RECONFIRMATION AND MOLECULAR CHARACTERIZATION OF MRSA & MRSE ISOLATES

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ABSTRACT

The treatment of diseases related to antibiotic resistance is becoming difficult due to the rapid development of antibiotic-resistant strains. Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus epidermidis* (MRSE) are some of the common antibiotic-resistant bacteria that are spreading rapidly in the hospitals and communities. The methicillin resistance of MRSA and MRSE is mainly due to the formation of penicillin binding protein 2a (PBP2a) encoded by *mec*A gene that is not inhibited by beta-lactam antibiotics. The *mec*A gene in MRSA is carried by staphylococcal cassette chromosome mec (SCC*mec*) and it classified into five major types; I-V. The aim of this study was to identify the types of SCC*mec* among the MRSA isolates, and to confirm the presence of *mec*A gene in the possible MRSE isolates as well as to determine the DNA extraction method that yield better quality and quantity of DNA. In this study, a total of 35 isolates isolated by Chuah (2016) from healthy individuals at INTI International University were analysed using confirmatory tests such as gram-staining, catalase test, MSA, Brilliance MRSA 2 Agar, and antibiotic susceptibility testing. Two DNA extraction methods; crude extraction and i-genomic BYF DNA Extraction Mini Kit were used in this study and compared. PCR was conducted on genomic DNA from possible MRSE isolates to detect the *mec*A gene. Furthermore, multiplex PCR was conducted on genomic DNA from confirmed MRSA isolates to identify the types of SCC*mec*. From the confirmatory tests, out of total 35 isolates, 4 were confirmed as MRSA and 6 isolates were possibly MRSE. This study also shows that i-genomic BYF DNA extraction Mini Kit yielded better quantity and quality of DNA than crude extraction based on the bands produced by agarose gel electrophoresis and the spectrophotometer analysis. Results from the PCR and SCC*mec* typing showed no amplification of *mec*A gene in all the possible MRSE isolates. Furthermore, the SCC*mec* types could not be identified from the MRSA isolates as no amplification of the specific genes. There are various factors could have contributed to the failure of detecting *mec*A gene and types of SCC*mec*.

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LIST OF ABBREVIATIONS

A	Absorbance
AIDS	Acquired Immune Deficeincy Syndrome
bp	Base pair
CRBSIs	Catheter-related bloodstream infections
CLSI	Clinical and Laboratory Standard Institute
CoNS	Coagulase Negative Staphylococci
CFU/mL	Colony forming units/millilitre
CA-MRSA	Community-Associated Methicillin-resistant S. *aureus*
˚C	Degree Celsius
dNTP	Deoxynucleotide triphosphate
DNA	Deoxyribonucleic acid
dH_2O	Distilled water
EDTA	Ethylenediaminetetraacetic acid
g	Gram
HA-MRSA	Healthcare-associated Methicillin-resistant S. *aureus*
hr	Hours
HIV	Human immunodeficiency virus
HCL	Hydrochloric acid
H_2O_2	Hydrogen Peroxide
kb	Kilobase
L	Liter
$MgCl_2$	Magnesium Chloride
Mg^{2+}	Magnesium ions
MSA	Mannitol Salt Agar

T_m	Melting temperature
MRSA	Methicillin-resistant *Staphylococcus aureus*
MRSE	Methicillin-resistant *Staphylococcus epidermidis*
MSSA	Methicillin-sensitive *Staphylococcus aureus*
µg	Microgram
µL	Microliter
µM	Micromolar
mL	Millilitre
mm	Millimetre
mM	Millimolar
min	Minutes
M	Molar
MDR	Multidrug resistance
MLST	Multilocus sequence typing
nm	Nanometre
OZ	Oxacillin
PVL	Panton-Valentine leucocidin
PBP	Penicillin binding protein
%	Percentage
PCR	Polymerase Chain Reaction
pH	Potential of hydrogen
PVE	Prosthetic valve endocarditis
PFGE	Pulsed-field gel electrophoresis
RFLP	Restriction fragment length polymorphism
rpm	Revolutions per minutes

RNA	Ribonucleic acid
s	Second
NaCl	Sodium Chloride
SCC*mec*	Staphylococcal cassette chromosome mec
S. *aureus*	*Staphylococcus aureus*
S. *epidermidis*	*Staphylococcus epidermidis*
UV	Ultraviolet
U	Units
V	Volt
v/v	Volume per volume
w/v	Weight per volume

CHAPTER 1

INTRODUCTION

Staphylococcus aureus is a common microflora that can be found on the skin. However, some of the strains of *S. aureus* can cause infections that vary from mild conditions such as cutaneous abscess, to life-threatening bacteremia and toxic shock syndrome (Vysakh & Jeya, 2013). The treatment of diseases caused by *S. aureus* is becoming difficult due to the rapid development of antibiotic-resistant strains. One of such is the methicillin-resistant *Staphylococcus aureus* (MRSA) which is spreading rapidly in the hospitals and the communities (Vysakh & Jeya, 2013). Another common microflora of the skin is the *Staphylococcus epidermidis*. However, some strains are also found to cause hospital-acquired infection (Cherifi et al., 2013). Studies show that as many as 70% of *S. epidermidis* in the environment have been found to be resistant to methicillin (Miragaia et al., 2002). However, limited studies have been done on MRSE.

