



**UNIVERSITI PUTRA MALAYSIA**

***DETECTION OF BETA-LACTAMASE GENES AND ANTIBIOTIC  
SUSCEPTIBILITY PROFILES OF *Staphylococcus aureus* ISOLATES IN  
A  
HOSPITAL IN MALAYSIA***

**BAKHTIYAR MAHMOOD HAMASALIH**

**FPSK(M) 2017 48**



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By

**BAKHTIYAR MAHMOOD HAMASALIH**

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfillment of the Requirements for the Degree of Master of Science**

**June 2017**

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## **DEDICATION**

I would like to dedicate this work to my beloved parents, family members and those who taught, motivated and helped me throughout my study. Without my parents support I could not accomplished this work.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

**DETECTION OF BETA-LACTAMASE GENES AND ANTIBIOTIC SUSCEPTIBILITY PROFILES OF *Staphylococcus aureus* ISOLATES IN A HOSPITAL IN MALAYSIA**

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**June 2017**

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**Faculty : Medicine and Health Sciences**

*Staphylococcus aureus* is a versatile pathogen causing a variety of infections. Resistance to  $\beta$ -lactam antibiotics among *S. aureus* isolates has been attributed to the bla system detection. The bla system consists of three main components which are *blaR1*, a sensor signal transducer gene, *blaZ*, structural gene encoding  $\beta$ -Lactamase enzyme, and *blaI*, a repressor gene. The aim of this study is to detect the presence of *blaR1* gene and to determine its relationship with phenotypic resistance of *S. aureus*. Identification of 128 isolates of *S. aureus* was carried out based on colony morphology, Gram stain, catalase and coagulase tests. All isolates were subjected to susceptibility test of commonly used antibiotics by disc diffusion method. Resistant strains toward cefoxitin were considered as phenotypic methicillin resistant *S. aureus* (MRSA) and were subjected to further determination of vancomycin Minimum Inhibitory Concentration (MIC) by E-test. *BlaR1* gene detection among all isolates was done by polymerase chain reaction (PCR). For phenotypic MRSA strains, resistance towards methicillin was further confirmed by *mecA* gene detection.

All non urine isolates were sensitive to vancomycin and rifampin via disc diffusion method. Followed by trimethoprim/sulfamethoxazole (n=122, 98.4%). Whereas, all urine isolates were sensitive to vancomycin, trimethoprim/sulfamethoxazole, gentamicin and nitrofurantoin. Widespread of resistance against penicillin group (n=107, 83.6%) was detected, followed by cefoxitin (n=32, 25%) reported as phenotypic MRSA. PCR revealed all (n=107, 83.6%) penicillin group resistant isolates harboured *blaR1* gene. Whereas, all (n=32, 100%) phenotypic MRSA strains harboured *mecA* gene. Five penicillin susceptible strains were positive for *blaR1* gene, four had slightly larger amplicon size compared to *blaR1* gene and one had similar amplicon size with the *blaR1* gene. Penicillin and oxacillin E-test, nitrocefin disc test, bla system genes and *mecA* gene detection were conducted for these five strains. Sequencing analysis was performed for these five penicillin susceptible strains.

Vancomycin E-test revealed two (n=2, 6.25%) MRSA strains were intermediate to vancomycin and one strain with reduced susceptibility (MIC 3 µg/mL). All five penicillin susceptible *blaR1* positive strains were susceptible to penicillin and oxacillin by E-test and negative for nitrocefin test. Only one strain (A56) was positive for *blaZ* and *mecA* gene. Sequencing and homology analysis of all five penicillin susceptible *blaR1* positive strains did not have any similarity with any *blaR1* gene sequence in NCBI website. However, four strains had 100% correspondence with *S. aureus* strain AUS0325 genome assembly. Conversely, sequencing result for *blaZ* gene of strain A56 and *blaR1* gene of representative penicillin resistant strains had 100% and 99% similarity with *S. aureus* strain N315 *blaZ* and *blaR1* genes respectively. Statistical analysis found significance association of *blaR1* gene with phenotypic resistant of *S. aureus* to beta lactam antibiotics. There was no significant association seen for other antibiotics. In conclusion, in this study *Staphylococcus aureus* strains were resistant to penicillin, followed by ceftazidime and erythromycin. All penicillin resistant strains harboured *blaR1* gene, and all ceftazidime resistant strains harboured *mecA* gene. There may be a role for *blaR1* gene detection among *S. aureus* to confirm beta lactam resistance, which would be useful to aid in managing infected patients efficiently.

