "Prospective comparison of methicillin-susceptible and methicillin-resistant community-associated Staphylococcus aureus infections in hospitalized patients," *Infection*, vol 54, no. 5, pp. 427–434, 2007.
7. Baird D. Staphylococcus: cluster-forming gram-positive cocci. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie & McCartney practical medical microbiology, 14th ed. New York: Churchill Livingstone; 1996. p. 245-61.
8. Herold BC, Immergluck LC, Maranan MC, Lauderdale DS, Gaskin RE, Boyle-Vavra S, *et al*. Community-acquired methicillin-resistant Staphylococcus aureus in children with no identified predisposing risk. JAMA 1998; 279 : 593-8.
9. McClure J, Conly JM, Lau V, Elsayed S, Louie T, Hutchins W, *et al*. Novel multiplex PCR assay for detection of the staphylococcal virulence marker panton-valentine leukocidin genes and simultaneous discrimination of methicillinsusceptible from -resistant staphylococci. J Clin Microbiol 2006; 44 : 1141-4
10. Oliveira DC, de Lencastre H. Multiplex PCR strategy for rapid-identification of structural types and variants of the mec element in methicillin-resistant Staphylococcus aureus. Antimicrob Agents Chemother 2002; 46 : 2155-61.
11. McClure J, Conly JM, Lau V, Elsayed S, Louie T, Hutchins W, *et al*. Novel multiplex PCR assay for detection of the staphylococcal virulence marker panton-valentine leukocidin genes and simultaneous discrimination of methicillinsusceptible from -resistant staphylococci. J Clin Microbiol 2006; 44: 1141-4.
12. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966; 45: 493-6.
13. Clinical and Laboratory Standards Institute. (CLSI) (2019) Performance standards for antimicrobial susceptibility testing, 29th informational supplement CLSI document M100-S29 (ISBN 978-1-68440 (-033-1)
14. S. V. Kazakova, J. C. Hageman, M. Matava *et al*., "A clone of methicillin-resistant Staphylococcus aureus among professional football players," *The New England Journal of Medicine*, vol. 352, no. 5, pp. 468–475, 2005.View at: Publisher Site | Google Scholar

15. A. Gravet, M. Rondeau, C. Harf-Monteil *et al*., "Predominant Staphylococcus aureus isolated from antibiotic-associated diarrhea is clinically relevant and produces enterotoxin A and the bicomponent toxin LukE-LukD," *Journal of Clinical Microbiology*, vol. 37, no. 12, pp. 4012–4019, 1999.View at: Google Scholar
16. G. Lina, Y. Piémont, F. Godail-Gamot *et al*., "Involvement of Panton-Valentine leukocidin—producing *Staphylococcus aureus* in primary skin infections and pneumonia," *Clinical Infectious Diseases*, vol. 29, no. 5, pp. 1128–1132, 1999.
17. S. A. Havaei, S. O. Moghadam, M. R. Pourmand, and J. Faghri, "Prevalence of genes encoding bi-component leukocidins among clinical isolates of methicillin-resistant Staphylococcus aureus," *Iranian Journal of Public Health*, vol. 39, no. 1, pp. 8–14, 2010.
18. L. G. Miller, F. Perdreau-Remington, G. Rieg *et al*., "Necrotizing fasciitis caused by community-associated methicillin-resistant: *Staphylococcus aureus* in Los Angeles," *The New England Journal of Medicine*, vol. 352, no. 14, pp. 1445–1453, 2005.
19. G. Lina, Y. Piémont, F. Godail-Gamot *et al*., "Involvement of Panton-Valentine leukocidin—producing *Staphylococcus aureus* in primary skin infections and pneumonia," *Clinical Infectious Diseases*, vol. 29, no. 5, pp. 1128–1132, 1999.
20. A. Holmes, M. Ganner, S. McGuane, T. L. Pitt, B. D. Cookson, and A. M. Kearns, "*Staphylococcus aureus* isolates carrying Panton-Valentine leucocidin genes in England and Wales: frequency, characterization, and association with clinical disease," *Journal of Clinical Microbiology*, vol. 43, no. 5, pp. 2384–2390, 2005.
21. I. M. Moussa and A. M. Shibl, "Molecular characterization of methicillin-resistant Staphylococcus aureus recovered from outpatient clinics in Riyadh, Saudi Arabia," *Saudi Medical Journal*, vol. 30, no. 5, pp. 611–617, 2009.
22. S. Afroz, N. Kobayashi, S. Nagashima, M. M. Alam, A. B. M. B. Hossain, and M. A. Rahman, "Genetic characterization of Staphylococcus aureus isolates carrying Panton Valentine Leukocidin genes in Bangladesh," *Japanese Journal of Infectious Diseases*, vol. 61, pp. 393–396, 2008.
23. H. Kaur, S. Purwar, A. Saini *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*, vol. 1, no. 2, pp. 43–51, 2012.
24. Srinivasan S, Sheela D, Mathew R, Bazroy J, Kanungo R. Risk factors and associated problems in the management of infections with methicillin resistant Staphylococcus aureus. Indian J Med Microbiol 2006; 24:182-185.
25. A. S. Rossney, A. C. Shore, P.M.Morgan, M. M. Fitzgibbon B. O'Connell, and D. C. Coleman, "The emergence and important 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*, vol. 45, no. 8, pp 2554–2563, 2007
26. W. J. Munckhof, G. R. Nimmo, J. Carney *et al*., "Methicillin susceptible, non-multiresistant methicillin resistant and multiresistant methicillin-resistant *Staphylococcus aureus* nfections: a clinical, epidemiological and microbiological comparative study," *European Journal of Clinical Microbiology and Infectious Diseases*, vol. 27, no. 5, pp. 355–364, 2008.

How to cite this article:

K Reena Devi *et al* (2023) 'Association of Panton Valentine Leukocidin (PVL) Genes with Methicillin Resistant Staphylococcus Aureus (MRSA): Tertiary Care Hospital Based Study in Manipur, India', *International Journal of Current Advanced Research*, 12(06), pp. 2171-2174. DOI: http://dx.doi.org/10.24327/ijcar.2023.2174.1476

****