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Research Report

Identification of SCC MEC Methicillin-Resistant Staphylococcus Aureus (MRSA) From Hospitals' Clinical Samples in Jambi using Polymerase Chain Reaction (PCR)

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ABSTRACT

Staphylococcal cassette chromosome mec (SCCmec) is one of the mobile genetic elements of Methicillin-Resistant Staphylococcus aureus (MRSA) that carries many resistance genes and allows SCCmec to move from one bacterium to another. Twelve types of SCCmec have been identified throughout the world. Identification of SCCmec type is needed to determine the pattern of MRSA resistance in a particular region. This study aimed to identify the type of SCCmec MRSA from clinical samples. Specifically, this study was conducted at the Biomolecular Laboratory of the Faculty of Medicine and Health Sciences of Jambi University in June 2018-February 2019. Culture was carried out on 100 clinical specimens of festering wound swabs from inpatients at hopitals in Jambi City. A total of 32 samples of Staphytect plus test positive were tested using Cefoxitin disc diffusion method and MecA Polymerase Chain Reaction (PCR). There were 14 samples identified as MRSA isolates, namely twelve samples (85.72%) of SCCmec type III, one sample (7.14%) of SCCmec type II, and one sample (7.14%) of SCCmec type IVb. The results were different from previous studies where all MRSA isolates (100%) in Indonesia were SCCmec type III, although most SCCmec types were still dominated by SCCmec type III. This study concludes that there has been a shift in the content of SCCmec in MRSA isolate originating from hospitals in Jambi city.

Keywords: *MRSA, MecA, SCCMec, genetic, resistance*

ABSTRAK

Staphylococcal cassette chromosome mec (SCCmec) merupakan salah satu elemen genetik yang mobile pada Methicillin Resistant Staphylococcus aureus (MRSA) yang membawa beberapa gen resistensi dan memungkinkan SCCmec berpindah dari satu bakteri ke bakteri lainnya. Terdapat dua belas tipe SCCmec yang telah teridentifi kasi di seluruh dunia. Identifi kasi tipe SCCmec sangat diperlukan untuk mengetahui pola resistensi MRSA di suatu wilayah tertentu. Penelitian ini bertujuan untuk mengidentifi kasi tipe SCCmec MRSA dari sampel klinik. Penelitian ini dilakukan di Laboratorium Biomolekuler Fakultas Kedokteran dan Ilmu Kesehatan Universitas Jambi pada bulan Juni 2018-Februari 2019. Kultur dilakukan terhadap 100 spesimen klinik berupa swab luka yang bernanah pada pasien yang dirawat inap di Rumah Sakit di Kota Jambi. Sebanyak 32 sampel yang positif pada Uji Staphytect plus diuji dengan Cefoxitin Disk Difusion Metode dan Polymerase Chain Reaction (PCR) MecA. Terdapat 14 sampel yang teridentifi kasi sebagai isolat MRSA. Sebanyak 12 sampel (85,72%) merupakan SCCmec tipe III, satu sampel (7,14%) SCCmec tipe II dan satu sampel (7,14%) SCCmec tipe IVb. Hasil penelitian ini berbeda dengan penelitian sebelumnya dimana seluruh (100%) galat MRSA di Indonesia merupakan SCCmec tipe III, meskipun tipe SCCmec terbanyak masih didominasi oleh SCCmec tipe III. Kesimpulan dari penelitian ini adalah mulai ditemukannya perubahan kandungan SCCmec pada isolat MRSA yang berasal dari rumah sakit di Kota Jambi.

Kata kunci: *MRSA, MecA, SCCMec, genetic, resistensi*

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INTRODUCTION

S. aureus is a common bacterial pathogen that causes minor to serious disease in human. *S. aureus* can be treated with methicillin (MSSA) and resistant to methicillin (MRSA). Infection of MRSA becomes an important concern throughout the world and associated with infection in both Hospital-acquired Methicillin-Resistant *Staphylococcus aureus* (HA-MRSA) and Community-acquired Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA).[1,2,3] Infection caused by MRSA keeps increasing year to year. According to research in Indonesia, the prevalence of MRSA is approximately 30–40%. The prevalence of MRSA in Cipto Mangunkusumo Hospital on 2010 and Abdul Moeloek Hospital Lampung on 2013 were 32% and 38%, respectively.[4,5]

The resistance of MRSA against beta-lactam antibiotic is encoded by the mecA gene. MecA gene is a part of the conserved MRSA genetic elements of the Staphylococcal cassette chromosome mec (SCCmec), encoding PBP2a or PBP2 mutants.[6,7] MecA gene is located in a genetic element called the Staphylococcal Cassette Chromosome (SCCmec). SCCmec is integrated into the chromosome of *S. aureus* at a unique site located near the *S. aureus* origin of replication. SCCmec is a mobile genetic element that carries many resistance genes and allows SCCmec to move from one bacterium to another.[8] Thirteen types of SCCmec have been identified throughout the world.[9]

