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The Characterization of mecA Gene and SCCmec Typing in Clinical Samples of MRSA

Siti Aisya Aswani and Aziyah Abdul-Aziz*

Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia

Corresponding author: aziyah960@uitm.edu.my

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ABSTRACT

MRSA is a major pathogen worldwide and its infections are associated with increased morbidity and mortality, in comparison with other *S. aureus* infections. The study was designed to characterize the *mecA* gene and staphylococcal cassette chromosome (*SCCmec*) in methicillin resistance *S. aureus* (MRSA). A total of 20 presumptive *S. aureus* strains were collected from one of the teaching hospitals in Selangor. Using standard biochemical tests, all the isolates were verified as *S. aureus*. When tested against cefoxitin, 80% of the isolates were confirmed as MRSA. All the MRSA isolates were further subjected to polymerase chain reaction (PCR) to detect the presence of *mecA* gene. Nine out of the 16 MRSA isolates (56%) were *mecA* positive, whereas the remaining four were *mecA* negative. The 16 MRSA with positive *mecA* gene were further subjected to *SCCmec* typing of type I, II, III, IV and V. The most frequent *SCCmec* types were type III (56%) followed by type II (33%), and type IV (11%). None of the isolates were of *SCCmec* type I or V. Our study indicates that *SCCmec* type III is predominant among the isolates which is in agreement with other studies conducted on clinical strains of MRSA.

Keywords: *MRSA; mecA gene; SCCmec type; Staphylococcus aureus; HA-MRSA Malaysia*

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a multidrug-resistant pathogen and involves in a wide spectrum of infections [1]. Some of the most common superficial infections caused by *S. aureus* include boils, impetigo and folliculitis. More serious infections caused by this organism include pneumonia, bacteremia, and infections of the bone and wounds [2]. These bacteria are present in up to 25 percent of healthy people and are even more common among those with skin, eye, nose, or throat infections [3]. The capability to survive within the living host makes *S. aureus* the leading pathogen not only within the genus but also one of the most threatening microorganisms [4].

The first antibiotic used to treat staphylococcal infection was penicillin. Penicillin resistant *S. aureus* was then treated by methicillin, which is a semi synthetic penicillin. However, the resistance to methicillin finally emerged in 1962 by a group of *S. aureus* strains called as methicillin resistance *S. aureus* (MRSA) [5].

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MRSA is a major pathogen worldwide and its infections are associated with increased morbidity and mortality, in comparison with other *S. aureus* infections. Over the past decade, the changing pattern of resistance in *S. aureus* has underscored the need for new antimicrobial agents [6]. MRSA can be divided into two types which are hospital acquired *S. aureus* (HAI) and community acquired *S. aureus* (CAI). HAI also known as nosocomial infections which develop from hospital environments usually during hospital visit or transfer among hospital staff. According to recent data from the Centers for Disease Control and Prevention in USA showed that majority of all health-care associated *S. aureus* infections are due to MRSA [7]. In a nationwide surveillance study of nosocomial bloodstream infections (BSI), investigators reported that *S. aureus* was the second most common organism causing BSI and that the proportion of MRSA isolates increased from 22% in 1995 to 57 % in 2001 [8].

Community acquired infection (CAI) is the infections involving community and it is now adapted to be more virulent with enhanced ability to invade and kill host cells [9]. CAI not only spread rapidly in communities but also into hospitals, approximately 2 days after admission [10]. Thus, CAI-MRSA strains can be a significant threat to the immuno- compromised patients in hospitals. Within two decades, ~50% of both HAI and CAI isolates were penicillin resistant protein due to the β-lactamase catalytic function [11]. Once confined to health care associated environments, MRSA has now migrated into the community as the first case of community acquired S. aureus was reported in the United States in 1980 [12].

In Malaysia, MRSA was introduced in the early 1970s as reviewed from the data taken from all the state in local hospital [13]. Ministry of Health Malaysia (MOH) stated that, about 21% out of 2389 cases of S. aureus infected were caused by MRSA [14]. Usage of antibiotics, period of hospitalisation and bedside invasive procedures are the most significant factors that are associated with the emergence of MRSA infections in Malaysia hospitals [15].

