Type of the Paper (Article)

Investigating the Correlation Between the Panton-Valentine Leukocidin (PVL) Gene and Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Community-Acquired Soft Tissue Infections

Mohammed S. Z. Ahmed[1*], Ahmed A. Wegdan[1], Enas M. Hefzy[1], Abdel-Rahman A. Mohamed[1]

[1] Medical Microbiology & Immunology Department, Faculty of Medicine, Fayoum University, Fayoum, 63511, Egypt.

* Correspondence: Mohammed S. Z. Ahmed, msz00@fayoum.edu.eg, Tel: (002) 01008963868.

Abstract

Introduction: MRSA, methicillin-resistant Staphylococcus aureus, poses a considerable threat to human health by causing infections in both community and healthcare environments. Among the numerous virulence factors possessed by S. aureus, the Panton-Valentine Leukocidin gene (PVL) stands out as particularly significant. The current study aimed to detect the prevalence of the PVL gene in community-acquired MRSA isolated from skin and soft tissue infections (SSTIs).

Subjects and Methods: Among 109 S. aureus isolates collected from SSTIs, MRSA isolates were detected by the cefoxitin disc diffusion method and cultured on an ORSAB (oxacillin resistance screening agar base) medium. Polymerase chain reaction (PCR) was used to detect the PVL gene in MRSA isolates.

Results: Forty-one MRSA (37.6%) isolates were detected among S. aureus isolated from community-acquired SSTIs by different phenotypic and genotypic methods. The majority of community-acquired MRSA cases (97.5%) tested positive for the pvl gene.

Conclusion: Our study revealed a significant prevalence of PVL among community-acquired MRSA isolates.

Keywords: MRSA; Staphylococcus aureus; PVL; SSTIs.

1. Introduction

Staphylococcus aureus (*S. aureus*) is a significant human pathogen that is linked to community- and hospital-acquired illnesses all over the world [1]. A potentially fatal staphylococcal infection's prognosis was improved by the discovery of penicillin, but after years of widespread use, resistance emerged due to the production of lactamases [2]. Despite the fact that methicillin is lactamase-resistant, methicillin-resistant S. aureus (MRSA) strains have been identified quickly following methicillin use [3]. Since 1960, MRSA, also known as hospital-associated MRSA (HA-MRSA), has been recognized as a virulent disease in hospitals [4]. The epidemiology of MRSA has changed since 1990 as a result of the emergence of community-acquired MRSA (CA-MRSA)

outside of hospitals [5]. The Panton-Valentine Leukocidin gene (PVL) is S. aureus's most serious toxin [6]. A phage-encoded two-component toxin that can be introduced into the S. aureus genome [7] Particularly in skin and soft tissue infections (SSTIs), PVL-toxin is regarded as the most significant marker of CA-MRSA [8].

The study was aimed at detecting the incidence of MRSA in S. aureus isolated from SSTIs and the PVL gene prevalence in CA-MRSA.

2. Subjects and methods

2.1.Identification of MRSA

The detection of MRSA was carried out across 109 *S. aureus* isolates obtained from SSTIs by the disk-diffusion method using a cefoxitin (30 g) disc and oxacillin resistance screening agar base (ORSAB) medium. Isolates that had an inhibition zone to Cefoxitin diameters of less than 21mm [9, 10] and/or characteristic growth on ORSAB medium (Oxoid Ltd., Hampshire, UK) were considered MRSA. ORSAB medium employs aniline blue to detect mannitol fermentation, which leads to the formation of vibrant blue colonies indicating the presence of S. aureus. ORSAB was enhanced with lithium chloride, polymyxin B, and oxacillin as per the instructions provided by the manufacturer and subsequently incubated at a temperature of 35–37 ℃ for 24 hours. MRSA grows on this medium and yields blue colonies [11].

2.2.Antibiotic susceptibility test

Antibiotic sensitivity tests were done for MRSA isolates by the Kurby-Bauer method. The antibacterial activities of antibiotics were assessed by measuring the inhibition zones in mm [12].

