การตรวจหายีนที่สร้างเอนไซม์ carbapenemases ในเชื้อ *Acinetobacter baumannii*

นางนภาวรรณ ปุณกบุตร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาจุลชีววิทยาทางการแพทย์ (สหสาขาวิชา)
บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2551
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

DETECTION OF CARBAPENEMASE GENES IN *ACINETOBACTER BAUMANNII*

Mrs. Napawan Punakabutra

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Medical Microbiology
(Interdisciplinary Program)
Graduate School
Chulalongkorn University
Academic Year 2008

Thesis Title	Detection of Carbapenemase Genes in *Acinetobacter baumannii*
By	Mrs. Napawan Punakabutra
Field of Study	Medical Microbiology
Thesis Principle Advisor	Tanittha Chatsuwan, Ph.D.
Thesis Co-advisor	Nibondh Udomsantisuk

Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master 's Degree

.....Dean of the Graduate School
(Associate Professor Pornpote Piumsomboon, Ph.D.)

THESIS COMMITTEE

.....Chairman
(Assistant Professor Anan Chongthaleong, M.D.)

.....Thesis Principle Advisor
(Tanittha Chatsuwan, Ph.D.)

.....Thesis Co-advisor
(Nibondh Udomsantisuk)

.....Member
(Associate Professor Dr. Aroonwadee Chanawong, Ph.D.)

นภาวรรณ ปุณกบุตร : การตรวจหายีนที่สร้างเอนไซม์ carbapenemases ในเชื้อ *Acinetobacter baumannii*. (Detection of Carbapenemase Genes in *Acinetobacter baumannii*) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : ดร. ธนิษฐา ฉัตรสุวรรณ , อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : อ. นิพนธ์ อุดมสันติสุข, 167 หน้า.

Carbapenems เป็นยาที่นิยมใช้ในการรักษาการติดเชื้อ *Acinetobacter baumannii* ที่ดื้อต่อยาหลายชนิด อย่างไรก็ตาม มีรายงานการดื้อยากลุ่ม carbapenems ในเชื้อชนิดนี้ทั่วโลก การศึกษานี้มีวัตถุประสงค์เพื่อตรวจหายีนที่สร้างเอนไซม์ carbapenemases ในเชื้อ *A. baumannii* และตรวจหาความชุกของการดื้อยา กลุ่ม carbapenems โดยทำการศึกษาในเชื้อ *A. baumannii* จำนวน 501 สายพันธุ์ซึ่งเพาะแยกได้จากผู้ป่วยในโรงพยาบาลจุฬาลงกรณ์ ระหว่างเดือนมกราคม ปี พ.ศ.2547 ถึงเดือนสิงหาคม ปี พ.ศ.2550 จากการทดสอบความไวรับของเชื้อด้วยวิธี Agar dilution พบว่า อัตราการดื้อยา imipenem และ meropenem คือ 82.4% และ 81.8% ตามลำดับ เชื้อที่ดื้อยากลุ่ม carbapenems ทุกสายพันธุ์มี carbapenemase activity เมื่อทดสอบโดยวิธี modified Hodge test ไม่พบเอนไซม์ Metallo-β-lactamases เมื่อทดสอบด้วยวิธี imipenem-EDTA double-disk synergy test การตรวจคัดกรองยีนที่สร้างเอนไซม์ carbapenemases ในเชื้อทั้งหมด 501 สายพันธุ์ได้แก่ $bla_{OXA\text{-}like}$, $bla_{IMP\text{-}like}$ และ $bla_{VIM\text{-}like}$ ด้วยวิธี PCR พบยีน $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$, $bla_{OXA\text{-}51\text{-}like}$ และ $bla_{OXA\text{-}58\text{-}like}$ แต่ไม่พบยีน $bla_{IMP\text{-}like}$ และ $bla_{VIM\text{-}like}$ โดยเชื้อ *A. baumannii* ทุกสายพันธุ์มียีน $bla_{OXA\text{-}51\text{-}like}$ ซึ่งสายพันธุ์ที่พบยีน $bla_{OXA\text{-}51\text{-}like}$ เพียงชนิดเดียวจะไวต่อยากลุ่ม carbapenems ในขณะที่สายพันธุ์ที่พบยีน $bla_{OXA\text{-}51\text{-}like}$ ร่วมกับยีน $bla_{OXA\text{-}like}$ ชนิดอื่นๆ ได้แก่ $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$ และ $bla_{OXA\text{-}58\text{-}like}$ จะดื้อต่อยา carbapenems การศึกษาครั้งนี้เชื้อ *A. baumannii* ที่ดื้อยากลุ่ม carbapenems พบยีน $bla_{OXA\text{-}51\text{-}like}$ ร่วมกับยีน $bla_{OXA\text{-}23\text{-}like}$ มากที่สุด (93.2%) เมื่อทำการวิเคราะห์ลำดับ นิวคลีโอไทด์ของยีน $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$, $bla_{OXA\text{-}51\text{-}like}$ และ $bla_{OXA\text{-}58\text{-}like}$ ในเชื้อ *A. baumannii* ชนิดละ 10 สายพันธุ์ พบว่าเป็นชนิด $bla_{OXA\text{-}23}$(100%) , $bla_{OXA\text{-}72}$(100%) และ $bla_{OXA\text{-}58}$(100%) ตามลำดับ พบความหลากหลายในยีนกลุ่ม $bla_{OXA\text{-}51\text{-}like}$ โดยพบเป็น $bla_{OXA\text{-}68}$ 5 สายพันธุ์ (50%), $bla_{OXA\text{-}66}$ 2 สายพันธุ์ (20%), $bla_{OXA\text{-}64}$ 1 สายพันธุ์ (10%), $bla_{OXA\text{-}67}$ 1 สายพันธุ์ (10%) และ $bla_{OXA\text{-}51\text{-}like}$ ชนิดใหม่ซึ่งยังไม่เคยมีรายงานมาก่อน 1 สายพันธุ์ (10%) โดยลำดับนิวคลีโอไทด์และกรดอะมิโนของเอนไซม์ชนิดใหม่นี้มีความเหมือนกับลำดับนิวคลีโอไทด์และกรดอะมิโนของ $bla_{OXA\text{-}51}$ และ OXA-51 99%

สาขาวิชา จุลชีววิทยาทางการแพทย์ ลายมือชื่อนิสิต.....
ปีการศึกษา 2551 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....
ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

4889088920 : MAJOR MEDICAL MICROBIOLOGY
KEY WORD: *ACINETOBACTER BAUMANNII* / CARBAPENEMASE / CARBAPENEM RESISTANT / OXA

NAPAWAN PUNAKABUTRA : DETECTION OF CARBAPENEMASE GENES IN *ACINETOBACTER BAUMANNII*. THESIS PRINCIPLE ADVISOR : TANITTHA CHATSUWAN, Ph.D., THESIS COADVISOR : NIBONDH UDOMSANTISUK, 167 pp.

Carbapenems are among the drugs of choice for treatment of multidrug-resistant *Acinetobacter baumannii* infections. However, emergence of carbapenem resistance in *A. baumannii* has been reported worldwide. The present study identified carbapenemase genes in *A. baumannii* and investigated the prevalence of carbapenem resistance of the 501 *A. baumannii* isolated from patients at the King Chulalongkorn Memorial Hospital, Bangkok during January 2004 to August 2007. The minimal inhibitory concentrations (MICs) of imipenem and meropenem were determined by agar dilution. Resistance rates were 82.4% and 81.8% for imipenem and meropenem, respectively. All carbapenem-resistant isolates had carbapenemase activity by modified Hodge test. Metallo-β-lactamases were not detected in any isolate when screened by imipenem-EDTA double-disk synergy test. Screening for carbapenemase genes in the 501 *A. baumannii* by PCR revealed the presence of $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$ and $bla_{\text{OXA-58-like}}$ and the absence of $bla_{\text{IMP-like}}$ and $bla_{\text{VIM-like}}$. All 501 *A. baumannii* isolates carried the $bla_{\text{OXA-51-like}}$ gene. The isolates carrying only $bla_{\text{OXA-51-like}}$ were susceptible to carbapenems whereas the isolates carrying $bla_{\text{OXA-51-like}}$ together with other $bla_{\text{OXA-like}}$ including $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$ were resistant to carbapenems. The presence of $bla_{\text{OXA-51-like}}$ together with $bla_{\text{OXA-23-like}}$ were detected in the majority of carbapenem-resistant *A. baumannii* isolates (93.2%), suggesting that $bla_{\text{OXA-23-like}}$ plays a major role in carbapenem resistance. DNA sequences of the entire $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$ and $bla_{\text{OXA-58-like}}$, each from 10 representative isolates, were analyzed. The results revealed that the $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$ from their 10 representative were $bla_{\text{OXA-23}}$, $bla_{\text{OXA-72}}$ and $bla_{\text{OXA-58}}$ respectively, whereas those of the $bla_{\text{OXA-51-like}}$ were $bla_{\text{OXA-68}}$ (5 isolates), $bla_{\text{OXA-66}}$ (2 isolates) and $bla_{\text{OXA-64}}$, $bla_{\text{OXA-67}}$ and a novel $bla_{\text{OXA-51-like}}$ (1 isolate each). Nucleotide and amino acid sequences of this novel enzyme showed 99% identity to $bla_{\text{OXA-51}}$ and OXA-51.

Field of study Medical Microbiology
Academic year 2008

Student's signature Napawan Punakabutra
Principle Advisor's signature Tanittha Chatsuwan
Co-advisor's signature Nibondh Udomsantisuk

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation and gratitude to the following whose gave me the possibility to complete my thesis: Tanittha Chatsuwan, Ph.D., my thesis advisor at the Division of Bacteriology, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, for her guidance, general suggestion, discussion, criticism, kindness and strong encouragement throughout the course of this study.

Nibondh Udomsantisuk, my co-advisor at the Division of Bacteriology, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, for his kindness, invaluable advice and strong encouragement throughout the period of this study.

Assistant Professor Anan Chongthaleong, M.D., the chairman of thesis committee, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, for his critisms.

My sincere gratitude is also give to the members of my thesis committee, Associate Professor Dr. Aroonwadee Chanawong, Ph.D., Department of Clinical Microbiology, Faculty of Associated Medical Sciences, Khon Kaen University, for her kindness, valuable comments helpful suggestion for the complement of this thesis.

This work was financially supported by grant from Ratchadapisek sompotch, Faculty of Medicine, Chulalongkorn University.

Sincere thanks go to all members in inter-department of Medical Microbiology, staff in the Department of Microbiology, Faculty of Medicine, Chulalongkorn University for their encouragement and helpful. My appreciation also goes to all of those whose names have not been mentioned, for helping me whether directly or indirectly to make this work a reality.

Finally, I am deeply indebted to my parents for their understanding and support during my study period.

CONTENTS PAGE

CHAPTER PAGE

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

A	adenosine
AC	amoxicillin
Ala (A)	alanine
Asn (N)	asparagine
Asp (D)	aspartic acid
Arg (R)	arginine
bp	base pair
C	cytidine
CO_2	carbon dioxide
CH	clarithromycin
CLSI	Clinical and Laboratory Standards Institute
°C	degree Celsius
Cys (C)	cysteine
dATP	deoxyadenosine 5'-triphosphate
dCTP	deoxycytidine 5'-triphosphate
ddATP	dideoxyadenosine 5'-triphosphate
ddCTP	dideoxycytidine 5'-triphosphate
ddGTP	dideoxyguanosine 5'-triphosphate
ddTTP	dideoxytymidine 5'-triphosphate
DDW	double distilled water
ddNTPs	dideonucleotide-tri-phosphate
dGTP	deoxyguanosine 5'-triphosphate
DNA	deoxynucleic acid
dNTPs	deoxynucleotide-tri-phosphate
dTTP	deoxytymidine 5'-triphosphate
DW	distilled water
EDTA	ethylenediamine tetraacetic acid
et al.	*et alii*
E-test	epsilometer test
g	gram
G	guanosine
Gly (G)	glycine

Glu (E)	glutamic acid
Gln (Q)	glutamine
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
hr	hour
His (H)	histidine
i.e.	id test
Ile (I)	isoleucine
Lys (K)	lysine
Leu (L)	leucine
M	molar
mg	milligram
$MgCl_2$	magnesium chloride
MIC	minimum inhibitory concentration
min	minute (s)
mL	milliliter
mM	millimolar
mmol	millimole
Met (M)	methionine
NaCl	sodium chloride
Na_2HPO_4	sodium phosphate dibasic, anhydrous
NaOH	sodium hydroxide
NARST	National Antimicrobial Resistance Surveillance Center Thailand
Phe (F)	phenylalanine
PCR	polymerase chain reaction
Pfu	*Pyrococcus furiosus*
pmol	picomol
Pro (P)	proline
sec	second
Ser (S)	serine
T	thymidine
TAE	tris-acetate-EDTA
Thr (T)	threonine

Tris	Tris-(hydroxymethyl)-aminoethane
Trp (W)	tryptophan
Tyr (Y)	tyrosine
U	unit
μg	microgram
μL	microliter
μM	micromolar
UV	ultraviolet
V	volt

CHAPTER I

INTRODUCTION

Acinetobacter baumannii is an opportunistic pathogen that is frequently involved in nosocomial infection. *A. baumannii* is mostly a cause of infection in all systems such as septicaemia, urinary tract infection, ventilator-associated pneumonia and skin infection following hospitalization of patients with more severe illness or immunocompromised patients. Carbapenems such as imipenem and meropenem are drugs of choice for treatment of *A. baumannii* infection, but carbapenem resistance in this species is now observed increasingly worldwide and also to be multidrug-resistance strains.

Carbapenems are member of β-lactam antibiotics, act cytostatically on bacteria by inactivating peptidoglycan transpeptidases known as penicillin-binding proteins (PBPs) that catalyse the cross-linking of the peptidoglycan polymers in the bacterial cell wall. Primary cause of carbapenem resistance in *A. baumannii* is β-lactamase production such as carbapenemase enzymes to destroy β-lactam ring structure of drug, resulting in loss of efficiencing. Other resistance mechanisms are attributed to reduced affinity of PBPs for carbapenems, increased efflux of the β-lactam antibiotics, decreased permeability of the outer membrane or to a combination of several mechanisms.

The β-lactamases can be classified into four different molecular groups, A, B, C and D according to amino acid sequence identities. Class A, C and D β-lactamases use a catalytically active serine residue for inactivation of the β-lactam drug, while molecular class B β-lactamases are metallo-enzymes requiring zinc for their catalytic activity. Carbapenemase production is the major mechanism of carbapenem resistance in *A. baumannii*. Most of these enzymes are members of class D β-lactamase (oxacillinase), followed by class B metallo-β-lactamase such as IMP-type and VIM-type and rarely found in class A β-lactamase (1).

Carbapenems such as imipenem and meropenem are drugs of choice for treating penicillin- and cephalosporin-resistant *A. baumannii*. Reports of carbapenem-resistance *A. baumannii* have been increasing over the last few years. In Taiwan, the proportion of *A. baumannii* resistant to carbapenems rose from 5.88% in 1993 to 21.5% in 2000 (2). In 1999, carbapenem-resistance *A. baumannii* was 53% in USA and 34% in Spain during 1997 to 1999 (3, 4). Furthermore, there were many reports about carbapenem-resistance *Acinetobacter* spp. worldwide such as Greece (5), Turkey (6, 7), Croatia (8), Italy, England (9), Germany, Poland, China, Brazil, France (10, 11), Argentina (12), Romania (13), etc.

Most of carbapenemase enzymes that found in *A. baumannii* are OXA-type carbapenemase (oxacillinase), whereas metallo-β-lactamases such as IMP-type and VIM-type have been rarely identified. First carbapenem-hydrolyzing oxacillinase (CHDLs) encoding gene was reported in *A. baumannii* in 1995. This enzyme, originally named ARI-1, was identified in Scotland and it was renamed OXA-23 following its genetic and biochemical reaction (14). After that, there were many reports about OXA-type carbapenemases produced by *A. baumannii* around the world. OXA enzymes can be subclassified into eight subgroups by amino acid identities. Four of the eight subgroups, OXA-23 like, OXA-24 like, OXA-51 like and OXA-58 like, have been identified in *A. baumannii*. The first subgroup encompasses the OXA-23, -27 and -49, which were reported in Brazil (15), Korea (16), China (17), Singapore (18), Scotland (14), South America (19), United Kingdom (20), Australia (21) and Tahiti (11). The second subgroup encompasses the OXA-24 to -26, -33, -40 and -72, which were reported in Spain (22), Belgium (23) and France (24). The Third subgroup consists of the OXA-51 family, including OXA-51, OXA-64 to -71, OXA-75 to -80, OXA-82 to -84, OXA-86 to -95, OXA-99, OXA-100, OXA-104, OXA-106 to -113, OXA-115 to -117, OXA-128, OXA-130 to -132. They were reported in Argentina (25-27), Spain (25), Hong Kong (25), Singapore (25), South Africa (25), France (28), Poland (29), United Kingdom (30), Greece (31, 32) and Turkey. The fourth subgroup encompasses the OXA-58, -96 and -97, which were reported in South Europe (13), France (33), Singapore (18) and Tunisia (34). For IMP-type, there were reported in *Acinetobacter* spp. IMP-1 were reported in Japan (35), Korea (36, 37) and Italy (38). IMP-2, IMP-4, IMP-5, IMP-6 and IMP-11 were

reported in Italy (39), Hong Kong (40), Protugal (41), Brazil (42) and Japan (43), respectively. For VIM-type, VIM-2 were reported in *A. baumannii* in Korea (36, 44). VIM-1 and VIM-2 were reported in *Acinetobacter* spp. in Italy (41) and Korea (36, 45), respectively whereas SIM-1 was reported in South Korea (46).

In Thailand, there was no evidence of carbapenem resistance in *Acineobacter* spp. at Siriraj Hospital in 1998 (47). In 1999, *Acinetobacter* spp. were 21% resistant to imipenem at Vajira Hospital (48). In 2007, imipenem resistance rate of *A. baumannii* collected from Chiang Mai University Hospital were 35.5% (49). At the same peroid, the imipenem resistance rate of 2,130 *A. baumannii* isolated from clinical specimens at Songklanagarind Hospital was 34.7%. The 61.9% of these imipenem-resistance *A. baumannii* isolates were isolated from respiratory tract, and frequently found in ICU. Moreover, 14.8% of all imipenem-resistance *A. baumannii* isolates were resistant to all routine tested antibiotics (50). In 2003, the imipenem resistance rate of *A. baumannii* was 61% at Police General Hospital (51). Sixty-eight percent of *A. baumannii* isolated from Siriraj Hospital in 2006 were resistant to carbapenems and 57% were resistant to all antimicrobials currently available in Thailand (52). The data from the National Antimicrobial Resistance Surveillance Center Thailand (NARST) showed that imipenem resistance in *Acinetobacter calcoaceticus-baumannii* complex increased from 2% in 1998 to 57% in 2006 (53).

The emergence of carbapenem resistance in *A. baumannii* has become a global concern including Thailand. In Thailand there are few studies on mechanisms of carbapenem resistance in *A. baumannii*. The purpose of this study is to determine carbapenemase genes including $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-58-like}}$, $bla_{\text{IMP-like}}$ and $bla_{\text{VIM-like}}$ in carbapenem-resistant *A. baumannii* and investigate the prevalence of carbapenem resistance in *A. baumannii* isolated from Thai patients between January 2004 and August 2007.

CHAPTER II

OBJECTIVES

I. To examine carbapenemase genes (bla_{OXA} , bla_{IMP} , bla_{VIM}) in carbapenem-resistant *A. baumannii.*

II. To investigate the prevalence of carbapenem resistance in *A. baumannii* isolated from Thai patients.

CHAPTER III

LITERATURE REVIEW

1. BACTERIOLOGY

Genus *Acinetobacter* is currently classified in the Phylum Proteobacteria, Class Gammaproteobacteria, Order Pseudomonadales, Family Moraxellaceae. There are three genera in this family including *Moraxella*, *Acinetobacter* and *Psychrobacter*. Species in genus *Acinetobacter* are strictly aerobic non lactose fermentative gram negative coccobacillus but occasionary are difficult to destain. Individual cell is 1 to 1.5 μm by 1.5 to 2.5 μm in size and frequently arrange in pairs. They are non motile, oxidase-negative, catalase positive and do not reduce nitrate to nitrite. Colonies on MacConkey agar are smooth, opaque, colorless or slightly pinkish colonies. All strains grow between 20°C and 30°C. Most strains have optimal temperature at 33-35°C and grow in defined media containing a single carbon and energy source. They use ammonium or nitrate salts as the source of nitrogen. D-Glucose is the only hexose utilized by some strains, whereas the pentose D-ribose, D-xylose and L-arabinose can also be utilized as carbon sources by some strains.

Studies based on DNA-DNA hybridization, showed that there were at least 32 genomic species and 17 of them have been assigned species name as described in Table 1. Genomospecies 1, 2, 3 and 13 of Tjernberg and Ursing are closely related and difficult to separate in the clinical laboratory, refered to as the *A. calcoaceticus-A. baumannii* complex (54). Strains belonging to genomic species 2 (*A. baumannii*), 3, and 13 have been found most frequently to be associated with hospital infection and epidemic outbreaks (55-57).

Acinetobacter spp. are generally considered to be nonphathogenic to healthy individuals. However, *A baumannii* persists in hospital environments and causes severe, life-threatening infections in compromised patients. This organism is resistant to muitiple antimicrobial agents and has high capacity for survival on most both

moist and dry environmental surfaces, including respiratory therapy equipment and human skin. It can also be isolated readily from nonclinical sources such as soil, water, sewage and a variety of different foodstuffs. In addition, this organism is also part of the normal oropharyngeal flora of a small number of healthy people and can proliferate to large numbers during hospitalization. These factors have led to an increased concern regarding hospital-acquired infections due to *A. baumannii*.

2. EPIDEMIOLOGY

2.1 Hospital-acquired *A. baumannii* infection

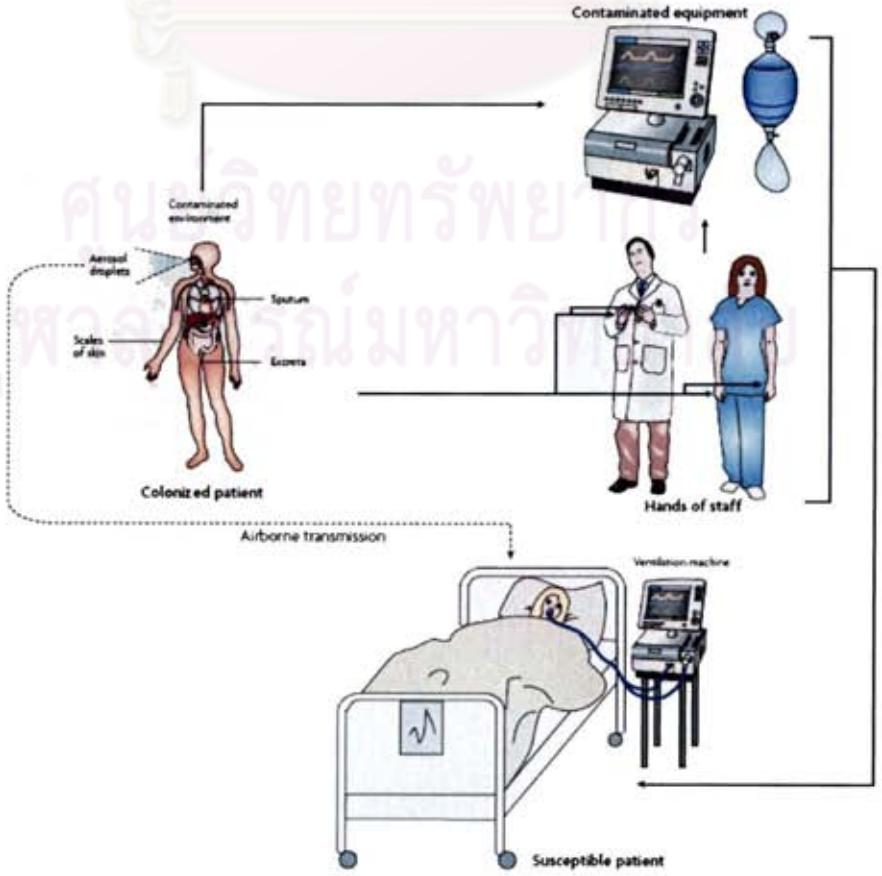
Hospital-acquired *A. baumannii* infection includes ventilator-associated pneumonia, skin and soft-tissue infections, wound infections, urinary-tract infections, secondary meningitis and bloodstream infections. The most frequent clinical manifestations of nosocomial *A. baumannii* are ventilator-associated pneumonia and bloodstream infections. A scheme that explains the dynamics of *A. baumannii* epidemiology in a hospital is shown by Figure 1 (58). *A. baumannii* can spread from the colonized patient to the environment and other susceptible patients via excreta, aerosol droplets and scale of skin. The most common mode of transmission is from the hands of medical staff. Organisms can survival in dry environment. During outbreak, it has been recovered from various patient's environments including bed curtains, furniture and medical equipment. The medical equipments such as mechanical ventilation, indwelling devices (intravascular catheters, urinary catheters and drainage tubes) are the most common contaminated by *A. baumannii*.

The potential risk factors for the acquisition of *A. baumannii* infection include host factors such as major surgery, major trauma especially in burn trauma, prematurity in newborns. The exposure related factors are previous stay in an ICU, the length of stay in hospital or ICU, residence in a unit that *A. baumannii* is endemic, exposure to contaminated medical equipment, the number of invasive procedures, previous antimicrobial therapy and deficiency in the implementation of infection

control guidelines. These factors explain the difficulty of controlling *A. baumannii* infection in hospitals.

The clinical impact of nosocomial *A. baumannii* infection has been debated. Cisneros *et al.* and Seifert *et al.* showed high mortality rates in patients who had *A. baumannii* bacteraemia or pneumonia (59, 60). Falagas *et al.* also concluded that an increase in attributable mortality ranging from 7.8 to 23% was associated with *A. baumannii* infection (61, 62). However, this organism mainly affects patients that severe underlying disease and prognosis. It has been argued that the mortality in patients with *A. baumannii* infection caused by their underlying disease rather than by *A. baumannii* infection. Study by Blot *et al.* showed no significant difference between the mortality rate of *A. baumannii* bacteraemia in ICU patients and control group that were 42.2% and 34.4%, respectively (P = 0.378) (63). Similar to the study by Garnacho *et al.*, there was no significant difference between the mortality rate of ventilator-associated pneumonia caused by *A. baumannii* and control group that were 40% and 28.3%, respectively (P = 0.17) (64).

Figure 1. Overview of the dynamics between patients, bacteria and the hospital environment (58).



2.2 Community-acquired *A. baumannii* infection

A. baumannii is an important cause of community-acquired pneumonia. The evidence of community-acquired *A. baumannii* infection is increasing. High incidence of bacteraemia and high mortality ranging from 40 to 64% (58). The incidence of community-acquired *A. baumannii* infection has been reported almost exclusively in tropical climates, especially in Southeast Asia and tropical Australia (65, 66). In Israel, *A. baumannii* showed a 31-fold increase when it was hospital-acquired bacteraemia and a 13-fold increase when it was community-acquired bacteraemia by an infection control surveillance program at the Assaf Harofeh Medical Center (AHMC) in 1997-2004 (67). In northern Australia, 10% of all community-acquired bacteremic pneumonia were caused by *A. bamannii* and 21% by gram-negative bacteria in 1992 (68). There were case reports of community-acquired pneumonia caused by *A. baumannii* from countries in tropical or subtropical regions such as Australia (68), Kuwait (69), Taiwan (70) and Thailand (71-73). The prevalence of infection occurred in warm and humid environment might be due to its appropriated condition for growth. The risk factors for community-acquired *Acinetobacter* pneumonia have been associated with underlying conditions such as alcoholism, smoking, chronic obstructive pulmonary disease (COPD) and diabetes mellitus.

3. PATHOGENESIS AND VIRULENCE FACTORS OF *ACINETOBACTER BAUMANNII*

Acinetobacter baumannii is an opportunistic pathogen. Colonization with *A. baumannii* is more common than infection. However it can be severe when an infection is developed. The factors that contribute to *A. baumannii* environment persistence and host infection and colonization are summarized in Figure 2 (58). Adherence to host cell such as bronchial epithelial cells and skin is the first step in the colonization process. *A. baumannii* can survive and grow on host skin and mucosal surface because it can resist to inhibitory agents and conditions that are exerted by these surfaces. In addition, it can resist to desiccation, disinfectants and

antibiotics and can use various substrates for growth, resulting in survival in the environment. Growth on mucosal surfaces and medical devices, such as intravascular catheters and endotracheal tubes, can result in biofilm formation, which enhanced the risk of bloodstream and airway infections (74). Biofilm formation might have been regulated by quorum sensing, the presence of which has been inferred from the detection of a genes that is involved in autoinducer production (75). Virulence factors that could have a role in *A. baumannii* are described below.

1. Adherence of bacterial cell to epithelial cell

Adherence of bacteria to epithelial cells is considered to be an essential first step in colonization and subsequent infection (76). This organism can survive and grow on host skin because organism can resist to inhibitory agents and protective conditions of skin (such as desiccation, low pH, the resident normal flora and toxic lipids). Organism also can survive and grow on mucus membrane because it can resist to the presence of mucus, lactoferrin, lactoperoxidase and the sloughing of cells (58). In addition, the pili and hydrophobic sugars in the O-side-chain moiety of lipopolysaccharide (LPS) might promote adherence to host cells (77-80). Studies by electron microscopy demonstrated that pili on the surface of *Acinetobacter* interact with human epithelial cell. Moreover, there were thread-like connections between these bacteria in early phase of biofilm formation (81).

2. Biofilm formation

Biofilm formation is important pathogenic feature, especially in intravascular line infections and ventilator-associated pneumonia. *A. baumannii* can grow on mucosal surfaces and medical devices such as intravascular catheters and endotracheal tubes by biofilm formation, resulting in enhancing the risk of infection in bloodstream and airways. Study by Rodriguez-Bano demonstrated that all catheters related urinary tract or bloodstream infections and shunt-related meningitis were caused by biofilm forming strains. They also found that previous aminoglycoside use was associated with biofilm-forming isolates (82). *A. baumannii* isolates carrying bla_{PER-1} showed a significantly higher ability for epithelial cell

adherence and biofilm formation than isolates without bla_{PER-1} (83). Similar to the study by Sechi *et al.*, PER-1 production in *A. baumannii* was found to be related to cell adhesion (84). In the other hand, treatment with EDTA caused a 55%-65% reduction in biofilm formation by detachment of biofilm cells and inhibit bacterial growth (83). Organisms that caused many device-related and chronic infections and formed biofilm in or on these devices were difficult to eradicate because organisms in biofilm were usually resistant to numerous antimicrobial agents and products of the immune system (85, 86). Similar to the study by Lee *et al.*, multidrug-resistant isolates of *A. baumannii* were reported to form large amounts of biofilm and showed a significant correlation with epithelial cells adherence (83). In addition, the ability of bacterial cells to transfer gene horizontally is increasing within biofilm communities, resulting in the spread of antibiotic resistance (86, 87). So, the high ability to colonize of *A. baumannii* combined with its resistance to multidrugs will contribute to the organism's survival and further dissemination in the hospital.

3. Potential toxic role of lipopolysaccharide

The lipopolysaccharide (LPS) is involved in resistance to complement in human serum and acts in synergy with the capsular exopolysaccharide. The endotoxic potential of *A. baumannii* LPS is able to stimulate inflammatory signaling via Toll-like receptor (TLRs). Whole UV-killed cells of *A. baumannii* could stimulate both TLR2- and TLR4-dependent signaling, whereas pure endotoxins induced signaling only TLR4 (88). In addition, capsular polysaccharide blocks the access of complement to microbial cell wall and prevent the triggering of the alternative pathway of complement activation. Stimulating in innate immune response by the endotoxins and capsular polysaccharide may contribute to the pathology of *A. baumannii* infection.

4. The outer membrane protein

The outer membrane protein A, AbOmpA, previously called Omp38, has been associated with the cytotoxicity induction. AbOmpA is a 38 kDa porin, which is a trimeric porin with a pore size of 1.3 nm and acts as a general diffusion pore. Choi

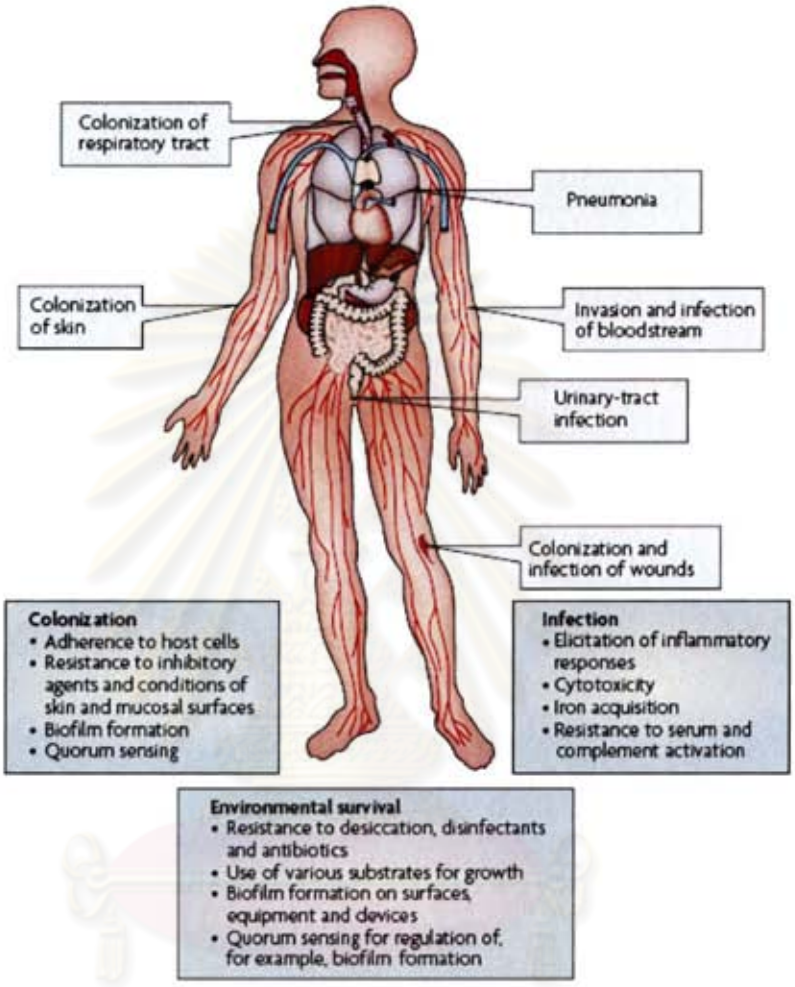
et al. found that this protein was a potent cytotoxin that induced apoptosis of human laryngeal epithelial cell, Hep-2 (89, 90). AbOmpA entered to the Hep-2 cell and was localized to the mitochondria, resulting in releasing of proapoptotic molecules such as cytochrome *c* and apoptosis-inducing factor (AIF) into the cytosol as a consequence of mitochondrial disintegration. This event activated caspase-3 followed by degradation of DNA approximately 180 bp in size. AIF activated caspase-independent apoptosis and degraded chromosomal DNA approximately 50 kb in size, which resulting in large-scale DNA fragmentation. Apoptosis of epithelial cells may disrupt the mucosal lining and resulting in invading of bacteria or bacterial products to deep tissues.

5. Siderophores synthesis

A. baumannii is able to utilize host resources for its survival in human. Iron is an important resource that is not readily available in human, rather it is found to be complexed with iron-binding molecules, including heme and lactoferrin (91, 92). Siderophores are low-molecular-mass ferric binding compounds that can uptake iron in iron-limiting conditions. The bacteria can survive and multiply under iron-limiting conditions by expressing this active system, siderophores synthesis. Siderophores are classified into four categories by their chemical structures (91, 93). Yamamoto *et al.* demonstrated the structure of acinetobactin, the siderophore secreted by *A. baumannii* 19606 had a phenolate group as the iron-binding site (94). Acinetobactin formed complex with ferric and this complex were transported into bacterial cell across the bacterial membrane via the FatA-like protein (95). Other bacteria that has siderophores synthesis is *Vibrio anguillarum*, which secreted anguibactin for iron acquisition.

Over all together, the ability of *A. baumannii* to adapt in variable conditions together with antibiotic resistance and effective stress-response mechanisms might explain the success of *A. baumannii* infection.

Figure 2. **The factors that contribute to *A. baumannii* environment persistence and host infection and colonization (58).**



4. DIAGNOSIS OF *A. BAUMANNII* INFECTION

Infection or colonization with *A. baumannii* is usually diagnosed by culture of blood, sputum, urine, wound, sterile body fluid, ect. on routine medium. There are several methods for identifying *A. baumannii*, including phenotypic and genotypic identification as described below.

Phenotypic Identification

A. baumannii is gram negative coccobacilli usually diploid form. Colonies growing on MacConkey agar may have a slightly pinkish tint. This organism does not produce cytochrome oxidases and oxidize glucose with production of acid. It can grow at 37°, 42° and 44°C. *A. baumannii* can decarboxylate arginine and use malonate as a carbon source. It does not produce urease enzyme and cannot reduce nitrate to nitrite and nitrogen gas. The biochemical scheme of genus *Acinetobacter* is shown in Table 3 (54). Phenotypic identification methods take a long time for identification and can not discriminate some genomospecies that are highly related (57, 96). Genomospecies 1 (*A. calcoaceticus*), 2 (*A. baumannii*), 3 and Tjernberg and Ursing 13 are often similar and belong to *A. calcoaceticus* - *A. baumannii* complex (ACB complex). These organisms cannot be differentiate by phenotypic tests. So, genotypic identification has been used for classifying the members of genus *Acinetobcter*.

Genotypic Identification

In 1986, Bouvet and Grimont classified the genus *Acinetobacter* to 12 DNA groups or genomospecies by DNA-DNA hybridization tests (97). Genomospecies 1, 2, 4, 5, 7 and 8/9 were designated *A. calcoaceticus, A. baumannii, A. haemolyticus, A. junii, A. johnsonii and A. lwoffii,* respectively. The others were designated by group number, genomospecies 3, 6 10, 11 and 12. In the following years, *A. radioresistens* was described and demonstrated to correspond to Bouvet and Grimont's genomospecies 12 by Nishimura *et al.* (98). By using DNA-DNA hybridization, Tjernberg and Ursing found three additional genomic species, genomospecies TU13, TU14 and TU15 in 1989 (99). At the same time, Bouvet and Jeanjean reported five genomospecies, BJ13 to BJ17, to the scheme of Bouvet and Grimont. DNA group 13 sensu Bouvet and Jeanjean (BJ13) corresponds to genomic species 14 sensu Tjernberg and Ursing (TU14). In addition, "Close to TU13" and "Between 1 and 3" were demonsatrated by Gerner-Smidt *et al.* in 1993 (96). To date, at least of 32 genomic species were identified and 17 of them have been assigned species name as described in Table 1 (58).

DNA-DNA hybridization is the standard method for genotypic identification but this technique is complex, time-consuming and cannot be performed in most clinical laboratories. So, many techniques were developed, including amplified ribosomal DNA restriction analysis (ARDRA) (100), the analysis of restriction fragment length polymorphisms (RFLPs) (101), ribotyping and restriction analysis of the 16S-23S ribosomal RNA Intergenic Spacer Sequences (102).

Table 1. Classification of the genus *Acinetobacter* (58)

Species	Source
Species that have valid names	
A. calcoaceticus (Genomospecies 1)	Soil and humans (including clinical specimens)
A. baumannii (Genomospecies 2)	Humans (including clinical specimens), soil, meat and vegetables
A. haemolyticus (Genomospecies 4)	Humans (including clinical specimens)
A. junii (Genomospecies 5)	Humans (including clinical specimens)
A. johnsonii (Genomospecies 7)	Humans (including clinical specimens) and animals
A. lwoffii (Genomospecies 8/9)	Humans (including clinical specimens) and animals
A. radioresistens (Genomospecies 12)	Humans (including clinical specimens), soil and cotton
A. ursingii	Humans (including clinical specimens)
A. schindleri	Humans (including clinical specimens)
A. parvus	Humans (including clinical specimens) and animals
A. baylyi	Activated sludge and soil
A. bouvetii	Activated sludge
A. towneri	Activated sludge
A. tandoii	Activated sludge
A. grimontii	Activated sludge
A. tjernbergiae	Activated sludge
A. gerneri	Activated sludge
Species that have provisional designations	
A. venetianus	Sea water
Genomospecies 3	Humans (including clinical specimens), soil and vegetables
Genomospecies 6	Humans (including clinical specimens)
Genomospecies 10	Humans (including clinical specimens), soil and vegetables
Genomospecies 11	Humans (including clinical specimens) and animals
Genomospecies 13BJ or 14TU	Humans (including clinical specimens)
Genomospecies 14BJ	Humans (including clinical specimens)
Genomospecies 15BJ	Humans (including clinical specimens)
Genomospecies 16	Humans (including clinical specimens) and vegetables
Genomospecies 17	Humans (including clinical specimens) and soil
Genomospecies 13TU	Humans (including clinical specimens)
Genomospecies 15TU	Humans (including clinical specimens)
Genomospecies between 1 and 3	Humans (clinical specimens)
Genomospecies close to 13TU	Humans (clinical specimens)

5. TREATMENT OF *A. BAUMANNII* INFECTION

Broad-spectrum cephalosporins, β-lactam-β-lactamase inhibitor combinations such as ampicillin combined with sulbactam, carbapenems that used alone or combination with an aminoglycoside and other combinations of a β-lactam with a fluoroquinolone or rifampin have been used for treatment of *A. baumannii* infections (103). Carbapenems are a class of β-lactam antibiotics with a broad spectrum of antibacterial activity, and have a structure which renders them highly resistant to β-lactamases. Carbapenems that usually use for treatment of *A. baumannii* infection are imipenem amd meropenem.

For infections caused by multidrug-resistant isolates, the most active agents are the polymyxins, polymyxin B and polymyxin E (colistin). These agents caused disruption of the integrity of bacterial cell membrane, leading to leakage of intracellular contents and cell death (104). They were abandoned in 1960s and 1970s but they have brought back into use again during the past few years for treatment of multidrug-resistant gram negative bacilli including *Acinetobacter*. Bronchoconstriction is the main side effect of inhaled colistin when used for treatment of ventilator-associated pneumonia (105). Garancho-Montero *et al.* suggested that intravenous polymyxins could be used successfully for the treatment of ventilator-associated *A. baumannii* pneumonia, with less nephrotoxicity (106). The cure rates for colistin were 57-77% among severely ill patients with multidrug-resistant *Acinetobacter* infections, including pneumonia, bacteremia, sepsis, intra-abdominal infection and central nervous system infection (107-110). Motaouakkil *et al.* demonstrated the successfully treatment of ventilator-associated pneumonia and bloodstream infections by combination of colistin and rifampin (111). Yoon *et al.* also demonstrated the synergy activities of polymyxin B combined with imipenem and rifampin against multidrug-resistant *A. baumannii* (112). It might be because of rapid permeable through the outer membrane of polymyxin B, allowing enhanced penetration and activity of both imipenem and rifampin. However, Saballs *et al.* found a high failure rate and emergence of rifampin resistance in 70% of carbapenem-resistant *Acinetobacter* infected patients treated

with rifampin plus imipenem (113). Bernabeu-Witlel *et al.* also demonstrated the activities of imipenem combined amikacin were worse than imipenem alone for treatment of impenem-resistant pneumonia in a guinea pig model (114).

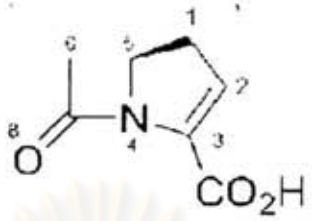
New glycylcycline antibiotic, tigecycline, has been reported to be active against some multidrug-resistant *A. baumannii* (115, 116). However, tigecycline-resistant *A. baumannii* isolated were reported recently (117-123). Peleg *et al.* and Ruzin *et al.* demonstrated that tigecycline resistance was associated with upregulation of chromosomally mediated efflux pumps (124). Cefoperazone combined with sulbactam, sulperazone, was also used to treat multidrug-resistant *Acinetobacter* spp. Lim *et al.* found 62% of cefoperazone-resistant *Acinetobacter* spp. were susceptible to cefoperazone-sulbactam (125).

6. CARBAPENEMS

Carbapenems are β-lactam antibiotics that contain a β-lactam ring in their structures. They differ from other β-lactams by having a hydroxyethyl side chain in trans-configuration at position 6 and a sulfur atom at position 1 of the structure has been replaced with a carbon atom, and hence the name of the group, the carbapenems (Figure 3.). The unique stereochemistry of the hydroxyethyl side chain confers stability against β-lactamases (126, 127).

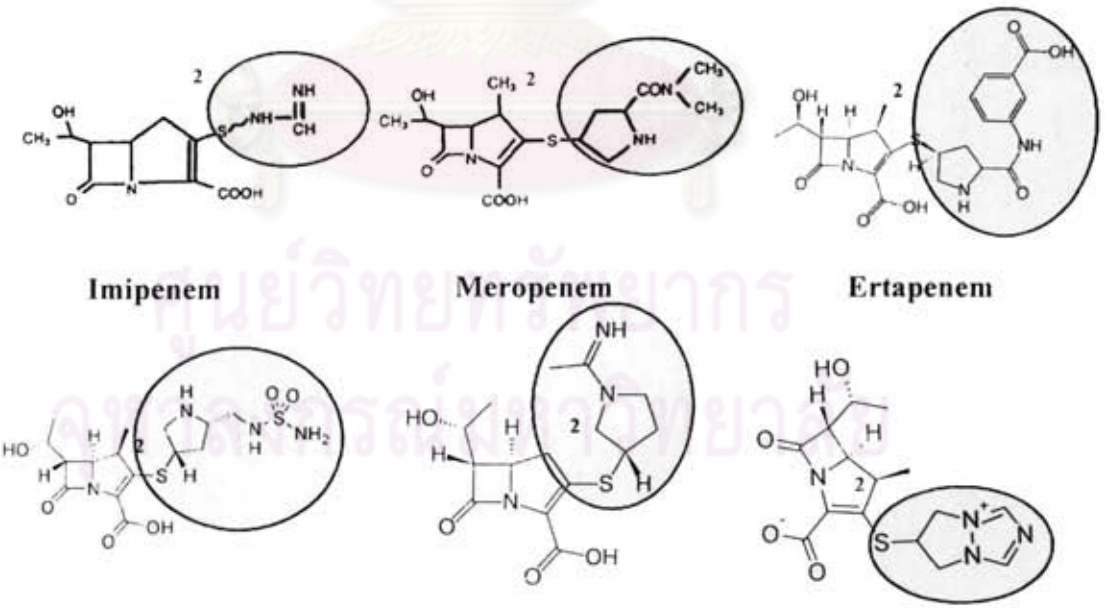
All β-lactam antibiotics are bactericidal, and act by inactivating cell wall enzymes such as transpeptidases which are members of the family of penicillin binding proteins (PBPs). These enzymes catalyze the cross-linking of the peptidoglycan polymers in the bacterial cell wall (128). β-Lactam antibiotics are similar to the D-alanyl-D-alanine of the pentapeptide which attached to N-acetylmuramic acid (NAM) unit in the nascent peptidoglycan layer. PBPs mistakenly use penicillin as a substrate for cell wall synthesis and transpeptidase is acylated. The acylated PBPs inhibit cell wall synthesis, while autolysis by cell wall autolytic continues. These events lead to permeably of water, rapidly take up fluid and eventually lyse of bacterial cells (129).

Figure 3. Structure of carbapenem backbone



Carbapenem agents currently available are imipenem (N-formimidoyl thienamycin), semisynthetic derivative of thienamycin, meropenem, ertapenem, doripenem, panipenem and biapenem. Imipenem and meropenem are the most clnical use. Structures of these carbapenems are shown in Figure 4., which differed in side chain at position 2.

Figure 4. Structures of carbapenems



Imipenem **Meropenem** **Ertapenem**

Doripenem **Panipenem** **Biapenem**

Imipenem is an intravenous β-lactam antibiotic developed in 1985. It is derivatives of thienamycin, which is produced by the *Streptomyces cattleya*. Nomenclature by International Union of Pure and Applied Chemistry (IUPAC) is (5R,6S)-3-[2-(aminomethylideneamino)ethylsulfanyl]-6-(1-hydroxyethyl)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid. Imipenem has a broad spectrum of activity against aerobic and anaerobic gram positive as well as gram negative bacteria. This agent remains very stable in the presence of β-lactamase (both penicillinase and cephalosporinase) produced by some bacteria, and is a strong inhibitor of β-lactamases from some gram negative bacteria that are resistant to most β-lactam antibiotics. Imipenem can be hydrolysed in the mammalian kidney by a dehydropeptidase enzyme. Cilastatin is a competitive, reversible and specific inhibitor of dehydropeptidase-I enzyme. So, imipenem is given with a dehydropeptidase inhibitor, cilastatin, to prevent the breakdown of antibiotic in the kidney (126, 130).

Meropenem is an ultra-broad-spectrum injectable antibiotic used to treat a wide variety of infection. This agent was developed by Sumitomo Pharmaceuticals, Japan and was approved by FDA in July, 1996. Systematic (IUPAC) name is 3-[5-(dimethylcarbamoyl) pyrrolidin-2-yl] sulfanyl-6- (1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0] hept-2-ene-2-carboxylic acid. It is bactericidal except against *Listeria monocytogenes* where it is bacteriostatic. This agent is also highly resistant to degradation by β-lactamases or cephalosporinases. The overall spectrum is similar to imipenem although it is more active against Enterobacteriaceae and less active against gram positive bacteria. It is also resistant to ESBL but more susceptible to metallo-β-lactamases (126).

Other carbapenems include ertapenem, doripenem, panipenem and biapenem. All carbapenems except imipenem have 1-β-methyl group in their structures, confer β-lactamase stability and resistance to inactivation by renal dehydropeptidases (131). Ertapenem is effective against gram negative bacteria and anaerobic bacteria, but not active against MRSA, ampicillin-resistant enterococci, *Ps. aeruginisa* and *Acinetobacter* spp (126, 132, 133). Doripenem was approved by the United States

Food and Drug Administration on October 12, 2007. This agent has a spectrum and potency against gram positive cocci similar to imipenem and ertapenem and gram negative activity similar to meropenem (two or four folds superior to imipenem) (126, 134). Panipenem is a parenteral carbapenem antibacterial agent which was launched in 1993. This agent has a broad spectrum of gram negative and gram positive aerobic and anaerobic bacteria, including *Streptococcus pneumoniae* and β-lactamase producing organisms. A panipenem is coadministered with betamipron to inhibit panipenem uptake into the renal tubule and prevent nephrotoxicity (126, 135, 136). Biapenem is a parenteral carbapenem that possessed antibacterial activities against a wide range of gram positive, gram negative and anaerobic bacteria (126, 135).

7. MECHANISMS OF CARBAPENEM RESISTANCE IN *A. BAUMANNII*

The emergences of multidrug resistance including aminoglycosides, quinolones and broad-spectrum beta-lactams in *A. baumannii* have been reported (137-140). Carbapenems have become the preferred treatment for serious *Acinetobacter* infections in many countries because of their stability against β-lactamases. However, resistance to carbapenems has been increasingly reported worldwide (141-144). A major contributing factor in the emergence of antibiotic-resistant *A. baumannii* is the acquisition and transferability of antibiotic resistance genes on plasmids, transposons and integrons. The presence of integrons related to epidemic strains in *Acinetobacter* spp (39, 46, 145-147). Three main classes of integrons have been described, class I, class II and class III differed by amino acid sequences of gene encoding integrase enzymes. Class I integrons have found predominantly in *A. baumanni* (146, 148, 149). Class II integrons hybrid with class I integron was reported in one *A. baumannii* isolate (150). The epidemic potential of *A. baumannii* may be linked to the presence of class I integrons that contain antibiotic resistance genes.

The most common mechanism found in carbapenem-resistant *A. baumannii* is production of carbapenemases, either chromosomally-encoded or plasmid

mediated. Other mechanisms include alteration of PBPs structure, decreased outer membrane permeability for taking up antibiotics and active efflux pumps for excreting antibiotics (151). Combination of these resistance mechanisms can occur. Mechanisms of carbapenem resistance in *A. baumannii* are described below.

1. Production of carbapenemase enzymes

The β-lactamases can be classified into four different molecular groups, A, B, C and D, according to amino acid sequence identities (152-154). β-Lactamase class A, C (AmpC) and D use a catalytically active serine residue for inactivation of the β-lactam drug (155), while class B β-lactamases are metallo-enzymes requiring zinc for their catalytic activity (156, 157). Classification scheme for β-lactamases is shown in Table 2. Carbapenemases belong to class A, B and D β-lactamases. Carbapenemases mostly found in carbapenem-resistant *A. baumannii* are OXA-type carbapenemases which belong to class D β-lactamase. Some of metallo-β-lactamases which belong to class B β-lactamase and have carbapenemase activity were reported.

1.1 OXA-type carbapenemase enzymes

OXA-type carbapenemase enzymes are major carbapenemases produced by *A. baumannii*. Walther-Rasmussen demonstrated that these enzymes were classified in class D β-lactamase which was attributed to the presence of three highly conserved active site elements (158, 159). The first element was the tetrad, Ser^{70} –X-X-Lys, where X represents a variable residue, containing the active site serine [Ser^{70} according to the DBL (class D β-lactamase) numbering]. The second element was Ser^{118} –X-Val/Ile. The third element was Lys^{216} –Thr/Ser-Gly element. Other conserved motifs in class D β-lactamases were the triad Tyr/Phe^{144}-Gly-Asn and the Trp^{232}-X-X-Gly. Mature OXA-type carbapenemases contain between 243 and 260 amino acid residues with molecular masses ranged from 23 to 35.5 kDa. The isoelectric points (pIs) of OXA-type carbapenemase enzymes that found in *A. baumannii* varied between 6.3 and 9.0 (11, 12, 14, 16, 22-26, 33, 160). These enzymes were referred to as oxacillinases because they hydrolyzed the isoxazolyl

penicillin oxacillin more efficiently than benzylpenicillin and usually hydrolyzed amoxicillin, methicillin, cephaloridine and some cephalothin. These enzymes hydrolyzed imipenem and meropenem weakly and did not hydrolyze extended spectrum cephalosporins and aztreonam. Their activities were inhibited more efficiently by clavulanic acid. In addition, most OXA-type carbapenemases were inhibited by NaCl. This was attributed to the presence of a Tyr residue in the Tyr^{144}-Gly-Asn motif. OXA-type carbapenemases containing a Phe-Gly-Asn element instead (OXA-23, -25, -26, -27, -40, -49, -72) were not or weakly inhibited by NaCl. By their nucleotide sequences identities, these enzymes could be subclassified into eight distinct subgroups but only four subgroups have been identified in *A. baumannii* (11, 12, 14, 16, 22-26, 33, 160). These enzymes were grouped and termed carbapenem-hydrolyzing oxacillinases (CHDLs). The identitites between subgroups ranged from 40 to 70% whereas the amino acid sequences of the members in the same group were more than 92.5% or differed by 1-5 amino acids as shown in Appendix G (159).

The first subgroup of OXA-type carbapenemases is formed by OXA-23, together with OXA-27 and OXA-49. Donald *et al.* and Afzal-Shah *et al.* demonstrated that these enzymes contained an open reading frame of 822 bp which translates into a protein of 273 amino acids and differed by 2-5 amino acids (14, 23). OXA-23, also named ARI-1 (an acronym of *Acinetobacter* resistant to imipenem), was the first OXA-type carbapenemase enzyme that were found in imipenem-resistant *A. baumannii* isolated from patient in Edinburgh, United Kingdom, in 1995. OXA-27 and OXA-49 were isolated from patients in Singapore and China, respectively. These enzymes contained the S-T-F-K tetrad at amino acid position 81 to 84 and S-X-V triplet at position 126-128, which was typical of class D β-lactamases. However, the Y-G-N motif at position 154-156 was replaced by F-G-N, resulting in weak inhibition by NaCl. OXA-23 was more active than OXA-27 against cephaloridine, whereas imipenem hydrolyzing activity was relatively weaker. Moreover, OXA-23 hydrolyzed oxacillin and ampicillin rapidly whereas OXA-27 had weak activity against both these compounds. The amino acid alignment of this subgroup is shown in Appendix G.

Table 2. Classification schemes for bacterial β-lactamases (161)

Functional group	Molecular class	Produced enzymes	Inhibited by		Representative enzymes
			CA[A]	EDTA[B]	
1	C	Cephalosporins	-	-	AmpC enzymes from Gram negative bacteria ; MIR-1
2a	A	Penicillins	+	-	Penicillinases from Gram positive bacteria
2b	A	Penicillins, cephalosporins	+	-	TEM-1, TEM-2, SHV-1
2be	A	Penicillins, narrow-spectrum and extended-spectrum cephalosporins, monobactam	+	-	TEM-3 to 26, SHV-2 to 6, *Klebsiella oxytoca* K1
2br	A	Penicillins	±[E]	-	TEM-30 to 36, TRC-1
2c	A	Penicillins, carbenicillin	+	-	PSE-1, -3, -4
2d	D	Penicillins, cloxacillin	±	-	OXA-1 to -11, PSE-2 (OXA-10)
2e	A	Cephalosporins	+	-	Inducible cephalosporinase from *P. vulgaris*
2f	A	Penicillins, cephalosporins, carbapenems	+	-	NMC-A from *E. cloacae*, Sme-1 from *S. marcescens*
3	B	Most β-lactams, including carbapenems	-	+	L1 from *Xanthomonas maltophilia*, CcrA from *B. fragilis*
4	ND[F]	Penicillins	-	ND	Penicillinases from *Pseudomonas cepacia*

[A]CA = clavulanic acid, [B]EDTA = ethylenediaminetetraacetic acid,
[E]± = low binding to CA, [F]ND = nondetermined.

