กลไกการดื้อยากลุ่ม carbapenems ในเชื้อ *Acinetobacter baumannii* ที่แยกได้จากผู้ป่วย

นางสาวพรรณธิพา สมานไทย

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MECHANISMS OF CARBAPENEM RESISTANCE IN *ACINETOBACTER BAUMANNII* CLINICAL ISOLATES

Miss Pantipa Samarnthai

A Thesis Submitted in Partial Fulfillment of the Requirements
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พรรณธิพา สมานไทย : กลไกการดื้อยากลุ่ม carbapenems ในเชื้อ *Acinetobacter baumannii* ที่แยกได้จากผู้ป่วย (Mechanisms of carbapenem resistance in *Acinetobacter baumannii*) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : อ.ดร. ธนิษฐา ฉัตรสุวรรณ, 192 หน้า.

Acinetobacter baumannii เป็นสาเหตุสำคัญของการติดเชื้อในโรงพยาบาล ยากลุ่ม carbapenems เป็นยาที่ใช้ในการรักษาการติดเชื้อ *A. baumannii* ที่ดื้อต่อยาหลายขนาน ปัจจุบันพบการดื้อต่อยา carbapenems ในอัตราสูงในหลายประเทศทั่วโลก การศึกษานี้มีวัตถุประสงค์เพื่อตรวจหาความชุกและกลไกการดื้อยากลุ่ม carbapenems ได้แก่ การสร้างเอนไซม์ carbapenemases การขับยาออกนอกเซลล์ และการลดลงหรือขาดหายไปของ outer membrane protein โดยทำการศึกษาในเชื้อ *A. baumannii* จำนวน 453 สายพันธุ์ ซึ่งแยกได้จากผู้ป่วยที่เข้ามารับการรักษาในโรงพยาบาลจุฬาลงกรณ์ ระหว่างปี พ.ศ. 2551-2553 พบอัตราการดื้อยา imipenem และ meropenem เป็นร้อยละ 88.7 เชื้อดื้อยาทุกสายพันธุ์ตรวจพบเอนไซม์ carbapenemases แต่ตรวจไม่พบเอนไซม์ metallo-β-lactamases การตรวจหายีนดื้อยา carbapenems ด้วยวิธี multiplex PCR พบยีน $bla_{\text{OXA-51-like}}$ ในเชื้อทุกสายพันธุ์ จากการศึกษาในเชื้อ *A. baumannii* ที่ดื้อยากลุ่ม carbapenems จำนวน 402 สายพันธุ์พบ $bla_{\text{OXA-51-like}}$ ร่วมกับ $bla_{\text{OXA-23-like}}$ ร้อยละ 99.8 $bla_{\text{OXA-51-like}}$ ร่วมกับ $bla_{\text{OXA-24-like}}$ ร้อยละ 0.2 และ $bla_{\text{OXA-51-like}}$ ร่วมกับ $bla_{\text{OXA-23-like}}$ และ $bla_{\text{OXA-58-like}}$ ร้อยละ 4 และตรวจไม่พบยีนที่สร้าง metallo-β-lactamases นอกจากนี้จากการตรวจหา IS*Aba1* บริเวณ upstream ของยีน $bla_{\text{OXA-23-like}}$ พบ IS*Aba1* ในเชื้อตัวแทนทุกสายพันธุ์ (38 สายพันธุ์) และพบ IS*Aba3* บริเวณ upstream ของยีน $bla_{\text{OXA-58-like}}$ ในเชื้อทั้งหมดที่นำมาศึกษา (4 สายพันธุ์) เมื่อทำการวิเคราะห์ลำดับนิวคลีโอไทด์ของยีน $bla_{\text{OXA-like}}$ จากตัวแทนเชื้อ *A. baumannii* พบว่ายีน $bla_{\text{OXA-23-like}}$ จากเชื้อ 15 สายพันธุ์เป็นชนิด $bla_{\text{OXA-23}}$ และยีน $bla_{\text{OXA-58-like}}$ จากเชื้อทั้งหมด 4 สายพันธุ์ เป็นชนิด $bla_{\text{OXA-58}}$ ส่วน $bla_{\text{OXA-51-like}}$ พบว่าเป็นชนิด $bla_{\text{OXA-65}}$ 4 สายพันธุ์ $bla_{\text{OXA-66}}$ 7 สายพันธุ์ และ $bla_{\text{OXA-69}}$ 1 สายพันธุ์ จากการวิเคราะห์ลำดับนิวคลีโอไทด์ของ IS*Aba1* บริเวณ upstream ของยีน $bla_{\text{OXA-23-like}}$ ในเชื้อ 5 สายพันธุ์ และ IS*Aba3* upstream $bla_{\text{OXA-58-like}}$ ในเชื้อทั้งหมด 4 สายพันธุ์ พบว่ามี -35 และ -10 sequence อยู่บน IS*Aba* การศึกษากลไกการขับยาออกนอกเซลล์โดยวิธีการใช้ carbonyl cyanide m-chlorophenylhydrazone (CCCP) ซึ่งเป็นสารยับยั้งการขับยาออกนอกเซลล์ไม่พบกลไกการขับยาออกจากเซลล์ การตรวจหา outer membrane protein ในเชื้อจำนวน 23 สายพันธุ์โดยวิธี SDS-PAGE พบการลดลงของ outer membrane protein ขนาด 43 kDa การศึกษาในครั้งนี้แสดงให้เห็นถึงอัตราการดื้อยากลุ่ม carbapenems ที่สูงในเชื้อ *A. baumannii* ที่แยกได้จากผู้ป่วยในประเทศไทยและกลไกการดื้อยา ได้แก่ การสร้างเอนไซม์ carbapenemases และการลดลงของ outer membrane proteins โดยกลไกการดื้อยาหลักคือการสร้างเอนไซม์ carbapenemase ชนิด OXA-23-like

สาขาวิชา....จุลชีววิทยาทางการแพทย์..... ลายมือชื่อนิสิต.....

ปีการศึกษา.....2553..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

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KEYWORDS: *ACINETOBACTER BAUMANNII* / CARBAPENEMASES / EFFLUX PUMP/ OUTER MEMBRANE PROTEIN

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ADVISOR :TANITTHA CHATSUWAN, Ph.D., 192 pp.

Acinetobacter baumannii is an important cause of nosocomial infection. Carbapenems are the first line drug for treatment of multidrug-resistant *A. baumannii* infections. Currently, carbapenem-resistant *A. baumannii* isolates have been increasingly reported worldwide. In this study, the prevalence and the mechanisms of carbapenem resistance in *A. baumannii* including the production of carbapenemases, the presence of efflux pump and the loss or decrease of outer membrane protein were investigated. A total of 453 *A. baumannii* isolated from patients at King Chulalongkorn Memorial Hospital between 2008-2010 were studied. The prevalence of imipenem and meropenem resistance were both 88.7%. Carbapenemase activity was found in all carbapenem-resistant *A. baumannii* isolates but metallo-β-lactamase enzymes were not detected in any isolates. Screening for the presence of carbapenemase genes by multiplex PCR revealed that $bla_{\text{OXA-51-like}}$ was detected in all isolates. Of the 402 carbapenem-resistant *A. baumannii* isolates, 99.8% carried $bla_{\text{OXA-51-like}}$ with $bla_{\text{OXA-23-like}}$, 0.2% haboured $bla_{\text{OXA-51-like}}$ with $bla_{\text{OXA-24-like}}$ and 4% had $bla_{\text{OXA-51-like}}$ with $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-58-like}}$. The metallo-β-lactamase genes were not found in any isolate. The IS*Aba1* was found in the upstream region of $bla_{\text{OXA-23}}$ in all 38 representative carbapenem-resistant *A. baumannii* isolates and IS*Aba3* was found upstream region of $bla_{\text{OXA-58}}$ in all 4 representative isolates. Sequencing analysis of entire $bla_{\text{OXA-like}}$ showed that all 15 representative $bla_{\text{OXA-23-like}}$ genes were $bla_{\text{OXA-23}}$ and all 4 $bla_{\text{OXA-58-like}}$ genes were $bla_{\text{OXA-58}}$. Twelve representative $bla_{\text{OXA-51-like}}$ genes were $bla_{\text{OXA-65}}$ (4 isolates), $bla_{\text{OXA-66}}$ (7 isolates) and $bla_{\text{OXA-69}}$ (1 isolate). Five representative IS*Aba1* upstream region $bla_{\text{OXA-23-like}}$ and all 4 IS*Aba3* upstream region $bla_{\text{OXA-58-like}}$ genes had -35 and -10 sequences. The presence of efflux pump mechanism by using carbonyl cyanide m-chlorophenylhydrazone (CCCP), the efflux pump inhibitor, was determined in all 453 isolates. No carbapenem-resistant *A. baumannii* isolates had this mechanism. Twenty-three *A. baumannii* isolates were determined for the loss or decrease of outer membrane protein. Five carbapenem-resistant isolates had decreased 43 kDa outer membrane protein. This study showed high rate of carbapenem-resistant *A. baumannii* isolates in Thailand and demonstrated that carbapenem resistance was attributed to the production of carbapenemases and the reduction of outer membrane protein. The main mechanism of resistance was the production of OXA-23-like carbapenemases.

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LIST OF ABBREVIATIONS

A	adenosine
AC	amoxicillin
Ala (A)	alanine
Asn (N)	asparagine
Asp (D)	aspartic acid
Arg (R)	arginine
bp	base pair
C	cytidine
CO2	carbon dioxide
CH	clarithromycin
CLSI	Clinical and Laboratory Standards Institute
^{o}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 emerging nosocomial pathogen that cause serious infections in various systems including pneumonia, wound infection, meningitis, bacteremia and urinary tract infection, especially for the patients in intensive care units (1). The risk factor of *A. baumannii* infection is prolong admission, prolong antibiotic use, underlying diseases and immunocompromised condition (2). Extensive use of antibiotics has contributed to the emergence of multidrug-resistant strains which resist to many classes of antibiotics including broad-spectrum β-lactams, aminoglycosides, fluoroquinolones and carbapenems (3). Carbapenems are the drug of choice for treatment of multidrug-resistant *A. baumannii* infections (4). Increasing numbers of carbapenem-resistant *A. baumannii* have been described in several regions, leading to limited therapeutic options (3).

Carbapenems are β-lactam antibiotic. They act like other members of β-lactams by inhibiting cross-linkage of cell wall synthesis. Carbapenems bind to PBPs and prevent the transpeptidation of peptidoglycan strand, leading to cell wall damage and bacterial death. Carbapenems that are usually used for treatment of *A. baumannii* include imipenem and meropenem. Increasing carbapenem resistance in *A. baumannii* has been reported worldwide. Many countries were reported carbapenem resistance in *A. baumannii*, including England (5), Greece (6), Italy (7), Taiwan (8), United Arab Emirates (9) Spain and Portugal (10), Mexico (11), Iran (12) and Thailand (13).

A. baumannii isolates resist to carbapenems through various mechanisms including production of carbapenemase enzymes to hydrolyze carbapenems, overexpression of efflux pump and decreased outer membrane permeability. The production of carbapenemase enzymes have been identified in carbapenem-resistant *A. baumannii*, including Class B β-lactamases (Metallo-β-lactamase) and Class D β-lactamases (OXA-type carbapenemase). Metallo-β-lactamase enzymes confer the high level of carbapenem resistance in *A. baumannii* isolates. Three groups of MBLs have been found in *A. baumannii* including IMP-like, VIM-like and SIM-1. IMP has been

reported in Japan (14), Italy (15), Brazil (16), Hongkong (17) and South Korea (18). VIM have been detected in Korea (18) and Greece (19). SIM-1 was identified in South Korea (20). The production of class D β-lactamases or OXA-type carbapenemases was the most prevalent mechanism of carbapenem resistance in *A. baumannii*. Although these enzymes hydrolyzed carbapenems in the lower level than metallo- β-lactamases, they spread into the wide range geographic area. There are four groups of OXA-type carbapenemases in *A. baumannii*. The first group is OXA-23. The members of this group are OXA-23, OXA-27 and OXA-49. OXA-23 was originally characterized as plasmid-encoded from Edinburgh, Scotland in 1985 (21). Currently, this enzyme was also encoded on the chromosome (22). OXA-23-producing *A. baumannii* isolates have been reported in Columbia (23), Argentina (24), Turkey (25), Bulgaria (26), China (27), Singapore, Korea, Thailand and India (28). OXA-24 is the second group of acquired CHDL genes. This group consists of OXA-24, OXA-25, OXA-26 and OXA-72. OXA-24 was frequently found in Spain and Portugal (10, 29), however, it was also detected in several countries such as Czech Republic (30), USA (31), Belgium (32), Taiwan (33), Thailand, Indonesia and Iran (12, 28). The third group is OXA-51, which is the intrinsic enzyme in *A. baumannii*. The fourth group is OXA-58, the members of this group are OXA-58, OXA-96 and OXA-97. OXA-58 was reported in Italy (34), France (35), Belgium (36), Greece (37), Tunesia (38), China and Thailand (28).

Overexpression of efflux pump is another mechanism of carbapenem resistance in *A. baumannii*. AdeABC efflux pump has been reported to confer carbapenem resistance in *A. baumannii*. Substrates of AdeABC efflux pump are tetracyclines, trimethoprim, chloramphenicol, aminoglicosides, fluoroquinolones, erythromycin, ethidium bromide, β-lactams and tigecycline. AdeABC efflux pump in *A. baumannii* was reported from Korea (39) and China (40) (41).

The loss or decrease of outer membrane protein is also the mechanism of carbapenem resistance in *A. baumannii*. Several studies demonstrated the loss or reduced outer membrane protein. There are three kinds of OMPs often reported for carbapenem resistance in *A. baumannii* including 29 kDa, 33-36 kDa and 43 kDa. The loss or reduction of 29 kDa was reported from France (42), Argentina (43). The loss

or decrease of 33-36 kDa was found in USA (44) and Brazil (45) and the loss or reduction of 43 kDa was reported from France (42).

In Thailand, there were several reports of the prevalence of carbapenem resistance in *A. baumannii*. In 2002, Siriraj Hospital reported that prevalence of carbapenem resistance in *A. baumannii* were 68% (13). At the Police General Hospital reported that resistance rates of imipenem was 61% in 2003 (46). Maharaj Nakorn Chiang Mai Hospital were 60% in 2005 (47). Songklanagarind Hospital was 34.7% in 2007 (48).

Carbapenem resistance in *A. baumannii* has become emerging issue worldwide, including Thailand. Few studies on mechanisms of carbapenem resistance *A. baumannii* in Thailand.The purpose study is to investigate the prevalence and the mechanisms of carbapenem resistance in *A. baumannii* including the production of carbapenemases, the presence of efflux pump and the loss or decrease of outer membrane proteins.

CHAPTER II

OBJECTIVES

I. To investigate the prevalence of carbapenem resistance in *A. baumannii* clinical isolates.

II. To study the mechanisms of carbapenem resistance in *A. baumannii* including production of carbapenemase enzymes, the presence of efflux pumps and the loss or decrease of outer membrane proteins.

CHAPTER III

LITERATURE REVIEW

1. BACTERIOLOGY

Acinetobacter baumannii is classified in the Phylum Proteobacteria, Class Gammaproteobacteria, Order Pseudomonadales, Family Moraxellaceae, Genus *Acinetobacter*. The genus *Acinetobacter* is strictly aerobic, Gram-negative coccobacilli that occasionally are difficult to destain. The colonies on MacConkey agar are smooth, opaque, sometimes mucoid and grayish pink colonies with diameter of 1-2 mm. For preliminary identification, they are non motile, catalase positive, oxidase negative and nitrate negative.