The following discs of antibiotics (Oxoid) were used: Cefoxitin (FOX) 30 µg, Amoxicillin/Clavulanic acid (AMC) 30 µg, Erythromycin (E) 15 µg, Tetracycline (TE) 30 µg, Linezolid (LNZ) 30 µg, Gentamycin (CN) 10 µg, Ciprofloxacin (CIP) 5 µg, Sulphamethoxazole/Trimethoprim (SXT) 25 µg, Imipenem (IPM) 10 µg, Cefotaxime (CTX) 30 µg, Cefepime (FEP) 30 µg [13, 14].

Strains that were identified as MRSA were stored in Eppendorf tubes with broth and glycerol tubes at -80°C for further molecular work.

2.3.Molecular Methods

DNA extraction

By using the boiling procedure reported by Zhang et al., (2012) DNA was extracted from MRSA strains as follows: MRSA plate cultures that were freshly grown overnight were necessary [15]. The day before PCR, a single colony of MRSA was chosen using a sterile culture stick, and a thick stripe of the bacteria was formed on a Mannitol salt agar plate and incubated for 18 hours at 37 ℃.

A 1.5 ml micro-centrifuge tube was filled with around 75 µl of sterile, distilled water. A small number of bacteria from the dense overnight streak was collected using a sterile culture stick. To create a cloudy solution, the bacteria were stirred in sterile

water. Every sample underwent this procedure.

To lyse the bacteria and liberate its nucleic acids, the tubes were heated in a dry heat block for 10 min at 95 °C. The samples were then taken out of the heat block and let to cool for five minutes. The samples were then centrifuged at 13,000 rpm for 1 minute to produce a clear supernatant that included nucleic acids and a pellet of cellular debris. For later usage, the DNA (clear supernatant) was kept in storage at -20 °C [16].

Detection of Pvl gene

The pvl gene products (LukS and LukF, respectively) were then amplified by PCR using a 433-bp overlap-region fragment of genomic DNA the primer sets that were used to find the pvl genes [17]. The following conditions were used for the amplifications: pre-denaturation for 2 minutes at 94°, then 25 cycles of 94° for 15 seconds, 55° for 15 seconds, and 72° for 15 seconds. and a final extension phase lasting five minutes at 72 °C [18]. The samples were examined on a 2% agarose gel stained with ethidium bromide in 0.5x TBE buffer, and the DNA bands were visualized using a UV transilluminator.

3. Results

By phenotypic methods using a disc diffusion approach with a 30 µg Cefoxitin disc and ORSAB medium (**Figure 1**), 41 MRSA (37.6%) isolates were detected from 109S. aureus isolated and identified from SSTIs.

Figure 1: MRSA Identification by using ORSAB.

In the current study, resistance rates were the highest with Cefoxitin (100%), Amoxicillin/Clavulanic Acid (100%), Cefotaxime (100%), and also (100%) with Cefepime, while the lowest resistance was with Ciprofloxacin (12.2), Sulphamethoxazole/Trimethoprim (12.2%), Imipenem (14.6%), and Linezolid (24.4%) (**Figure 2; Table 1**).

