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Prevalence of SCC*mec* Types I, II, III, and *pvl* gene among Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolated from clinical specimens in Sanglah General Hospital

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ABSTRACT

Background: *Methicillin-Resistant Staphylococcus aureus* (MRSA) is a big challenge for health services worldwide which causes infections both in healthcare and community. Healthcare-associated MRSA (HA-MRSA) strains are shown to be resistant to beta-lactam antibiotics and several non-beta lactam antibiotics. At the same time, the community-associated MRSA (CA-MRSA) tends to be resistant to beta-lactam antibiotics. MRSA carried *staphylococcal cassette chromosome* (SCC*mec*) types I, II, III, IV, and V. SCC*mec* types I, II, and III were predominantly found in HA-MRSA *strain* while SCC*mec* types IV and V predominantly found in CA-MRSA strains. Furthermore, the *panton valentine leukocidine* (*pvl*) gene is commonly found in CA-MRSA strains. Therefore, this study aimed to determine the prevalence of SCC*mec* types I, II, III, and *pvl* gene in MRSA isolated from clinical specimens in Sanglah General Hospital.

Methods: This study was a cross-sectional descriptive study. MRSA was isolated from clinical specimens (sputum, wounds, tissue, blood, etc.) from January 2020 to July 2021 and identified by the Vitek 2 Compact (Biomerieux, France) at the Clinical Microbiology Laboratory of Sanglah Hospital. Prevalence of SCC*mec* and *pvl* gene using PCR. Data were analyzed using Microsoft Excel version 2010 for Windows.

Results: Most of the specimens (69.56%) were wound. Seventeen (73.91%) out of 23 MRSA isolates were positive for the SCC*mec* III and *pvl* gene, while none was positive for the SCC*mec* I and SCC*mec* II. About 19 (82.60%) isolates were resistant to two or more non-beta-lactam antibiotics.

Conclusions: The isolates of MRSA in this study were predominantly isolated from wound specimens, with the most prevalent genetic element being SCC*mec* III. In this study, although most MRSA isolates carried SCC*mec* III that suggested as HA-MRSA, however, most of the strains harbored the *pvl* gene. This interesting phenomenon needs to be further elucidated.

Keywords: MRSA, PCR, *Panton-Valentine Leukocidine*, *Staphylococcal cassette chromosome mec*.
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INTRODUCTION

Antibiotics research has revolutionized the medical world, resulting in breakthroughs across a broad spectrum of clinical medicine.[1] However, misuse of antibiotics can lead to the emergence of multidrug-resistant organisms (MDROs). Usually, MDROs are associated with the incidence of healthcare-associated infections (HAIs). However, some MDROs are increasingly becoming the cause of disease in the community. The spread of MDROs in the community leads to an increase in the population at risk and increases the number of infections caused by MDROs.[2] Various types of MDROs have been found in the community, including *Methicillin-Resistant Staphylococcus aureus* (MRSA), *Vancomycin-Resistant Enterococci* (VRE), *Extended Spectrum Beta-Lactamase* (ESBL) producing Enterobacteriaceae, *Carbapenem-Resistant Enterobacteriaceae* (CRE), *Carbapenem-Resistant Pseudomonas aeruginosa* (CR-PA), and *Carbapenem-Resistant Acinetobacter baumanii* (CR-AB).[2]

Methicillin-resistant Staphylococcus aureus (MRSA) is a *Staphylococcus aureus* strain resistant to beta-lactam antibiotics. MRSA can be classified molecularly into Community-Associated MRSA (CA-MRSA) strains and healthcare-associated MRSA (HA-MRSA) strains.[3] Most HA-MRSA strain carries SCC*mec* elements types I, II, or III. Meanwhile, CA-MRSA isolates have SCC*mec* type IV or type V. HA-MRSA tends to be resistant to many antibiotics than CA-MRSA, especially non-beta lactam antibiotics such as erythromycin, tetracycline, clindamycin, norfloxacin, ciprofloxacin, and cotrimoxazole.[4-7] At the same time,

CA-MRSA shows better sensitivity to non-beta-lactam antibiotics. In addition, CA-MRSA often carries the Panton-Valentine Leukocidine (*pvl*) gene.[8] PVL is a type of cytotoxin that can cause mild skin infections, pneumonia, and sepsis. PVL is cytotoxic to neutrophils and lesser to monocytes and macrophages, not to lymphocytes.[9]

The prevalence of HA-MRSA from several hospitals in Indonesia varies from 8% to - 32%.[10,11] SCC*mec* III was predominantly found (80%) in HA-MRSA isolates in Indonesia, while the prevalence of SCC*mec* II was around 7%.[12,13] The prevalence of *pvl* genes in Indonesia varies in different regions. A study at Cipto Mangunkusumo Hospital found that 8.3% of isolates harbored *pvl* genes while the prevalence of *pvl* genes among clinical isolates at Denpasar was 46.5%.[14,15]

There is a lack of study to identify SCC*mec* elements and *pvl* genes from clinical isolates in Bali. Therefore, this study aims to determine the prevalence of the SCC*mec* I, II, and III elements and *pvl* genes of MRSA isolated from clinical specimens in Sanglah General Hospital.