The current taxonomy of *Acinetobacter* spp. based on DNA-DNA hybridization studies demonstrated that 33 genomic species have been described. Twenty of 33 genomic species have been given valid species names as shown in Table 1. *A. calcoaceticus* (*Acinetobacter* genomic species 1), *A. baumannii* (*Acinetobacter* genomic species 2), *Acinetobacter* genomic species 3, and *Acinetobacter* genomic species 13TU, are closely related and difficult to differentiate from other species by phenotypic methods. These groups are given the name as *A. calcoaceticus-A. baumannii* complex (49). *A. baumannii*, *Acinetobacter* genomic species 3 and *Acinetobacter* genomic species 13TU have been reported to be associated with nosocomial infection (50-52), whereas *A. calcoaceticus*, an environment species, was hardly found in serious infection.

Table 1. The taxonomy of the genus *Acinetobacter*

Species	Source	Reference
Species that have valid names		
A. calcoaceticus (Gen.sp 1)	Soil and humans	ATCC23055
A. baumannii (Gen.sp 2)	Humans, soil, meat and vegetables	ATCC19606
A. haemolyticus (Gen.sp 4)	Humans	ATCC 17906
A. junii (Gen.sp 5)	Humans	ATCC 17908
A. johnsonii (Gen.sp 7)	Humans and animals	ATCC 17909
A. lwoffii (Gen.sp 8/9)	Humans and animals	ACTC 15309
A. radioresistens (Gen.sp 12)	Humans, soil and cotton	IAM 13186
A. ursingii	Humans	NIPH137
A. schindleri	Humans	NIPH1034
A. parvus	Humans and animals	NIPH384
A. baylyi	Activated sludge and soil	DSM 14961
A. bouvetii	Activated sludge	DSM 14964
A. towneri	Activated sludge	DSM 14962
A. tandoii	Activated sludge	DSM 14970
A. grimontii	Activated sludge	DSM 14968
A. tjernbergiae	Activated sludge	DSM 14971
A. gerneri	Activated sludge	DSM 14967
A. venetianus	Seawater	CCUG 45561
A. beijerinckii	Humans, animal, soil and water	MR 22
A. gyllenbergii	Human	RUH 422T
Species that have provisional designations		
A. venetianus	Sea water	ATCC 31012
Gen.sp 3	Humans, soil and vegetables	ATCC 19004
Gen.sp 6	Humans	ATCC 17979
Gen.sp 10	Humans, soil and vegetables	ATCC 17924
Gen.sp 11	Humans and animals	ATCC 11171
Gen.sp 13BJ or 14TU	Humans	ATCC 17905
Gen.sp 14BJ	Humans	CCUG 14816
Gen.sp 15BJ	Humans	SEIP 23.78
Gen.sp 16	Humans and vegetables	ATCC 17988
Gen.sp 17	Humans and soil	SEIP Ac87.314
Gen.sp 13TU	Humans	ATCC 17903
Gen.sp 15TU	Humans	M 151a

Species	Source	Reference
Gen.sp between 1 and 3	Humans	10095
Gen.sp close to 13TU	Humans	10090

(Adapt from L. Dijkshoorn et al., 2007 (53), Y. Peleg *et al*., 2008 (3) and A. Nemec *et al*., 2009 (54))

2. PATHOGENESIS AND VIRULENCE FACTOR

A. baumannii colonization is more common than infection. However, it can be develop to severe infection. The precise mechanism associated with an establishment and progression of *A. baumannii* infection are unclear. Biofilm formation, lipopolysaccaharides (LPS) and outer membrane protein A have been described as virulence factors. *A. baumannii* can adhere to both biological such as bronchial epithelial cell and skin or abiotic surface such as urinary catheter and intravascular tube which is able to biofilm formation, which is an initial step to colonization, drug-resistant contribution and invasion to host cell. Resistance to the condition or inhibitory agents from the surface of mucosa and skin is the reason for survival of *A. baumannii* in host cell. *A. baumannii* can also survive in environment by resistance to desiccation, disinfectants and antibiotics. The virulence factor of *A. baumannii* are described below.

Biofilm formation

Biofilm is a community of multiple bacterial cells on both biotic and abiotic surface. This structure renders bacteria resistant to antibiotics and allows them to invade the host response and persistence on medically surface such as urinary catheter and endotracheal tube. The study of Rodriguez-Bano *et al*. showed that biofilm forming strains of *A. baumannii* caused catheter-related urinary tract infections, bloodstream infections and shunt-related meningitis (55). Biofilm is influenced by environmental factors including

nutrient availability, bacterial surface components, macromolecular secretions and quorum sensing. It was reported that cell adhesiveness and biofilm formation on plastic in *A. baumannii* isolates habouring bla_{PER-1} is higher than isolates without bla_{PER-1} (56).

Outer membrane protein

The outer membrane protein A (AbOmpA) is a potential virulence factor of *A. baumannii*. It has been described to induce mitochondrial apoptosis. It is a 38 kDa porin. The structure is trimeric porin with a pore size of 1.3 nm and function as a general diffusion pore (57). AbOmpA localized to the mitochondria, leading to a release of cytochrome c and apoptosis-inducing factor (AIF) releasing into cytosol that mediates caspase dependent and AIF-dependent apoptosis in epithelial cells. Approximately 180 bp was degraded by activation of caspase-3 and chromosomal DNA approximately 50 kb was degraded by AIF which resulted in large scale DNA fragmentation. Apoptosis of epithelial cells by AbOmpA may disrupt the mucosal lining and allow invading of bacteria into the deep tissues (58).

Siderophores synthesis

A. baumannii can survive in the environment and the host cell by utilize nutrient resource such as iron. In human host, iron form complexed to hemoglobin, hemosiderin and transferin. Bacteria obtain iron for survival and multiplication in human hosts by secreting siderophore. Siderophores are low-molecular weight ferric ion, a specific chelating agent which was secreted by microorganism in iron-deficient conditions (59). Actis *et al*. demonstrated that all 12 *A. baumannii* isolates grew under the iron-limiting conditions, indicating that they secreted an iron-regulated siderophore into the growth medium (60). The type of siderophore was the catechol type, 2,3-dihydroxybenzoic acid (DHBA) (60). Yamamoto *et al*. reported the structure of acinetobactin, the siderophore which was isolated from

A. baumannii ATCC 19606. This siderophore had both catechol and hydroxamate functional groups (61).

3. NOSOCOMIAL INFECTION AND COMMUNITY-ACQUIRED INFECTION

Acinetobacter spp. have been identified from environment such as water, soil, sludge and human skin. Nevertheless, *A. baumannii* is frequently isolated from clinical specimens and hospital environment. It can be found rarely from other environmetntal samples. The habitat of *A. baumannii* and other species of *Acinetobacter* are shown in Table 1.

Nosocomial infection

A. baumannii can spread from colonized patients to hospital environment and susceptible patients by air droplet and scales of skin. This organism resists to disinfectants and dry environment which allow them to long-term persistence in the hospital and occurrence of outbreak of infection. The susceptible patients received organism through various route such as equipments, hands of staff and ventilation machine.

A. baumannii is opportunistic pathogen that caused various type of infections including ventilator-associated pneumonia, wound infection, urinary tract infection, meningitis and bacteremia. The risk factors of *A. baumannii* infections occur in patients with serious underlying diseases, major surgery, prolonged-hospitalization and treatment with broad-spectrum antibiotics. The infections are associated with exposure to contaminated medical equipment such as intravascular catheter, urinary catheter and endotracheal tube. Ventilator-associated pneumonia and bloodstream infection are the most frequent clinical manifestations of nosocomial *A. baumannii* infection.

Community-acquired infections

Community-acquired infections of *A. baumannii* is less common than hospital-acquired infections. Acute pneumonia is the most frequent community-acquired infection caused by *A. baumannii*, followed by bacteraemia. Community-acquired *A. baumannii* pneumonia typically occurs in patients with underlying condition such as alcoholism, diabetes, cancer and chronic obstructive pulmonary disease (62, 63). Community-acquired pneumonia have a high incident of bacteremia and high mortality rate of 40-60% (62, 64, 65). Many reports of community-acquired pneumonia has been described from tropical or subtropical areas including Australia (62), Turkey (66), Kuwait (67), Papua New Guinea (68), Taiwan (69) and Thailand (70).

4. *A. BAUMANNII* IDENTIFICATION

2.1 Phenotypic identification

In routine laboratories, the conventional methods such as Gram stain, colony morphology and biochemical tests are used for identification of *A. baumannii* isolates. They are Gram-negative coccobacilli, non-motile, non-fermenting, oxidase-negative and catalase-negative. The colonies on MacConkey are opaque, smooth, sometimes mucoid. The colour of colonies is pale yellow to grayish pink. *A. baumannii* cannot produce urease and cannot reduce nitrate to nitrite and nitrogen gas. They can grow at 37^{o}, 42^{o} and 44^{o}C (71) and use malonate and decarboxylate arginine as a carbon source. *A. calcoaceticus-A. baumannii* complex cannot be separated by phenotypic methods (49). The genotypic methods have been brought to discriminate for this species.

2.2 Genotypic identification

DNA-DNA hybridization is the gold standard for identification of *Acinetobacter* spp. (72) but this method is laborious and only avaliable in reference laboratories. The alternative genotypic methods have been developed including ribotyping (73), amplified fragment length polymorphism (AFLP) analysis (74),

amplified 16S ribosomal DNA restriction analysis (ARDRA) (75) and restriction analysis of the 16S-23S rRNA intergenic spacer sequences (76).

5. TREATMENT OF *A. BAUMANNII* INFECTION

Multidrug resistance *A. baumannii* have been reported increasingly during the last two decades. The very broad-spectrum β-lactam antibiotics such as carbapenems were presented in 1985. They have been the most important antibiotics for the treatment of multidrug-resistant *A. baumannii* for many years. Unfortunately, carbapenem-resistant *A. baumannii* is now observed increasingly worldwide. Many therapeutic options were brought for treatment of infectious by carbapenem-resistant *A. baumannii*.

3.1 Monotherapy

Sulbactam, a β-lactamase inhibitors, has intrinsic antibiotic activity against *Acinetobacter* (77-79) by binding to PBP2 (80). Sulbactam was used alone or combined with ampicillin and cefoperazone. Urban *et al*. studied a small group of treatment using ampicillin-sulbactam in outbreak multidrug-resistant *A. baumannii*. Nine of 10 patients had shown clinical response (81). This result is similar to Corbella *et al.* that demonstrated the treatment of 41 patients with ampicillin-sulbactam or sulbactam alone to *A. baumannii* infections. Twenty-nine in 41 patients were cured or clinically improved (82). Nevertheless, failure treatment studies with ampicillin-sulbactam also have been reported (83, 84).

Colistin (polymyxin E) is an old antibiotic of the polymyxin family, which is quickly bactericidal to Gram-negative bacteria. The action of colistin is interfering with outer cytoplasmic membrane, leading to increasing permeability and cell death. It was first presented in 1952. This antibiotic was used for the treatment of infections caused by Gram-negative bacilli until 1980s because of nephrotoxicity and neurotoxicity effects. Because of limited therapeutic options, colistin has returned to be use for treatment of *A. baumannii* infections, especially in ICU. Falagas *et al*.

showed clinical efficacy ranging from 55% to >80% (85). Kim *et al*. demonstrated the successful of treatment in carbapenem-resistant *A. baumannii* isolates by combination of colistin plus aminoglycoside with or without rifampin (86). Colistin cured rate or clinical improvement in severe patients of multidrug-resistant *Acinetobacter* infections in many sites of infection such as intra-abdominal, central nervous system, sepsis, bacteremia and pneumonia were 57-77%. However, Levin *et al.* showed low level of improvement rate of 25% in multidrug-resistant patients with pneumonia (87). The alteration of OMPs was the mechanism of colistin resistance (88).

Tigecycline is a new glycylcycline agent approved by FDA in 2005. It is a bacteriostatic agent, which disturbs bacterial protein synthesis. Tigecycline has a broader spectrum than classical tetracycline because it was not effect by tetracycline specific resistant-mechanisms, *tet*(A-E), *tet*(K), *tet*(O) and *tet*(M). However, tigecycline-resistant *A. baumannii* isolates have increasingly been reported (89-91). Upregulation of chromosomally mediated efflux pumps involved in tigecycline resistance in *A. baumannii* (92, 93).

3.2 Combination therapy

The combination therapy is used to improve efficacy and prevent the emergence of resistance. Two or three antimicrobial agents used to combine and study in vitro, animal model and clinical trials. Tigecycline enhanced activity when combined with colistin, amikin, imipenem and levofloxacin but reduced efficacy when combined with piperacillin/tazobactam (94). Colistin, when combined with ceftazidime, minocycline, rifampicin and imipenem, have been shown synergistic result. However, Montero *et al*. reported conflict result between mouse model and clinical study in rifampin plus imipenem (95). Joly-Guillou *et al.* demonstrated a lack of enhanced the efficacy of levofloxacin with imipenem or amikacin in a mouse pneumonia model (96). Although, many information of combination therapies have been reported but a lack of controlled clinical studies make it difficult to evaluate the synergistic results.

6. CARBAPENEMS

Carbapenems are a class of β-lactam antibiotics. The structure of carbapenems contains the nitrogen and one carbon on the β-lactam ring fused to a five-member thiazolidinic secondary ring (Figure 1). They differ from penicillins, the classical β-lactam by the replacement of a carbon atom for sulfur at position 1, and hence the name of the group, the carbapenems. The second different structure is the addition of double bond between carbon atoms 2 and 3 in the secondary ring. The hydroxyethyl side chain in trans configuration at position 6 is unique for carbapenems and confers stability against β-lactamases (97, 98)

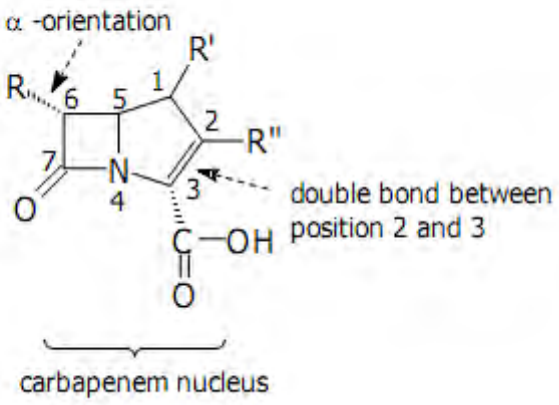
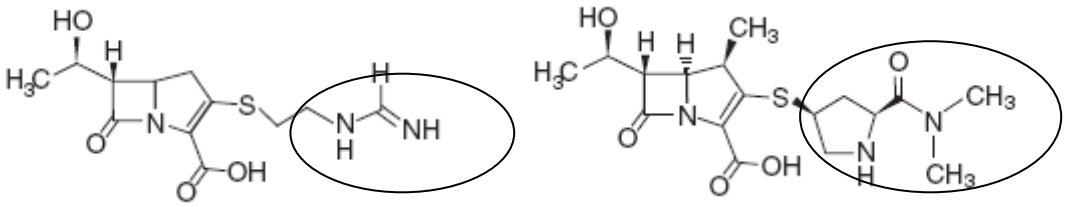


Figure 1. Core structure of carbapenems

Carbapenems act like all β-lactam antibiotics. They are bactericidal that function by inhibiting cross-linkage of cell wall synthesis. Penicillin binding proteins (PBPs) such as transpeptidases are the enzymes that catalyze transpeptidation of peptidoglycan in bacterial cell wall. The structure of β-lactam antibiotics analogues to the D-alanyl-D-alanine of the pentapeptide which attached to N-acetylmuramic acid (NAM). PBPs mistakenly use β-lactam antibiotics as a substrate for cell wall synthesis. The transpeptidase is acylated that prevent complete cell wall synthesis. The loss in cell wall integrity leads to lysis of bacterial cell. Thienamycin was the first carbapenem discovered in 1976 by the soil organism *Streptomyces cattleya*. This antibiotic had broad spectrum of antibiotic activity against Gram-positive and Gram-negative organisms but unstable in clinical use (99).