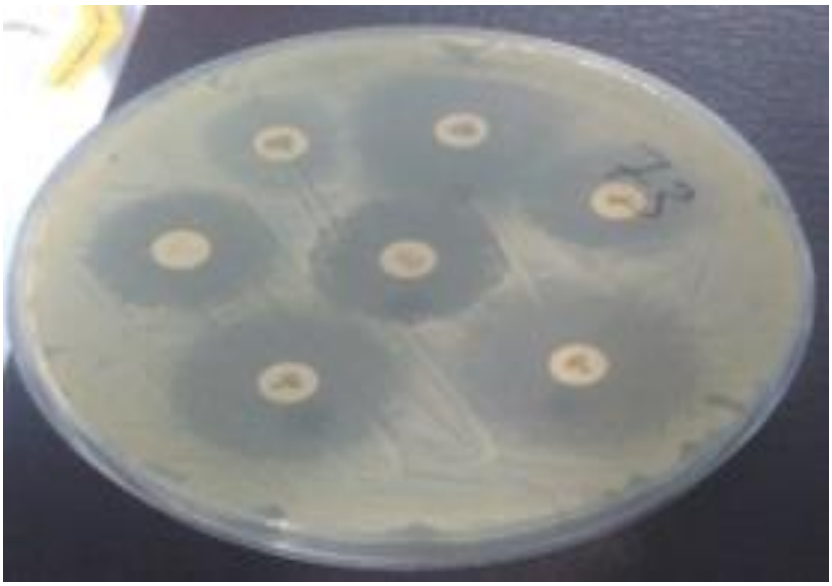

Figure 2: Antibiotic susceptibility among MRSA.

Table 1: Antibiotic susceptibility among MRSA.

Variables	Resistance	Intermediate	Sensitive
Cefoxitin (FOX)	41 (100%)	0 (0%)	0 (0%)
Erythromycin (E)	9 (22%)	22 (53.7%)	10 (24.4%)
Tetracycline (TE)	20 (48.8%)	7 (17.1%)	14 (34.1%)
Gentamycin (CN)	10 (24.4%)	19 (46.3%)	12 (29.3%)
Ciprofloxacin (CIP)	26 (63.4%)	3 (7.3%)	12 (29.3%)
Sulphamethoxazole/Trimethoprim (SXT)	5 (12.2%)	20 (48.8%)	16 (39%)
Linezolid (LNZ)	10 (24.4%)	0 (0%)	31 (75.6%)
Amoxicillin/ Clavulanic acid (AMC)	41 (100%)	0 (0%)	0 (0%)
Imipenem (IPM)	6 (14.6%)	2 (4.9%)	33 (80.5%)
Cefotaxime (CTX)	41 (100%)	0 (0%)	0 (0%)
Cefepime (FEP)	41 (100%)	0 (0%)	0 (0%)

Almost all CA-MRSA (97.5%) were PVL. positive (**Figure 3**), with an amplicon size of 433 bp.

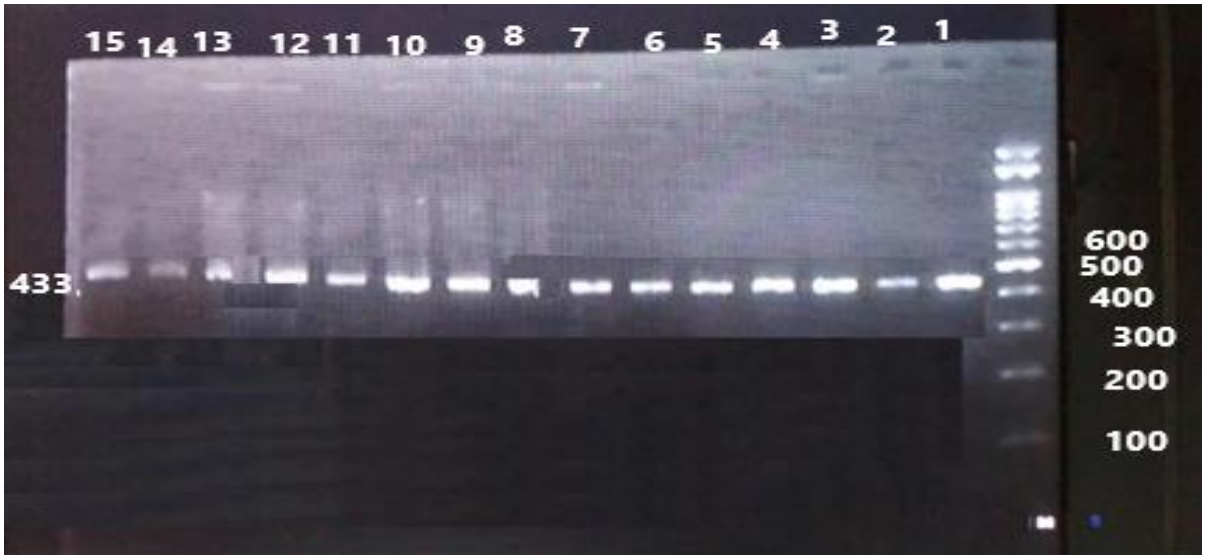

Figure 3: PVL gene of MRSA. All samples (15 samples) are positive (band at 433).