Staphylococcal cassette chromosome (*SCCmec*) is one of the mobile genetic elements in Staphylococcus that involved in the transfers of virulence and resistance [16]. *SCCmec* act as a mobile element which comprise of the *mec* gene complex, the *ccr* gene complex, the junkyard (J) regions and insertion sequence [10]. The typing of the *SCCmec* is according to the combination of the *ccr* gene complex and the *mec* gene complex [17]. Up to date, there are about 13 types of SCCmec (I -XIII) in Staphylococcus aureus based on *SCCmec*Finder website [18]. *SCCmec* type I, II and III are the most common *SCCmec* types in HAI while, *SCCmec* type IV is a common *SCCmec* type among CAI [19].

Research on *SCCmec* is not very intensive especially in Malaysia. The prevalence of MRSA and its antimicrobial resistance pattern, as well as *SCCmec* types in Malaysia remains unclear. Studies at the genetic level are important to understand the development of the resistance and the mechanism involved. This study is designed to determine the relation of *mecA* gene with different types of *SCCmec* within local hospital in Malaysia. The characterization and distribution of the *SCCmec* type of MRSA in local hospital will provide data which could be used to correlate them with the ability to transmit and cause infections.

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EXPERIMENTAL

Materials

Twenty presumptive isolates of *S. aureus* were collected randomly from one of the teaching hospitals in Selangor. All isolates were subjected to several tests which include Gram staining, mannitol fermentation, catalase test, coagulase test (Prolex™ Staph Latex Kit) and grown on BPA to confirm their identity. *S. aureus* ATCC 33591 and ATCC 33862 was used as positive and negative control respectively.

Identification of MRSA

Identification of MRSA were tested for its cefoxitin resistance by using the Kirby- Bauer disk diffusion method. All pure cultures were inoculated into Mueller-Hinton broth (MHB) and incubated overnight at 37°C, 150 rpm. Following that, the bacterial culture was inoculated into fresh MHB at 1:100 dilutions and grown until it reached the log phase. The bacterial culture was adjusted to a standard turbidity of 0.5 McFarland before inoculated onto Mueller-Hinton agar (MHA) plate. Cefoxitin (30µg) (Oxoid) antibiotic discs are placed onto the agar. Following overnight incubation, the zone of inhibition was interpreted according to Clinical and Laboratory Standards Institute (CLSI 2016).

Amplification of *mecA* gene by PCR

All the clinical samples were subjected to PCR amplification for *mec*A gene detection. The bacterial culture was grown overnight in BHI. Extracted volume of 20µl of the culture was mixed with an equal amount of 1X TE buffer pH8.0 and heated at 100°C thermo block for 10 minutes to extract the DNA. The mixture was centrifuged, and the supernatant was used immediately for PCR. The *mec*A specific primer pairs used were *mec*A1 5′- GTAGAAATGACTGAACGTCCGATGA-3′ and *mec*A2 5′-CCAATTCCACATTGTTTCGGTCTAA-3′ which resulted in the amplification of a 310-bp fragment [20]. The product of the PCR reaction was examined by electrophoresis using 1.5% (w/v) agarose gel stained with gel red and was visualized with a UV transilluminator (Bio-Rad).

Identification of SCC*mec* Typing

The MRSA strains were further subjected to SCC*mec* typing via PCR amplification method. The primer sets that are used for the assignment of SCC*mec* elements are as in previous study [17]. An aliquot of 2 µl of DNA was added to 23 µl of PCR mixture containing 10X PCR buffer, 50mM $MgCl_2$, 2.5 mM deoxynucleoside triphosphate (dATP, dUTP, dGTP, and dCTP), 5U/µl *Taq* DNA polymerase and 10µM for each primer. The PCR amplicons was visualized using a UV transilluminator (Bio-Rad) after electrophoresis on a 2% agarose gel containing 0.5 µg/ml gel red.