Imipenem **Meropenem**

Figure 2. Structure of carbapenem antibiotics

Imipenem, *N*-formimidoyl thienamycin is a derivative of thienamycin developed in 1985 (Figure 2). Systematic (IUPAC) name is (5R,6S)-3-[2-(aminomethylideneamino)ethylsulfanyl]-6-(1-hydroxyethyl)-7-oxo-1azabicyclo[3.2.0] hept-2-ene-2-carboxylic acid. Imipenem has a broad spectrum of activity against Gram positive, Gram negative aerobic and anaerobic bacteria. Imipenem is hydrolyzed by dehydropeptidase I (DHP-I) at renal brush border of mammalian kidney. For use in clinically, imipenem is coadministered with cilastatin, the DHP-I inhibitor in equal amount for preventing the reducing level of imipenem and the production of nephrotoxic compound (98).

Meropenem is another derivative of thienamycin. This antibiotic was approved by FDA in 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. The structure of meropenem differs from imipenem by addition of a 1-β methyl at carbon atoms 1 and having a pyrrolidinyl substitution at the 2 position (Figure 2). The presence of 1-β methyl at C1 made meropenem stable to dehydropeptidase I (DHP-I) degradation and the substitution of C2 side chains probably accounts for the higher activity against Gram-negative bacteria in comparison with imipenem. In general, meropenem is more active against Gram-negative and less active against Gram-positive bacteria than imipenem (98).

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

Multidrug resistance in *A. baumannii* such as aminoglycosides, quinolones and β-lactam antibiotics have been increasingly reported. Carbapenems, a broad-spectrum antibiotics have been the most important agents for treatment of multidrug-resistant *A. baumannii* infection. Carbapenem resistance in *A. baumannii* has been reported increasingly worldwide. *A. baumannii* can develope carbapenem resistance through various mechanisms.

3.1 Carbapenemase production

The production of carbapenemases has been found in carbapenem-resistant *A. baumannii* including Class B carbapenemases (Metallo-β-lactamase) enzymes and Class D β-lactamases (OXA-type carbapenemase enzymes). Class D β-lactamases are mostly found in carbapenem-resistant *A. baumannii* isolates as a main mechanism (100).

3.1.1 Class B carbapenemases (Metallo-β-lactamases)

Metallo-β-lactamase enzymes such as IMP (Imipenem-hydrolyzing β-lactamase), VIM (Verona integron-encoded metallo-β-lactamase), SIM-1 enzymes (*S*eoul imipenemase), SPM-1 (Sao Paulo Metallo-β-lactamase) and GIM-1 (German IMipenemase) have been identified to various Gram-negative bacteria. IMP-1 enzyme was first isolated from *P. aeruginosa* from Japan in 1988 (101). VIM was first identified in carbapenem-resistant *P. aeruginosa* clinical isolate from Verona, Italy in 1997 (102). These enzymes have less than 40% amino acid identity with IMP enzymes. SPM-1 was first isolated in *P. aeruginosa* from Sao Paulo, Brazil. These enzyme shares 35.5% amino acid homology to IMP-1 (103). GIM-1 was isolated in 2002 from Germany (104). GIM had approximately 30% identity to VIM, 43% to IMPs and 29% to SPM. SIM-1 was first discovered in Seoul. It was more closely related to IMP-type enzymes than other MBLs by presenting 64 to 69% amino acid identities to IMP-type. Most acquired MBL genes was found in class-1 integron often

form part of the gene cassettes together with other antibiotic resistance genes especially aminoglycoside-modifying enzymes. Only three groups of enzymes have been recognized in *A. baumannii* including IMP, VIM and SIM-1. The enzymes hydrolyzed various β-lactam antibiotics such as penicillins, narrow- to expanded-spectrum cephalosporins and carbapenems but not for aztreonam. Although these enzymes are less commonly identified in *A. baumannii* than OXA-type carbapenemases but their hydrolytic efficiency are significant much more higher than OXA-type 100-to 1000-fold (100). The MBL enzymes are resistant to inhibit by β-lactam inhibitors such as clavulanate and tazobactam. The enzymes are susceptible to EDTA inhibition so it can use this property to detect MBL production by using E-test strips containing imipenem with or without EDTA (105).

Table 2. Metallo-β-lactamase enzymes identified in *A. baumannii*

Enzyme	Discovery in	Accession no.	Geographical origin
IMP-1	1999	HM036079.1	Italy, Japan, South Korea
IMP-2	2000	AJ243491	Italy, Japan
IMP-4	2000	AF244145	Hongkong
IMP-5	2002	AF290912	Portugal
IMP-6	2003	AB040994	Brazil
IMP-11	2001	AB074436	Japan
VIM-1	2007	EF690696	Greece
VIM-2	2002	AF324464	South Korea
SIM-1	2005	AY887066	South Korea

Adapt from Poirel and Nordmann., 2006 (100)

3.1.2 Class D-β-lactamases (OXA-type carbapenemase enzymes)

Class D β-lactamases or OXA-type carbapenemases are active-serine-site enzymes. These enzymes have three highly conserved active-site elements. The first element is tetrad, Ser^{70}-X-X-Lys, X represents a variable residue, containing the active site serine. The second element is Ser^{118} -X-Val/Ile and the third are the triad Tyr/Phe^{144}-Gly-Asn and the tetrad Trp^{232}-X-X-Gly. OXA-type carbapenemases contain between 243 and 260 amino acid residues with molecular mass ranging from 23 to 35.5 kDa. The isoelectric points (pIs) of the enzymes vary from 5.1 to 9.0. Phenotypically, most of the class D enzymes belong to group 2d of the Bush functional classification scheme for β-lactamase. These enzymes were first defined as oxacillinases because they commonly hydrolyze cloxacillin and oxacillin faster than benzylpenicillin but not all class D β-lactamase have this characteristic. OXA-type carbapenemases hydrolysze penicillin (benzylpenicillin, ampicillin, ticarcillin and piperacillin) and narrow-spectrum cephalosporins but weakly or not hydrolyze extended-spectrum cephalosporins and aztreonam. Imipenem and meropenem were hydrolyzed weakly. These enzymes are usually not inhibited by clavulanic acid, sulbactam and tazobactam whereas they may be inhibited in vitro by NaCl. This property is not shared by other classes of β-lactamase. The activities of most class D β-lactamases were totally inhibited by NaCl at a concentration of 100 mM. It has been attributed to the presence of a Tyr residue at position 144. The replacement of a Tyr residue by a Phe at position 144 makes a resistance to NaCl inhibition. The carbapenem-hydrolyzing class D β-lactamases (CHDLs) can be subclassified into nine main subgroups based on amino acid homologies. Four of nine subgroups have been identified in *A. baumannii*.

The first subgroup of CHDLs is OXA-23, originally named ARI-1 (*Acinetobacter* resistant to imipenem) identified as plasmid-encoded from Edinburg Scotland in 1985 (21). The other members are OXA-27 and OXA-49. OXA-23 shares 56% amino-acid identity with OXA-51. The progenitor of the $bla_{\text{OXA-23}}$ was *A. radioresistens* (106). The $bla_{\text{OXA-23}}$ has also been detected in chromosomally located *Proteus mirabilis* isolated from France (107).

The second subgroup encompasses the OXA-24, OXA-25, OXA-26 and OXA-72. OXA-24 originally identified as chromosomally encoded in carbapenem-resistant *A. baumannii* isolated from Spain (108). This group shares 63% and 60% amino-acid homology with OXA-51 and OXA-23, respectively.

The third subgroup of CHDLs is represented by OXA-51, the intrinsic gene in *A. baumannii*. The other members of this group are OXA-64 to OXA-71, OXA-75 to OXA-80, OXA-82 to OXA-84, OXA-86 to OXA-95, OXA-98 to OXA-99, OXA-100, OXA-104, OXA-106 to OXA-113, OXA-115 to OXA-117, OXA-128 and OXA-130 to OXA-132. OXA-51 shares 56%, 63% and 59% with OXA-23, OXA-24 and OXA-58, respectively (109).

The forth subgroup consists of the OXA-58, which was first identified in a multidrug-resistant *A. baumannii* from France (35). The cluster includes OXA-96 and OXA-97. This enzyme group was weakly related to other oxacillinases, OXA-58 shares 48%, 47% and 59% amino acid identity with OXA-23, OXA-24 and OXA-51, respectively. The $bla_{\text{OXA-58}}$ has been identified in different species such as *Acinetobacter junii* from Australia and Romania.

3.1.3 Insertion sequences

Insertion sequences (IS) are the smallest mobile genetic elements which a size generally between 0.8-2.5 kb. They had a simple genetic organization containing the direct repeats (DRs) which are the target site duplication length between 2 and 14 bp., inverted-repeat left (IRL) and inverted-repeat right (IRR) length between 10 and 40 bp. and one or two open reading frames encoding transposases (Figure 3). Insertion sequences had no other genes exceptional transposases which function only for transposition (110, 111). Insertion sequences associated with bla_{OXA} that enhance downstream of bla_{OXA}.

IS*Aba1* is a member of the IS*4* family of insertion sequences and possesses two open reading frames, encoding 189 and 178 amino-acid transposases. It had two 16-bp imperfect inverted repeat and 9-bp duplicated repeat (112). The length of

IS*Aba1* is 1180 bp. IS*Aba1* has been found upstream of *bla*$_{OXA-23}$, *bla*$_{OXA-51}$ and *bla*$_{OXA-58}$ in *A.baumannii*.

IS*Aba2* belongs to the IS*3* family, 1306 bp long. It possesses two open reading frames encoding 318 and 903 amino acid transposases, 25-bp perfect inverted repeats and generates 5-bp target site duplication upon transposition. IS*Aba2* has been found upstream of *bla*$_{OXA-58}$ (113).

IS*Aba3* belongs to the IS*1* family, is 800 bp long, possesses inverted repeats of 24 bp and generates 3-bp duplicated repeats. Its transposase is made of an open reading frame, encoding 145 amino acids. IS*Aba3* has been reported upstream of *bla*$_{OXA-58}$ (113).

IS*Aba4*, the insertion sequence that belongs to the IS*982* family is 975 bp long. It possesses 18-bp inverted repeats and an open reading frame encodes 292 amino acid transposase (112). IS*Aba4* has been found upstream of *bla*$_{OXA-23}$ in *A.baumannii*.

OXA-24 group, not consist to insertion sequences but they can mobile to other plasmid by conserved inverted repeat sequences which homologous to XerC/XerD binding sites. These sequences were presented upstream and downstream of *bla*$_{OXA-24}$.

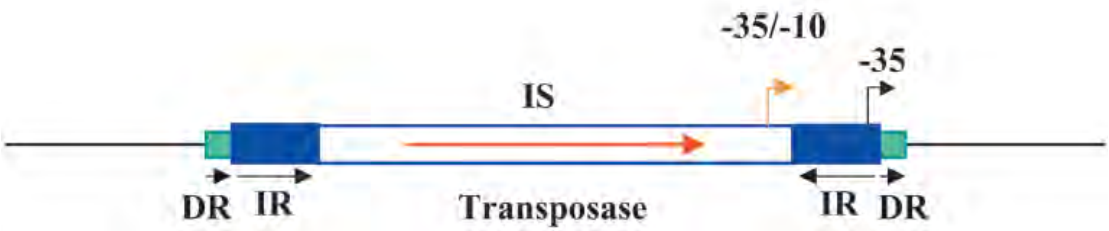


Figure 3. Structure of insertion sequence (IS) (110)

3.2 Reduced outer membrane protein permeability

In Gram-negative bacteria such as *A. baumannii*, the cell envelope is composes of outer membrane and inner membrane separated by periplasm. Outer membrane performs the important role to protect bacteria from hazardous agents such as enzymes and antibiotics but some nutrients can pass the membrane by diffusion through porins. Porins are the protein that form water-filled channels allowing the transport of small hydrophilic substrates. Many bacteria developed survival strategies by their diverse structure and regulation of porin expression in antibiotic pressure.

The reduction or decrease of outer membrane proteins is one of carbapenem resistance mechanism in *A. baumannii*. There are several OMPs have been described to loss or decrease in carbapenem-resistant *A. baumannii*. In 1996, Clark reported that decreased expression of 33-36 kDa OMP involved in imipenem-resistant *A. baumannii* isolates. Bou *et al*. demonstrated the reduction of 22 kDa and 33 kDa in imipenem and meropenem-resistant *A. baumannii* isolates in 2000. Siroy *et al*. studied the channel-forming properties of heat-modifiable 29 kDa OMP or CarO in imipenem and meropenem-resistant *A. baumannii* isolates by Mass spectrometry in 2005. CarO was found together with 25 kDa protein but only CarO form to protein chanel. Imipenem binding-site was not detected in CarO, suggesting that this chanel function as an unspecific monomeric chanel (114). Del Mar Tomas *et al* studied that cloning 33-36 kDa to carbapenem-resistant *A. baumannii* isolates was able to restore carbapenem susceptible. Dupon demonstrated 43 kDa which was homologous to OprD in *P. aeruginosa* (42).

3.3 Overexpression of efflux pumps

Efflux pump is an universal mechanism found in all cells. This mechanism plays an important role to remove metabolic compounds and toxins. In bacteria, efflux pump genes are located on the chromosome or on transmissible genetic elements, such as plasmids. Multidrug resistance in bacteria has often been associated with overexpression of this system. Bacterial multidrug efflux pumps were classified into five families, containing the ATP binding cassette (ABC) family, the major facilitator superfamily (MFS), the multidrug and toxic compound extrusion (MATE) family, the small multidrug resistance (SMR) family and the resistance-nodulation-division (RND) family (115). Efflux pumps that are described in *A. baumannii* including RND family, MFS and MATE (116). The RND family is the efflux pump that associated with clinically significant resistance to antibiotics in Gram-negative bacteria. The structure is organized as three-component systems including a transporter protein which is located in cytoplasmic membrane; a membrane fusion protein and an outer membrane porin. These pumps use proton gradient as an energy to efflux chemical compounds. The RND family pumps are susceptible to efflux pump inhibitors such as phenylalanyl-arginyl-β-naphthylamide (PAβN), 1-(1-naphthylmethyl)-piperazine (NMP), Carbonyl cyanide m-chlorophenylhydrazone (CCCP) (117).The efflux pump found in carbapenem-resistant *A. baumannii* is AdeABC efflux pump, a member of the RND family.The substrates for this pump including β-lactams, aminoglycosides, fluoroquinolones, erythromycin, tetracyclines, trimethoprim and chloramphenicol (116). The AdeABC efflux pump is a tripartite efflux machinery which is homologous to MexAB-OprM in *P. aeruginosa* (Figure 5). AdeB is a multidrug transporter comprising of 12 transmembrane segments, AdeA is a membrane fusion protein and AdeC is an outer membrane protein (118). The *adeABC* genes form an operon which are contiguous and directly oriented (Figure 4). This efflux pump is regulated by two regulatory genes such as *adeR* (regulator) and *adeS* (sensor kinase).These genes are localized upstream the *adeABC* genes and transcribed in the opposite direction (119). Distruption of AdeB by insertion sequence led to loss of efflux pump function and multidrug resistance (118). But not for the case of AdeC, the resistance level of antibiotics was not changed because AdeAB may be able to recruit other OMPs to form a functional complex (119).