4. Discussion

The incidence of CA-MRSA increased in the early years of this century [19]. MRSA is found in SSTIs due to the exposure of deep tissues to the pathogen [20]. The objective of this research was to analyse the incidence of MRSA among *S. aureus* strains isolated from SSTIs and determine the incidence of the PVL gene within the community-acquired MRSA isolates.

In the present research, resistance rates were the highest with cefoxitin (100%), amoxicillin/clavulanic acid (100%), cefotaxime (100%), and cefepime (100%), while the lowest resistance was with ciprofloxacin (12.2), sulphamethoxazole/ trimethoprim (12.2%), imipenem (14.6%), and linezolid (24.4%). This disagrees with the results of Zuma et al. (2017), who found the resistance rates for Ciprofloxacin were high (60.6%) and 78.7% for Erythromycin, while the lowest was observed with Gentamycin (16.4%), Tetracycline, and Linezolid (4.9%) [21]. The resistance rate was low for Sulphamethoxazole/ Trimethoprim in the current study (12.2%); in the study by Zuma et al. (2017), the resistance rate was 11.5% [21].

The PVL, which is a powerful serious cytotoxin and essential virulence factor of *S. aureus*, was first reported in 1932. This toxin can disrupt leukocyte membranes, cause marked tissue necrosis, and lead to enhanced virulence [22]. PVL-producing MRSA can cause mild SSTIs, but necrotizing pneumonia and severe cases of sepsis were also

documented [23]. CA-MRSA containing the PVL gene has spread worldwide, with various incidences all over the world [24].

PVL is present in most CA-MRSA and is rarely isolated from HA-MRSA [25]. In the current research, we found the PVL gene in 97.5% of the studied CA-MRSA This agrees with Bhatta et al. (2016) who considered the PVL gene an important marker of community-acquired strains, as they found 75 PVL-positive out of 83 CA-MRSA isolates with a positive predictive value of 95% [25]. Our findings were much lower than those of Moghadam et al. (2017), where PVL-positive MRSA strains were 52.94% [26]. 20% of S. aureus isolates from SSTIs were PVL-positive, according to data from the United Kingdom [27]. This is a worrying indicator, as the prevalence rate of PVL-positive S. aureus has increased from less than 2% [28].

Conclusion

PVL-positive MRSA is a rapidly emerging phenomenon, as we found the PVL gene in 98% of the studied CA-MRSA isolated from SSTIs.

Ethical approval and consent to participate: The study protocol was approved by the Research Ethics Committee, Faculty of Medicine at Fayoum University, Egypt, number 16 (Code: D68). Adequate precautions have been taken to safeguard data confidentiality during data collection, storage, analysis, and dispensation.

Funding: This research is not funded.

Conflicts of Interest: All authors declare no conflict of interest.