The second subgroup consists of OXA-40/24, 25, 26, 33 and OXA-72. Walther-Rasmussen demonstrated that these enzymes shared 60% amino acid identity to OXA-23. The members of this cluster vary from 1-5 amino acids (159). The alignment of amino acid sequences of this subgroup are shown in Appendix G. Bou *et al.*, Heritier *et al.* and Afzal-Shah *et al.* demonstrated that these enzymes contained 825 bp open reading frame encoding a 274 amino acid protein (22-24). Conserved motif, serine-threonine-phenylalanine-lysine tetrad (S-T-F-K), indicated

serine β-lactamases. The typical motifs of the OXA-24 enzyme, tyrosine-glycine-asparagine (Y-G-N) and lysine-threonine-arginine (K-T-G) were replaced by F-G-N and K-S-G, respectively. OXA-24 differed from other oxacillinase by lacking hydrolytic activity against oxacillin, cloxacillin and methicillin but showed moderate hydrolysis of imipenem and meropenem. Although OXA-24 had FGN motif but the enzymatic activity was inhibited by chloride ions. In addition, it was also inhibited by sulbactam, tazobactam and clavulanic acid. OXA-24, -25, -26 and -40 had pIs (isoelectric points) of 9.0, 7.9, 8.0 and 8.6, respectively. The pIs of OXA-25 and OXA-26 were low because of the presence of additional glutamate residues in their structures. OXA-40 had a narrow-spectrum hydrolytic profile including most penicillins. Hydrolysis of imipenem was low, whereas hydrolysis of meropenem was not detected. Both OXA-24 and OXA-25 were identified in carbapenem-resistant *A. baumannii* isolated from Spain (22, 23). OXA-26 and OXA-40 were identified in carbapenem-resistant *A. baumannii* isolated from Belgium and France, respectively (22, 24). OXA-33 and OXA-72 were identified in carbapenem-resistant *A. baumannii* isolated from Protugal and Thailand, which were unpublished.

The third subgroup is OXA-51 family enzymes including OXA-51, OXA-64 to -71, OXA-75 to -80, OXA-82 to -84, OXA-86 to -95, OXA-99, OXA-100, OXA-104, OXA-106 to -113, OXA-115 to -117, OXA-128, OXA-130 to -132. The members of this family diverged by 1-15 amino acids (159). OXA-51 shares 56%, 63% and 59% with OXA-23, OXA-24 and OXA-58, respectively. The alignment of amino acid sequences of this subgroup is shown in Appendix G. Many studies demonstrated that $bla_{\text{OXA-51-like}}$ genes were naturally harboured by *A. baumannii* (27, 28, 30). Brown *et al.* demonstrated that the amino acid sequences contained the conserved serine active site motif S-T-F-K (DBL numbering 70-73) (26). These enzymes had a Thr → Ser change in the K-T-G motif (DBL numbering 216-218). This K-S-G motif was found as in OXA-40/24, 25, 26, 33, 72, 58, 96 and 97. A unique Val → Ile change was in the S-X-V motif, which was not present in the other class D carbapenemases. Moreover, these enzymes also retained the Y-G-N motif (DBL numbering 144-146). OXA-51 could hydrolyze both oxacillin and cloxacillin, and hydrolyzed ampicillin effectively. It was not hydrolyzed cephalosporins except cephaloridine. Slow hydrolysis of imipenem was detected.

OXA-51 activity was inhibited by sulbactam and chloride ions and weakly inhibited by clavulanate. The pI of this enzyme was 7.0 (26, 159).

The fourth subgroup consists of OXA-58, together with OXA-96 and OXA-97. These enzymes shared 47% amino acid identities with OXA-23 and OXA-24 and 59% amino acid identities with OXA-51 group (159). The alignment of amino acid sequences of this subgroup is shown in Appendix G. Poirel *et al.* demonstrated that these enzymes contained a 843-bp open reading frame which encoded a 280 amino-acid protein (33). A serine-threonine-phenylalanine-lysine tetrad tetrad (S-T-F-K) was at position 70, K-S-G elememt is at position 216-218. The Y-G-N element at position 144-146 was not replaced by F-G-N motif. OXA-58 had a narrow-spectrum hydrolysis profile, including penicillins and oxacillin. Hydrolysis of imipenem was low, while hydrolysis of meropenem was not detected but this enzyme hydrolyzed imipenem twice as much as OXA-40. In addition, OXA-58 had some hydrolytic activity against cefpirome, whereas hydrolytic activity against ceftazidime, cefotaxime and cefepime was not detected. Their activity was weakly inhibited by clavulanic acid, tazobactam and sulbactam, and well inhibited by NaCl. OXA-58 was identified in carbapenem-resistant *A. baumannii* isolated from France and Greece (143, 144). OXA-96 and OXA-97 were identified in carbapenem-resistant *A. baumannii* isolated from Singapore and Tunisia, respectively (18, 34). Alignments of nucleotide sequences and amino-acid sequences of the four main groups of OXA-type carbapenemase enzymes identified in *A. baumannii* are shown in Appendix F and G., respectively.

Turton *et al.* demonstrated that the activity of OXA-type carbapenemases also depended on insertion sequence (IS) element that found upstream of bla_{OXA} gene. It might play a role in bla_{OXA} expression by providing promoter sequences (162). There were many reports indicated that IS*Aba1* element was found upstream of bla_{OXA51} and bla_{OXA23} , showed high-level resistance to carbapenems (162, 163). In addition, Poirel *et al.* demonstrated that IS*Aba2*, IS*Aba3* and IS*18* were shown to provide promoter sequences enhancing bla_{OXA58} expression (164).

1.2 Metallo-β-lactamase enzymes

Metallo-β-lactamase enzymes (MBL, class B β-lactamase) such as IMP (Imipenem-hydrolyzing β-lactamase), VIM (Verona integron-encoded metallo-β-lactamase) and SIM-1 enzymes (*S*eoul *im*ipenemase) were identified rarely in *A. baumannii* (18, 35-42, 45, 46, 165-167). These MBL-encoding genes are embedded in class-1 integron structures and form part of the gene cassettes together with other antibiotic resistance genes especially aminoglycoside-modifying enzymes. IMP-1 enzyme was first isolated from nosocomial isolates of *Serratia marcescens*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* from Japan (168). The amino acid sequences of IMP-2 enzyme was 85% identity to IMP-1 (39). Both, bla_{IMP} and bla_{VIM} genes were also found on conjugative plasmids (32, 168-170). VIM-1 enzyme was first isolated in a carbapenem-resistant *Pseudomonas aeruginosa* clinical isolate (VR-143/97) from an Italian patient at the Verona University Hospital (Northern Italy) (171). Lauretti *et al.* demonstrated that bla_{VIM} encoded a 266-amino acid protein. The G+C content of the bla_{VIM} was 56%, being higher than that of the bla_{IMP} cassette (40%) (171). The IMP-like and VIM-like confered a high level of carbapenemase resistance in *A. baumannii* isolates (18, 39, 172). The hydrolytic efficiency of these MBL against carbapenems was much higher (100 to 1,000-fold) than that of the OXA-type carbapenemases (151). These enzymes hydrolyzed many β-lactam substrates including penicillins, narrow- to expanded-spectrum cephalosporins including cephamycins and carbapenems. Hydrolysis of aztreonam was not detected. The activities of these enzymes were not inhibited by β-lactam inhibitor such as clavulanate and tazobactam were inhibited by EDTA. It is helpful for identification of MBL production such as using Etest strips containing imipenem with or without EDTA and EDTA-disk synergy test (173). Lee *et al.* demonstrated that SIM-1 enzyme was also encoded by a class 1 integron-borne gene cassette and was more closely related to IMP-type enzymes than other MBLs by exhibiting 64 to 69% amino acid identities to IMP-type MBLs. The theoretical molecular weight and pI of the mature SIM-1 protein were 25,439 kDa and 7.28, respectively. Both IMP-1 and SIM-1 also hydrolyzed broad array of β-lactams, including penicillins, narrow- to expanded-spectrum cephalosporins and carbapenems (46).

2. Alteration of the target site

Target sites of β-lactam antibiotics are PBPs. Changing in structure of PBP structure confers reduced affinity for binding between bacteria and these antibiotics (174). Fernandez-Cuenca *et al.* found that production of oxacillinase and the absence of PBP2 were associated with carbapenem resistance (175). Similarly, Gehrlein *et al.* reported that PBP alterations were responsible for imipenem resistance in *A. baumannii* isolates (176).

3. Reduction in permeability of outer membrane proteins.

Resistance to carbapenems may also be explained by other mechanisms such as porin loss. Porins are proteins able to form channels allowing the transport of molecules across lipid bilayer membranes. Variation in their structures and regulation of porin expression in the presence of antibiotics are survival strategies that have been developed by many bacteria including *A. baumannii*. The small number and size of porins or outer membrane proteins (OMP) could explain the decrease in permeability to antimicrobial agents (177).

Gribun *et al.* demonstrated the major OMP of *A. baumannii* was the heat-modifiable protein, HMP-AB (178). This protein consisted of 346 amino acids with a molecular mass of 35,636 Da and belongs to the OmpA family. Mussi *et al.* and Siroy *et al.* demonstrated that a heat-modifiable 29 kDa outer-membrane protein, CarO, was involved in taking up carbapenem antibiotics in *A. baumannii* (179, 180). The CarO gene encoded a 247-amino acids protein which had a typical N-terminal signal sequence and a predicted trans-membrane β-barrel topology. Resistance to carbapenem in multidrug-resistant *A. baumannii* isolates was associated with loss of this outer-membrane protein. Fernandez-Guenca *et al.* also found that resistance to carbapenems was related to the absence of an OMP of 22.5 kDa (175). Tomas *et al.* demonstrated that losing of a 33- to 36- kDa outer membrane protein was associated with carbapenem resistance in *A. baumannii* isolated from Spain (181). This protein had amino acid sequence similar to typical of gram-negative bacterial porins. Dupont *et al.* also demonstrated that a 43 kDa protein was associated with imipenem

resistance in *A. baumannii* (182). Mechanism of carbapenem resistance in *A. baumannii* may cause by combine mechanisms such as carbapenemase production and decreased permeability of drugs.

4. Efflux system

Efflux pump activity is to export multiple structurally-distinct classes of antimicrobial agents out of bacteria. Efflux transporters are expressed in all living cells, protecting them from toxic effects of organic chemicals. Multidrug resistance in bacteria has often been associated with overexpression of these transporters. Magnet *et al.* demonstrated AdeABC efflux system in *A. baumannii*. It belongs to the resistance-nodulation-division (RND) family (183). This efflux system played a role in aminoglycoside resistance and was also responsible for decreased susceptibility to chloramphenical, fluoroquinolones, cefotaxime and trimethiprim. Heritier *et al.* demonstrated a synergic effect when AdeABC system was combined with production of carbapenem-hydrolyzing oxacillinase enzymes. This resulted in increasing of resistance level of carbapenems in *A. baumanni* (10).

8. EPIDEMIOLOGY OF CARBAPENEM RESISTANCE IN *A. BAUMANNII*

Carbapenems have been used for treatment of serious nosocomial infection caused by *Acinetobacter* spp. in many countries, resulting in better activity than other antimicrobial agents. However, many reports on carbapenem resistance are increasing around the world, including Europe, America and Asia (141-144). In Spain, there were many studies to investigate the prevalence of imipenem resistance *A. baumannii*. Martin-Lozano *et al.* showed imipenem resistance in *A. baumannii* was 34% in 2002 (4). At the same time, Betriu *et al.* demonstrated the 28.1% of *A. baumannii* isolated from 12 Spanish medical centers were resistant to imipenem (184). Between 1991 and 2001, Cisneros *et al.* demonstrated carbapenem-resistant *A. baumannii* isolates was increasing from 0% to 50% in Seville (185). In Greece, imipenem resistance in *A. baumannii* isolated from ICUs, medical wards

and surgical wards rose from 0% to 91%, 8% to 71% and 5% to 71%, respectively, between 1996 and 2006 (139). At the same country, Souli *et al.* also demonstrated the 84% of bacteraemic *A. baumannii* isolated from ICUs were resistant to imipenem in 2005 (186). Data from Meropenem Yearly Susceptibility Test Information Collections program (MYSTIC) in 1997-2000, showed that 490 *A. baumannii* isolates were resistant to meropenem 30%, 34% and 23% in Italy, Turkey and UK, respectively, whereas isolates were resistant to imipenem 22%, 38% and 22% respectively (9). By the Ministry of Health National Antimicrobial Resistance Surveillance Net (Mohnarin) in 2008, imipenem and panipenem resistance in *A. baumannii* were 10.4% and 14.5%, respectively in China (187). In 2008, Nemec *et al.* found carbapenem resistance in 23 out of 108 isolates of *A. baumannii* in Czech Republic (188).

In USA, carbapenem resistance in *A. baumannii* isolates were 53% in 1999 (3). Qi *et al.* found imipenem-resistant *A. baumannii* isolates increased from 52% to 96% in 2005 to 2007 in Chicago (189). In Shanghai and Hong Kong, both imipenem and meropenem-resistant rates of *A. baumannii* collected from Shanghai were 6.3% while those from Hong Kong were 2.7% and 10.8%, respectively (138). In China, national resistance surveillance data in 1996-2002 from intensive care units (ICUs) of 19 hospitals showed that imipenem resistance rate in *Acinetobacter* spp. was 5% in 1996 to 2002 (190). At the same country, another surveillance program showed carbapenem resistance rate increased from 4.5% in 2003 to 18.2% in 2004 (191).

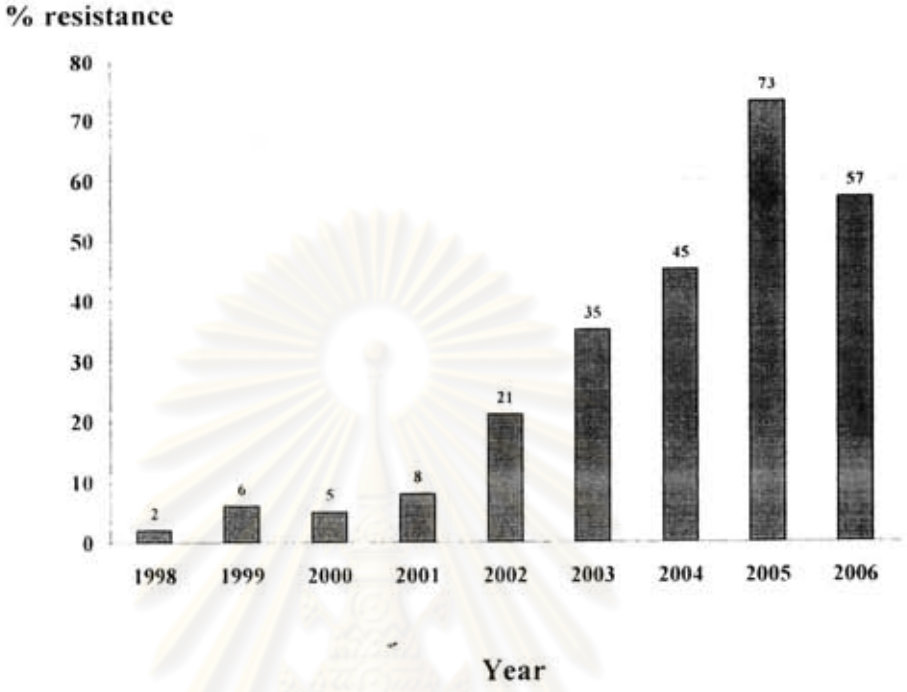
Most of carbapenemase enzymes found in *A. baumannii* were OXA-type carbapenemases (oxacillinases). IMP-type and VIM-type were rarely reported (35-46). ARI-1 was the first enzyme that reported in *A. baumannii* in 1995 (14). This enzyme was renamed OXA23 following its genetics and biochemical characterization. After that, there have been many reports about OXA-type carbapenemases in *Acinetobacter* spp. worldwide. OXA-type carbapenemases found in *A. baumannii* were classified into 4 subgroups depending on their amino acid sequences (159). The first subgroup, OXA23 group enzymes were identified in *A. baumannii* isolated from Brazil, Spain, Korea, China, Singapore, Scotland and Tahiti (15-17, 23). Second subgroup, OXA24 group enzymes, were identified in

Spain (22, 23), Belgium (23) and France (24). The third subgroup, OXA-51 family enzymes, were found in Argentina (25-27), Spain, Hong Kong, Singapore, South Africa (25), France (28), Poland (29), United Kingdom (30), Greece (31, 32) and Turkey . The fourth subgroup, OXA-58, were reported in *A. baumannii* isolated from South Europe (13), France (33), Singapore (18) and Tunisia (34).

Besides OXA-type carbapenemases, metallo-β-lactamases, IMP-type and VIM-type, were identified in *A. baumannii* isolates. IMP-1 was reported in Japan (35), Korea (36, 37) and Italy (38). IMP-2, IMP-4, IMP-5, IMP-6 and IMP-11 were reported in Italy (39), Hong Kong (40), Protugal (41), Brazil (42) and Japan (43), respectively. VIM-type, VIM-2 were found in *A. baumannii* in Korea (36, 44). VIM-1 and VIM-2 were reported in *Acinetobacter* spp. in Italy (41) and Korea (36, 45), respectively whereas SIM-1 was reported in South Korea (46).

In Thailand, there was no evidence of carbapenem resistance *Acineobacter* spp. from the study at Siriraj Hospital in 1998 (47). In 1999, 21% of *Acinetobacter* spp. isolates were resistant to imipenem at Vajira Hospital (48). In 2003, the imipenem resistance rate of *A. baumannii* was 61% at Police General Hospital (51). Sixty-eight percents of *A. baumannii* isolated from Siriraj Hospital in 2006 were resistant to carbapenems and 57% were resistant to all antimicrobials currently available in Thailand (52). In 2007, imipenem resistance rate of *A. baumannii* collected from Chiang Mai University Hospital were 35.5% (49). At the same peroid, the imipenem resistance rate of 2,130 *A. baumannii* isolated from clinical specimens at Songklanagarind Hospital was 34.7%. The 61.9% of these imipenem-resistant *A. baumannii* isolates were from respiratory tract, and 49.9% from ICU. Moreover, 14.8% of all imipenem-resistant *A. baumannii* isolates were resistant to all routine tested antibiotics, including gentamicin, amikacin, ampicillin, cephalothin, cefoxitin, ceftazidime, ceftriaxone, meropenem, sulperazone, ciprofloxacin and cotrimoxazole (50). Moreover, the data from the National Antimicrobial Resistance Surveillance Center Thailand (NARST) showed that imipenem resistance in *Acinetobacter calcoaceticus-baumannii* complex increased from 2% in 1998 to 57% in 2006 as shown in Figure 5 (53).

Figure 5. Imipenem resistance rates of *Acinetobacter calcoaceticus-baumannii* complex during 1998-2006 (NARST) (53)



CHAPTER IV

MATERIALS AND METHODS

The chemical agents used in this study were molecular biology grade. Name list of all media, chemical reagents materials, instruments and reagents are shown in Appendix I.

PART I : BACTERIAL STRAINS

1. *Acinetobacter baumannii* isolates

Five hundred and one isolates of *A. baumannii* were collected from Department of Microbiology, King Chulalongkorn Memorial Hospital, Bangkok during January 2004 to August 2007. Each was from a different patient. They were identified as *A. baumannii* according to the Manual of Clinical Microbiology and Koneman's Color Atlas and Textbook of Diagnostic Microbiology (54, 131).

2. Quality control strain for bacterial identification

A. baumannii ATCC 19606

3. Quality control strains for MIC determination

Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212

CULTURE PRESERVATION

All culture isolates were grown entirely on tryptic soy agar (BBL, Becton Dickinson and Company, Cockeysville, MD) at 37°C. The overnight cultures were

transferred to microcentrifuge tubes of 1 ml trypticase soy broth containing 10% glycerol and mix-well suspensions were kept at -70°C until required.

PART II BACTERIAL IDENTIFICATION

A. baumannii isolates were identified by conventional method including colonial morphology, gram stain and biochemical tests.

1. Colonial Morphology

A. baumannii is a lactose non-fermenter. Colonies are between 0.5-2 mm in diameter , smooth, opaque, colorless or slightly pink on MacConkey agar.

2. Gram stain

A. baumannii is a gram-negatiye coccobacillus. Individual cell is 1 to 1.5 μm by 1.5 to 2.5 μm in size and frequently arranged in pairs.

3. Biochemical test

A single colony was picked and inoculated to testing media. The biochemical test lists and interpretation criteria for *A. baumannii* are described below and summarized in Table 3 (54). *A. baumannii* ATCC 19606 was used as a reference control for bacterial identification.

3.1 Triple Sugar Iron medium test (TSI)

Tryple sugar iron medium is the nutrient agar containing glucose, lactose and sucrose (1:10:10). There are thiosulfate and iron salt for the detection of hydrogen sulfide production. Phenol red is the indicator used to determine acidity or alkalinity in the medium. For TSI testing, tested strains were stabbed by needle into butt and streaked on the surface of slant, and then incubated at 37°C for 24 h.

Interpretation

- The organisms that can ferment only glucose but not lactose and sucrose, are recorded as K/A (alkaline (purple) slant / acid (yellow) butt). This may be with gas production.
- The organisms that can ferment glucose, lactose and/or sucrose, are recorded as A/A (acid (yellow) butt / acid (yellow) slant). This may be with gas production.
- The organisms that cannot ferment any sugars, are recorded as K/N (alkaline (purple) slant / neutral (no change) butt).
- A black precipitation in the butt indicates the production of H_2S.

A. baumannii shows K/N (alkaline slant / neutral butt) on TSI medium.

3.2 Oxidase test

The oxidase test detects the presence of cytochrome oxidase. This enzyme can oxidize the substrate N,N,N',N'-tetramethyl-*p*-phenylenediamine dihydrochloride (Sigma, U.S.A) to indophenol and produce a purple color. To test for oxidase, tested strains were transferred with a loop onto filter paper soaked with oxidase reagent. A dark purple color will appear within 5-10 seconds if the microorganism has cytochrome oxidase. *A. baumannii* does not produce cytochrome oxidase, and shows a negative result for oxidase test.

3.3 Urease test

The purpose of the urease test is to detect whether a microorganism possesses the enzyme urease which hydrolyzes urea. The urea agar contains phenol red indicator, which turns pink at an alkaline pH. When urea is hydrolyzed, it releases ammonia, which causes an alkaline reaction and a vivid pink color develops. Urease activity was detected by inoculating a loopfull of the organism onto slope of the urea agar slant, and then incubated at 37°C for 24 h. *A. baumannii* cannot produce urease enzyme, and shows a negative result for urease test.

3.4 Growth at 37°C, 42°C and 44°C

To determine the ability to grow at 37°C, 42°C and 44°C, the organism was inoculated on 2 plates of tryptic soy agar and in a tube of tryptic soy broth. The plates were incubated at 37°C and 42°C in the incubators. A tube of tryptic soy broth was incubated at 44°C in the waterbath. Growth was observed after 24 h of incubation. *A. baumannii* can grow at 37°C, 42°C and 44°C.

3.5 Hemolysis on sheep blood agar

The organism was inoculated on sheep blood agar plate. The plate was incubated at 37°C for 24 h. Hydrolysis of red blood on sheep blood agar was interpreted as positive result. *A. baumannii* shows no hemolysis on sheep blood agar and gives a negative result.

3.6 Oxidation / Fermentation of dextrose

Fermentaion is an anaerobic process, while oxidation is aerobic process. The main difference between fermentative and oxidative metabolism of carbohydrate is the requirement for atmospheric oxygen and initial phosphorylation. Fermentation requires initial phosphorylation of glucose prior to degradation to relatively strong mixed acids, where as oxidation is a strict aerobic process involving direct oxidation of a nonphosphorylated glucose molecule. Fermentation produces higher acidity than oxidation. Medium used to determine the oxidative or fermentative metabolism was OF basal medium (BBL). The tested organism was inoculated in a pair of OF tubes by stabbing into the medium to approximately ¼ inch from bottom. One tube was overlaid with approximately 1.0-2.0 mL of sterile melted petrolatum. The tubes were incubated at 35°C for 48 h. The medium contains bromthymol blue which will turn to yellow because of acid production from metabolic pathway of the organism while blue or green means that carbohydrate cannot be used. Oxidation will occur in an opened tube, while fermentation will occur in tube covered with sterile melted petrolatum. *A. baumannii* can use dextrose by oxidation. Acid production changes the color of the medium from green to yellow in the opened tube.

3.7 Arginine decarboxylase

To measure the enzymatic ability of an organism to decarboxylate arginine to form an amine. Tested strains were inoculated into arginine decarboxylase medium and were covered with sterile melted petrolatum. The tubes were incubated at 37°C for 24 h. If the organism can use arginine for their metabolism, colour of the medium will turn to violet by alkalinity while the organism that cannot use arginine, the medium will change to yellow by acidity. *A. baumannii* can decarboxylate arginine and shows a positive result in arginine decarboxylase medium.

3.8 Malonate utilization

Malonate broth was used to determine an organism's ability to use sodium malonate as the sole source of carbon. Bromthymol blue is an indicator. The color of the medium changes from light blue to deep Prussian blue color by alkalinity when the organism uses malonate as a carbon source, interpreted as positive result. No color change or yellow is interpreted as negative result. Tested strains were inoculated into the medium by a loop, and then incubated at 37°C for 24 h. *A. baumannii* can use malonate as a carbon source and gives a positive result for malonate utilization.

3.9 Nitrate reducion

To determine the ability of an organism to reduce nitrate to nitrite or nitrogen gas, tested organism was heavily inoculated into nitrate broth and incubated at 35°C for 24 h. Nitrate reagents, reagent A (α-Naphthylamine) and reagent B (sulfanilic acid), were added before attempting an interpretation. If the tested medium turns to pink to a deep red color within 1-2 min, it means that nitrate was reduced to nitrite by the organism. No color development means that nitrite is not present and zinc dust will be added. Pink to deep red occurred within 5-10 min confirms negative result. This means that nitrate is not reduced by the organism. No color development means that the organism can reduce nitrate to nitrite and then further reduce nitrite to nitrogen gas (N_2). Ammonia is tested by adding a few drops of Nessler's reagent.

Positive result shows a deep orange color. *A. baumannii* cannot reduce nitrate to nitrite or nitrogen gas and shows a negative result for nitrate reduction test.

Table 3. Biochemical schemes of genus *Acinetobacter* (54)

ORGANISM	GENOMO-SPECIES	UREASE	NITRATE REDUCTION	GROWTH AT			HEMOLYSIS OF SHEEP BLOOD	OXIDIZES DEXTROSE	ARGININE DECARBOXY-LASE	MALONATE UTILIZATION
				37°C	42°C	44°C				
Acinetobacter johnsonii	7	–	–	–	–	–	–	–	V (35)	V (13)
Acinetobacter baumannii	2	–	–	+	+	+[a]	–	+	+	+
Acinetobacter haemolyticus	4	–	–	+	–	–	+	V (52)	+	–
Acinetobacter spp.	6	–	–	+	–	–	+	V (66)	+	–
Acinetobacter spp.	10	–	–	+	–	–	–	+	–	–
Acinetobacter calcoaceticus	1	–	–	+	–	–	–	+	+	+
Acinetobacter spp.	3	–	–	+	+	–	–	+	+	V (87)
Acinetobacter spp.	12	–	–	+	–	–	–	V (33)	+	+
Acinetobacter junii	5	–	–	+	+	–	–	–	+	–
Acinetobacter lwoffii	8/9	–	–	+	–	–	–	–	–	–
Acinetobacter spp.	11	–	–	+	–	–	–	–	–	–

Compiled from

[a] Must also be OF dextrose positive

+, 90% or more strains positive; –, 90% or more strains negative; V, 11-89% of strains positive; Numbers in parentheses are percentages of strains giving positive reactions.

PART III : ANTIMICROBIAL SUSCEPTIBILITY TEST

All 501 *A. baumannii* isolates were tested for antimicrobial susceptibility against imipenem and meropenem. The minimal inhibitory concentrations (MICs) of carbapenems were examined by agar dilution technique on Muller-Hinton agar as recommended by CLSI (192). The MIC is the lowest concentration of antimicrobial agents required to inhibit the growth of a microorganism *in vitro*.

MIC determination by agar dilution

Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as reference controls for MIC determination. Standardization of inoculum was prepared from a pure overnight culture in tryptic soy broth. The turbidity of cell suspension was adjusted to 0.5 McFarland (approximately $1.5x10^8$ CFU/mL) in 0.85% NaCl. After adjusting the turbidity of inoculum, the suspension was diluted 10-fold to yield the final inoculum suspension. The inoculum suspension was used for inoculation within 15 minutes. After that, the 500 µL of inoculum suspension was transferred to the multi-point inoculator wells. The inoculum suspension was inoculated on plate with two-fold serial dilution of each imipenem (Merck & Co., U.S.A.) and meropenem (AstraZeneca UK Limited, United Kingdom) starting at 0.015-≥256 mg/L. (The concentration of antimicrobial for use in agar dilution are shown in Table 4). Before inoculation on the plate containing the lowest concentration of antibiotic, the growth control was inoculated on the plate without antibiotic. The inoculum-spots were allowed to dry and inverted the plates before incubated at 37°C for 18-24 h. The final inoculum on the agar was approximately 10^4 CFU/spot. After 24 hours of incubation, the MIC was read as the lowest concentration of antimicrobial agent without visible growth as shown in Figure 6. A faint haze, pinpoint colonies or growth of a single colony were ignored. The MIC breakpoint criteria recommended by CLSI are shown in the Table 5. Acceptable MIC limits for quality control strains are listed in Table 6.

Table 4. **Scheme for preparing dilutions of antimicrobial agents to be used in agar dilution susceptibility tests (192).**

Step	Concentration (mg/L)	Source	Volume (mL)	Diluent (mL)	Intermediate concentration (mg/L)	Final concentration at 1:10 dilution in agar (mg/L)	Log_2
	5,120	Stock	-	-	5,120	512	9
1	5,120	Stock	2	2	2,560	256	8
2	5,120	Stock	1	3	1,280	128	7
3	5,120	Stock	1	7	640	64	6
4	640	Step 3	2	2	320	32	5
5	640	Step 3	1	3	160	16	4
6	640	Step 3	1	7	80	8	3
7	80	Step 6	2	2	40	4	2
8	80	Step 6	1	3	20	2	1
9	80	Step 6	1	7	10	1	0
10	10	Step 9	2	2	5	0.5	-1
11	10	Step 9	1	3	2.5	0.25	-2
12	10	Step 9	1	7	1.25	0.125	-3

Figure 6. Inoculum plate of agar dilution method

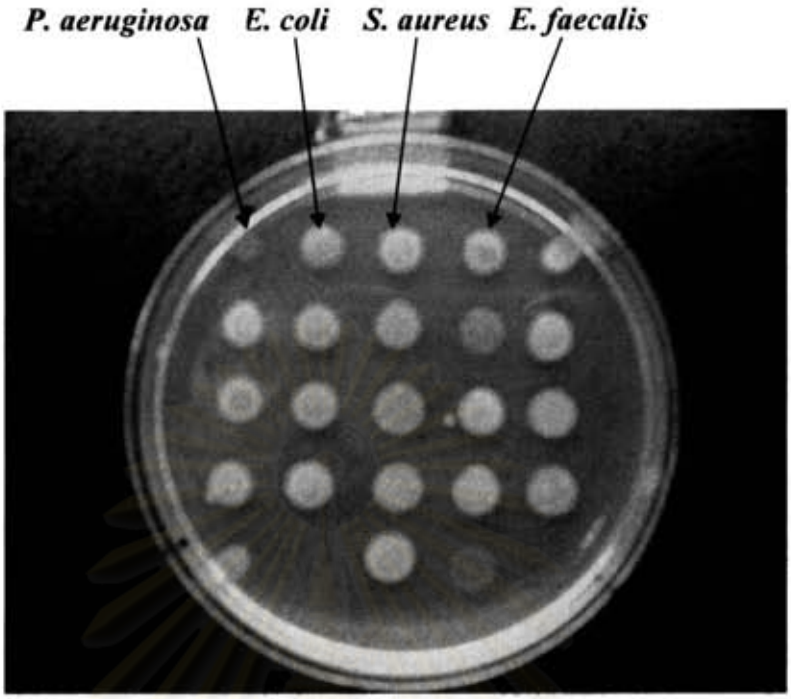


Table 5. MIC Interpretive Standards for *Acinetobacter* spp. (192)

Antimicrobial agents	MIC (mg/L) Interpretive Standard		
	Susceptible	Intermediate	Resistant
Imipenem	≤ 4	8	≥ 16
Meropenem	≤ 4	8	≥ 16

Table 6. Acceptable limits of MIC (mg/L) for quality control strains (192).

Antimicrobial agents	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Staphylococcus aureus* ATCC 29213	*Enterococcus faecalis* ATCC 29212
Imipenem	0.06-0.25	1-4	0.015-0.06	0.5-2
Meropenem	0.008-0.06	0.25-1	0.03-0.12	2-8

PART IV : DETERMINATION OF CARBAPENEMASE

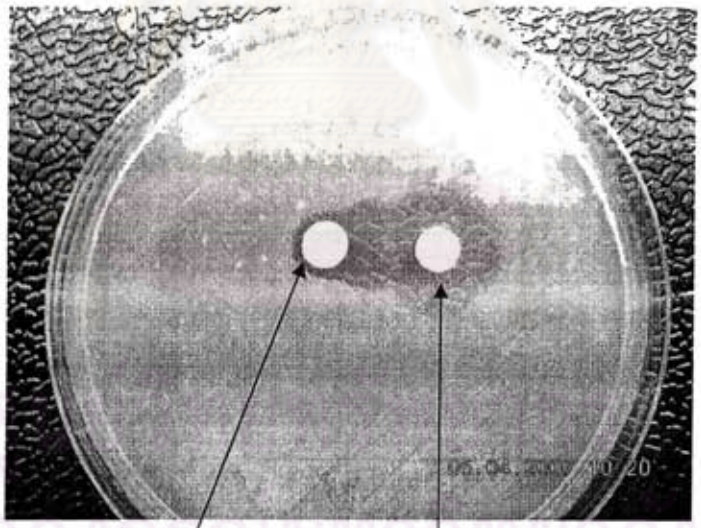
Modified Hodge test is the method for screening carbapenemase-producing strains (173). The surface of a Mueller-Hinton agar plate was inoculated evenly by using a cotton swab with an overnight culture suspension of *Escherichia coli* ATCC 25922, which was adjusted to one-tenth turbidity of 0.5 McFarland. After brief drying, a 10-μg imipenem disk was placed at the center of the plate, and imipenem-resistant isolates from the overnight culture plates were streaked heavily from the edge of the disk to the periphery of the plate. The Mueller-Hinton agar plate was incubated at 35°C for 24 h. The presence of a distorted inhibition zone after overnight incubation was interpreted as modified Hodge test positive (Figure 7.).

PART V : DETERMINATION OF METALLO-β-LACTAMASE

EDTA-disk synergy test was used to screen metallo-β-lactamase-producing isolates (173). An overnight culture of the tested strain was suspended to the turbidity of 0.5 McFarland and inoculated onto a Mueller-Hinton agar plate by using a cotton swab. After drying, a 10-μg imipenem disk and a blank filter paper disk were placed 10 mm apart from edge to edge. Ten microlitres of 0.5 M EDTA solution was then applied to the blank disk, which resulted in approximately 1.5 mg/disk. After overnight incubation at 35°C, the presence of an enlarged zone of inhibition was interpreted as EDTA-synergy test positive (Figure 8.).

Figure 7. **Distorted inhibition zone of *E. coli* by Modified Hodge test**

Figure 8. **Enlarged inhibition zone of tested isolate by EDTA-disk synergy test**



PART VI : SCREENING OF GENES ENCODING OXA- , IMP- AND VIM-TYPE CARBAPENEMASES BY PCR AND DNA SEQUENCING OF ENTIRE *BLA*-OXA GENE

1. Preparation of genomic DNA

1.1 Boiling method

Bacterial DNA was prepared by suspending 4-5 colonies of pure culture in 100 μL of sterile deionized distilled water and boiled at 100°C for 15 min. The suspension was centrifuged at 13,000 rpm at room temperature for 5 min. Supernatant was stored at -20°C.

1.2 QIAamp DNA Mini kit

The extraction and purification of *A. baumannii* DNA for amplifying entire $bla_{\text{OXA51-like}}$ genes was performed by QIAamp® DNA Mini kit (QIAGEN, Germany), according to the manufacturer's directions. *A. baumannii* colonies were scraped from the culture plate with inoculation loop and suspended with 180 μL of ALT. Twenty microliters of proteinase K were added, mixed by vortexing, and incubated at 56°C until bacterial cells were completely lysed. The samples were then added by 200 μL of buffer AL. After well mixing, the suspensions were heated at 70°C for 10 min, and briefly centrifuged to remove drops from inside the lid. The samples were added by 200 μL of ethanol and mixed by pulse-vortexing for 15 sec. The supernatants were then transferred into spin columns and centrifuged at 8,000 rpm for 1 min. The QIAamp spin columns were placed in clean 2 mL collection tubes and the tubes containing the filtrate were discarded. QIAamp spin columns were carefully opened and 500 μL of buffer AW1 were added and centrifuged at 8,000 rpm for 1 min. The filtrates were then discarded. QIAamp spin columns were washed with 500 μL of AW2 and centrifuged at 14,000 rpm for 3 minutes. QIAamp spin columns were placed in clean 1.5 mL microcentrifuge tube and added with 200 μL of buffer AE. The samples were incubated at room temperature for 1 minute and

centrifuged at 8,000 rpm for 1 minute. Extracted DNA samples were stored at –20 °C.

2. Preparation of plasmid DNA

The extraction of *A. baumannii* plasmid DNA for amplifying entire $bla_{OXA23\text{-like}}$, $bla_{OXA24\text{-like}}$, $bla_{OXA58\text{-like}}$ genes was performed by Aurum™ Plasmid Mini Kit (BIO-RAD, USA). The DNA was purified according to the manufacturer's directions. *A. baumannii* were cultured in Luria-Bertani broth (Pronadisa, Spain) and measure the density of bacterial cells up to 12 OD.mL (OD600). Bacterial cells were transferred to a 1.5 mL capped microcentrifuge tube and centrifuged at 12,000 g for 1 min. The supernatant was removed. The 250 µL of resuspension solution were added and mixed by vortexing. The 250 µL of lysis solution were added to the sample and mixed by inverting the capped tube briskly 6-8 times. After that, the 350 µL of neutralizing solution were added to the sample and mixed by inverting the capped tube briskly 6-8 times. Then the sample was centrifuged for 5 min. A compact white debris pellet was formed at the bottom of the tube, while supernatant or cleared lysate contained the plasmid DNA. The cleared lysate was transferred into a plasmid mini column which was inserted in a 2 mL capless wash tube and the column was centrifuged for 1 min. The filtrate was removed from the tube. The column was replaced into the same wash tube. Then, 750 µL of wash solution were added to the column and centrifuged for 1 min. The wash solution was discarded from the tube and the column was replaced into the same wash tube and centrifuged for 1 additional minute to remove residual wash solution. Finally, the plasmid mini column was transferred to a 1.5 mL capped microcentrifuge tube and 50 µL of elution solution was added onto the membrane stack at the base of the column and allowed for 1 min. for saturating of solution to the membrane. Then, the column was centrifuged for 1 min. to elute the plasmid. The eluted plasmid DNA samples were stored at 4°C.

3. Screening for the presence of $bla_{\text{OXA-like}}$, $bla_{\text{IMP-like}}$ and $bla_{\text{VIM-like}}$

The presence of carbapenemase genes including $bla_{\text{OXA-like}}$, $bla_{\text{IMP-like}}$ and $bla_{\text{VIM-like}}$ was screened by PCR. The PCR primers of $bla_{\text{OXA-like}}$, $bla_{\text{IMP-like}}$ and $bla_{\text{VIM-like}}$ genes are demonstrated in Table 7.

Table 7. Primers used for amplification of $bla_{\text{OXA-like}}$, $bla_{\text{IMP-like}}$ and $bla_{\text{VIM-like}}$ and size of expected PCR products.

Specific for	Primer	Primer sequence	Location	Product size (bp)	Reference
$bla_{\text{OXA23-like}}$	OXA23-F	5'-GATCGGATTGGAGAACCAGA-3'	261-761	501	(193)
	OXA23-R	5'-ATTTCTGACCGCATTTCCAT-3'			
$bla_{\text{OXA24-like}}$	OXA24-F	5'-GGTTAGTTGGCCCCCTTAAA-3'	500-748	249	
	OXA24-R	5'-AGTTGAGCGAAAAGGGGATT-3'			
$bla_{\text{OXA51-like}}$	OXA51-F	5'-TAATGCTTTGATCGGCCTTG-3'	255-607	353	
	OXA51-R	5'-TGGATTGCACTTCATCTTGG-3'			
$bla_{\text{OXA58-like}}$	OXA58-F	5'-AAGTATTGGGGCTTGTGCTG-3'	39-637	599	
	OXA58-R	5'-CCCCTCTGCGCTCTACATAC-3'			
$bla_{\text{IMP-like}}$	IMP-F	5'-CTACCGCAGCAGAGTCTTTG-3'	47-633	587	(194)
	IMP-R	5'-AACCAGTTTTGCCTTACCAT-3'			
$bla_{\text{VIM-like}}$	VIM-F	5'-TTTGGTCGCATATCGCAACG-3'	158-657	500	(195)
	VIM-R	5'-CCATTCAGCCAGATCGGCAT-3'			

3.1 Amplification of the $bla_{\text{OXA-like}}$ by multiplex PCR

The presence of $bla_{\text{OXA23-like}}$, $bla_{\text{OXA24-like}}$, $bla_{\text{OXA51-like}}$ and $bla_{\text{OXA58-like}}$ was screened using OXA23-F, OXA23-R, OXA24-F, OXA24-R, OXA51-F, OXA51-R, OXA58-F and OXA58-R primers. In a 25 μL PCR reaction mixture, the amplification reaction was as follows : 1X PCR buffer , 1.5 mM $MgCl_2$, 200 μM dNTP (Fermentas, USA), 1.25 pmol of each primer except 0.625 pmol of OXA58-F and OXA58-R, and 0.625 U *Taq* polymerase (Fermentas, USA) and 1 μL of DNA template from boiling method. The amplification conditions were, initial denaturation at 94°C for 5 minutes, 30 cycles of 94°C for 25 seconds, 52°C for 40 seconds and 72°C for 50 seconds, and a final elongation at 72°C for 6 minutes

(193). The PCR products of $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$ and $bla_{\text{OXA-58-like}}$, were 501 bp, 249 bp, 353 bp and 599 bp, respectively.

3.2 Amplification of the $bla_{\text{IMP-like}}$

The presence of $bla_{\text{IMP-like}}$ was screened using IMP-F and IMP-R primers. In a 25 μL PCR reaction mixture, the amplification reaction was as follows : 1X PCR buffer , 1.5 mM $MgCl_2$, 200 μM dNTP (Fermentas, USA), 1.25 pmol of each primer, and 0.625 U *Taq* polymerase (Fermentas, USA) and 1 μL of DNA template from boiling method. The amplification conditions were, initial denaturation at 94°C for 2 minutes, 30 cycles of 94°C for 1 minute, 55°C for 1 minute and 72°C for 1.5 minutes, and a final elongation at 72°C for 10 minutes (194). The PCR product of $bla_{\text{IMP-like}}$ was 587 bp.

3.3 Amplification of the $bla_{\text{VIM-like}}$

The presence of $bla_{\text{VIM-like}}$ was screened using VIM-F and VIM-R primers. In a 25 μL PCR reaction mixture, the amplification reaction was as follows : 1X PCR buffer , 1.5 mM $MgCl_2$, 200 μM dNTP (Fermentas, USA), 1.25 pmol of each primer, and 0.625 U *Taq* polymerase (Fermentas, USA) and 1 μL of DNA template from boiling method. The amplification conditions were, initial denaturation at 95°C for 5 minutes, 30 cycles of 95°C for 30 seconds, 66°C for 1 minute and 72°C for 1 minute, and a final elongation at 72°C for 10 minutes (195). The PCR product of $bla_{\text{VIM-like}}$ was 502 bp.

4. Amplification and DNA sequencing of entire $bla_{\text{OXA-like}}$ genes

4.1 Amplification of entire $bla_{\text{OXA-like}}$ genes

Primers for amplification of entire $bla_{\text{OXA23-like}}$, $bla_{\text{OXA24-like}}$, $bla_{\text{OXA51-like}}$, $bla_{\text{OXA58-like}}$ are demonstrated in Table 8. Ten *A. baumannii* isolates which carried either of these genes and had different levels of MIC to imipenem were selected for amplification of $bla_{\text{OXA23-like}}$, $bla_{\text{OXA24-like}}$, $bla_{\text{OXA51-like}}$ and $bla_{\text{OXA58-like}}$.

4.1.1 Amplification of entire *bla*$_{\text{OXA-23-like}}$ and *bla*$_{\text{OXA-58-like}}$ genes

OXA23-F and OXA23-R primers were used for amplification of the entire *bla*$_{\text{OXA23-like}}$ gene whereas OXA58-F and OXA58-R primers were for entire *bla*$_{\text{OXA58-like}}$ gene amplification. Amplification of entired *bla*$_{\text{OXA-23-like}}$ and *bla*$_{\text{OXA-58-like}}$ used the same conditions. The 50 μL PCR mixture contained ; 1X *Pfu* buffer , 2.0 mM $MgSO_4$, 200 μM dNTP (Fermentas, USA), 5 pmol of each primer, 4 U *Pfu* DNA polymerase (Fermentas, USA), and 3 μL of plasmid DNA template. The amplification conditions were, initial denaturation at 94°C for 5 minutes and 30 cycles of 94°C for 25 seconds, 52°C for 40 seconds, 72°C for 50 seconds and final elongation at 72 °C for 6 minutes. The PCR products of entire *bla*$_{\text{OXA-23-like}}$ and *bla*$_{\text{OXA-58-like}}$ were 1,062 bp and 933 bp, respectively.

4.1.2 Amplification of entire *bla*$_{\text{OXA-51-like}}$ genes

OXA51-F and OXA51-R primers were used for amplification of entire *bla*$_{\text{OXA51-like}}$ gene. The 50 μL PCR mixture contained ; 1X *Pfu* buffer , 2.0 mM $MgSO_4$, 200 μM dNTP (Fermentas, USA) , 5 pmol of each primer, 4 U *Pfu* DNA polymerase (Fermentas, USA), and 3 μL of extracted DNA template. The amplification conditions were, initial denaturation at 95°C for 5 minutes and 35 cycles of 95°C for 30 seconds, 52°C for 1 minute, 72°C for 90 seconds and final elongation at 72 °C for 10 minutes. The PCR product of entire *bla*$_{\text{OXA-51-like}}$ was 825 bp.

4.1.3 Amplification of entire *bla*$_{\text{OXA-24-like}}$ genes

OXA24-F and OXA24-R primers were used for amplification of the entire *bla*$_{\text{OXA24-like}}$ gene. The 50 μL PCR mixture contained ; 1X PCR buffer , 1.5 mM $MgCl_2$, 200 μM dNTP (Fermentas, USA) , 2.5 pmol of each primer, 1.25 U *Taq* polymerase (Fermentas, USA), and 1 μL of plasmid DNA template. The amplification conditions were, initial denaturation at 94°C for 4 minutes and 30 cycles of 94°C for 1 minute, 52°C for 1 minute, 72°C for 2 minutes and final

elongation at 72 °C for 10 minutes. The PCR product of entire $bla_{\text{OXA-24-like}}$ was 1,021 bp.

Table 8. Sequences of the oligonucleotides used as primers for amplifications of entire *bla*$_{OXA\text{-}like}$ gene and DNA sequencing.

Primer	Primer sequence	Product size (bp)	Reference
PCR primers			
entire *bla*$_{OXA23\text{-}like}$			
OXA23-likeF	5'-GATGTGTCATAGTATTCGTCG-3'	1,065	
OXA23-likeR	5'-TCACAACAACTAAAAGCACTG-3'		
entire *bla*$_{OXA24\text{-}like}$			(23)
OXA24-likeF	5'-GTACTAATCAAAGTTGTGAA-3'	1,021	
OXA24-likeR	5'-TTCCCCTAACATGAATTTGT-3'		
entire *bla*$_{OXA51\text{-}like}$			
OXA51-likeF	5'-TACGCCAATCCATACAGCAA-3'	1,416	(GenBank accession no.CU468230)
OXA51-likeR	5'-GCTTGACGCTGCTTTTTACC-3'		
entire *bla*$_{OXA58\text{-}like}$			
OXA58-likeF	5'-TTATCAAAATCCAATCGGC-3'	933	(143)
OXA58-likeR	5'-TAACCTCAAACTTCTAATTC-3'		
Sequencing primers			
For entire *bla*$_{OXA23\text{-}like}$			
OXA23-likeF	5'-GATGTGTCATAGTATTCGTCG-3'		
OXA23-likeR	5'-TCACAACAACTAAAAGCACTG-3'		(23)
For entire *bla*$_{OXA24\text{-}like}$			
OXA24-likeF	5'-GTACTAATCAAAGTTGTGAA-3'		
OXA24-likeR	5'-TTCCCCTAACATGAATTTGT-3'		
OXA21-likeR1	5'-AGTTGAGCGAAAAGGGGATT-3'		
For entire *bla*$_{OXA51\text{-}like}$			
OXA51-likeF	5'-TACGCCAATCCATACAGCAA-3'		(GenBank accession no.CU468230)
OXA51-likeFM	5'-TAATGCTTTGATCGGCCTTG-3'		(193)
OXA51-likeFM1	5'-ATGAACATTAAAGCACTC-3'		(GenBank accession
OXA51-likeR1	5'-CTATAAAATACCTAATTGTTC-3'		no.EU977569)
For entire *bla*$_{OXA58\text{-}like}$			
OXA58-likeF	5'-TTATCAAAATCCAATCGGC-3'		(143)
OXA58-likeR	5'-TAACCTCAAACTTCTAATTC-3'		
OXA58-likeFM	5'-AAGTATTGGGGCTTGTGCTG-3'		(193)

5. Analysis of PCR Product

The PCR products were analyzed on 1.2 % agarose gels (Pronadisa, Spain) containing 0.5 μg/mL ethidium bromide (Sigma, USA) in TBE buffer. PCR products were mixed with 6X of gel loading buffer (20% ficoll, 0.05% bromophenol blue). The electrophoresis was carried out at 100 volts for 60 minutes. The amplified products were visualized on a UV light transilluminator. A 100 base pair DNA ladder (Fermentas, USA) and a 100 base pair plus DNA ladder (Fermentas, USA) were used as DNA size markers.

6. Purification of PCR product

The PCR products were purified by QIAquick PCR purification kit as described by the manufacturer (QIAGEN, GmbH, Germany). The QIAquick system is combination of spin-column technology with the selective binding properties of a uniquely- designed silica-gel membrane. DNA is absorbed to the silica- membrane in the presence of high salt while contaminants pass through the column. Five volumes of buffer PB (Contains quanidine hydrochloride and isopropanol) were added to 1 volume of PCR product sample. After well mixing, the suspension was transferred into QIAquick spin column in a provided 2 mL collection tube and centrifuged at 13,000 rpm for 60 sec. The flow–through was discarded and 750 μL of buffer PE (washing buffer) were added to the column. The column was centrifuged at 13,000 rpm for 60 sec. The flow-through was discarded from the tube and column was replaced into the same tube and centrifuged for 1 additional minute to remove residual wash solution. QIAquick spin columns were placed in a clean 1.5 mL microcentrifuge tube and added with 50 μL of buffer EB (elution buffer). The samples were incubated at room temperature for 1 minute and centrifuged at 13,000 rpm for 60 sec. The purified PCR product was stored at -20 ^{0}C. The concentration of DNA was determined by spectrophotometer (BIO RAD, U.S.A).

7. Preparation of sequencing reactions

Purified PCR products were sequenced under BigDye terminator cycling conditions using 3730xl DNA analyzer (Applied Biosystems, USA) by Macrogen Inc., Korea. Sequencing oligonucleotide primers are shown in Table 4.

8. Sequencing analysis

The nucleotide sequences and the deduced protein sequences were analyzed with the software available over the Internet at the National Center for Biotechnology Information (http: // www.ncbi.nlm.nih.gov/BLAST), Multalin (www.toulouse.inra.fr/multalin.html) and ExPASy (www.expasy.org).

CHAPTER V

RESULTS

PART I : BACTERIAL STRAINS

A total of 501 isolates of *A. baumannii* were used in the present study. The clinical isolates collected in 2004, 2005, 2006 and 2007 were 194, 177, 16 and 114 isolates, respectively. Two hundred and eighty-four (56.7%) isolates and 217 (43.3%) isolates were isolated from male and female, respectively. Two hundred and fifty-nine (51.7%) specimens and 242 (48.3%) specimens were collected from patients in ICUs and other hospital wards (non-ICUs), respectively. The clinical isolates were recovered from blood 69 (13.8%) isolates, body fluid 20 (4%) isolates, cerebrospinal fluid 1 (0.2%) isolate, sputum 238 (47.5%) isolates, respiratory aspirate 73 (14.6%) isolates, pus 17 (3.4%) isolates, tissue 14 (2.8%) isolates, urine 52 (10.4%) isolates and other specimen 17 (3.4%) isolates.

PART II : BACTERIAL IDENTIFICATION

Biochemical patterns of 501 *A. baumannii* isolates are shown in Table 9. A total of 501 isolates were identified as *A. baumannii* based on colonial morphology, cell morphology and biochemical characteristics. *A. baumannii* isolates were gram-negative coccobacilli usually diploid form. Colonies on MacConkey agar were lactose non-fermenting, 0.5-2 mm in diameter, smooth, opaque, colorless or slightly pink. Biochemical characteristics included alkaline slant / neutral butt on triple sugar iron agar, no hemolysis on sheep blood agar, oxidase negative, urease negative, non motile and negative for nitrate reduction. It grew at 37°, 42° and 44°C and oxidized dextrose. There were six biochemical patterns in 501 isolates differed by citrate, malonate and arginine utilization as shown in Table 9. Of the 501 isolates, 287 (57.3%) were belonged to biochemical pattern B and 178 (35.5%) were belonged to biochemical pattern D. Twenty-two

(4.4%) , 11 (2.2%) , 2 (0.4%) and 1 (0.2%) were belonged to biochemical pattern C, A, E and F, respectively. The results suggested that most of *A. baumannii* isolates were belonged to biochemical pattern B and D.

Table 9. Biochemical patterns of 501 *A. baumannii* isolates

Biochemical pattern	Triple sugar iron	Oxidase test	Motility	Urease	Nitrate reduction	Growth at			Hemolysis of sheep blood	Oxidize dextrose	Arginine decarboxylase	Malonate utilization	Citrate utilization	% of isolates (n)
						37°C	42°C	44°C						
A	K/N[a]	–	–	–	–	+	+	+	–	+	+	+	+	2.2 (11)
B	K/N	–	–	–	–	+	+	+	–	+	–	–	+	57.3 (287)
C	K/N	–	–	–	–	+	+	+	–	+	+	–	+	4.4 (22)
D	K/N	–	–	–	–	+	+	+	–	+	–	+	+	35.5 (178)
E	K/N	–	–	–	–	+	+	+	–	+	–	–	–	0.4 (2)
F	K/N	–	–	–	–	+	+	+	–	+	–	+	–	0.2 (1)

[a] Alkaline slant / neutral butt

PART III : ANTIMICROBIAL SUSCEPTIBILITY TEST

The minimal inhibitory concentrations (MICs) of carbapenems in the 501 *A. baumannii* isolates were examined by agar-dilution technique on Muller-Hinton agar. The MIC results of these *A. baumannii* isolates are shown in Appendix D. MIC breakpoints of imipenem and meropenem were ≥ 16 mg/L. Susceptibility and resistance rates of the 501 *A. baumannii* isolates to carbapenems are shown in Table 10. Prevalence of imipenem and meropenem resistance was 82.4% (413 isolates) and 81.8% (410 isolates), respectively. MICs required to inhibit 50% (MIC_{50}) of isolates for imipenem and meropenem were 64 and 32 mg/L, respectively. MICs required to inhibit 90% (MIC_{90}) of isolates for imipenem and meropenem were 256 and 64 mg/L, respectively. Distributions of the MICs for imipenem and meropenem are shown in Figure 9. and 10. It was demonstrated that MICs of imipenem-susceptible in *A. baumannii* isolates ranged from 0.12 to 4 mg/L. Most of imipenem-susceptible isolates (96.6%) had imipenem MICs of ≥ 0.5 mg/L. The majority of imipenem-resistant isolates (82.8%) had imipenem MICs of ≥ 64 mg/L. Meropenem-susceptible isolates had meropenem MIC range of 0.12 to 4 mg/L. The majority of meropenem-susceptible isolates had meropenem MIC of ≥ 0.25 mg/L. Three isolates were intermediately resistant. Most of meropenem-resistant isolates (77.4%) had meropenem MIC range of 16 to 64 mg/L. Peaks of imipenem and meropenem MIC were at 64 mg/L and 32 mg/L, respectively.

Carbapenem resistance rates of *A. baumannii* isolated from patients in ICUs and non-ICUs are shown in Figure 11. Imipenem resistance of ICU and non-ICU isolates was 90.3% (234/259 isolates) and 74.0% (179/242 isolates), respectively, whereas meropenem resistance was 89.6% (232/259 isolates) and 73.5% (178/242 isolates), respectively. MIC_{50} and MIC_{90} values of imipenem for both ICU and non-ICU isolates were 64 and 256 mg/L, respectively. MIC_{50} values of meropenem for ICU and non-ICU isolates were 32 and 16 mg/L, respectively. MIC_{90} values of meropenem for both ICU and non-ICU isolates were 64 mg/L. Distributions of the MICs for imipenem and meropenem of ICU and non-ICU isolates are shown in Figure 12. and 13. It was demonstrated that peaks of imipenem MIC of ICUs and non-ICUs isolates were at 64 mg/L and 128 mg/L, respectively. Peaks of

meropenem MIC of ICUs and non-ICUs isolates were at 16 mg/L and 32 mg/L, respectively. The MICs of imipenem-susceptible in ICU isolates ranged from 0.12 to 4 mg/L. Most of imipenem-susceptible in ICU isolates (88%) had imipenem MICs of 0.5-2 mg/L. The majority of imipenem-resistant in ICU isolates (89.2%) had imipenem MICs of $\geq$ 32 mg/L. Meropenem-susceptible isolates had meropenem MIC range of 0.12 to 4 mg/L. The majority of meropenem-susceptible isolates (96%) had meropenem MIC of 0.25 to 2 mg/L. The majority of meropenem-resistant isolates (83.8%) had imipenem MICs of 16 to 64 mg/L.

The MICs of imipenem-susceptible in non-ICU isolates ranged from 0.12 to 4 mg/L. Most of imipenem-susceptible in non-ICU isolates (93.6%) had imipenem MICs of 0.5 to 2 mg/L. The majority of imipenem-resistant non-ICU isolates (95.5%) had imipenem MICs of 32 to 256 mg/L. Meropenem-susceptible in non-ICU isolates had meropenem MIC range of 0.12 to 4 mg/L. The majority of meropenem-susceptible in non-ICU isolates (90.5%) had meropenem MIC of 0.25 to 2 mg/L. The majority of meropenem-resistant in non-ICU isolates (96.1%) had imipenem MICs of 16 to 64 mg/L. By statistical analysis using Chi-square test, imipenem and meropenem resistance rates of *A. baumannii* isolated from patients in ICUs were significantly higher than those from non-ICUs ($P < 0.01$).