The components of SCCmec are recombinase genes (ccr complexes), mec complex genes, additional resistant genes, and insertion sequences (IS).[8,10] Differences between SCCmec are determined by variations in the ccr complex and the mec complex. SCCmec type I about 39 kb, in the 1960s era, has a composition of type 1 ccr complex and class B mec complex. SCCmec type II about 52 kb, dominant in the 1980s era, has a composition of type 2 ccr complex and the class A mec complex. SCCmec type III about 67 kb, dominant in the 1980s, has the composition of the type 3 ccr complex and the class A mec complex. SCCmec type IV (a and b) about 20.9–24,3 kb, found in 2002, has a composition of type 2 ccr complex and class B mec complex.[4,5,6]

Various findings of MRSA patterns in the last decade have shown the changes in distribution, sensitivity to various antibiotics, and possible changes in the SCCmec type.[11,12] Identification of SCCmec type is needed to determine the pattern of MRSA resistance in a particular region. Based on the previous description, it is important to identify the type of SCCmec MRSA from clinical samples.

MATERIALS AND METHODS

This study was a cross-sectional study. This study was conducted in the Biomolecular Laboratory of the Faculty of Medicine and Health Sciences in Jambi University from June 2018 to February 2019.

A hundred samples of swabs from festering wound were collected from three secondary referral hospitals in Jambi (Raden Mattaher hospital, dr. Bratanata hospital, and Kambang hospital). The swabs were incubated at 30 ºC on Mannitol Salt Agar (MSA) for 18-24 hours, the yellowish colony would be confirmed by Gram staining. Gram-positive coccus bacteria were tested using Staphytect plus Test DR 850 M (Oxoid) to detect clumping factor, protein A and type 5 and 8 capsules of polysaccharide.

Positive samples were tested for resistance to cefoxitin antibiotics by using the disc diffusion method in Mueller Hinton (MH) Agar. The susceptibility testing was conducted as a standard of CLSI 2011.[13] Identification of *MecA* gene and the type of SCCmec were using Polymerase Chain Reaction (PCR). Primers used are shown in Table 1.

Preparation of Bacterial DNA Samples, PCR Mec A and PCR SCCmec

DNA samples 5 µl of bacterial suspension (0.5 Mc Farland) from yellowish colonies were incubated at 30°C 18-24 hours on MSA. PCR was performed in a final volume of 25 µl consisting of 5 µl of DNA samples, 10 µl of 2x GoTaq green master mix (Promega), 2 µl 1mM forward primer (Mec A1), 2 µl 1mM reverse primer (Mec A2) and 6 µl of nuclease-free water. Positive control and negative control were S. aureus ATCC 43300 and S. aureus ATCC 25923. The mixture was denatured at 94°C for 5 minutes followed by 30 cycles, 94°C for 45 seconds, 72°C for 90 seconds, and 72°C for 10 minutes. DNA was amplified with a thermocycler (Thermo scientific, USA).

Multiplex PCR SCCmec was carried out on positive samples of MecA gene to detect SCCmec chromosomes. Primers used are shown in Table 1. PCR was performed in a final volume of 25 µl consisting of 5 µl of DNA samples, 12.5 µl of 2x GoTaq green master mix (Promega), 0.5 µl 1 mM of forward primer, 0.5 µl 1mM of reverse primer (SCCmec primers type I, II, III, IVa, and IVb) and 2.5 µl nuclease-free water. PCR to identify the type of SCCmec began with an initial denaturation at 94°C for 5 minutes followed by 10 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, extension at 72°C for 90 seconds, then continued with 25 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 45 seconds, extension at 72°C for 90 seconds, and final extension 72°C 10 minutes. The amplicons were visualized in 0.8% agarose stained using Sybr safe DNA (Invitrogen), and images were obtained using a gel documentation system.

RESULTS AND DISCUSSION

A total of 100 festering wound swab samples were obtained from hospitalized patients in Raden Mattaher hospital, dr. Bratanata hospital, and Kambang hospital. Thirty-two samples were positive *S. aureus* through staphytect plus test. There were 14 isolates of MRSA based on cefoxitin resistance in disc diffusion method and PCR mecA positive (Figure 1).

Multiplex PCR was performed on 14 MRSA isolates to identify the type of SCCmec in the samples. There were 12 samples (85.72%) of SCCmec type III, 1 sample (7.14%) of SCCmec type II, and 1 sample (7.14%) of SCCmec type IVb (Figure 2).