Figure 4. Schematic organization of the *ade* gene cluster (120)

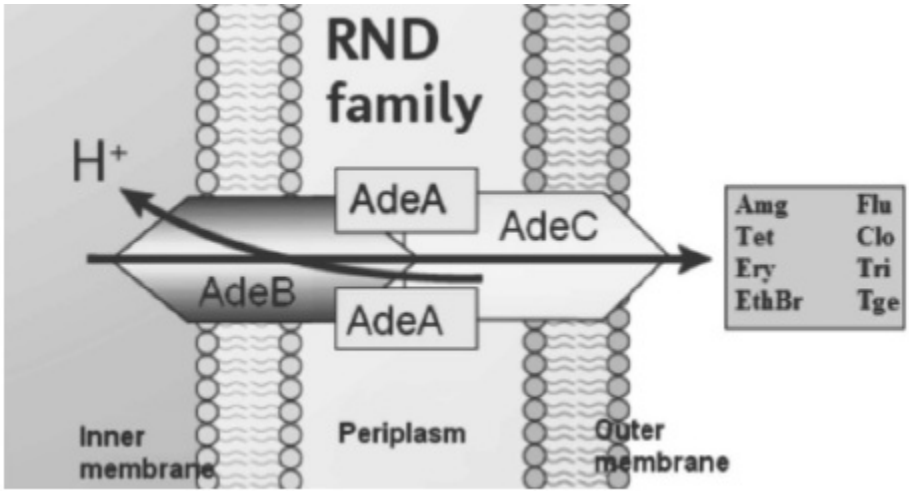


Figure 5. The structure of multidrug resistance RND family pump (120)

3.4 Alteration of penicillin-binding proteins

The alteration of penicillin-binding proteins (PBPs) is another mechanism of carbapenem resistance in *A. baumannii*. PBPs are the target sites of β-lactam antibiotics. The change of PBP structure reduce affinity for binding between antibiotics and bacteria. Gehrlein *et al* demonstrated in 1991 that the mutant carbapenem-resistant *A. baumannii* produced 24 kDa in high level, whereas produced low level of other PBPs (121). In 2003, Fernandez-Cuenca *et al*. demonstrated that reduction of PBP2, production of β-lactamase enzymes and loss of 22.5 kDa OMP were related to carbapenem resistance in *A. baumannii* (122).

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

Carbapenems are reliable drug for treatment of multidrug resistance in *A. baumannii* infections for many years. Currently, carbapenem resistance in *A. baumannii* has been reported worldwide. The MYSTIC (Meropenem Year Susceptibility Test Information Collection) program reported that rate of imipenem and meropenem resistance in *A. baumannii* in European countries increased from 16% and 18%, respectively in 1997-2000 (123) to 42.5% and 43.4%, respectively in 2006 (124). Between 2000 and 2006, prevalence of carbapenem resistance in *A. baumannii* was 48% in Turkey (125). During 2003-2008, imipenem resistance rate of *A. baumannii* increased from 1% to 58% in USA (126). In 2008, resistance rates of imipenem and meropenem in Taiwan were 42% and 38%, respectively (127). At the same period, carbapenem resistance in *A. baumannii* was 50% in Iran (12), 21.3% in Czech Republic and high rate of resistance was reported to be 70.1% in Greece (128). Between 2006 and 2009, meropenem resistance rate of *A. baumannii* in Mexico was 59% (11). In Brazil, imipenem and meropenem resistance rates increased from 5.13% and 0%, respectively in 2004 to 77.27% and 80%, respectively in 2008 (129). In 2011, imipenem and meropenem resistance rates in *A. baumannii* were 54% and 56%, respectively in Iran. In Thailand, the study from Police General Hospital showed that imipenem resistance rate of *A. baumannii* was 61% in 2003 (46). Carbapenems

resistance rate of *A. baumannii* was 60% in 2005 at Maharaj Nakorn Chiang Mai Hospital (47) and 68% in 2006 at Siriraj Hospital (13). In 2007, imipenem resistantce rate of *A. baumannii* isolated from Songklanagarind Hospital was 34.7% (48).

Carbapenemase enzymes have been identified in carbapenem-resistant *A. baumannii*, including Class B β-lactamases (Metallo-β-lactamase) and Class D β-lactamases (OXA-type carbapenemase).The most prevalence of carbapenemase enzyme was OXA-type carbapenemases, whereas, metallo-β-lactamases have been sporadically reported in *A. baumannii*. OXA-type carbapenemase enzymes were classified to 4 subgroups including OXA-23, OXA-24, OXA-51 and OXA-58. OXA-23-producing *A. baumannii* isolates have been reported from Columbia (23), French Polynesia (130), Bulgaria (26), China (27), Turkey (25) and Thailand (131). OXA-24-producing *A. baumannii* isolates were identified in Spain (132), Portugal (10), Czech Republic (30), USA (31), Belgium (32), Taiwan (33), Thailand, Indonesia and Iran (12, 28). OXA-51 group was the intrinsic enzyme in *A. baumannii*. OXA-58-producing *A. baumannii* isolates were identified in France (35), Italy (34), Greece (37), Tunesia (38), Belgium (36), China and Thailand (28).

Metallo-β-lactamase enzymes found in *A. baumannii* including IMP-like, VIM-like and SIM-1. IMP has been reported in Japan (14), Italy (15), Hongkong (17) South Korea (18) and Brazil (16). VIM have been detected in Korea (18) and Greece (19). SIM-1 was identified in South Korea (20).

CHAPTER IV

MATERIALS AND METHODS

Methodology Scheme

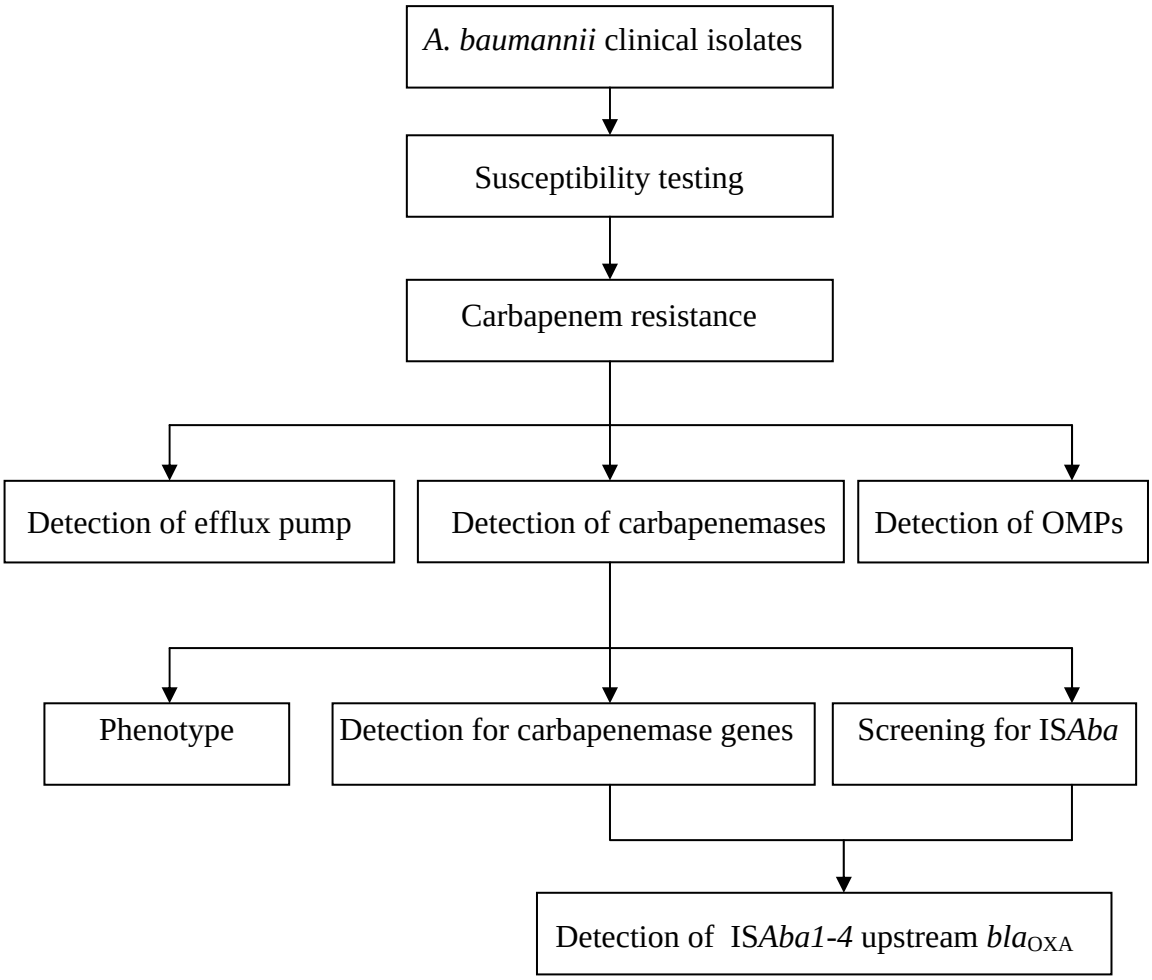


Figure 6. Methodology Scheme

PART I : BACTERIAL STRAINS

1. *Acinetobacter baumannii* isolates

A total of 453 isolates of *Acinetobacter baumannii* were collected between March 2008 and April 2010 at the Department of Microbiology, King Chulalongkorn Memorial Hospital, Bangkok, Thailand. The specimens included blood, CSF, body fluid, pus, tissue, sputum, respiratory aspirate and urine.

2. Quality control strain for bacterial identification

Acinetobacter baumannii ATCC 19606 was used as quality control for identification and susceptible strain for OMP profile.

3. Quality control strains for MIC determination

Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as antimicrobial susceptible control.

4. Quality control strains for efflux pump

Acinetobacter baumannii Aci65 obtained from Dr. Padungsri Dubbs, Mahidol University, Thailand.

5. Culture preservation

All *Acinetobacter baumannii* isolates were grown on trypticase soy agar (Oxiod, UK) at 37^{o}C for 18-24 hours. Keeping the colonies for a long time at 70^{o}C by suspended in cryogenic vials containing 20% glycerol in trypticase soy broth.

PART II : *ACINETOBACTER BAUMANNII* IDENTIFICATION

All clinical isolates of *Acinetobacter baumannii* were identified by conventional method including colony morphology, Gram stain and biochemical tests.

1. Colony morphology

The colony morphology of *Acinetobacter baumannii* is 1 to 2 mm, domed, opaque, smooth and sometimes mucoid. On MacConkey agar the colony is colorless or greyish-pink

2. Cell morphology

Gram stain for cell morphology of *Acinetobacter baumannii* is Gram negative coccobacilli, typically 1.0 to 1.5 μm by 1.5 to 2.5 μm.

3. Biochemical tests

3.1 Triple Sugar Iron medium test (TSI)

Triple Sugar Iron medium test (TSI) is used to determine fermentation of glucose, lactose, sucrose and H_2S production. TSI contains three sugars such as glucose, lactose and sucrose (1:10:10). Phenol red is the indicator for acid or alkaline. Acid production is indicated by change of phenol red from red to yellow. Ferrous ions is the indicator for H_2S production by the appearance of a black precipitate.

Test procedure : Pure colony was streaked and stabbed into slant and butt of TSI agar. Incubated TSI tube at 37^{o}C for 24 hours.

Interpretation :

- A/A (Acid slant/ acid butt) : the organisms that can ferment glucose, and either lactose or sucrose and this may be with gas production.
- K/A (alkaline slant / acid butt) : the organisms that can ferment only glucose but not ferment lactose and sucrose. This may be with gas production.
- K/N or N/N (alkaline slant / neutral butt) or (neutral slant / neutral butt) : the organisms that cannot ferment all of three sugars.

- H_2S production : a black precipitation in the butt.
- *A.baumannii* shows K/N (alkaline slant/ neutral butt) on TSI agar.

3.2 Motility

The test for motility is used to observe the ability of organism to move by flagella through a semisolid medium.

Test procedure : Pure colony is stabbed into a semisolid motility medium only half way down the center of the tube and incubate at 37^{o}C for 24 hours.

Interpretation

- Motility test positive : the organisms produce a cloudiness in medium or produce a diffuse viel of growth.

- Motility test negative : the organisms do not grow beyond the line of stab.

- *A. baumannii* cannot motile.

3.3 Urease test

The urease test is used to detect an organism that possesses the enzyme urease which hydrolyzes urea. Phenol red is an indicator, which turns pink at an alkaline pH. When urea is hydrolyzed, ammonia is split during incubation which makes an alkaline reaction, producing a pink-red colour.

Test procedure : Pure colony is streaked on slope of the urea agar slant and incubate at 37^{o}C for 24 hours.

Interpretation

- Urease test positive : The urea agar is changed to pink-red color.

- Urease test negative : The urea agar remains plae yellowish-orange.

- *A. baumannii* shows negative result for urease test.

3.4 Citrate test

The citrate test is used to detect an organism that able to utilize ammonium dihydrogen phosphate and sodium citrate as the sources of nitrogen and carbon. Bromthymol blue is an indicator, which turn from green to blue at an alkaline reaction.

Test procedure : Pure colony is streaked onto slope of the simmons citrate agar slant and incubate at 37^{o}C for 24 hours.

Interpretation

- Citrate test positive : The simmons citrate agar is changed to blue color.

- Citrate test negative : The simmons citrate agar remains green.

- *A. baumannii* shows positive result for citrate test.

3.5 Oxidase test

The oxidase test is used to detect an organism that possesses the cytochrome oxidase enzyme. This enzyme can oxidize the substrate N,N,Ń,Ń-tetramethyl-*p*-phenylenediamine dihydrochloride to indophenol and produce a purple colour.

Test procedure : Pure colony is transferred onto filter paper soaked with oxidase reagent.

Interpretation

- Oxidase test positive : A dark purple colour appears within 10 seconds on filter paper.
- Oxidase test negative : No colour development on the filter paper within 10 seconds.

- *A. baumannii* shows a negative result for oxidase test.

3.6 Hemolysis on sheep blood agar

The organism was inoculated on sheep blood agar plate and incubated at 37^{o}C for 24 hours.

Interpretation

- Positive result : Hydrolysis of red blood on sheep blood agar.

- Negative result : No hemolysis on sheep blood agar.

- *A. baumannii* shows no hemolysis on sheep blood agar.

3.7 Growth at 37^{o}C, 42^{o}C and 44^{o}C

To determine the ability to grow at 37^{o}C, 42^{o}C and 44^{o}C, the organism was inoculated on tryptic soy agar and incubated at 37^{o}C, 42^{o}C and 44^{o}C . The ability to grow was observed after 24 hours of incubation. *A. baumannii* can grow at 37^{o}C, 42^{o}C and 44^{o}C.

PART III : ANTIMICROBIAL SUSCEPTIBILITY TESTING

The minimal inhibitory concentrations (MICs) of imipenem (Merck & Co., U.S.A.) and meropenem (AstraZeneca UK Limited, United Kingdom) were determined by agar dilution technique and interpreted according to the guidelines of Clinical and Laboratory Standards Institute. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as quality controls.