By comparison between isolates from sterile sites and non sterile sites, prevalence of imipenem resistance was 83.5% (81/97 isolates) and 82.2% (332/404 isolates), respectively, whereas prevalence of meropenem resistance was 82.5% (80/97 isolates) and 81.7% (330/404 isolates), respectively. MIC_{50} and MIC_{90} values of imipenem for isolates from both sterile sites and non sterile sites were 64 and 256 mg/L, respectively. MIC_{50} and MIC_{90} values of meropenem for isolates from both sterile sites and non sterile sites were 32 and 64 mg/L, respectively. Distributions of the MICs for imipenem and meropenem of isolates from sterile sites and non sterile sites are shown in Figure 14. and 15. Peaks of imipenem MIC of isolates from both sterile sites and non sterile sites were at 64 mg/L. Peaks of meropenem MIC of isolates from sterile sites and non sterile sites were at 16 mg/L and 32 mg/L, respectively. Most of imipenem-resistant in isolates from sterile site (82.2%) had imipenem MICs of >32 mg/L. The majority of meropenem-resistant in

isolates from sterile site (74.4%) had meropenem MICs of 16 to 64 mg/L. Most of imipenem-resistant in isolates from non-sterile site (67.7%) had imipenem MICs of >64 mg/L. The majority of meropenem-resistant in isolates from non-sterile site (78.1%) had meropenem MICs of 16 to 64 mg/L. By statistical analysis using Chi-square test, there was no significant difference in imipenem and meropenem resistance rates in *A. baumannii* isolated from sterile and non sterile clinical specimens (P = 0.758).

Table 10. Carbapenem MICs and prevalence of carbapenem resistance in 501 *A. baumannii* isolates.

Antimicrobial agents	MICs (mg/L)			Resistance (%)
	Range	MIC_{50}	MIC_{90}	
Imipenem	0.12->256	64	256	413 (82.4)
Meropenem	0.12->256	32	64	410 (81.8)

Figure 9. Distribution of imipenem MICs

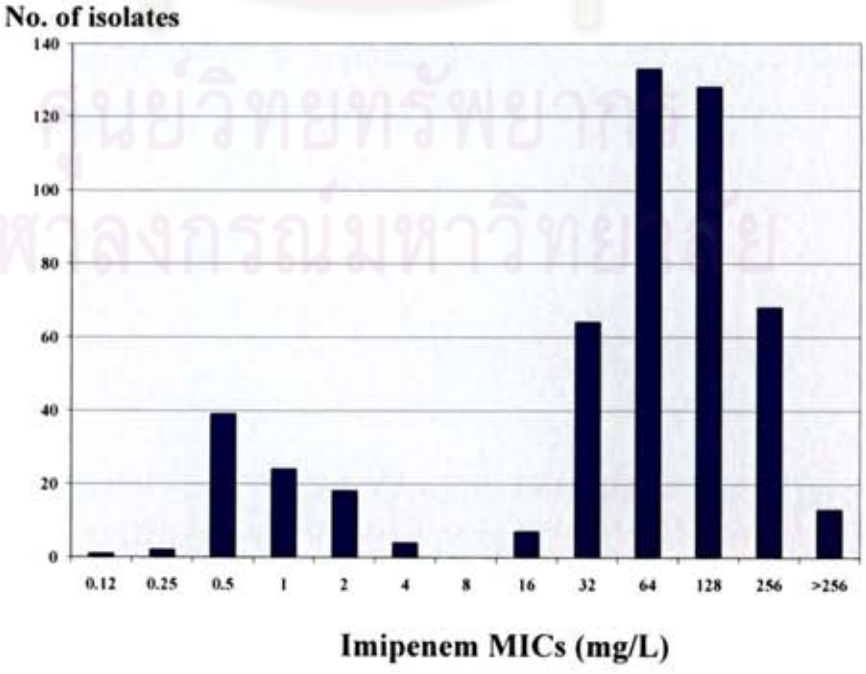


Figure 10. Distribution of meropenem MICs.

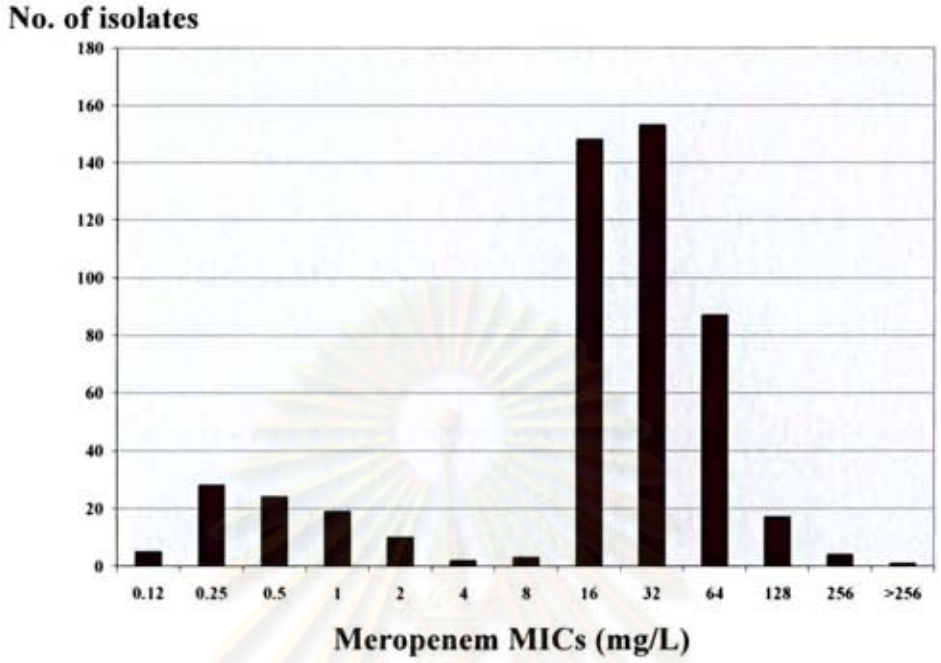


Figure 11. Prevalence of carbapenem resistance in ICU and non-ICU *A. baumannii* isolates

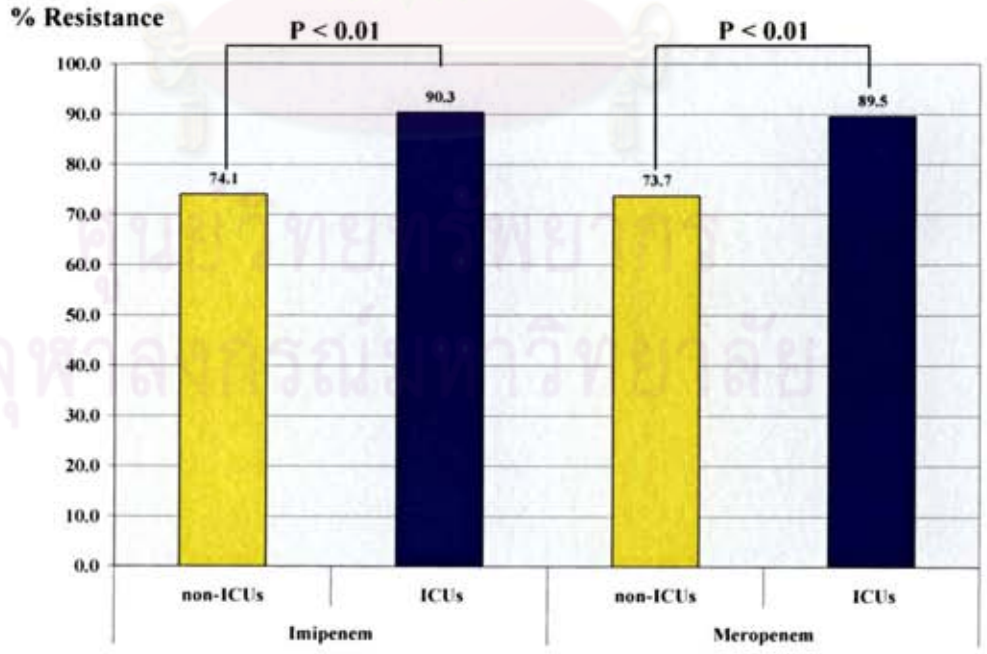


Figure 12. Distributions of imipenem MICs of ICU and non-ICU isolates

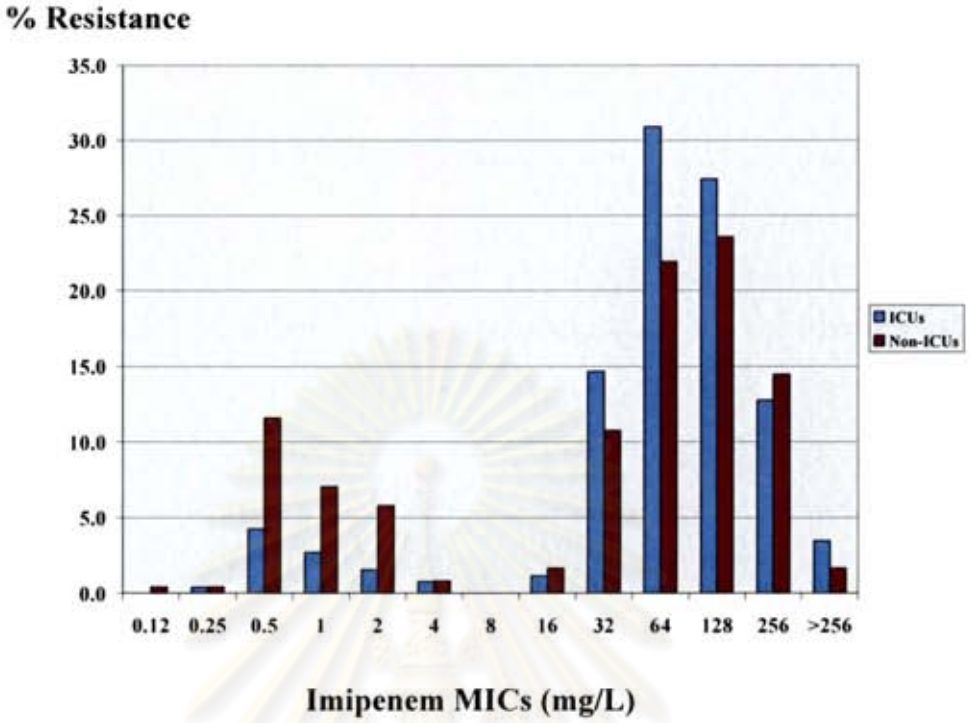


Figure 13. Distributions of the meropenem MICs of ICU and non-ICU isolates

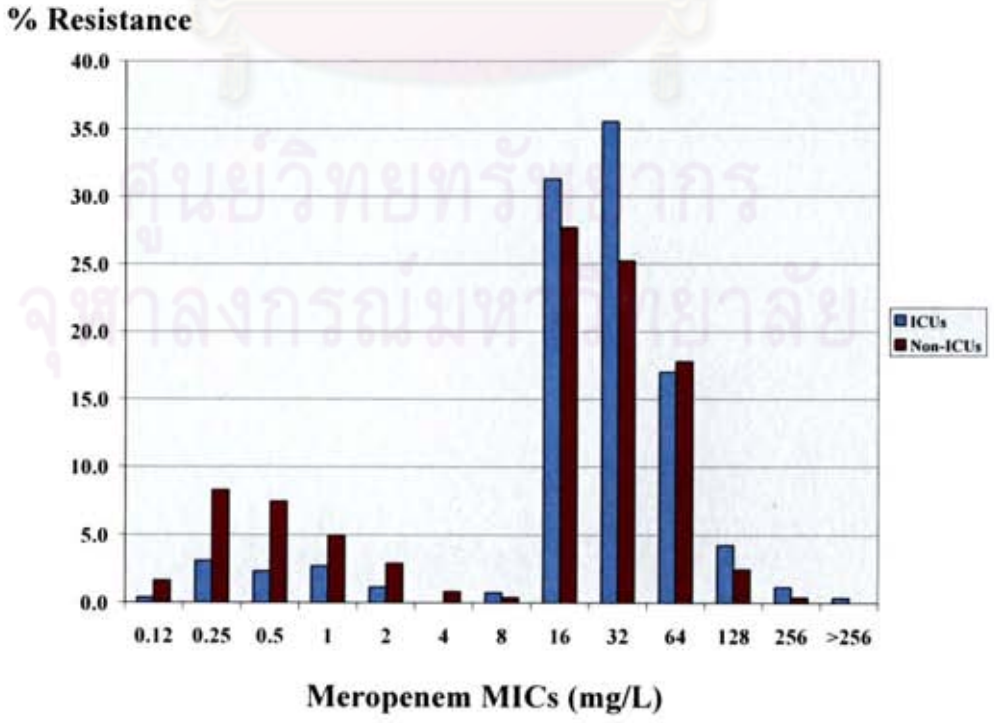


Figure 14. Distributions of imipenem MICs of isolates from sterile sites and non sterile sites

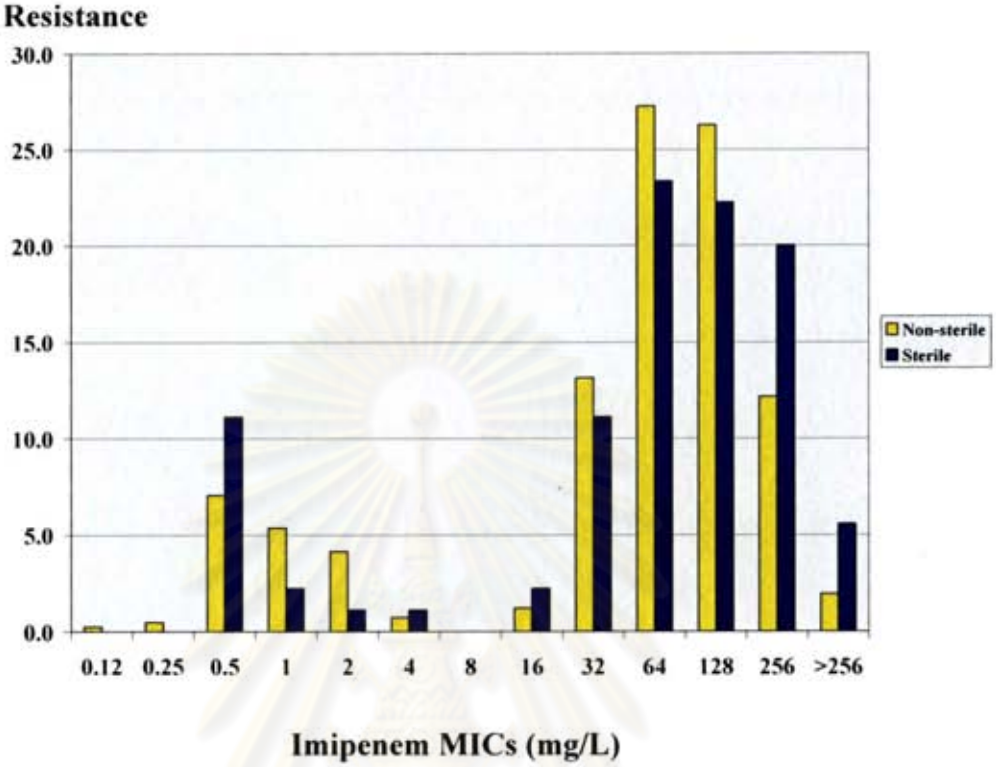
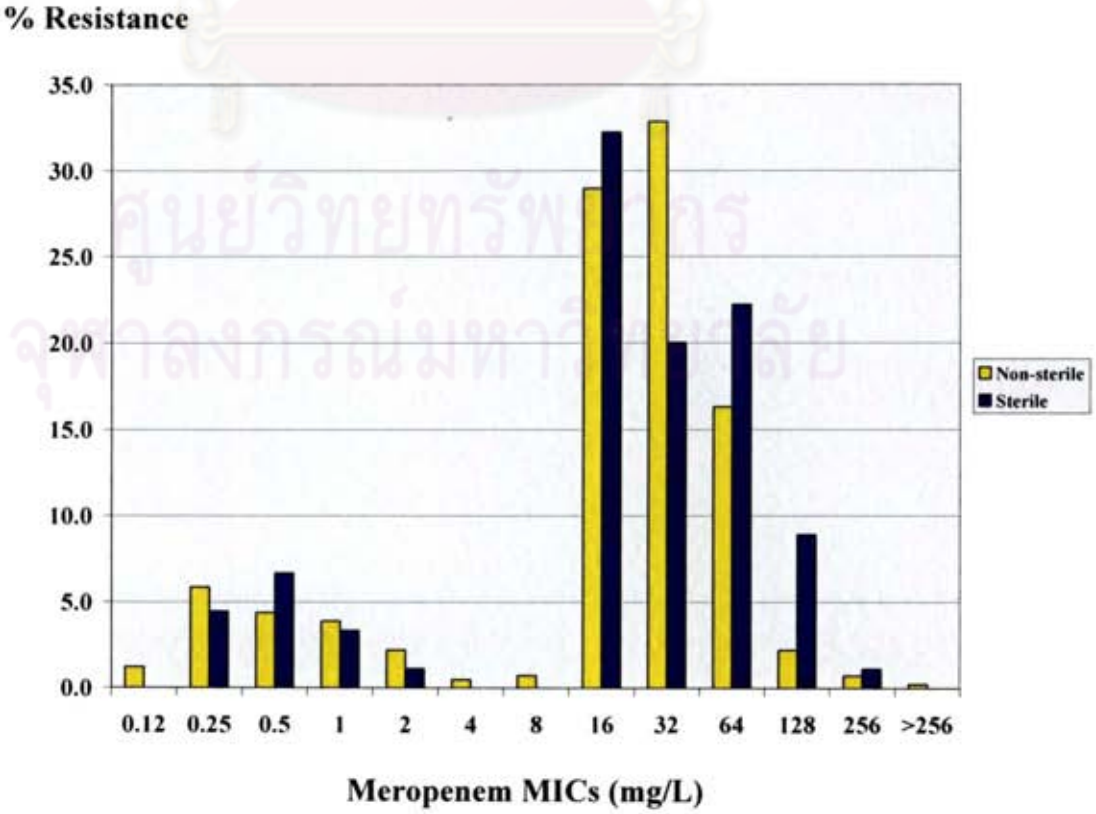


Figure 15. Distributions of meropenem MICs of isolates from sterile sites and non sterile sites



PART IV : DETERMINATION OF CARBAPENEMASE ACTIVITY

Detection of carbapenemase activity was determined by modified Hodge test. The carbapenemase activities of the 501 *A. baumannii* isolates are shown in Appendix D. Four hundred and thirteen (82.4 %) isolates had carbapenemase activity. Comparisons between MICs of carbapenems in *A. baumannii* isolates and the presence of carbapenemase activity are shown in Figures 16 and 17. The MIC ranges of imipenem and meropenem of isolates with carbapenemase activity were 16->256 mg/L and 8->256 mg/L, respectively, whereas the MIC ranges of imipenem and meropenem of isolates with no carbapenemase activity were 0.12-4 mg/L. All 413 isolates showing carbapenemase activity, were resistant to imipenem (MIC $\geq$ 16 mg/L) but 410 (99.3%) isolates were resistant to meropenem (MIC $\geq$ 16 mg/L) and 3 (0.7%) isolates were intermediately resistant to meropenem (MIC = 8 mg/L).

Figure 16. Comparison between the presence of carbapenemase activity and imipenem MIC.

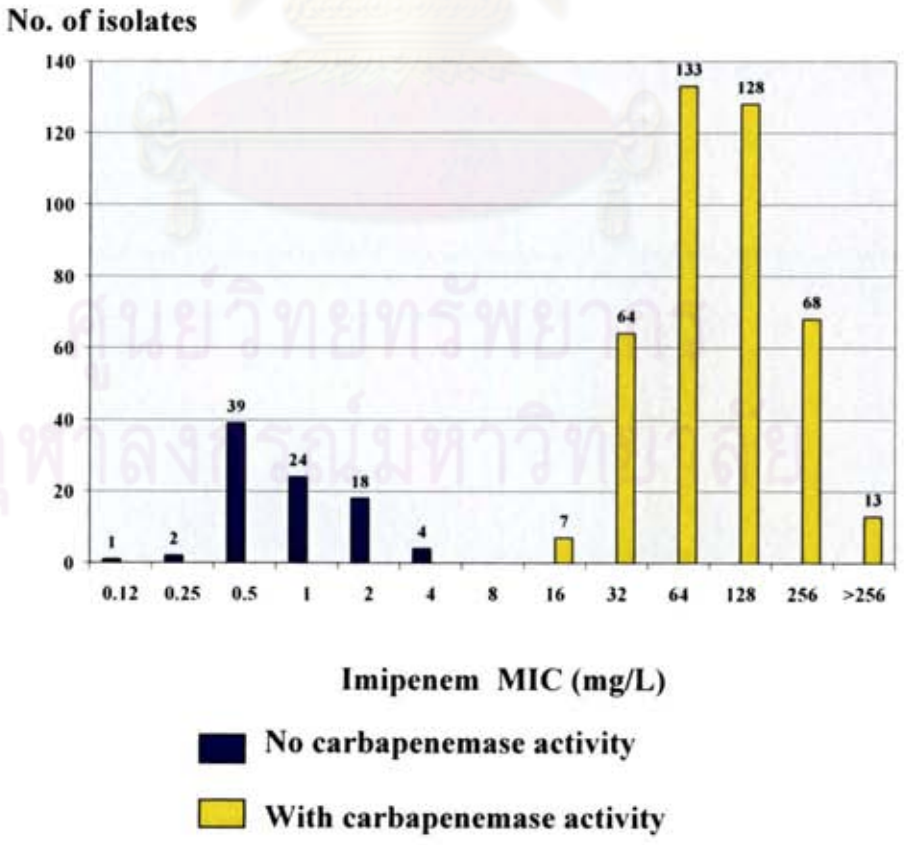
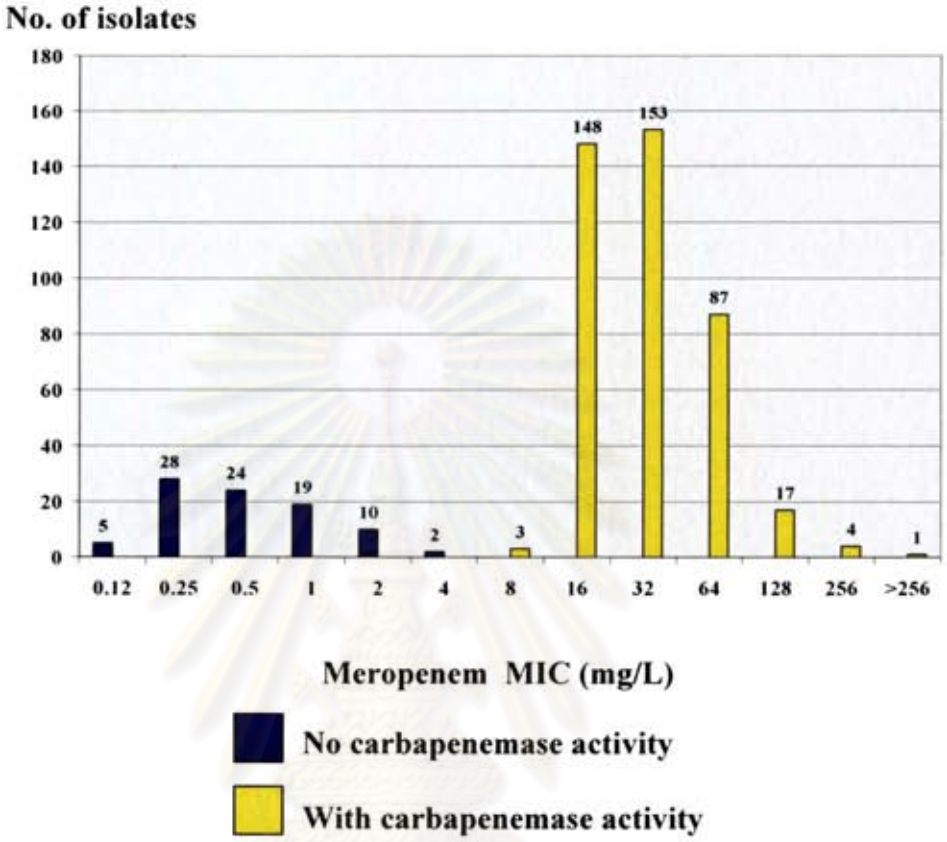


Figure 17. Comparison between the presence of carbapenemase activity and meropenem MIC.



PART V : DETERMINATION OF METALLO-β-LACTAMASES

Determination of metallo-β-lactamase was performed by EDTA-disk synergy test by using imipenem and EDTA disks. Metallo-β-lactamase activity of the 501 *A. baumannii* isolates are shown in Appendix D. No metallo-β-lactamase was detected in any isolates.

PART VI : SCREENING OF GENES ENCODING OXA- , IMP- AND VIM-TYPE CARBAPENEMASES BY PCR AND DNA SEQUENCING OF ENTIRE *BLA*-OXA GENE

Screening for the presence of $bla_{\text{OXA-like}}$, $bla_{\text{IMP-like}}$ and $bla_{\text{VIM-like}}$

All *A. baumannii* isolates were screened for the presence of carbapenemase genes, $bla_{\text{OXA-like}}$, $bla_{\text{IMP-like}}$ and $bla_{\text{VIM-like}}$, with primers specific for each type by multiplex PCR. The expected PCR products of $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-58-like}}$, $bla_{\text{IMP-like}}$ and $bla_{\text{VIM-like}}$ were 501 bp, 249 bp, 353 bp, 599 bp, 587 bp and 500 bp, respectively (Figure 18.). The presence of $bla_{\text{OXA-like}}$, $bla_{\text{IMP-like}}$ and $bla_{\text{VIM-like}}$ in the 501 *A. baumannii* isolates are shown in Appendix D. The $bla_{\text{OXA-51-like}}$ was detected in all isolates. Eighty-eight (17.6%) isolates had only $bla_{\text{OXA-51-like}}$. Three hundred and fifty-six (71.1%) isolates had both $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-51-like}}$ Four (0.4%) isolates carried both $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-51-like}}$. Six (1.2%) isolates had $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$. Seven (1.4%) isolates harbored $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-58-like}}$. Twenty-four (4.8%) isolates carried $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$. Sixteen (3.2%) isolates had all $bla_{\text{OXA-like}}$ types, $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$. The results are summarized in Table 14.

Of the 413 imipenem-resistant *A. baumannii* isolates, 385 (93.2%) isolates had $bla_{\text{OXA23-like}}$ Fifty (12.1%) isolates carried $bla_{\text{OXA24-like}}$. Forty-seven (11.4%) isolates had $bla_{\text{OXA-58-like}}$. The results are summarized in Table 15.

The distribution of carbapenemase genes and imipenem and meropenem MICs in 501 *A. baumannii* isolates are shown in Figures 19 and 20 and Table 14. The isolates carrying only $bla_{\text{OXA51-like}}$ showed low-level MICs to imipenem and meropenem (MIC range of 0.12 to 4 mg/L). MIC_{50} of these isolates for imipenem and meropenem were 1 and 0.5 mg/L, respectively, whereas MIC_{90} for both imipenem and meropenem were 2 mg/L. Isolates harboring $bla_{\text{OXA51-like}}$ and $bla_{\text{OXA23-like}}$ had imipenem and meropenem MIC range of 16 to >256 mg/L and 8 to 256 mg/L, respectively. MIC_{50} of these isolates for imipenem and meropenem were

64 and 32 mg/L, respectively, whereas MIC_{90} were 256 and 64 mg/L, respectively. Isolates carrying $bla_{OXA51\text{-}like}$ and $bla_{OXA24\text{-}like}$ had imipenem and meropenem MIC of $\geq$ 256 mg/L and 64 to 256 mg/L, respectively. MIC_{50} of these isolates for imipenem and meropenem were 256 and 128 mg/L, respectively, whereas MIC_{90} were >256 and 256 mg/L, respectively.

Isolates carrying $bla_{OXA51\text{-}like}$, $bla_{OXA23\text{-}like}$ and $bla_{OXA24\text{-}like}$ had imipenem MIC range of 128 to $\geq$ 256 mg/L, and meropenem MIC range of 64 to 128 mg/L. Isolates carrying $bla_{OXA51\text{-}like}$,$bla_{OXA23\text{-}like}$ and $bla_{OXA58\text{-}like}$ had imipenem MIC range of 32 to 128 mg/L and meropenem MIC range of 16 to 32 mg/L. Isolates harboring $bla_{OXA51\text{-}like}$, $bla_{OXA24\text{-}like}$ and $bla_{OXA58\text{-}like}$ had imipenem MIC range of 128 to 256 mg/L and meropenem MIC range of 32 to $\geq$256 mg/L. Isolates carrying $bla_{OXA51\text{-}like}$, $bla_{OXA23\text{-}like}$, $bla_{OXA24\text{-}like}$ and $bla_{OXA58\text{-}like}$ had imipenem MIC range of 128 to >256 mg/L and meropenem MIC range of 32 to 128 mg/L. It was demonstrated that most isolates carrying $bla_{OXA\text{-}24\text{-}like}$ had high level of carbapenem resistance (MIC range of 128 to >256 mg/L).

Carbapenemase activity was found in isolates carrying $bla_{OXA\text{-}51\text{-}like}$ together with other $bla_{OXA\text{-}like}$ genes including $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$ and $bla_{OXA\text{-}58\text{-}like}$ whereas no carbapenemase activity was detected in those carrying only $bla_{OXA\text{-}51\text{-}like}$.

Comparison between type of clinical specimen, carbapenem MIC and carbapenemase genes are shown in Table 11. Isolates from sterile site, including body fluid, CSF and blood had imipenem and meropenem MIC range of 0.5->256 mg/L and 0.25-256 mg/L, respectively. Isolates from non-sterile site, including sputum, urine, respiration aspirate, pus, tissue had both imipenem and meropenem MIC range of 0.12->256 mg/L. Most isolates from sterile and non-sterile site carried $bla_{OXA\text{-}51\text{-}like}$ together with $bla_{OXA\text{-}23\text{-}like}$. The results suggested that $bla_{OXA\text{-}23\text{-}like}$ disseminated in both invasive and non-invasive clinical isolates.

Comparison between ICU isolates, non-ICU isolates and carbapenemase genes are shown in Table 12. Of the 259 isolates from ICU, 203 (78.4%) carried

bla$_{OXA-51-like}$ together with *bla*$_{OXA-23-like}$ One (0.4%) carried *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-24-like}$. Six (2.3%) had *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-23-like}$ and *bla*$_{OXA-24-like}$ Six (2.3%) carried *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-23-like}$ and *bla*$_{OXA-58-like}$ Eight (3.1%) had *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-24-like}$ and *bla*$_{OXA-58-like}$. Ten (3.7%) carried *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-23-like}$, *bla*$_{OXA-24-like}$ and *bla*$_{OXA-58-like}$. Twenty-five (9.7%) had only *bla*$_{OXA-51-like}$ Of the 242 isolates from non-ICU, 153 (63.2%) carried *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-23-like}$ Three (1.2%) carried *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-24-like}$. One (0.4%) had *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-23-like}$ and *bla*$_{OXA-58-like}$ Sixteen (6.6%) carried *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-24-like}$ and *bla*$_{OXA-58-like}$. Six (2.5%) had *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-23-like}$, *bla*$_{OXA-24-like}$ and *bla*$_{OXA-58-like}$. Sixty-three (26%) carried only *bla*$_{OXA-51-like}$ The results suggested that *bla*$_{OXA-51-like}$ and *bla*$_{OXA-23-like}$ was the major carbapenemase genes found in ICU and non-ICU *A. baumannii* isolates.

Correlation between biochemical patterns of *A. baumannii* isolates and OXA-type carbapenemase genes in 413 carbapenem-resistant *A. baumannii* isolates are shown in Table 13. Of the 8 isolates with biochemical pattern A, 6 (75%) carried *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-23-like}$, whereas one (12.5%) carried *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-24-like}$ and one (12.5%) had *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-23-like}$ and *bla*$_{OXA-24-like}$. Of the 242 isolates with biochemical pattern B, 199 (82.2%) had *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-23-like}$, whereas 3 (1.2%) isolates had *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-24-like}$ and 3 (1.2%) had *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-23-like}$ and *bla*$_{OXA-24-like}$. Seven (2.9%) had *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-23-like}$ and *bla*$_{OXA-58-like}$, 20 (8.3%) had *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-24-like}$ and *bla*$_{OXA-58-like}$ and 10 (4.1%) had *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-23-like}$ and *bla*$_{OXA-24-like}$ and *bla*$_{OXA-58-like}$. All isolates with biochemical pattern C (15 isolates), E (1 isolate) and F (1 isolate) carried *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-23-like}$. Of the 146 isolates with biochemical pattern D, 134 (91.8%) had *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-23-like}$, whereas 2 (1.4%) had *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-23-like}$ and *bla*$_{OXA-24-like}$. Four (2.7%) had *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-24-like}$ and *bla*$_{OXA-58-like}$ and 6 (4.1%) had *bla*$_{OXA-51-like}$ together with *bla*$_{OXA-23-like}$ and *bla*$_{OXA-24-like}$ and *bla*$_{OXA-58-like}$ The results suggested *bla*$_{OXA-51-like}$

together with $bla_{\text{OXA-23-like}}$ was the most prevalent carbapenemase genes found in all biochemical patterns.

Table 11. Carbapenem MICs and OXA-type carbapenemase genes of 501 *A. baumannii* isolates by type of clinical specimens

Type of specimen	No. of isolates	MICs (mg/L)						Presence of $bla_{OXA\text{-}like}$						
		Imipenem			Meropenem			OXA-51-like	OXA-51-like, OXA-23-like	OXA-51-like, OXA-24-like	OXA-51-like, OXA-23-like, OXA-24-like	OXA-51-like, OXA-23-like, OXA-58-like	OXA-51-like, OXA-24-like, OXA-58-like	OXA-51-like, OXA-23-like, OXA-24-like, OXA-58-like
		Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}							
Blood	69	0.5->256	128	256	0.25-256	32	128	13	47		1		6	2
Body fluid	20	0.5->256	64	128	0.25-64	16	64	3	17					
CSF	1	64			16				1					
Sputum	238	0.12->256	64	256	0.12->256	32	64	36	169	2	4	7	10	10
respiratory aspirate*	73	0.25->256	64	256	0.25-128	32	64	14	55	1			1	2
Pus	17	0.5->256	64	256	0.25-128	32	64	5	11	1				
Urine	52	0.5-256	64	256	0.25-64	16	64	11	31		1		7	2
Tissue	14	0.25-128	32	128	0.25-32	16	32	3	11					
Other	17	0.5-256	64	256	0.5-64	16	64	3	14					

* Bronchial larvage, endotrachial secretion included

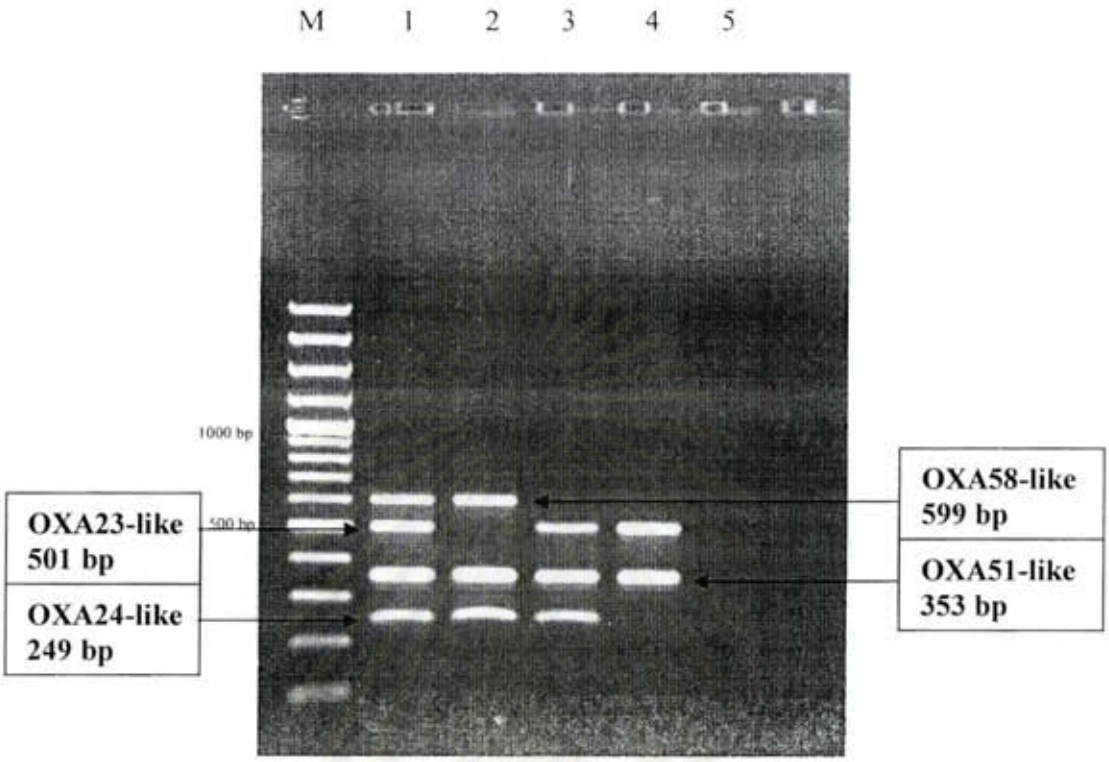
Table 12. Carbapenem MICs and OXA-type carbapenemase genes of 501 *A. baumannii* isolates by wards

Type of ward	No. of isolates	MICs (mg/L)						No. of isolates carried $bla_{OXA\text{-}like}$ (%)						
		Imipenem			Meropenem			OXA-51-like	OXA-51-like, OXA-23-like	OXA-51-like, OXA-24-like	OXA-51-like, OXA-23-like, OXA-24-like	OXA-51-like, OXA-23-like, OXA-58-like	OXA-51-like, OXA-24-like, OXA-58-like	OXA-51-like, OXA-23-like, OXA-24-like, OXA-58-like
		Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}							
ICU	259	0.25->256	64	256	0.12->256	32	64	25 (9.7)	203 (78.4)	1 (0.4)	6 (2.3)	6 (2.3)	8 (3.1)	10 (3.7)
Non-ICU	242	0.12->256	64	256	0.12-256	16	64	63 (26)	153 (63.2)	3 (1.2)		1 (0.4)	16 (6.6)	6 (2.5)

Table 13. **Correlation of biochemical patterns and OXA-type carbapenemase genes in 413 carbapenem-resistant *A. baumannii* isolates**

OXA-type carbapenemase gene	% of isolates in biochemical patterns (n)					
	A (8)	**B (242)**	**C (15)**	**D (146)**	**E (1)**	**F (1)**
OXA-51-like, OXA-23-like	75 (6)	82.2 (199)	100 (15)	91.8 (134)	100 (1)	100 (1)
OXA-51-like, OXA-24-like	12.5 (1)	1.2 (3)				
OXA-51-like, OXA-23-like, OXA-24-like	12.5 (1)	1.2 (3)		1.4 (2)		
OXA-51-like, OXA-23-like, OXA-58-like		2.9 (7)				
OXA-51-like, OXA-24-like, OXA-58-like		8.3 (20)		2.7 (4)		
OXA-51-like, OXA-23-like, OXA-24-like, OXA-58-like		4.1 (10)		4.1 (6)		

Figure 18. PCR products of OXA-type carbapenemase genes by multiplex PCR.



Lane M	Marker
Lane 1	$bla_{\text{OXA58-like}}$, $bla_{\text{OXA23-like}}$, $bla_{\text{OXA51-like}}$, $bla_{\text{OXA24-like}}$
Lane 2	$bla_{\text{OXA58-like}}$, $bla_{\text{OXA51-like}}$, $bla_{\text{OXA24-like}}$
Lane 3	$bla_{\text{OXA23-like}}$, $bla_{\text{OXA51-like}}$, $bla_{\text{OXA24-like}}$
Lane 4	$bla_{\text{OXA23-like}}$, $bla_{\text{OXA51-like}}$

Table 14. OXA-type carbapenemase genes in the 501 *A. baumannii* isolates.

Carbapenemase activity	$bla_{OXA\text{-}like}$	No. of isolates (%)	Imipenem MIC (mg/L)			Meropenem MIC (mg/L)		
			Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Negative	$bla_{OXA51\text{-}like}$	88 (17.6)	0.12-4	1	2	0.12-4	0.5	2
Positive	$bla_{OXA51\text{-}like}$, $bla_{OXA23\text{-}like}$	356 (71.1)	16->256	64	256	8-256	32	64
Positive	$bla_{OXA51\text{-}like}$, $bla_{OXA24\text{-}like}$	4 (0.8)	≥256	256	>256	64-256	128	256
Positive	$bla_{OXA51\text{-}like}$, $bla_{OXA23\text{-}like}$, $bla_{OXA24\text{-}like}$	6 (1.2)	128->256	256	>256	64->256	64	>256
Positive	$bla_{OXA51\text{-}like}$, $bla_{OXA23\text{-}like}$, $bla_{OXA58\text{-}like}$	7 (1.4)	32-128	64	128	16-32	32	32
Positive	$bla_{OXA51\text{-}like}$, $bla_{OXA24\text{-}like}$, $bla_{OXA58\text{-}like}$	24 (4.8)	128-256	256	256	32-256	64	128
Positive	$bla_{OXA51\text{-}like}$, $bla_{OXA23\text{-}like}$, $bla_{OXA24\text{-}like}$, $bla_{OXA58\text{-}like}$	16 (3.2)	128->256	256	256	32-128	64	128

Table 15. OXA-type carbapenemase genes associated with carbapenem resistance in the 413 imipenem-resistant *A. baumannii* isolates.

$bla_{OXA\text{-}like}$	No. of isolates (%)
$bla_{OXA23\text{-}like}$	385 (93.2)
$bla_{OXA24\text{-}like}$	50 (12.1)
$bla_{OXA58\text{-}like}$	47 (11.4)

Figure 19. Distribution of genes encoding OXA-type carbapenemases and imipenem MICs.

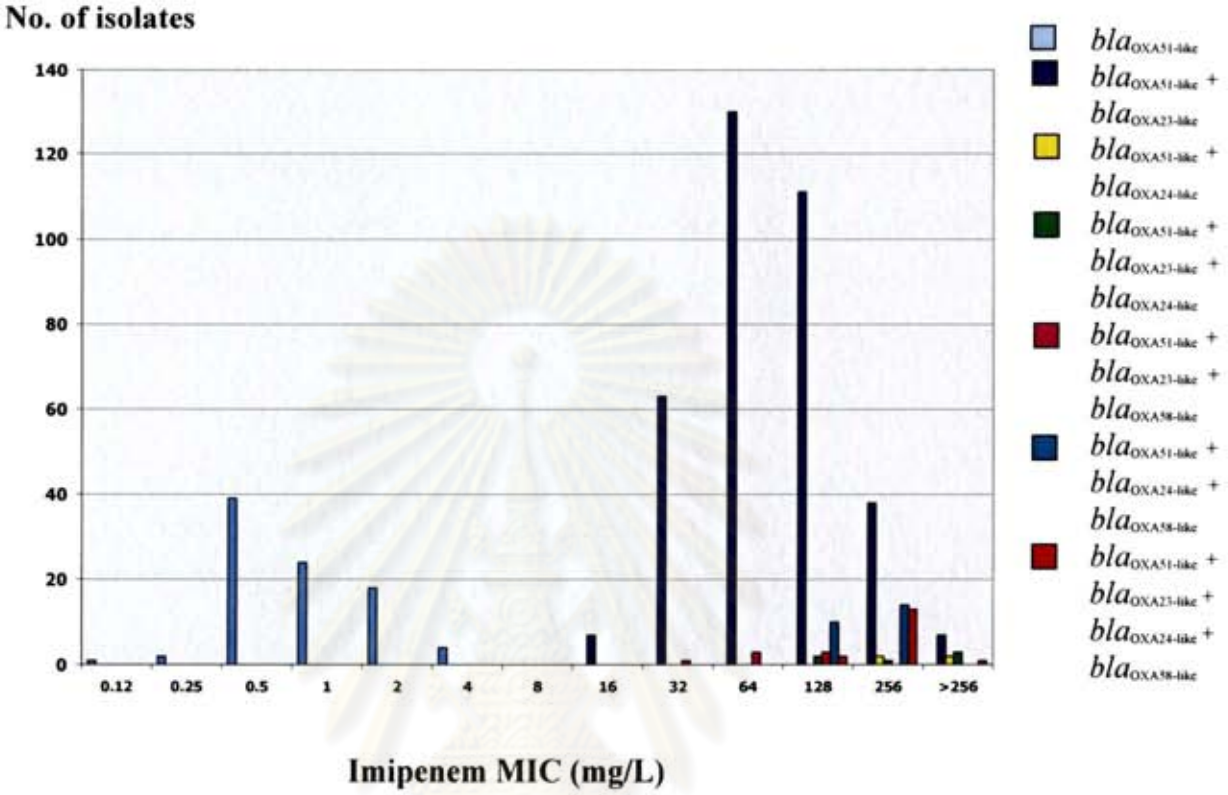
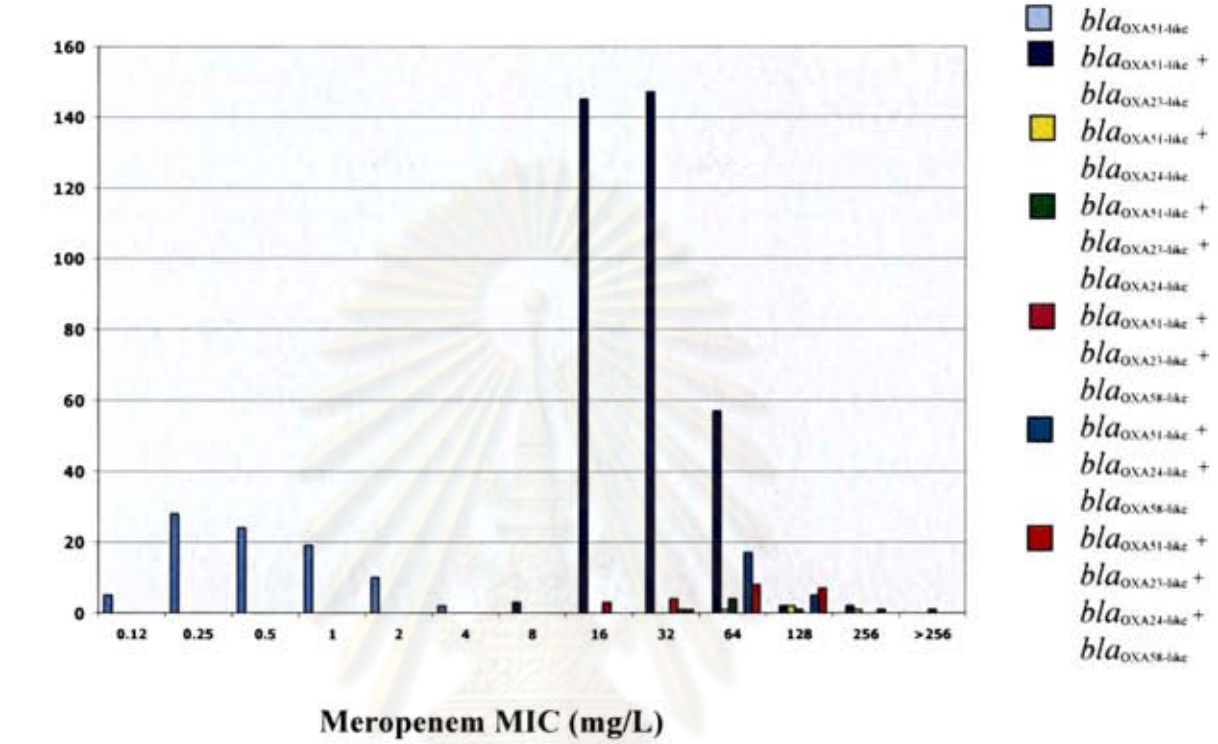


Figure 20. Distribution of genes encoding OXA-type carbapenemases and meropenem MICs.



DNA sequencing of entire bla_{OXA} genes

Entire $bla_{OXA23\text{-like}}$, $bla_{OXA24\text{-like}}$, $bla_{OXA51\text{-like}}$ and $bla_{OXA58\text{-like}}$ genes were amplified by PCR and sequenced in ten *A. baumannii* isolates carrying different $bla_{OXA\text{-like}}$ genes. Each $bla_{OXA\text{-like}}$ gene was amplified by PCR and then sequenced. DNA sequences were analysed by the software available over the Internet at the National Center for Biotechnology Information (http: // www.ncbi.nlm.nih.gov/BLAST), Multalin (www.toulouse.inra.fr/multalin.html), and ExPASy (www.expasy.org). The results are summarized in Table 16. Similar to studies by Walther *et al.*, Donald *et al.*, Afzal *et al.*, Bou *et al.*, Heritier *et al.*, Brown *et al.* and Poirel *et al.*, the nucleotide sequences of all $bla_{OXA\text{-like}}$ genes were belonged to class D β-lactamases which were attributed to the presence of three highly conserved active site elements (14, 22-24, 26, 33, 159). The first element contained the conserved serine active site motif S-T-F-K (DBL numbering 70-73).

The second element was the S-X-V triad (DBL numbering 118-120), which valine (V) was replaced by an isoleucine (I) in OXA-51-like enzymes. The third element was the K-T-G triad (DBL numbering 216-218) in OXA-23-like enzymes whereas it was replaced by K-S-G triad in OXA-24-like, OXA-51-like and OXA-58-like enzymes. The other conserved motif, Y-G-N motif (DBL numbering 144-146) was retained in OXA-51-like and OXA-58-like whereas it was replaced by F-G-N in OXA-23-like and OXA-24-like enzymes. (Figure 21.)

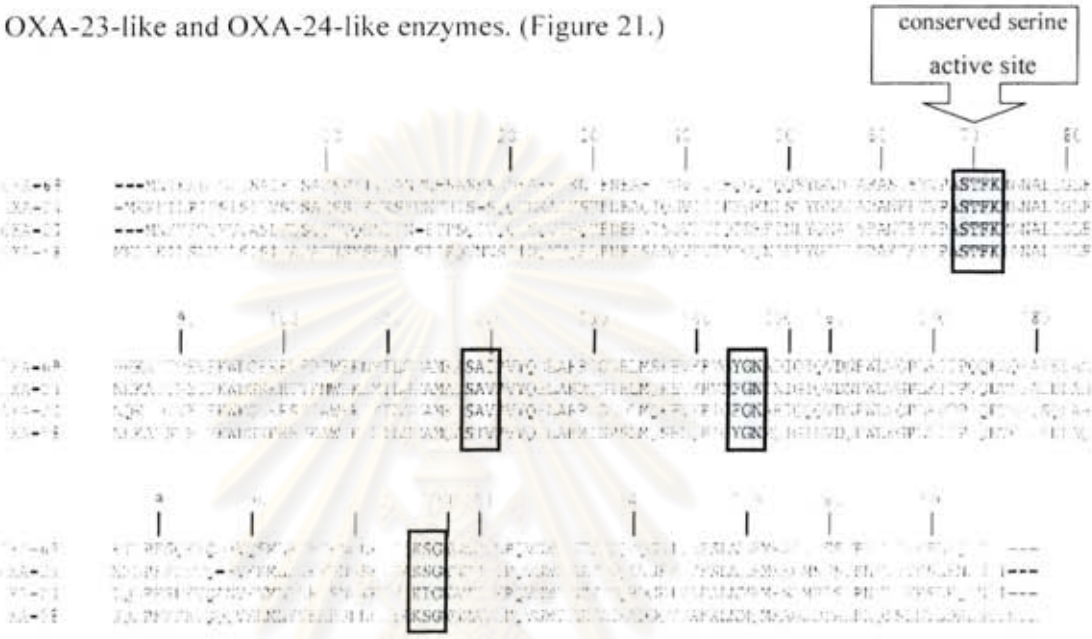


Figure 21. Alignment of amino acid sequences of four main groups of carbapenem-hydrolyzing class D β-lactamases (CHDLs) identified in *A. baumannii*, including OXA-23, OXA-24 and OXA-58 as representatives of the three groups of acquired CHDLs, and OXA-51 as a representative of the naturally occurring CHDLs. The conserved residues for oxacillinases are shaded and conserved residues for class D β-lactamases are blocked. Numbering of β-lactamases is according to Ambler class D β-lactamase numbering DBL (151).

All 10 representative *A. baumannii* isolates that were selected for $bla_{\text{OXA-23-like}}$ sequencing, A66, A67, 72, A266, A74, A150, A472, A446, A433 and A519, had imipenem MIC range of 16->256 mg/L. These isolates carried various

combinations of $bla_{\text{OXA-like}}$ genes (Table 16.1). DNA sequence analysis of the 1,065-bp fragments revealed an open reading frame of 822 bp, encoding a 271-amino-acid protein, which showed 100% nucleotide and amino acid sequences identity to $bla_{\text{OXA-23}}$ and OXA-23 enzyme, respectively (GenBank accession no. AJ132105 and CAB69042, respectively). OXA-23 enzyme shared 99% amino acid sequence identity to OXA-27 and OXA-49. Alignments of nucleotide sequences of OXA-23 and those from ten representative *A. baumannii* isolates are shown in Appendix E.

All 10 representative *A. baumannii* isolates that were selected for $bla_{\text{OXA-24-like}}$ sequencing, A150, A180, A471, A472, A242, A146, A519, A177, A288 and A446, had imipenem MIC range of 128->256 mg/L. These isolates carried various combinations of $bla_{\text{OXA-like}}$ genes (Table 16.2.). DNA sequence analysis of the 1,021-bp fragments revealed an open reading frame of 825 bp, encoding a 274-amino-acid protein, which showed 100% nucleotide and amino acid sequence identity to $bla_{\text{OXA-72}}$ and OXA-72 enzyme, respectively (GenBank accession no.AY739646 and AAU86900, respectively). OXA-72 enzyme shared 99% amino acid sequence identity to OXA-26, OXA-33 and OXA-40 (OXA24) whereas shared 98% amino acid sequence identity to OXA-25. Alignments of nucleotide sequences of OXA-24 and those from ten representative *A. baumannii* isolates are shown in Appendix E.

All 10 representative *A. baumannii* isolates that were selected for $bla_{\text{OXA-58-like}}$ sequencing, A266, A188, A262, A292, A180, A150, A471, A472, A242 and A446, had imipenem MIC range of 32-256 mg/L. These isolates carried various combinations of $bla_{\text{OXA-like}}$ genes (Table 16.4). DNA sequence analysis of the 933-bp fragments revealed an open reading frame of 841 bp, encoding a 280-amino-acid protein, which showed 100% nucleotide and amino acid sequence identity to $bla_{\text{OXA-58}}$ and OXA-58, respectively (GenBank accession no. AY570763 and AAT95987, respectively). This enzyme shared 99% amino acid sequence identity to OXA-96 and OXA-97. Alignments of nucleotide sequences of OXA-58 and those from ten representative *A. baumannii* isolates are shown in Appendix E.

All 10 representative *A. baumannii* isolates that were selected for $bla_{\text{OXA-51-like}}$ sequencing, A80, A405, A433, A74, A66, A266, A150, A471, A472 and A242, had imipenem MIC range of 0.5->256 mg/L. These isolates carried various combinations of $bla_{\text{OXA-like}}$ genes (Table 16.3.). DNA sequence analysis of the 1,416-bp amplicons revealed an open reading frame of 825 bp, encoding a 274-amino-acid protein, which showed 100% nucleotide and amino acid sequence identity to $bla_{\text{OXA-68}}$ and OXA-68 (5 isolates), $bla_{\text{OXA-66}}$ and OXA-66 (2 isolates), $bla_{\text{OXA-64}}$ and OXA-64 (1 isolate), $bla_{\text{OXA-67}}$ and OXA-67 (1 isolate) and showed 99% nucleotide sequence identity to $bla_{\text{OXA-51}}$ and OXA-51 (1 isolate).

The $bla_{\text{OXA-68}}$ was found in 5 *A. baumannii* isolates, A266, A150, A471, A472 and A242. A266 carrying $bla_{\text{OXA-51-like}}$ together with $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-58-like}}$ had imipenem MIC of 32 mg/L. A150, A471, A472 and A242 carrying $bla_{\text{OXA-51-like}}$ together with $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$ had imipenem MIC of 256 mg/L. OXA-68 shared 99% amino acid sequence identity to OXA-77, OXA-91, OXA-117 and OXA-128. Alignments of nucleotide sequences of $bla_{\text{OXA-68}}$ (GenBank accession no.AY750910) and those from five representative *A. baumannii* isolates are shown in Appendix E.

The $bla_{\text{OXA-66}}$ was presented in 2 *A. baumannii* isolates, A405 and A433. A405 carrying only $bla_{\text{OXA-51-like}}$, had imipenem MIC of 4 mg/L whereas A433 carrying $bla_{\text{OXA-51-like}}$ together with $bla_{\text{OXA-23-like}}$ had imipenem MIC of >256 mg/L. OXA-66 shared 99% amino acid sequence identity to OXA-65, OXA-76, OXA-79, OXA-80, OXA-82, OXA-83, OXA-84, OXA-88, OXA-90, OXA-109 and OXA-115. Alignments of nucleotide sequences of $bla_{\text{OXA-66}}$ (GenBank accession no.AY750909) and those from two representative *A. baumannii* isolates are shown in Appendix E.

The $bla_{\text{OXA-64}}$ was identified in one *A. baumannii* isolate, A74.This isolate carried $bla_{\text{OXA-51-like}}$ together with $bla_{\text{OXA-23-like}}$ and had imipenem MIC of 64 mg/L. OXA-64 shared 99% amino acid sequence identity to OXA-71 and 98% amino acid sequence identity to OXA-51, OXA-65, OXA-66, OXA-67, OXA-88, OXA-90, OXA-91, OXA-95, OXA-99, OXA-100, OXA-104, OXA-106, OXA-111 and

OXA-113. Alignments of nucleotide sequences of bla_{OXA-64} (GenBank accession no.AY750907) and those from A74 are shown in Appendix E.

The bla_{OXA-67} was found in one *A. baumannii* isolate, A66. This isolate carried $bla_{OXA-51-like}$ together with $bla_{OXA-23-like}$ and had imipenem MIC of 16 mg/L. OXA-67 shared 99% amino acid sequence identity to OXA-51, OXA-64, OXA-86, OXA-87, OXA-89 and OXA-100. Alignments of nucleotide sequences of bla_{OXA-67} (GenBank accession no.DQ491200) and those from A66 are shown in Appendix E.

