UNIVERSITI PUTRA MALAYSIA

PREVALENCE OF METALLO-BETA-LACTAMASE PRODUCING Pseudomonas aeruginosa IN A TERTIARY CARE HOSPITAL IN KUALA LUMPUR, MALAYSIA

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By

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

PREVALENCE OF METALLO-BETA-LACTAMASE PRODUCING *Pseudomonas aeruginosa* IN A TERTIARY CARE HOSPITAL IN KUALA LUMPUR, MALAYSIA

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May 2016

Chairman : Niazlin Mohd Taib, PhD
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Introduction: Carbapenems are the primary choice of treatment for nosocomial *Pseudomonas aeruginosa* infections. However, the emergence of carbapenem resistance due to the production of metallo-β-lactamases (MBLs) is a worldwide concern. **Objective:** To determine the prevalence of *P. aeruginosa* producing metallo-β-lactamase isolated from clinical isolates at Hospital Kuala Lumpur (HKL), Malaysia. **Method:** A total of 83 *P. aeruginosa* isolates were collected and tested for metallo-β-lactamase production using a phenotypic combined disc method and genotypic detection by polymerase chain reaction (PCR) and random amplified polymorphic DNA (RAPD). **Results:** Of all the isolates, 52% were found to be imipenem resistant *P. aeruginosa* and from that, 65% of the isolates showed positive result by phenotypic imipenem-EDTA combined disc test in which there were an increase in inhibition zone on imipenem with EDTA as compared to imipenem alone. MBL genes were detected from polymerase chain reaction (PCR) in 35% of total isolates (strains producing bla_{IMP} were 45% followed by bla_{VIM}, bla_{SPM} and bla_{GIM} with 34%, 14% and 7%, respectively. RAPD analysis showed a total of 8 clusters among the 83 *P. aeruginosa* clinical isolates. **Conclusion:** Our study highlight that bla_{IMP} and bla_{VIM} were predominantly present among the *P. aeruginosa* clinical isolates from Hospital Kuala Lumpur. **Keywords:** *Pseudomonas aeruginosa*, imipenem resistance, metallo-β-lactamases (MBLs), bla_{IMP}, bla_{VIM}, bla_{SPM} and bla_{GIM}

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

PREVALENS METALLO-BETA-LACTAMASE *Pseudomonas aeruginosa* DI HOSPITAL RAWATAN TERTIER DI KUALA LUMPUR, MALAYSIA.

Oleh

SITI NUR ATIQAH BINTI HAJI IDRIS

Mei 2016

Pengerusi : Niazlin Mohd Taib
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Pengenalan: Carbapenems adalah pilihan utama rawatan untuk jangkitan nosokomial *Pseudomonas aeruginosa*. Walau bagaimanapun, kemunculan rintangan terhadap carbapenem disebabkan oleh penghasilan metallo-β-lactamases (MBLs) adalah satu kebimbangan di seluruh dunia. **Objektif:** Untuk menentukan prevalen *P. aeruginosa* yang menghasilkan metallo-β-lactamase daripada pencilan klinikal di Hospital Kuala Lumpur (HKL), Malaysia. **Kaedah:** Sebanyak 83 pencilan *P. aeruginosa* telah dikumpulkan dan diuji untuk penghasilan metallo-β-lactamase menggunakan kaedah fenotip penggabungan cakera dan pengesanan genotip oleh tindak balas polymerase berantai (PCR) dan DNA polimorfik amplifikasi rawak (RAPD). **Keputusan:** Dari semua pencilan, 52% *P. aeruginosa* didapati rintang kepada imipenem dan daripada itu, 65% daripada pencilan menunjukkan keputusan positif selepas melalui ujian menggunakan kaedah fenotip imipenem-EDTA penggabungan cakera di mana terdapat peningkatan pada ukuran zon perencat imipenem yang ditambahkan EDTA berbanding zon imipenem sahaja. Gen MBL telah dikesan melalui ujian tindak balas polimerase berantai (PCR) pada 35% daripada jumlah pencilan (strain yang menghasilkan bla_{IMP} adalah 45% diikuti oleh bla_{VIM}, bla_{SPM} *dan* bla_{GIM} dengan 34%, 14% dan 7%, masing-masing mengikut urutan. Analisis RAPD menunjukkan sejumlah 8 kluster diperoleh daripada 83 sampel klinikal *P.aeruginosa*. **Kesimpulan:** Daripada kemuncak kajian kami mendapati yang bla_{IMP} dan bla_{VIM} merupakan yang paling dominan dalam pencilan klinikal *P. aeruginosa* dari Hospital Kuala Lumpur **Katakunci:** *Pseudomonas aeruginosa,* rintangan kepada Imipenem, metallo-β-lactamases (MBLs), bla_{IMP}, bla_{VIM}, bla_{SPM} *dan* bla_{GIM}