MICs were determined on Mueller-Hinton agar (BBL, Becton Dickinson and Company, Coskeysville, MD). Preparing the inoculum from a pure overnight culture in tryptic soy broth (BBL, Becton Dickinson and Company, Coskeysville, MD) and the turbidity was adjusted to a 0.5 McFarland standard (approximately $1.5x10^8$ CFU/ml) in 0.85% NaCl. Then, the suspension was diluted 10-fold to yield the final inoculum suspension and the 1 ml of inoculum suspension was transferred to the multi-point inoculator wells which deliver 1-2 µl of inoculum suspension to agar dilution plates made the final inoculums was approximately 10^4 CFU/spot. Inoculate the bacterial suspension on Mueller-Hinton agar containing two-fold dilution of antimicrobial agent concentration from 0.015 to $\geq$ 256 µg/ml. The concentration of antimicrobial agents used in agar dilution are shown in Table 3. Mueller-Hinton agar without antimicrobial agent were used as growth control. Plates were incubated at 35-37°C for 18-24 hours. The MIC is the lowest concentration of antimicrobial agents that inhibit the growth of a microorganism (Figure 7).

The MICs interpretation used breakpoint criteria recommended by CLSI (Clinical and Laboratory Standards Institute, 2008) are shown in the Table 4 and Acceptable MIC limits for quality control strains are listed in Table 5.

Table 3. Scheme for preparing dilutions of antimicrobial agents to used in agar dilution susceptibility tests.

Antimicrobial solution							
Step	Conc. µg/ml	Source	Vol. (ml)	Diluent (ml)	Intermediate Concentration (mg/l)	Final conc. at 1:10 dilution in agar (µg/ml)	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
13	1.25	Step 10	2	2	0.625	0.0625	-4
14	1.25	Step 9	1	3	0.3125	0.03125	-5
15	1.25	Step 9	1	7	0.15625	0.015625	-6

Note: This table is modified from Ericsson HM. Sherris JC. Antibiotic sensitivity testing. Report of an international collaborative study. (Acta Pathol Microbiol Scand.1971; 217 (suppl B): 1-98).

A **B**

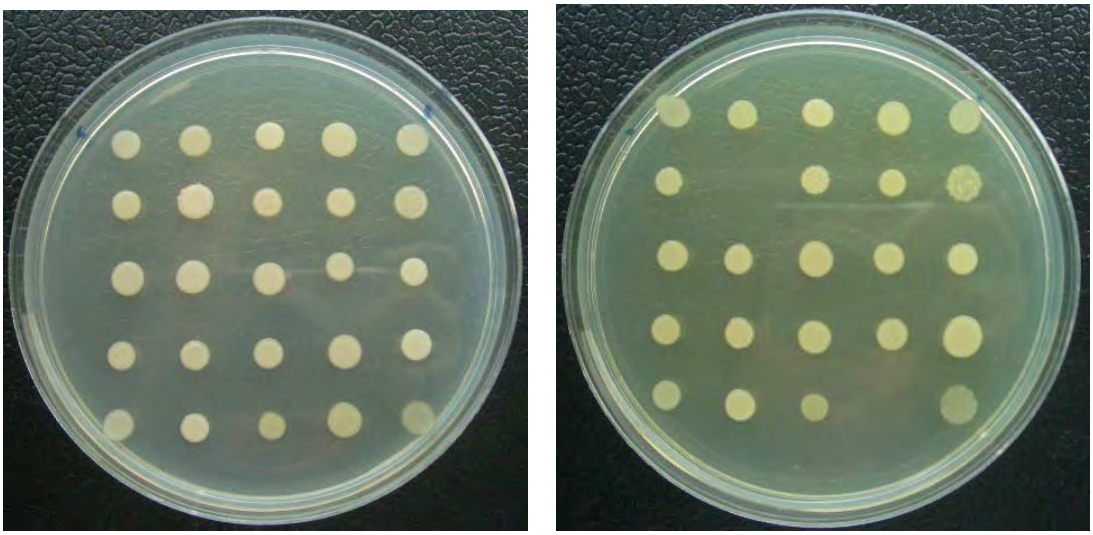


Figure 7. The inoculum plate by agar dilution method for *A. baumannii* isolates: A, control plate (no antibiotic) ; B, MIC plate with imipenem

Table 4. MIC interpretive standards for *Acinetobacter* spp.

Antimicrobial agent	MIC Interpretive Standard (µg/ml)		
	Susceptible	**Intermediate**	**Resistant**
Imipenem	≤ 4	8	≥16
Meropenem	≤ 4	8	≥16

Table 5. Acceptable limits for quality control strains used to monitor accuracy of MICs

Antimicrobial agents	MIC (µg/ml)			
	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 : PHENOTYPIC DETECTION OF CARBAPENEMASES

Carbapenemase-producing strains in 453 *A. baumannii* isolates were screening by Modified Hodge test. *E. coli* ATCC 25922 was suspended in 0.85% saline to the turbidity of 0.5 Mcfarland and inoculated onto a Mueller-Hinton agar plate. After brief drying, a 10-µg imipenem disk (BBL, Coskeysville, MD) was placed in the center of the plate and test strains were streaked from the edge of the disk to the periphery of the plate. The plate was incubated at 35-37ºC for 24 hours. Modified Hodge test positive interpreted by the presence of a distorted inhibition zone after overnight incubation (Figure 8).

Figure 8. Detection of carbapenemase phenotype by Modified Hodge test. The distorted inhibition zone of *E. coli* showed a positive of carbapenemase activity.

PART V : PHENOTYPIC DETECTION OF METALLO-β-LACTAMASES

The method that used for screening metallo-β-lactamases is EDTA-disk synergy test. All 453 *A. baumannii* isolates were screened for metallo-β-lactamases. The bacterial isolate was suspended in 0.85% saline to the turbidity of 0.5 Mcfarland and inoculated onto a Mueller-Hinton agar plate. A 10-µg imipenem disk was placed 10 mm from a blank filter paper disk soaked in 10 µl of 0.5 M EDTA solution (concentration approximately 1.5 mg/disk) in a center of each plate. The plate was incubated at 35-37°C for 24 hours. After incubation period, EDTA-synergy test positive was interpreted by the presence of an enlarged inhibition zone (Figure 9). *P.aeruginosa* carrying bla_{VIM} gene is used as control for MBL enzyme.

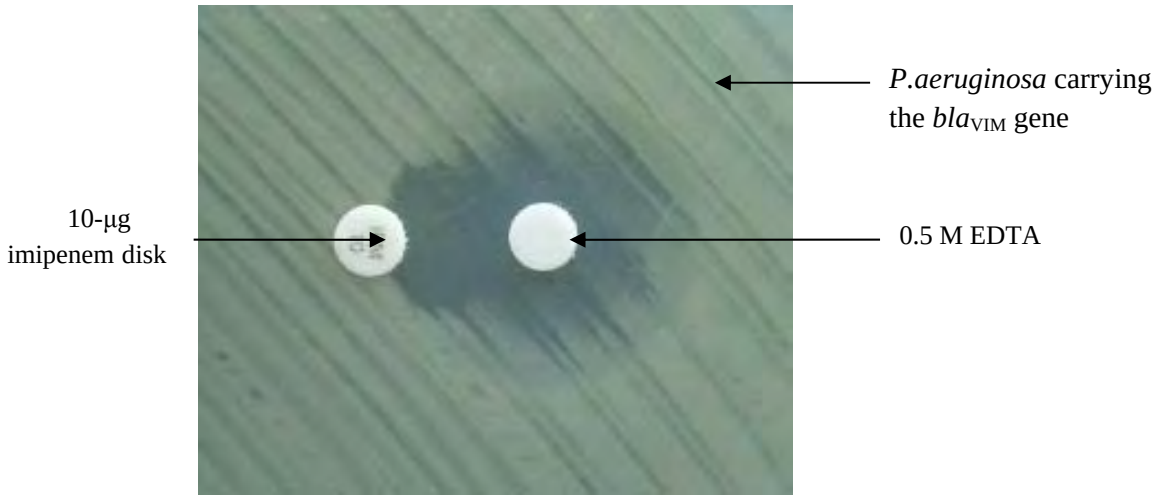


Figure 9. Detection of MBLs by EDTA-disk synergy test. The enlarged inhibition zone of tested isolate showed a positive of metallo-β-lactamase activity.

PART VI : SCREENING FOR THE PRESENCE OF METALLO-β-LACTAMASE GENES

A total of 453 *A. baumannii* isolates were detected for MBL genes by multiplex PCR.

1. DNA preparation

Genomic DNA was prepared by boiling method, three to five pured culture colonies were suspended in 100 µl of nuclease-free water and boiled at 100°C for 10 minutes. The bacterial suspensions were centrifuged at 12,000 rpm for 5 minutes at room temperature. The supernatant was used as the DNA template for PCR experiments and stored at -20°C.

2. Primers

The presence of bla_{IMP}, bla_{VIM}, $bla_{GIM,}$ bla_{SPM} and bla_{SIM} was screened by multiplex PCR using the primers as described in Table 6. All except one primer were designed by Ellington *et al*. (133). SIM-R1 was designed by this thesis.

Table 6. Primers of multiplex PCR used for amplification of *bla* genes encoded metallo-β-lactamases

Gene	Primer	Sequence	Product size (bp)	Reference
bla_{IMP}	IMP-F	5'-GGAATAGAGTGGCTTAAYTCT C-3'	188	(133)
	IMP-R	5'-CCAAACYACTASGTTATCT-3'		
bla_{VIM}	VIM-F	5'-GATGGTGTTTGGTCG CATA-3'	390	
	VIM-R	5'-CGAATGCGCAGCACCAG-3'		
bla_{GIM}	GIM-F	5'-TCGACACACCTTGGTCTGAA-3'	477	
	GIM-R	5'-AACTTCCAACTTTGCCATGC-3'		
bla_{SPM}	SPM-F	5'-AAAATCTGGGTACGCAAACG-3'	271	
	SPM-R	5'-ACATTATCCGCTGGAACAGG-3'		
bla_{SIM}	SIM-F	5'-TACAAGGGATTCGGCATCG-3'	304	
	SIM-R1	5'- CCAACCAAAAGCTCTCTTTATC -3'		This study

3. Amplification of the MBLs genes by multiplex PCR

Metallo-β-lactamases genes were amplified by multiplex PCR using IMP-F, IMP-R, VIM-F, VIM-R, GIM-F, GIM-R, SPM-F, SPM-R, SIM-F and SIM-R1 primers. The PCR was performed in a volume 25 µl containing 1X *Taq* buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTPs (Fermentas, USA), 5 pmol of IMP-F, IMP-R primers, 2 pmol of VIM-F, VIM-R primers, 0.625 pmol of GIM-F, GIM-R, SPM-F, SPM-R, SIM-F and SIM-R1 primers, 0.625 U *Taq* polymerase (Fermentas, USA) and 2 µl of DNA template from boiling method. Cycling conditions were an initial denaturation step at 94°C for 5 minutes, amplification step at 36 cycles of 94°C for 30 seconds, 52°C for 30 seconds, 72°C for 50 seconds and a final extension at 72°C for 5 minutes. The PCR products of bla_{IMP}, bla_{VIM}, bla_{GIM}, bla_{SPM} and bla_{SIM} were 188 bp, 390 bp, 477 bp, 271 bp and 304 bp respectively.

4. Analysis of amplified DNA

The PCR products were analyzed on 1% agarose gels electrophoresis (Pronadisa, Spain) in 0.5X TBE buffer containing 0.5 µg/ml of ethidium bromide (Sigma, USA). Six microliters of PCR products were mixed with 3 µl of loading buffer (20% ficoll, 0.05% bromphenol blue). The electrophoresis was carried out at 100 volts for 60 minutes. The amplified products were visualized and photographed under UV light transilluminator. A 100 bp DNA ladder plus (Fermentus, USA) was used as a DNA size marker.

5. Quality control

The clinical strains of *P. aeruginosa* harbouring bla_{IMP} and bla_{VIM} were used as positive controls. The bla_{IMP} was obtained from Associate Professor Dr. Aroonwadee Chanawong, Khon Kaen University, Thailand.

PART VII : SCREENING FOR THE PRESENCE OF OXA-TYPE CARBAPENEMASE GENES

A total of 453 *A. baumannii* isolates were detected for OXA-type carbapenemase genes by multiplex PCR.

1. DNA preparation

Genomic DNA was prepared by boiling method, four to five pured culture colonies were suspended in 100 µl of nuclease-free water and boiled at 100°C for 10 minutes. The bacterial suspensions were centrifuged at 12,000 rpm for 5 minutes at room temperature. The supernatant was used as the DNA template for PCR experiments and stored at -20°C.

2. Primers

The presence of $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$, $bla_{\text{OXA-51}}$ and $bla_{\text{OXA-58}}$ were screened by multiplex PCR using the primers OXA23-F, OXA23-R, OXA24-F, OXA24-R, OXA51-F, OXA51-R, OXA58-F and OXA58-R as described by Woodford *et al*. (134) in Table 7.

Table 7. Primers of multiplex PCR used for amplification of *bla* genes encoded OXA-type carbapenemase

Gene	Primer	Sequence	Product size (bp)	references
$bla_{\text{OXA-23}}$	OXA23-F	5'-GATCGGATTGGAGAACCAGA-3'	501	(134)
	OXA23-R	5'-ATTTCTGACCGCATTTCCAT-3'		
$bla_{\text{OXA-24}}$	OXA24-F	5'-GGTTAGTTGGCCCCCTTAAA-3'	249	
	OXA24-R	5'-AGTTGAGCGAAAAGGGGATT-3'		
$bla_{\text{OXA-51}}$	OXA51-F	5'-TAATGCTTTGATCGGCCTTG-3'	353	
	OXA51-R	5'-TGGATTGCACTTCATCTTGG-3'		
$bla_{\text{OXA-58}}$	OXA58-F	5'-AAGTATTGGGGCTTGTGCTG-3'	599	
	OXA58-R	5'-CCCCTCTGCGCTCTACATAC-3'		

3. Amplification of the *bla*$_{\text{OXA-like}}$ genes by multiplex PCR

OXA-type carbapenemase genes were amplified by multiplex PCR using OXA23-F, OXA23-R, OXA24-F, OXA24-R, OXA51-F, OXA51-R, OXA58-F and OXA58-R primers. The PCR was performed in a volume 25 µl containing 1X *Taq* buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTPs (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. Cycling conditions were an initial denaturation step at 94°C for 5 minutes, amplification step at 30 cycles of 94°C for 25 seconds, 52°C for 40 seconds, 72°C for 50 seconds and a final extension at 72°C for 6 minutes. The PCR products of *bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-24}}$, *bla*$_{\text{OXA-51}}$ and *bla*$_{\text{OXA-58}}$ were 501 bp, 249 bp, 353 bp and 599 bp, respectively.

4. Analysis of amplified DNA

The PCR products were analyzed on 1% agarose gels electrophoresis (Pronadisa, Spain) in 0.5X TBE buffer containing 0.5 µg/ml of ethidium bromide (Sigma, USA). Six microliters of PCR products were mixed with 3 µl of loading buffer (20% ficoll, 0.05% bromphenol blue). The electrophoresis was carried out at 100 volts for 60 minutes. The amplified products were visualized and photographed under UV light transilluminator. A 100 bp DNA ladder plus (Fermentus, USA) was used as a DNA size marker.