The methicillin resistance of MRSA is due to the formation of penicillin binding protein 2a (PBP2a) encoded by the *mec*A gene that is not inhibited by the beta-lactam antibiotics. The *mec*A gene is carried in the staphylococcal cassette chromosome mec (SCC*mec*) which is a mobile genetic element. This SCC*mec* enables the classification of MRSA into five major types (I-V) (Bhutia, Singh, Adhikari, & Biswas, 2015). Larger SCC*mec* elements types I, II and III (34-67kb) are usually carried by HA-MRSA whereas the smaller SCC*mec* element type IV and type V (21 to 25kb) are mainly found in CA-MRSA (Bhutia et al., 2015). Smaller SCC*mec* element (IV & V) carry genes that confer resistance only towards beta-lactam antibiotics, while larger SCC*mec* element (I, II, & III) have genes that confer resistance to multiple classes of antibiotics. This is because the latter (I-III) have additional integrated antibiotic resistant genes that enable them to resist many antibiotics (Eed, Ghonaim, Hussein, Saber, & Khalifa, 2015).

The SCC*mec* typing is used for epidemiological studies to investigate the types of MRSA in an environment and also to assist in the treatment process (Turlej, Hryniewicz, & Empel, 2011). This is because the results from the molecular typing can help to estimate the class of antibiotics that are ineffective against the respective MRSA

strains. In addition, this molecular typing method provides a rapid result compared to the tedious conventional method, the antibiotic susceptibility testing. Apart from that, molecular typing is also sensitive and provide reasonably accurate result to determine the most appropriate choice of antibiotic treatment for MRSA related diseases. Thus, molecular typing assay can be used as potential diagnostic tool in hospital.

Chuah (2016), isolated 35 possible MRSA and MRSE isolates from INTI International University. The MRSA isolates were confirmed using the Brilliance MRSA 2 agar but no study was done to determine the type of MRSA isolates and the possible MRSE isolates could not be further confirmed. Thus, this study was aimed to (1) Reconfirmation of MRSA & MRSE isolates through biochemical tests and antibiotic susceptibility testing. (2) Determine the types of SCC*mec* carried by the MRSA isolates isolated by Chuah (2016) using SCC*mec* typing and to (3) confirm the presence of *mec*A gene in the possible MRSE isolates. (4) This study was also to determine qualitatively the DNA extraction method that is more efficient in yield and quality of extracted DNA.

CHAPTER 2

LITERATURE REVIEW

2.1 ANTIBIOTIC-RESISTANT BACTERIA: MRSA & MRSE

2.1.1 Methicillin-resistant *Staphylococcus aureus* (MRSA)

Staphylococcus aureus is a common bacteria found on the skin and in the nostrils of human (Office of National Statistics, 2013). If *S. aureus* is able to enter into the body through the broken skin or other pathways, it can cause disease that varies from mild to life-threatening (Vysakh & Jeya, 2013). Most *S. aureus* infection are pyogenic, and some cause fever and local tissue destruction (Green et al., 2012). MRSA infections show the characteristics of pus production and pain at the site of infection and is often accompanied by swelling and inflammation (Green et al., 2012). Example of mild infection caused by MRSA are cutaneous such as an abscess (Figure 1).

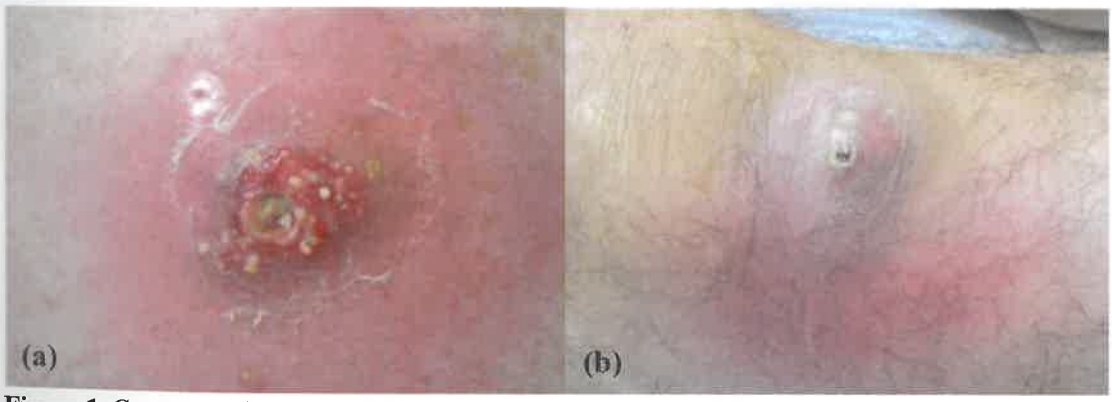

Figure 1. Cutaneous abscess on different body parts caused by MRSA. (Green et al., 2012)

Cutaneous MRSA wounds are frequently found on injured skin. These cutaneous MRSA wounds can lead to more serious complications such as cellulitis, erysipelas and folliculitis if the wounds are left untreated (Green et al., 2012). More severe form of MRSA infections include osteomyelitis, pyomyositis, purpura fulminans and necrotizing fasciitis (Figure 2)(Green et al., 2012). Bacteremia and endocarditis caused by MRSA are associated with relatively high mortality (Boucher, Miller, & Razonable, 2010). Studies also shows that HIV patients, burn victims and cancer