References

1. Samsudin S, Al- Talib H and Zain ZM. Distribution of Panton-Valentine Leukocidin Positive Staphylococcus aureus Nasal Carriers among Patients and Nurses in Tertiary Hospital-Malaysia: *Staphylococcus aureus* Nasal Carriers among Patients and Nurses, ILKKM Journal of Medical and Health Sciences. 2021; 3 (1): 1- 4.
2. Miller LG, Quan C, Shay A, Mostafaie K, Bharadwa K, Tan N, Matayoshi K, Cronin J, Tan J, Tagudar G, Bayer AS. A prospective investigation of outcomes after hospital discharge for endemic, community-acquired methicillin-resistant and -susceptible *Staphylococcus aureus* skin infection. Clin Infect Dis. 2007;44(4):483-492. doi: 10.1086/511041.
3. Madhava Charyulu E, Gnanamani A, Mandal AB. Identification and Discrimination of Methicillin Resistant *Staphylococcus aureus* Strains Isolated from Burn Wound Sites Using PCR and Authentication with MALDI-TOF-MS. Indian J Microbiol. 2012;52(3):337-345. doi: 10.1007/s12088-011-0245-8.
4. Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etienne J, Johnson SK, Vandenesch F, Fridkin S, O'Boyle C, Danila RN, Lynfield R. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA. 2003;290(22):2976-2984. doi: 10.1001/jama.290.22.2976.
5. Brennan GI, Shore AC, Corcoran S,

Tecklenborg S, Coleman DC, O'Connell B. Emergence of hospital- and community-associated panton-valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* genotype ST772-MRSA-V in Ireland and detailed investigation of an ST772-MRSA-V cluster in a neonatal intensive care unit. J Clin Microbiol. 2012;50(3):841-847. doi: 10.1128/JCM.06354-11.

6. Skov RL, Jensen KS. Community-associated meticillin-resistant Staphylococcus aureus as a cause of hospital-acquired infections. J Hosp Infect. 2009;73(4):364-370. doi: 10.1016/j.jhin.2009.07.004.
7. Shrestha B, Singh W, Raj VS, Pokhrel BM, Mohapatra TM. High prevalence of Panton-Valentine leukocidin (PVL) genes in nosocomial-acquired Staphylococcus aureus isolated from tertiary care hospitals in Nepal. Biomed Res Int. 2014;2014:790350. doi: 10.1155/2014/790350.
8. Shashindran N, Nagasundaram N, Thappa DM, Sistla S. Can Panton Valentine Leukocidin Gene And Clindamycin Susceptibility Serve As Predictors of Community Origin of MRSA From Skin and Soft Tissue Infections? J Clin Diagn Res. 2016;10(1):DC01-DC04. doi: 10.7860/JCDR/2016/14531.7036.
9. Felten A, Grandry B, Lagrange PH, Casin I. Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA): a disk diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-screen latex agglutination test. J Clin Microbiol. 2002;40(8):2766-2771. doi: 10.1128/JCM.40.8.2766-2771.2002.
10. Clinical and laboratory standards institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI document M. wayne, PA. 2019; 22: 99- 122.
11. Ben Nsira S, Dupuis M, Leclercq R. Evaluation of MRSA Select, a new chromogenic medium for the detection of nasal carriage of methicillin-resistant *Staphylococcus aureus.* Int J Antimicrob Agents. 2006;27(6):561-564. doi: 10.1016/j.ijantimicag.2006.03.011.
12. Patton T, Barrett J, Brennan J, Moran N. Use of a spectrophotometric bioassay for determination of microbial sensitivity to manuka honey. J Microbiol Methods. 2006;64(1):84-95. doi: 10.1016/j.mimet.2005.04.007.
13. Clinical and laboratory standards institute (CLSI). Performance standards for antimicrobial susceptibility testing: 22 informational supplemented. CLSI document M. wayne, PA. 2012; 22: 100-22.
14. Clinical and laboratory standards institute (CLSI). Performance standards for antimicrobial susceptibility testing: 22 informational supplemented. CLSI document M. wayne, PA. 2015; 22: 100-22.
15. Zhang K, McClure JA, Conly JM. Enhanced multiplex PCR assay for typing of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*. Mol Cell Probes. 2012;26(5):218-221. doi: 10.1016/j.mcp.2012.04.002.
16. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant Staphylococcus aureus. J Clin Microbiol. 2005;43(10):5026-5033. doi: 10.1128/JCM.43.10.5026-5033.2005.
17. Lina G, Piémont 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(5):1128-1132. doi: 10.1086/313461.