5. Quality control

The clinical strains of *A. baumannii* harbouring *bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-24}}$, *bla*$_{\text{OXA-51}}$ and *bla*$_{\text{OXA-58}}$ were used as positive control strains.

PART VIII : SCREENING FOR THE PRESENCE OF IS*ABA*

A total of 49 *A. baumannii* isolates were selected by the different of MIC level (MIC range 0.25 to >256). They were screened for the presence of IS*Aba1*, IS*Aba2*, IS*Aba3* and IS*Aba4* with primers specific for each type of IS*Aba* by PCR.

1. DNA preparation

Genomic DNA was prepared by boiling method, four to five pured culture colonies were suspended in 100 µl of nuclease-free water and boiled at 100°C for 10 minutes. The bacterial suspensions were centrifuged at 12,000 rpm for 5 minutes at room temperature. The supernatant was used as the DNA template for PCR experiments and stored at -20°C.

2. Screening for IS*Aba1-4*

The presence of ISA*ba1*, ISA*ba2*, ISA*ba3* and ISA*ba4* was screened by polymerase chain reaction using the primers described in Table 8.

Table 8. Primers for amplification of IS*Aba*

Element	Primer	Sequence	Product size (bp)	References
ISA*ba1*	ISA*ba1*-F	5'- CACGAATGCAGAAGTTG-3'	549	(135)
	ISA*ba1*-R	5'- CGACGAATACTATGACAC-3'		
ISA*ba2*	ISA*ba2*-F	5'- CATCATAGTGACAGAGGTGTGC-3'	268	This study
	ISA*ba2*-R	5'- AAGGTGACACATAACCTAGTGC-3'		
ISA*ba*3	ISA*ba*3-F	5'- CAATCAAATGTCCAACCTGC-3'	403	(113)
	ISA*ba*3-R	5'- CGTTTACCCCAAACATAAGC-3'		
ISA*ba4*	ISA*ba4*-F	5'- ATTTGAACCCATCTATTGGC-3'	612	(112)
	ISA*ba4*-R	5'- ACTCTCATATTTTTTCTTGG-3'		

3. Amplification of the IS*Aba1-4* by PCR

IS*Aba1*, IS*Aba2*, IS*Aba3* and IS*Aba4* were amplified by PCR using ISA*ba1*-F, ISA*ba1*-R, ISA*ba2*-F, ISA*ba2*-R, ISA*ba*3-F, ISA*ba*3-R, ISA*ba4*-F and ISA*ba4*-R. The PCR was performed in a volume 25 µl containing 1X *Taq* buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTPs (Fermentas, USA), 1 pmol of each primers, 0.625 U *Taq* polymerase (Fermentas, USA) and 1 µl of DNA template from boiling method. Cycling conditions were an initial denaturation step at 95°C for 5 minutes, amplification step at 35 cycles of 95°C for 30 seconds, 52°C for 30 seconds, 72°C for 1 minutes and a final extension at 72°C for 10 minutes. The PCR products of ISA*ba1*-F, ISA*ba1*-R, ISA*ba2*-F, ISA*ba2*-R, ISA*ba*3-F, ISA*ba*3-R. ISA*ba4*-F, ISA*ba4*-R were 549 bp, 268 bp, 403 bp and 612 bp respectively.

4. Analysis of amplified DNA

The PCR products were analyzed on 1% agarose gels electrophoresis (Pronadisa, Spain) in 0.5X TBE buffer containing 0.5 µg/ml of ethidium bromide (Sigma, USA). Six microliters of PCR products were mixed with 3 µl of loading buffer (20% ficoll, 0.05% bromphenol blue). The electrophoresis was carried out at 100 volts for 60 minutes. The amplified products were visualized and photographed under UV light transilluminator. A 100 bp DNA ladder plus (Fermentus, USA) was used as a DNA size marker.

5. Quality control

The clinical strains of *A. baumannii* harbouring IS*Aba1*, IS*Aba2* and IS*Aba3* were used as positive control strains.

PART IX: SCREENING FOR THE PRESENCE OF IS*ABA* UPSTREAM *BLA*$_{OXA}$ GENES

A total of 43 *A. baumannii* isolates carrying *bla*$_{OXA}$ were investigated for IS*Aba* in the upstream region of *bla* genes by PCR using the forward primer specific for IS*Aba* and the reverse primers specific for *bla*$_{OXA}$ genes.

1. DNA preparation

Genomic DNA was prepared by boiling method, four to five pured culture colonies were suspended in 100 µl of nuclease-free water and boiled at 100°C for 10 minutes. The bacterial suspensions were centrifuged at 12,000 rpm for 5 minutes at room temperature. The supernatant was used as the DNA template for PCR experiments and stored at -20°C.

2. Primers

The presence of IS*Aba* upstream of *bla* genes was screened using IS*Aba* for forward and reverse primers and *bla* genes for reverse primers. The primers for screening of IS*Aba* upstream of of *bla* genes are shown in Table 9.

Table 9. Primers for amplification of IS*Aba* upstream of *bla*$_{OXA}$ genes

Element	Primer	Sequence
IS*Aba1*	IS*Aba1*-F	5'- CACGAATGCAGAAGTTG-3'
	IS*Aba1*-R	5'- CGACGAATACTATGACAC-3'
IS*Aba2*	IS*Aba2*-F	5'- CATCATAGTGACAGAGGTGTGC-3'
	IS*Aba2*-R	5'- AAGGTGACACATAACCTAGTGC-3'
IS*Aba*3	IS*Aba*3-F	5'- CAATCAAATGTCCAACCTGC-3'
	IS*Aba*3-R	5'- CGTTTACCCCAAACATAAGC-3'
bla$_{OXA-23}$	OXA23-R	5'-ATTTCTGACCGCATTTCCAT-3'
bla$_{OXA-24}$	OXA24-R	5'-AGTTGAGCGAAAAGGGGATT-3'
bla$_{OXA-51}$	OXA51-R	5'-TGGATTGCACTTCATCTTGG-3'
bla$_{OXA-58}$	OXA58-R	5'-CCCCTCTGCGCTCTACATAC-3'

3. Amplification of IS*Aba* upstream of *bla*$_{\text{OXA-like}}$ genes by PCR

The PCR was performed in 50 µl PCR reaction mixture containing 1X *Taq* buffer, 3 mM $MgCl_2$, 0.2 mM dNTPs (Fermentas, USA), 1 pmol of IS*Aba* and *bla* genes primers , 0.625 U *Taq* polymerase (Fermentas, USA) and 2 µl of DNA template from boiling method. Cycling conditions were an initial denaturation step at 95°C for 1 minute, amplification step at 35 cycles of 95°C for 30 seconds, 50°C for 30 seconds, 72°C for 1 minute and a final extension at 72°C for 10 minutes.

4. Analysis of amplified DNA

The PCR products were analyzed on 1% agarose gels electrophoresis (Pronadisa, Spain) in 0.5X TBE buffer containing 0.5 µg/ml of ethidium bromide (Sigma, USA). Six microliters of PCR products were mixed with 3 µl of loading buffer (20% ficoll, 0.05% bromphenol blue). The electrophoresis was carried out at 100 volts for 60 minutes. The amplified products were visualized and photographed under UV light transilluminator. A 100 bp DNA ladder plus (Fermentus, USA) was used as a DNA size marker.

PART X : ANALYSIS OF ENTIRE *BLA*$_{OXA}$ AND IS*ABA* UPSTREAM *BLA*$_{OXA}$ BY PCR AND DNA SEQUENCING

A. baumannii isolates carrying *bla* genes were characterized by PCR of entire *bla* genes and IS*Aba* upstream region and automated DNA sequencing.

1. DNA preparation

1.1 Plasmid DNA extraction

A. baumannii was extracted plasmid DNA for amplify entire *bla*$_{OXA\text{-}23\text{-}like}$ and *bla*$_{OXA\text{-}58\text{-}like}$ genes by Plasmid Mini Kit (GmbH & Co. KG, Germany). *A. baumannii* was cultured in Luria-Bertani broth (Pronadisa, Spain) until the density 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 11,000 g for 30 seconds then remove the supernatant. The 250 µl of resuspension solution were added and then, mixed by vortexing. The sample was added by 250 µl of lysis solution and mixed by inverting 6-8 times. The 350 µl of neutralizing solution were added to the sample and mixed by inverting 6-8 times. The sample was centrifuged for 5-10 minutes. The plasmid DNA in clear supernatant was transferred into a plasmid mini column and was centrifuged for 1 minute. The filtrate was removed from the tube and replaced into the same wash tube. The column was added by 750 µl of wash solution and centrifuged for 1 minute. The wash solution was removed from the tube and the column was replaced into the same wash tube. Then, the tube was centrifuged for 1 additional minute to remove residual wash solution. The plasmid mini column was transferred to a 1.5 ml microcentrifuge tube and the 50 µl of elution solution was added onto the base of the column and incubated for 1 minute at room temperature. After that, the column was centrifuged for 1 minute to elute the plasmid DNA. The plasmid DNA samples were stored at -20°C.

1.2 Boiling method

Genomic DNA was prepared by boiling method, four to five pured culture colonies were suspended in 100 µl of nuclease-free water and boiled at 100°C for 10 minutes. The bacterial suspensions were centrifuged at 12,000 rpm for 5 minutes at room temperature. The supernatant was used as the DNA template for PCR experiments and stored at -20°C.

2. Primers for PCR and DNA sequencing

2.1 Primers for entire *bla*$_{\text{OXA-like}}$ genes

The primers for PCR and sequencing of entire *bla*$_{\text{OXA-23-like}}$, *bla*$_{\text{OXA-51-like}}$, *bla*$_{\text{OXA-58-like}}$ are shown in Table 10.

Table 10. Primers for PCR and DNA sequencing entire *bla* genes

Specific for	Primers	Primer sequence	Product size (bp)	Reference
PCR primers				
entire $bla_{\text{OXA23-like}}$	OXA23-likeF	5'-GATGTGTCATAGTATTCGTCG-3'	1065	(136)
	OXA23-likeR	5'-TCACAACAACTAAAAGCACTG-3'		
entire $bla_{\text{OXA24-like}}$	OXA24-likeF	5'-GTACTAATCAAAGTTGTGAA-3'	1021	
	OXA24-likeR	5'-TTCCCCTAACATGAATTTGT-3'		
entire $bla_{\text{OXA51-like}}$	OXA51-likeF	5'-TACGCCAATCCATACAGCAA-3'	1416	(GenBank accession no.CU468230)
	OXA51-likeR	5'-GCTTGACGCTGCTTTTTACC-3'		
entire $bla_{\text{OXA58-like}}$	OXA58-likeF	5'-TTATCAAAATCCAATCGGC-3'	933	(137)
	OXA58-likeR	5'-TAACCTCAAACTTCTAATTC-3'		
Sequencing primers				
entire $bla_{\text{OXA23-like}}$	OXA23-likeF	5'-GATGTGTCATAGTATTCGTCG-3'		(138)
	OXA23-likeR	5'-TCACAACAACTAAAAGCACTG-3'		
entire $bla_{\text{OXA24-like}}$	OXA24-likeF	5'-GTACTAATCAAAGTTGTGAA-3'		
	OXA24-likeR	5'-TTCCCCTAACATGAATTTGT-3'		
	OXA21-likeR1	5'-AGTTGAGCGAAAAGGGGATT-3'		
entire $bla_{\text{OXA51-like}}$	OXA51-likeF	5'-TACGCCAATCCATACAGCAA-3'		(GenBank accession no.CU46823) **(134)** (GenBank accession no.EU97756)
	OXA51-likeFM	5'-TAATGCTTTGATCGGCCTTG-3'		
	OXA51-likeFM1	5'-ATGAACATTAAAGCACTC-3'		
	OXA51-likeR1	5'-CTATAAAATACCTAATTGTTC-3'		
entire $bla_{\text{OXA58-like}}$	OXA58-likeF	5'-TTATCAAAATCCAATCGGC-3'		(137)
	OXA58-likeR	5'-TAACCTCAAACTTCTAATTC-3'		
	OXA58-likeFM	5'-AAGTATTGGGGCTTGTGCTG-3'		(134)

2.2 Primers for ISA*ba* upstream *bla*$_{\text{OXA-like}}$ genes

The primers for PCR and sequencing of ISA*ba* upstream *bla*$_{\text{OXA-like}}$ are shown in Table 11.

Table 11. Sequence of the oligonucleotides used as primers for PCR and DNA sequencing *bla* genes and ISA*ba* upstream region

Specific for	Primer	Sequence
ISA*ba1*-*bla*$_{\text{OXA-23}}$	ISA*ba1*-F	5'- CACGAATGCAGAAGTTG-3'
	OXA23-R	5'-ATTTCTGACCGCATTTCCAT-3'
ISA*ba3*- *bla*$_{\text{OXA-58}}$	ISA*ba*3-R	5'- CGTTTACCCCAAACATAAGC-3'
	OXA58-R	5'-CCCCTCTGCGCTCTACATAC-3'

3. Amplification of entire *bla*$_{\text{OXA-like}}$ genes

3.1 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*$_{\text{OXA-23-like}}$ gene while OXA58-F and OXA58-R primers were for entire *bla*$_{\text{OXA-58-like}}$ gene amplification. The PCR was performed in 50 µl PCR reaction mixture containing 1X *taq* buffer, 3 mM $MgCl_2$, 200 µM dNTP (Fermentas, USA), 5 pmol of each primer, 1.25 U *Taq* polymerase (Fermentas, USA), and 3 µl of DNA template. Cycling conditions were an initial denaturation step at 95°C for 5 minutes, amplification step at 30 cycles of 95°C for 25 seconds, 52°C for 40 seconds, 72°C for 50 seconds and a final extension 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.

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

The amplification of entire *bla*$_{\text{OXA51-like}}$ gene was used OXA51-F and OXA51-R as primers. The PCR was performed in 50 µl PCR reaction mixture containing 1X *taq* buffer, 3 mM $MgCl_2$, 200 µM dNTP (Fermentas, USA), 5 pmol of each primer, 1.25 U *Taq* polymerase (Fermentas, USA), and 3 µl of DNA template. Cycling conditions were an initial denaturation step at 95°C for 5 minutes, amplification step at 35 cycles of 95°C for 30 seconds, 52°C for 1 minute, 72°C for 90 seconds and a final extension at 72°C for 10 minutes. The PCR product of entire *bla*$_{\text{OXA-51-like}}$ was 825 bp.

4. Amplification of IS*Aba* upstream of *bla*$_{\text{OXA-like}}$ genes by PCR

The PCR was performed in 50 µl PCR reaction mixture containing 1X *Taq* buffer, 3 mM $MgCl_2$, 0.2 mM dNTPs (Fermentas, USA), 1 pmol of IS*Aba* and *bla* genes primers , 0.625 U *Taq* polymerase (Fermentas, USA) and 2 µl of DNA template from boiling method. Cycling conditions were an initial denaturation step at 95°C for 1 minute, amplification step at 35 cycles of 95°C for 30 seconds, 50°C for 30 seconds, 72°C for 1 minute and a final extension at 72°C for 10 minutes.

5. Analysis of amplified DNA

The PCR products were analyzed on 1% agarose gels electrophoresis (Pronadisa, Spain) in 0.5X TBE buffer containing 0.5 µg/ml of ethidium bromide (Sigma, USA). Six microliters of PCR products were mixed with 3 µl of loading buffer (20% ficoll, 0.05% bromphenol blue). The electrophoresis was carried out at 100 volts for 60 minutes. The amplified products were visualized and photographed under UV light transilluminator. A 100 bp DNA ladder plus (Fermentus, USA) was used as a DNA size marker.