**Keywords:** Antibiotic resistance, *blaR1*, *S. aureus*, MRSA

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

**PENGESANAN GEN-GEN BETA-LACTAMASE DAN PROFIL  
KERINTANGAN BAKTERIA *Staphylococcus aureus* YANG DIASINGKAN  
DARI SEBUAH HOSPITAL DI MALAYSIA**

Oleh

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*Staphylococcus aureus* adalah patogen serba boleh yang menyebabkan pelbagai jenis jangkitan. Kerintangan terhadap antibiotik kumpulan beta-lactam ( $\beta$ -lactam) telah dikaitkan dengan pengesanan sistem bla, yang terdiri daripada tiga komponen utama iaitu gen *blaR1* sejenis transduser gen yang memberi isyarat untuk kerintangan, gen *blaZ* yang mengekod struktur enzim beta-lactamase ( $\beta$ -lactamase), dan gen *blaI* sebagai gen repressor. Tujuan kajian ini adalah untuk mengesan kehadiran gen *blaR1* dan untuk menentukan hubungannya dengan kerintangan antibiotik beta lactam oleh *S. aureus*. Sebanyak 128 *S. aureus* telah dikaji, dan ujian fenotipik dilakukan berdasarkan morfologi koloni, Gram stain, ujian enzim katalase dan enzim koagulase. Ujian kerintangan antibiotik yang biasa digunakan dalam suasana klinikal dengan kaedah cakera penyebaran turut dilakukan. Kerintangan terhadap sefoksitin dianggap sebagai kerintangan fenotipik terhadap metisilin, iaitu methicillin resistance *S. aureus* (MRSA). Selanjutnya bagi fenotipik MRSA, pengesanan kerintangan terhadap vankomisin dilakukan melalui E-test (CLSI, 2016). Tindak balas rantai polimerase (PCR) telah dilakukan untuk mengesan kehadiran gen *blaR1* bagi semua *S. aureus*. Bagi fenotipik MRSA pula, pengesanan gen *mecA* dilakukan. Penisilin dan oksasilin E-test, ujian cakera nitrocefin, pengesanan gen sistem bla dan pengesanan gen *mecA* telah dijalankan bagi lima *S. aureus* yang tiada kerintangan terhadap penisilin tetapi positif untuk gen *blaR1*. Analisa urutan genetik juga dilakukan untuk lima bakteria *S. aureus* tersebut.

Ujian kerintangan antibiotik melalui kaedah cakera penyebaran menunjukkan semua *S. aureus* daripada sampel selain air kencing adalah sensitif kepada vankomisin, rifampin (n =124, 100%), diikuti oleh trimethoprim/sulfamethoxazole (n =122, 98.4%). Kesemua *S. aureus* daripada sampel air kencing adalah sensitif terhadap vankomisin, trimethoprim/sulfamethoxazole, gentamisin dan nitrofurantoin (n =4, 100%). Peratusan kerintangan terhadap penisilin adalah agak tinggi (n =107, 83.6%),

dan diikuti oleh sefoksitin (n =32, 25%). Vankomisin E-test menunjukkan dua (n =2, 6.25%) strain MRSA mempunyai kerintangan intermediat untuk vankomisin dan satu strain mempunyai suseptibiliti berkurangan (MIC 3 µg/mL). Gen *blaR1* telah dikesan dalam 107 (83.6%) *S. aureus* yang mempunyai kerintangan terhadap penisilin termasuk dua strain yang diasingkan daripada air kencing. Semua (n =32, 100%) strain fenotipik MRSA mempunyai gen *mecA*. Kesemua lima strain *S. aureus* yang sensitif terhadap penisilin yang mempunyai gen *blaR1* masih sensitif terhadap penisilin dan oksasilin melalui E-test, dan negatif untuk ujian nitrocefirin. Hanya satu strain (A56) yang positif untuk gen *blaZ* dan *mecA*. Analisis homologi urutan genetik lima strain *S. aureus* yang masih sensitif terhadap penisilin, tidak mempunyai persamaan dengan mana-mana urutan gen *blaR1* dalam laman web NCBI. Walaubagaimanapun, empat strain mempunyai 100% persamaan dengan genom *S. aureus* AUS0325. Sebaliknya, hasil penjujukan untuk gen *blaZ* bagi A56 dan gen *blaR1* bagi strain wakil mempunyai persamaan 100% dan 99% dengan gen *blaZ* dan gen *blaR1* *S. aureus* N315 masing-masing. Analisis statistik mendapati kewujudan gen *blaR1* adalah berhubungkait dengan kerintangan fenotipik *S. aureus* terhadap antibiotik beta-lactam. Tidak ada hubungan yang signifikan dilihat untuk kumpulan antibiotik lain. Kesimpulannya, majoriti *Staphylococcus aureus* dalam kajian ini mempunyai kerintangan terhadap penisilin, diikuti oleh sefoksitin dan eritromisin. Semua strain yang mempunyai kerintangan terhadap penisilin mempunyai gen *blaR1*, dan semua strain yang mempunyai kerintangan terhadap sefoksitin mempunyai gen *mecA*. Peranan untuk mengesan gen *blaR1* bagi strain *S. aureus* untuk mengesahkan kerintangan beta-lactam secara PCR, adalah lebih cepat dan berpotensi untuk membantu doktor merawat jangkitan *S. aureus* dengan lebih efisien.