Novel $bla_{OXA-51-like}$ was found in isolate A80 with imipenem MIC of 0.5 mg/L. Nucleotide and amino acid sequences showed 99% identity to bla_{OXA-51} and OXA-51 enzyme. The nucleotide sequences differed from bla_{OXA-51} (GenBank accession no.AJ309734) by two nucleotides. Cytosine (C) was replaced by adenosine (A) at position 152 and cytosine (C) was replaced by guanine (G) at position 388. This enzyme differed from OXA-51 (GenBank accession no.ABD47672) by two amino acids. The threonine (T) was replaced by lysine (K) at position 51 and the proline (P) was replaced by alanine (A) at position 130. All amino-acid changes were located outside the class D carbapenemase motifs. It shared 99% amino acid sequence identity to OXA-132, OXA-99, OXA-111, OXA-64 and OXA-95. The alignment of amino acid sequences of the novel OXA-51-like and these enzymes are shown in Figure 22. This novel enzyme shared 98% amino acid sequence identity to OXA-67, OXA-70, OXA-71, OXA-86, OXA-87, OXA-88, OXA-89, OXA-90, OXA-91, OXA-93, OXA-100, OXA-106, OXA-113, OXA-117 and OXA-130. Alignments of amino acid and nucleotide sequences of OXA-51 subgroup and those from A80 isolate are shown in Figure 23 and Appendix E, respectively.

Figure 22. Alignments of amino acid sequences of a novel OXA-51-like enzyme from A80 and OXA-51-like enzymes (OXA-51, OXA-64, OXA-95, OXA-99, OXA-111 and OXA-132) that shared 99% similarlity.

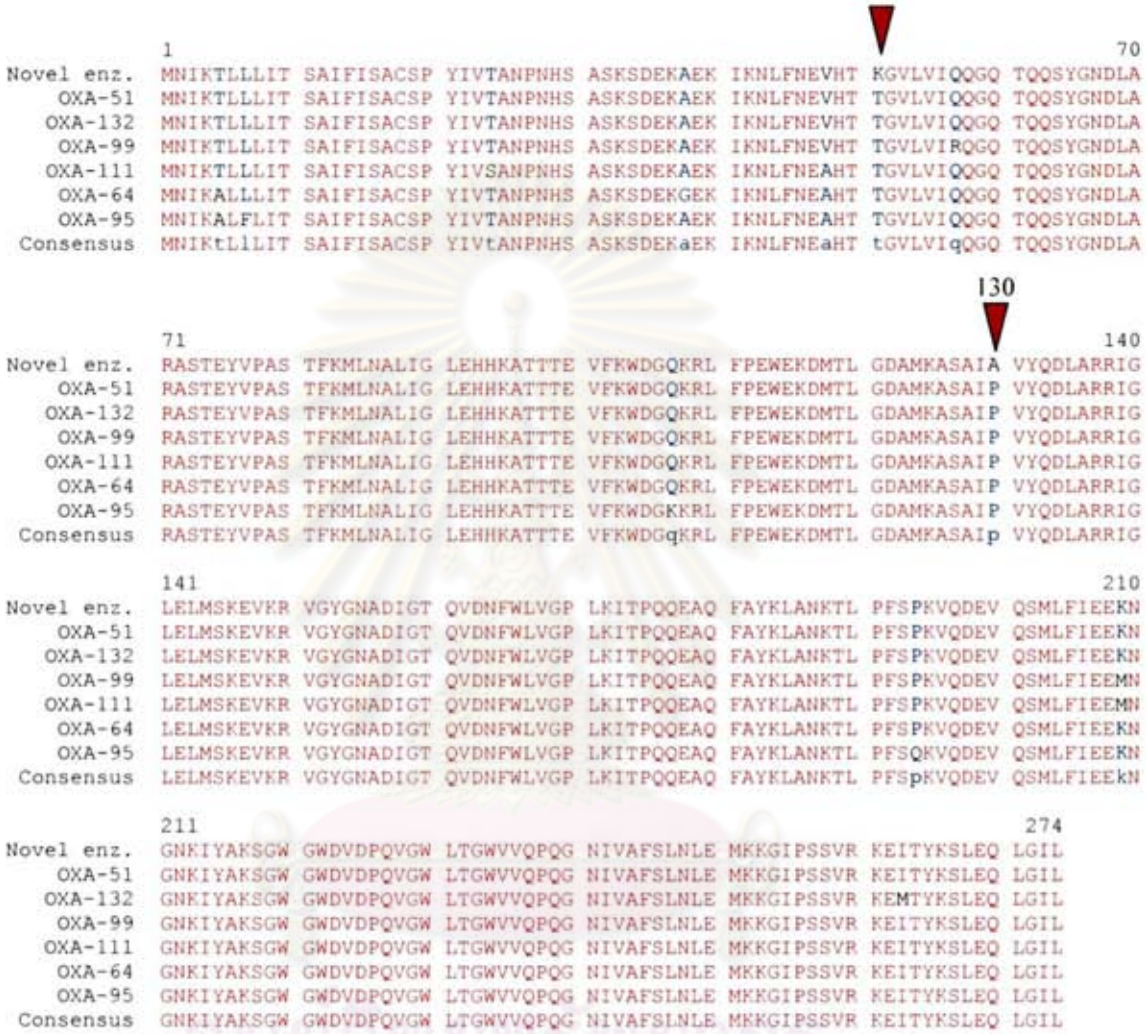


Table 16. Sequencing analysis of entire *bla*$_{\text{OXA-like}}$ genes.

Table 16.1. Sequencing analysis of *bla* $_{\text{OXA-23-like}}$ genes from 10 representative isolates.

Isolates	Imipenem MIC (mg/L)	Presence of *bla* $_{\text{OXA-like}}$ by multiplex PCR				*bla*$_{\text{OXA-23-type}}$ gene
		bla $_{\text{OXA-51-like}}$	*bla* $_{\text{OXA-23-like}}$	*bla* $_{\text{OXA-24-like}}$	*bla* $_{\text{OXA-58-like}}$	
A66	16	+	+	-	-	*bla*$_{\text{OXA-23}}$
A67	32	+	+	-	-	*bla*$_{\text{OXA-23}}$
A72	32	+	+	-	-	*bla*$_{\text{OXA-23}}$
A266	32	+	+	-	+	*bla*$_{\text{OXA-23}}$
A74	64	+	+	-	-	*bla*$_{\text{OXA-23}}$
A150	256	+	+	+	+	*bla*$_{\text{OXA-23}}$
A472	256	+	+	+	+	*bla*$_{\text{OXA-23}}$
A446	256	+	+	+	+	*bla*$_{\text{OXA-23}}$
A433	>256	+	+	-	-	*bla*$_{\text{OXA-23}}$
A519	>256	+	+	+	-	*bla*$_{\text{OXA-23}}$

Table 16.2. Sequencing analysis of *bla* $_{\text{OXA-24-like}}$ genes from 10 representative isolates.

Isolates	Imipenem MIC (mg/L)	Presence of *bla* $_{\text{OXA-like}}$ by multiplex PCR				*bla*$_{\text{OXA-24-type}}$ gene
		bla $_{\text{OXA-51-like}}$	*bla* $_{\text{OXA-24-like}}$	*bla* $_{\text{OXA-23-like}}$	*bla* $_{\text{OXA-58-like}}$	
A180	128	+	+	-	+	*bla*$_{\text{OXA-72}}$
A288	128	+	+	-	+	*bla*$_{\text{OXA-72}}$
A177	256	+	+	-	-	*bla*$_{\text{OXA-72}}$
A146	256	+	+	-	-	*bla*$_{\text{OXA-72}}$
A150	256	+	+	+	+	*bla*$_{\text{OXA-72}}$
A471	256	+	+	+	+	*bla*$_{\text{OXA-72}}$
A472	256	+	+	+	+	*bla*$_{\text{OXA-72}}$
A242	256	+	+	+	+	*bla*$_{\text{OXA-72}}$
A446	256	+	+	+	+	*bla*$_{\text{OXA-72}}$
A519	>256	+	+	+	-	*bla*$_{\text{OXA-72}}$

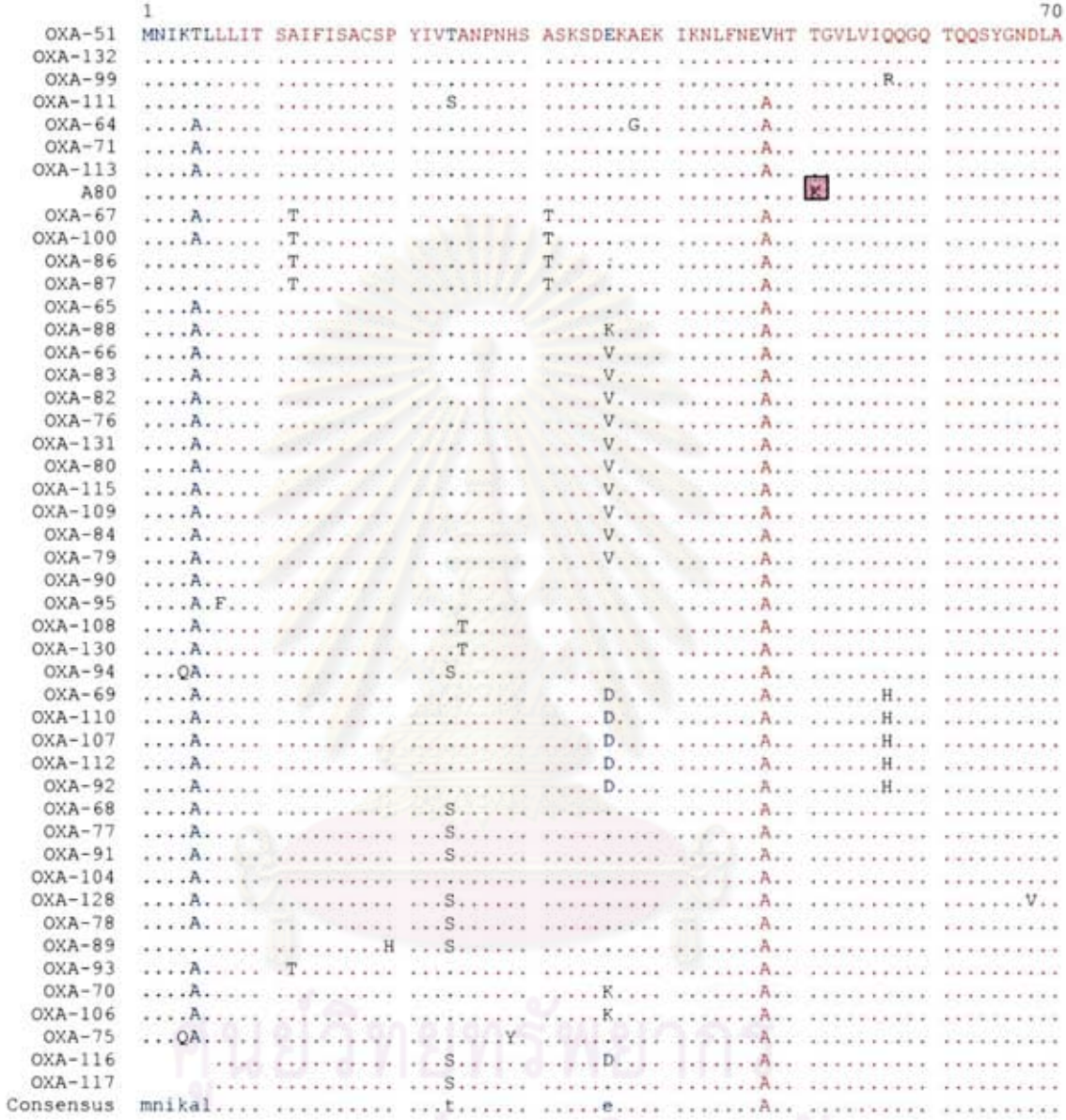
Table 16.3. Sequencing analysis of *bla* $_{\text{OXA-51-like}}$ genes from 10 representative isolates.

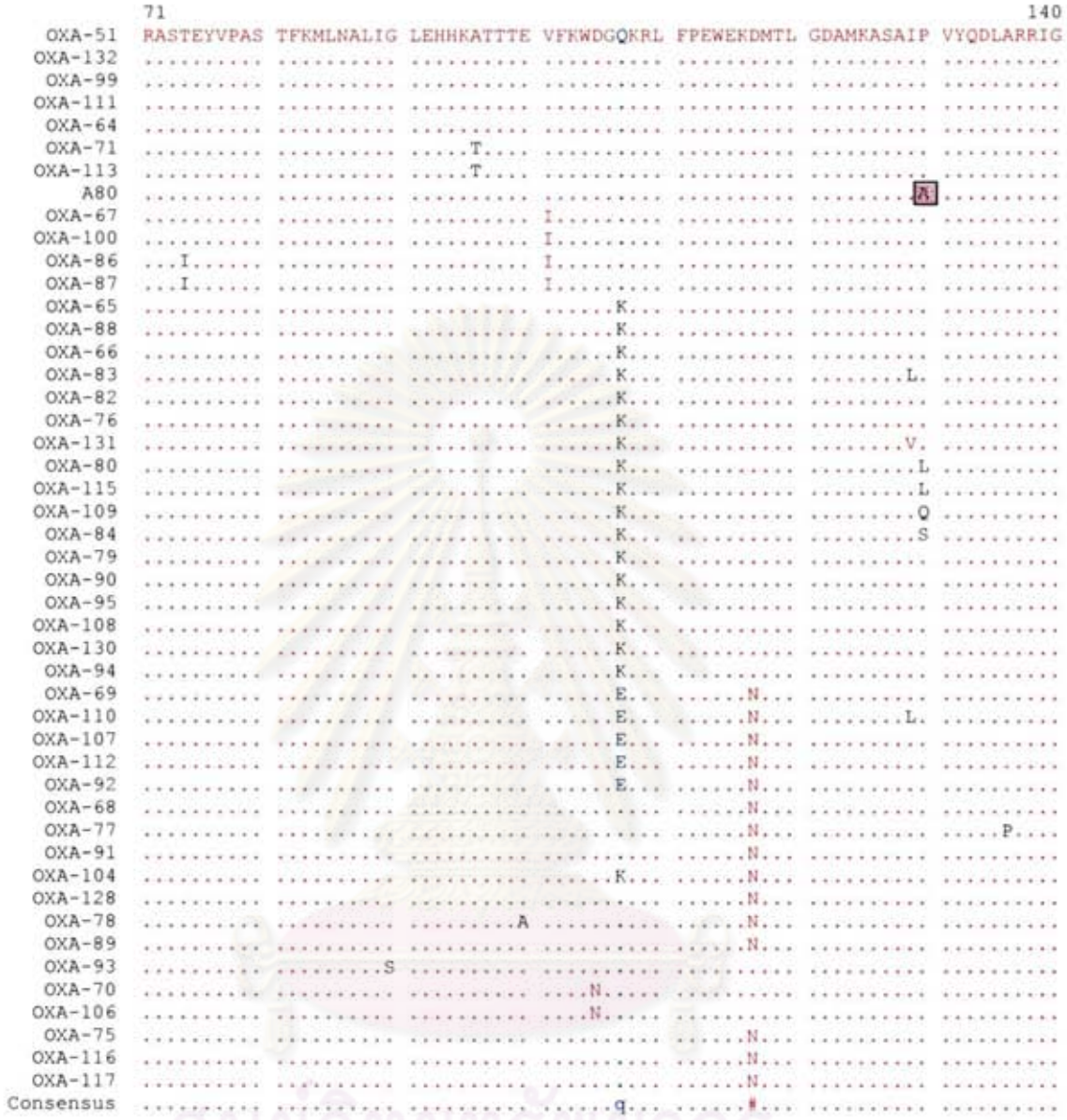
Isolates	Imipenem MIC (mg/L)	Presence of *bla* $_{\text{OXA-like}}$ by multiplex PCR				*bla*$_{\text{OXA-51-type}}$ gene
		bla $_{\text{OXA-51-like}}$	*bla* $_{\text{OXA-23-like}}$	*bla* $_{\text{OXA-24-like}}$	*bla* $_{\text{OXA-58-like}}$	
A80	0.5	+	-	-	-	novel *bla*$_{\text{OXA-51-like}}$
A405	4	+	-	-	-	*bla*$_{\text{OXA-66}}$
A66	16	+	+	-	-	*bla*$_{\text{OXA-67}}$
A266	32	+	+	-	+	*bla*$_{\text{OXA-68}}$
A74	64	+	+	-	-	*bla*$_{\text{OXA-64}}$
A150	256	+	+	+	+	*bla*$_{\text{OXA-68}}$
A471	256	+	+	+	+	*bla*$_{\text{OXA-68}}$
A472	256	+	+	+	+	*bla*$_{\text{OXA-68}}$
A242	256	+	+	+	+	*bla*$_{\text{OXA-68}}$
A433	>256	+	+	-	-	*bla*$_{\text{OXA-66}}$

Table 16.4. Sequencing analysis of *bla* $_{OXA-58-like}$ genes from 10 representative isolates.

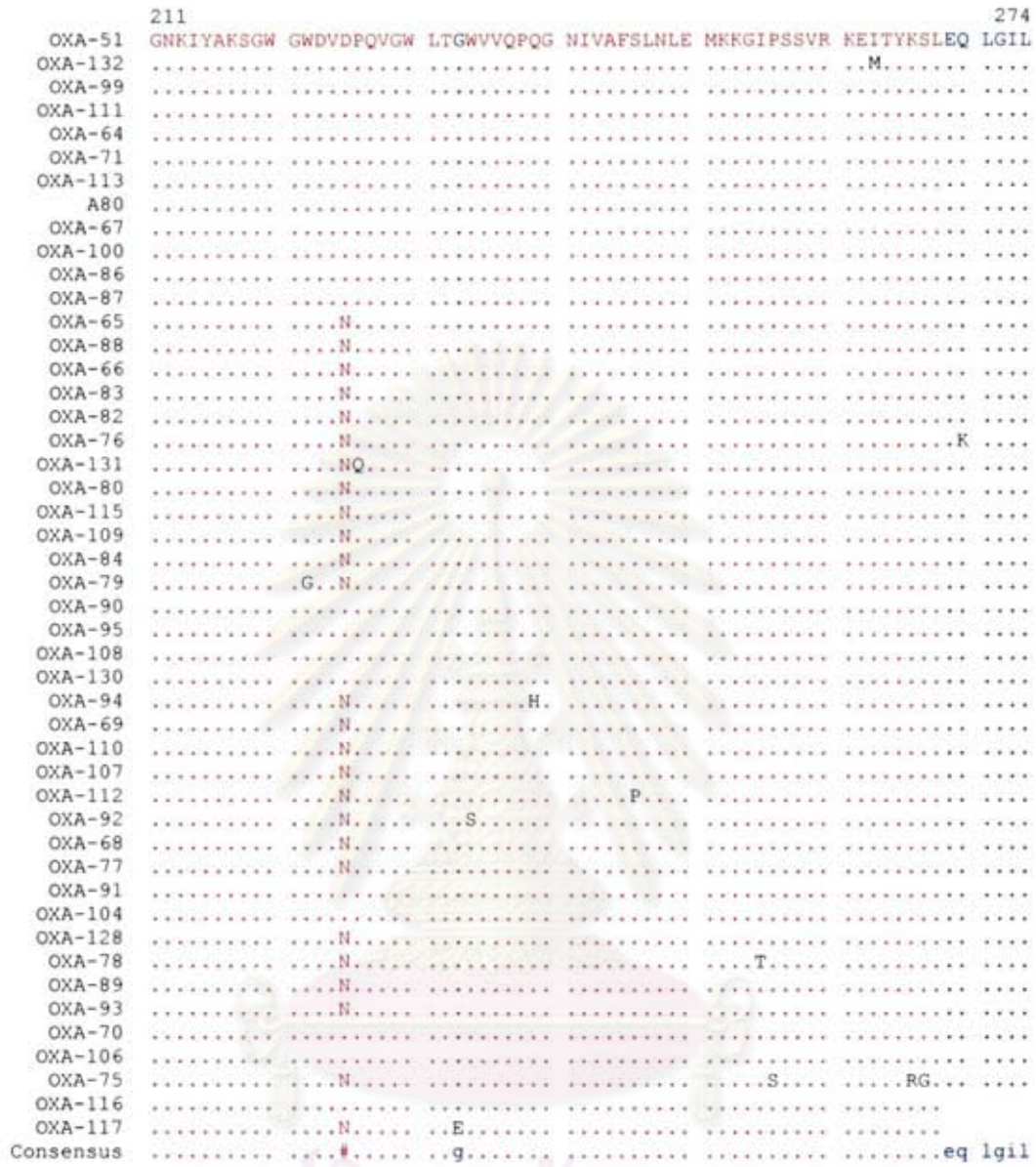
Isolates	Imipenem MIC (mg/L)	Presence of *bla* $_{OXA-like}$ by multiplex PCR				*bla*$_{OXA-58-type}$ gene
		bla $_{OXA-51-like}$	*bla* $_{OXA-58-like}$	*bla* $_{OXA-23-like}$	*bla* $_{OXA-24-like}$	
A266	32	+	+	+	-	*bla*$_{OXA-58}$
A188	64	+	+	+	-	*bla*$_{OXA-58}$
A262	64	+	+	+	-	*bla*$_{OXA-58}$
A292	64	+	+	+	-	*bla*$_{OXA-58}$
A180	128	+	+	-	+	*bla*$_{OXA-58}$
A150	256	+	+	+	+	*bla*$_{OXA-58}$
A471	256	+	+	+	+	*bla*$_{OXA-58}$
A472	256	+	+	+	+	*bla*$_{OXA-58}$
A242	256	+	+	+	+	*bla*$_{OXA-58}$
A446	256	+	+	+	+	*bla*$_{OXA-58}$

Figure 23. **Alignments of amino acid sequences of OXA-51 subgroup and that from A80 isolate.**





```
           141                                                                    210
OXA-51     LELMSKEVKR VGYGNADIGT QVDNFWLVGP LKITPQQEAQ FAYKLANKTL PFSPKVQDEV QSMLFIEEKN
OXA-132    .......... .......... .......... .......... .......... .......... ..........
OXA-99     .......... .......... .......... .......... .......... .......... ........M.
OXA-111    .......... .......... .......... .......... .......... .......... ........M.
OXA-64     .......... .......... .......... .......... .......... .......... ..........
OXA-71     .......... .......... .......... .......... .......... .......... ..........
OXA-113    .......... .......... ......V... .......... .......... .......... ..........
A80        .......... .......... .......... .......... .......... .......... ..........
OXA-67     .......... .......... .......... .......... .......... ...L...... ..........
OXA-100    .......... .......... .......... .......... .......... ...Q...... ..........
OXA-86     .......... .......... .......... .......... .......... ...L...... ..........
OXA-87     .......... .......... .......... .......... .......... ...L.A.... ..........
OXA-65     .......... .......... .......... .......... .......... ...Q...... ..........
OXA-88     .......... .......... .......... .......... .......... ...Q...... ..........
OXA-66     .......... .......... .......... .......... .......... ...Q...... ..........
OXA-83     .......... .......... .......... .......... .......... ...Q...... ..........
OXA-82     .......... .......... ......V... .......... .......... ...Q...... ..........
OXA-76     .......... .......... .......... .......... .......... ...Q...... ..........
OXA-131    .......... .......... .......... .......... .......... ...Q...... ..........
OXA-80     .......... .......... .......... .......... .......... ...Q...... ..........
OXA-115    .......... .......... ......V... .......... .......... ...Q...... ..........
OXA-109    .......... .......... .......... .......... .......... ...Q...... ..........
OXA-84     .......... .......... .......A.. .......... .......... ...Q...... ..........
OXA-79     .......... .......... .......... .......... .......... ...Q...... ..........
OXA-90     .......... .......... .......... .......... .......... ...Q...... ..........
OXA-95     .......... .......... .......... .......... .......... ...Q...... ..........
OXA-108    .......... .....T.... ......V... .......... .......... ...Q...... ..........
OXA-130    .......... .....T.... .......... .......... .......... ...Q...... ..........
OXA-94     .......... .......... .......... .......... .......... ...Q...... ..........
OXA-69     .......... .......... .......... .......... .......... ...Q...... ..........
OXA-110    .......... .......... .......... .......... .......... ...Q...... ..........
OXA-107    .......... .......... ......V... .......... .......... ...Q...... ..........
OXA-112    .......... .......... .......... .......... .......... ...Q...... ..........
OXA-92     .......... .......... .......... .......... .......... ...Q...... ..........
OXA-68     .....N.... .......... .......... .......... .......... ...QE..... ..........
OXA-77     .....N.... .......... .......... .......... .......... ...Q...... ..........
OXA-91     .....N.... .......... .......... .......... .......... ...Q...... ..........
OXA-104    .....N.... .......... .......... .......... .......... ...Q...... ..........
OXA-128    .....N.... .......... .......... .......... .......... ...QE..... ..........
OXA-78     .....N.... .......... .......... .......... .......... ...Q...... ..........
OXA-89     .....N.... .......... .......... .......... .T........ ...Q...... ..........
OXA-93     .....N.... .......... .......... .......... .......... ...Q...... ..........
OXA-70     .....N.... .......... .......... .......... .......... ...Q...H.. ..........
OXA-106    .....N.... .......... .......... .......... .......... ...Q...... ..........
OXA-75     .....N.... I......... .......... .......... .......... ...Q...... ..........
OXA-116    .....N.... .......... .......... .......... .......... ...Q...... ..........
OXA-117    .....N.... .......... .......... .......... .......... ...Q...... ..........
Consensus  .....k.... .......... .......... .......... .......... ...q...... ..........
```



CHAPTER VI

DISCUSSION

Antibiotic-resistant *A. baumannii* is a major problem worldwide because it has become resistant to almost all currently available antimicrobial agents. Carbapenems are a class of β-lactam antibiotics with a broad spectrum of antibacterial activity, and have a structure which renders them highly resistant to β-lactamase. In 2007, imipenem resistantce rate of *A. baumannii* collected from Chiang Mai University Hospital and Songklanagarind Hospital were 35.5% and 34.7%, respectively (49, 50). Among imipenem-resistant *A. baumannii* isolated from Songklanagarind Hospital, 61.9% were from respiratory tract, 49.9% from ICU and 14.8% were resistant to all routine tested antibiotics. In 2003, the imipenem resistance rate of *A. baumannii* was 61% at Police General Hospital, whereas those was 68% at Siriraj Hospital in 2006 (51, 52). Among carbapenem-resistant isolates, 57% were resistant to all antimicrobials currently available in Thailand. The data from the National Antimicrobial Resistance Surveillance Center Thailand (NARST) showed that imipenem resistance in *Acinetobacter calcoaceticus-baumannii* complex increased from 2% in 1998 to 57% in 2006 (53). In this study, prevalence of carbapenem resistance in *A. baumannii* isolated from patients at King Chulalongkorn Memorial Hospital, Bangkok during January 2004 to August 2007 was 82.4% for imipenem and 81.8% for meropenem, which were higher than those reported by other studies in Thailand. This may be because King Chulalongkorn Memorial Hospital is a referral hospital. Transferring of severe patients from other hospitals may be resulting in increasing in the prevalence of carbapenem resistance in *A. baumannii*.

The carbapenem resistance rates from this study were also higher than other countries. In 2005, both imipenem and meropenem resistance rates of *A. baumannii* collected from Shanghai were 6.3% while those from Hong Kong were 2.7% and 10.8%, respectively (138). Another surveillance program showed carbapenem-resistant rate increased from 4.5% in 2003 to 18.2% in 2004 (191). The carbapenem-resistant rates in 3,601 isolates of *A. baumannii* from more than 311

U.S. hospitals surveyed by the NCCLS, increased from 9% in 1995 to 40% in 2004 (196). In 2008, carbapenem-resistant rate in *A. baumanii* isolated from Greece was 70.1% (197). High prevalence of carbapenem resistance on this study may be because of high consumption of carbapenem for treatment of *A. baumannii* and other bacteria.

For infections caused by carbapenem-resistant *A. baumannii* isolates, antibiotic choices may be quite limited because this organism always resist to multiple antibiotics. Antimicrobial combination therapy appears to be a reasonable alternative for treatment of carbapenem-resistant *A. baumannii* isolates. Combination of imipenem and amikacin, colistin and rifampin, imipenem combined with rifampin and colistin are suggested (111, 112). Tigecycline was used for treatment of this organism, but high level of tigecycline resistance were recently reported (117, 119, 123). In addition, sulperazone may be used to treat carbapenem-resistant *A. baumannii* isolates (125).

The imipenem resistance rates of *A. baumannii* isolates in ICUs and non ICUs were 90.3% and 74.1%, whereas meropenem resistance rates of those were 89.5% and 73.7%, respectively. The carbapenem resistance rate in *A. baumannii* isolates from ICUs was significantly higher than that in non ICUs ($P<0.01$). High carbapenem resistance rate in ICUs is similar to study by Bogaerts *et al.* and Souli *et al.* that found the carbapenem resistance rate of *A. baumannii* in ICUs were 70.6% and 84%, respectively (186, 198). In contrast to this study, Sinha *et al.* found that meropenem resistance rate of *Acinetobacter* spp. in ICUs and other wards were 42.8% and 57.2%, respectively (199). In this study, the carbapenem resistance in ICUs was higher than non-ICUs, which similar to study by Falagas *et al.* that found imipenem resistance rate of *A. baumannii* in ICUs medical wards was 91% whereas those in medical wards and surgical wards were 71% (139). Higher carbapenem resistance rate of *A. baumannii* isolates from ICUs may be because carbapenem-resistant isolates are selected by increased use of carbapenems in severe patients. *A. baumannii* can colonize in the environment in ICU such as contaminated equipment including ventilator, bed rail, pillow and bed. Transmission of microorganism also occurred via hand of staff. In addition, transferring of severed

and trauma patients from other hospitals also associated to the high prevalence of carbapenem resistance in ICUs.

Identification and elimination of common source of *A. baumannii* reservoir are most important for infection control of *A. baumannii* in the hospital. Outbreak managements required an active infection-control programme for patients and medical personel, including contact isolation to prevent transmission as well as active surveillance cultures to identify *A. baumannii*-colonized patients. Improved hand hygiene, cleaning and disinfection of the environment, including equipment decontamination, cohort nursing, the isolation of patients in single room, the restriction of access to the ICU, control of antibiotic usage, improving staffing ratio and closing a ward for cleaning and disinfection, can be used to control the spread of *A. baumannii* in hospital.

In our study, $bla_{\text{OXA-51-like}}$ was detected in all carbapenem-susceptible and -resistant *A. baumannii* isolates. This is similar to the studies of Turton *et al.*, Merkier *et al.*, Heritier *et al.* and Wang *et al.* which reported that $bla_{\text{OXA-51-like}}$ were found in all carbapenem-susceptible and resistant *A. baumannii* isolates (27, 28, 30, 200). The results suggested that OXA-51-like enzymes were very poor carbapenemases and may be natural and chromosomally encoded in all *A. baumannii* isolates. However, Tsakris *et al.* and Koh *et al.* found $bla_{\text{OXA-51-like}}$ in 90.9% and 77.3% of *A. baumannii* isolates, respectively (18, 31).

The OXA-51 was first described in imipenem-resistant *A. baumannii* clones from Argentina and was the largest and most diverse collection of class D carbapenemases in *A. baumannii* (26). To date, forty-six enzymes of this group have been reported. In the present study, the variations were found in $bla_{\text{OXA-51-like}}$ group with 50% of $bla_{\text{OXA-68}}$, 20% of $bla_{\text{OXA-66}}$, 10% each for $bla_{\text{OXA-64}}$, $bla_{\text{OXA-67}}$ and a novel $bla_{\text{OXA-51-like}}$. OXA-64, OXA-67 and OXA-68 were reported in *A. baumannii* isolated from South Africa, Argentina and Spain, respectively (25), whereas OXA-66 was reported in Spain, Greece and China (25, 197, 200). The sequences of entire $bla_{\text{OXA-51-like}}$ in the isolate A80 was 99% similar to $bla_{\text{OXA-51}}$ and differed by two nucleotides, resulting in two amino acid changes at positions 51 and

130. This novel enzyme has conserved sequences of class D β-lactamases. However, biochemical properties and susceptibility patterns of this enzyme have to be further investigated.

The presence of $bla_{\text{OXA-23-like}}$ were detected in the majority of our carbapenem-resistant *A. baumannii* isolates (93.2%). The results suggested that $bla_{\text{OXA-23-like}}$ plays an important role in carbapenem resistance in this study. Similar to many studies, $bla_{\text{OXA-23-like}}$ was predominant in carbapenem-resistant *A. baumannii* isolates (11, 18, 37, 193, 201). Wang *et al.* found $bla_{\text{OXA-23-like}}$ in 97.7% of imipenem-resistant *A. baumannii* isolates, and both Zong *et al.* and Stoeva *et al.* found in 100% of imipenem-resistant *A. baumannii* isolates (200-202). Imipenem MICs of these isolates varied from 16 to >256 mg/L. This may be due to the involvement of other resistance mechanisms such as impermeability of outer membrane proteins or loss of porin and efflux system. Heritier *et al.* found that overexpression of AdeABC efflux pump associated with expression of these oxacillinases induced a higher level of carbapenem resistance (10).

In this study, $bla_{\text{OXA-23}}$ in carbapenem-resistant *A. baumannii* was amplified and sequenced from plasmid DNA. There were many reports demonstrated that $bla_{\text{OXA-23}}$ is plasmid-encoded (14, 163). Villegas reported that $bla_{\text{OXA-23}}$ was chromosomal encoded in three of four clones of carbapenem-resistant *A. baumannii* isolates and plasmid encoded in one clone (19). In addition, Wang *et al.*, Meric *et al.* and Stoeva *et al.* also found that $bla_{\text{OXA-23}}$ was chromosomally encoded (200, 201, 203). Therefore, the gene location of this gene has to be further investigated.

OXA-23 group consists of OXA-23, OXA-27 and OXA-49. In the present study, $bla_{\text{OXA-23}}$ was detected in all ten representative *A. baumannii* isolates by sequencing of entired $bla_{\text{OXA-23-like}}$. OXA-23 enzyme was first described in an imipenem-resistant *A. baumannii* isolated from Scotland in 1985 (14). From late 2003 to the end of 2005, the dissemination of two carbapenem-resistant clones with OXA-23 carbapenemase at multiple hospitals in London and Southeast England were reported (20). OXA-23-producing in carbapenem-resistant *A. baumannii* isolates have also been reported in Brazil (100%) and French Polynesia (100%),

China (100%) and Korea (69%) (11, 15, 16, 202). Wang *et al.* also found OXA-23-producing in 97.7% of imipenem-resistant *A. baumannii* (200). In addition, this enzyme was also reported in Singapore (18), South America (19) and Australia (21). OXA-27 was reported in one isolate of carbapenem-resistant *A. baumannii* isolated from Singapore (23).

The MIC level to imipenem in strains carrying $bla_{\text{OXA-51-like}}$ together with $bla_{\text{OXA-24-like}}$ were to ≥256 mg/L. The MIC range for isolates carrying $bla_{\text{OXA-24-like}}$ together other bla_{OXA} were 128->256 mg/L for imipenem, 32->256 mg/L for meropenem. These suggested that $bla_{\text{OXA-24-like}}$ confered a high level of MICs to carbapenems and played an importance role in carbapenem resistance in these isolates. Afzal-Shah *et al.* demonstrated that the hydrolytic activity to imipenem of OXA-25 and OXA-26 were higher than those of OXA-27, so leading to higher imipenem MIC than others (23).

The OXA-24 cluster consists of OXA-24, OXA-25, OXA-26, OXA-33, OXA-40 and OXA-72. The OXA-24 enzyme was first described in carbapenem-resistant *A. baumannii* isolated from Spain (22). The nucleotide sequences of this enzyme were corrected in 2006 by the same researchers, resulting in amino acid sequences identical to OXA-40 described in 2003 (204). In the present study, OXA-72 was detected in all ten representative *A. baumannii* isolates. OXA-72 was also detected in one isolate of imipenem-resistant *Acinetobacter* genomospecies 3 and *Acinetobacter* spp. isolated from China (200).

There were many reports previously demonstrated that $bla_{\text{OXA-24}}$ is chromosomally encoded (22, 24). However, Lolans *et al.* investigated the $bla_{\text{OXA-40}}$ gene location and found that it was on both chromosome and plasmid in imipenem-resistant *A. baumannii* isolates (117). In this study, $bla_{\text{OXA-24}}$ in carbapenem-resistant *A. baumannii* was amplified and sequenced from plasmid DNA. It could not be amplified from chromosomal DNA. So, the gene location of this gene has to confirm by other techniques such as Southern blot analysis.

In this study, there were isolate carrying $bla_{\text{OXA-58-like}}$ together with $bla_{\text{OXA-51-like}}$ and other bla_{OXA}. So, the activity of OXA-58 could not be discussed. Tsakris *et al.* found that *A. baumannii* isolates carrying $bla_{\text{OXA-58}}$ showed low level of imipenem MIC (31). This is similar to the study by Poirel *et al.* that OXA-58 showed low hydrolysis to imipenem (33). In addition, Heritier *et al.* demonstrated that OXA-58 exhibited weak carbapenemase activity and played a role in carbapenem resistance in *A. baumannii* when $bla_{\text{OXA-58}}$ was highly expressed (10). Bertini *et al.* also found that the increase in carbapenem resistance level was associated with an increased production of OXA-58 by multiple copies of the $bla_{\text{OXA-58}}$ gene (205).

The OXA-58 group consists of OXA-58, OXA-96 and OXA-97. In the present study, OXA-58 was detected in all ten representative *A. baumannii* isolates. This enzyme was first described in carbapenem-resistant *A. baumannii* isolated from France in 2005 (33). At the same time, it was detected in seven out of eight carbapenem-resistant *A. baumannii* by Heritier *et al.* (143). Marque *et al.* found *A. baumannii* carrying $bla_{\text{OXA-58}}$ gene in 22 out of 42 carbapenem-resistant *A. baumannii* isolated from France, Spain, Turkey and Romania (13). Both $bla_{\text{OXA-58}}$ and $bla_{\text{OXA-51}}$ were detected in many *A. baumannii* isolated from ICU in Greece (31, 144). Coelho *et al.* also found *Acinetobacter* spp. carrying $bla_{\text{OXA-58}}$ and $bla_{\text{OXA-51}}$ isolated from Argentina, Kuwait and United Kingdom over a 10 year period in 2006 (206). The $bla_{\text{OXA-58}}$ also was detected in 7 imipenem-resistant *A. baumannii* isolated from China (200). Between October 2003 and June 2004, $bla_{\text{OXA-58}}$ was detected in 44 of 45 *A. baumannii* isolated from Italy (140). In addition, between April 2005 and March 2007, this gene also was detected in 26 of 31 *A. baumannii* isolated from ICU patients in Greece (207). The $bla_{\text{OXA-96}}$ was detected in one imipenem-resistant *A. baumannii* isolated from Singapore (18). Poirel *et al.* found $bla_{\text{OXA-97}}$ in 19 of 39 carbapenem-resistant *A. baumannii* isolated from Tunisia (34).

Among carbapenem-resistant *A. baumannii* isolates, 4 isolates carried $bla_{\text{OXA-23-like}}$ together with $bla_{\text{OXA-58-like}}$, 4 isolates carried $bla_{\text{OXA-24-like}}$ together with $bla_{\text{OXA-58-like}}$ and 4 isolates carried $bla_{\text{OXA-23-like}}$ together with $bla_{\text{OXA-24-like}}$ and

bla$_{OXA\text{-}58\text{-}like}$ were isolated from patients at the same period of time and the same ward. The results suggested that clonal spread of carbapenem-resistant *A. baumannii* may involve and should be further investigated by genotyping such as pulse field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD).

Unlike OXA-type carbapenemase, metallo β-lactamases-producing *A. baumannii* is less than OXA-type-producing isolates. In this study, the metallo-β-lactamase was not detected in all 501 *A. baumannii* isolates by EDTA-disk synergy test and by PCR amplification of *bla*$_{IMP\text{-}like}$ and *bla*$_{VIM\text{-}like}$. This was in contrast to the study by Sung *et al.* that found high prevalence of metallo-β-lactamase-producing *A. baumannii* isolates (208). Among carbapenemase-producing *A. baumannii*, 69.6% was metallo-β-lactamase whereas 30.4% was OXA-type carbapenemase. IMP-1 and VIM-2 were found in 93.7% and 6.3% of metallo β-lactamase-producing isolates whereas OXA-23 was detected in all carbapenemase-producing isolates. In contrast, Koh *et al.* reported that OXA-type carbapenemase-producing *A. baumannii* was found higher than metallo-β-lactamase-producing isolates. OXA-51-like was found in 77.3% of *A. baumanni* isolates and OXA-23 was found in 91.1% of carbapenemase-producing *A. baumannii* isolates whereas IMP-4 was found in 9.1% of those isolates (18). This study suggests none or rare incidence of metallo-β-lactamase dissemination in carbapenem-hydrolyzing *A. baumannii* isolates in this study.

CHAPTER VII

CONCLUSION

Carbapenems are among the drugs of choice for treatment of multidrug-resistant *Acinetobacter baumannii* infections. However, emergence of carbapenem resistance in *A. baumannii* has been reported worldwide. In the present study, the imipenem and meropenem-resistant rates in 501 *A. baumannii* were 82.4% and 81.8%, respectively. MIC_{50} for imipenem and meropenem were 64 and 32 mg/L, respectively. MIC_{90} for imipenem and meropenem were 256 and 64 mg/L, respectively. Imipenem-resistant rates of ICUs and non-ICUs isolates were 90.3% and 74.1%, respectively, whereas meropenem-resistant rates were 89.5% and 73.7%, respectively. Imipenem-resistant rates of isolates from sterile and non-sterile specimens were 83.5% and 82.2%, respectively, while meropenem-resistant rates of those were 82.5% and 81.7%, respectively. Carbapenem-resistant rates of *A. baumannii* isolated from patients in ICUs were significantly higher than those in non-ICUs ($P < 0.01$) whereas there was no significant difference in carbapenem-resistant rates in *A. baumannii* isolated from sterile and non sterile clinical specimens ($P = 0.758$).

All carbapenem-resistant *A. baumannii* had carbapenemase activity. Metallo-β-lactamases were not detected in any isolate. The strains with carbapenemase activity were 100% resistant to imipenem whereas 99.3% were resistant and 0.7% were intermediately resistant to meropenem. Screening for carbapenemase genes by multiplex PCR revealed the presence of $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$ and $bla_{\text{OXA-58-like}}$ and the absence of $bla_{\text{IMP-like}}$ and $bla_{\text{VIM-like}}$. All 501 *A. baumannii* isolates carried the $bla_{\text{OXA51-like}}$ gene. The isolates carrying only $bla_{\text{OXA51-like}}$ were susceptible to carbapenems whereas the isolates carrying $bla_{\text{OXA-51-like}}$ together with other $bla_{\text{OXA-like}}$ including $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$ were resistant to carbapenems. Imipenem MIC of isolates carrying $bla_{\text{OXA-24-like}}$ together with other $bla_{\text{OXA-like}}$ showed a high level of carbapenem MIC(128->256 mg/L). The presence of $bla_{\text{OXA-23-like}}$ were detected in

the majority of carbapenem-resistant *A. baumannii* isolates (93.2%), suggesting that $bla_{\text{OXA-23-like}}$ plays a major role in carbapenem resistance.

Sequences of ten entire $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$ and $bla_{\text{OXA-58-like}}$ were analyzed. The results revealed that $bla_{\text{OXA-23}}$, $bla_{\text{OXA-72}}$ and $bla_{\text{OXA-58}}$ were identified in 100% of $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-58-like}}$ groups, respectively. The variations were found in $bla_{\text{OXA-51-like}}$ group with 50% of $bla_{\text{OXA-68}}$, 20% of $bla_{\text{OXA-66}}$, 10% each for $bla_{\text{OXA-64}}$, $bla_{\text{OXA-67}}$ and a novel $bla_{\text{OXA-51-like}}$. This novel enzyme was found in the isolate A80, which has not yet been reported. Nucleotide and amino acid sequences of this novel enzyme showed 99% identity to $bla_{\text{OXA-51}}$ and OXA-51. This study demonstrated high prevalence of carbapenem resistance in *A. baumannii* isolates and showed that $bla_{\text{OXA-23-like}}$ was the major carbapenemase gene associated with carbapenem resistance in our *A. baumannii*-resistant isolates.

REFERENCES

(1)Van Looveren, M., and Goossens, H. Antimicrobial resistance of *Acinetobacter* spp. in Europe. Clin Microbiol Infect 2004;10(8):684-704.

(2)Hsueh, P. R., et al. Pandrug-resistant *Acinetobacter baumannii* causing nosocomial infections in a university hospital, Taiwan. Emerg Infect Dis 2002;8(8):827-32.

(3)Landman, D., et al. Citywide clonal outbreak of multiresistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in Brooklyn, NY: the preantibjotic era has returned. Arch Intern Med 2002;162(13):1515-20.

(4)Martin-Lozano, D., et al. Comparison of a repetitive extragenic palindromic sequence-based PCR method and clinical and microbiological methods for determining strain sources in cases of nosocomial *Acinetobacter baumannii* bacteremia. J Clin Microbiol 2002;40(12):4571-5.

(5)Maniatis, A. N.; Pournaras, S.; Orkopoulou, S.; Tassios, P. T., and Legakis, N. J. Multiresistant *Acinetobacter baumannii* isolates in intensive care units in Greece. Clin Microbiol Infect 2003;9(6):547-53.

(6)Gunseren, F., et al. A surveillance study of antimicrobial resistance of gram-negative bacteria isolated from intensive care units in eight hospitals in Turkey. J Antimicrob Chemother 1999;43(3):373-8.

(7)Aksaray, S., et al. Surveillance of antimicrobial resistance among gram-negative isolates from intensive care units in eight hospitals in Turkey. J Antimicrob Chemother 2000;45(5):695-9.

(8)Tambic, T.; Kalenic, S., and Jankovic, V. Surveillance for antimicrobial resistance in Croatia. Emerg Infect Dis 2002;8(1):14-8.

(9)Turner, P. J., and Greenhalgh, J. M. The activity of meropenem and comparators against *Acinetobacter* strains isolated from European hospitals, 1997-2000. Clin Microbiol Infect 2003;9(6):563-7.

(10)Heritier, C.; Poirel, L.; Lambert, T., and Nordmann, P. Contribution of acquired carbapenem-hydrolyzing oxacillinases to carbapenem resistance in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2005;49(8):3198-202.

(11)Naas, T.; Levy, M.; Hirschauer, C.; Marchandin, H., and Nordmann, P. Outbreak of carbapenem-resistant *Acinetobacter baumannii* producing the carbapenemase OXA-23 in a tertiary care hospital of Papeete, French Polynesia. J Clin Microbiol 2005;43(9):4826-9.

(12)Afzal-Shah, M.; Villar, H. E., and Livermore, D. M. Biochemical characteristics of a carbapenemase from an *Acinetobacter baumannii* isolate collected in Buenos Aires, Argentina. J Antimicrob Chemother 1999;43(1):127-31.

(13)Marque, S., et al. Regional occurrence of plasmid-mediated carbapenem-hydrolyzing oxacillinase OXA-58 in *Acinetobacter* spp. in Europe. J Clin Microbiol 2005;43(9):4885-8.

(14)Donald, H. M.; Scaife, W.; Amyes, S. G., and Young, H. K. Sequence analysis of ARI-1, a novel OXA beta-lactamase, responsible for imipenem resistance in *Acinetobacter baumannii* 6B92. Antimicrob Agents Chemother 2000;44(1):196-9.

(15)Dalla-Costa, L. M., et al. Outbreak of carbapenem-resistant *Acinetobacter baumannii* producing the OXA-23 enzyme in Curitiba, Brazil. J Clin Microbiol 2003;41(7):3403-6.

(16)Jeon, B. C., et al. Investigation of a nosocomial outbreak of imipenem-resistant *Acinetobacter baumannii* producing the OXA-23 beta-lactamase in korea. J Clin Microbiol 2005;43(5):2241-5.

(17)Yu, Y. S., et al. Typing and characterization of carbapenem-resistant *Acinetobacter calcoaceticus-baumannii* complex in a Chinese hospital. J Med Microbiol 2004;53(Pt 7):653-6.

(18)Koh, T. H.; Sng, L. H.; Wang, G. C.; Hsu, L. Y., and Zhao, Y. IMP-4 and OXA beta-lactamases in *Acinetobacter baumannii* from Singapore. J Antimicrob Chemother 2007;59(4):627-32.

(19)Villegas, M. V., et al. Dissemination of *Acinetobacter baumannii* clones with OXA-23 Carbapenemase in Colombian hospitals. Antimicrob Agents Chemother 2007;51(6):2001-4.

(20)Coelho, J. M., et al. Occurrence of carbapenem-resistant *Acinetobacter baumannii* clones at multiple hospitals in London and Southeast England. J Clin Microbiol 2006;44(10):3623-7.

(21)Valẹnzuela, J. K., et al. Horizontal gene transfer in a polyclonal outbreak of carbapenem-resistant *Acinetobacter baumannii*. J Clin Microbiol 2007;45(2):453-60.

(22)Bou, G.; Oliver, A., and Martinez-Beltran, J. OXA-24, a novel class D beta-lactamase with carbapenemase activity in an *Acinetobacter baumannii* clinical strain. Antimicrob Agents Chemother 2000;44(6):1556-61.

(23)Afzal-Shah, M.; Woodford, N., and Livermore, D. M. Characterization of OXA-25, OXA-26, and OXA-27, molecular class D beta-lactamases associated with carbapenem resistance in clinical isolates of *Acinetobacter baumannii*. Antimicrob Agents Chemother 2001;45(2):583-8.

(24) Heritier, C.; Poirel, L.; Aubert, D., and Nordmann, P. Genetic and functional analysis of the chromosome-encoded carbapenem-hydrolyzing oxacillinase OXA-40 of *Acinetobacter baumannii*. Antimicrob Agents Chemother 2003;47(1):268-73.

(25)Brown, S., and Amyes, S. G. The sequences of seven class D beta-lactamases isolated from carbapenem-resistant *Acinetobacter baumannii* from four continents. Clin Microbiol Infect 2005;11(4):326-9.

(26)Brown, S.; Young, H. K., and Amyes, S. G. Characterisation of OXA-51, a novel class D carbapenemase found in genetically unrelated clinical strains of *Acinetobacter baumannii* from Argentina. Clin Microbiol Infect 2005;11(1):15-23.

(27)Merkier, A. K., and Centron, D. bla(OXA-51)-type beta-lactamase genes are ubiquitous and vary within a strain in *Acinetobacter baumannii*. Int J Antimicrob Agents 2006;28(2):110-3.

(28)Heritier, C., et al. Characterization of the naturally occurring oxacillinase of *Acinetobacter baumannii*. Antimicrob Agents Chemother 2005;49(10):4174-9.

(29)Wroblewska, M. M.; Towner, K. J.; Marchel, H., and Luczak, M. Emergence and spread of carbapenem-resistant strains of *Acinetobacter baumannii* in a tertiary-care hospital in Poland. Clin Microbiol Infect 2007;13(5):490-6.

(30)Turton, J. F., et al. Identification of *Acinetobacter baumannii* by detection of the $bla_{\text{OXA-51-like}}$ carbapenemase gene intrinsic to this species. J Clin Microbiol 2006;44(8):2974-6.

(31)Tsakris, A.; Ikonomidis, A.; Pournaras, S.; Spanakis, N., and Markogiannakis, A. Carriage of OXA-58 but not of OXA-51 beta-lactamase gene correlates with carbapenem resistance in *Acinetobacter baumannii*. J Antimicrob Chemother 2006;58(5):1097-9.

(32)Ikonomidis, A., et al. Emergence of carbapenem-resistant Enterobacter cloacae carrying VIM-4 metallo-beta-lactamase and SHV-2a extended-spectrum beta-lactamase in a conjugative plasmid. Microb Drug Resist 2007;13(4):221-6.

(33)Poirel, L., et al. OXA-58, a novel class D {beta}-lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2005;49(1):202-8.

(34)Poirel, L.; Mansour, W.; Bouallegue, O., and Nordmann, P. Carbapenem-resistant *Acinetobacter baumannii* isolates from Tunisia producing the OXA-58-like carbapenem-hydrolyzing oxacillinase OXA-97. Antimicrob Agents Chemother 2008;52(5):1613-7.

(35)Takahashi, A., et al. Detection of carbapenemase-producing *Acinetobacter baumannii* in a hospital. J Clin Microbiol 2000;38(2):526-9.

(36)Lee, K., et al. VIM- and IMP-type metallo-beta-lactamase-producing *Pseudomonas*spp. and *Acinetobacter* spp. in Korean hospitals. Emerg Infect Dis 2003;9(7):868-71.

(37)Jeong, S. H., et al. Outbreaks of imipenem-resistant *Acinetobacter baumannii* producing carbapenemases in Korea. J Microbiol 2006;44(4):423-31.

(38)Cornaglia, G., et al. Appearance of IMP-1 metallo-beta-lactamase in Europe. Lancet 1999;353(9156):899-900.

(39)Riccio, M. L., et al. Characterization of the metallo-beta-lactamase determinant of *Acinetobacter baumannii* AC-54/97 reveals the existence of $bla_{(IMP)}$ allelic variants carried by gene cassettes of different phylogeny. Antimicrob Agents Chemother 2000;44(5):1229-35.

(40)Chu, Y. W., et al. IMP-4, a novel metallo-beta-lactamase from nosocomial *Acinetobacter* spp. collected in Hong Kong between 1994 and 1998. Antimicrob Agents Chemother 2001;45(3):710-4.

(41)Nordmann, P., and Poirel, L. Emerging carbapenemases in Gram-negative aerobes. Clin Microbiol Infect 2002;8(6):321-31.

(42)Gales, A. C.; Tognim, M. C.; Reis, A. O.; Jones, R. N., and Sader, H. S. Emergence of an IMP-like metallo-enzyme in an *Acinetobacter baumannii* clinical strain from a Brazilian teaching hospital. Diagn Microbiol Infect Dis 2003;45(1):77-9.

(43)Walsh, T. R.; Toleman, M. A.; Poirel, L., and Nordmann, P. Metallo-beta-lactamases: the quiet before the storm? Clin Microbiol Rev 2005;18(2):306-25.

(44)Yum, J. H., et al. Molecular characterization of metallo-beta-lactamase-producing *Acinetobacter baumannii* and *Acinetobacter* genomospecies 3 from Korea: identification of two new integrons carrying the $bla_{(VIM-2)}$ gene cassettes. J Antimicrob Chemother 2002;49(5):837-40.

(45)Oh, E. J., et al. Prevalence of metallo-beta-lactamase among *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in a Korean university hospital and comparison of screening methods for detecting metallo-beta-lactamase. J Microbiol Methods 2003;54(3):411-8.

(46)Lee, K., et al. Novel acquired metallo-beta-lactamase gene, bla(SIM-1), in a class 1 integron from *Acinetobacter baumannii* clinical isolates from Korea. Antimicrob Agents Chemother 2005;49(11):4485-91.

(47)Aswapokee, N. Antimicrobial resistance pattern of *Acinetobacter* spp. Infect Dis Antimicrob Agents 1998;15(2):43-47.

(48)Likhitwatanakit, D. A Study of antimicrobial susceptibility of aerobic Gram-negative bacilli in 3 intensive care units in Vajira Hospital. Vajira Med J 1999;43(3):219-224.

(49)Leepethacharat, K., and Peninnah, O. *Acinetobacter baumannii* infection and colonization among Pediatric Patients at Chiang Mai University Hospital. Infect Dis Antimicrob Agents 2007;24:63-73.

(50)Jamulitrat, S.; Thongpiyapoom, S., and Suwalak, N. An Outbreak of imipenem-resistance *Acinetobacter baumannii* at Songklanagarind Hospital : The risk factors and patient prognosis. J Med Assoc Thai 2007;90(10):2181-91.

(51)Peanutsaha, S. Antimicrobial resistance pattern of *Acinetobacter* spp. in Police General Hospital. The Thai Police Med J 2003;29:19-26.

(52)Keerasuntonpong, A.; Samakeenich, C.; Tribuddharat, C., and Thamlikitkul, V. Epidemiology of *Acinetobacter baumannii* infections in Siriraj Hospital 2002. Siriraj Med J 2006;58(8):951-954.

(53)National Antimicrobial Resistance Surveillance Center , T. Antibiogram 2006. In: http://narst.dmsc.moph.go.th/; 2007.

(54)Winn, W. C., et al. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. sixth ed; 2006, p.361.

(55)Bouvet, P. J., and Grimont, P. A. Identification and biotyping of clinical isolates of *Acinetobacter*. Ann Inst Pasteur Microbiol 1987;138(5):569-78.

(56)Dijkshoorn, L., et al. Correlation of typing methods for *Acinetobacter* isolates from hospital outbreaks. J Clin Microbiol 1993;31(3):702-5.

(57)Gerner-Smidt, P., and Tjernberg, I. *Acinetobacter* in Denmark: II. Molecular studies of the *Acinetobacter* calcoaceticus-*Acinetobacter baumannii* complex. Apmis 1993;101(11):826-32.

(58)Dijkshoorn, L.; Nemec, A., and Seifert, H. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. Nat Rev Microbiol 2007;5(12):939-51.

(59)Seifert, H.; Strate, A., and Pulverer, G. Nosocomial bacteremia due to *Acinetobacter baumannii*. Clinical features, epidemiology, and predictors of mortality. Medicine (Baltimore) 1995;74(6):340-9.

(60)Cisneros, J. M., et al. Bacteremia due to *Acinetobacter baumannii*: epidemiology, clinical findings, and prognostic features. Clin Infect Dis 1996;22(6):1026-32.

(61)Falagas, M. E.; Kopterides, P., and Siempos, II. Attributable mortality of *Acinetobacter baumannii* infection among critically ill patients. Clin Infect Dis 2006;43(3):389; author reply 389-90.

(62)Falagas, M. E., and Rafailidis, P. I. Attributable mortality of *Acinetobacter baumannii*: no longer a controversial issue. Crit Care 2007;11(3):134.

(63)Blot, S.; Vandewoude, K., and Colardyn, F. Nosocomial bacteremia involving *Acinetobacter baumannii* in critically ill patients: a matched cohort study. Intensive Care Med 2003;29(3):471-5.

(64)Garnacho, J.; Sole-Violan, J.; Sa-Borges, M.; Diaz, E., and Rello, J. Clinical impact of pneumonia caused by *Acinetobacter baumannii* in intubated patients: a matched cohort study. Crit Care Med 2003;31(10):2478-82.