MATERIAL AND METHODS

This cross-sectional study was conducted in the Clinical Microbiology Laboratory Faculty of Medicine, Universitas Udayana, Bali, Indonesia. A purposive sample of 23 glycerol stock of MRSA isolates collected from January 2020 to July 2021 in the Clinical Microbiology Laboratory Sanglah General Hospital was used in this study. Identification of MRSA and the antibiotics susceptibility testing were performed by using VITEK 2 Compact (Biomerieux, France). The inclusion criteria included isolated from clinical specimens such as sputum, wounds, tissue, or blood from January 2020 to July 2021 at Clinical Microbiology Laboratory Faculty of Medicine, Universitas Udayana, Bali, Indonesia.

DNA isolation was performed by using the boiling method. In this method, the bacterial colony was placed in a tube by using a 200 µl Tris-EDTA buffer as a solution. The suspension was then boiled at 100°C for ten minutes. Then, the suspension was centrifuged at 13,000 x g for one minute. This boiling method takes twelve minutes to proceed.[16] Uniplex PCR was carried out using kit mix PCR GoTaq® Green Master Mix (Promega) to detect SCC*mec* types I, II, III, and *pvl* gene using primers according to the previous studies.[17,18] Primers concentration for this study was SCC*mec* I (0.8 µM), SCC*mec* II and SCC*mec* III (0.5 µM), and *pvl* gene (0.3 µM). Amplification was carried out in a thermocycler machine (Biometra, USA). PCR steps were started with initial denaturation conditions of 95°C for 2 minutes followed by 35 cycles with denaturation temperature of 95°C for one minute, annealing temperature at 54°C (SCC*mec* I), 49°C (SCC*mec* II), 50°C (SCC*mec* III), 60°C (*pvl* gene) for one minute each, extension at 72-74°C for one minute, and final extension at 72-74°C for five minutes. The amplicons were visualized with 1.5% agarose gel stained using gel red (Biotium) then the images were obtained by UV visualization. Data were analyzed using Microsoft Excel version 2010 for Windows.

RESULTS

There were twenty-three MRSA isolates used in this study. Three isolates were isolated from blood (13.04%), sixteen isolates from the wound (including wound swab, tissue, and pus) (69.56%), one isolate from the double lumen (4.35%), and three isolates from sputum (13.04%), as shown in Table 2 (Table 1).

The analysis results of VITEK 2 Compact (Biomerieux, France) showed that all isolates were resistant to beta-lactam antibiotics, and 19 (82.60%) out of 23 isolates had resistance non-beta-lactam antibiotics. The antibiotic susceptibility pattern of MRSA can be seen in Figure 1.

Uniplex PCR results showed that 17 isolates (73.91%) were positive for SCC*mec* III and the *pvl* gene, no isolate was positive for SCC*mec* I and SCC*mec* II. The wound specimen type found the most positive results of SCC*mec* III (47.82%) and *pvl* gene (52.17%). The PCR results based on specimen distribution can be seen in Table 2.

Of the six negative isolates to SCC*mec* I, II, and III, three were sensitive to non-beta-lactam antibiotics. One isolate had resistance to quinolones (ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin), gentamicin, tetracycline, and rifampicin. An isolate had similar resistance to the quinolones, gentamicin, in addition to trimethoprim/sulfamethoxazole (TMP/SMX). The other isolate had resistance to macrolides. Meanwhile, one isolate was positive for SCC*mec* III, which was sensitive to all non-beta-lactam antibiotics. About 17 isolates were positive to the *pvl* gene; however, only four were susceptible to non-beta-lactam antibiotics. Meanwhile, the rest were resistant to more than two non-beta-lactam antibiotics (Figure 2).

Table 1. Distribution of MRSA isolates based on specimen type.

