**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 mec* (SCCmeC) types I, II, III, IV, and V. SCCmeC types I, II, and III were predominantly found in HA-MRSA strain while SCCmeC types IV and V predominantly found in CA-MRSA strains. Furthermore, the *panton valentine leukocidin* (*pvl*) gene is commonly found in CA-MRSA strains. Therefore, this study aimed to determine the prevalence of SCCmeC 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 SCCmeC 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 SCCmeC III and *pvl* gene, while none was positive for the SCCmeC I and SCCmeC 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 SCCmeC III. In this study, although most MRSA isolates carried SCCmeC 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 Leukocidin*, *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. 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. 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 baumannii* (CR-AB).

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. Most HA-MRSA strain carries SCCmeC elements types I, II, or III. Meanwhile, CA-MRSA isolates have SCCmeC 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. At the same time,
CA-MRSA shows better sensitivity to non-beta-lactam antibiotics. In addition, CA-MRSA often carries the Panton-Valentine Leukocidin (pvl) gene. PVL is a type of cytolysin that can cause mild skin infections, pneumonia, and sepsis. PVL is cytoxic to neutrophils and lesser to monocytes and macrophages, not to lymphocytes.

The prevalence of HA-MRSA from several hospitals in Indonesia varies from 8% to 32%. SCCmec III was predominantly found (80%) in HA-MRSA isolates in Indonesia, while the prevalence of SCCmec II was around 7%. 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%.

There is a lack of study to identify SCCmec elements and pvl genes from clinical isolates in Bali. Therefore, this study aims to determine the prevalence of the SCCmec 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. Uniplex PCR was carried out using kit mix PCR GoTaq Green Master Mix (Promega) to detect SCCmec types I, II, III, and pvl gene using primers according to the previous studies. Primers concentration for this study was SCCmec I (0.8 µM), SCCmec II and SCCmec 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 (SCCmec I), 49°C (SCCmec II), 50°C (SCCmec 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 SCCmec III and the pvl gene, no isolate was positive for SCCmec I and SCCmec II. The wound specimen type found the most positive results of SCCmec 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 SCCmec 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 SCCmec 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.

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Isolates (N=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood, n (%)</td>
<td>3 (13.04%)</td>
</tr>
<tr>
<td>Wound, n (%)</td>
<td>16 (69.56%)</td>
</tr>
<tr>
<td>Double lumen, n (%)</td>
<td>1 (4.35%)</td>
</tr>
<tr>
<td>Sputum, n (%)</td>
<td>3 (13.04%)</td>
</tr>
</tbody>
</table>

### Figure 1. MRSA Susceptibility Percentage to Non-Beta Lactam Antibiotics.
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. 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. A study conducted at Dr. Saiful Anwar Hospital found that predominant isolates of MRSA from pus were 49%, this results similar to this study.

This study carried out the polymerase chain reaction on 23 MRSA isolates. It was found that 17 isolates (73.91%) had SCCmec type III and pvl genes. At the same time, for SCCmec I and SCCmec 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 SCCmec III (89%), while SCCmec IV was 11%. Research in Jambi showed similar findings where the prevalence of SCCmec III was 85.72%, SCCmec II was 7.14%, and SCCmec IV was 7.14%. 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%.

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.

The antigens detected in 16 isolates were SCCmec III and 7 were SCCmec IV. SCCmec III gene-positive in MRSA isolates based on specimen type.

**DISCUSSION**

The isolates of MRSA in this study were predominantly isolated from wound specimens, with the most prevalent genetic element being SCCmec III. In this study, 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. A similar study at Universitas Padjajaran also showed that MRSA was resistant to gentamicin (89%), erythromycin (89%), norfloxacin (89%), and vancomycin (2%). 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%).

This study showed that the predominant strain was the HA-MRSA, indicated by the SCCmec type III element detected in 17 isolates. The other six isolates negative to SCCmec 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 SCCmec III.

Although there were no positive controls for confirming SCCmecIII and pvl gene PCR, we overcame this limitation by using the PCR marker’s size to determine the base pair size of SCCmec 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 SCCmec III. In this study,
although most MRSA isolates carried SCCmec 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.

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