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Thank You.
SITI NUR ATIQAH HAJI IDRIS
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I certify that a Thesis Examination Committee has met on 16 May 2016 to conduct the final examination of Siti Nur Atiqah binti Haji Idris on her thesis entitled "Prevalence of Metallo-Beta-Lactamase Producing *Pseudomonas aeruginosa* in a Tertiary Care at Hospital Kuala Lumpur, 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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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AST	Antibiotic susceptibility test
ATCC	American Type Culture Collection
Bp	base pairs
CA	Cetrimide agar
CAZ	Ceftazidime
CDC	Center for Disease Control
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standard Institute
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetra-acetic acid
et al	Others
FEP	Cefepime
GEN	Gentamicin
GIM	German Imipenemase
HKL	Hospital Kuala Lumpur
I	Intermediate
IMP	Imipenem
IMP	Imipenemase
LB	Luria Bertani
MBLs	Metallo-β-lactamases
MDR	Multidrug resistant
MDRPa	Multidrug resistant *P. Aeruginosa*
MEM	Meropenem
MHA	Mueller Hinton agar

ml	Millilitre
mm	Millimetre
n	Frequency
NDM	New-Delhi MBL
NMRR	National Medical Research Register
NNIS	National Nosocomial Infection Surveillance
OF	Oxidative-fermentative
PCR	Polymerase chain reaction
PFGE	Pulse field gel electrophoresis
pH	Numeric scale used to specify the acidity or alkalinity of an aqueous solution.
R	Resistant
RAPD	Random amplified polymorphic DNA
S	Sensitive
SIM	Seoul Imipenemase
SPM	Sao Paulo MBL
TBE	Tris-borate-EDTA
TZP	Piperacillin-tazobactam
UPM	Universiti Putra Malaysia
USA	United State of America
V	Volt
VIM	Verona Integron-encoded MBL
µg	Microgram
µl	Micro litre
µm	Micrometre
µM	Micro molar

°C	Degree Celsius
%	Percentage
≥	Greater and equal
<	Less than
+ve	Positive

CHAPTER 1

INTRODUCTION

1.1 Research background

Pseudomonas aeruginosa (*P. aeruginosa*) is known as a motile rod shaped and aerobe Gram-negative bacteria. They are found almost everywhere, in soil, water, plants and animals. It has minimal nutritional requirements but its high growth capability explains why it can easily disseminate in the surrounding (Ryan and Ray, 2010). *Pseudomonas aeruginosa* is a known opportunistic pathogen responsible for a major problem in healthcare setting. *P. aeruginosa* is one of the most common bacteria isolated from nosocomial infections (Cornaglia *et al.,* 2000) such as ventilator-associated pneumonia and burn, catheter related, urinary tract infection and bacterimia (Martin and Yost, 2011). For such infections, antimicrobial therapy may become a challenging task because *P. aeruginosa* naturally resistant to many drugs and have the ability to develop further resistance mechanism towards multiple classes of antimicrobial agents, even during the course of a treatment.

Till the emergence of carbapenemases, carbapenems including imipenem and meropenem were the drugs of choice for treatment of infections caused by Gram negative bacteria which resistant to other β-lactam agents because of their broad spectra activity and stability to hydrolysis by most β-lactamases including extended-spectrum β-lactamases (Yan *et al.,* 2001). However, the increasing use of these compounds has resulted in the emergence of carbapenems-resistant *P. aeruginosa* isolates, thus limiting the treatment options (Zavascki *et al.,* 2006; Falagas *et al.,* 2006). Several mechanisms are involved in *P. aeruginosa* resistance to antimicrobial agents, such as chromosomal expression of resistance encoding genes, β-lactamase production, efflux pumps and decrease in membrane permeability. However, most carbapenems resistance is due to impermeability, which arises via loss of the OprD (d2) porin, but surprisingly carbapenems hydrolyzing metallo-β-lactamases (MBLs) are increasingly reported (Livermore *et al.,* 2000). Carbapenem resistance due to MBL production in *P. aeruginosa* has been reported worldwide in which they are capable to hydrolyze not only carbapenems, but also all β-lactam antibiotics except aztreonam.