Specimen type	Isolates (N=23)
Blood, n (%)	3 (13.04%)
Wound, n (%)	16 (69.56%)
Double lumen, n (%)	1 (4.35%)
Sputum, n (%)	3 (13.04%)

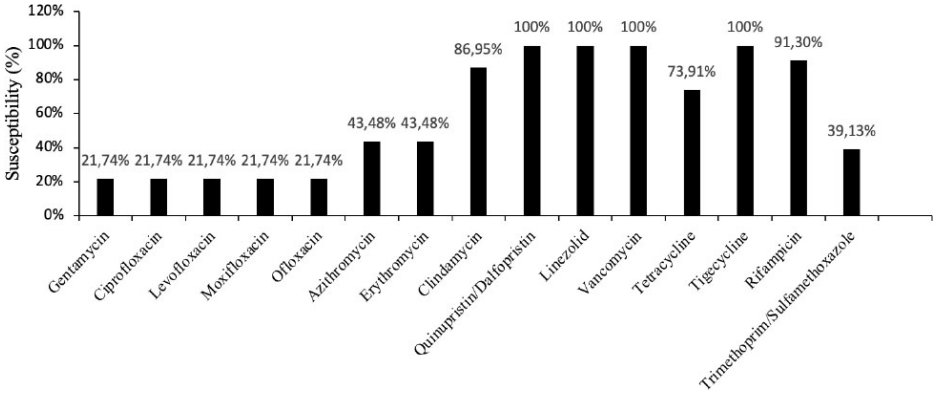

Figure 1. MRSA Susceptibility Percentage to Non-Beta Lactam Antibiotics.

Table 2. Distribution of SCC*mec* III and *pvl* gene-positive in MRSA isolates based on specimen type.

Specimen type	SCC*mec* III Positive Isolate (N=23)	*pvl* gene Positive Isolate (N=23)
Blood, n (%)	2 (8.7%)	3 (13.04%)
Wound, n (%)	11 (47.82%)	12 (52.17%)
Double Lumen, n (%)	1 (4.35%)	0 (0%)
Sputum, n (%)	3 (13.04%)	2 (8.7%)
Total	**17 (73.91%)**	**17 (73.91%)**

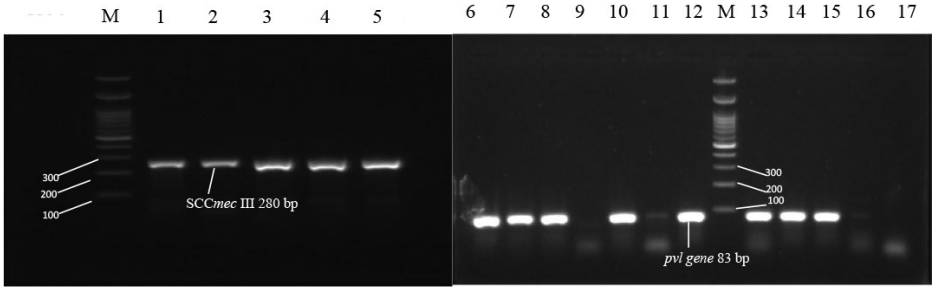

Figure 2. Uniplex PCR for SCC*mec* III (280 bp) (left) and *pvl* gene (83 bp) (right) detection (Lane 1, 2, 3, 4, and 5: positive for SCC*mec* III, Lane 6, 7, 8, 10, 12, 13, 14, and 15: positive for *pvl* gene, Lane M: Marker 100 bp DNA Ladder, Geneaid).

DISCUSSION

MRSA has been known as one of the causes of HAIs in almost all hospitals worldwide since the 1980s. The predominant clinical manifestations of MRSA infection are skin furuncles and impetigo in children. This infection can metastasize to other tissues, such as osteomyelitis, arthritis, endocarditis, brain abscess, lung, kidney, and mammary glands.[19] This study found that MRSA isolates were predominantly from wounds (69.56%). This finding was similar to the study at Dr. Cipto Mangunkusumo Hospital, where the predominant isolates from pus were 25 (34.7%) of 72 isolates.[20] A study conducted at Dr. Saiful Anwar Hospital found that predominant isolates of MRSA from pus were 49%, this results similar to this study.[21]

This study carried out the polymerase chain reaction on 23 MRSA isolates. It was found that 17 isolates (73.91%) had SCC*mec* type III and *pvl* genes. At the same time, for SCC*mec* I and SCC*mec* II, there was no positive isolate tested. Studies at Universitas Padjajaran and Universitas Sriwijaya also found similar results, which showed that the predominant type was SCC*mec* III (89%), while SCC*mec* IV was 11%.[13] Research in Jambi showed similar findings where the prevalence of SCC*mec* III was 85.72%, SCC*mec* II was 7.14%, and SCC*mec* IV was 7.14%.[12] However, different findings were found in the majority of the *pvl* gene. Research at Sanglah Hospital and Puskesmas in Denpasar found a *pvl* prevalence of 46.5%, and at Cipto Mangunkusumo Hospital found were 8.3%.[15,16]

This study found that 19 isolates (82.60%) were resistant to non-beta-lactam antibiotics, of which 78% isolates were resistant to gentamicin, ciprofloxacin, levofloxacin, ofloxacin, moxifloxacin. In addition, 60% of isolates were resistant to TMP/SMX, 56% were resistant to azithromycin, and erythromycin, 26% resistant to tetracycline, 13% resistant to clindamycin, and 8% to rifampicin. Fortunately, all isolates (100%) were still sensitive to quinupristin/dalfopristin, linezolid, vancomycin, and tigecycline.