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RESULTS AND DISCUSSION

All 20 presumptive isolates taken from various clinical samples were identified as *S. aureus* through several standard biochemical tests. In hospital setting, genotypic test could not be performed due to high number of samples. Therefore, they are relying on antibiotics disk diffusion method for MRSA confirmation. About 16 out of 20 (80%) *S. aureus* isolates were resistant against cefoxitin and was confirmed as MRSA according to guidelines of the Clinical and Laboratory Standards Institute (CLSI 2016) on MHA. A total four of the isolates were determined to be methicillin susceptible *S. aureus* (MSSA) since the isolates were not resistance to cefoxitin. This study supports the fact that cefoxitin is more specific than other antibiotics towards detection of methicillin resistance [21]. The resistant of isolates towards cefoxitin, is due to expression of penicillin binding protein 2α (PBP2α) encoded by the *mec*A gene, which has a low binding affinity to all β-lactam antibiotics [22]. Therefore, cefoxitin was used as heteroresistance detector as it is a stronger inducer of PBP2α. All the MRSA isolates were further confirmed as MRSA by amplification of the *mec*A gene.

A product of 310 bp of size was considered as a positive band for *mecA* gene screening via PCR as shown in Figure 1. Analysis of phenotypic tests for MRSA detection in comparison with PCR was done. Based on the analysis, only nine out of the 16 samples of MRSA isolates carry *mecA* gene, whereas the other seven were MRSA that do not harbor *mec*A gene. Some researcher suggests that MRSA isolates that do not harbor *mec*A is due to mutation in penicillin-binding protein gene [23]. This suggestion is also supported by other study [24,25]. The Olayinka group proposed that the absence of *mec*A could be due to hyper production of type A-β- lactamase [26]. This study provided clear evidence that molecular techniques alone are not reliable for the characterization of MRSA isolates.

All 9 MRSA isolates with *mec*A gene were classified into SCC*mec* subtypes; 3 samples harbored SCC*mec* type II, 5 SCC*mec* type III, whereas Type IV was identified in just one case. No types I and V were detected as shown in Figure 2. SCC*mec* Type III was the most frequently detected SCC*mec* type in this study, followed by Type II. In agreement to the previous study, HA-MRSA isolates typically have SCC*mec* subtypes I–III [27]. One study reported that SCC*mec* type III (90%) is the predominant SCC*mec* in Malaysia as well as in hospitals in most Asian countries except Korea and Japan [2]. Only one MRSA sample was identified as SCC*mec* Type IV. In contrast, CA-MRSA mainly contains SCCmec type IV and SCCmec type V [28]. However, 87% of patient samples in one of the teaching hospitals in Switzerland belonged to SCC*mec* types IV and V. Surprisingly, classical nosocomial SCC*mec* types I and II represented a minority, whereas SCC*mec* type III was completely absent [29]. In addition, SCC*mec* element is more variable than other SCC*mec* types due to its small size, enables it to spread easily among S. *aureus* strain. The presence of SCC*mec* Type IV in Malaysia strengthens the case for the epidemic nature of this clone and also suggests that it may easily spread to other Asian countries [13].

CONCLUSIONS

In summary, the antibiotic resistance of clinical isolates *S. aureus* against cefoxitin was successfully determined. Detection of *mecA* gene by PCR is a good tool for rapid characterization of MRSA strains. However, further investigation regarding their resistance phenomena are required. The SCC*mec* Typing of the MRSA strains that are positive for *mecA* gene was also successfully identified. Research on

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SCC*mec* in terms of molecular epidemiology data is still insufficient in Malaysia. A comprehensive study should be enhanced and established to have an in depth understanding of the impact of SCC*mec* type on antibiotic resistance gene in methicillin-resistant *S. aureus* (MRSA) infections especially in Malaysia's healthcare institutions. Furthermore, data on the SCC*mec* typing for MRSA in local hospitals is lacking. The genetic understanding based on SCC*mec* typing can help to better understand the epidemiology of MRSA locally.

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