Metallo-β-lactamases (MBLs) belong to Ambler class B and have the ability to hydrolyze a wide variety of β-lactam agents, such as penicillin, cephalosporin and carbapenems (Livermore *et al.,* 2000). The first MBLs enzymes were IMP-1 which was initially found in *S. marcescens* in Japan (1991), VIM-1 originally detected in Italy (1997), SPM-1 first detected in Brazil (1997), and finally GIM-1 detected in Germany (2002) (Nordmann *et al.,* 2002; Poirel *et al.,* 2004) and since then has been described from various part of the world. The enzymes require zinc for their catalytic activity and inhibited by metal chelator such as EDTA and thiol-based compounds (Livermore *et al.,* 2000).

The genes responsible for the productions of MBLs are typically part of an integron structure and are carried on transferable plasmids but can also be part of the chromosome (Poirel *et al.,* 2002). Acquired MBLs can be divided into four categories according to their molecular structures, namely, the IMP, VIM, GIM and SPM types (Pitout *et al.,* 2005). The most common and widespread acquired MBLs especially around Asia are those of the IMP and VIM types, which later exhibit a worldwide distribution and for which several allelic variants are known (Doosti *et al.,* 2013).

This study is carried out to screen the present of metallo-β-lactamases (MBLs) in the *P. aeruginosa* clinical isolates from a major government referral hospital; Hospital Kuala Lumpur, Malaysia. Phenotypic detection of MBLs was done by Imipenem-EDTA combined disc method. In order to confirm the prevalence of MBL genes in *P. aeruginosa* clinical strains, polymerase chain reaction (PCR) technique is used to identify the MBL genotypes including bla_{IMP}, bla_{VIM}, bla_{GIM} and bla_{SPM}.

Since MBLs have become one of the major factor of resistant towards β-lactams especially carbapenems over the past few decades, the identification of MBL genotyping is very important in order to improve our current antimicrobial therapy. Genotypic identification by Random Amplified of Polymorphic DNA (RAPD) analysis was used to screen for genetic diversity. Finally, Gel Compar II software (Applied Maths) was used to analyze the results obtained in the study.

1.2 Problem statement

Nosocomial infections caused by *P. aeruginosa* presenting resistance to β-lactam antibiotics are one of the most challenging targets for antimicrobial therapy, leading to substantial increase in mortality rates in hospitals worldwide (Polloto *et al.,* 2012). The emergences of carbapenems resistant due to the production of metallo-β-lactamase (MBL) genes are global concern.

However, in Malaysia, there are very limited studies about the presence of genes codifying MBLs among carbapenems resistance *P. aeruginosa* isolates.

1.3 Significant of study

The findings of this study will provide clinician with the updates report on *Pseudomonas aeruginosa* isolates producing metallo-β-lactamases (MBLs) and their association to the increasing of carbapenems resistance.

The information obtained will be used as the guidelines for the selection of appropriate antimicrobial agents in the future in order to prevent wider spread of this worrisome resistance determinant.

1.4 Objectives

1.4.1 General objective

To characterize the presence of metallo-β-lactamases (MBLs) in *P. aeruginosa* from clinical specimens at Hospital Kuala Lumpur (HKL) and their associated factors (patient's socio-demography) as well as clinical factor.

1.4.2 Specific objectives

1) To determine the antibiotic susceptibility patterns of the *P. aeruginosa* clinical isolates.

2) To determine distribution of *P. aeruginosa* isolates in relation to demographic and clinical factors.

3) To determine the distribution of carbapenems resistant in *P. aeruginosa* clinical isolates.

4) To detect MBL-producing *P. aeruginosa* isolates by imipenem-EDTA combined disc method.

5) To detect the production of MBLs in *P. aeruginosa* isolates by polymerase chain reaction (PCR)

6) To investigate the genetic relatedness of *P. aeruginosa* clinical isolates by Random amplified polymorphic DNA (RAPD-PCR)

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