AMPLIFICATION OF *mec*A GENE AND SCC*mec* IN MRSA ISOLATES ISOLATED FROM HEALTHY INDIVIDUALS IN NILAI, NEGERI SEMBILAN.

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THIS DISSERTATION IS SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF BACHELOR OF BIOTECHNOLOGY (HONOURS)

FACULTY OF HEALTH AND LIFE SCIENCES
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ABSTARCT

Resistance in methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus epidermidis* is due to the presence of *mec*A gene which encodes for penicillin-binding protein (PBP2a). PBP2a is able to prevent the action of methicillin and enables the bacteria to synthesize peptidoglycan and grow. The *mec*A gene is found in the staphylococcal cassette chromosome *mec* (SCC*mec*) element. Chuah (2016) isolated possible MRSA and MRSE isolates from healthy individuals in Nilai. This was followed by an attempt by Kum (2017) to amplify the SCC*mec* elements and the *mec*A gene of the MRSA and MRSE isolates respectively but was unsuccessful. Thus, the aim of this study was to amplify the SCC*mec* types in the MRSA isolates by optimizing the PCR reactions. These isolates were cultured and confirmed through several confirmatory tests as well as the antibiotic susceptibility assay using cefoxitin. Based on the zone of inhibition, isolate A/2016M/14 was found to be resistant towards cefoxitin. No MRSE was identified. The DNA of this resistant isolate was extracted using the crude extraction method and subjected to PCR. Isolate A/2016M/14 was confirmed to be MRSA as the *mec*A gene and type II SCC*mec* element were successfully amplified. Since, the *mec*A gene and the type II SCC*mec* elements were successfully amplified using the recommended conditions, no optimization of amplification was done. The DNA sequence analysis through BLAST also confirmed that isolate A/2016M/14 was MRSA with type II SCC*mec* element. The result of BLAST showed that the sequence amplified using KDP primers was in consensus with the DNA sequence encoding for type II SCC*mec* with 99 % similarities. Hence, it can be confirmed that isolate A/2016M/14 is hospital-acquired MRSA as it carries the type II SCC*mec*. The human carrier of isolate A/2016M/14 could have been treated with antibiotics or could have come in contact with another MRSA carrier or could have contracted it from a clinical setting or from the environment. This carrier could transmit this resistant strain to other individuals in INTI International University. Therefore, every individuals should practice good hygiene in order to minimize the risk of infection caused by MRSA.

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LIST OF ABBREVIATIONS

bp	Base Pair
CLSI	Clinical and Laboratory Institute
CoNS	Coagulase Negative Staphylococci
CFU/ mL	Colony forming units/ millilitre
CA-MRSA	Community-acquired Methicillin-resistan *S.aureus*
HA-MRSA	Hospital-acquired methicillin-resistant S. *aureus*
°C	Degree Celsius
dNTP	Deoxynucleotide triphosphate
DNA	Deoxyribonucleic acid
dH_2O	Deionized water
EDTA	Ethylenediaminetetraacetic acid
g	Gram
hr	Hours
H_2O_2	Hydrogen peroxide
kb	Kilobase
L	Litre
$MgCl_2$	Magnesium chloride
Mg^{2+}	Magnesium ions
MSA	Mannitol salt agar
T_m	Melting temperature
MRSA	Methicillin-resistant *S. aureus*
MRSE	Methicillin-resistant *S. epidermidis*
MSSA	Methicillin-susceptible *S. aureus*
MSSE	Methicillin-susceptible *S. epidermidis*
µg	Microgram
µL	Microlitre
µM	Micromolar
mL	Millilitre
mm	Millimetre
mM	Millimolar

min	Minutes
M	Molar
MLST	Multilocus sequence typing
nm	Nanometre
PBP	Penicillin-binding protein
%	Percentage
PCR	Polymerase Chain Reaction
pH	Potential of hydrogen
PFGE	Pulsed-field gel electrophoresis
rpm	Revolution per minutes
RNA	Ribonucleic acid
s	Second
SCC*mec*	Staphylococcal cassette chromosome *mec*
S. aureus	*Staphylococcus aureus*
S. epidermidis	*Staphylococcus epidermidis*
V	Volt
v/v	Volume per volume
w/v	Weight per volume

CHAPTER 1

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a nosocomial pathogen but has also emerged as a community pathogen (Anand, Agrawal, Kumar, & Kapila, 2009). Infections caused by the resistant bacteria is becoming a major concern not only because of its methicillin-resistance, but also because of its ability to form biofilm. These characteristics increase their survival rate in the environment, causing the treatments for the related infections to be tougher (Bhattacharya, Wozniak, Stoodley, & Hall-Stoodley, 2015). According to CDC (2015), more than 80,000 MRSA-related cases were reported in 2011 and 11,825 of them caused death in the reported cases.