6. Purification of PCR product

The PCR products were purified by Invisorb Fragment cleanup kit as described by the manufacturer (Invitek GmbH,Germany). For binding the PCR-fragments, add 250 µl binding buffer to the PCR sample and mix very well by vortexing. Transfer the complete sample onto a Spin Filter and centrifuge for 3 minutes at 12,000 rpm. For elution of the PCR-fragments, place the Spin Filter into a new 1.5 ml tube and add 30 µl elution buffer onto the center of the Spin Filter. After that, incubate for 1 minute at room temperature then the tube was centrifuged for 1 minute at 10,000 rpm. The concentration of DNA was measured by spectrophotometer (BIO RAD, Smart Spec tm 3000, U.S.A) and approximately adjusted to 50-100 ng/µl for preparation of sequencing reaction and the purified PCR products were stored at -20^{o}C.

7. Preparation of sequencing reaction

Purified PCR products were sequenced by the chain termination method at the Macrogen Inc. (Seoul, Korea). Sequencing was conducted under BigDyeTM terminator cycling conditions. The reacted products were purified by ethanol precipitation and running using automatic sequencer, Applied Biosystems DNA sequencer model 3730xI (Rochester NY, USA).

8. Sequencing analysis

The nucleotide sequences and 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), ExPASy (www.expasy.org/) and multiple sequence alignment of sequences were analyzed by Multilin (http://bioinfo.genopole-toulouse.prd.fr/multalin/multalin.html) or Bioedit program.

PART XI: DETECTION OF CARBAPENEM EFFLUX PUMP PHENOTYPE

The mechanism of carbapenem efflux pump in 453 *A. baumannii* isolates was determined by inhibitory effects of carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), the efflux pump inhibitor. Susceptibility testing was carried out using agar dilution method. MIC changes were observed in either the presence or the absence of CCCP (Sigma, USA) at concentrations of 12.5 μM. The Mueller Hinton agar containing the two-fold dilutions of imipenem and meropenem (0.015-256 μg/ml) were inoculated with $5x10^4$ CFU/ml of each isolate. Plates were incubated for 20-24 hour at 37^oC. The positive of phenotype efflux pump was detected by the MIC of imipenem and meropenem decreased at least 4-fold. MICs in the the presence of CCCP *A. baumannii* Aci65 with efflux pump positive (decreased 4-fold) were used as positive control and the plate CCCP without antibiotics was used as control.

PART XII : OUTER MEMBRANE PROTEIN ANALYSIS

A total of 23 *A. baumannii* isolates including 2 susceptible isolates and 21 resistant isolates, *A. baumannii* ATCC 19606 were selected by different types of $bla_{\text{OXA-like}}$ and the level of MIC for imipenem and meropenem. The outer membrane protein (OMPs) fractions were prepared by the *N*-lauroyl sacrosinate method and analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis.

1. Cell envelope preparation and OMP extraction.

Inoculation of *A. baumannii* isolates in Luria-Bertani broth 100 ml for 18-24 hours at 37^{o}C in shaker incubator. After that, centrifuge for 30 minutes at 5,000 rpm at 4^{o}C. The pellet was resuspended in 10 mmol/l Tris HCl pH 8.0 10 ml and cells were breaked by sonication for 5 minutes. The cell debris was removed by centrifugation at 5000 rpm at 4^{o}C for 45 minutes. The supernatant was transferred and centrifuged for 20000 rpm at 4^{o}C for 1 hour then resuspensed in DDW 150 µl. The suspension was treated by 2% sarkosyl, incubated for 20 minutes and then centrifuge for 35000 rpm at 4^{o}C for 90 minutes. The pellet was resuspended in 100 µl of 10 mmol/l Tris HCl pH 8.0 and stored at -70°C until further use.

2. SDS-PAGE

Prepare 12% polyacrylamide gel for separate gel and 4% polyacrylamide gel for stacking gel. Electrophoresis was performed at a constant amphere of 30 mA for 80 minutes and the gel was stained with Coomassie Brilliant Blue.

CHAPTER V

RESULTS

PART I : BACTERIAL STRAINS

A total of 453 *A. baumannii* isolates were used in this study. They were collected from clinical specimens at the Department of Microbiology, King Chulalongkorn Memorial Hospital, Bangkok, Thailand between March 2008 and April 2010. Each isolate was from different patient. Two hundred and sixty-nine (59.4%) isolates were collected from male and 184 (40.6%) isolates were from female. The range of age was 8 days to 99 years old. Two hundred and three (44.8%) were obtained from ICUs and 255 (55.2%) were from non-ICUs. For the sites of specimens, 58 (12.8%) isolates were from sterile sites including blood, CSF and body fluid and 395 (87.2%) isolates were from non-sterile sites including sputum, urine, pus, tissue, respiratory aspirate and other specimen. The results are shown in Table 12.

Table 12. Clinical specimen of 453 *A. baumannii* isolates

Source	Specimen	No. of isolates	Total (%)
Sterile site n= 58 (12.8%)	Body fluid	35	7.7
	Blood	21	4.6
	CSF	2	0.4
Non-sterile site n= 395 (87.2%)	Sputum	197	43.5
	Urine	90	19.9
	Pus	30	6.6
	Tissue	12	2.6
	Respiratory aspirate	43	9.5
	Other	23	5.1

All clinical isolates were identified as *A. baumannii* based on conventional methods such as colony morphology, Gram stain and biochemical characteristics. The colony morphology of *A. baumannii* isolates on MacConkey agar was lactose non-ferment, 1 to 2 mm in diameter, colorless or greyish-pink, smooth and sometimes mucoid. Gram stain showed gram negative coccobacilli, usually in diploid form. For biochemical characteristics, the isolates showed neutral slant/neutral butt on triple sugar iron agar, non motile, urease negative, citrate positive, oxidase negative, oxidized glucose and non hemolysis on sheep blood agar. For ability to grow, the isolates grew at 37^{o}C, 42^{o}C and 44^{o}C.

PART II : ANTIMICROBIAL SUSCEPTIBILITY TESTING

The antimicrobial susceptibility testing to carbapenems in *A. baumannii* was determined by agar dilution method on Mueller-Hinton agar. The MIC is the lowest concentration of antibiotics required to inhibit the growth of a microorganism *in vitro*. The MIC_{50} and MIC_{90} are the lowest concentration of antibiotics required to inhibit 50% and 90% of isolates tested, respectively. MIC breakpoints of imipenem and meropenem are ≥ 16 µg/ml. The results of susceptibility testing and resistance rates of imipenem and meropenem against 453 *A. baumannii* isolates are shown in Table 13 and appendix D.

Prevalence of imipenem resistance was 88.7% (402/453). Imipenem MIC ranged from 0.5 to >256 µg/ml. MIC_{50} and MIC_{90} were 128 µg/ml and 256 µg/ml, respectively. Distribution of the imipenem MIC for 453 *A. baumannii* isolates is shown in Figure 10. It was demonstrated that 11.3% (51/453) of imipenem-susceptible *A. baumannii* isolates had MICs ranged from 0.5 to 4 µg/ml and most of imipenem-susceptible isolates (72.5 %, 37/51) had imipenem MICs of 1 and 2 µg/ml. Imipenem-resistant isolates had MICs ranged from 32 to >256 µg/ml and the majority of imipenem-resistant isolates (79.1%, 318/402) had imipenem MICs of 128 to 256 µg/ml. Similar to imipenem, prevalence of meropenem resistance was 88.7% (402/453). The meropenem MIC ranged from 0.25 to 256 µg/ml. MIC_{50} and MIC_{90} were 64 µg/ml and 128 µg/ml, respectively. Distribution of the meropenem MICs for 453 *A. baumannii* isolates is shown in Figure 11. It was shown that 11.3% (51/453) of MICs of meropenem-susceptible isolates had the MIC ranging from 0.25 to 4 µg/ml. Most of meropenem-susceptible isolates (92.2%, 47/51) had meropenem MIC of 0.25 to 2 µg/ml. Meropenem-resistant isolates had MICs ranged from 16 to 256 µg/ml and the majority of meropenem-resistant isolates (80.8%, 325/402) had imipenem MICs of 32 to 64 µg/ml.

The resistance rates of imipenem and meropenem in *A. baumannii* isolates from ICUs and non-ICUs were studied. The comparison of carbapenem-resistant rate between ICUs and non-ICUs isolates are shown in Figure 12. Imipenem resistance in ICU and non-ICU isolates were 88.7% (180/203) and 88.8 % (222/250), respectively. MIC_{50} and MIC_{90} of imipenem resistance in both ICUs and non-ICUs were 128 µg/ml and 256 µg/ml, respectively. Similar to imipenem resistance rates, meropenem resistance rates in ICU and non-ICU isolates were 88.7% (180/203) and 88.8 % (222/250), respectively. MIC_{50} and MIC_{90} of meropenem resistance in both ICU and non-ICU isolates were 64 µg/ml and 128 µg/ml, respectively (Table 14). Distribution of imipenem MIC in ICU and non-ICU isolates are demonstrated in Figure 13. The MIC range of imipenem-susceptible isolates from ICU and non-ICU were 0.5 to 2 µg/ml. The majority of imipenem-susceptibile isolates in ICU (82.6%, 19/23) and non-ICU (85.7%, 24/28) had imipenem MIC ranged from 1 to 2 µg/ml and 1 to 4 µg/ml, respectively. Whereas, the MIC range of imipenem-resistant isolates in ICU and non-ICU had imipenem MIC ranging from 32 to >256 µg/ml. Most of imipenem-resistant isolates from ICU (85%, 153/180) and non-ICU (93.7%, 208/222) had imipenem MIC ranging from 128 to 256 and 64 to 256 µg/ml, respectively. Distribution of the MIC of meropenem in ICU and non-ICU isolates are shown in Figure 14. It was demonstrated that meropenem-susceptible isolates from ICU and non-ICU had meropenem MIC ranging 0.25 to 2 µg/ml. Most of meropenem-susceptible isolates from ICU (82.6%, 19/23) and non-ICU (85.7%, 24/28) had meropenem MIC of 0.25 to 0.5 µg/ml and 0.25 to 1 µg/ml, respectively. In addition, MICs of meropenem-resistant isolates from ICU and non-ICU had the MIC range of 16 to 256 µg/ml. The majority of meropenem-resistant strains from both ICU (81.1%, 146/180) and non-ICU (80.6%, 179/222) had meropenem MIC of 16 to 128 µg/ml.

Table 13. The susceptibility of imipenem and meropenem against 453 *A. baumannii* isolates.

Antimicrobial agents	MICs (µg/ml)			Susceptibility (%)		
	MIC50	MIC90	Range	S	I	R
Imipenem	128	256	0.5- >256	11.3 (51/453)	0	88.7 (402/453)
Meropenem	64	128	0.25-256	11.3 (51/453)	0	88.7 (402/453)

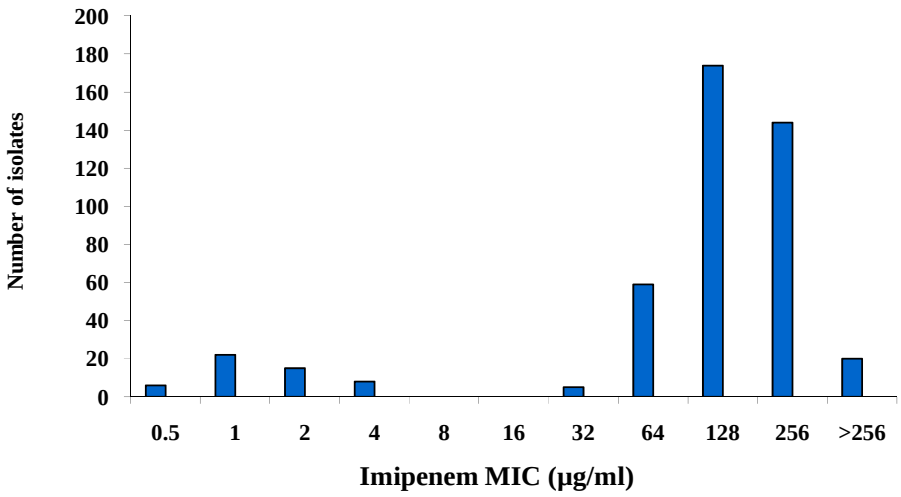


Figure 10. Distribution of imipenem MICs among 453 *A. baumannii* isolates

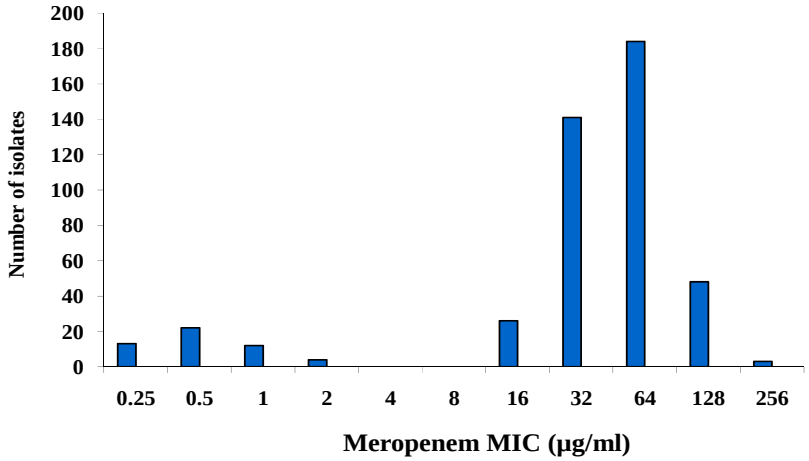


Figure 11. Distribution of meropenem MICs among 453 *A. baumannii* isolates

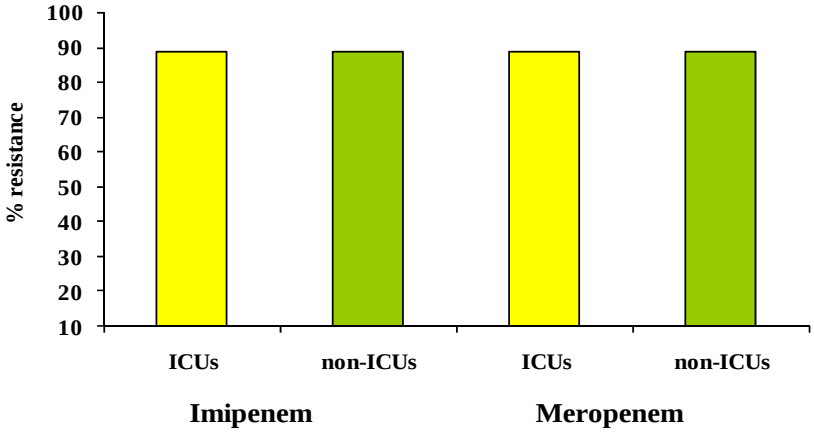


Figure 12. Comparison of carbapenem resistance in 453 *A. baumannii* isolates from ICUs and non-ICUs