(65)Chen, M. Z., et al. Severe community-acquired pneumonia due to *Acinetobacter baumannii*. Chest 2001;120(4):1072-7.

(66)Anstey, N. M., et al. Community-acquired bacteremic *Acinetobacter* pneumonia in tropical Australia is caused by diverse strains of *Acinetobacter baumannii*, with carriage in the throat in at-risk groups. J Clin Microbiol 2002;40(2):685-6.

(67)Marchaim, D., et al. Surveillance cultures and duration of carriage of multidrug-resistant *Acinetobacter baumannii*. J Clin Microbiol 2007;45(5):1551-5.

(68) Anstey, N. M.; Currie, B. J., and Withnall, K. M. Community-acquired *Acinetobacter* pneumonia in the Northern Territory of Australia. Clin Infect Dis 1992;14(1):83-91.

(69)Achar, K. N.; Johny, M.; Achar, M. N., and Menon, N. K. Community-acquired bacteraemic *Acinetobacter* pneumonia with survival. Postgrad Med J 1993;69(818):934-7.

(70)Yang, C. H.; Chen, K. J., and Wang, C. K. Community-acquired *Acinetobacter* pneumonia: a case report. J Infect 1997;35(3):316-8.

(71)Vathesatogkit, P.; Charoenphan, P.; Saenghirunvattana, S.; Kiatboonsri, S., and Sathapatayavongs, B. Community-acquired *Acinetobacter* pneumonia in Thailand. Report of 5 cases. J Med Assoc Thai 1987;70(2):96-101.

(72)Krisanapan, S.; Naphathorn, P., and Kaewprom, P. Community acquired *Acinetobacter* pneumonia: report of two cases. Southeast Asian J Trop Med Public Health 1989;20(3):497-8.

(73)Danchaivijitr, S.; Dhiraputra, C.; Rongrungruang, Y.; Worajitr, M., and Jintanothaitavorn, D. Antimicrobial susceptibility of community and hospital acquired bacteria. J Med Assoc Thai 2005;88 Suppl 10:S14-25.

(74)Tomaras, A. P.; Dorsey, C. W.; Edelmann, R. E., and Actis, L. A. Attachment to and biofilm formation on abiotic surfaces by *Acinetobacter baumannii*: involvement of a novel chaperone-usher pili assembly system. Microbiol 2003;149(Pt 12):3473-84.

(75)Smith, M. G., et al. New insights into *Acinetobacter baumannii* pathogenesis revealed by high-density pyrosequencing and transposon mutagenesis. Genes Dev 2007;21(5):601-14.

(76)Beachey, E. H. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surface. J Infect Dis 1981;143(3):325-45.

(77)Haseley, S. R.; Holst, O., and Brade, H. Structural and serological characterisation of the O-antigenic polysaccharide of the lipopolysaccharide from *Acinetobacter* strain 90 belonging to DNA group 10. Eur J Biochem 1997;245(2):470-6.

(78)Haseley, S. R.; Holst, O., and Brade, H. Structural and serological characterisation of the O-antigenic polysaccharide of the lipopolysaccharide from *Acinetobacter* haemolyticus strain ATCC 17906. Eur J Biochem 1997;244(3):761-6.

(79)Haseley, S. R.; Pantophlet, R.; Brade, L.; Holst, O., and Brade, H. Structural and serological characterisation of the O-antigenic polysaccharide of the lipopolysaccharide from *Acinetobacter* junii strain 65. Eur J Biochem 1997;245(2):477-81.

(80)Vinogradov, E. V., et al. Structural and serological characterisation of the O-specific polysaccharide from lipopolysaccharide of *Acinetobacter* calcoaceticus strain 7 (DNA group 1). Eur J Biochem 1997;243(1-2):167-73.

(81)Lee, J. C., et al. Adherence of *Acinetobacter baumannii* strains to human bronchial epithelial cells. Res Microbiol 2006;157(4):360-6.

(82)Rodriguez-Bano, J., et al. Biofilm formation in *Acinetobacter baumannii*: associated features and clinical implications. Clin Microbiol Infect 2008;14(3):276-8.

(83)Lee, H. W., et al. Capacity of multidrug-resistant clinical isolates of *Acinetobacter baumannii* to form biofilm and adhere to epithelial cell surfaces. Clin Microbiol Infect 2008;14(1):49-54.

(84)Sechi, L. A., et al. PER-1 type beta-lactamase production in *Acinetobacter baumannii* is related to cell adhesion. Med Sci Monit 2004;10(6):BR180-4.

(85)Costerton, J. W.; Stewart, P. S., and Greenberg, E. P. Bacterial biofilms: a common cause of persistent infections. Science 1999;284(5418):1318-22.

(86)Donlan, R. M., and Costerton, J. W. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 2002;15(2):167-93.

(87)Bergogne-Berezin, E., and Towner, K. J. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin Microbiol Rev 1996;9(2):148-65.

(88)Erridge, C.; Moncayo-Nieto, O. L.; Morgan, R.; Young, M., and Poxton, I. R. *Acinetobacter baumannii* lipopolysaccharides are potent stimulators of human monocyte activation via Toll-like receptor 4 signalling. J Med Microbiol 2007;56(Pt 2):165-71.

(89)Choi, C. H., et al. Outer membrane protein 38 of *Acinetobacter baumannii* localizes to the mitochondria and induces apoptosis of epithelial cells. Cell Microbiol 2005;7(8):1127-38.

(90)Choi, C. H., et al. *Acinetobacter baumannii* outer membrane protein A targets the nucleus and induces cytotoxicity. Cell Microbiol 2008;10(2):309-19.

(91)Neilands, J. B. Microbial iron compounds. Annu Rev Biochem 1981;50:715-31.

(92)Gray-Owen, S. D., and Schryvers, A. B. Bacterial transferrin and lactoferrin receptors. Trends Microbiol 1996;4(5):185-91.

(93)Neilands, J. B. Siderophores: structure and function of microbial iron transport compounds. J Biol Chem 1995;270(45):26723-6.

(94)Yamamoto, S.; Okujo, N., and Sakakibara, Y. Isolation and structure elucidation of acinetobactin, a novel siderophore from *Acinetobacter baumannii*. Arch Microbiol 1994;162(4):249-54.

(95)Dorsey, C. W.; Beglin, M. S., and Actis, L. A. Detection and analysis of iron uptake components expressed by *Acinetobacter baumannii* clinical isolates. J Clin Microbiol 2003;41(9):4188-93.

(96)Gerner-Smidt, P.; Tjernberg, I., and Ursing, J. Reliability of phenotypic tests for identification of *Acinetobacter* species. J Clin Microbiol 1991;29(2):277-82.

(97)Bouvet, P. J. M., and Grimont, P. A. D. Taxonomy of the genus *Acinetobacter baumannii* sp. nov., *Acinetobacter haemolyticus* sp. nov., *Acinetobacter johnsonii* sp. nov., and *Acinetobacter junii* sp. nov. and emended

descriptions of *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii*. Int. J. Syst. Bacteriol. 1986;36:228-240.

(98)Nishimura, Y.; Kairiyama, E.; Shimadzu, M., and Iizuka, H. Characterization of a radiation-resistant *Acinetobacter*. Z Allg Mikrobiol 1981;21(2):125-30.

(99)Tjernberg, I., and Ursing, J. Clinical Strains of *Acinetobacter* classified by DNA-DNA hybridization APMIS 1989;97:595-605.

(100)Vaneechoutte, M., et al. Identification of *Acinetobacter* genomic species by amplified ribosomal DNA restriction analysis. J Clin Microbiol 1995;33(1):11-5.

(101)Nowak, A., and Kur, J. Differentiation of seventeen genospecies of *Acinetobacter* by multiplex polymerase chain reaction and restriction fragment length polymorphism analysis. Mol Cell Probes 1996;10(6):405-11.

(102)Chang, H. C., et al. Species-level identification of isolates of the *Acinetobacter* calcoaceticus-*Acinetobacter baumannii* complex by sequence analysis of the 16S-23S rRNA gene spacer region. J Clin Microbiol 2005;43(4):1632-9.

(103)Munoz-Price, L. S., and Weinstein, R. A. *Acinetobacter* infection. N Engl J Med 2008;358(12):1271-81.

(104)Falagas, M. E., and Kasiakou, S. K. Colistin: the revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. Clin Infect Dis 2005;40(9):1333-41.

(105)Falagas, M. E., and Kasiakou, S. K. Toxicity of polymyxins: a systematic review of the evidence from old and recent studies. Crit Care 2006;10(1):R27.

(106)Garnacho-Montero, J., et al. Treatment of multidrug-resistant *Acinetobacter baumannii* ventilator-associated pneumonia (VAP) with intravenous colistin: a comparison with imipenem-susceptible VAP. Clin Infect Dis 2003;36(9):1111-8.

(107)Levin, A. S., et al. Intravenous colistin as therapy for nosocomial infections caused by multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Clin Infect Dis 1999;28(5):1008-11.

(108)Garnacho-Montero, J., et al. *Acinetobacter baumannii* ventilator-associated pneumonia: epidemiological and clinical findings. Intensive Care Med 2005;31(5):649-55.

(109)Holloway, K. P.; Rouphael, N. G.; Wells, J. B.; King, M. D., and Blumberg, H. M. Polymyxin B and doxycycline use in patients with multidrug-resistant *Acinetobacter baumannii* infections in the intensive care unit. Ann Pharmacother 2006;40(11):1939-45.

(110)Kallel, H., et al. Colistin as a salvage therapy for nosocomial infections caused by multidrug-resistant bacteria in the ICU. Int J Antimicrob Agents 2006;28(4):366-9.

(111)Motaouakkil, S., et al. Colistin and rifampicin in the treatment of nosocomial infections from multiresistant *Acinetobacter baumannii*. J Infect 2006;53(4):274-8.

(112)Yoon, J.; Urban, C.; Terzian, C.; Mariano, N., and Rahal, J. J. In vitro double and triple synergistic activities of Polymyxin B, imipenem, and rifampin against multidrug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 2004;48(3):753-7.

(113)Saballs, M., et al. Rifampicin/imipenem combination in the treatment of carbapenem-resistant *Acinetobacter baumannii* infections. J Antimicrob Chemother 2006;58(3):697-700.

(114)Bernabeu-Wittel, M., et al. Pharmacokinetic/pharmacodynamic assessment of the in-vivo efficacy of imipenem alone or in combination with amikacin for the treatment of experimental multiresistant *Acinetobacter baumannii* pneumonia. Clin Microbiol Infect 2005;11(4):319-25.

(115)Pachon-Ibanez, M. E.; Jimenez-Mejias, M. E.; Pichardo, C.; Llanos, A. C., and Pachon, J. Activity of tigecycline (GAR-936) against *Acinetobacter baumannii* strains, including those resistant to imipenem. Antimicrob Agents Chemother 2004;48(11):4479-81.

(116)Seifert, H.; Stefanik, D., and Wisplinghoff, H. Comparative in vitro activities of tigecycline and 11 other antimicrobial agents against 215 epidemiologically defined multidrug-resistant *Acinetobacter baumannii* isolates. J Antimicrob Chemother 2006;58(5):1099-100.

(117)Lolans, K.; Rice, T. W.; Munoz-Price, L. S., and Quinn, J. P. Multicity outbreak of carbapenem-resistant *Acinetobacter baumannii* isolates producing the carbapenemase OXA-40. Antimicrob Agents Chemother 2006;50(9):2941-5.

(118)Rice, L. B. Challenges in identifying new antimicrobial agents effective for treating infections with *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Clin Infect Dis 2006;43 Suppl 2:S100-5.

(119)Insa, R.; Cercenado, E.; Goyanes, M. J.; Morente, A., and Bouza, E. In vitro activity of tigecycline against clinical isolates of *Acinetobacter baumannii* and *Stenotrophomonas maltophilia*. J Antimicrob Chemother 2007;59(3):583-5.

(120)Jones, R. N., et al. Multicenter studies of tigecycline disk diffusion susceptibility results for *Acinetobacter* spp. J Clin Microbiol 2007;45(1):227-30.

(121)Navon-Venezia, S.; Leavitt, A., and Carmeli, Y. High tigecycline resistance in multidrug-resistant *Acinetobacter baumannii*. J Antimicrob Chemother 2007;59(4):772-4.

(122)Peleg, A. Y.; Adams, J., and Paterson, D. L. Tigecycline Efflux as a Mechanism for Nonsusceptibility in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2007;51(6):2065-9.

(123)Reid, G. E.; Grim, S. A.; Aldeza, C. A.; Janda, W. M., and Clark, N. M. Rapid development of *Acinetobacter baumannii* resistance to tigecycline. Pharmacotherapy 2007;27(8):1198-201.

(124)Ruzin, A.; Keeney, D., and Bradford, P. A. AdeABC multidrug efflux pump is associated with decreased susceptibility to tigecycline in *Acinetobacter* calcoaceticus-*Acinetobacter baumannii* complex. J Antimicrob Chemother 2007;59(5):1001-4.

(125)Lim, V. K., and Cheong, Y. M. In-vitro activity of cefoperazone-sulbactam combination against cefoperazone resistant clinical isolates in a Malaysian general hospital. Malays J Pathol 1995;17(2):73-6.

(126)Kinoshita, S., and Kumagai, S. [Antimicrobial activity of carbapenem antibiotics against gram-negative bacilli]. Jpn J Antibiot 1998;51(9):551-60.

(127)Kanazawa, K.; Nouda, H.; Sumita, Y., and Sunagawa, M. Structure-activity relationships of carbapenems to the antagonism of the antipseudomonal activity of other beta-lactam agents and to the beta-lactamase inducibility in *Pseudomonas aeruginosa*: effects of 1beta-methyl group and C-2 side chain. J Antibiot (Tokyo) 1999;52(2):142-9.

(128)Waxman, D. J., and Strominger, J. L. Penicillin-binding proteins and the mechanism of action of beta-lactam antibiotics. Annu Rev Biochem 1983;52:825-69.

(129)Spratt, B. Properties of the penicillin-binding proteins of *Escherichia coli* K12. Eur. J. Biochem. 1977;72(2):341-52.

(130)Clissold, S. P.; Todd, P. A., and Campoli-Richards, D. M. Imipenem/cilastatin. A review of its antibacterial activity, pharmacokinetic properties and therapeutic efficacy. Drugs 1987;33(3):183-241.

(131)Schreckenberger, P. C., and Graevenitz, A. Manual of Clinical Microbiology. seventh ed; 1999, p.539-560.

(132)Livermore, D. M.; Sefton, A. M., and Scott, G. M. Properties and potential of ertapenem. J Antimicrob Chemother 2003;52(3):331-44.

(133)Livermore, D. M.; Mushtaq, S., and Warner, M. Selectivity of ertapenem for *Pseudomonas aeruginosa* mutants cross-resistant to other carbapenems. J Antimicrob Chemother 2005;55(3):306-11.

(134)Mushtaq, S.; Ge, Y., and Livermore, D. M. Doripenem versus *Pseudomonas aeruginosa* in vitro: activity against characterized isolates, mutants, and transconjugants and resistance selection potential. Antimicrob Agents Chemother 2004;48(8):3086-92.

(135)Mouton, J. W.; Touzw, D. J.; Horrevorts, A. M., and Vinks, A. A. Comparative pharmacokinetics of the carbapenems: clinical implications. Clin Pharmacokinet 2000;39(3):185-201.

(136)Sunagawa, M.; Kanazawa, K., and Nouda, H. [Antipseudomonal activity of carbapenem antibiotics]. Jpn J Antibiot 2000;53(7):479-511.

(137)Ruiz, J., et al. Evolution of resistance among clinical isolates of *Acinetobacter* over a 6-year period. Eur J Clin Microbiol Infect Dis 1999;18(4):292-5.

(138)Ling, T. K.; Ying, C. M.; Lee, C. C., and Liu, Z. K. Comparison of antimicrobial resistance of *Acinetobacter baumannii* clinical isolates from Shanghai and Hong Kong. Med Princ Pract 2005;14(5):338-41.

(139)Falagas, M. E.; Mourtzoukou, E. G.; Polemis, M., and Vatopoulos, A. C. Trends in antimicrobial resistance of *Acinetobacter baumannii* clinical isolates from hospitalised patients in Greece and treatment implications. Clin Microbiol Infect 2007;13(8):816-9.

(140)Zarrilli, R., et al. Molecular epidemiology of a clonal outbreak of multidrug-resistant *Acinetobacter baumannii* in a university hospital in Italy. Clin Microbiol Infect 2007;13(5):481-9.

(141)Lopez-Otsoa, F., et al. Endemic carbapenem resistance associated with OXA-40 carbapenemase among *Acinetobacter baumannii* isolates from a hospital in northern Spain. J Clin Microbiol 2002;40(12):4741-3.

(142)del Mar Tomas, M., et al. Hospital outbreak caused by a carbapenem-resistant strain of *Acinetobacter baumannii*: patient prognosis and risk-factors for colonisation and infection. Clin Microbiol Infect 2005;11(7):540-6.

(143)Heritier, C.; Dubouix, A.; Poirel, L.; Marty, N., and Nordmann, P. A nosocomial outbreak of *Acinetobacter baumannii* isolates expressing the carbapenem-hydrolysing oxacillinase OXA-58. J Antimicrob Chemother 2005;55(1):115-8.

(144)Pournaras, S., et al. Outbreak of multiple clones of imipenem-resistant *Acinetobacter baumannii* isolates expressing OXA-58 carbapenemase in an intensive care unit. J Antimicrob Chemother 2006;57(3):557-61.

(145)Gallego, L., and Towner, K. J. Carriage of class 1 integrons and antibiotic resistance in clinical isolates of *Acinetobacter baumannii* from northern Spain. J Med Microbiol 2001;50(1):71-7.

(146)Liu, S. Y., et al. Integron-associated imipenem resistance in *Acinetobacter baumannii* isolated from a regional hospital in Taiwan. Int J Antimicrob Agents 2006;27(1):81-4.

(147)Gu, B., et al. Prevalence and characterization of class 1 integrons among *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates from patients in Nanjing, China. J Clin Microbiol 2007;45(1):241-3.

(148)Zarrilli, R., et al. Molecular epidemiology of sequential outbreaks of *Acinetobacter baumannii* in an intensive care unit shows the emergence of carbapenem resistance. J Clin Microbiol 2004;42(3):946-53.

(149)Turton, J. F., et al. Detection and typing of integrons in epidemic strains of *Acinetobacter baumannii* found in the United Kingdom. J Clin Microbiol 2005;43(7):3074-82.

(150)Ploy, M. C.; Denis, F.; Courvalin, P., and Lambert, T. Molecular characterization of integrons in *Acinetobacter baumannii*: description of a hybrid class 2 integron. Antimicrob Agents Chemother 2000;44(10):2684-8.

(151)Poirel, L., and Nordmann, P. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. Clin Microbiol Infect 2006;12(9):826-36.

(152)Ambler, R. P. The structure of beta-lactamases. Philos Trans R Soc Lond B Biol Sci 1980;289(1036):321-31.

(153)Jaurin, B., and Grundstrom, T. ampC cephalosporinase of Escherichia coli K-12 has a different evolutionary origin from that of beta-lactamases of the penicillinase type. Proc Natl Acad Sci U S A 1981;78(8):4897-901.

(154)Huovinen, P.; Huovinen, S., and Jacoby, A. Sequence of PSE-2 beta-lactamase. Antimicrob Agents Chemother 1998;32:134-36.

(155)Lamotte-Brasseur, J.; Knox, J., and Kelly, J. The structures and catalytic mechanisms of active-site serine beta-lactamases. Biotech Genet Eng Rev 1994;12:189-230.

(156)Wang, Z.; Fast, W.; Valentine, A. M., and Benkovic, S. J. Metallo-beta-lactamase: structure and mechanism. Curr Opin Chem Biol 1999;3(5):614-22.

(157)Majiduddin, F. K.; Materon, I. C., and Palzkill, T. G. Molecular analysis of beta-lactamase structure and function. Int J Med Microbiol 2002;292(2):127-37.

(158)Couture, F.; Lachapelle, J., and Levesque, R. C. Phylogeny of LCR-1 and OXA-5 with class A and class D beta-lactamases. Mol Microbiol 1992;6(12):1693-705.

(159)Walther-Rasmussen, J., and Hoiby, N. OXA-type carbapenemases. J Antimicrob Chemother 2006;57(3):373-83.

(160)Paton, R., et al. ARI 1: beta-lactamase-mediated imipenem resistance in *Acinetobacter baumannii*. Int J Antimicrob Agents 1993;2(2):81-7.

(161)Bush, K.; Jacoby, G. A., and Medeiros, A. A. A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 1995;39(6):1211-33.

(162)Turton, J. F., et al. The role of ISAba1 in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. FEMS Microbiol Lett 2006;258(1):72-7.

(163)Corvec, S.; Poirel, L.; Naas, T.; Drugeon, H., and Nordmann, P. Genetics and expression of the carbapenem-hydrolyzing oxacillinase gene blaOXA-23 in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2007;51(4):1530-3.

(164)Poirel, L., and Nordmann, P. Genetic structures at the origin of acquisition and expression of the carbapenem-hydrolyzing oxacillinase gene blaOXA-58 in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2006;50(4):1442-8.

(165)Shibata, N., et al. PCR typing of genetic determinants for metallo-beta-lactamases and integrases carried by gram-negative bacteria isolated in Japan, with focus on the class 3 integron. J Clin Microbiol 2003;41(12):5407-13.

(166)Lee, K., et al. Metallo-beta-lactamase-producing Gram-negative bacilli in Korean Nationwide Surveillance of Antimicrobial Resistance group hospitals in 2003: continued prevalence of VIM-producing *Pseudomonas*spp. and increase of IMP-producing *Acinetobacter* spp. Diagn Microbiol Infect Dis 2004;50(1):51-8.

(167)Walsh, T. R. The emergence and implications of metallo-beta-lactamases in Gram-negative bacteria. Clin Microbiol Infect 2005;11 Suppl 6:2-9.

(168)Watanabe, M.; Iyobe, S.; Inoue, M., and Mitsuhashi, S. Transferable imipenem resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1991;35(1):147-51.

(169)Luzzaro, F., et al. Emergence in Klebsiella pneumoniae and Enterobacter cloacae clinical isolates of the VIM-4 metallo-beta-lactamase encoded by a conjugative plasmid. Antimicrob Agents Chemother 2004;48(2):648-50.

(170)Loli, A., et al. Sources of diversity of carbapenem resistance levels in Klebsiella pneumoniae carrying blaVIM-1. J Antimicrob Chemother 2006;58(3):669-72.

(171)Lauretti, L., et al. Cloning and characterization of blaVIM, a new integron-borne metallo-beta-lactamase gene from a *Pseudomonas aeruginosa* clinical isolate. Antimicrob Agents Chemother 1999;43(7):1584-90.

(172)Da Silva, G. J., et al. Molecular characterization of bla(IMP-5), a new integron-borne metallo-beta-lactamase gene from an *Acinetobacter baumannii* nosocomial isolate in Portugal. FEMS Microbiol Lett 2002;215(1):33-9.

(173)Lee, K., et al. Modified Hodge and EDTA-disk synergy tests to screen metallo-beta-lactamase-producing strains of *Pseudomonas*and *Acinetobacter* species. Clin Microbiol Infect 2001;7(2):88-91.

(174)Sefton, A. M. Mechanisms of antimicrobial resistance: their clinical relevance in the new millennium. Drugs 2002;62(4):557-66.

(175)Fernandez-Cuenca, F., et al. Relationship between beta-lactamase production, outer membrane protein and penicillin-binding protein profiles on the activity of carbapenems against clinical isolates of *Acinetobacter baumannii*. J Antimicrob Chemother 2003;51(3):565-74.

(176)Gehrlein, M.; Leying, H.; Cullmann, W.; Wendt, S., and Opferkuch, W. Imipenem resistance in *Acinetobacter* baumanii is due to altered penicillin-binding proteins. Chemotherapy 1991;37(6):405-12.

(177)Vila, J.; Marti, S., and Sanchez-Cespedes, J. Porins, efflux pumps and multidrug resistance in *Acinetobacter baumannii*. J Antimicrob Chemother 2007;59(6):1210-5.

(178)Gribun, A.; Nitzan, Y.; Pechatnikov, I.; Hershkovits, G., and Katcoff, D. J. Molecular and structural characterization of the HMP-AB gene encoding a pore-forming protein from a clinical isolate of *Acinetobacter baumannii*. Curr Microbiol 2003;47(5):434-43.

(179)Limansky, A. S.; Mussi, M. A., and Viale, A. M. Loss of a 29-kilodalton outer membrane protein in *Acinetobacter baumannii* is associated with imipenem resistance. J Clin Microbiol 2002;40(12):4776-8.

(180)Mussi, M. A.; Limansky, A. S., and Viale, A. M. Acquisition of resistance to carbapenems in multidrug-resistant clinical strains of *Acinetobacter baumannii*: natural insertional inactivation of a gene encoding a member of a novel family of beta-barrel outer membrane proteins. Antimicrob Agents Chemother 2005;49(4):1432-40.

(181)del Mar Tomas, M., et al. Cloning and functional analysis of the gene encoding the 33- to 36-kilodalton outer membrane protein associated with carbapenem resistance in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2005;49(12):5172-5.

(182)Dupont, M.; Pages, J. M.; Lafitte, D.; Siroy, A., and Bollet, C. Identification of an OprD homologue in *Acinetobacter baumannii*. J Proteome Res 2005;4(6):2386-90.

(183)Magnet, S.; Courvalin, P., and Lambert, T. Resistance-nodulation-cell division-type efflux pump involved in aminoglycoside resistance in *Acinetobacter baumannii* strain BM4454. Antimicrob Agents Chemother 2001;45:3375-3380.

(184)Betriu, C., et al. In vitro activities of tigecycline (GAR-936) against recently isolated clinical bacteria in Spain. Antimicrob Agents Chemother 2002;46(3):892-5.

(185)Cisneros, J. M., and Rodriguez-Bano, J. Nosocomial bacteremia due to *Acinetobacter baumannii*: epidemiology, clinical features and treatment. Clin Microbiol Infect 2002;8(11):687-93.

(186)Souli, M., et al. In vitro activity of tigecycline against multiple-drug-resistant, including pan-resistant, gram-negative and gram-positive clinical isolates from Greek hospitals. Antimicrob Agents Chemother 2006;50(9):3166-9.

(187)Xiao, Y. H.; Wang, J., and Li, Y. Bacterial resistance surveillance in China: a report from Mohnarin 2004-2005. Eur J Clin Microbiol Infect Dis 2008;27(8):697-708.

(188)Nemec, A., et al. Emergence of carbapenem resistance in *Acinetobacter baumannii* in the Czech Republic is associated with the spread of multidrug-resistant strains of European clone II. J Antimicrob Chemother 2008;62(3):484-9.

(189)Qi, C.; Malczynski, M.; Parker, M., and Scheetz, M. H. Characterization of genetic diversity of carbapenem-resistant *Acinetobacter baumannii* clinical strains collected from 2004 to 2007. J Clin Microbiol 2008;46(3):1106-9.

(190)Wang, H., and Chen, M. Surveillance for antimicrobial resistance among clinical isolates of gram-negative bacteria from intensive care unit patients in China, 1996 to 2002. Diagn Microbiol Infect Dis 2005;51(3):201-8.

(191)Wang, H., et al. Antimicrobial resistance analysis among nosocomial gram-negative bacilli from 10 teaching hospitals in China. Chin. J. Lab. Med. 2005;28:1295-1303.

(192)Wikler, M. A., et al. Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement; 2008.

(193)Woodford, N., et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int J Antimicrob Agents 2006;27(4):351-3.

(194)Senda, K., et al. PCR detection of metallo-beta-lactamase gene (blaIMP) in gram-negative rods resistant to broad-spectrum beta-lactams. J Clin Microbiol 1996;34(12):2909-13.

(195)Hujer, K. M., et al. Analysis of antibiotic resistance genes in multidrug-resistant *Acinetobacter* sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. Antimicrob Agents Chemother 2006;50(12):4114-23.

(196)Carey, R. B.; Banerjee, S. N., and Srinivasan, A. Multidrug-resistant *Acinetobacter* infections, 1995-2004. Presented at the 46th Interscience Conference on Antimicrobial Agents and Chemotherapy, Sanfrancisco 2006;September 27-30.

(197)Ikonomidis, A.; Ntokou, E.; Maniatis, A. N.; Tsakris, A., and Pournaras, S. Hidden VIM-1 metallo-beta-lactamase phenotypes among *Acinetobacter baumannii* clinical isolates. J Clin Microbiol 2008;46(1):346-9.

(198)Bogaerts, P., et al. Outbreak of infection by carbapenem-resistant *Acinetobacter baumannii* producing the carbapenemase OXA-58 in Belgium. J Clin Microbiol 2006;44(11):4189-92.

(199)Sinha, M., and Srinivasa, H. Mechanisms of resistance to carbapenems in meropenem- resistant *Acinetobacter* isolates from clinical samples. Indian J Med Microbiol 2007;25(2):121-5.

(200)Wang, H., et al. Molecular epidemiology of clinical isolates of carbapenem-resistant *Acinetobacter* spp. from Chinese hospitals. Antimicrob Agents Chemother 2007;51(11):4022-8.

(201)Stoeva, T.; Higgins, P. G.; Bojkova, K., and Seifert, H. Clonal spread of carbapenem-resistant OXA-23-positive *Acinetobacter baumannii* in a Bulgarian university hospital. Clin Microbiol Infect 2008;14(7):723-7.

(202)Zong, Z.; Lu, X.; Valenzuela, J. K.; Partridge, S. R., and Iredell, J. An outbreak of carbapenem-resistant *Acinetobacter baumannii* producing OXA-23 carbapenemase in western China. Int J Antimicrob Agents 2008;31(1):50-4.

(203)Meric, M., et al. Emergence and spread of carbapenem-resistant *Acinetobacter baumannii* in a tertiary care hospital in Turkey. FEMS Microbiol Lett 2008;282(2):214-8.

(204)Bou, G.; Oliver, A., and Martinez-Beltran, J. (Erratum) OXA-24 a novel class D beta-lactamase with carbapenemase activity in an *Acinetobacter baumannii* clinical strain. Antimicrob Agents Chemother 2006;50(6):2280.

(205)Bertini, A., et al. Multicopy blaOXA-58 gene as a source of high-level resistance to carbapenems in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2007;51(7):2324-8.

(206)Coelho, J.; Woodford, N.; Afzal-Shah, M., and Livermore, D. Occurrence of OXA-58-like carbapenemases in *Acinetobacter* spp. collected over 10 years in three continents. Antimicrob Agents Chemother 2006;50(2):756-8.

(207)Tsakris, A., et al. Clusters of imipenem-resistant *Acinetobacter baumannii* clones producing different carbapenemases in an intensive care unit. Clin Microbiol Infect 2008;14(6):588-94.

(208)Sung, J. Y., et al. [Dissemination of IMP-1 and OXA type beta-lactamase in carbapenem-resistant *Acinetobacter baumannii*]. Korean J Lab Med 2008;28(1):16-23.

APPENDICES

APPENDIX A

REAGENTS AND INSTRUMENTS

A. REAGENTS

Absolute ethanol	(Merck, Germany)
Agarose	(Biorad, USA)
Bacto agar	(Difco, USA)
Boric acid	(Sigma, USA)
Bromthymol blue	(Fluka, Germany)
dNTPs	(Promega, USA)
EDTA	(Amresco, USA)
Ethidium bromide	(Amresco, USA)
Glacial acetic acid	(Merck, Germany)
Miniral oil	(Sigma, USA)
N,N,Ń,Ń-tetramethyl-*p*-phenylenediamine dihydrochloride	(Sigma, U.S.A)
NaCl	(Merck, Germany)
$NaHCO_3$	(Merck, Germany)
$Na_2HPO_4*2H_2O$	(Sigma, USA)
Pfu DNA Polymerase	(Fermentas, USA)
Taq DNA Polymerase	(Fermentas, USA)
Tris	(Amresco, USA)
100 bp DNA ladder	(Fermentas, USA)
100 bp plus DNA ladder	(Fermentas, USA)

B. INSTRUMENTS

ABI prism 310 automated sequencer	(Perkin Elmer, USA)
Automatic pipette	(Gilson, Lyon, France)
Camera Gel Doc™ MZL	(BIO-RAD, USA)
Incubator	(Forma Scientific, USA)
Perkin Elmer GeneAmp PCR system 9600	(Perkin Elmer, USA)
Microcentrifuge	(Eppendorf, USA)
Spectrophotometer	(BIO-RAD, USA)
Water bath	(Memmert, USA)

APPENDIX B

MEDIA AND ANTIBIOTIC SOLUTION PREPARATION

1. Sheep blood agar (Oxoid, England)

Suspend Tryptone Soya agar 40 grams in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Hold the medium until ~50 °C , then add 5% sheep blood into the medium before pour plate. Once the medium is prepared, store at 4°C.

2. MacConkey agar

Suspend 51.5 grams in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Once the medium is prepared, store at 4°C.

3. Muller-Hinton II agar (Difco, France)

Suspend 38 grams of the dehydrated medium in 1,000 ml of distilled water. Dissolve by heating with frequent agitation until complete dissolution. Adjust final volume to 1,000 ml. Sterilize at 121°C (15 lbs. sp) for 15 minutes. Once the medium is prepared, store at 4°C.

4. Tryptic Soy Broth (Oxoid, England)

Suspend 30 grams in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Once the medium is prepared, store at 4°C.

5. LB broth (Pronadisa, Spain)

Suspend 20 grams of the dehydrated medium in 900 ml of distilled water. Dissolve by heating with frequent agitation until complete dissolution. Adjust final volume to 1,000 ml. Sterilize at 121°C (15 lbs. sp) for 15 minutes. Once the medium is prepared, store at 4°C.

6. Tryptone Soya agar (Oxoid, England)

Suspend 40 grams in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Once the medium is prepared, store at 4°C.

7. Triple Sugar Iron agar (Oxoid, England)

Suspend 65 grams in 1 litre of distilled water. Bring to the boil to dissolve completely. Mix well and distribute into containers. Sterilize by autoclaving at 121°C for 15 minutes. Allow to set as slopes with 2.5 cm butts. Once the medium is prepared, store at 4°C.

8. OF Basal Medium (Difco, France)

Suspend 9.4 grams of the dehydrated medium in 900 ml of distilled water. Dissolve by heating with frequent agitation until complete dissolution. Adjust final volume to 1,000 ml. Add 1% carbohydrate (Glucose). Sterilize at 121°C (15 lbs. sp) for 15 minutes. Once the medium is prepared, store at 4°C.

9. Decarboxylase Base Moeller (Difco, France)

Suspend 10.5 grams of the dehydrated medium in 1,000 ml of distilled water. Dissolve by heating with frequent agitation until complete dissolution. Add 10 grams L-amino acid (Arginine) and dissolve. Sterilize at 121°C (15 lbs. sp) for 15 minutes. Once the medium is prepared, store at 4°C.

10. Nitrate Broth (Difco, France)

Suspend 9 grams in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Once the medium is prepared, store at 4°C.

11. Malonate broth (BBL, USA)

Suspend 9.8 grams in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Once the medium is prepared, store at 4°C.

12. Urea Medium (BBL, USA)

Solution A: for	20 ml
Urea agar base (BBL)	2.9 g
Urea	4 g
Distilled water	20 ml

Dissolve in 20 ml of distilled water. Adjust to pH 6.2 Sterilize by filtration (use a 0.22 μm filter).

Solution B: for	80 ml
Bacto agar (Difco)	0.5 g
Distilled water	80 ml

Add the ingredient to 80 ml of distilled water; heat with stirring until the agar is dissolved. Sterilize by autoclaving at 121°C, 15 ponds/inch^2 pressure, for 15 minutes. Mix solution A, 20 ml with solution B, 80 ml. Aliquot into sterile 1.5 microtube (1 ml/tube). Test the sterility of Urease medium by incubate tubes at 37°C for 24 hours. Store tubes in refrigerator at 4°C until used.

13. Sterile saline solution

Sodium Chloride	8.5 g/L
Distilled water	1 L

Sterilze by autoclaving at 121°C, 15 pounds/inch^2 pressure, for minutes. Store at room temperature.

14. Antibiotic solution preparation

Imipenem, stock concentration 512 mg/L

- Prepare a stock solution; dissolve 0.1024 g in 10 ml distilled water

Meropenem, stock concentration 512 mg/L

- Prepare a stock solution, dissolve 0.0512 g in 10 ml distilled water.

APPENDIX C

REAGENTS PREPARATION

1. 10x Tris-borate buffer (TBE)

Tris base	108 g/L
Boric acid	55 g/L
0.5 M EDTA (pH 8.0)	40 ml

Adjust volume to 1 liter with distilled water. The solution was mixed and sterilized by autoclaving at 121°C for 15 min.

2. 0.5 M EDTA (pH 8.0)

Disodium ethylene diamine tetra-acelate $2H_2O$	186.1 g/L
Distilled water	1 L

Adjust pH to 8.0 and volume to 1 liter. Store at room temperature for no longer than 1 year.

3. 10x TE buffer

Tris	12.11 g/L
0.5 M EDTA	20 ml

Adjust to pH 8.0 by adding conc. HCl. Adjust volume to 1,000 ml and sterilized by autoclaving at 121°C for 15 min.

4. 1 % Agarose gel

Agarose	0.4 g
1x TBE	40 ml

Dissolve by heating in microwave oven and occasional mix unit no granules of agarose are visible.

5. 5x Loading buffer 100 ml

Tris HCl	0.6 g
EDTA	1.68 g
SDS	0.5 g

Bromphenol Blue	0.1 g
Sucrose	40 g

Adjust volume to 100 ml with distilled water. Mix the solution, aliquot into 1.5 microtubes and store at 4°C.

Reagent for DNA Extraction

1.1 Protease K

Reconstituted of protease K (lyophilized) with 1.25 ml protease solvent, stored at –20°C

1.2 Buffer AL (Ready to used)

1.3 Buffer AW1

Buffer AW1 is supplied as a concentrate. Before using for the first time, add the 25 ml of ethanol (96-100%) to buffer AW1 concentrate as indicated on the bottle.

1.4 Buffer AW2

Buffer AW2 is supplied as a concentrate. Before using for the first time, add the 30 ml of ethanol (96-100%) to buffer AW2 concentrate as indicated on the bottle.

1.5 Buffer AE (Ready to used)

Reagent for PCR product purification

Buffer PB (Ready to used)

Buffer PE

Buffer PE is supplied as a concentrate. Before using for the first time, add the 55 ml of ethanol (96-100%) to buffer PE concentrate as indicated on the bottle.

APPENDIX D

THE RESULTS OF ALL TESTS IN THIS STUDY

Results of carbapenem susceptibility, carbapenemase activity and the presence of carbapenemase genes of 501 *A. baumannii* isolates.

No	Strains	MICs (mg/L)		Carbapenemase activity		Presence of carbapenemase genes					
		IMP	MER	M-Hodge test	EDTA disk synergy test	$bla_{OXA23\text{-}like}$	$bla_{OXA24\text{-}like}$	$bla_{OXA51\text{-}like}$	$bla_{OXA58\text{-}like}$	$bla_{IMP\text{-}like}$	$bla_{VIM\text{-}like}$
1	*A. baumannii* ATCC 19606	2	2	-	-	-	-	+	-	-	-
2	*E. coli* ATCC 25922	0.25	0.06	ND	ND	ND	ND	ND	ND	ND	ND
3	*Ps. aeruginosa* ATCC 27853	2	0.5	ND	ND	ND	ND	ND	ND	ND	ND
4	*S. aureus* ATCC 29213	0.06	0.06	ND	ND	ND	ND	ND	ND	ND	ND
5	*E. faecalis* ATCC 29212	1	4	ND	ND	ND	ND	ND	ND	ND	ND
6	P4*	64	64	+	+	-	-	-	-	-	+
7	AB 018	0.5	0.25	-	-	-	-	+	-	-	-
8	AB 044	256	128	+	-	+	-	+	-	-	-
9	AB 055	64	16	+	-	+	-	+	-	-	-
10	AB 060	2	2	-	-	-	-	+	-	-	-
11	AB 061	128	32	+	-	+	-	+	-	-	-
12	AB 063	128	32	+	-	+	-	+	-	-	-
13	AB 065	>256	256	+	-	-	+	+	-	-	-
14	AB 066	>256	>256	+	-	+	+	+	-	-	-
15	AB 067	128	32	+	-	+	-	+	-	-	-
16	AB 071	2	2	-	-	-	-	+	-	-	-
17	AB 072	128	32	+	-	+	-	+	-	-	-
18	AB 075	128	16	+	-	+	-	+	-	-	-
19	AB 076	0.5	1	-	-	-	-	+	-	-	-
20	AB 084	32	16	+	-	+	-	+	-	-	-
21	AB 091	0.5	0.12	-	-	-	-	+	-	-	-
22	AB 092	128	32	+	-	+	-	+	-	-	-
23	AB 093	128	64	+	-	+	-	+	-	-	-
24	AB 094	128	64	+	-	+	-	+	-	-	-
25	AB 096	64	64	+	-	+	-	+	-	-	-
26	AB 098	0.5	0.12	-	-	-	-	+	-	-	-
27	AB 099	0.5	0.5	-	-	-	-	+	-	-	-
28	AB 102	64	64	+	-	+	-	+	-	-	-
29	AB 103	128	64	+	-	+	-	+	-	-	-
30	AB 104	64	32	+	-	+	-	+	-	-	-
31	AB 105	64	64	+	-	+	-	+	-	-	-
32	AB 106	128	64	+	-	+	-	+	-	-	-
33	AB 107	256	64	+	-	+	-	+	-	-	-
34	AB 108	256	16	+	-	+	-	+	-	-	-
35	AB 109	64	8	+	-	+	-	+	-	-	-
36	AB 111	0.5	0.25	-	-	-	-	+	-	-	-
37	AB 113	2	2	-	-	-	-	+	-	-	-
38	AB 114	32	16	+	-	+	-	+	-	-	-
39	AB 115	128	64	+	-	+	-	+	-	-	-
40	AB 116	1	0.5	-	-	-	-	+	-	-	-
41	A 001	16	16	+	-	+	-	+	-	-	-
42	A 002	64	16	+	-	+	-	+	-	-	-

Results of carbapenem susceptibility, carbapenemase activity and the presence of carbapenemase genes of *A. baumannii.* (cont.)

No	Strains	MICs (mg/L)		Carbapenemase activity		Presence of carbapenemase genes					
		IMP	MER	M-Hodge test	EDTA disk synergy test	$bla_{OXA23\text{-}like}$	$bla_{OXA24\text{-}like}$	$bla_{OXA51\text{-}like}$	$bla_{OXA58\text{-}like}$	$bla_{IMP\text{-}like}$	$bla_{VIM\text{-}like}$
43	A 003	0.5	0.25	-	-	-	-	+	-	-	-
44	A 004	64	16	+	-	+	-	+	-	-	-
45	A 005	64	16	+	-	+	-	+	-	-	-
46	A 006	64	16	+	-	+	-	+	-	-	-
47	A 007	2	2	-	-	-	-	+	-	-	-
48	A 008	256	64	+	-	+	-	+	-	-	-
49	A 010	64	16	+	-	+	-	+	-	-	-
50	A 011	128	32	+	-	+	-	+	-	-	-
51	A 012	128	32	+	-	+	-	+	-	-	-
52	A 013	128	64	+	-	+	-	+	-	-	-
53	A 014	64	32	+	-	+	-	+	-	-	-
54	A 015	64	32	+	-	+	-	+	-	-	-
55	A 016	64	16	+	-	+	-	+	-	-	-
56	A 017	32	16	+	-	+	-	+	-	-	-
57	A 018	64	16	+	-	+	-	+	-	-	-
58	A 019	16	16	+	-	+	-	+	-	-	-
59	A 021	64	16	+	-	+	-	+	-	-	-
60	A 022	32	16	+	-	+	-	+	-	-	-
61	A 023	128	64	+	-	+	+	+	-	-	-
62	A 024	64	16	+	-	+	-	+	-	-	-
63	A 025	0.5	0.25	-	-	-	-	+	-	-	-
64	A 026	64	16	+	-	+	-	+	-	-	-
65	A 027	128	64	+	-	+	-	+	-	-	-
66	A 028	2	2	-	-	-	-	+	-	-	-
67	A 030	128	64	+	-	+	-	+	-	-	-
68	A 031	128	64	+	-	+	-	+	-	-	-
69	A 032	64	32	+	-	+	-	+	-	-	-
70	A 033	128	64	+	-	-	+	+	+	-	-
71	A 034	64	32	+	-	+	-	+	-	-	-
72	A 035	1	0.25	-	-	-	-	+	-	-	-
73	A 036	1	0.5	-	-	-	-	+	-	-	-
74	A 037	0.5	0.25	-	-	-	-	+	-	-	-
75	A 038	128	64	+	-	+	-	+	-	-	-
76	A 040	64	16	+	-	+	-	+	-	-	-
77	A 041	1	0.5	-	-	-	-	+	-	-	-
78	A 042	2	1	-	-	-	-	+	-	-	-
79	A 043	32	16	+	-	+	-	+	-	-	-
80	A 044	64	32	+	-	+	-	+	-	-	-
81	A 045	32	16	+	-	+	-	+	-	-	-
82	A 046	128	32	+	-	+	-	+	-	-	-
83	A 047	64	16	+	-	+	-	+	-	-	-
84	A 048	32	16	+	-	+	-	+	-	-	-
85	A 049	4	1	-	-	-	-	+	-	-	-
86	A 051	64	16	+	-	+	-	+	-	-	-
87	A 052	64	16	+	-	+	-	+	-	-	-
88	A 053	0.5	0.5	-	-	-	-	+	-	-	-
89	A 054	256	64	+	-	+	-	+	-	-	-
90	A 055	256	256	+	-	-	+	+	+	-	-
91	A 056	1	0.25	-	-	-	-	+	-	-	-
92	A 057	128	16	+	-	+	-	+	-	-	-
93	A 058	1	0.25	-	-	-	-	+	-	-	-
94	A 059	32	16	+	-	+	-	+	-	-	-
95	A 060	2	2	-	-	-	-	+	-	-	-
96	A 064	64	16	+	-	+	-	+	-	-	-
97	A 065	64	16	+	-	+	-	+	-	-	-
98	A 066	16	16	+	-	+	-	+	-	-	-
99	A 067	32	16	+	-	+	-	+	-	-	-
100	A 068	0.5	2	-	-	-	-	+	-	-	-
101	A 070	128	64	+	-	+	+	+	+	-	-

Results of carbapenem susceptibility, carbapenemase activity and the presence of carbapenemase genes of *A. baumannii.* (cont.)

No	Strains	MICs (mg/L)		Carbapenemase activity		Presence of carbapenemase genes					
		IMP	MER	M-Hodge test	EDTA disk synergy test	$bla_{OXA23\text{-}like}$	$bla_{OXA24\text{-}like}$	$bla_{OXA51\text{-}like}$	$bla_{OXA58\text{-}like}$	$bla_{IMP\text{-}like}$	$bla_{VIM\text{-}like}$
102	A 071	1	1	-	-	-	-	+	-	-	-
103	A 072	32	16	+	-	+	-	+	-	-	-
104	A 074	64	32	+	-	+	-	+	-	-	-
105	A 075	64	32	+	-	+	-	+	-	-	-
106	A 077	32	16	+	-	+	-	+	-	-	-
107	A 078	0.5	0.5	-	-	-	-	+	-	-	-
108	A 079	32	32	+	-	+	-	+	-	-	-
109	A 080	0.5	0.5	-	-	-	-	+	-	-	-
110	A 082	0.5	0.5	-	-	-	-	+	-	-	-
111	A 083	256	64	+	-	+	-	+	-	-	-
112	A 084	256	128	+	-	-	+	+	+	-	-
113	A 085	0.5	0.25	-	-	-	-	+	-	-	-
114	A 086	1	0.25	-	-	-	-	+	-	-	-
115	A 087	64	16	+	-	+	-	+	-	-	-
116	A 088	128	64	+	-	+	-	+	-	-	-
117	A 089	64	32	+	-	+	-	+	-	-	-
118	A 090	32	8	+	-	+	-	+	-	-	-
119	A 091	128	32	+	-	+	-	+	-	-	-
120	A 092	128	32	+	-	+	-	+	-	-	-
121	A 093	128	16	+	-	+	-	+	-	-	-
122	A 094	128	64	+	-	-	+	+	+	-	-
123	A 095	128	32	+	-	+	-	+	-	-	-
124	A 096	0.5	0.25	-	-	-	-	+	-	-	-
125	A 098	128	32	+	-	+	-	+	-	-	-
126	A 100	1	0.25	-	-	-	-	+	-	-	-
127	A 101	128	16	+	-	+	-	+	-	-	-
128	A 104	256	64	+	-	+	-	+	-	-	-
129	A 106	>256	64	+	-	+	-	+	-	-	-
130	A 107	128	32	+	-	+	-	+	-	-	-
131	A 108	256	64	+	-	+	-	+	-	-	-
132	A 109	128	64	+	-	-	+	+	+	-	-
133	A 110	256	128	+	-	+	+	+	+	-	-
134	A 111	64	16	+	-	+	-	+	-	-	-
135	A 112	64	16	+	-	+	-	+	-	-	-
136	A 113	128	64	+	-	-	+	+	+	-	-
137	A 114	128	64	+	-	+	-	+	-	-	-
138	A 115	64	16	+	-	+	-	+	-	-	-
139	A 116	128	32	+	-	+	-	+	-	-	-
140	A 118	0.5	0.25	-	-	-	-	+	-	-	-
141	A 119	64	16	+	-	+	-	+	-	-	-
142	A 120	64	16	+	-	+	-	+	-	-	-
143	A 121	64	16	+	-	+	-	+	-	-	-
144	A 122	32	16	+	-	+	-	+	-	-	-
145	A 123	32	16	+	-	+	-	+	-	-	-
146	A 124	64	16	+	-	+	-	+	-	-	-
147	A 125	64	16	+	-	+	-	+	-	-	-
148	A 126	64	16	+	-	+	-	+	-	-	-
149	A 127	0.12	0.12	-	-	-	-	+	-	-	-
150	A 128	64	16	+	-	+	-	+	-	-	-
151	A 129	128	32	+	-	+	+	+	+	-	-
152	A 130	64	16	+	-	+	-	+	-	-	-
153	A 131	128	16	+	-	+	-	+	-	-	-
154	A 133	128	32	+	-	+	-	+	-	-	-
155	A 134	128	32	+	-	+	-	+	-	-	-
156	A 135	64	32	+	-	+	-	+	-	-	-
157	A 136	256	64	+	-	-	+	+	+	-	-
158	A 137	256	64	+	-	-	+	+	+	-	-
159	A 139	128	32	+	-	+	-	+	-	-	-
160	A 140	32	16	+	-	+	-	+	-	-	-

Results of carbapenem susceptibility, carbapenemase activity and the presence of carbapenemase genes of *A. baumannii.* (cont.)

No	Strains	MICs (mg/L)		Carbapenemase activity		Presence of carbapenemase genes					
		IMP	MER	M-Hodge test	EDTA disk synergy test	$bla_{OXA23\text{-}like}$	$bla_{OXA24\text{-}like}$	$bla_{OXA51\text{-}like}$	$bla_{OXA58\text{-}like}$	$bla_{IMP\text{-}like}$	$bla_{VIM\text{-}like}$
161	A 141	>256	256	+	-	+	-	+	-	-	-
162	A 142	64	16	+	-	+	-	+	-	-	-
163	A 144	256	64	+	-	-	+	+	+	-	-
164	A 145	64	16	+	-	+	-	+	-	-	-
165	A 146	>256	128	+	-	-	+	+	-	-	-
166	A 147	32	32	+	-	+	-	+	-	-	-
167	A 148	128	64	+	-	+	-	+	-	-	-
168	A 149	128	32	+	-	+	-	+	-	-	-
169	A 150	256	64	+	-	+	+	+	+	-	-
170	A 151	32	16	+	-	+	-	+	-	-	-
171	A 152	128	32	+	-	+	-	+	-	-	-
172	A 153	1	0.5	-	-	-	-	+	-	-	-
173	A 155	128	32	+	-	+	-	+	-	-	-
174	A 156	64	64	+	-	+	-	+	-	-	-
175	A 157	128	64	+	-	+	-	+	-	-	-
176	A 158	64	32	+	-	+	-	+	-	-	-
177	A 159	0.5	0.25	-	-	-	-	+	-	-	-
178	A 160	128	32	+	-	+	-	+	-	-	-
179	A 161	32	32	+	-	+	-	+	-	-	-
180	A 162	16	32	+	-	+	-	+	-	-	-
181	A 163	32	32	+	-	+	-	+	-	-	-
182	A 164	128	32	+	-	+	-	+	-	-	-
183	A 165	128	64	+	-	-	+	+	+	-	-
184	A 166	2	2	-	-	-	-	+	-	-	-
185	A 167	256	64	+	-	-	+	+	-	-	-
186	A 169	64	16	+	-	+	-	+	-	-	-
187	A 170	128	32	+	-	+	-	+	-	-	-
188	A 171	32	16	+	-	+	-	+	-	-	-
189	A 172	32	16	+	-	+	-	+	-	-	-
190	A 176	0.25	0.25	-	-	-	-	+	-	-	-
191	A 177	256	128	+	-	-	+	+	-	-	-
192	A 178	2	1	-	-	-	-	+	-	-	-
193	A 179	128	32	+	-	+	-	+	-	-	-
194	A 180	128	128	+	-	-	+	+	+	-	-
195	A 181	64	16	+	-	+	-	+	-	-	-
196	A 183	64	32	+	-	+	-	+	-	-	-
197	A 184	2	1	-	-	-	-	+	-	-	-
198	A 185	64	32	+	-	+	-	+	-	-	-
199	A 186	128	32	+	-	+	-	+	-	-	-
200	A 187	128	16	+	-	+	-	+	-	-	-
201	A 188	64	32	+	-	+	-	+	+	-	-
202	A 189	64	32	+	-	+	-	+	-	-	-
203	A 190	64	16	+	-	+	-	+	-	-	-
204	A 191	64	32	+	-	+	-	+	-	-	-
205	A 194	128	32	+	-	+	-	+	-	-	-
206	A 196	128	32	+	-	+	-	+	-	-	-
207	A 198	128	64	+	-	-	+	+	+	-	-
208	A 199	64	32	+	-	+	-	+	-	-	-
209	A 200	64	32	+	-	+	-	+	-	-	-
210	A 201	64	32	+	-	+	-	+	-	-	-
211	A 202	64	32	+	-	+	-	+	-	-	-
212	A 203	16	16	+	-	+	-	+	-	-	-
213	A 204	32	16	+	-	+	-	+	-	-	-
214	A 205	64	32	+	-	+	-	+	-	-	-
215	A 206	32	16	+	-	+	-	+	-	-	-
216	A 207	32	16	+	-	+	-	+	-	-	-
217	A 208	32	16	+	-	+	-	+	-	-	-
218	A 209	32	16	+	-	+	-	+	-	-	-
219	A 210	64	16	+	-	+	-	+	-	-	-
220	A 211	32	16	+	-	+	-	+	-	-	-

Results of carbapenem susceptibility, carbapenemase activity and the presence of carbapenemase genes of *A. baumannii.* (cont.)

No	Strains	MICs (mg/L)		Carbapenemase activity		Presence of carbapenemase genes					
		IMP	MER	M-Hodge test	EDTA disk synergy test	$bla_{OXA23\text{-}like}$	$bla_{OXA24\text{-}like}$	$bla_{OXA51\text{-}like}$	$bla_{OXA58\text{-}like}$	$bla_{IMP\text{-}like}$	$bla_{VIM\text{-}like}$
221	A 212	128	32	+	-	+	-	+	-	-	-
222	A 213	64	16	+	-	+	-	+	-	-	-
223	A 214	64	16	+	-	+	-	+	-	-	-
224	A 215	32	16	+	-	+	-	+	-	-	-
225	A 216	64	16	+	-	+	-	+	-	-	-
226	A 217	64	32	+	-	+	-	+	-	-	-
227	A 218	64	32	+	-	+	-	+	-	-	-
228	A 219	16	16	+	-	+	-	+	-	-	-
229	A 220	32	16	+	-	+	-	+	-	-	-
230	A 221	32	16	+	-	+	-	+	-	-	-
231	A 222	64	16	+	-	+	-	+	-	-	-
232	A 223	2	1	-	-	-	-	+	-	-	-
233	A 226	128	32	+	-	+	-	+	-	-	-
234	A 228	32	16	+	-	+	-	+	-	-	-
235	A 229	64	32	+	-	+	-	+	-	-	-
236	A 230	32	16	+	-	+	-	+	-	-	-
237	A 231	32	16	+	-	+	-	+	-	-	-
238	A 232	32	16	+	-	+	-	+	-	-	-
239	A 233	2	1	-	-	-	-	+	-	-	-
240	A 234	128	64	+	-	+	-	+	-	-	-
241	A 235	32	16	+	-	+	-	+	-	-	-
242	A 236	128	64	+	-	+	-	+	-	-	-
243	A 237	1	1	-	-	-	-	+	-	-	-
244	A 238	256	64	+	-	+	+	+	+	-	-
245	A 240	32	16	+	-	+	-	+	-	-	-
246	A 241	64	16	+	-	+	-	+	-	-	-
247	A 242	256	64	+	-	+	+	+	+	-	-
248	A 243	64	16	+	-	+	-	+	-	-	-
249	A 244	64	16	+	-	+	-	+	-	-	-
250	A 245	32	16	+	-	+	-	+	-	-	-
251	A 246	32	16	+	-	+	-	+	-	-	-
252	A 247	128	32	+	-	+	-	+	-	-	-
253	A 249	256	64	+	-	+	-	+	-	-	-
254	A 250	128	32	+	-	+	-	+	+	-	-
255	A 251	16	32	+	-	+	-	+	-	-	-
256	A 252	0.5	1	-	-	-	-	+	-	-	-
257	A 253	128	32	+	-	+	-	+	-	-	-
258	A 257	64	32	+	-	+	-	+	-	-	-
259	A 258	128	32	+	-	+	-	+	-	-	-
260	A 259	64	16	+	-	+	-	+	-	-	-
261	A 260	64	16	+	-	+	-	+	-	-	-
262	A 262	64	16	+	-	+	-	+	+	-	-
263	A 263	64	32	+	-	+	-	+	-	-	-
264	A 265	256	64	+	-	+	+	+	+	-	-
265	A 266	32	16	+	-	+	-	+	+	-	-
266	A 267	2	1	-	-	-	-	+	-	-	-
267	A 268	64	16	+	-	+	-	+	-	-	-
268	A 269	32	16	+	-	+	-	+	-	-	-
269	A 270	128	32	+	-	+	-	+	-	-	-
270	A 271	256	64	+	-	+	+	+	-	-	-
271	A 272	32	16	+	-	+	-	+	-	-	-
272	A 274	64	16	+	-	+	-	+	-	-	-
273	A 275	64	16	+	-	+	-	+	-	-	-
274	A 277	64	32	+	-	+	-	+	-	-	-
275	A 278	64	16	+	-	+	-	+	-	-	-
276	A 280	64	16	+	-	+	-	+	-	-	-
277	A 281	0.5	0.5	-	-	-	-	+	-	-	-
278	A 285	128	32	+	-	+	-	+	-	-	-
279	A 286	64	16	+	-	+	-	+	-	-	-
280	A 288	128	64	+	-	-	+	+	+	-	-

Results of carbapenem susceptibility, carbapenemase activity and the presence of carbapenemase genes of *A. baumannii.* (cont.)