The antibiotics still recommended for MRSA management include quinupristin/dalfopristin, linezolid, vancomycin, and tigecycline. Meanwhile, quinolones, aminoglycosides, and macrolides have decreased sensitivity for MRSA therapy. The first line antibiotic therapy that can treat MRSA infection is vancomycin, and the second line antibiotic is linezolid.[22] The antimicrobial susceptibility patterns of HA-MRSA and CA-MRSA strains are commonly different. According to the CDC, HA-MRSA is resistant to commonly used antibiotics such as erythromycin, clindamycin, quinolones, and tetracyclines. While the CA-MRSA strain has resistance only to beta-lactam antibiotics and erythromycin and, in some instances, is also resistant to quinolones.[23] A similar study at Universitas Padjajaran also showed that MRSA was resistant to gentamicin (89%), erythromycin (89%), norfloxacin (89%), and vancomycin (2%)[13]. A study conducted at H. Adam Malik Hospital Medan also showed the phenomenon of resistance to non-beta-lactam antibiotics such as resistance to ciprofloxacin and levofloxacin (97.5%), clindamycin, and erythromycin (45%), gentamicin (87.5%), tetracycline (62.5%), vancomycin (15%), TMP/SMX (27.5%), moxifloxacin (95%), and linezolid (2.5%).[24]

This study showed that the predominant strain was the HA-MRSA, indicated by the SCC*mec* type III element detected in 17 isolates. The other six isolates negative to SCC*mec* I, II, and III probably derived from the CA-MRSA strain. Commonly *pvl* gene associated with CA-MRSA strain, however in this study, *pvl* gene could not be used as an indicator of CA-MRSA strain solely because there was no difference in the pattern of antibiotic sensitivity between positive and negative strains *pvl* gene, and it was also found that some isolates with *pvl* gene also had SCC*mec* III.[8] Although there were no positive controls for confirming SCC*mec*III and *pvl* gene PCR, we overcame this limitation by using the PCR marker's size to determine the base pair size of SCC*mec* and *pvl* gene amplicons.

CONCLUSIONS

The isolates of MRSA in this study were predominantly isolated from wound specimens, with the most prevalent genetic element being SCC*mec* III. In this study,

although most MRSA isolates carried SCC*mec* III that suggested as HA-MRSA, however, most of the strains harbored the *pvl* gene. This interesting phenomenon needs to be further elucidated.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

Research Ethics Committee, Faculty of Medicine, Udayana University, Denpasar, Bali, Indonesia, permitted this study with numbers: 356/UN14.2.2.VII.14/LT/2021.

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AUTHOR CONTRIBUTIONS

All authors equally contribute to the study from the conceptual framework, literature search, data acquisition, data analysis, manuscript preparation until reporting the study results through publication