18. Francis JS, Doherty MC, Lopatin U, Johnston CP, Sinha G, Ross T, Cai M, Hansel NN, Perl T, Ticehurst JR, Carroll K, Thomas DL, Nuermberger E, Bartlett JG. Severe community-onset pneumonia in healthy adults caused by methicillin-resistant *Staphylococcus aureus* carrying the Panton-Valentine leukocidin genes. Clin Infect Dis. 2005;40(1):100-107. doi: 10.1086/427148.
19. Principi N, Argentiero A, Neglia C, Gramegna A, Esposito S. New Antibiotics for the Treatment of Acute Bacterial Skin and Soft Tissue Infections in Pediatrics. Pharmaceuticals (Basel). 2020;13(11):333. doi: 10.3390/ph13110333.
20. Silva V, Almeida F, Carvalho JA, Castro AP, Ferreira E, Manageiro V, Tejedor-Junco MT, Caniça M, Igrejas G, Poeta P. Emergence of community-acquired methicillin-resistant Staphylococcus aureus EMRSA-15 clone as the predominant cause of diabetic foot ulcer infections in Portugal. Eur J Clin Microbiol Infect Dis. 2020;39(1):179-186. doi: 10.1007/s10096-019-03709-6.
21. Zuma AVP, Lima DF, Assef APDC, Marques EA, Leão RS. Molecular characterization of methicillin-resistant *Staphylococcus aureus* isolated from blood in Rio de Janeiro displaying susceptibility profiles to non-β-lactam antibiotics. Braz J Microbiol. 2017;48(2):237-241. doi: 10.1016/j.bjm.2016.09.016.
22. Shrestha B, Singh W, Raj VS, Pokhrel BM, Mohapatra TM. High prevalence of Panton-Valentine leukocidin (PVL) genes in nosocomial-acquired *Staphylococcus aureus* isolated from tertiary care hospitals in Nepal. Biomed Res Int. 2014;2014:790350. doi: 10.1155/2014/790350.
23. Maltezou HC, Giamarellou H. Community-acquired methicillin-resistant Staphylococcus aureus infections. Int J Antimicrob Agents 2006; 27(2):87-96.
24. Ellington MJ, Ganner M, Warner M, Cookson BD, Kearns AM. Polyclonal multiply antibiotic-resistant methicillin-resistant *Staphylococcus aureus* with Panton-Valentine leucocidin in England. J Antimicrob Chemother. 2010;65(1):46-50. doi: 10.1093/jac/dkp386.
25. Bhatta DR, Cavaco LM, Nath G, Kumar K, Gaur A, Gokhale S, Bhatta DR. Association of Panton Valentine Leukocidin (PVL) genes with methicillin resistant Staphylococcus aureus (MRSA) in Western Nepal: a matter of concern for community infections (a hospital based prospective study). BMC Infect Dis. 2016;16:199. doi: 10.1186/s12879-016-1531-1.
26. Ohadian Moghadam S, Modoodi Yaghooti M, Pourramezan N, Pourmand MR. Molecular characterization and antimicrobial susceptibility of the CA-MRSA isolated from healthcare workers, Tehran, Iran. Microb Pathog. 2017;107:409-412. doi: 10.1016/j.micpath.2017.04.027.
27. Shallcross LJ, Williams K, Hopkins S, Aldridge RW, Johnson AM, Hayward AC. Panton-Valentine leukocidin associated staphylococcal disease: a cross-sectional study at a London hospital, England. Clin Microbiol Infect. 2010;16(11):1644-1648. doi: 10.1111/j.1469-0691.2010.03153.x.
28. Holmes A, Ganner M, McGuane S, Pitt TL, Cookson BD, Kearns AM. Staphylococcus aureus isolates carrying Panton-Valentine leucocidin genes in England and Wales: frequency, characterization, and association with clinical disease. J Clin Microbiol. 2005;43(5):2384-2390. doi: 10.1128/JCM.43.5.2384-2390.2005.