No	Strains	MICs (mg/L)		Carbapenemase activity		Presence of carbapenemase genes					
		IMP	MER	M-Hodge test	EDTA disk synergy test	$bla_{OXA23\text{-}like}$	$bla_{OXA24\text{-}like}$	$bla_{OXA51\text{-}like}$	$bla_{OXA58\text{-}like}$	$bla_{IMP\text{-}like}$	$bla_{VIM\text{-}like}$
281	A 289	128	32	+	-	+	-	+	+	-	-
282	A 290	1	1	-	-	-	-	+	-	-	-
283	A 291	128	32	+	-	+	-	+	+	-	-
284	A 292	64	16	+	-	+	-	+	+	-	-
285	A 293	2	1	-	-	-	-	+	-	-	-
286	A 296	64	16	+	-	+	-	+	-	-	-
287	A 297	0.5	0.5	-	-	-	-	+	-	-	-
288	A 298	128	32	+	-	+	-	+	-	-	-
289	A 300	128	32	+	-	+	-	+	-	-	-
290	A 302	64	16	+	-	+	-	+	-	-	-
291	A 303	128	64	+	-	-	+	+	+	-	-
292	A 305	32	32	+	-	+	-	+	-	-	-
293	A 307	32	16	+	-	+	-	+	-	-	-
294	A 308	0.5	0.25	-	-	-	-	+	-	-	-
295	A 309	0.5	0.5	-	-	-	-	+	-	-	-
296	A 310	32	16	+	-	+	-	+	-	-	-
297	A 311	256	64	+	-	+	-	+	-	-	-
298	A 312	64	32	+	-	+	-	+	-	-	-
299	A 314	0.25	0.25	-	-	-	-	+	-	-	-
300	A 315	64	32	+	-	+	-	+	-	-	-
301	A 316	64	32	+	-	+	-	+	-	-	-
302	A 317	64	32	+	-	+	-	+	-	-	-
303	A 318	128	32	+	-	+	-	+	-	-	-
304	A 320	64	16	+	-	+	-	+	-	-	-
305	A 321	64	32	+	-	+	-	+	-	-	-
306	A 322	128	32	+	-	+	-	+	-	-	-
307	A 323	256	64	+	-	+	+	+	+	-	-
308	A 324	64	16	+	-	+	-	+	-	-	-
309	A 325	128	32	+	-	+	-	+	-	-	-
310	A 327	64	16	+	-	+	-	+	-	-	-
311	A 328	64	16	+	-	+	-	+	-	-	-
312	A 329	64	32	+	-	+	-	+	-	-	-
313	A 332	32	16	+	-	+	-	+	-	-	-
314	A 334	64	32	+	-	+	-	+	-	-	-
315	A 335	128	32	+	-	+	-	+	-	-	-
316	A 337	32	32	+	-	+	-	+	-	-	-
317	A 338	0.5	0.5	-	-	-	-	+	-	-	-
318	A 339	128	32	+	-	+	-	+	-	-	-
319	A 341	0.5	2	-	-	-	-	+	-	-	-
320	A 342	128	32	+	-	+	-	+	-	-	-
321	A 343	128	32	+	-	+	-	+	-	-	-
322	A 345	32	16	+	-	+	-	+	-	-	-
323	A 346	64	16	+	-	+	-	+	-	-	-
324	A 347	128	32	+	-	+	-	+	-	-	-
325	A 348	32	16	+	-	+	-	+	-	-	-
326	A 350	1	0.25	-	-	-	-	+	-	-	-
327	A 351	128	32	+	-	+	-	+	-	-	-
328	A 352	32	32	+	-	+	-	+	-	-	-
329	A 354	128	32	+	-	+	-	+	-	-	-
330	A 356	128	32	+	-	+	-	+	-	-	-
331	A 357	1	0.25	-	-	-	-	+	-	-	-
332	A 358	1	0.12	-	-	-	-	+	-	-	-
333	A 360	64	32	+	-	+	-	+	-	-	-
334	A 362	64	16	+	-	+	-	+	-	-	-
335	A 364	2	2	-	-	-	-	+	-	-	-
336	A 365	64	64	+	-	+	-	+	-	-	-
337	A 368	32	32	+	-	+	-	+	-	-	-
338	A 369	128	32	+	-	+	-	+	-	-	-
339	A 370	128	32	+	-	+	-	+	-	-	-
340	A 371	128	32	+	-	+	-	+	-	-	-

Results of carbapenem susceptibility, carbapenemase activity and the presence of carbapenemase genes of *A. baumannii.* (cont.)

No	Strains	MICs (mg/L)		Carbapenemase activity		Presence of carbapenemase genes					
		IMP	MER	M-Hodge test	EDTA disk synergy test	$bla_{OXA23\text{-}like}$	$bla_{OXA24\text{-}like}$	$bla_{OXA51\text{-}like}$	$bla_{OXA58\text{-}like}$	$bla_{IMP\text{-}like}$	$bla_{VIM\text{-}like}$
341	A 372	64	16	+	-	+	-	+	-	-	-
342	A 373	64	64	+	-	+	-	+	-	-	-
343	A 374	32	16	+	-	+	-	+	-	-	-
344	A 375	128	64	+	-	+	-	+	-	-	-
345	A 377	256	128	+	-	-	+	+	+	-	-
346	A 378	256	64	+	-	+	-	+	-	-	-
347	A 380	2	4	-	-	-	-	+	-	-	-
348	A 381	32	32	+	-	+	-	+	-	-	-
349	A 382	256	64	+	-	-	+	+	+	-	-
350	A 383	128	64	+	-	+	-	+	-	-	-
351	A 384	256	32	+	-	-	+	+	+	-	-
352	A 385	1	0.5	-	-	-	-	+	-	-	-
353	A 386	256	32	+	-	+	-	+	-	-	-
354	A 387	64	32	+	-	+	-	+	-	-	-
355	A 388	256	32	+	-	+	-	+	-	-	-
356	A 389	256	64	+	-	+	+	+	+	-	-
357	A 390	128	32	+	-	+	-	+	-	-	-
358	A 392	0.5	1	-	-	-	-	+	-	-	-
359	A 393	256	32	+	-	+	-	+	-	-	-
360	A 394	128	32	+	-	+	-	+	-	-	-
361	A 397	256	64	+	-	+	-	+	-	-	-
362	A 398	2	0.5	-	-	-	-	+	-	-	-
363	A 399	128	32	+	-	+	-	+	-	-	-
364	A 400	0.5	0.5	-	-	-	-	+	-	-	-
365	A 401	256	64	+	-	-	+	+	+	-	-
366	A 402	256	128	+	-	+	+	+	+	-	-
367	A 403	128	32	+	-	+	-	+	-	-	-
368	A 404	64	32	+	-	+	-	+	-	-	-
369	A 405	4	4	-	-	-	-	+	-	-	-
370	A 408	32	16	+	-	+	-	+	-	-	-
371	A 409	32	16	+	-	+	-	+	-	-	-
372	A 410	128	32	+	-	+	-	+	-	-	-
373	A 412	256	64	+	-	+	-	+	-	-	-
374	A 415	128	32	+	-	+	-	+	-	-	-
375	A 416	64	32	+	-	+	-	+	-	-	-
376	A 419	0.5	0.25	-	-	-	-	+	-	-	-
377	A 420	128	32	+	-	+	-	+	-	-	-
378	A 421	32	16	+	-	+	-	+	-	-	-
379	A 422	1	0.5	-	-	-	-	+	-	-	-
380	A 423	256	64	+	-	+	+	+	+	-	-
381	A 424	64	16	+	-	+	-	+	-	-	-
382	A 425	256	128	+	-	-	+	+	+	-	-
383	A 426	256	128	+	-	-	+	+	+	-	-
384	A 427	256	64	+	-	-	+	+	+	-	-
385	A 428	256	64	+	-	+	-	+	-	-	-
386	A 429	256	64	+	-	-	+	+	+	-	-
387	A 430	0.5	0.25	-	-	-	-	+	-	-	-
388	A 431	256	64	+	-	+	-	+	-	-	-
389	A 432	>256	32	+	-	+	-	+	-	-	-
390	A 433	>256	64	+	-	+	-	+	-	-	-
391	A 434	128	16	+	-	+	-	+	-	-	-
392	A 435	256	64	+	-	-	+	+	+	-	-
393	A 436	256	64	+	-	+	-	+	-	-	-
394	A 437	128	64	+	-	-	+	+	+	-	-
395	A 438	64	16	+	-	+	-	+	-	-	-
396	A 439	256	128	+	-	+	-	+	-	-	-
397	A 440	32	16	+	-	+	-	+	-	-	-
398	A 441	64	32	+	-	+	-	+	-	-	-
399	A 442	64	16	+	-	+	-	+	-	-	-
400	A 443	64	16	+	-	+	-	+	-	-	-

Results of carbapenem susceptibility, carbapenemase activity and the presence of carbapenemase genes of *A. baumannii.* (cont.)

No	Strains	MICs (mg/L)		Carbapenemase activity		Presence of carbapenemase genes					
		IMP	MER	M-Hodge test	EDTA disk synergy test	$bla_{OXA23\text{-}like}$	$bla_{OXA24\text{-}like}$	$bla_{OXA51\text{-}like}$	$bla_{OXA58\text{-}like}$	$bla_{IMP\text{-}like}$	$bla_{VIM\text{-}like}$
401	A 444	128	64	+	-	+	-	+	-	-	-
402	A 446	256	128	+	-	+	+	+	+	-	-
403	A 449	32	16	+	-	+	-	+	-	-	-
404	A 450	64	32	+	-	+	-	+	-	-	-
405	A 453	64	16	+	-	+	-	+	-	-	-
406	A 455	32	16	+	-	+	-	+	-	-	-
407	A 456	32	16	+	-	+	-	+	-	-	-
408	A 458	128	16	+	-	+	-	+	-	-	-
409	A 459	128	16	+	-	+	-	+	-	-	-
410	A 460	64	64	+	-	+	-	+	-	-	-
411	A 461	64	16	+	-	+	-	+	-	-	-
412	A 462	64	16	+	-	+	-	+	-	-	-
413	A 465	128	64	+	-	+	-	+	-	-	-
414	A 466	128	16	+	-	+	-	+	-	-	-
415	A 467	128	16	+	-	+	-	+	-	-	-
416	A 468	64	32	+	-	+	-	+	-	-	-
417	A 469	128	64	+	-	+	-	+	-	-	-
418	A 470	256	128	+	-	+	+	+	+	-	-
419	A 471	256	128	+	-	+	+	+	+	-	-
420	A 472	256	128	+	-	+	+	+	+	-	-
421	A 473	1	0.5	-	-	-	-	+	-	-	-
422	A 474	0.5	0.25	-	-	-	-	+	-	-	-
423	A 475	32	8	+	-	+	-	+	-	-	-
424	A 479	64	16	+	-	+	-	+	-	-	-
425	A 480	0.5	0.5	-	-	-	-	+	-	-	-
426	A 481	64	32	+	-	+	-	+	-	-	-
427	A 482	64	32	+	-	+	-	+	-	-	-
428	A 483	0.5	0.5	-	-	-	-	+	-	-	-
429	A 486	64	32	+	-	+	-	+	-	-	-
430	A 487	64	16	+	-	+	-	+	-	-	-
431	A 488	0.5	1	-	-	-	-	+	-	-	-
432	A 489	32	16	+	-	+	-	+	-	-	-
433	A 493	1	0.12	-	-	-	-	+	-	-	-
434	A 494	0.5	0.5	-	-	-	-	+	-	-	-
435	A 495	64	32	+	-	+	-	+	-	-	-
436	A 496	0.5	1	-	-	-	-	+	-	-	-
437	A 497	32	32	+	-	+	-	+	-	-	-
438	A 498	32	16	+	-	+	-	+	-	-	-
439	A 499	64	16	+	-	+	-	+	-	-	-
440	A 500	>256	64	+	-	+	-	+	-	-	-
441	A 502	1	0.25	-	-	-	-	+	-	-	-
442	A 503	64	64	+	-	+	-	+	-	-	-
443	A 504	0.5	0.25	-	-	-	-	+	-	-	-
444	A 505	0.5	0.25	-	-	-	-	+	-	-	-
445	A 506	64	32	+	-	+	-	+	-	-	-
446	A 507	64	32	+	-	+	-	+	-	-	-
447	A 508	0.5	1	-	-	-	-	+	-	-	-
448	A 510	128	32	+	-	+	-	+	-	-	-
449	A 511	64	16	+	-	+	-	+	-	-	-
450	A 512	128	64	+	-	+	+	+	-	-	-
451	A 513	64	32	+	-	+	-	+	-	-	-
452	A 514	0.5	0.25	-	-	-	-	+	-	-	-
453	A 515	128	64	+	-	+	-	+	-	-	-
454	A 516	256	64	+	-	+	-	+	-	-	-
455	A 517	128	32	+	-	+	-	+	-	-	-
456	A 518	256	32	+	-	+	-	+	-	-	-
457	A 519	>256	64	+	-	+	+	+	-	-	-
458	A 522	128	16	+	-	+	-	+	-	-	-
459	A 523	128	32	+	-	+	-	+	-	-	-
460	A 524	128	32	+	-	+	-	+	-	-	-

Results of carbapenem susceptibility, carbapenemase activity and the presence of carbapenemase genes of *A. baumannii.* (cont.)

No	Strains	MICs (mg/L)		Carbapenemase activity		Presence of carbapenemase genes					
		IMP	MER	M-Hodge test	EDTA disk synergy test	$bla_{OXA23\text{-}like}$	$bla_{OXA24\text{-}like}$	$bla_{OXA51\text{-}like}$	$bla_{OXA58\text{-}like}$	$bla_{IMP\text{-}like}$	$bla_{VIM\text{-}like}$
461	A 526	128	32	+	-	+	-	+	-	-	-
462	A 527	128	32	+	-	+	-	+	-	-	-
463	A 528	128	16	+	-	+	-	+	-	-	-
464	A 529	256	32	+	-	+	-	+	-	-	-
465	A 530	128	32	+	-	+	-	+	-	-	-
466	A 531	128	32	+	-	+	-	+	-	-	-
467	A 532	128	32	+	-	+	-	+	-	-	-
468	A 535	128	32	+	-	+	-	+	-	-	-
469	A 536	4	0.5	-	-	-	-	+	-	-	-
470	A 537	>256	128	+	-	+	+	+	-	-	-
471	A 538	128	16	+	-	+	-	+	-	-	-
472	A 539	256	32	+	-	+	-	+	-	-	-
473	A 540	1	0.5	-	-	-	-	+	-	-	-
474	A 541	256	32	+	-	+	-	+	-	-	-
475	A 542	64	16	+	-	+	-	+	-	-	-
476	A 543	128	32	+	-	+	-	+	-	-	-
477	A 544	128	32	+	-	+	-	+	-	-	-
478	A 546	1	0.25	-	-	-	-	+	-	-	-
479	A 548	256	64	+	-	+	-	+	-	-	-
480	A 549	1	0.25	-	-	-	-	+	-	-	-
481	A 551	128	32	+	-	+	-	+	-	-	-
482	A 554	256	64	+	-	+	-	+	-	-	-
483	A 557	128	16	+	-	+	-	+	-	-	-
484	A 558	2	0.5	-	-	-	-	+	-	-	-
485	A 559	>256	128	+	-	+	+	+	+	-	-
486	A 560	128	32	+	-	+	-	+	-	-	-
487	A 562	256	32	+	-	+	-	+	-	-	-
488	A 563	256	32	+	-	+	-	+	-	-	-
489	A 564	4	1	-	-	-	-	+	-	-	-
490	A 565	1	1	-	-	-	-	+	-	-	-
491	A 566	64	16	+	-	+	-	+	-	-	-
492	A 567	256	64	+	-	+	-	+	-	-	-
493	A 568	256	32	+	-	+	-	+	-	-	-
494	A 569	256	32	+	-	+	-	+	-	-	-
495	A 570	128	32	+	-	+	-	+	-	-	-
496	A 571	256	32	+	-	+	-	+	-	-	-
497	A 573	64	16	+	-	+	-	+	-	-	-
498	A 574	256	64	+	-	+	-	+	-	-	-
499	A 575	128	32	+	-	+	-	+	-	-	-
500	A 576	256	32	+	-	+	-	+	-	-	-
501	A 577	64	32	+	-	+	-	+	-	-	-

+ = positive , - = negative

[a] P4 = *Pseudomonas aeruginosa* (lab strain) that carried $bla_{VIM\text{-}like}$

APPENDIX E

NUCLEOTIDE SEQUENCES ALIGNMENT OF SELECTED 10 *A. BAUMANNII* ISOLATES

1. OXA-23 GROUP

```
           1                                                                                                    120
OXA-23     ATGAATAAAT ATTTTACTTG CTATGTGGTT GCTTCTCTTT TTCTTTCTGG TTGTACGGTT CAGCATAATT TAATAAATGA AACCCCGAGT CAGATTGTTC AAGGACATAA TCAGGTGATT
A66        ATGAATAAAT ATTTTACTTG CTATGTGGTT GCTTCTCTTT TTCTTTCTGG TTGTACGGTT CAGCATAATT TAATAAATGA AACCCCGAGT CAGATTGTTC AAGGACATAA TCAGGTGATT
A67        ATGAATAAAT ATTTTACTTG CTATGTGGTT GCTTCTCTTT TTCTTTCTGG TTGTACGGTT CAGCATAATT TAATAAATGA AACCCCGAGT CAGATTGTTC AAGGACATAA TCAGGTGATT
A72        ATGAATAAAT ATTTTACTTG CTATGTGGTT GCTTCTCTTT TTCTTTCTGG TTGTACGGTT CAGCATAATT TAATAAATGA AACCCCGAGT CAGATTGTTC AAGGACATAA TCAGGTGATT
A266       ATGAATAAAT ATTTTACTTG CTATGTGGTT GCTTCTCTTT TTCTTTCTGG TTGTACGGTT CAGCATAATT TAATAAATGA AACCCCGAGT CAGATTGTTC AAGGACATAA TCAGGTGATT
A74        ATGAATAAAT ATTTTACTTG CTATGTGGTT GCTTCTCTTT TTCTTTCTGG TTGTACGGTT CAGCATAATT TAATAAATGA AACCCCGAGT CAGATTGTTC AAGGACATAA TCAGGTGATT
A150       ATGAATAAAT ATTTTACTTG CTATGTGGTT GCTTCTCTTT TTCTTTCTGG TTGTACGGTT CAGCATAATT TAATAAATGA AACCCCGAGT CAGATTGTTC AAGGACATAA TCAGGTGATT
A472       ATGAATAAAT ATTTTACTTG CTATGTGGTT GCTTCTCTTT TTCTTTCTGG TTGTACGGTT CAGCATAATT TAATAAATGA AACCCCGAGT CAGATTGTTC AAGGACATAA TCAGGTGATT
A446       ATGAATAAAT ATTTTACTTG CTATGTGGTT GCTTCTCTTT TTCTTTCTGG TTGTACGGTT CAGCATAATT TAATAAATGA AACCCCGAGT CAGATTGTTC AAGGACATAA TCAGGTGATT
A433       ATGAATAAAT ATTTTACTTG CTATGTGGTT GCTTCTCTTT TTCTTTCTGG TTGTACGGTT CAGCATAATT TAATAAATGA AACCCCGAGT CAGATTGTTC AAGGACATAA TCAGGTGATT
A519       ATGAATAAAT ATTTTACTTG CTATGTGGTT GCTTCTCTTT TTCTTTCTGG TTGTACGGTT CAGCATAATT TAATAAATGA AACCCCGAGT CAGATTGTTC AAGGACATAA TCAGGTGATT
Consensus  ATGAATAAAT ATTTTACTTG CTATGTGGTT GCTTCTCTTT TTCTTTCTGG TTGTACGGTT CAGCATAATT TAATAAATGA AACCCCGAGT CAGATTGTTC AAGGACATAA TCAGGTGATT

           121                                                                                                  240
OXA-23     CATCAATACT TTGATGAAAA AAACACCTCA GGTGTGCTGG TTATTCAAAC AGATAAAAAA ATTAATCTAT ATGGTAATGC TCTAAGCCGC GCAAATACAG AATATGTGCC AGCCTCTACA
A66        CATCAATACT TTGATGAAAA AAACACCTCA GGTGTGCTGG TTATTCAAAC AGATAAAAAA ATTAATCTAT ATGGTAATGC TCTAAGCCGC GCAAATACAG AATATGTGCC AGCCTCTACA
A67        CATCAATACT TTGATGAAAA AAACACCTCA GGTGTGCTGG TTATTCAAAC AGATAAAAAA ATTAATCTAT ATGGTAATGC TCTAAGCCGC GCAAATACAG AATATGTGCC AGCCTCTACA
A72        CATCAATACT TTGATGAAAA AAACACCTCA GGTGTGCTGG TTATTCAAAC AGATAAAAAA ATTAATCTAT ATGGTAATGC TCTAAGCCGC GCAAATACAG AATATGTGCC AGCCTCTACA
A266       CATCAATACT TTGATGAAAA AAACACCTCA GGTGTGCTGG TTATTCAAAC AGATAAAAAA ATTAATCTAT ATGGTAATGC TCTAAGCCGC GCAAATACAG AATATGTGCC AGCCTCTACA
A74        CATCAATACT TTGATGAAAA AAACACCTCA GGTGTGCTGG TTATTCAAAC AGATAAAAAA ATTAATCTAT ATGGTAATGC TCTAAGCCGC GCAAATACAG AATATGTGCC AGCCTCTACA
A150       CATCAATACT TTGATGAAAA AAACACCTCA GGTGTGCTGG TTATTCAAAC AGATAAAAAA ATTAATCTAT ATGGTAATGC TCTAAGCCGC GCAAATACAG AATATGTGCC AGCCTCTACA
A472       CATCAATACT TTGATGAAAA AAACACCTCA GGTGTGCTGG TTATTCAAAC AGATAAAAAA ATTAATCTAT ATGGTAATGC TCTAAGCCGC GCAAATACAG AATATGTGCC AGCCTCTACA
A446       CATCAATACT TTGATGAAAA AAACACCTCA GGTGTGCTGG TTATTCAAAC AGATAAAAAA ATTAATCTAT ATGGTAATGC TCTAAGCCGC GCAAATACAG AATATGTGCC AGCCTCTACA
A433       CATCAATACT TTGATGAAAA AAACACCTCA GGTGTGCTGG TTATTCAAAC AGATAAAAAA ATTAATCTAT ATGGTAATGC TCTAAGCCGC GCAAATACAG AATATGTGCC AGCCTCTACA
A519       CATCAATACT TTGATGAAAA AAACACCTCA GGTGTGCTGG TTATTCAAAC AGATAAAAAA ATTAATCTAT ATGGTAATGC TCTAAGCCGC GCAAATACAG AATATGTGCC AGCCTCTACA
Consensus  CATCAATACT TTGATGAAAA AAACACCTCA GGTGTGCTGG TTATTCAAAC AGATAAAAAA ATTAATCTAT ATGGTAATGC TCTAAGCCGC GCAAATACAG AATATGTGCC AGCCTCTACA
```

```
           241                                                                                                                                                  360
OXA-23     TTTAAAATGT TGAATGCCCT GATCGGATTG GAGAACCAGA AAACGGATAT TAATGAAATA TTTAAATGGA AGGGCGAGAA AAGGTCATTT ACCGCTTGGG AAAAAGACAT GACACTAGGA
A66        TTTAAAATGT TGAATGCCCT GATCGGATTG GAGAACCAGA AAACGGATAT TAATGAAATA TTTAAATGGA AGGGCGAGAA AAGGTCATTT ACCGCTTGGG AAAAAGACAT GACACTAGGA
A67        TTTAAAATGT TGAATGCCCT GATCGGATTG GAGAACCAGA AAACGGATAT TAATGAAATA TTTAAATGGA AGGGCGAGAA AAGGTCATTT ACCGCTTGGG AAAAAGACAT GACACTAGGA
A72        TTTAAAATGT TGAATGCCCT GATCGGATTG GAGAACCAGA AAACGGATAT TAATGAAATA TTTAAATGGA AGGGCGAGAA AAGGTCATTT ACCGCTTGGG AAAAAGACAT GACACTAGGA
A266       TTTAAAATGT TGAATGCCCT GATCGGATTG GAGAACCAGA AAACGGATAT TAATGAAATA TTTAAATGGA AGGGCGAGAA AAGGTCATTT ACCGCTTGGG AAAAAGACAT GACACTAGGA
A74        TTTAAAATGT TGAATGCCCT GATCGGATTG GAGAACCAGA AAACGGATAT TAATGAAATA TTTAAATGGA AGGGCGAGAA AAGGTCATTT ACCGCTTGGG AAAAAGACAT GACACTAGGA
A150       TTTAAAATGT TGAATGCCCT GATCGGATTG GAGAACCAGA AAACGGATAT TAATGAAATA TTTAAATGGA AGGGCGAGAA AAGGTCATTT ACCGCTTGGG AAAAAGACAT GACACTAGGA
A472       TTTAAAATGT TGAATGCCCT GATCGGATTG GAGAACCAGA AAACGGATAT TAATGAAATA TTTAAATGGA AGGGCGAGAA AAGGTCATTT ACCGCTTGGG AAAAAGACAT GACACTAGGA
A446       TTTAAAATGT TGAATGCCCT GATCGGATTG GAGAACCAGA AAACGGATAT TAATGAAATA TTTAAATGGA AGGGCGAGAA AAGGTCATTT ACCGCTTGGG AAAAAGACAT GACACTAGGA
A433       TTTAAAATGT TGAATGCCCT GATCGGATTG GAGAACCAGA AAACGGATAT TAATGAAATA TTTAAATGGA AGGGCGAGAA AAGGTCATTT ACCGCTTGGG AAAAAGACAT GACACTAGGA
A519       TTTAAAATGT TGAATGCCCT GATCGGATTG GAGAACCAGA AAACGGATAT TAATGAAATA TTTAAATGGA AGGGCGAGAA AAGGTCATTT ACCGCTTGGG AAAAAGACAT GACACTAGGA
Consensus  TTTAAAATGT TGAATGCCCT GATCGGATTG GAGAACCAGA AAACGGATAT TAATGAAATA TTTAAATGGA AGGGCGAGAA AAGGTCATTT ACCGCTTGGG AAAAAGACAT GACACTAGGA

           361                                                                                                                                                  480
OXA-23     GAAGCCATGA AGCTTTCTGC AGTCCCAGTC TATCAGGAAC TTGCGCGACG TATCGGTCTT GATCTCATGC AAAAAGAAGT AAAACGTATT GGTTTCGGTA ATGCTGAAAT TGGACAGCAG
A66        GAAGCCATGA AGCTTTCTGC AGTCCCAGTC TATCAGGAAC TTGCGCGACG TATCGGTCTT GATCTCATGC AAAAAGAAGT AAAACGTATT GGTTTCGGTA ATGCTGAAAT TGGACAGCAG
A67        GAAGCCATGA AGCTTTCTGC AGTCCCAGTC TATCAGGAAC TTGCGCGACG TATCGGTCTT GATCTCATGC AAAAAGAAGT AAAACGTATT GGTTTCGGTA ATGCTGAAAT TGGACAGCAG
A72        GAAGCCATGA AGCTTTCTGC AGTCCCAGTC TATCAGGAAC TTGCGCGACG TATCGGTCTT GATCTCATGC AAAAAGAAGT AAAACGTATT GGTTTCGGTA ATGCTGAAAT TGGACAGCAG
A266       GAAGCCATGA AGCTTTCTGC AGTCCCAGTC TATCAGGAAC TTGCGCGACG TATCGGTCTT GATCTCATGC AAAAAGAAGT AAAACGTATT GGTTTCGGTA ATGCTGAAAT TGGACAGCAG
A74        GAAGCCATGA AGCTTTCTGC AGTCCCAGTC TATCAGGAAC TTGCGCGACG TATCGGTCTT GATCTCATGC AAAAAGAAGT AAAACGTATT GGTTTCGGTA ATGCTGAAAT TGGACAGCAG
A150       GAAGCCATGA AGCTTTCTGC AGTCCCAGTC TATCAGGAAC TTGCGCGACG TATCGGTCTT GATCTCATGC AAAAAGAAGT AAAACGTATT GGTTTCGGTA ATGCTGAAAT TGGACAGCAG
A472       GAAGCCATGA AGCTTTCTGC AGTCCCAGTC TATCAGGAAC TTGCGCGACG TATCGGTCTT GATCTCATGC AAAAAGAAGT AAAACGTATT GGTTTCGGTA ATGCTGAAAT TGGACAGCAG
A446       GAAGCCATGA AGCTTTCTGC AGTCCCAGTC TATCAGGAAC TTGCGCGACG TATCGGTCTT GATCTCATGC AAAAAGAAGT AAAACGTATT GGTTTCGGTA ATGCTGAAAT TGGACAGCAG
A433       GAAGCCATGA AGCTTTCTGC AGTCCCAGTC TATCAGGAAC TTGCGCGACG TATCGGTCTT GATCTCATGC AAAAAGAAGT AAAACGTATT GGTTTCGGTA ATGCTGAAAT TGGACAGCAG
A519       GAAGCCATGA AGCTTTCTGC AGTCCCAGTC TATCAGGAAC TTGCGCGACG TATCGGTCTT GATCTCATGC AAAAAGAAGT AAAACGTATT GGTTTCGGTA ATGCTGAAAT TGGACAGCAG
Consensus  GAAGCCATGA AGCTTTCTGC AGTCCCAGTC TATCAGGAAC TTGCGCGACG TATCGGTCTT GATCTCATGC AAAAAGAAGT AAAACGTATT GGTTTCGGTA ATGCTGAAAT TGGACAGCAG

           481                                                                                                                                                  600
OXA-23     GTTGATAATT TCTGGTTGGT AGGACCATTA AAGGTTACGC CTATTCAAGA GGTAGAGTTT GTTTCCCAAT TAGCACATAC ACAGCTTCCA TTTAGTGAAA AAGTGCAGGC TAATGTAAAA
A66        GTTGATAATT TCTGGTTGGT AGGACCATTA AAGGTTACGC CTATTCAAGA GGTAGAGTTT GTTTCCCAAT TAGCACATAC ACAGCTTCCA TTTAGTGAAA AAGTGCAGGC TAATGTAAAA
A67        GTTGATAATT TCTGGTTGGT AGGACCATTA AAGGTTACGC CTATTCAAGA GGTAGAGTTT GTTTCCCAAT TAGCACATAC ACAGCTTCCA TTTAGTGAAA AAGTGCAGGC TAATGTAAAA
A72        GTTGATAATT TCTGGTTGGT AGGACCATTA AAGGTTACGC CTATTCAAGA GGTAGAGTTT GTTTCCCAAT TAGCACATAC ACAGCTTCCA TTTAGTGAAA AAGTGCAGGC TAATGTAAAA
A266       GTTGATAATT TCTGGTTGGT AGGACCATTA AAGGTTACGC CTATTCAAGA GGTAGAGTTT GTTTCCCAAT TAGCACATAC ACAGCTTCCA TTTAGTGAAA AAGTGCAGGC TAATGTAAAA
A74        GTTGATAATT TCTGGTTGGT AGGACCATTA AAGGTTACGC CTATTCAAGA GGTAGAGTTT GTTTCCCAAT TAGCACATAC ACAGCTTCCA TTTAGTGAAA AAGTGCAGGC TAATGTAAAA
A150       GTTGATAATT TCTGGTTGGT AGGACCATTA AAGGTTACGC CTATTCAAGA GGTAGAGTTT GTTTCCCAAT TAGCACATAC ACAGCTTCCA TTTAGTGAAA AAGTGCAGGC TAATGTAAAA
A472       GTTGATAATT TCTGGTTGGT AGGACCATTA AAGGTTACGC CTATTCAAGA GGTAGAGTTT GTTTCCCAAT TAGCACATAC ACAGCTTCCA TTTAGTGAAA AAGTGCAGGC TAATGTAAAA
A446       GTTGATAATT TCTGGTTGGT AGGACCATTA AAGGTTACGC CTATTCAAGA GGTAGAGTTT GTTTCCCAAT TAGCACATAC ACAGCTTCCA TTTAGTGAAA AAGTGCAGGC TAATGTAAAA
A433       GTTGATAATT TCTGGTTGGT AGGACCATTA AAGGTTACGC CTATTCAAGA GGTAGAGTTT GTTTCCCAAT TAGCACATAC ACAGCTTCCA TTTAGTGAAA AAGTGCAGGC TAATGTAAAA
A519       GTTGATAATT TCTGGTTGGT AGGACCATTA AAGGTTACGC CTATTCAAGA GGTAGAGTTT GTTTCCCAAT TAGCACATAC ACAGCTTCCA TTTAGTGAAA AAGTGCAGGC TAATGTAAAA
Consensus  GTTGATAATT TCTGGTTGGT AGGACCATTA AAGGTTACGC CTATTCAAGA GGTAGAGTTT GTTTCCCAAT TAGCACATAC ACAGCTTCCA TTTAGTGAAA AAGTGCAGGC TAATGTAAAA
```

```
           601                                                                                                                  720
OXA-23     AATATGCTTC TTTTAGAAGA GAGTAATGGC TACAAAATTT TTGGAAAGAC TGGTTGGGCA ATGGATATAA AACCACAAGT GGGCTGGTTG ACCGGCTGGG TTGAGCAGCC AGATGGAAAA
A66        AATATGCTTC TTTTAGAAGA GAGTAATGGC TACAAAATTT TTGGAAAGAC TGGTTGGGCA ATGGATATAA AACCACAAGT GGGCTGGTTG ACCGGCTGGG TTGAGCAGCC AGATGGAAAA
A67        AATATGCTTC TTTTAGAAGA GAGTAATGGC TACAAAATTT TTGGAAAGAC TGGTTGGGCA ATGGATATAA AACCACAAGT GGGCTGGTTG ACCGGCTGGG TTGAGCAGCC AGATGGAAAA
A72        AATATGCTTC TTTTAGAAGA GAGTAATGGC TACAAAATTT TTGGAAAGAC TGGTTGGGCA ATGGATATAA AACCACAAGT GGGCTGGTTG ACCGGCTGGG TTGAGCAGCC AGATGGAAAA
A266       AATATGCTTC TTTTAGAAGA GAGTAATGGC TACAAAATTT TTGGAAAGAC TGGTTGGGCA ATGGATATAA AACCACAAGT GGGCTGGTTG ACCGGCTGGG TTGAGCAGCC AGATGGAAAA
A74        AATATGCTTC TTTTAGAAGA GAGTAATGGC TACAAAATTT TTGGAAAGAC TGGTTGGGCA ATGGATATAA AACCACAAGT GGGCTGGTTG ACCGGCTGGG TTGAGCAGCC AGATGGAAAA
A150       AATATGCTTC TTTTAGAAGA GAGTAATGGC TACAAAATTT TTGGAAAGAC TGGTTGGGCA ATGGATATAA AACCACAAGT GGGCTGGTTG ACCGGCTGGG TTGAGCAGCC AGATGGAAAA
A472       AATATGCTTC TTTTAGAAGA GAGTAATGGC TACAAAATTT TTGGAAAGAC TGGTTGGGCA ATGGATATAA AACCACAAGT GGGCTGGTTG ACCGGCTGGG TTGAGCAGCC AGATGGAAAA
A446       AATATGCTTC TTTTAGAAGA GAGTAATGGC TACAAAATTT TTGGAAAGAC TGGTTGGGCA ATGGATATAA AACCACAAGT GGGCTGGTTG ACCGGCTGGG TTGAGCAGCC AGATGGAAAA
A433       AATATGCTTC TTTTAGAAGA GAGTAATGGC TACAAAATTT TTGGAAAGAC TGGTTGGGCA ATGGATATAA AACCACAAGT GGGCTGGTTG ACCGGCTGGG TTGAGCAGCC AGATGGAAAA
A519       AATATGCTTC TTTTAGAAGA GAGTAATGGC TACAAAATTT TTGGAAAGAC TGGTTGGGCA ATGGATATAA AACCACAAGT GGGCTGGTTG ACCGGCTGGG TTGAGCAGCC AGATGGAAAA
Consensus  AATATGCTTC TTTTAGAAGA GAGTAATGGC TACAAAATTT TTGGAAAGAC TGGTTGGGCA ATGGATATAA AACCACAAGT GGGCTGGTTG ACCGGCTGGG TTGAGCAGCC AGATGGAAAA

           721                                                                                                  822
OXA-23     ATTGTCGCTT TTGCATTAAA TATGGAAATG CGGTCAGAAA TGCCGGCATC TATACGTAAT GAATTATTGA TGAAATCATT AAAACAGCTG AATATTATTT AA
A66        ATTGTCGCTT TTGCATTAAA TATGGAAATG CGGTCAGAAA TGCCGGCATC TATACGTAAT GAATTATTGA TGAAATCATT AAAACAGCTG AATATTATTT AA
A67        ATTGTCGCTT TTGCATTAAA TATGGAAATG CGGTCAGAAA TGCCGGCATC TATACGTAAT GAATTATTGA TGAAATCATT AAAACAGCTG AATATTATTT AA
A72        ATTGTCGCTT TTGCATTAAA TATGGAAATG CGGTCAGAAA TGCCGGCATC TATACGTAAT GAATTATTGA TGAAATCATT AAAACAGCTG AATATTATTT AA
A266       ATTGTCGCTT TTGCATTAAA TATGGAAATG CGGTCAGAAA TGCCGGCATC TATACGTAAT GAATTATTGA TGAAATCATT AAAACAGCTG AATATTATTT AA
A74        ATTGTCGCTT TTGCATTAAA TATGGAAATG CGGTCAGAAA TGCCGGCATC TATACGTAAT GAATTATTGA TGAAATCATT AAAACAGCTG AATATTATTT AA
A150       ATTGTCGCTT TTGCATTAAA TATGGAAATG CGGTCAGAAA TGCCGGCATC TATACGTAAT GAATTATTGA TGAAATCATT AAAACAGCTG AATATTATTT AA
A472       ATTGTCGCTT TTGCATTAAA TATGGAAATG CGGTCAGAAA TGCCGGCATC TATACGTAAT GAATTATTGA TGAAATCATT AAAACAGCTG AATATTATTT AA
A446       ATTGTCGCTT TTGCATTAAA TATGGAAATG CGGTCAGAAA TGCCGGCATC TATACGTAAT GAATTATTGA TGAAATCATT AAAACAGCTG AATATTATTT AA
A433       ATTGTCGCTT TTGCATTAAA TATGGAAATG CGGTCAGAAA TGCCGGCATC TATACGTAAT GAATTATTGA TGAAATCATT AAAACAGCTG AATATTATTT AA
A519       ATTGTCGCTT TTGCATTAAA TATGGAAATG CGGTCAGAAA TGCCGGCATC TATACGTAAT GAATTATTGA TGAAATCATT AAAACAGCTG AATATTATTT AA
Consensus  ATTGTCGCTT TTGCATTAAA TATGGAAATG CGGTCAGAAA TGCCGGCATC TATACGTAAT GAATTATTGA TGAAATCATT AAAACAGCTG AATATTATTT AA
```

2. OXA-24 GROUP

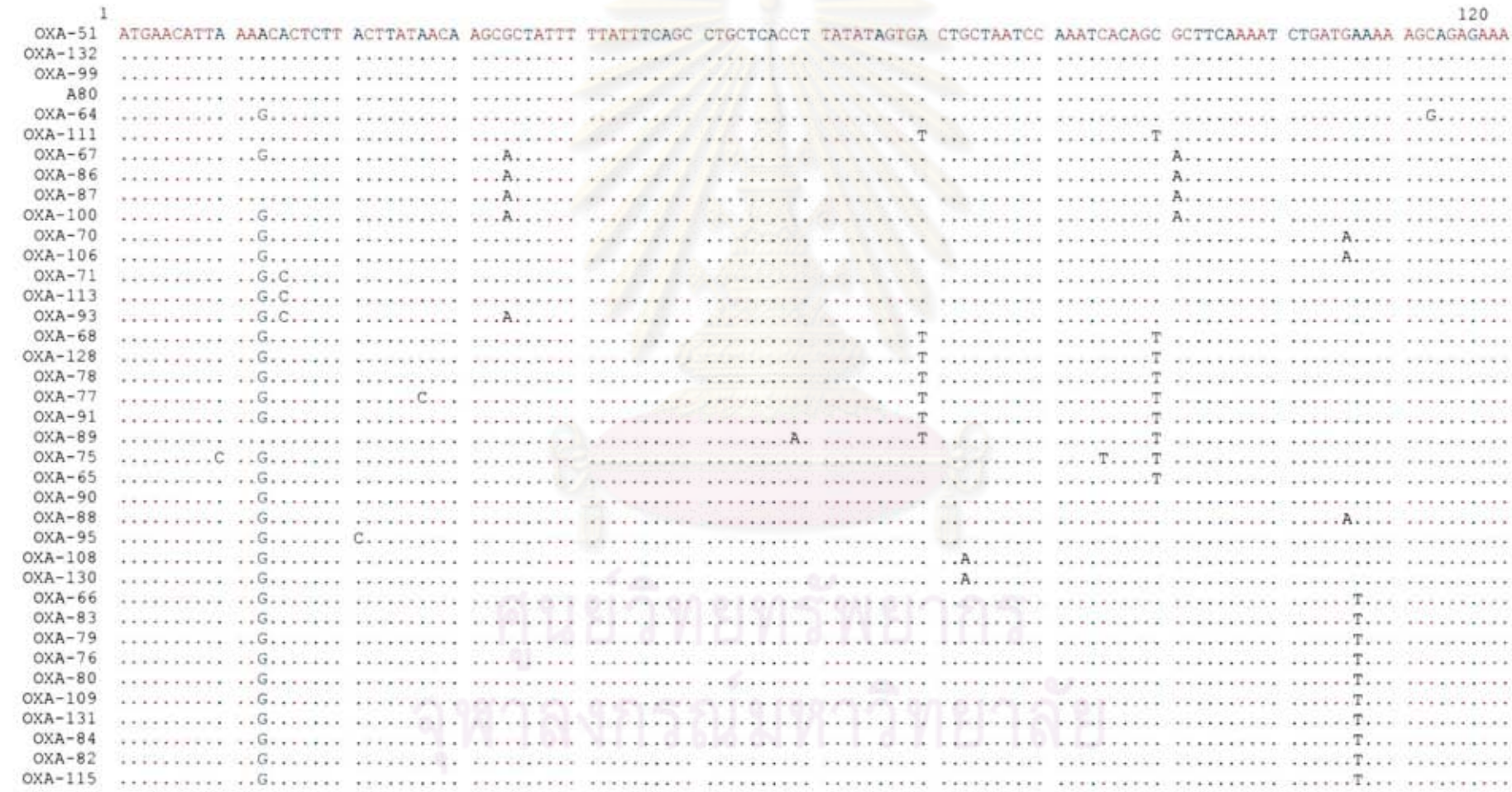
```
          1                                                                                                                   120
OXA-72    ATGAAAAAAT TTATACTTCC TATATTCAGC ATTTCTATTC TAGTTTCTCT CAGTGCATGT TCATCTATTA AAACTAAATC TGAAGATAAT TTTCATATTT CTTCTCAGCA ACATGAAAAA
A146      ATGAAAAAAT TTATACTTCC TATATTCAGC ATTTCTATTC TAGTTTCTCT CAGTGCATGT TCATCTATTA AAACTAAATC TGAAGATAAT TTTCATATTT CTTCTCAGCA ACATGAAAAA
A150      ATGAAAAAAT TTATACTTCC TATATTCAGC ATTTCTATTC TAGTTTCTCT CAGTGCATGT TCATCTATTA AAACTAAATC TGAAGATAAT TTTCATATTT CTTCTCAGCA ACATGAAAAA
A177      ATGAAAAAAT TTATACTTCC TATATTCAGC ATTTCTATTC TAGTTTCTCT CAGTGCATGT TCATCTATTA AAACTAAATC TGAAGATAAT TTTCATATTT CTTCTCAGCA ACATGAAAAA
A180      ATGAAAAAAT TTATACTTCC TATATTCAGC ATTTCTATTC TAGTTTCTCT CAGTGCATGT TCATCTATTA AAACTAAATC TGAAGATAAT TTTCATATTT CTTCTCAGCA ACATGAAAAA
A242      ATGAAAAAAT TTATACTTCC TATATTCAGC ATTTCTATTC TAGTTTCTCT CAGTGCATGT TCATCTATTA AAACTAAATC TGAAGATAAT TTTCATATTT CTTCTCAGCA ACATGAAAAA
A288      ATGAAAAAAT TTATACTTCC TATATTCAGC ATTTCTATTC TAGTTTCTCT CAGTGCATGT TCATCTATTA AAACTAAATC TGAAGATAAT TTTCATATTT CTTCTCAGCA ACATGAAAAA
A446      ATGAAAAAAT TTATACTTCC TATATTCAGC ATTTCTATTC TAGTTTCTCT CAGTGCATGT TCATCTATTA AAACTAAATC TGAAGATAAT TTTCATATTT CTTCTCAGCA ACATGAAAAA
A471      ATGAAAAAAT TTATACTTCC TATATTCAGC ATTTCTATTC TAGTTTCTCT CAGTGCATGT TCATCTATTA AAACTAAATC TGAAGATAAT TTTCATATTT CTTCTCAGCA ACATGAAAAA
A472      ATGAAAAAAT TTATACTTCC TATATTCAGC ATTTCTATTC TAGTTTCTCT CAGTGCATGT TCATCTATTA AAACTAAATC TGAAGATAAT TTTCATATTT CTTCTCAGCA ACATGAAAAA
A519      ATGAAAAAAT TTATACTTCC TATATTCAGC ATTTCTATTC TAGTTTCTCT CAGTGCATGT TCATCTATTA AAACTAAATC TGAAGATAAT TTTCATATTT CTTCTCAGCA ACATGAAAAA
Consensus ATGAAAAAAT TTATACTTCC TATATTCAGC ATTTCTATTC TAGTTTCTCT CAGTGCATGT TCATCTATTA AAACTAAATC TGAAGATAAT TTTCATATTT CTTCTCAGCA ACATGAAAAA

          121                                                                                                                 240
OXA-72    GCTATTAAAA GCTATTTTGA TGAAGCTCAA ACACAGGGTG TAATTATTAT TAAAGAGGGT AAAAATCTTA GCACCTATGG TAATGCTCTT GCACGAGCAA ATAAAGAATA TGTCCCTGCA
A146      GCTATTAAAA GCTATTTTGA TGAAGCTCAA ACACAGGGTG TAATTATTAT TAAAGAGGGT AAAAATCTTA GCACCTATGG TAATGCTCTT GCACGAGCAA ATAAAGAATA TGTCCCTGCA
A150      GCTATTAAAA GCTATTTTGA TGAAGCTCAA ACACAGGGTG TAATTATTAT TAAAGAGGGT AAAAATCTTA GCACCTATGG TAATGCTCTT GCACGAGCAA ATAAAGAATA TGTCCCTGCA
A177      GCTATTAAAA GCTATTTTGA TGAAGCTCAA ACACAGGGTG TAATTATTAT TAAAGAGGGT AAAAATCTTA GCACCTATGG TAATGCTCTT GCACGAGCAA ATAAAGAATA TGTCCCTGCA
A180      GCTATTAAAA GCTATTTTGA TGAAGCTCAA ACACAGGGTG TAATTATTAT TAAAGAGGGT AAAAATCTTA GCACCTATGG TAATGCTCTT GCACGAGCAA ATAAAGAATA TGTCCCTGCA
A242      GCTATTAAAA GCTATTTTGA TGAAGCTCAA ACACAGGGTG TAATTATTAT TAAAGAGGGT AAAAATCTTA GCACCTATGG TAATGCTCTT GCACGAGCAA ATAAAGAATA TGTCCCTGCA
A288      GCTATTAAAA GCTATTTTGA TGAAGCTCAA ACACAGGGTG TAATTATTAT TAAAGAGGGT AAAAATCTTA GCACCTATGG TAATGCTCTT GCACGAGCAA ATAAAGAATA TGTCCCTGCA
A446      GCTATTAAAA GCTATTTTGA TGAAGCTCAA ACACAGGGTG TAATTATTAT TAAAGAGGGT AAAAATCTTA GCACCTATGG TAATGCTCTT GCACGAGCAA ATAAAGAATA TGTCCCTGCA
A471      GCTATTAAAA GCTATTTTGA TGAAGCTCAA ACACAGGGTG TAATTATTAT TAAAGAGGGT AAAAATCTTA GCACCTATGG TAATGCTCTT GCACGAGCAA ATAAAGAATA TGTCCCTGCA
A472      GCTATTAAAA GCTATTTTGA TGAAGCTCAA ACACAGGGTG TAATTATTAT TAAAGAGGGT AAAAATCTTA GCACCTATGG TAATGCTCTT GCACGAGCAA ATAAAGAATA TGTCCCTGCA
A519      GCTATTAAAA GCTATTTTGA TGAAGCTCAA ACACAGGGTG TAATTATTAT TAAAGAGGGT AAAAATCTTA GCACCTATGG TAATGCTCTT GCACGAGCAA ATAAAGAATA TGTCCCTGCA
Consensus GCTATTAAAA GCTATTTTGA TGAAGCTCAA ACACAGGGTG TAATTATTAT TAAAGAGGGT AAAAATCTTA GCACCTATGG TAATGCTCTT GCACGAGCAA ATAAAGAATA TGTCCCTGCA

          241                                                                                                                 360
OXA-72    TCAACATTTA AGATGCTAAA TGCTTTAATC GGGCTAGAAA ATCATAAAGC AACAACAAAT GAGATTTTCA AATGGGATGG TAAAAAAAGA ACTTATCCTA TGTGGGAGAA AGATATGACT
A146      TCAACATTTA AGATGCTAAA TGCTTTAATC GGGCTAGAAA ATCATAAAGC AACAACAAAT GAGATTTTCA AATGGGATGG TAAAAAAAGA ACTTATCCTA TGTGGGAGAA AGATATGACT
A150      TCAACATTTA AGATGCTAAA TGCTTTAATC GGGCTAGAAA ATCATAAAGC AACAACAAAT GAGATTTTCA AATGGGATGG TAAAAAAAGA ACTTATCCTA TGTGGGAGAA AGATATGACT
A177      TCAACATTTA AGATGCTAAA TGCTTTAATC GGGCTAGAAA ATCATAAAGC AACAACAAAT GAGATTTTCA AATGGGATGG TAAAAAAAGA ACTTATCCTA TGTGGGAGAA AGATATGACT
A180      TCAACATTTA AGATGCTAAA TGCTTTAATC GGGCTAGAAA ATCATAAAGC AACAACAAAT GAGATTTTCA AATGGGATGG TAAAAAAAGA ACTTATCCTA TGTGGGAGAA AGATATGACT
A242      TCAACATTTA AGATGCTAAA TGCTTTAATC GGGCTAGAAA ATCATAAAGC AACAACAAAT GAGATTTTCA AATGGGATGG TAAAAAAAGA ACTTATCCTA TGTGGGAGAA AGATATGACT
A288      TCAACATTTA AGATGCTAAA TGCTTTAATC GGGCTAGAAA ATCATAAAGC AACAACAAAT GAGATTTTCA AATGGGATGG TAAAAAAAGA ACTTATCCTA TGTGGGAGAA AGATATGACT
A446      TCAACATTTA AGATGCTAAA TGCTTTAATC GGGCTAGAAA ATCATAAAGC AACAACAAAT GAGATTTTCA AATGGGATGG TAAAAAAAGA ACTTATCCTA TGTGGGAGAA AGATATGACT
A471      TCAACATTTA AGATGCTAAA TGCTTTAATC GGGCTAGAAA ATCATAAAGC AACAACAAAT GAGATTTTCA AATGGGATGG TAAAAAAAGA ACTTATCCTA TGTGGGAGAA AGATATGACT
A472      TCAACATTTA AGATGCTAAA TGCTTTAATC GGGCTAGAAA ATCATAAAGC AACAACAAAT GAGATTTTCA AATGGGATGG TAAAAAAAGA ACTTATCCTA TGTGGGAGAA AGATATGACT
A519      TCAACATTTA AGATGCTAAA TGCTTTAATC GGGCTAGAAA ATCATAAAGC AACAACAAAT GAGATTTTCA AATGGGATGG TAAAAAAAGA ACTTATCCTA TGTGGGAGAA AGATATGACT
Consensus TCAACATTTA AGATGCTAAA TGCTTTAATC GGGCTAGAAA ATCATAAAGC AACAACAAAT GAGATTTTCA AATGGGATGG TAAAAAAAGA ACTTATCCTA TGTGGGAGAA AGATATGACT
```