**Kata kunci:** rintangan antibiotik, *blaR1*, *S. aureus*, MRSA



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I certify that a Thesis Examination Committee has met on 15 June 2017 to conduct the final examination of Bakhtiyar Mahmood Hamasalih on his thesis entitled "Detection of Beta-Lactamase Genes and Antibiotic Susceptibility Profiles of *Staphylococcus aureus* Isolates in a Hospital in Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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## LIST OF ABBREVIATIONS

AMP	Ampicillin
AST	Antimicrobial Susceptibility Test
ATCC	American Type Culture Collection
bp	Base pair
ccr	Chromosomal cassette recombinant
CDC	Centre for Disease Control and Prevention
CLI	Clindamycin
CLSI	Clinical Laboratory Standards Institute
CONS	Coagulase negative staphylococcus
CRF	Coagulase reacting factor
DNA	Deoxyribonucleic acid
dsDNA	Double stranded DNA
EARSS	European Antimicrobial Surveillance System
EDTA	Ethylene Diamine Tetra acetic Acid
ERM	Erythromycin
E-test	Epsilometer test
FA	Fusidic acid
FOX	Cefoxitin
g	Gram
GM	Gentamycin
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
HA-MRSA	Hospital acquired MRSA
ICUs	Intensive Care Units
L	Litre
LZD	Linezolid
Mbp	Mega base pairs
MDR	Multidrug-Resistant

mg	Milligram
MGEs	Mobile genetic elements
MIC	Minimum Inhibitory Concentration
mL	Millilitre
MRSA	Methicillin Resistant <i>Staphylococcus aureus</i>
MSA	Mannitol salt agar
MSCRAMMs	Microbial surface components recognizing adhesive matrix molecules
MSSA	Methicillin Sensitive <i>Staphylococcus aureus</i>
NC	Negative control
NCBI	National Centre for Biotechnology Information
ng	Nanogram
NIT	Nitrofurantoin
NNIS	National Nosocomial Infection Surveillance
NSAR	National Surveillance of Antibiotic Resistance
ORFs	Open reading frames
PAP-AUC	Population analysis profile – area under curve
PBP	Penicillin binding protein
PBP <sub>a</sub>	Alternative penicillin binding protein
PC	Positive control
PCN	Penicillin
PCR	Polymerase Chain Reaction
PVL	Panton Valentine Leukocidine
RA	Rifampin
SCC <sub>mec</sub>	Staphylococcal Cassette Chromosome <i>mec</i> element
SPSS	Statistical Package for the Social Science
SSTI	Skin and Soft Tissue Infection
SXT	Trimethoprim / Sulfamethoxazole
TBE	Tris Borate-EDTA

TSB	Trypticase soy broth
TSST-1	Toxic shock syndrome toxin-1
UK	United Kingdom
UPM	Universiti Putra Malaysia
US	United State
UV	Ultra violet
VAN	Vancomycin
VISA	Vancomycin intermediate <i>Staphylococcus aureus</i>
VRSA	Vancomycin Resistant <i>Staphylococcus aureus</i>
µg	Micro gram
µL	Microlitre
µm	Micromoler

# CHAPTER 1

## INTRODUCTION

### 1.1 Background of the Study

*Staphylococcus aureus* as a major pathogen of humans is an issue of global healthcare concern. Although considered as an opportunistic pathogen, as it colonizes its host asymptomatically, it occasionally causes a range of infections from the relatively less severe minor skin and soft tissue infections (SSTI) to the life-threatening scalded skin syndrome, bacteraemia and pneumonia. Originally, to a variety of *S. aureus* infections, penicillin was the choice drug. However, the emergence of beta lactamase producing strains in 1942 made them penicillin-resistant. Nowadays, over 95% of *S. aureus* from humans are proved to be penicillin-resistant (Fuda *et al.*, 2005; Tang *et al.*, 2014).