Methicilin resistance in MRSA and MRSE is due to the presence of *mec*A gene in the staphylococcal cassette chromosomes *mec* element (SCC*mec*) (Aklilu, Nurhardy, Mokhtat, Zahirul, & Siti Rokiah, 2016). The *mec*A gene in staphylococci encode for a protein penicillin-binding protein 2a (PBP2a), which confers low binding affinity to beta-lactam antibiotics. Thus, antibiotics such as penicillin, methicillin and others are unable to inhibit the synthesis of peptidoglycan hence, unable to kill the cells (Ballhausen, Kriegeskorte, Schleimer, Peters, & Becker, 2014).

There are two major types of MRSA which are hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA). Larger SCC*mec* element of types I, II, and III are usually found in HA-MRSA whereas, the smaller SCC*mec* elements types IV and V are found in CA-MRSA (Bhutia, Singh, Adhikari, & Biswas, 2015). Larger SCC*mec* types (I, II, and II) confers resistance to multiple classes of antibiotics apart from β-lactam as a result of additional drug resistance genes integrated in the SCC*mec* element. On the other hand, smaller SCC*mec* types (IV and V) carry genes that confer resistance to β-lactam antibiotics only (Oliveira, Tomsz, & Lencastre, 2001). This explains why HA-MRSA which carry types I, II, and III SCC*mec* elements is

more resistant to antibiotics compared to CA-MRSA which carry types IV and V of SCC*mec* elements.

MRSA is related to high mortality and morbidity, hence, having a rapid molecular diagnosis helps in controlling infections. One of such is by using the SCC*mec* typing (Monecke et al., 2016). This techniques helps to determine the types of SCC*mec* elements found in the isolates so that the resistance pattern of the bacteria can be known. Thus, the types of antibiotics that could be used to treat diseases caused by such bacteria can be determined (Kum, 2017).

Chuah (2016) has isolated MRSA and possible MRSE strains from the nasal cavity and axillae of healthy individuals in Nilai. Kum (2017) attempted to identify the types of MRSA and MRSE isolates by amplifying the SCC*mec* element and *mec*A gene respectively. However, this attempt was unsuccessful due to several reasons. Therefore, the aim of this study was to optimize the PCR conditions (if needed) for the amplification of SCC*mec* elements in MRSA and *mec*A gene in the MRSE isolated by Chuah (2016). This study was also aimed to identify the types of SCC*mec* element in MRSA and to confirm the presence of *mec*A gene is MRSE.

CHAPTER 2

LITERATURE REVIEW

2.1 RESISTANCE IN STAPHYLOCOCCAL SPECIES

2.1.1 Methicillin-resistant *Staphylococcus aureus* (MRSA)

Staphylococcus aureus is a bacterium that is commonly found on skin and the nasal cavity (Coughlan et al., 2013). Generally, *S. aureus* does not cause diseases unless introduced into the body of the host through wound or skin injury (Kathryn, 2012). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a type of *S. aureus* which is resistant to antibiotics that are usually used in the treatment for staphylococcal-related infections (Coughlan et al., 2013). Some examples of antibiotics that MRSA is resistant to are penicillin, vancomycin, and linezolid (Kaur, & Chate, 2015).

Wounds infected by MRSA look similar to minor skin infections, such as pimple, abscess, or boils (Figure 1). Individuals suffering from MRSA infection may have symptoms such as dizziness, chest pain, aches, chills, high fever and other symptoms (NHS Choices, 2015). Besides, MRSA infection isn't always localized but can spread to organs such as lungs or to the circulatory system causing pneumonia and bloodstream infection (Wendt et al., 2014).

Places which are crowded where skin contact occurs frequently, and environment with poor hygiene are at risk of MRSA outbreaks (DeNoon, 2007). Examples of such environments are health-care facilities, prisons, schools and others. The risk of outbreak increases as carriers of MRSA do not necessarily to have signs of infection (CDC, 2015). According to CDC (2015), studies have shown that two in every 100 people are carriers of MRSA and may be asymptomatic.