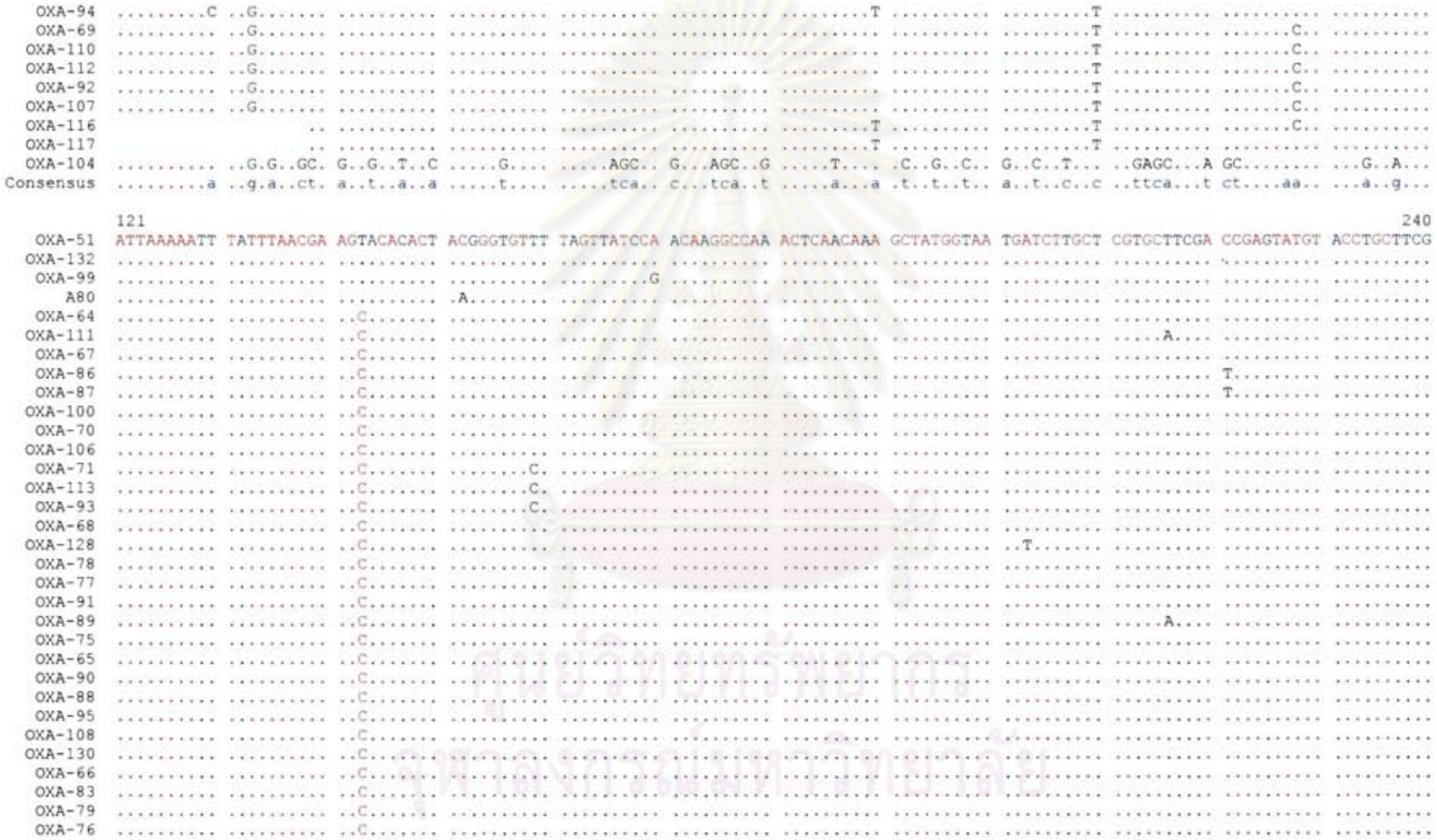
```
           361                                                                                                  480
OXA-72     TTAGGTGAGG CAATGGCATT GTCAGCAGTT CCAGTATATC AAGAGCTTGC AAGACGGACT GGCCTAGAGC TAATGCAGAA AGAAGTAAAG CGGGTTAATT TTGGAAATAC AAATATTGGA
A146       TTAGGTGAGG CAATGGCATT GTCAGCAGTT CCAGTATATC AAGAGCTTGC AAGACGGACT GGCCTAGAGC TAATGCAGAA AGAAGTAAAG CGGGTTAATT TTGGAAATAC AAATATTGGA
A150       TTAGGTGAGG CAATGGCATT GTCAGCAGTT CCAGTATATC AAGAGCTTGC AAGACGGACT GGCCTAGAGC TAATGCAGAA AGAAGTAAAG CGGGTTAATT TTGGAAATAC AAATATTGGA
A177       TTAGGTGAGG CAATGGCATT GTCAGCAGTT CCAGTATATC AAGAGCTTGC AAGACGGACT GGCCTAGAGC TAATGCAGAA AGAAGTAAAG CGGGTTAATT TTGGAAATAC AAATATTGGA
A180       TTAGGTGAGG CAATGGCATT GTCAGCAGTT CCAGTATATC AAGAGCTTGC AAGACGGACT GGCCTAGAGC TAATGCAGAA AGAAGTAAAG CGGGTTAATT TTGGAAATAC AAATATTGGA
A242       TTAGGTGAGG CAATGGCATT GTCAGCAGTT CCAGTATATC AAGAGCTTGC AAGACGGACT GGCCTAGAGC TAATGCAGAA AGAAGTAAAG CGGGTTAATT TTGGAAATAC AAATATTGGA
A288       TTAGGTGAGG CAATGGCATT GTCAGCAGTT CCAGTATATC AAGAGCTTGC AAGACGGACT GGCCTAGAGC TAATGCAGAA AGAAGTAAAG CGGGTTAATT TTGGAAATAC AAATATTGGA
A446       TTAGGTGAGG CAATGGCATT GTCAGCAGTT CCAGTATATC AAGAGCTTGC AAGACGGACT GGCCTAGAGC TAATGCAGAA AGAAGTAAAG CGGGTTAATT TTGGAAATAC AAATATTGGA
A471       TTAGGTGAGG CAATGGCATT GTCAGCAGTT CCAGTATATC AAGAGCTTGC AAGACGGACT GGCCTAGAGC TAATGCAGAA AGAAGTAAAG CGGGTTAATT TTGGAAATAC AAATATTGGA
A472       TTAGGTGAGG CAATGGCATT GTCAGCAGTT CCAGTATATC AAGAGCTTGC AAGACGGACT GGCCTAGAGC TAATGCAGAA AGAAGTAAAG CGGGTTAATT TTGGAAATAC AAATATTGGA
A519       TTAGGTGAGG CAATGGCATT GTCAGCAGTT CCAGTATATC AAGAGCTTGC AAGACGGACT GGCCTAGAGC TAATGCAGAA AGAAGTAAAG CGGGTTAATT TTGGAAATAC AAATATTGGA
Consensus  TTAGGTGAGG CAATGGCATT GTCAGCAGTT CCAGTATATC AAGAGCTTGC AAGACGGACT GGCCTAGAGC TAATGCAGAA AGAAGTAAAG CGGGTTAATT TTGGAAATAC AAATATTGGA

           481                                                                                                  600
OXA-72     ACACAGGTCG ATAATTTTTG GTTAGTTGGC CCCCTTAAAA TTACACCAGT ACAAGAAGTT AATTTTGCCG ATGACCTTGC ACATAACCGA TTACCTTTTA AATTAGAAAC TCAAGAAGAA
A146       ACACAGGTCG ATAATTTTTG GTTAGTTGGC CCCCTTAAAA TTACACCAGT ACAAGAAGTT AATTTTGCCG ATGACCTTGC ACATAACCGA TTACCTTTTA AATTAGAAAC TCAAGAAGAA
A150       ACACAGGTCG ATAATTTTTG GTTAGTTGGC CCCCTTAAAA TTACACCAGT ACAAGAAGTT AATTTTGCCG ATGACCTTGC ACATAACCGA TTACCTTTTA AATTAGAAAC TCAAGAAGAA
A177       ACACAGGTCG ATAATTTTTG GTTAGTTGGC CCCCTTAAAA TTACACCAGT ACAAGAAGTT AATTTTGCCG ATGACCTTGC ACATAACCGA TTACCTTTTA AATTAGAAAC TCAAGAAGAA
A180       ACACAGGTCG ATAATTTTTG GTTAGTTGGC CCCCTTAAAA TTACACCAGT ACAAGAAGTT AATTTTGCCG ATGACCTTGC ACATAACCGA TTACCTTTTA AATTAGAAAC TCAAGAAGAA
A242       ACACAGGTCG ATAATTTTTG GTTAGTTGGC CCCCTTAAAA TTACACCAGT ACAAGAAGTT AATTTTGCCG ATGACCTTGC ACATAACCGA TTACCTTTTA AATTAGAAAC TCAAGAAGAA
A288       ACACAGGTCG ATAATTTTTG GTTAGTTGGC CCCCTTAAAA TTACACCAGT ACAAGAAGTT AATTTTGCCG ATGACCTTGC ACATAACCGA TTACCTTTTA AATTAGAAAC TCAAGAAGAA
A446       ACACAGGTCG ATAATTTTTG GTTAGTTGGC CCCCTTAAAA TTACACCAGT ACAAGAAGTT AATTTTGCCG ATGACCTTGC ACATAACCGA TTACCTTTTA AATTAGAAAC TCAAGAAGAA
A471       ACACAGGTCG ATAATTTTTG GTTAGTTGGC CCCCTTAAAA TTACACCAGT ACAAGAAGTT AATTTTGCCG ATGACCTTGC ACATAACCGA TTACCTTTTA AATTAGAAAC TCAAGAAGAA
A472       ACACAGGTCG ATAATTTTTG GTTAGTTGGC CCCCTTAAAA TTACACCAGT ACAAGAAGTT AATTTTGCCG ATGACCTTGC ACATAACCGA TTACCTTTTA AATTAGAAAC TCAAGAAGAA
A519       ACACAGGTCG ATAATTTTTG GTTAGTTGGC CCCCTTAAAA TTACACCAGT ACAAGAAGTT AATTTTGCCG ATGACCTTGC ACATAACCGA TTACCTTTTA AATTAGAAAC TCAAGAAGAA
Consensus  ACACAGGTCG ATAATTTTTG GTTAGTTGGC CCCCTTAAAA TTACACCAGT ACAAGAAGTT AATTTTGCCG ATGACCTTGC ACATAACCGA TTACCTTTTA AATTAGAAAC TCAAGAAGAA

           601                                                                                                  720
OXA-72     GTTAAAAAAA TGCTTCTAAT TAAAGAAGTA AATGGTAGTA AGATTTATGC AAAAAGTGGA TGGGGAATGG ATGTTACTCC ACAGGTAGGT TGGTTGACTG GTTGGGTGGA GCAAGCTAAT
A146       GTTAAAAAAA TGCTTCTAAT TAAAGAAGTA AATGGTAGTA AGATTTATGC AAAAAGTGGA TGGGGAATGG ATGTTACTCC ACAGGTAGGT TGGTTGACTG GTTGGGTGGA GCAAGCTAAT
A150       GTTAAAAAAA TGCTTCTAAT TAAAGAAGTA AATGGTAGTA AGATTTATGC AAAAAGTGGA TGGGGAATGG ATGTTACTCC ACAGGTAGGT TGGTTGACTG GTTGGGTGGA GCAAGCTAAT
A177       GTTAAAAAAA TGCTTCTAAT TAAAGAAGTA AATGGTAGTA AGATTTATGC AAAAAGTGGA TGGGGAATGG ATGTTACTCC ACAGGTAGGT TGGTTGACTG GTTGGGTGGA GCAAGCTAAT
A180       GTTAAAAAAA TGCTTCTAAT TAAAGAAGTA AATGGTAGTA AGATTTATGC AAAAAGTGGA TGGGGAATGG ATGTTACTCC ACAGGTAGGT TGGTTGACTG GTTGGGTGGA GCAAGCTAAT
A242       GTTAAAAAAA TGCTTCTAAT TAAAGAAGTA AATGGTAGTA AGATTTATGC AAAAAGTGGA TGGGGAATGG ATGTTACTCC ACAGGTAGGT TGGTTGACTG GTTGGGTGGA GCAAGCTAAT
A288       GTTAAAAAAA TGCTTCTAAT TAAAGAAGTA AATGGTAGTA AGATTTATGC AAAAAGTGGA TGGGGAATGG ATGTTACTCC ACAGGTAGGT TGGTTGACTG GTTGGGTGGA GCAAGCTAAT
A446       GTTAAAAAAA TGCTTCTAAT TAAAGAAGTA AATGGTAGTA AGATTTATGC AAAAAGTGGA TGGGGAATGG ATGTTACTCC ACAGGTAGGT TGGTTGACTG GTTGGGTGGA GCAAGCTAAT
A471       GTTAAAAAAA TGCTTCTAAT TAAAGAAGTA AATGGTAGTA AGATTTATGC AAAAAGTGGA TGGGGAATGG ATGTTACTCC ACAGGTAGGT TGGTTGACTG GTTGGGTGGA GCAAGCTAAT
A472       GTTAAAAAAA TGCTTCTAAT TAAAGAAGTA AATGGTAGTA AGATTTATGC AAAAAGTGGA TGGGGAATGG ATGTTACTCC ACAGGTAGGT TGGTTGACTG GTTGGGTGGA GCAAGCTAAT
A519       GTTAAAAAAA TGCTTCTAAT TAAAGAAGTA AATGGTAGTA AGATTTATGC AAAAAGTGGA TGGGGAATGG ATGTTACTCC ACAGGTAGGT TGGTTGACTG GTTGGGTGGA GCAAGCTAAT
Consensus  GTTAAAAAAA TGCTTCTAAT TAAAGAAGTA AATGGTAGTA AGATTTATGC AAAAAGTGGA TGGGGAATGG ATGTTACTCC ACAGGTAGGT TGGTTGACTG GTTGGGTGGA GCAAGCTAAT
```

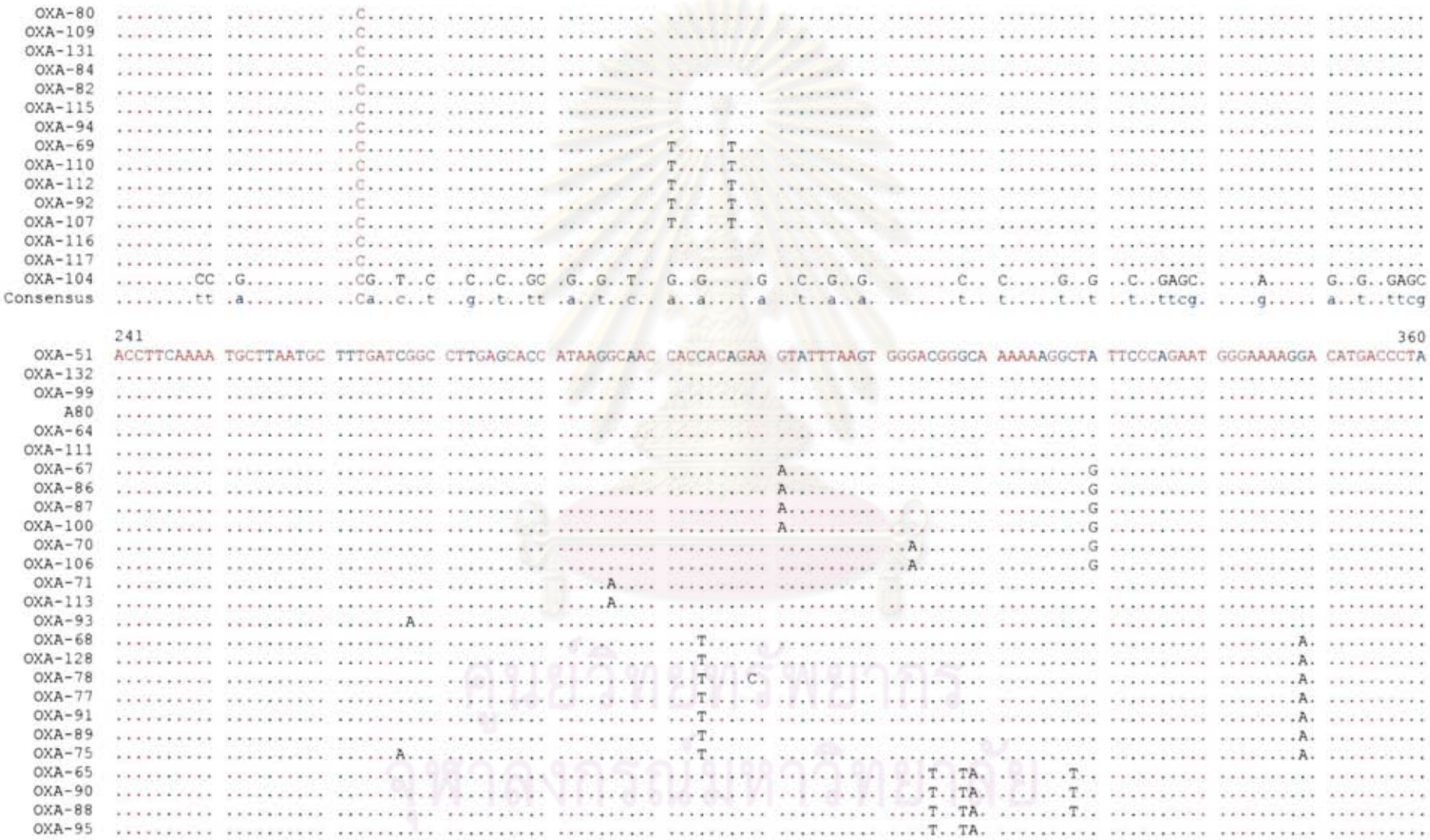
```
          721                                                                                                                                828
OXA-72    GGAAAAAAAA TCCCCTTTTC GCTCAACTTA GAAATGAAAG AAGGAATGTC TGGTTCTATT CGTAATGAAA TTACTTATAA GTCGCTAGAA AATCTTGGAA TCATTTAA
A146      GGAAAAAAAA TCCCCTTTTC GCTCAACTTA GAAATGAAAG AAGGAATGTC TGGTTCTATT CGTAATGAAA TTACTTATAA GTCGCTAGAA AATCTTGGAA TCATTTAA
A150      GGAAAAAAAA TCCCCTTTTC GCTCAACTTA GAAATGAAAG AAGGAATGTC TGGTTCTATT CGTAATGAAA TTACTTATAA GTCGCTAGAA AATCTTGGAA TCATTTAA
A177      GGAAAAAAAA TCCCCTTTTC GCTCAACTTA GAAATGAAAG AAGGAATGTC TGGTTCTATT CGTAATGAAA TTACTTATAA GTCGCTAGAA AATCTTGGAA TCATTTAA
A180      GGAAAAAAAA TCCCCTTTTC GCTCAACTTA GAAATGAAAG AAGGAATGTC TGGTTCTATT CGTAATGAAA TTACTTATAA GTCGCTAGAA AATCTTGGAA TCATTTAA
A242      GGAAAAAAAA TCCCCTTTTC GCTCAACTTA GAAATGAAAG AAGGAATGTC TGGTTCTATT CGTAATGAAA TTACTTATAA GTCGCTAGAA AATCTTGGAA TCATTTAA
A288      GGAAAAAAAA TCCCCTTTTC GCTCAACTTA GAAATGAAAG AAGGAATGTC TGGTTCTATT CGTAATGAAA TTACTTATAA GTCGCTAGAA AATCTTGGAA TCATTTAA
A446      GGAAAAAAAA TCCCCTTTTC GCTCAACTTA GAAATGAAAG AAGGAATGTC TGGTTCTATT CGTAATGAAA TTACTTATAA GTCGCTAGAA AATCTTGGAA TCATTTAA
A471      GGAAAAAAAA TCCCCTTTTC GCTCAACTTA GAAATGAAAG AAGGAATGTC TGGTTCTATT CGTAATGAAA TTACTTATAA GTCGCTAGAA AATCTTGGAA TCATTTAA
A472      GGAAAAAAAA TCCCCTTTTC GCTCAACTTA GAAATGAAAG AAGGAATGTC TGGTTCTATT CGTAATGAAA TTACTTATAA GTCGCTAGAA AATCTTGGAA TCATTTAA
A519      GGAAAAAAAA TCCCCTTTTC GCTCAACTTA GAAATGAAAG AAGGAATGTC TGGTTCTATT CGTAATGAAA TTACTTATAA GTCGCTAGAA AATCTTGGAA TCATTTAA
Consensus GGAAAAAAAA TCCCCTTTTC GCTCAACTTA GAAATGAAAG AAGGAATGTC TGGTTCTATT CGTAATGAAA TTACTTATAA GTCGCTAGAA AATCTTGGAA TCATTTAA
```

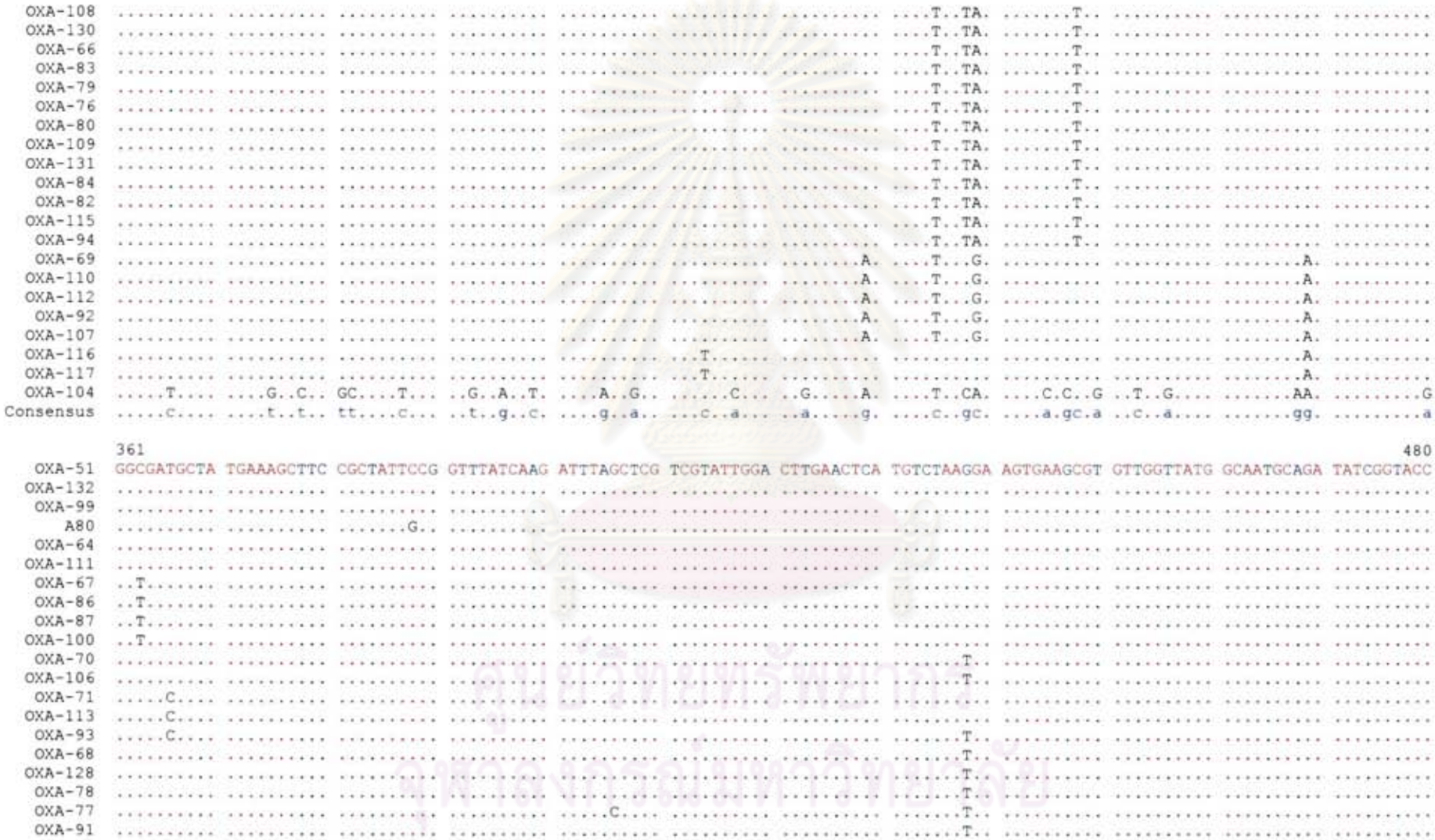
3. OXA-51 GROUP

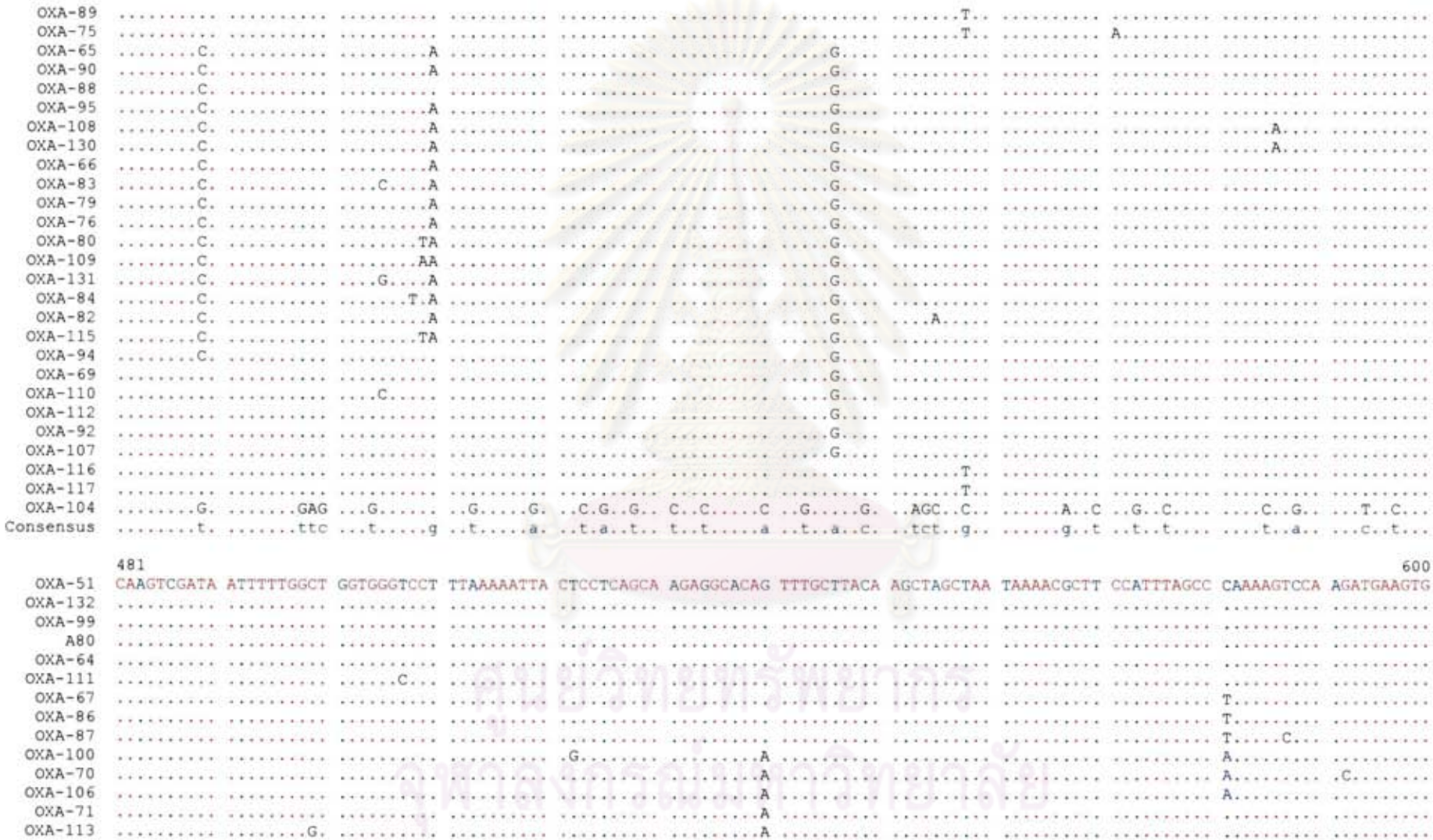
- A80

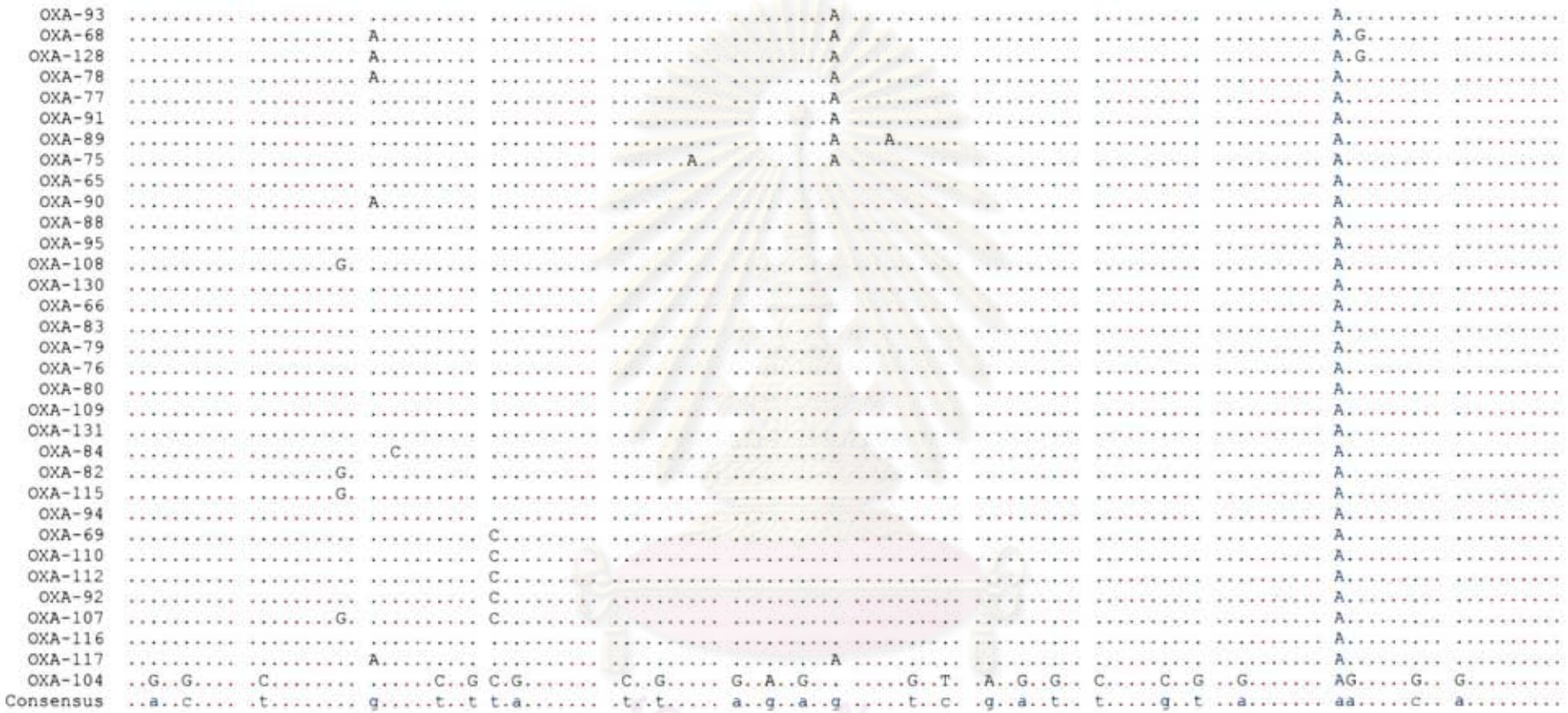


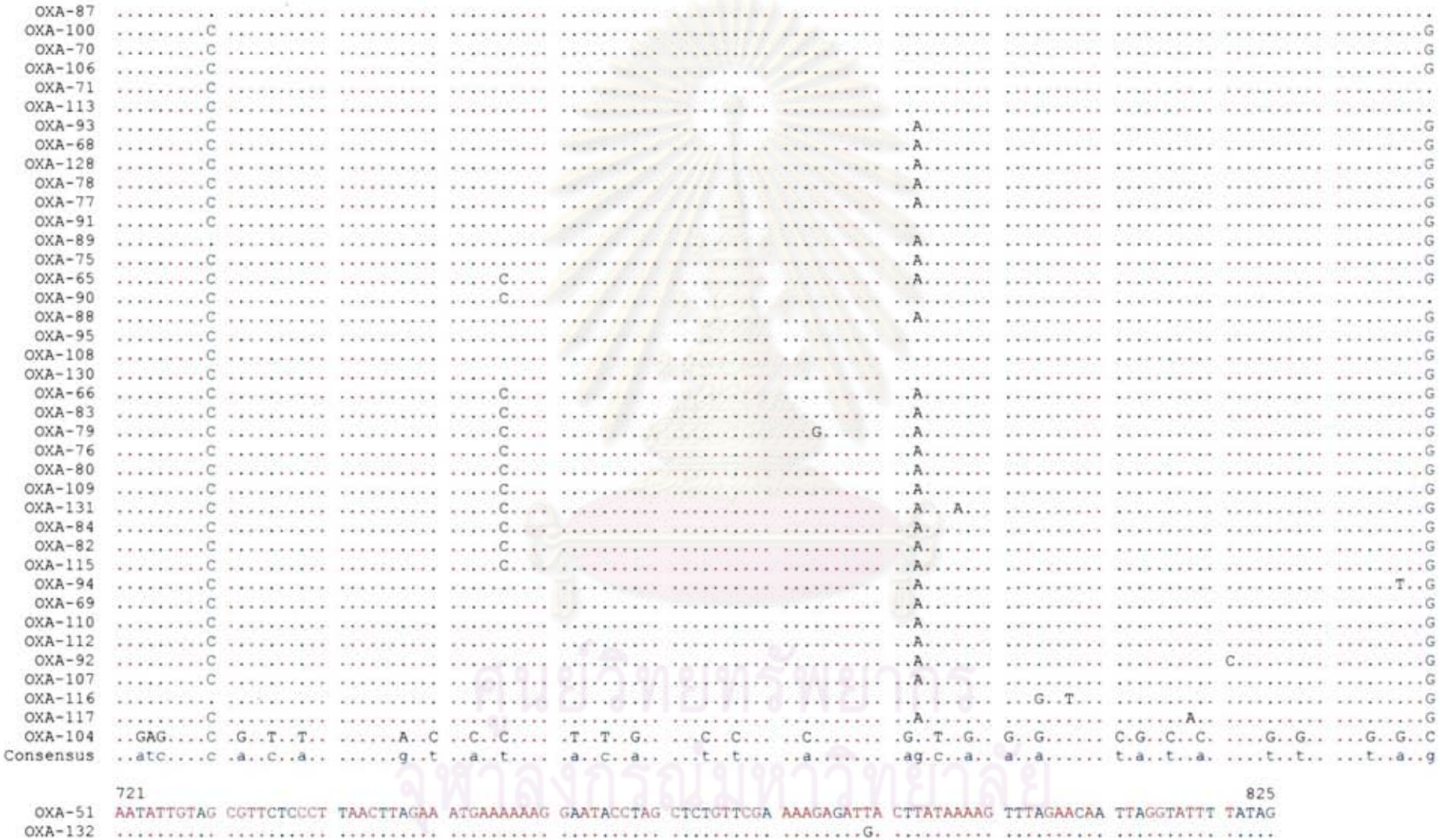

OXA-94 .....C ..G..... ....T ....T ....
OXA-69 ....G..... ....T ....C.. .....
OXA-110 ....G..... ....T ....C.. .....
OXA-112 ....G..... ....T ....C.. .....
OXA-92 ....G..... ....T ....C.. .....
OXA-107 ....G..... ....T ....C.. .....
OXA-116 .. ..T ....T ....C.. .....
OXA-117 .. ..T ....T ....
OXA-104 ....G.G..GC. G..G..T..C .....G.... .....AGC.. G...AGC..G .....T..... .C..G..C.. G..C..T... ..GAGC...A GC..... ...G..A...
Consensus .....a ..g.a..ct. a..t..a..a .....t.... .....tca.. c...tca..t .....a...a .t..t..t.. a..t..c..c ..ttca...t ct....aa.. ...a..g...
121 240
OXA-51 ATTAAAAATT TATTTAACGA AGTACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG
OXA-132 ....
OXA-99 ....G ....
A80 .... .A..... ....
OXA-64 ....C..... ....
OXA-111 ....C..... ....A.... ....
OXA-67 ....C..... ....
OXA-86 ....C..... .... T..... .....
OXA-87 ....C..... .... T..... .....
OXA-100 ....C..... ....
OXA-70 ....C..... ....
OXA-106 ....C..... ....
OXA-71 ....C..... .....C. ....
OXA-113 ....C..... .....C. ....
OXA-93 ....C..... .....C. ....
OXA-68 ....C..... ....
OXA-128 ....C..... ....T..... ....
OXA-78 ....C..... ....
OXA-77 ....C..... ....
OXA-91 ....C..... ....
OXA-89 ....C..... ....A.... ....
OXA-75 ....C..... ....
OXA-65 ....C..... ....
OXA-90 ....C..... ....
OXA-88 ....C..... ....
OXA-95 ....C..... ....
OXA-108 ....C..... ....
OXA-130 ....C..... ....
OXA-66 ....C..... ....
OXA-83 ....C..... ....
OXA-79 ....C..... ....
OXA-76 ....C..... ....

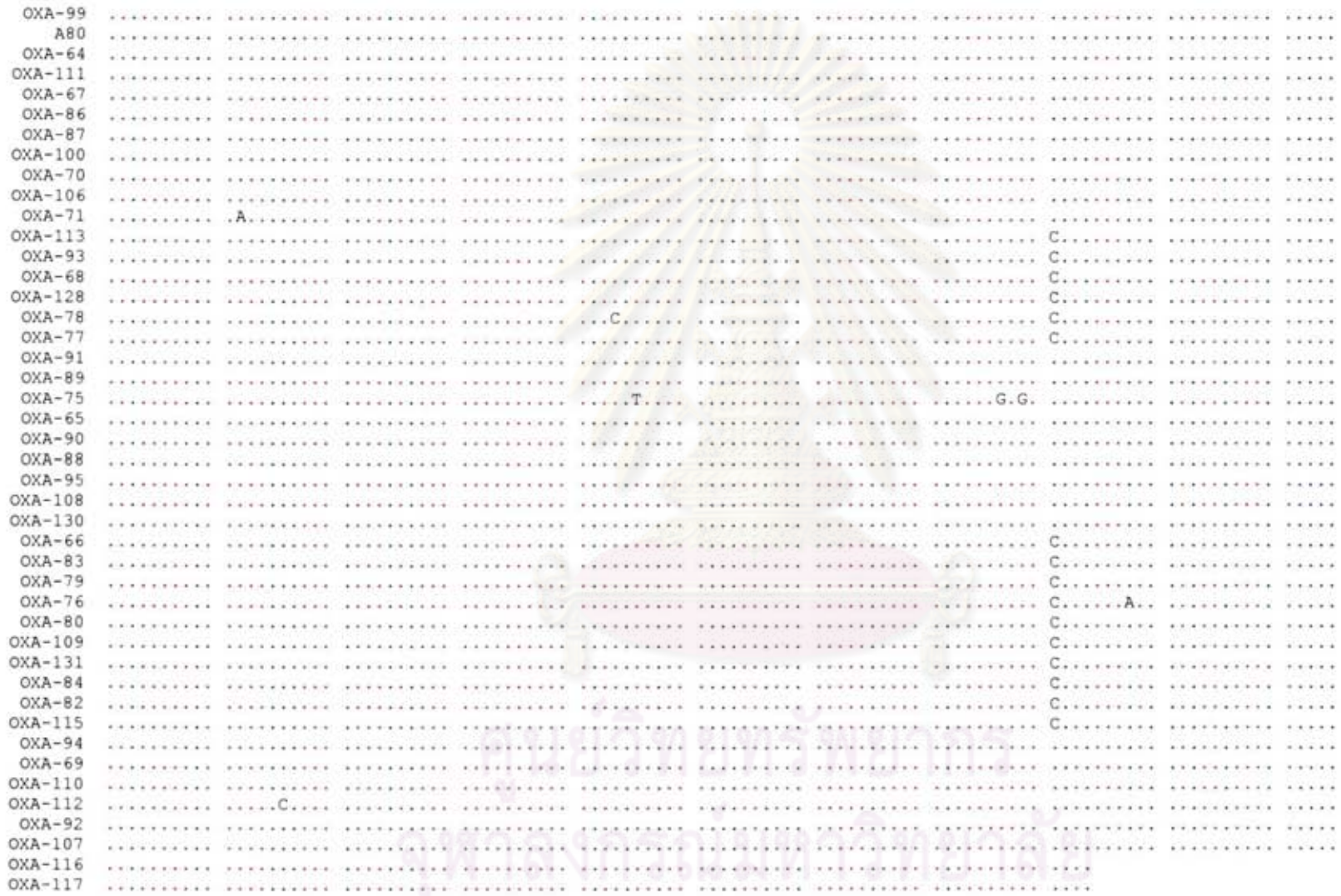



OXA-108 ....T..TA. ....T.. ....
OXA-130 ....T..TA. ....T.. ....
OXA-66 ....T..TA. ....T.. ....
OXA-83 ....T..TA. ....T.. ....
OXA-79 ....T..TA. ....T.. ....
OXA-76 ....T..TA. ....T.. ....
OXA-80 ....T..TA. ....T.. ....
OXA-109 ....T..TA. ....T.. ....
OXA-131 ....T..TA. ....T.. ....
OXA-84 ....T..TA. ....T.. ....
OXA-82 ....T..TA. ....T.. ....
OXA-115 ....T..TA. ....T.. ....
OXA-94 ....T..TA. ....T.. ....
OXA-69 ....A. ....T...G. ....A. .....
OXA-110 ....A. ....T...G. ....A. .....
OXA-112 ....A. ....T...G. ....A. .....
OXA-92 ....A. ....T...G. ....A. .....
OXA-107 ....A. ....T...G. ....A. .....
OXA-116 ....T..... ....A. .....
OXA-117 ....T..... ....A. .....
OXA-104 .....T.... ....G..C.. GC....T... ..G..A..T. ....A..G.. .....C... ..G.....A. ....T..CA. ....C.C..G ..T..G.... .....AA. .....G
Consensus .....c.... ....t..t.. tt....c... ..t..g..c. ....g..a.. ...c..a... ..a.....g. ....c..gc. ....a.gc.a ..c..a.... .....gg. .....a
361 480
OXA-51 GGCGATGCTA TGAAAGCTTC CGCTATTCCG GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAACTCA TGTCTAAGGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC
OXA-132 ....
OXA-99 ....
A80 ....G.. ....
OXA-64 ....
OXA-111 ....
OXA-67 ..T..... ....
OXA-86 ..T..... ....
OXA-87 ..T..... ....
OXA-100 ..T..... ....
OXA-70 ....T.. ....
OXA-106 ....T.. ....
OXA-71 .....C.... ....
OXA-113 .....C.... ....
OXA-93 .....C.... ....T.. ....
OXA-68 ....T.. ....
OXA-128 ....T.. ....
OXA-78 ....T.. ....
OXA-77 ....C.... ....T.. ....
OXA-91 ....T.. ....


481
600
OXA-51 CAAGTCGATA ATTTTTGGCT GGTGGGTCCT TTAAAAATTA CTCCTCAGCA AGAGGCACAG TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC CAAAAGTCCA AGATGAAGTG
OXA-89
OXA-75
OXA-65
OXA-90
OXA-88
OXA-95
OXA-108
OXA-130
OXA-66
OXA-83
OXA-79
OXA-76
OXA-80
OXA-109
OXA-131
OXA-84
OXA-82
OXA-115
OXA-94
OXA-69
OXA-110
OXA-112
OXA-92
OXA-107
OXA-116
OXA-117
OXA-104
Consensus
OXA-132
OXA-99
A80
OXA-64
OXA-111
OXA-67
OXA-86
OXA-87
OXA-100
OXA-70
OXA-106
OXA-71
OXA-113


OXA-93 ....A .... A..... .....
OXA-68 .... A..... ....A .... A.G.... .....
OXA-128 .... A..... ....A .... A.G.... .....
OXA-78 .... A..... ....A .... A..... .....
OXA-77 ....A .... A..... .....
OXA-91 ....A .... A..... .....
OXA-89 ....A ...A.. .... A..... .....
OXA-75 ....A.. .....A .... A..... .....
OXA-65 .... A..... .....
OXA-90 .... A..... .... A..... .....
OXA-88 .... A..... .....
OXA-95 .... A..... .....
OXA-108 ....G. .... A..... .....
OXA-130 .... A..... .....
OXA-66 .... A..... .....
OXA-83 .... A..... .....
OXA-79 .... A..... .....
OXA-76 .... A..... .....
OXA-80 .... A..... .....
OXA-109 .... A..... .....
OXA-131 .... A..... .....
OXA-84 ....C..... .... A..... .....
OXA-82 ....G. .... A..... .....
OXA-115 ....G. .... A..... .....
OXA-94 .... A..... .....
OXA-69 .... C..... .... A..... .....
OXA-110 .... C..... .... A..... .....
OXA-112 .... C..... .... A..... .....
OXA-92 .... C..... .... A..... .....
OXA-107 ....G. ..... C..... .... A..... .....
OXA-116 .... A..... .....
OXA-117 .... A..... ....A .... A..... .....
OXA-104 ..G..G.... .C..... .....C..G C.G..... .C..G..... G..A..G... .....G..T. .A..G..G.. C.....C..G ..G..... AG.....G.. G.....
Consensus ..a..c.... .t..... g.....t..t t.a..... .t..t..... a..g..a..g .....t..c. .g..a..t.. t.....g..t ..a..... aa.....c.. a.....
601 720
OXA-51 CAATCCATGT TATTCATAGA AGAAAAGAAT GGAAATAAAA TATACGCAAA AAGTGGTTGG GGATGGGATG TAGACCCACA AGTAGGCTGG TTAACTGGAT GGGTTGTTCA GCCTCAAGGA
OXA-132 ....
OXA-99 ....T.... ....
A80 ....
OXA-64 ....G
OXA-111 ....T.... ....
OXA-67 ....
OXA-86 ....




OXA-99 ....
A80 ....
OXA-64 ....
OXA-111 ....
OXA-67 ....
OXA-86 ....
OXA-87 ....
OXA-100 ....
OXA-70 ....
OXA-106 ....
OXA-71 .....A..... ....
OXA-113 .... C..... ....
OXA-93 .... C..... ....
OXA-68 .... C..... ....
OXA-128 .... C..... ....
OXA-78 ....C..... .... C..... ....
OXA-77 .... C..... ....
OXA-91 ....
OXA-89 ....
OXA-75 ....T..... ....G.G. ....
OXA-65 ....
OXA-90 ....
OXA-88 ....
OXA-95 ....
OXA-108 ....
OXA-130 ....
OXA-66 .... C..... ....
OXA-83 .... C..... ....
OXA-79 .... C..... ....
OXA-76 .... C.....A.. ....
OXA-80 .... C..... ....
OXA-109 .... C..... ....
OXA-131 .... C..... ....
OXA-84 .... C..... ....
OXA-82 .... C..... ....
OXA-115 .... C..... ....
OXA-94 ....
OXA-69 ....
OXA-110 ....
OXA-112 .....C..... ....
OXA-92 ....
OXA-107 ....
OXA-116 ....
OXA-117 ....

```
OXA-104   ..C.....G. ....TAG... G...C.G... .......... .C..T..G.. .AGC..G..C .....A.... .C........ CC.G.....G C.G..C...C .G
Consensus ..t.....a. ....ctc... t...t.a... .......... .a..a..t.. .tct..t..a .....g.... .t........ tt.a...c.a t.a..t...t .atag
```

- **A74**

```
          1                                                                                                          120
OXA-64    ATGAACATTA AAGCACTCTT ACTTATAACA AGCGCTATTT TTATTTCAGC CTGCTCACCT TATATAGTGA CTGCTAATCC AAATCACAGC GCTTCAAAAT CTGATGAAAA AGGAGAGAAA
A74       ATGAACATTA AAGCACTCTT ACTTATAACA AGCGCTATTT TTATTTCAGC CTGCTCACCT TATATAGTGA CTGCTAATCC AAATCACAGC GCTTCAAAAT CTGATGAAAA AGGAGAGAAA
Consensus ATGAACATTA AAGCACTCTT ACTTATAACA AGCGCTATTT TTATTTCAGC CTGCTCACCT TATATAGTGA CTGCTAATCC AAATCACAGC GCTTCAAAAT CTGATGAAAA AGGAGAGAAA

          121                                                                                                        240
OXA-64    ATTAAAAATT TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG
A74       ATTAAAAATT TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG
Consensus ATTAAAAATT TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG

          241                                                                                                        360
OXA-64    ACCTTCAAAA TGCTTAATGC TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACCACAGAA GTATTTAAGT GGGACGGGCA AAAAAGGCTA TTCCCAGAAT GGGAAAAGGA CATGACCCTA
A74       ACCTTCAAAA TGCTTAATGC TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACCACAGAA GTATTTAAGT GGGACGGGCA AAAAAGGCTA TTCCCAGAAT GGGAAAAGGA CATGACCCTA
Consensus ACCTTCAAAA TGCTTAATGC TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACCACAGAA GTATTTAAGT GGGACGGGCA AAAAAGGCTA TTCCCAGAAT GGGAAAAGGA CATGACCCTA

          361                                                                                                        480
OXA-64    GGCGATGCTA TGAAAGCTTC CGCTATTCCG GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAACTCA TGTCTAAGGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC
A74       GGCGATGCTA TGAAAGCTTC CGCTATTCCG GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAACTCA TGTCTAAGGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC
Consensus GGCGATGCTA TGAAAGCTTC CGCTATTCCG GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAACTCA TGTCTAAGGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC

          481                                                                                                        600
OXA-64    CAAGTCGATA ATTTTTGGCT GGTGGGTCCT TTAAAAATTA CTCCTCAGCA AGAGGCACAG TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC CAAAAGTCCA AGATGAAGTG
A74       CAAGTCGATA ATTTTTGGCT GGTGGGTCCT TTAAAAATTA CTCCTCAGCA AGAGGCACAG TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC CAAAAGTCCA AGATGAAGTG
Consensus CAAGTCGATA ATTTTTGGCT GGTGGGTCCT TTAAAAATTA CTCCTCAGCA AGAGGCACAG TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC CAAAAGTCCA AGATGAAGTG

          601                                                                                                        720
OXA-64    CAATCCATGT TATTCATAGA AGAAAAGAAT GGAAATAAAA TATACGCAAA AAGTGGTTGG GGATGGGATG TAGACCCACA AGTAGGCTGG TTAACTGGAT GGGTTGTTCA GCCTCAAGGG
A74       CAATCCATGT TATTCATAGA AGAAAAGAAT GGAAATAAAA TATACGCAAA AAGTGGTTGG GGATGGGATG TAGACCCACA AGTAGGCTGG TTAACTGGAT GGGTTGTTCA GCCTCAAGGG
Consensus CAATCCATGT TATTCATAGA AGAAAAGAAT GGAAATAAAA TATACGCAAA AAGTGGTTGG GGATGGGATG TAGACCCACA AGTAGGCTGG TTAACTGGAT GGGTTGTTCA GCCTCAAGGG

          721                                                                                                  825
OXA-64    AATATTGTAG CGTTCTCCCT TAACTTAGAA ATGAAAAAAG GAATACCTAG CTCTGTTCGA AAAGAGATTA CTTATAAAAG TTTAGAACAA TTAGGTATTT TATAG
A74       AATATTGTAG CGTTCTCCCT TAACTTAGAA ATGAAAAAAG GAATACCTAG CTCTGTTCGA AAAGAGATTA CTTATAAAAG TTTAGAACAA TTAGGTATTT TATAG
Consensus AATATTGTAG CGTTCTCCCT TAACTTAGAA ATGAAAAAAG GAATACCTAG CTCTGTTCGA AAAGAGATTA CTTATAAAAG TTTAGAACAA TTAGGTATTT TATAG
```

- **A405 and A433**

```
           1                                                                                                   120
   OXA-66  ATGAACATTA AAGCACTCTT ACTTATAACA AGCGCTATTT TTATTTCAGC CTGCTCACCT TATATAGTGA CTGCTAATCC AAATCACAGC GCTTCAAAAT CTGATGTAAA AGCAGAGAAA
     A405  ATGAACATTA AAGCACTCTT ACTTATAACA AGCGCTATTT TTATTTCAGC CTGCTCACCT TATATAGTGA CTGCTAATCC AAATCACAGC GCTTCAAAAT CTGATGTAAA AGCAGAGAAA
     A433  ATGAACATTA AAGCACTCTT ACTTATAACA AGCGCTATTT TTATTTCAGC CTGCTCACCT TATATAGTGA CTGCTAATCC AAATCACAGC GCTTCAAAAT CTGATGTAAA AGCAGAGAAA
Consensus  ATGAACATTA AAGCACTCTT ACTTATAACA AGCGCTATTT TTATTTCAGC CTGCTCACCT TATATAGTGA CTGCTAATCC AAATCACAGC GCTTCAAAAT CTGATGTAAA AGCAGAGAAA

           121                                                                                                 240
   OXA-66  ATTAAAAATT TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG
     A405  ATTAAAAATT TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG
     A433  ATTAAAAATT TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG
Consensus  ATTAAAAATT TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG

           241                                                                                                 360
   OXA-66  ACCTTCAAAA TGCTTAATGC TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACCACAGAA GTATTTAAGT GGGATGGTAA AAAAAGGTTA TTCCCAGAAT GGGAAAAGGA CATGACCCTA
     A405  ACCTTCAAAA TGCTTAATGC TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACCACAGAA GTATTTAAGT GGGATGGTAA AAAAAGGTTA TTCCCAGAAT GGGAAAAGGA CATGACCCTA
     A433  ACCTTCAAAA TGCTTAATGC TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACCACAGAA GTATTTAAGT GGGATGGTAA AAAAAGGTTA TTCCCAGAAT GGGAAAAGGA CATGACCCTA
Consensus  ACCTTCAAAA TGCTTAATGC TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACCACAGAA GTATTTAAGT GGGATGGTAA AAAAAGGTTA TTCCCAGAAT GGGAAAAGGA CATGACCCTA

           361                                                                                                 480
   OXA-66  GGCGATGCCA TGAAAGCTTC CGCTATTCCA GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAGCTCA TGTCTAAGGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC
     A405  GGCGATGCCA TGAAAGCTTC CGCTATTCCA GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAGCTCA TGTCTAAGGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC
     A433  GGCGATGCCA TGAAAGCTTC CGCTATTCCA GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAGCTCA TGTCTAAGGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC
Consensus  GGCGATGCCA TGAAAGCTTC CGCTATTCCA GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAGCTCA TGTCTAAGGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC

           481                                                                                                 600
   OXA-66  CAAGTCGATA ATTTTTGGCT GGTGGGTCCT TTAAAAATTA CTCCTCAGCA AGAGGCACAG TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC AAAAAGTCCA AGATGAAGTG
     A405  CAAGTCGATA ATTTTTGGCT GGTGGGTCCT TTAAAAATTA CTCCTCAGCA AGAGGCACAG TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC AAAAAGTCCA AGATGAAGTG
     A433  CAAGTCGATA ATTTTTGGCT GGTGGGTCCT TTAAAAATTA CTCCTCAGCA AGAGGCACAG TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC AAAAAGTCCA AGATGAAGTG
Consensus  CAAGTCGATA ATTTTTGGCT GGTGGGTCCT TTAAAAATTA CTCCTCAGCA AGAGGCACAG TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC AAAAAGTCCA AGATGAAGTG

           601                                                                                                 720
   OXA-66  CAATCCATGC TATTCATAGA AGAAAAGAAT GGAAACAAAA TATACGCAAA AAGTGGTTGG GGATGGGATG TAAACCCACA AGTAGGCTGG TTAACTGGAT GGGTTGTTCA GCCTCAAGGG
     A405  CAATCCATGC TATTCATAGA AGAAAAGAAT GGAAACAAAA TATACGCAAA AAGTGGTTGG GGATGGGATG TAAACCCACA AGTAGGCTGG TTAACTGGAT GGGTTGTTCA GCCTCAAGGG
     A433  CAATCCATGC TATTCATAGA AGAAAAGAAT GGAAACAAAA TATACGCAAA AAGTGGTTGG GGATGGGATG TAAACCCACA AGTAGGCTGG TTAACTGGAT GGGTTGTTCA GCCTCAAGGG
Consensus  CAATCCATGC TATTCATAGA AGAAAAGAAT GGAAACAAAA TATACGCAAA AAGTGGTTGG GGATGGGATG TAAACCCACA AGTAGGCTGG TTAACTGGAT GGGTTGTTCA GCCTCAAGGG

           721                                                                                          825
   OXA-66  AATATTGTAG CGTTCTCCCT TAACTTAGAA ATGAAAAAAG GAATACCTAG CTCTGTTCGA AAAGAGATTA CTTATAAAAG CTTAGAACAA TTAGGTATTT TATAG
     A405  AATATTGTAG CGTTCTCCCT TAACTTAGAA ATGAAAAAAG GAATACCTAG CTCTGTTCGA AAAGAGATTA CTTATAAAAG CTTAGAACAA TTAGGTATTT TATAG
     A433  AATATTGTAG CGTTCTCCCT TAACTTAGAA ATGAAAAAAG GAATACCTAG CTCTGTTCGA AAAGAGATTA CTTATAAAAG CTTAGAACAA TTAGGTATTT TATAG
Consensus  AATATTGTAG CGTTCTCCCT TAACTTAGAA ATGAAAAAAG GAATACCTAG CTCTGTTCGA AAAGAGATTA CTTATAAAAG CTTAGAACAA TTAGGTATTT TATAG
```

- **A66**

```
           1                                                                                                    120
   OXA-67  ATGAACATTA AAGCACTCTT ACTTATAACA AGCACTATTT TTATTTCAGC CTGCTCACCT TATATAGTGA CTGCTAATCC AAATCACAGC ACTTCAAAAT CTGATGAAAA AGCAGAGAAA
     A66   ATGAACATTA AAGCACTCTT ACTTATAACA AGCACTATTT TTATTTCAGC CTGCTCACCT TATATAGTGA CTGCTAATCC AAATCACAGC ACTTCAAAAT CTGATGAAAA AGCAGAGAAA
Consensus  ATGAACATTA AAGCACTCTT ACTTATAACA AGCACTATTT TTATTTCAGC CTGCTCACCT TATATAGTGA CTGCTAATCC AAATCACAGC ACTTCAAAAT CTGATGAAAA AGCAGAGAAA

           121                                                                                                  240
   OXA-67  ATTAAAAATT TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG
     A66   ATTAAAAATT TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG
Consensus  ATTAAAAATT TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG

           241                                                                                                  360
   OXA-67  ACCTTCAAAA TGCTTAATGC TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACCACAGAA ATATTTAAGT GGGACGGGCA AAAAAGGCTG TTCCCAGAAT GGGAAAAGGA CATGACCCTA
     A66   ACCTTCAAAA TGCTTAATGC TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACCACAGAA ATATTTAAGT GGGACGGGCA AAAAAGGCTG TTCCCAGAAT GGGAAAAGGA CATGACCCTA
Consensus  ACCTTCAAAA TGCTTAATGC TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACCACAGAA ATATTTAAGT GGGACGGGCA AAAAAGGCTG TTCCCAGAAT GGGAAAAGGA CATGACCCTA

           361                                                                                                  480
   OXA-67  GGTGATGCTA TGAAAGCTTC CGCTATTCCG GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAACTCA TGTCTAAGGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC
     A66   GGTGATGCTA TGAAAGCTTC CGCTATTCCG GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAACTCA TGTCTAAGGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC
Consensus  GGTGATGCTA TGAAAGCTTC CGCTATTCCG GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAACTCA TGTCTAAGGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC

           481                                                                                                  600
   OXA-67  CAAGTCGATA ATTTTTGGCT GGTGGGTCCT TTAAAAATTA CTCCTCAGCA AGAGGCACAG TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC TAAAAGTCCA AGATGAAGTG
     A66   CAAGTCGATA ATTTTTGGCT GGTGGGTCCT TTAAAAATTA CTCCTCAGCA AGAGGCACAG TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC TAAAAGTCCA AGATGAAGTG
Consensus  CAAGTCGATA ATTTTTGGCT GGTGGGTCCT TTAAAAATTA CTCCTCAGCA AGAGGCACAG TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC TAAAAGTCCA AGATGAAGTG

           601                                                                                                  720
   OXA-67  CAATCCATGT TATTCATAGA AGAAAAGAAT GGAAATAAAA TATACGCAAA AAGTGGTTGG GGATGGGATG TAGACCCACA AGTAGGCTGG TTAACTGGAT GGGTTGTTCA GCCTCAAGGA
     A66   CAATCCATGT TATTCATAGA AGAAAAGAAT GGAAATAAAA TATACGCAAA AAGTGGTTGG GGATGGGATG TAGACCCACA AGTAGGCTGG TTAACTGGAT GGGTTGTTCA GCCTCAAGGA
Consensus  CAATCCATGT TATTCATAGA AGAAAAGAAT GGAAATAAAA TATACGCAAA AAGTGGTTGG GGATGGGATG TAGACCCACA AGTAGGCTGG TTAACTGGAT GGGTTGTTCA GCCTCAAGGA

           721                                                                                          825
   OXA-67  AATATTGTAG CGTTCTCCCT TAACTTAGAA ATGAAAAAAG GAATACCTAG CTCTGTTCGA AAAGAGATTA CTTATAAAAG TTTAGAACAA TTAGGTATTT TATAG
     A66   AATATTGTAG CGTTCTCCCT TAACTTAGAA ATGAAAAAAG GAATACCTAG CTCTGTTCGA AAAGAGATTA CTTATAAAAG TTTAGAACAA TTAGGTATTT TATAG
Consensus  AATATTGTAG CGTTCTCCCT TAACTTAGAA ATGAAAAAAG GAATACCTAG CTCTGTTCGA AAAGAGATTA CTTATAAAAG TTTAGAACAA TTAGGTATTT TATAG
```