The *blaZ* is a gene that encodes for the *S. aureus* beta lactamase enzyme. The *blaZ* gene and its repressor *blaI* together with the *blaRI*, a signal transducer sensor protein, form an aggregate or cluster either within the bacterial chromosome or on a plasmid (Pence *et al.*, 2015; Holden *et al.*, 2004). In a situation where the organism is not exposed to a beta lactam antibiotic the *blaZ* becomes repressed by the *blaI* which is a DNA repressor. This is brought about by binding to the DNA conserved motif TACA/TGTA, that is located in the *blaZ* promoter region (Llarrull *et al.*, 2010; Safo *et al.*, 2005).

The drugs of choice for the treatment of *S. aureus* infections were initially the beta lactam antibiotics, but a slightly different strain of this organism, the methicillin resistant *Staphylococcus aureus* (MRSA) emerged in 1961, and proved resistant to all the members of whole class of the  $\beta$ -lactam. For more than half a century, MRSA has been a clinical problem all over the world. The set of genes within the operons *bla* and *mec* are the molecular basis for the incidentally inducible wide resistance of the MRSA. The protein *blaRI* (or the cognate *mecRI*) is a transducer of  $\beta$ -lactam antibiotic signal/sensor. It sends the information on the antibiotic presence in the cytoplasmic surrounding, using a process not clearly defined or understood (Boudreau *et al.*, 2015).

The strains of MRSA are importantly studied pathogens especially in the hospitals (McClure *et al.*, 2006; Trindade *et al.*, 2005). These bacterial pathogens are presently isolated from both hospital patients and members of community (Kowalski *et al.*, 2005; Vourli *et al.*, 2007). Many clinical studies have shown that the strains of MRSA are more virulent than their susceptible counterparts, the methicillin susceptible *S. aureus* (MSSA), on the basis of mortality rates (Rozgonyi *et al.*, 2007). The number of dead patients due to MRSA infections is greatly higher than that due to MSSA (Melzer *et al.*, 2003). In MRSA, mediation of resistance to methicillin is brought about by the presence of beta lactam antibiotic low affinity 78-kDa penicillin binding protein

PBP2 (or PBP2a). The gene *mecA* encodes for PBP2a (Moreillon, 2008). The location for *mecA* gene is a mobile genetic element (from 21kb to 67kb) known as staphylococcal cassette chromosome *mec* elements (SCC*mec*) (Ammons *et al.*, 2010). Penicillin binding proteins, the PBPs, normally have strong affinity for the  $\beta$ -lactam ring. However, in the strains of MRSA another PBP, the PBP2a is generated by the presence of *mecA* gene. This PBP2a has a very low affinity for binding to the beta-lactam antibiotics. This consequently leads to some beta lactam antibiotics like methicillin losing their ability to destroy the bacterial cell wall (Malachowa & DeLeo, 2010).

The identification and detection of *S. aureus* or MRSA in the laboratory is performed by either conventional or molecular methods, both methods have their own advantages and disadvantages depending on the equipments and expertise available in the laboratory setting (Tan, 2003). More research on *S. aureus* or MRSA needs to be conducted to expedite its diagnosis and appropriate clinical management.

## **1.2 Problem Statement**

The rise in drug virulent strains of *S. aureus* poses a challenge for clinicians to treat patients. *BlaR1* as a sensor/inducer gene play a central role in increasing resistant and limited choice of antibiotics for treatment of *S. aureus* infections in hospital setting. *BlaZ* gene detection was recommended by (CLSI, 2016). Why *blaR1* was chosen because this study embarks on the potential for *blaR1* gene detection to discriminate between  $\beta$ -lactam resistance and sensitivity of clinical *S. aureus* isolates. The aim of this study is to provide a confirmation of resistance to  $\beta$ -lactam antibiotics among clinical isolates of *S. aureus* by detecting *blaR1* gene. Laboratory detection of *blaR1* gene will confirm the resistance of *S. aureus* against  $\beta$ -lactam antibiotics which would be useful for clinicians to efficiently manage patients infected with phenotypic penicillin susceptible strain of *S. aureus* in the hospital.

## **1.3 Objectives of the Study**

### **1.3.1 General Objective:**

The general objective of this study is to detect the presence of *blaR1* gene among clinical isolates of *Staphylococcus aureus* and to determine its relationship with phenotypic antibiotic resistance of the bacteria.

### 1.3.2 Specific Objectives

1. To determine antimicrobial susceptibility pattern of *Staphylococcus aureus* strain isolated from Hospital Serdang from June to November 2016.
2. To detect the presence of *blaR1* gene among clinical isolates of *Staphylococcus aureus*.
3. To determine the association between the presence of *blaR1* gene with *Staphylococcus aureus* antimicrobial susceptibility pattern.
4. To detect the presence of *mecA* gene, and other bla system genes among MRSA and penicillin susceptible *Staphylococcus aureus* strains.





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