The SCC*mec* types distribution were depended on geographical manner. Most MRSA isolates from Eastern and Middle Eastern countries hospitals contain SCC*mec* type III.[15] This SCC*mec* type is common in some South East Asia countries hospitals such as Thailand, Singapore, Indonesia and Malaysia.[16] Different with some South East Asian countries, MRSA isolates from

Table 1. Sequence of oligonucleotide primers.[14]

Target Gene	Primer	Nucleotide sequence (5'-3')	Amplicon (bp)
MecA gene	MecA1	GTA GAA ATG ACT GAA CGT CCG ATA A	310
	MecA2	CCA ATT CCA CAT TGT TTC GGT CTA A	
SCCmec I	I-F	GCTTTA AAG AGT GTC GTT ACA GG	613
	I-R	GTTCTCTCATAGTATGACGTCC	
SCCmec II	II-F	CGTTGAAGATGATGAAGCG	398
	II-R	CGAAATCAATGGTTAATGGACC	
SCCmec III	III-F	CCATATTGTGTACGATGCG	280
	III-R	CCTTAGTTGTCGTAACAGATCG	
SCCmec IVa	IVa-F	GCCTTATTCGAAGAAACCG	776
	IVa-R	CTACTCTTCTGAAAAGCGTCG	
SCCmec IVb	IVb-F	TCTGGAATTACTTCAGCTGC	493
	IVb-R	AAACAATATTGCTCTCCCTC	

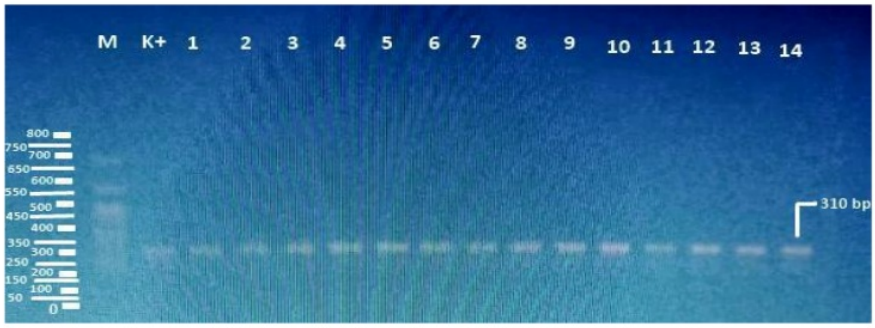

Figure 1. Agarose gel electrophoresis of PCR product amplified from MecA gene (310 bp). M is DNA marker; K(+) is positive control, Lane 1-14 are MecA fragments.

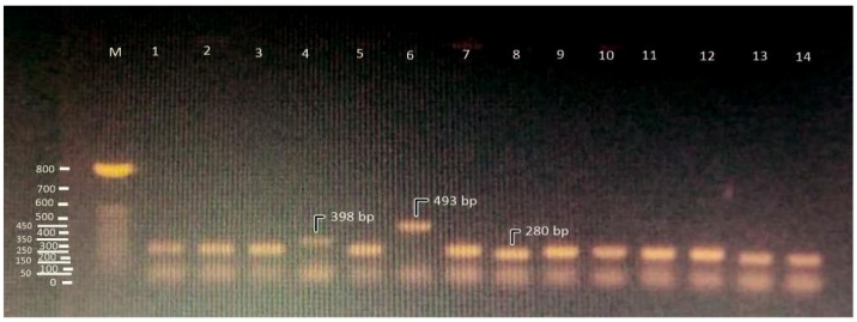

Figure 2. Agarose gel electrophoresis of PCR product amplified from SCCmec type. M is DNA marker; Lane 1-3,5,7-14 are SCC*mec* type III fragments (280 bp). Lane 4 is SCC*mec* type II fragment (398 bp). Lane 6 is SCC*mec* type IVb fragment (493 bp).

Korea and Japan predominantly contain SCC*mec* type II.[16] While some European countries MRSA isolates contain SCC*mec* type IV.[17]

In this study, the majority of SCCmec types was type III (85.72%). These results were consistent with studies conducted in seven countries in Asia including Indonesia and studies conducted in Iran where SCCmec type III was the most common in MRSA isolates.[16,18,19]

In addition to SCCmec type III, this study also found a small proportion of MRSA isolates contained SCCmec type II and type IVb. SCCmec type I, II, and III were the commonly found types in hospitals (HA-MRSA), while SCCmec type IV and V were the commonly found types in communities (CA-MRSA).[20,21,22]

SCCmec type II also found in Jakarta, a study mentioned that the majority of MRSA isolates in hospitals were SCCmec type II.[23] While SCCmec type IV also found in Denpasar (12.5%) and Malaysia (3.18%) among MRSA isolates in hospitals.[24,25] This means that there has been a shift in the content of SCCmec in MRSA isolates in Indonesia. The discovery of SCCmec type IV in the Hospital raises concerns because this type is more mobile, generally causes more severe clinical symptoms, and is more difficult in the selection of suitable antibiotics.[21,24] In comparison to other SCCmec elements, SCCmec IV is small in size and more variable, which has possibly enabled it to spread easily within *S. aureus*.

CONCLUSIONS

Based on the results revealed in this study, there has been a change in the type of SCCmec in MRSA isolates from hospitals. Therefore, it is

recommended to conduct further research with a larger sample size, both from hospitals and communities to identify the SCCmec type and its relationship to patterns of sensitivity to antibiotics. Keeping in view, the finding of SCCmec type IV in Jambi should be investigated, whether it is a circulator or a persisting invader. Further molecular analysis of these MRSA isolates by pulsed-field gel electrophoresis or MLST (Multi Locus Sequence Typing) may provide much useful information regarding the origin and the epidemiology of local isolates.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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