- **A266, A150, A242, A471 and A472**

```
         1                                                                                                                    120
OXA-68   ATGAACATTA AAGCACTCTT ACTTATAACA AGCGCTATTT TTATTTCAGC CTGCTCACCT TATATAGTGT CTGCTAATCC AAATCACAGT GCTTCAAAAT CTGATGAAAA AGCAGAGAAA
A266     ATGAACATTA AAGCACTCTT ACTTATAACA AGCGCTATTT TTATTTCAGC CTGCTCACCT TATATAGTGT CTGCTAATCC AAATCACAGT GCTTCAAAAT CTGATGAAAA AGCAGAGAAA
A242     ATGAACATTA AAGCACTCTT ACTTATAACA AGCGCTATTT TTATTTCAGC CTGCTCACCT TATATAGTGT CTGCTAATCC AAATCACAGT GCTTCAAAAT CTGATGAAAA AGCAGAGAAA
A472     ATGAACATTA AAGCACTCTT ACTTATAACA AGCGCTATTT TTATTTCAGC CTGCTCACCT TATATAGTGT CTGCTAATCC AAATCACAGT GCTTCAAAAT CTGATGAAAA AGCAGAGAAA
A471     ATGAACATTA AAGCACTCTT ACTTATAACA AGCGCTATTT TTATTTCAGC CTGCTCACCT TATATAGTGT CTGCTAATCC AAATCACAGT GCTTCAAAAT CTGATGAAAA AGCAGAGAAA
A150     ATGAACATTA AAGCACTCTT ACTTATAACA AGCGCTATTT TTATTTCAGC CTGCTCACCT TATATAGTGT CTGCTAATCC AAATCACAGT GCTTCAAAAT CTGATGAAAA AGCAGAGAAA
Consensus ATGAACATTA AAGCACTCTT ACTTATAACA AGCGCTATTT TTATTTCAGC CTGCTCACCT TATATAGTGT CTGCTAATCC AAATCACAGT GCTTCAAAAT CTGATGAAAA AGCAGAGAAA

         121                                                                                                                  240
OXA-68   ATTAAAAATT TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG
A266     ATTAAAAATT TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG
A242     ATTAAAAATT TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG
A472     ATTAAAAATT TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG
A471     ATTAAAAATT TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG
A150     ATTAAAAATT TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG
Consensus ATTAAAAATT TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG

         241                                                                                                                  360
OXA-68   ACCTTCAAAA TGCTTAATGC TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACTACAGAA GTATTTAAGT GGGACGGGCA AAAAAGGCTA TTCCCAGAAT GGGAAAAGAA CATGACCCTA
A266     ACCTTCAAAA TGCTTAATGC TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACTACAGAA GTATTTAAGT GGGACGGGCA AAAAAGGCTA TTCCCAGAAT GGGAAAAGAA CATGACCCTA
A242     ACCTTCAAAA TGCTTAATGC TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACTACAGAA GTATTTAAGT GGGACGGGCA AAAAAGGCTA TTCCCAGAAT GGGAAAAGAA CATGACCCTA
A472     ACCTTCAAAA TGCTTAATGC TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACTACAGAA GTATTTAAGT GGGACGGGCA AAAAAGGCTA TTCCCAGAAT GGGAAAAGAA CATGACCCTA
A471     ACCTTCAAAA TGCTTAATGC TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACTACAGAA GTATTTAAGT GGGACGGGCA AAAAAGGCTA TTCCCAGAAT GGGAAAAGAA CATGACCCTA
A150     ACCTTCAAAA TGCTTAATGC TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACTACAGAA GTATTTAAGT GGGACGGGCA AAAAAGGCTA TTCCCAGAAT GGGAAAAGAA CATGACCCTA
Consensus ACCTTCAAAA TGCTTAATGC TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACTACAGAA GTATTTAAGT GGGACGGGCA AAAAAGGCTA TTCCCAGAAT GGGAAAAGAA CATGACCCTA

         361                                                                                                                  480
OXA-68   GGCGATGCTA TGAAAGCTTC CGCTATTCCG GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAACTCA TGTCTAATGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC
A266     GGCGATGCTA TGAAAGCTTC CGCTATTCCG GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAACTCA TGTCTAATGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC
A242     GGCGATGCTA TGAAAGCTTC CGCTATTCCG GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAACTCA TGTCTAATGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC
A472     GGCGATGCTA TGAAAGCTTC CGCTATTCCG GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAACTCA TGTCTAATGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC
A471     GGCGATGCTA TGAAAGCTTC CGCTATTCCG GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAACTCA TGTCTAATGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC
A150     GGCGATGCTA TGAAAGCTTC CGCTATTCCG GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAACTCA TGTCTAATGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC
Consensus GGCGATGCTA TGAAAGCTTC CGCTATTCCG GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAACTCA TGTCTAATGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC
```

```
           481                                                                                                  600
OXA-68     CAAGTCGATA ATTTTTGGCT AGTGGGTCCT TTAAAAATTA CTCCTCAGCA AGAGGCACAA TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC AAGAAGTCCA AGATGAAGTG
A266       CAAGTCGATA ATTTTTGGCT AGTGGGTCCT TTAAAAATTA CTCCTCAGCA AGAGGCACAA TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC AAGAAGTCCA AGATGAAGTG
A242       CAAGTCGATA ATTTTTGGCT AGTGGGTCCT TTAAAAATTA CTCCTCAGCA AGAGGCACAA TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC AAGAAGTCCA AGATGAAGTG
A472       CAAGTCGATA ATTTTTGGCT AGTGGGTCCT TTAAAAATTA CTCCTCAGCA AGAGGCACAA TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC AAGAAGTCCA AGATGAAGTG
A471       CAAGTCGATA ATTTTTGGCT AGTGGGTCCT TTAAAAATTA CTCCTCAGCA AGAGGCACAA TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC AAGAAGTCCA AGATGAAGTG
A150       CAAGTCGATA ATTTTTGGCT AGTGGGTCCT TTAAAAATTA CTCCTCAGCA AGAGGCACAA TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC AAGAAGTCCA AGATGAAGTG
Consensus  CAAGTCGATA ATTTTTGGCT AGTGGGTCCT TTAAAAATTA CTCCTCAGCA AGAGGCACAA TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC AAGAAGTCCA AGATGAAGTG

           601                                                                                                  720
OXA-68     CAATCCATGC TATTCATAGA AGAAAAGAAT GGAAATAAAA TATACGCAAA AAGTGGTTGG GGATGGGATG TAAACCCACA AGTAGGCTGG TTAACTGGAT GGGTTGTTCA GCCTCAAGGG
A266       CAATCCATGC TATTCATAGA AGAAAAGAAT GGAAATAAAA TATACGCAAA AAGTGGTTGG GGATGGGATG TAAACCCACA AGTAGGCTGG TTAACTGGAT GGGTTGTTCA GCCTCAAGGG
A242       CAATCCATGC TATTCATAGA AGAAAAGAAT GGAAATAAAA TATACGCAAA AAGTGGTTGG GGATGGGATG TAAACCCACA AGTAGGCTGG TTAACTGGAT GGGTTGTTCA GCCTCAAGGG
A472       CAATCCATGC TATTCATAGA AGAAAAGAAT GGAAATAAAA TATACGCAAA AAGTGGTTGG GGATGGGATG TAAACCCACA AGTAGGCTGG TTAACTGGAT GGGTTGTTCA GCCTCAAGGG
A471       CAATCCATGC TATTCATAGA AGAAAAGAAT GGAAATAAAA TATACGCAAA AAGTGGTTGG GGATGGGATG TAAACCCACA AGTAGGCTGG TTAACTGGAT GGGTTGTTCA GCCTCAAGGG
A150       CAATCCATGC TATTCATAGA AGAAAAGAAT GGAAATAAAA TATACGCAAA AAGTGGTTGG GGATGGGATG TAAACCCACA AGTAGGCTGG TTAACTGGAT GGGTTGTTCA GCCTCAAGGG
Consensus  CAATCCATGC TATTCATAGA AGAAAAGAAT GGAAATAAAA TATACGCAAA AAGTGGTTGG GGATGGGATG TAAACCCACA AGTAGGCTGG TTAACTGGAT GGGTTGTTCA GCCTCAAGGG

           721                                                                                  825
OXA-68     AATATTGTAG CGTTCTCCCT TAACTTAGAA ATGAAAAAAG GAATACCTAG CTCTGTTCGA AAAGAGATTA CTTATAAAAG CTTAGAACAA TTAGGTATTT TATAG
A266       AATATTGTAG CGTTCTCCCT TAACTTAGAA ATGAAAAAAG GAATACCTAG CTCTGTTCGA AAAGAGATTA CTTATAAAAG CTTAGAACAA TTAGGTATTT TATAG
A242       AATATTGTAG CGTTCTCCCT TAACTTAGAA ATGAAAAAAG GAATACCTAG CTCTGTTCGA AAAGAGATTA CTTATAAAAG CTTAGAACAA TTAGGTATTT TATAG
A472       AATATTGTAG CGTTCTCCCT TAACTTAGAA ATGAAAAAAG GAATACCTAG CTCTGTTCGA AAAGAGATTA CTTATAAAAG CTTAGAACAA TTAGGTATTT TATAG
A471       AATATTGTAG CGTTCTCCCT TAACTTAGAA ATGAAAAAAG GAATACCTAG CTCTGTTCGA AAAGAGATTA CTTATAAAAG CTTAGAACAA TTAGGTATTT TATAG
A150       AATATTGTAG CGTTCTCCCT TAACTTAGAA ATGAAAAAAG GAATACCTAG CTCTGTTCGA AAAGAGATTA CTTATAAAAG CTTAGAACAA TTAGGTATTT TATAG
Consensus  AATATTGTAG CGTTCTCCCT TAACTTAGAA ATGAAAAAAG GAATACCTAG CTCTGTTCGA AAAGAGATTA CTTATAAAAG CTTAGAACAA TTAGGTATTT TATAG
```

4. OXA-58 GROUP

```
           1                                                                                                                    120
OXA-58     ATGAAATTAT TAAAAATATT GAGTTTAGTT TGCTTAAGCA TAAGTATTGG GGCTTGTGCT GAGCATAGTA TGAGTCGAGC AAAAACAAGT ACAATTCCAC AAGTGAATAA CTCAATCATC
A266       ATGAAATTAT TAAAAATATT GAGTTTAGTT TGCTTAAGCA TAAGTATTGG GGCTTGTGCT GAGCATAGTA TGAGTCGAGC AAAAACAAGT ACAATTCCAC AAGTGAATAA CTCAATCATC
A446       ATGAAATTAT TAAAAATATT GAGTTTAGTT TGCTTAAGCA TAAGTATTGG GGCTTGTGCT GAGCATAGTA TGAGTCGAGC AAAAACAAGT ACAATTCCAC AAGTGAATAA CTCAATCATC
A242       ATGAAATTAT TAAAAATATT GAGTTTAGTT TGCTTAAGCA TAAGTATTGG GGCTTGTGCT GAGCATAGTA TGAGTCGAGC AAAAACAAGT ACAATTCCAC AAGTGAATAA CTCAATCATC
A472       ATGAAATTAT TAAAAATATT GAGTTTAGTT TGCTTAAGCA TAAGTATTGG GGCTTGTGCT GAGCATAGTA TGAGTCGAGC AAAAACAAGT ACAATTCCAC AAGTGAATAA CTCAATCATC
A471       ATGAAATTAT TAAAAATATT GAGTTTAGTT TGCTTAAGCA TAAGTATTGG GGCTTGTGCT GAGCATAGTA TGAGTCGAGC AAAAACAAGT ACAATTCCAC AAGTGAATAA CTCAATCATC
A150       ATGAAATTAT TAAAAATATT GAGTTTAGTT TGCTTAAGCA TAAGTATTGG GGCTTGTGCT GAGCATAGTA TGAGTCGAGC AAAAACAAGT ACAATTCCAC AAGTGAATAA CTCAATCATC
A180       ATGAAATTAT TAAAAATATT GAGTTTAGTT TGCTTAAGCA TAAGTATTGG GGCTTGTGCT GAGCATAGTA TGAGTCGAGC AAAAACAAGT ACAATTCCAC AAGTGAATAA CTCAATCATC
A292       ATGAAATTAT TAAAAATATT GAGTTTAGTT TGCTTAAGCA TAAGTATTGG GGCTTGTGCT GAGCATAGTA TGAGTCGAGC AAAAACAAGT ACAATTCCAC AAGTGAATAA CTCAATCATC
A262       ATGAAATTAT TAAAAATATT GAGTTTAGTT TGCTTAAGCA TAAGTATTGG GGCTTGTGCT GAGCATAGTA TGAGTCGAGC AAAAACAAGT ACAATTCCAC AAGTGAATAA CTCAATCATC
A188       ATGAAATTAT TAAAAATATT GAGTTTAGTT TGCTTAAGCA TAAGTATTGG GGCTTGTGCT GAGCATAGTA TGAGTCGAGC AAAAACAAGT ACAATTCCAC AAGTGAATAA CTCAATCATC
Consensus  ATGAAATTAT TAAAAATATT GAGTTTAGTT TGCTTAAGCA TAAGTATTGG GGCTTGTGCT GAGCATAGTA TGAGTCGAGC AAAAACAAGT ACAATTCCAC AAGTGAATAA CTCAATCATC

           121                                                                                                                  240
OXA-58     GATCAGAATG TTCAAGCGCT TTTTAATGAA ATCTCAGCTG ATGCTGTGTT TGTCACATAT GATGGTCAAA ATATTAAAAA ATATGGCACG CATTTAGACC GAGCAAAAAC AGCTTATATT
A266       GATCAGAATG TTCAAGCGCT TTTTAATGAA ATCTCAGCTG ATGCTGTGTT TGTCACATAT GATGGTCAAA ATATTAAAAA ATATGGCACG CATTTAGACC GAGCAAAAAC AGCTTATATT
A446       GATCAGAATG TTCAAGCGCT TTTTAATGAA ATCTCAGCTG ATGCTGTGTT TGTCACATAT GATGGTCAAA ATATTAAAAA ATATGGCACG CATTTAGACC GAGCAAAAAC AGCTTATATT
A242       GATCAGAATG TTCAAGCGCT TTTTAATGAA ATCTCAGCTG ATGCTGTGTT TGTCACATAT GATGGTCAAA ATATTAAAAA ATATGGCACG CATTTAGACC GAGCAAAAAC AGCTTATATT
A472       GATCAGAATG TTCAAGCGCT TTTTAATGAA ATCTCAGCTG ATGCTGTGTT TGTCACATAT GATGGTCAAA ATATTAAAAA ATATGGCACG CATTTAGACC GAGCAAAAAC AGCTTATATT
A471       GATCAGAATG TTCAAGCGCT TTTTAATGAA ATCTCAGCTG ATGCTGTGTT TGTCACATAT GATGGTCAAA ATATTAAAAA ATATGGCACG CATTTAGACC GAGCAAAAAC AGCTTATATT
A150       GATCAGAATG TTCAAGCGCT TTTTAATGAA ATCTCAGCTG ATGCTGTGTT TGTCACATAT GATGGTCAAA ATATTAAAAA ATATGGCACG CATTTAGACC GAGCAAAAAC AGCTTATATT
A180       GATCAGAATG TTCAAGCGCT TTTTAATGAA ATCTCAGCTG ATGCTGTGTT TGTCACATAT GATGGTCAAA ATATTAAAAA ATATGGCACG CATTTAGACC GAGCAAAAAC AGCTTATATT
A292       GATCAGAATG TTCAAGCGCT TTTTAATGAA ATCTCAGCTG ATGCTGTGTT TGTCACATAT GATGGTCAAA ATATTAAAAA ATATGGCACG CATTTAGACC GAGCAAAAAC AGCTTATATT
A262       GATCAGAATG TTCAAGCGCT TTTTAATGAA ATCTCAGCTG ATGCTGTGTT TGTCACATAT GATGGTCAAA ATATTAAAAA ATATGGCACG CATTTAGACC GAGCAAAAAC AGCTTATATT
A188       GATCAGAATG TTCAAGCGCT TTTTAATGAA ATCTCAGCTG ATGCTGTGTT TGTCACATAT GATGGTCAAA ATATTAAAAA ATATGGCACG CATTTAGACC GAGCAAAAAC AGCTTATATT
Consensus  GATCAGAATG TTCAAGCGCT TTTTAATGAA ATCTCAGCTG ATGCTGTGTT TGTCACATAT GATGGTCAAA ATATTAAAAA ATATGGCACG CATTTAGACC GAGCAAAAAC AGCTTATATT

           241                                                                                                                  360
OXA-58     CCTGCATCTA CATTTAAAAT TGCCAATGCA CTAATTGGTT TAGAAAATCA TAAAGCAACA TCTACAGAAA TATTTAAGTG GGATGGAAAG CCACGTTTTT TTAAAGCATG GGACAAAGAT
A266       CCTGCATCTA CATTTAAAAT TGCCAATGCA CTAATTGGTT TAGAAAATCA TAAAGCAACA TCTACAGAAA TATTTAAGTG GGATGGAAAG CCACGTTTTT TTAAAGCATG GGACAAAGAT
A446       CCTGCATCTA CATTTAAAAT TGCCAATGCA CTAATTGGTT TAGAAAATCA TAAAGCAACA TCTACAGAAA TATTTAAGTG GGATGGAAAG CCACGTTTTT TTAAAGCATG GGACAAAGAT
A242       CCTGCATCTA CATTTAAAAT TGCCAATGCA CTAATTGGTT TAGAAAATCA TAAAGCAACA TCTACAGAAA TATTTAAGTG GGATGGAAAG CCACGTTTTT TTAAAGCATG GGACAAAGAT
A472       CCTGCATCTA CATTTAAAAT TGCCAATGCA CTAATTGGTT TAGAAAATCA TAAAGCAACA TCTACAGAAA TATTTAAGTG GGATGGAAAG CCACGTTTTT TTAAAGCATG GGACAAAGAT
A471       CCTGCATCTA CATTTAAAAT TGCCAATGCA CTAATTGGTT TAGAAAATCA TAAAGCAACA TCTACAGAAA TATTTAAGTG GGATGGAAAG CCACGTTTTT TTAAAGCATG GGACAAAGAT
A150       CCTGCATCTA CATTTAAAAT TGCCAATGCA CTAATTGGTT TAGAAAATCA TAAAGCAACA TCTACAGAAA TATTTAAGTG GGATGGAAAG CCACGTTTTT TTAAAGCATG GGACAAAGAT
A180       CCTGCATCTA CATTTAAAAT TGCCAATGCA CTAATTGGTT TAGAAAATCA TAAAGCAACA TCTACAGAAA TATTTAAGTG GGATGGAAAG CCACGTTTTT TTAAAGCATG GGACAAAGAT
A292       CCTGCATCTA CATTTAAAAT TGCCAATGCA CTAATTGGTT TAGAAAATCA TAAAGCAACA TCTACAGAAA TATTTAAGTG GGATGGAAAG CCACGTTTTT TTAAAGCATG GGACAAAGAT
A262       CCTGCATCTA CATTTAAAAT TGCCAATGCA CTAATTGGTT TAGAAAATCA TAAAGCAACA TCTACAGAAA TATTTAAGTG GGATGGAAAG CCACGTTTTT TTAAAGCATG GGACAAAGAT
A188       CCTGCATCTA CATTTAAAAT TGCCAATGCA CTAATTGGTT TAGAAAATCA TAAAGCAACA TCTACAGAAA TATTTAAGTG GGATGGAAAG CCACGTTTTT TTAAAGCATG GGACAAAGAT
Consensus  CCTGCATCTA CATTTAAAAT TGCCAATGCA CTAATTGGTT TAGAAAATCA TAAAGCAACA TCTACAGAAA TATTTAAGTG GGATGGAAAG CCACGTTTTT TTAAAGCATG GGACAAAGAT
```

```
           361                                                                                                                   480
   OXA-58  TTTACTTTGG GCGAAGCCAT GCAAGCATCT ACAGTGCCTG TATATCAAGA ATTGGCACGT CGTATTGGTC CAAGCTTAAT GCAAAGTGAA TTGCAACGTA TTGGTTATGG CAATATGCAA
     A266  TTTACTTTGG GCGAAGCCAT GCAAGCATCT ACAGTGCCTG TATATCAAGA ATTGGCACGT CGTATTGGTC CAAGCTTAAT GCAAAGTGAA TTGCAACGTA TTGGTTATGG CAATATGCAA
     A446  TTTACTTTGG GCGAAGCCAT GCAAGCATCT ACAGTGCCTG TATATCAAGA ATTGGCACGT CGTATTGGTC CAAGCTTAAT GCAAAGTGAA TTGCAACGTA TTGGTTATGG CAATATGCAA
     A242  TTTACTTTGG GCGAAGCCAT GCAAGCATCT ACAGTGCCTG TATATCAAGA ATTGGCACGT CGTATTGGTC CAAGCTTAAT GCAAAGTGAA TTGCAACGTA TTGGTTATGG CAATATGCAA
     A472  TTTACTTTGG GCGAAGCCAT GCAAGCATCT ACAGTGCCTG TATATCAAGA ATTGGCACGT CGTATTGGTC CAAGCTTAAT GCAAAGTGAA TTGCAACGTA TTGGTTATGG CAATATGCAA
     A471  TTTACTTTGG GCGAAGCCAT GCAAGCATCT ACAGTGCCTG TATATCAAGA ATTGGCACGT CGTATTGGTC CAAGCTTAAT GCAAAGTGAA TTGCAACGTA TTGGTTATGG CAATATGCAA
     A150  TTTACTTTGG GCGAAGCCAT GCAAGCATCT ACAGTGCCTG TATATCAAGA ATTGGCACGT CGTATTGGTC CAAGCTTAAT GCAAAGTGAA TTGCAACGTA TTGGTTATGG CAATATGCAA
     A180  TTTACTTTGG GCGAAGCCAT GCAAGCATCT ACAGTGCCTG TATATCAAGA ATTGGCACGT CGTATTGGTC CAAGCTTAAT GCAAAGTGAA TTGCAACGTA TTGGTTATGG CAATATGCAA
     A292  TTTACTTTGG GCGAAGCCAT GCAAGCATCT ACAGTGCCTG TATATCAAGA ATTGGCACGT CGTATTGGTC CAAGCTTAAT GCAAAGTGAA TTGCAACGTA TTGGTTATGG CAATATGCAA
     A262  TTTACTTTGG GCGAAGCCAT GCAAGCATCT ACAGTGCCTG TATATCAAGA ATTGGCACGT CGTATTGGTC CAAGCTTAAT GCAAAGTGAA TTGCAACGTA TTGGTTATGG CAATATGCAA
     A188  TTTACTTTGG GCGAAGCCAT GCAAGCATCT ACAGTGCCTG TATATCAAGA ATTGGCACGT CGTATTGGTC CAAGCTTAAT GCAAAGTGAA TTGCAACGTA TTGGTTATGG CAATATGCAA
Consensus  TTTACTTTGG GCGAAGCCAT GCAAGCATCT ACAGTGCCTG TATATCAAGA ATTGGCACGT CGTATTGGTC CAAGCTTAAT GCAAAGTGAA TTGCAACGTA TTGGTTATGG CAATATGCAA
```

```
           481                                                                                                                   600
   OXA-58  ATAGGCACGG AAGTTGATCA ATTTTGGTTG AAAGGGCCTT TGACAATTAC ACCTATACAA GAAGTAAAGT TTGTGTATGA TTTAGCCCAA GGGCAATTGC CTTTTAAACC TGAAGTTCAG
     A266  ATAGGCACGG AAGTTGATCA ATTTTGGTTG AAAGGGCCTT TGACAATTAC ACCTATACAA GAAGTAAAGT TTGTGTATGA TTTAGCCCAA GGGCAATTGC CTTTTAAACC TGAAGTTCAG
     A446  ATAGGCACGG AAGTTGATCA ATTTTGGTTG AAAGGGCCTT TGACAATTAC ACCTATACAA GAAGTAAAGT TTGTGTATGA TTTAGCCCAA GGGCAATTGC CTTTTAAACC TGAAGTTCAG
     A242  ATAGGCACGG AAGTTGATCA ATTTTGGTTG AAAGGGCCTT TGACAATTAC ACCTATACAA GAAGTAAAGT TTGTGTATGA TTTAGCCCAA GGGCAATTGC CTTTTAAACC TGAAGTTCAG
     A472  ATAGGCACGG AAGTTGATCA ATTTTGGTTG AAAGGGCCTT TGACAATTAC ACCTATACAA GAAGTAAAGT TTGTGTATGA TTTAGCCCAA GGGCAATTGC CTTTTAAACC TGAAGTTCAG
     A471  ATAGGCACGG AAGTTGATCA ATTTTGGTTG AAAGGGCCTT TGACAATTAC ACCTATACAA GAAGTAAAGT TTGTGTATGA TTTAGCCCAA GGGCAATTGC CTTTTAAACC TGAAGTTCAG
     A150  ATAGGCACGG AAGTTGATCA ATTTTGGTTG AAAGGGCCTT TGACAATTAC ACCTATACAA GAAGTAAAGT TTGTGTATGA TTTAGCCCAA GGGCAATTGC CTTTTAAACC TGAAGTTCAG
     A180  ATAGGCACGG AAGTTGATCA ATTTTGGTTG AAAGGGCCTT TGACAATTAC ACCTATACAA GAAGTAAAGT TTGTGTATGA TTTAGCCCAA GGGCAATTGC CTTTTAAACC TGAAGTTCAG
     A292  ATAGGCACGG AAGTTGATCA ATTTTGGTTG AAAGGGCCTT TGACAATTAC ACCTATACAA GAAGTAAAGT TTGTGTATGA TTTAGCCCAA GGGCAATTGC CTTTTAAACC TGAAGTTCAG
     A262  ATAGGCACGG AAGTTGATCA ATTTTGGTTG AAAGGGCCTT TGACAATTAC ACCTATACAA GAAGTAAAGT TTGTGTATGA TTTAGCCCAA GGGCAATTGC CTTTTAAACC TGAAGTTCAG
     A188  ATAGGCACGG AAGTTGATCA ATTTTGGTTG AAAGGGCCTT TGACAATTAC ACCTATACAA GAAGTAAAGT TTGTGTATGA TTTAGCCCAA GGGCAATTGC CTTTTAAACC TGAAGTTCAG
Consensus  ATAGGCACGG AAGTTGATCA ATTTTGGTTG AAAGGGCCTT TGACAATTAC ACCTATACAA GAAGTAAAGT TTGTGTATGA TTTAGCCCAA GGGCAATTGC CTTTTAAACC TGAAGTTCAG
```

```
           601                                                                                                                   720
   OXA-58  CAACAAGTGA AAGAGATGTT GTATGTAGAG CGCAGAGGGG AGAATCGTCT ATATGCTAAA AGTGGCTGGG GAATGGCTGT AGACCCGCAA GTGGGTTGGT ATGTGGGTTT TGTTGAAAAG
     A266  CAACAAGTGA AAGAGATGTT GTATGTAGAG CGCAGAGGGG AGAATCGTCT ATATGCTAAA AGTGGCTGGG GAATGGCTGT AGACCCGCAA GTGGGTTGGT ATGTGGGTTT TGTTGAAAAG
     A446  CAACAAGTGA AAGAGATGTT GTATGTAGAG CGCAGAGGGG AGAATCGTCT ATATGCTAAA AGTGGCTGGG GAATGGCTGT AGACCCGCAA GTGGGTTGGT ATGTGGGTTT TGTTGAAAAG
     A242  CAACAAGTGA AAGAGATGTT GTATGTAGAG CGCAGAGGGG AGAATCGTCT ATATGCTAAA AGTGGCTGGG GAATGGCTGT AGACCCGCAA GTGGGTTGGT ATGTGGGTTT TGTTGAAAAG
     A472  CAACAAGTGA AAGAGATGTT GTATGTAGAG CGCAGAGGGG AGAATCGTCT ATATGCTAAA AGTGGCTGGG GAATGGCTGT AGACCCGCAA GTGGGTTGGT ATGTGGGTTT TGTTGAAAAG
     A471  CAACAAGTGA AAGAGATGTT GTATGTAGAG CGCAGAGGGG AGAATCGTCT ATATGCTAAA AGTGGCTGGG GAATGGCTGT AGACCCGCAA GTGGGTTGGT ATGTGGGTTT TGTTGAAAAG
     A150  CAACAAGTGA AAGAGATGTT GTATGTAGAG CGCAGAGGGG AGAATCGTCT ATATGCTAAA AGTGGCTGGG GAATGGCTGT AGACCCGCAA GTGGGTTGGT ATGTGGGTTT TGTTGAAAAG
     A180  CAACAAGTGA AAGAGATGTT GTATGTAGAG CGCAGAGGGG AGAATCGTCT ATATGCTAAA AGTGGCTGGG GAATGGCTGT AGACCCGCAA GTGGGTTGGT ATGTGGGTTT TGTTGAAAAG
     A292  CAACAAGTGA AAGAGATGTT GTATGTAGAG CGCAGAGGGG AGAATCGTCT ATATGCTAAA AGTGGCTGGG GAATGGCTGT AGACCCGCAA GTGGGTTGGT ATGTGGGTTT TGTTGAAAAG
     A262  CAACAAGTGA AAGAGATGTT GTATGTAGAG CGCAGAGGGG AGAATCGTCT ATATGCTAAA AGTGGCTGGG GAATGGCTGT AGACCCGCAA GTGGGTTGGT ATGTGGGTTT TGTTGAAAAG
     A188  CAACAAGTGA AAGAGATGTT GTATGTAGAG CGCAGAGGGG AGAATCGTCT ATATGCTAAA AGTGGCTGGG GAATGGCTGT AGACCCGCAA GTGGGTTGGT ATGTGGGTTT TGTTGAAAAG
Consensus  CAACAAGTGA AAGAGATGTT GTATGTAGAG CGCAGAGGGG AGAATCGTCT ATATGCTAAA AGTGGCTGGG GAATGGCTGT AGACCCGCAA GTGGGTTGGT ATGTGGGTTT TGTTGAAAAG
```

```
           721                                                                                                                  840
    OXA-58 GCAGATGGGC AAGTGGTGGC ATTTGCTTTA AATATGCAAA TGAAAGCTGG TGATGATATT GCTCTACGTA AACAATTGTC TTTAGATGTG CTAGATAAGT TGGGTGTTTT TCATTATTTA
      A266 GCAGATGGGC AAGTGGTGGC ATTTGCTTTA AATATGCAAA TGAAAGCTGG TGATGATATT GCTCTACGTA AACAATTGTC TTTAGATGTG CTAGATAAGT TGGGTGTTTT TCATTATTTA
      A446 GCAGATGGGC AAGTGGTGGC ATTTGCTTTA AATATGCAAA TGAAAGCTGG TGATGATATT GCTCTACGTA AACAATTGTC TTTAGATGTG CTAGATAAGT TGGGTGTTTT TCATTATTTA
      A242 GCAGATGGGC AAGTGGTGGC ATTTGCTTTA AATATGCAAA TGAAAGCTGG TGATGATATT GCTCTACGTA AACAATTGTC TTTAGATGTG CTAGATAAGT TGGGTGTTTT TCATTATTTA
      A472 GCAGATGGGC AAGTGGTGGC ATTTGCTTTA AATATGCAAA TGAAAGCTGG TGATGATATT GCTCTACGTA AACAATTGTC TTTAGATGTG CTAGATAAGT TGGGTGTTTT TCATTATTTA
      A471 GCAGATGGGC AAGTGGTGGC ATTTGCTTTA AATATGCAAA TGAAAGCTGG TGATGATATT GCTCTACGTA AACAATTGTC TTTAGATGTG CTAGATAAGT TGGGTGTTTT TCATTATTTA
      A150 GCAGATGGGC AAGTGGTGGC ATTTGCTTTA AATATGCAAA TGAAAGCTGG TGATGATATT GCTCTACGTA AACAATTGTC TTTAGATGTG CTAGATAAGT TGGGTGTTTT TCATTATTTA
      A180 GCAGATGGGC AAGTGGTGGC ATTTGCTTTA AATATGCAAA TGAAAGCTGG TGATGATATT GCTCTACGTA AACAATTGTC TTTAGATGTG CTAGATAAGT TGGGTGTTTT TCATTATTTA
      A292 GCAGATGGGC AAGTGGTGGC ATTTGCTTTA AATATGCAAA TGAAAGCTGG TGATGATATT GCTCTACGTA AACAATTGTC TTTAGATGTG CTAGATAAGT TGGGTGTTTT TCATTATTTA
      A262 GCAGATGGGC AAGTGGTGGC ATTTGCTTTA AATATGCAAA TGAAAGCTGG TGATGATATT GCTCTACGTA AACAATTGTC TTTAGATGTG CTAGATAAGT TGGGTGTTTT TCATTATTTA
      A188 GCAGATGGGC AAGTGGTGGC ATTTGCTTTA AATATGCAAA TGAAAGCTGG TGATGATATT GCTCTACGTA AACAATTGTC TTTAGATGTG CTAGATAAGT TGGGTGTTTT TCATTATTTA
 Consensus GCAGATGGGC AAGTGGTGGC ATTTGCTTTA AATATGCAAA TGAAAGCTGG TGATGATATT GCTCTACGTA AACAATTGTC TTTAGATGTG CTAGATAAGT TGGGTGTTTT TCATTATTTA

           841
    OXA-58 TAA
      A266 TAA
      A446 TAA
      A242 TAA
      A472 TAA
      A471 TAA
      A150 TAA
      A180 TAA
      A292 TAA
      A262 TAA
      A188 TAA
 Consensus TAA
```

APPENDIX F

AMINO ACID SEQUENCES ALIGNMENT OF OXA-TYPE CARBAPENEMASE AND METALLO β-LACTAMASES

OXA-TYPE CARBAPENEMASES

1. OXA-23 GROUP

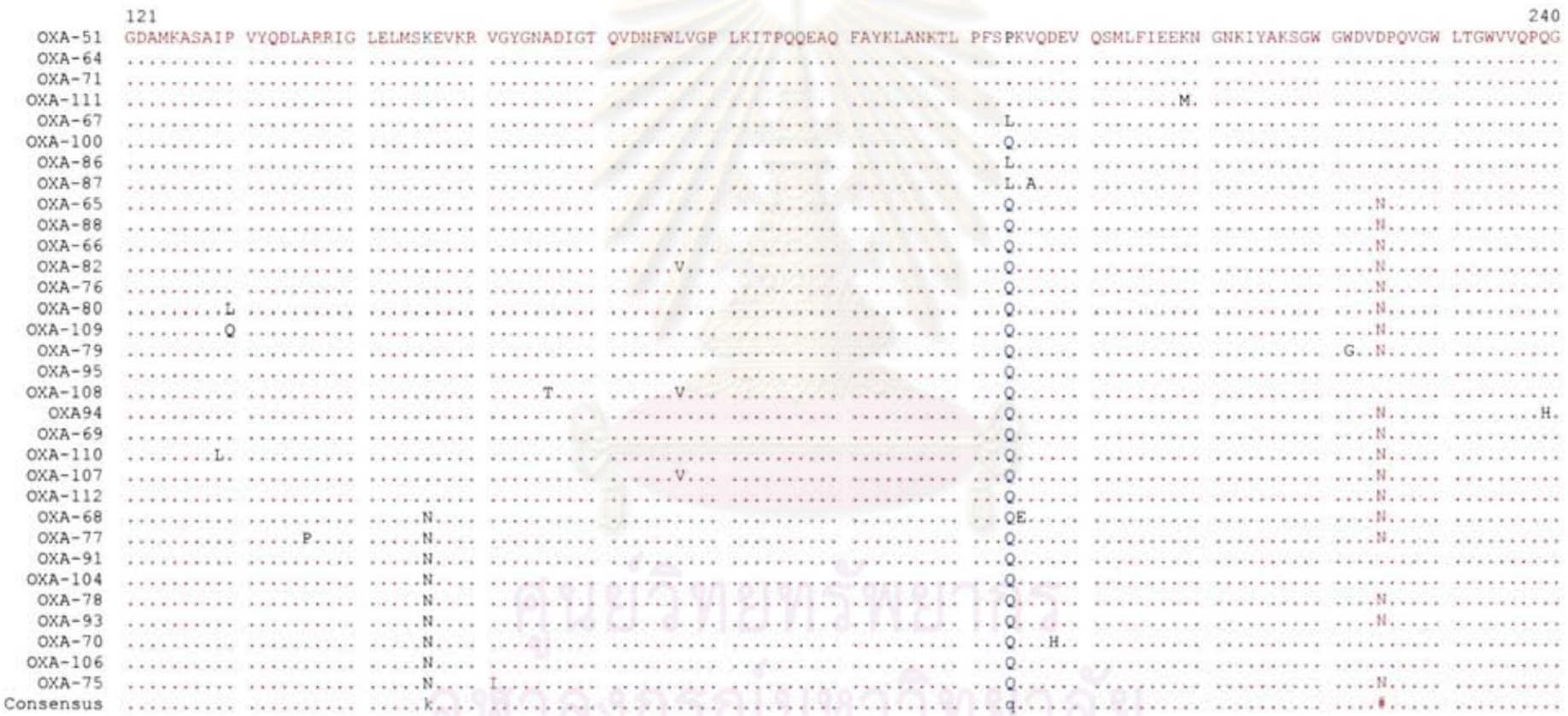
```
          1                                                                                                                   120
OXA-23    MNKYFTCYVV ASLFLSGCTV QHNLINETPS QIVQGHNQVI HQYFDEKNTS GVLVIQTDKK INLYGNALSR ANTEYVPAST FKMLNALIGL ENQKTDINEI FKWKGEKRSF TAWEKDMTLG
OXA-27    .......... .......... .......... .......... .......... .......... .......... .......... .......... ....A..... .......... ..........
OXA-49    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... ....t..... .......... ..........

          121                                                                                                                 240
OXA-23    EAMKLSAVPV YQELARRIGL DLMQKEVKRI GFGNAEIGQQ VDNFWLVGPL KVTPIQEVEF VSQLAHTQLP FSEKVQANVK NMLLLEESNG YKIFGKTGWA -MDIKPQVGW LTGWVEQPDG
OXA-27    .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... -......... ..........
OXA-49    .......... .......... .......E.. .......... .......... .......... .......... .......... .......... .......... A......... ..........
Consensus .......... .......... .......k.. .......... .......... .......... .......... .......... .......... .......... .......... ..........

          241                                 274
OXA-23    KIVAFALNME MRSEMPASIR NELLMKSLKQ LNII
OXA-27    .......K.. .......... .......... ....
OXA-49    .......... .......... .......... ....
Consensus .......n.. .......... .......... ....
```

2. OXA-24 GROUP

```
          1                                                                                                                    120
    OXA24 MKKFILPIFS ISILVSLSAC SSIKTKSEDN FHISSQQHEK AIKSYFDEAQ TQGVIIIKEG KNLSTYGNAL ARANKEYVPA STFKMLNALI GLENHKATTN EIFKWDGKKR TYPMWEKDMT
   OXA-33 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
   OXA-40 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
   OXA-26 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
   OXA-72 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
   OXA-25 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          121                                                                                                                  240
    OXA24 LGEAMALSAV PVYQELARRT GLELMQKEVK RVNFGNTNIG TQVDNFWLVG PLKITPVQEV NFADDLAHNR LPFKLETQEE VKKMLLIKEV NGSKIYAKSG WGMGVTPQVG WLTGWVEQAN
   OXA-33 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
   OXA-40 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
   OXA-26 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
   OXA-72 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ...D...... ..........
   OXA-25 .......... .......... .......... .......... .......... .......... .......... .......... .E........ .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .k........ .......... ...g...... ..........

          241                                 275
    OXA24 GKKIPFSLNL EMKEGMSGSI RNEITYKSLE NLGII
   OXA-33 .......... .......... .......... .....
   OXA-40 .......... .......... .......... .....
   OXA-26 .......... ......T... .......... .....
   OXA-72 .......... .......... .......... .....
   OXA-25 .......... .......... .......L.. .....
Consensus .......... ......s... .......s.. .....
```

3. OXA-51 GROUP

```
           1                                                                                                                      120
OXA-51     MNIKTLLLIT SAIFISACSP YIVTANPNHS ASKSDEKAEK IKNLFNEVHT TGVLVIQQGQ TQQSYGNDLA RASTEYVPAS TFKMLNALIG LEHHKATTTE VFKWDGQKRL FPEWEKDMTL
OXA-64     ....A..... .......... .......... .......G.. .......A.. .......... .......... .......... .......... .......... .......... ..........
OXA-71     ....A..... .......... .......... .......... .......A.. .......... .......... .......... .......... .....T.... .......... ..........
OXA-111    .......... .......... ...S...... .......... .......A.. .......... .......... .......... .......... .......... .......... ..........
OXA-67     ....A..... .T........ .......... T......... .......A.. .......... .......... .......... .......... .......... I......... ..........
OXA-100    ....A..... .T........ .......... T......... .......A.. .......... .......... .......... .......... .......... I......... ..........
OXA-86     .......... .T........ .......... T......... .......A.. .......... .......... ...I...... .......... .......... I......... ..........
OXA-87     .......... .T........ .......... T......... .......A.. .......... .......... ...I...... .......... .......... I......... ..........
OXA-65     ....A..... .......... .......... .......... .......A.. .......... .......... .......... .......... .......... ......K... ..........
OXA-88     ....A..... .......... .......... .....K.... .......A.. .......... .......... .......... .......... .......... ......K... ..........
OXA-66     ....A..... .......... .......... .....V.... .......A.. .......... .......... .......... .......... .......... ......K... ..........
OXA-82     ....A..... .......... .......... .....V.... .......A.. .......... .......... .......... .......... .......... ......K... ..........
OXA-76     ....A..... .......... .......... .....V.... .......A.. .......... .......... .......... .......... .......... ......K... ..........
OXA-80     ....A..... .......... .......... .....V.... .......A.. .......... .......... .......... .......... .......... ......K... ..........
OXA-109    ....A..... .......... .......... .....V.... .......A.. .......... .......... .......... .......... .......... ......K... ..........
OXA-79     ....A..... .......... .......... .....V.... .......A.. .......... .......... .......... .......... .......... ......K... ..........
OXA-95     ....A.F... .......... .......... .......... .......A.. .......... .......... .......... .......... .......... ......K... ..........
OXA-108    ....A..... .......... ....T..... .......... .......A.. .......... .......... .......... .......... .......... ......K... ..........
OXA94      ...QA..... .......... ...S...... .......... .......A.. .......... .......... .......... .......... .......... ......K... ..........
OXA-69     ....A..... .......... .......... .....D.... .......A.. ......H... .......... .......... .......... .......... ......E... ......N...
OXA-110    ....A..... .......... .......... .....D.... .......A.. ......H... .......... .......... .......... .......... ......E... ......N...
OXA-107    ....A..... .......... .......... .....D.... .......A.. ......H... .......... .......... .......... .......... ......E... ......N...
OXA-112    ....A..... .......... .......... .....D.... .......A.. ......H... .......... .......... .......... .......... ......E... ......N...
OXA-68     ....A..... .......... ...S...... .......... .......A.. .......... .......... .......... .......... .......... .......... ......N...
OXA-77     ....A..... .......... ...S...... .......... .......A.. .......... .......... .......... .......... .......... .......... ......N...
OXA-91     ....A..... .......... ...S...... .......... .......A.. .......... .......... .......... .......... .......... .......... ......N...
OXA-104    ....A..... .......... .......... .......... .......A.. .......... .......... .......... .......... .......... ......K... ......N...
OXA-78     ....A..... .......... ...S...... .......... .......A.. .......... .......... .......... .......... .........A .......... ......N...
OXA-93     ....A..... .T........ .......... .......... .......A.. .......... .......... .......... .........S .......... .......... ..........
OXA-70     ....A..... .......... .......... .....K.... .......A.. .......... .......... .......... .......... .......... ....N..... ..........
OXA-106    ....A..... .......... .......... .....K.... .......A.. .......... .......... .......... .......... .......... ....N..... ..........
OXA-75     ...QA..... .......... ........Y. .......... .......A.. .......... .......... .......... .......... .......... .......... ......N...
Consensus  ...ka..... .a........ ...t...... a....e.... .......A.. .......... .......... .......... .......... .......... !.....q... ......#...
```



```
          241                                 274
  OXA-51  NIVAFSLNLE MKKGIPSSVR KEITYKSLEQ LGIL
  OXA-64  .......... .......... .......... ....
  OXA-71  .......... .......... .......... ....
 OXA-111  .......... .......... .......... ....
  OXA-67  .......... .......... .......... ....
 OXA-100  .......... .......... .......... ....
  OXA-86  .......... .......... .......... ....
  OXA-87  .......... .......... .......... ....
  OXA-65  .......... .......... .......... ....
  OXA-88  .......... .......... .......... ....
  OXA-66  .......... .......... .......... ....
  OXA-82  .......... .......... .......... ....
  OXA-76  .......... .......... .........K ....
  OXA-80  .......... .......... .......... ....
 OXA-109  .......... .......... .......... ....
  OXA-79  .......... .......... .......... ....
  OXA-95  .......... .......... .......... ....
 OXA-108  .......... .......... .......... ....
   OXA94  .......... .......... .......... ....
  OXA-69  .......... .......... .......... ....
 OXA-110  .......... .......... .......... ....
 OXA-107  .......... .......... .......... ....
 OXA-112  .....P.... .......... .......... ....
  OXA-68  .......... .......... .......... ....
  OXA-77  .......... .......... .......... ....
  OXA-91  .......... .......... .......... ....
 OXA-104  .......... .......... .......... ....
  OXA-78  .......... ....T..... .......... ....
  OXA-93  .......... .......... .......... ....
  OXA-70  .......... .......... .......... ....
 OXA-106  .......... .......... .......... ....
  OXA-75  .......... .....S.... .....RG... ....
Consensus .......... .......... .......... ....
```

4. OXA-58 GROUP

```
          1                                                                                                                   120
   OXA-58 MKLLKILSLV CLSISIGACA EHSMSRAKTS TIPQVNNSII DQNVQALFNE ISADAVFVTY DGQNIKKYGT HLDRAKTAYI PASTFKIANA LIGLENHKAT STEIFKWDGK PRFFKAWDKD
   OXA-96 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
   OXA-97 .......... .......... .......... .......... .......... ..G....... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... ..a....... .......... .......... .......... .......... .......... ..........

          121                                                                                                                 240
   OXA-58 FTLGEAMQAS TVPVYQELAR RIGPSLMQSE LQRIGYGNMQ IGTEVDQFWL KGPLTITPIQ EVKFVYDLAQ GQLPFKPEVQ QQVKEMLYVE RRGENRLYAK SGWGMAVDPQ VGWYVGFVEK
   OXA-96 .......... .......... .......... .......... M......... .......... .......... .......... .......... .......... .......... ..........
   OXA-97 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... i......... .......... .......... .......... .......... .......... .......... ..........

          241                                 280
   OXA-58 ADGQVVAFAL NMQMKAGDDI ALRKQLSLDV LDKLGVFHYL
   OXA-96 .......... .......... .......... ..........
   OXA-97 .......... .......... .......... ..........
Consensus .......... .......... .......... ..........
```

AMINO ACID SEQUENCES ALIGNMENT OF 4 SUBGROUPS IN OXA-TYPE CARBAPENEMASE

```
           1                                                                                                   120
   OXA-23    MNKYFTCY VVASLFLSGC TVQHNLINET PSQI-VQGH- -NQVIHQYFD EKNTSGVLVI QTDKKINLYG NALSRANTEY VPASTFKMLN ALIGLENQKT DINEIFKWKG EKRSFTAWEK
    OXA24  MKKFILPIFS ISILVSLSAC SSIKTKSEDN FHIS-SQQH- -EKAIKSYFD EAQTQGVIII KEGKNLSTYG NALARANKEY VPASTFKMLN ALIGLENHKA TTNEIFKWDG KKRTYPMWEK
   OXA-51    MNIKTLLL ITSAIFISAC SPYIVTANPN HSASKSDEK- -AEKIKNLFN EVHTTGVLVI QQGQTQQSYG NDLARASTEY VPASTFKMLN ALIGLEHHKA TTTEVFKWDG QKRLFPEWEK
   OXA-58   MKLLKILSL VCLSISIGAC AEHSMSRAKT STIPQVNNSI IDQNVQALFN EISADAVFVT YDGQNIKKYG THLDRAKTAY IPASTFKIAN ALIGLENHKA TSTEIFKWDG KPRFFKAWDK
Consensus  ..k..k.l.l !..sisisaC .........t ..i..v#... ..q.!..lF# E..t.gV.!i ..gqni..YG n.L.RA.teY !PASTFKmlN ALIGLEnhKa t.tE!FKWdG kkR.%.aW#K

           121                                                                                                 240
   OXA-23  DMTLGEAMKL SAVPVYQELA RRIGLDLMQK EVKRIGFGNA EIGQQVDNFW LVGPLKVTPI QEVEFVSQLA HTQLPFSEKV QANVKNMLLL EESNGYKIFG KTGWAMDIKP QVGWLTGWVE
    OXA24  DMTLGEAMAL SAVPVYQELA RRTGLELMQK EVKRVNFGNT NIGTQVDNFW LVGPLKITPV QEVNFADDLA HNRLPFKLET QEEVKKMLLI KEVNGSKIYA KSGWGMGVTP QVGWLTGWVE
   OXA-51  DMTLGDAMKA SAIPVYQDLA RRIGLELMSK EVKRVGYGNA DIGTQVDNFW LVGPLKITPQ QEAQFAYKLA NKTLPFSPKV QDEVQSMLFI EEKNGNKIYA KSGWGWDVDP QVGWLTGWVV
   OXA-58  DFTLGEAMQA STVPVYQELA RRIGPSLMQS ELQRIGYGNM QIGTEVDQFW LKGPLTITPI QEVKFVYDLA QGQLPFKPEV QQQVKEMLYV ERRGENRLYA KSGWGMAVDP QVGWYVGFVE
Consensus  DmTLG#AM.a Sa!PVYQ#LA RRiGl.LMqk EvkR!g%GN. #IGt#VD#FW LvGPLk!TPi QEv.FvydLA ..qLPFkpev Q.#Vk.ML.. ee.ngnki%a KsGWgm.!dP QVGWltGWVe

           241                                     281
   OXA-23  QPDGKIVAFA LNMEMRSEMP ASIRNELLMK SLKQLNII
    OXA24  QANGKKIPFS LNLEMKEGMS GSIRNEITYK SLENLGII
   OXA-51  QPQGNIVAFS LNLEMKKGIP SSVRKEITYK SLEQLGIL
   OXA-58  KADGQVVAFA LNMQMKAGDD IALRKQLSLD VLDKLGVFHY L
Consensus  qa#G..!aFa LN$#Mk.g.. .s.Rk#l..k sL..Lg!... .
```

METALLO β-LACTAMASES

IMP-TYPE CARBAPENEMASES

```
           1                                                                                                                  120
    IMP-1  MSKLSVFFIF LFCSIATAAE SLPDLKIEKL DEGVYVHTSF EEVNGWGVVP KHGLVVLVNA EAYLIDTPFT AKDTEKLVTW FVERGYKIKG SISSHFHSDS TGGIEWLNSR SIPTYASELT
    IMP-6  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
    IMP-4  .......... .......... P......... .......... .......... ........D. .......... .......... .......... .......... .........Q ..........
    IMP-5  ....F...M. .....TA... .......... .......... .......... .........T .......... .......... .......... .......... .........Q ..........
    IMP-2  .K..F.LCVC FL...TA.GA R......... E......... .........S .........T D......... .T......N. .......... T......... .........Q ..........
   IMP-11  .K..F.LC.. ..L..TASG. V......... E....L.... ...S.....T .........N D......... N.......A. ..G..FT... .V........ .........Q ..........
Consensus  .k..f.lcif lfc..taage .......... #....v.... ...n...... ........#. #......... ak........ ..e..%k... s!........ .........q ..........

           121                                                                                                                240
    IMP-1  NELLKKDGKV QATNSFSGVN YWLVKNKIEV FYPGPGHTPD NVVVWLPERK ILFGGCFIKP YGLGNLGDAN IEAWPKSAKL LKSKYGKAKL VVPSHSEVGD ASLLKLTLEQ AVKGLNESKK
    IMP-6  .......... .......... .......... .......... .......... .......... .......... .......... .......... ...G...... .......... ..........
    IMP-4  .......... ..K...G... .......... .......... .L........ .......... .......... L......... .I........ .......A.. .......... ..........
    IMP-5  .......... ..K.....AS .....K.... .......... ........NR V......V.. .......... V......... .M........ .......... .....R.... ..........
    IMP-2  .......... ..K......S .......... ........Q. ........K. .......V.. D......... L.........I .M...V.... ..S....I.. .....R.W.. ..........
   IMP-11  ......N... .........S .........I ........Q. ........N. .......V.. D.....D... LK.......I .M........ ..SG...I.N .......W.. .....K....
Consensus  ......#... ..k...s.vs .....n...! ........q. .v.......k !......!.. d.....g... le.......i .m...g.... ..ss...i.# .....l.w.. .....n....

           241
    IMP-1  PSKPSN
    IMP-6  ......
    IMP-4  ...L..
    IMP-5  ......
    IMP-2  ..Q...
   IMP-11  .LL...
Consensus  .s.p..
```

VIM-TYPE CARBAPENEMASES

```
           1                                                                                                                   120
    VIM-1  MLKVISSLLV YMTASVMAVA SPLAHSGEPS GEYPTVNEIP VGEVRLYQIA DGVWSHIATQ SFDGAVYPSN GLIVRDGDEL LLIDTAWGAK NTAALLAEIE KQIGLPVTRA VSTHFHDDRV
    VIM-2  .F.LL.K... .L...I..I. ....F.VDS. ......S... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .l.li.k... .$...!..!. ....h.g#p. ......n... .......... .......... .......... .......... .......... .......... .......... ..........

           121                                                                                                                 240
    VIM-1  GGVDVLRAAG VATYASPSTR RLAEAEGNEI PTHSLEGLSS SGDAVRFGPV ELFYPGAAHS TDNLVVYVPS ANVLYGGCAV HELSSTSAGN VADADLAEWP TSVERIQKHY PEAEVVIPGH
    VIM-2  .......... .......... ....V..... .......... .......... .......... .......... .S.......I Y...R..... .......... ..I....Q.. ...QF.....
Consensus  .......... .......... ....a..... .......... .......... .......... .......... .n.......! h...r..... .......... ..!....q.. ...#f.....

           241                       266
    VIM-1  GLPGGLDLLQ HTANVVKAHK NRSVAE
    VIM-2  .........K ..T......T ....V.
Consensus  .........q ..a......k ....a.
```

AMINO ACID SEQUENCES ALIGNMENT OF METALLO β-LACTAMASES

```
           1                                                                                                                    120
    IMP-1       MSKLSV FFIFLFCSIA --TAAESLP- ---------- --DLKIEKLD EGVYVHTSFE EVNGWGVVPK HGLVVLVNAE AYLIDTPFTA KDTEKLVTWF VER-GYKIKG SISSHFHSDS
    SIM-1       MRTLLI LCLFGTLNTA --FAEEAQP- ---------- --DLKIEKIE EGIYLHTSFQ EYKGFGIVKK QGLVVLDNHK AYLIDTPASA GDTEKLVNWL EKN-DFTVNG SISTHFHDDS
    GIM-1       MKNVLV FLILLVALPA --LAQGHKP- ---------- ---LEVIKIE DGVYLHTSFK NIEGYGLVDS NGLVVLDNNQ AYIIDTPWSE EDTKLLLSWA TDR-GYQVMA SISTHSHEDR
    VIM-1  MLKVISSLLV YMTASVMAVA SPLAHSGEPS GEYPTVNEIP VGEVRLYQIA DGVWSHIATQ SFDG-AVYPS NGLIVRDGDE LLLIDTAWGA KNTAALLAEI EKQIGLPVTR AVSTHFHDDR
    SPM-1       MNSPKS RALLGFMGAF CLLLVAGAPL SAKSSDHVDL PYNLTATKID SDVFVVTDRD FYSSNVLVAK ----MLDGT- VVIVSSPFEN LGTQTLMDWV AKTMKPKKVV AINTHFHLDG
Consensus  ....m.s.lv ...l..m..a ..la..g.P. .......... ...l...ki. .g!..ht... ...g..lv.k .gl.vldg.. ..i!dtp... ..T..L..w. .k..g..v.. a!stHfH.D.

           121                                                                                                                  240
    IMP-1  TGGIEWLNSR SIPTYASELT NELLKKDGKV Q--------- ---------- -----ATNSF SGVNYW---L VKNKIEVFYP GPGHTPDNVV VWLPERKILF GGCFIKPY-- -GLGNLGDAN
    SIM-1  TAGIEWLNTK SIPTYASKLT NELLNKNGKT Q--------- ---------- -----AKHSF DKESFW---L VKNKIEIFYP GPGHTQDNEV VWIPNKKILF GGCFIKPN-- -GLGNLSDAN
    GIM-1  TAGIKLLNSK SIPTYTSELT KKLLAREGKP V--------- ---------- -----PTHYF KDDEFT---L GNGLIELYYP GAGHTEDNIV AWLPKSKILF GGCLVRSHEW EGLGYVGDAS
    VIM-1  VGGVDVLRAA GVATYASPST RRLAEAEGNE I--------- ---------- -----PTHSL EGLSSSGDAV RFGPVELFYP GAAHSTDNLV VYVPSANVLY GGCAVHELSS TSAGNVADAD
    SPM-1  TGGNEIYKKM GAETWSSDLT KQLRLEENKK DRIKAAEFYK NEDLKRRILS SHPVPADNVF DLKQGKVFSF SNELVEVSFP GPAHSPDNVV VYFPKKKLLF GGCMIKPKE- --LGYLGDAN
Consensus  tgG.e.l... g..Ty.S.lT k.L...#gk. .......... .......... .....ath.f .......... .n.l!E..%P GpaHs.DN.V vy.Pk.k.L% GGC.!kp.e. ..lGylgDAn

           241                                                  298
    IMP-1  IEAWPKSAKL LKSKYGKAKL VVPSHSEVGD ASLLKLTLEQ AVKGLNESKK PSKPSN
    SIM-1  LEAWPGSAKK MISKYSKAKL VIPSHSEIGD ASLLKLTWEQ AIKGLNESKS KPPLIN
    GIM-1  ISSWADSIKN IVSKKYPIQM VVPGHGKVGS SDILDHTIDL AESASNKLMQ PTAEASAD
    VIM-1  LAEWPTSVER IQKHYPEAEV VIPGHGLPGG LDLLQHTANV VKAHKNRSVA E
    SPM-1  VKAWPDSARR L--KKFDAKI VIPGHGEWGG PEMVNKTIKV AEKAVGEMRL
Consensus  ..aWpdSa.r ...kk..ak. V!PgHge.Gg ...l..Ti.v aeka.ne... ......
```

1. 64 codon on DNA

First Position	Second Position				Third Position
	T	C	A	G	
T	F	S	Y	C	T
	F	S	Y	C	C
	L	S	STOP CODON	STOP CODON	A
	L	S	STOP CODON	W	G
C	L	P	H	R	T
	L	P	H	R	C
	L	P	Q	R	A
	L	P	Q	R	G
A	I	T	N	S	T
	I	T	N	S	C
	I	T	K	R	A
	M	T	K	R	G
G	V	A	D	G	T
	V	A	D	G	C
	V	A	E	G	A
	V	A	E	G	G

Abbreviation: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, trytophan; Y, tyrosine

BIOGRAPHY

Mrs. Napawan Punakabutra was born on December 31, 1974 in Bangkok, Thailand. She graduated with the Bachelor degree of Science (Microbiology) from the Faculty of Science, Kasetsart University in 1996. She is currently a member of Bacteriology Unit, Department of Microbiology, King Chulalongkorn Memorial Hospital and was given the opportunity to pursue her MS degree in the Inter-Department of Medical Microbiology, Faculty of Graduate School, Chulalongkorn University since 2005.