REFERENCES

1. Marston HD, Dixon DM, Knisely JM, Palmore TN, Fauci AS. Antimicrobial Resistance. JAMA. 2016;316(11):1193-1204.
2. van Duin D, Paterson DL. Multidrug-Resistant Bacteria in the Community: Trends and Lessons Learned. Infect Dis Clin North Am. 2016;30(2):377-390.
3. Lakhundi S, Zhang K. Methicillin-Resistant Staphylococcus aureus: Molecular Characterization, Evolution, and Epidemiology. Clin Microbiol Rev. 2018;31(4):e00020-18.
4. Taherirad A, Jahanbakhsh R, Shakeri F, Anvary S, Ghaemi EA. Staphylococcal Cassette Chromosome mec Types Among Methicillin-Resistant Staphylococcus aureus in Northern Iran. Jundishapur J Microbiol. 2016;9(8):e33933.
5. Chen CJ, Huang YC. New epidemiology of Staphylococcus aureus infection in Asia. Clin Microbiol Infect. 2014;20(7):605-623.
6. Gayatri AA, Utama S, Somia A, Merati TP. MRSA infection in patients hospitalized at Sanglah Hospital: a case series. Acta Med Indones. 2015;47(1):50-55.
7. Santika IKJ, Pinatih KJP, Fatmawati NND. Pola Kepekaan Methicillin-Resistant Staphylococcus aureus terhadap Antibiotika di RSUP Sanglah pada Agustus 2013 - Oktober 2013. E-Jurnal Medika Udayana. 2014;3(7):1-8.
8. David MZ, Daum RS. Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev. 2010;23(3):616-687.
9. Spaan AN, Henry T, van Rooijen WJM, Perret M, Badiou C, Aerts PC, et al. The staphylococcal toxin Panton-Valentine Leukocidin targets human C5a receptors. Cell Host Microbe. 2013;13(5):584-594.
10. Santosaningsih D, Santoso S, Budayanti NS, Kuntaman K, Lestari ES, Farida H, et al. Epidemiology of Staphylococcus aureus harboring the mecA or Panton-Valentine leukocidin genes in hospitals in Java and Bali, Indonesia. Am J Trop Med Hyg. 2014;90(4):728-34.
11. Kuntaman K, Hadi U, Setiawan F, Koendori EB, Rusli M, Santosaningsih D, et al. Prevalence of Methicillin Resistant Staphylococcus Aureus from Nose and Throat of Patients on Admission to Medical Wards of Dr Soetomo Hospital, Surabaya, Indonesia. Southeast Asian J Trop Med Public Health. 2016;47(1):66-70.
12. Humaryanto, Hanina, Lipinwati, Simanjuntak CA. Identification of SCCmec Methicillin-Resistant Staphylococcus Aureus (MRSA) in Jambi using Polymerase Chain Reaction (PCR). Indonesian Journal of Tropical and Infectious Disease. 2020;8(2):77-82.
13. Sudigdoadi, S. Analisis Tipe Staphylococcal Cassette Chromosome mec (SCCmec) Isolat Methicillin-Resistant Staphylococcus aureus (MRSA). Majalah Kedokteran Bandung. 2010;42(4):149–154.
14. Linosefa L, Lestari DC, Kusumaningrum A, Karuniawati A, Yasmon A. Prevalensi Isolat Mrsa Penghasil Panton-Valentine Leukocidin Pada Pasien Icu Rumah Sakit Tersier. Majalah Kedokteran Andalas. 2016;39(1):1-10.
15. Budayanti NS, Rusyati LM, Bagus I, Dwija IBP, Puspasari NMI, Adiguna MS. Deteksi Gen Panton-Valentine Leukocidin Dari Staphylococcus Aureus Pada Pasien Infeksi Kulit. MDVI. 2010;41(2):54–59.
16. Wibawa IBGA, Darwinata AE, Fatmawati NND, Budayanti NS. Rapid Detection of Methicillin Resistant Staphylococci Using Multiplex PCR With Boiling Method for DNA Isolation. J Heal Sci Med. 2017;1(2):8–10.
17. 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.
18. Deurenberg RH, Vink C, Driessen C, Bes M, London N, Etienne J, Stobberingh EE. Rapid detection of Panton-Valentine leukocidin from clinical isolates of Staphylococcus aureus strains by real-time PCR. FEMS Microbiol Lett. 2004;240(2):225-228.
19. DeLeo FR, Diep BA, Otto M. Host defense and pathogenesis in Staphylococcus aureus infections. Infect Dis Clin North Am. 2009;23(1):17-34.
20. Liana P. Gambaran Kuman Methicilin Resistant Staphylococcus Aureus (MRSA) di Laboratorium Mikrobiologi Departemen Patologi Klinik Rumah Sakit Dr. Cipto Mangunkusumo (RSCM) Periode Januari-Desember 2010. Jurnal Kedokteran dan Kesehatan. 2014;46(3):171-175.
21. Erikawati D, Santosaningsih D, Santoso S. Tingginya Prevalensi MRSA pada Isolat Klinik Periode 2010- 2014 di RSUD Dr. Saiful Anwar Malang, Indonesia. Jurnal Kedokteran Brawijaya. 2016;29(2):149–156.
22. Cong Y, Yang S, Rao X. Vancomycin resistant Staphylococcus aureus infections: A review of case updating and clinical features. J Adv Res. 2019;21:169-176.
23. Sastre A, Roberts PF, Presutti RJ. A practical guide to community-acquired MRSA. J Fam Pract. 2013;62(11):624-629.
24. Nasution GS. Deteksi Gen Resisten mecA Pada Isolat Bakteri Staphylococcus aureus yang Tergolong MRSA Dari Hasil Pemeriksaan VITEK 2 Compact [Tesis]. Universitas Sumatera Utara: Medan. 2017.