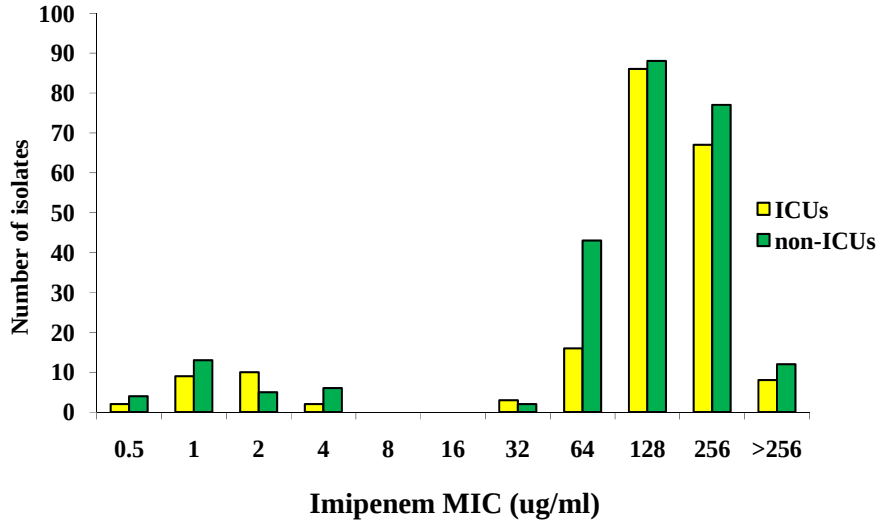


Figure 13. Distributions of imipenem MICs among 453 *A. baumannii* isolates from ICUs and non-ICUs

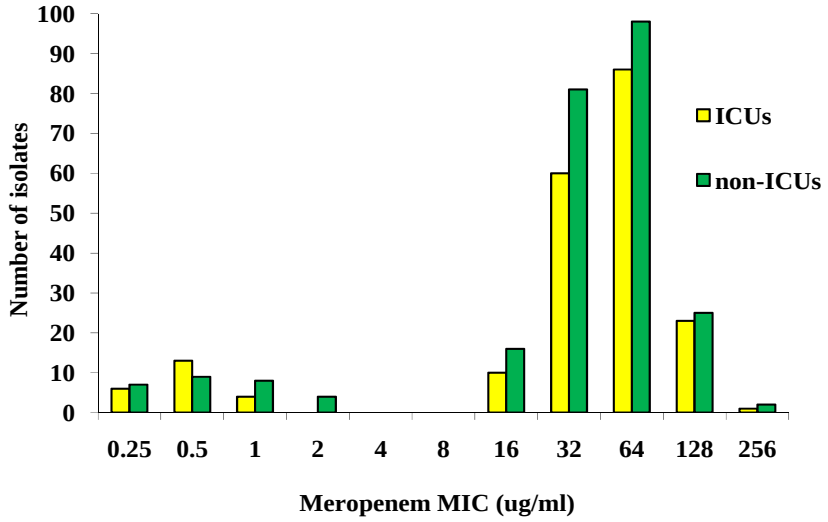


Figure 14. Distributions of meropenem MICs among 453 *A. baumannii* isolates from ICUs and non-ICUs

Table 14. Correlation between carbapenemase genes and MICs of *A. baumannii* isolates from ICUs and non-ICUs

Ward	No. of isolates	No. of isolates habouring $bla_{\text{OXA-like}}$ (%)				MIC (ug/ml)					
						Imipenem			Meropenem		
		OXA-51-like	OXA-51-like, OXA-23- like	OXA-51-like, OXA-24- like	OXA-51 like, OXA-23- like, OXA-58 -like	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
ICU	203	23 (11.3)	179 (88.2)	0	1 (0.5)	128	256	0.5->256	64	128	0.25-256
Non-ICU	250	28 (11.2)	218 (87.2)	1 (0.4)	3 (1.2)	128	256	0.5->256	64	128	0.25-256

PART III : DETECTION OF CARBAPENEMASE PHENOTYPE

The carbapenemase phenotype was detected by modified Hodge test. A total of 453 *A. baumannii* isolates were detected for carbapenemase activity. Carbapenemase activity of all isolates are shown in Appendix D. Four hundred and two (88.7%) were positive for carbapenemase activity. The range of imipenem MIC for carbapenemase activity negative and positive were 0.5 to 4 µg/ml and 32 to > 256 µg/ml, respectively. The MIC range of meropenem for carbapenemase activity negative and positive were 0.25 to 2 µg/ml and 16 to 256 µg/ml, respectively. All of 402 *A. baumannii* isolates with carbapenemase activity were resistant to imipenem and meropenem. Distribution of MICs of imipenem and meropenem MICs in *A. baumannii* isolates and the presence of carbapenemase activity are shown in Figures 15 and 16.

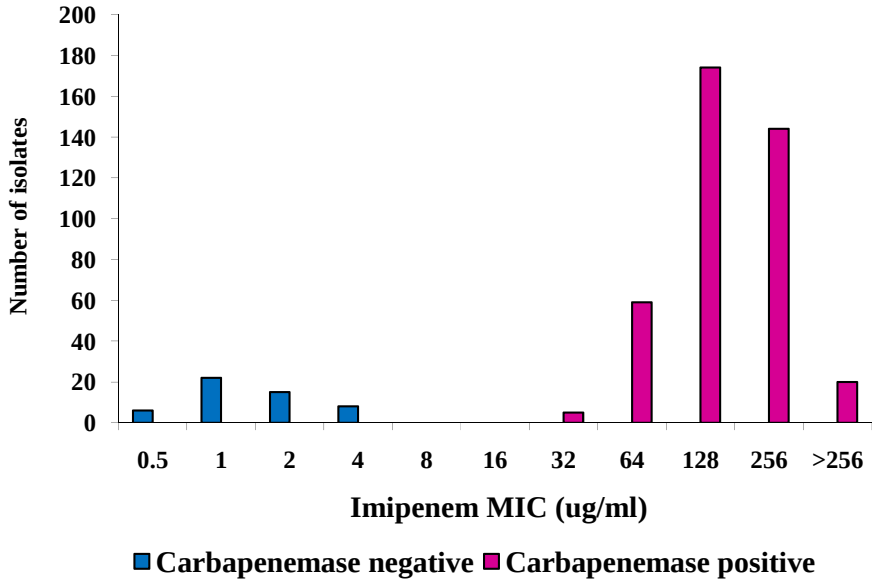


Figure 15. Distribution of imipenem MICs and carbapenemase activity

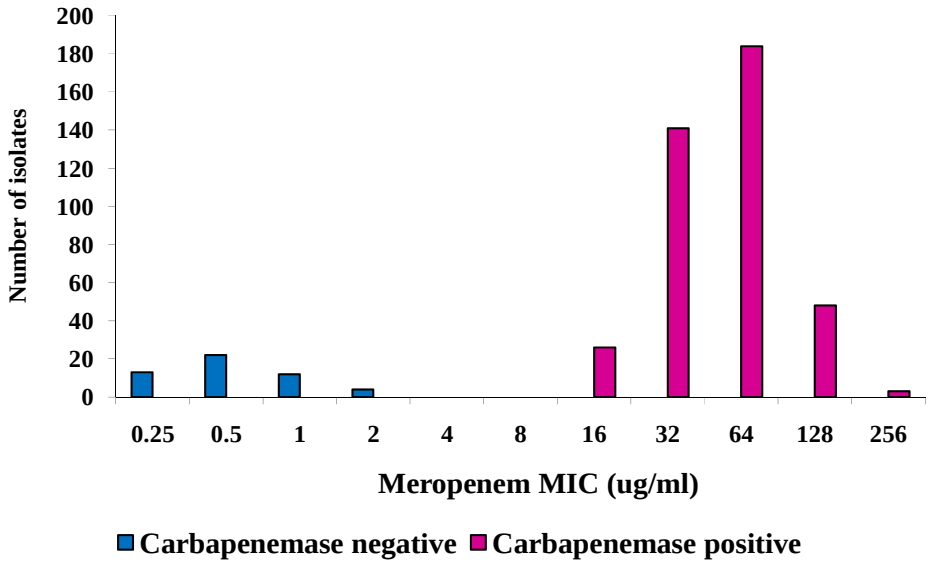


Figure 16. Distribution of meropenem MICs and carbapenemase activity

PART IV : DETECTION OF METALLO-β-LACTAMASES PHENOTYPE

The metallo-β-lactamase activity was detedcted by EDTA-disk synergy test. A total of 453 *A. baumannii* were tested for metallo-β-lactamase activity and the results are shown in Appendix D. All of 453 *A. baumannii* were negative for metallo-β-lactamase activity.

PART V: SCREENING FOR THE PRESENCE OF METALLO-β-LACTAMASE GENES

All of 453 *A. baumannii* isolates were screened for the presence of metallo-β-lactamase genes including $bla_{\text{IMP-like}}$, $bla_{\text{VIM-like}}$, $bla_{\text{SIM-1}}$, $bla_{\text{SPM-1}}$ and $bla_{\text{GIM-1.}}$ The expected size of PCR products of $bla_{\text{IMP-like}}$, $bla_{\text{VIM-like}}$, $bla_{\text{SIM-1}}$, $bla_{\text{SPM-1}}$ and $bla_{\text{GIM-1}}$ were 188 bp, 390 bp, 477 bp, 271bp and 304 bp, respectively. Metallo-β-lactamase genes were not detected in any isolates. The result of screening for $bla_{\text{IMP-like}}$, $bla_{\text{VIM-like}}$, $bla_{\text{SIM-1}}$, $bla_{\text{SPM-1}}$ and $bla_{\text{GIM-1}}$ are shown in appendix D.

PART VI : SCREENING FOR THE PRESENCE OF OXA-TYPE CARBAPENEMASE GENES

A total of 453 *A. baumannii* isolates were screened for OXA-type carbapenemase genes including *bla*$_{\text{OXA-23-like}}$, *bla*$_{\text{OXA-24-like}}$, *bla*$_{\text{OXA-51-like}}$ and *bla*$_{\text{OXA-58-like}}$ by multiplex PCR. The expected size of PCR products of *bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-24}}$, *bla*$_{\text{OXA-51}}$ and *bla*$_{\text{OXA-58}}$ were 501 bp, 249 bp, 353 bp, 599 bp, respectively (Figure 17). The result of screening for *bla*$_{\text{OXA}}$ are presented in appendix D. All isolates were positive for *bla*$_{\text{OXA-51}}$. Four hundred and one (88.5%, 401/453) were positive for *bla*$_{\text{OXA-23}}$. One (0.2%, 1/453) isolate were positive for *bla*$_{\text{OXA-24}}$. Four (0.9%, 4/453) were positive for *bla*$_{\text{OXA-58.}}$

Among 402 carbapenem-resistant *A. baumannii* isolates, 401 (99.8%) had *bla*$_{\text{OXA-23}}$, 1 (0.2%,) carried *bla*$_{\text{OXA-24}}$, 4 (1%) isolates haboured *bla*$_{\text{OXA-58}}$. The results are presented in Table 16. Of 453 isolates, 51 (11.3%) carried only *bla*$_{\text{OXA-51.}}$ These isolates had no carbapenemase activity. Three hundred and ninety-seven isolates (87.6%) carried *bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-51}}$, 1 (0.2%) haboured *bla*$_{\text{OXA-24}}$ and *bla*$_{\text{OXA-51}}$, 4 (1%) had *bla*$_{\text{OXA-23,}}$ *bla*$_{\text{OXA-51}}$ and *bla*$_{\text{OXA-58}}$. All of which had carbapenemase activity. The results are summarized in Table 15.

The MIC range of imipenem in *A. baumannii* isolates carrying only *bla*$_{\text{OXA-51}}$ were 0.5 to 4 µg/ml. MIC_{50} and MIC_{90} were 1 µg/ml and 4 µg/ml, respectively. The isolates that haboured *bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-51}}$ had imipenem MIC ranging from 32 to >256 µg/ml. MIC_{50} and MIC_{90} were 128 µg/ml and 256 µg/ml, respectively. Only one isolate habouring *bla*$_{\text{OXA-24}}$ and *bla*$_{\text{OXA-51}}$ had imipenem MIC of >256 µg/ml. All 4 isolates carrying *bla*$_{\text{OXA-23,}}$ *bla*$_{\text{OXA-51}}$ and *bla*$_{\text{OXA-58}}$ had imipenem MIC of 64 µg/ml. The distribution of imipenem MIC and *bla*$_{\text{OXA-like}}$ gene are presented in Table 15 and Figure 18.

The isolates habouring only *bla*$_{OXA-51}$ had low-level meropenem MIC as for imipenem MIC. The meropenem MIC ranged from 0.25 to 2 µg/ml. The isolates habouring *bla*$_{OXA-23}$ and *bla*$_{OXA-51}$ had MIC of meropenem ranged from 16 to 256 µg/ml. MIC_{50} and MIC_{90} were 64 µg/ml and 128 µg/ml, respectively. Only 1 isolate carrying *bla*$_{OXA-24}$ and *bla*$_{OXA-51}$ had meropenem MIC of 256 µg/ml. The isolates carrying *bla*$_{OXA-23,}$ *bla*$_{OXA-51}$ and *bla*$_{OXA-58}$ had meropenem MIC range of 16 µg/ml to 32 µg/ml. MIC_{50} and MIC_{90} were 16 µg/ml and 32 µg/ml, respectively. The distribution of meropenem MIC and *bla*$_{OXA-like}$ gene are presented in Table 15 and Figure 19.

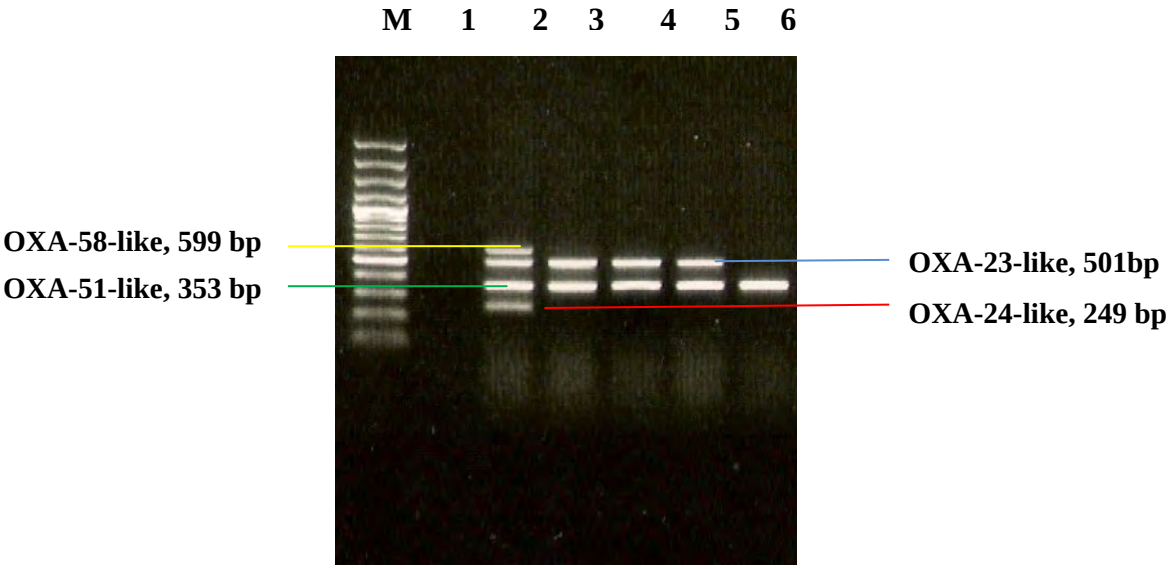


Figure 17. Multiplex PCR analysis of *bla*$_{OXA-like}$: M, 100-bp plus DNA ladder; lane 1 negative control (sterile DDW); lane 2, *bla*$_{OXA23-like,}$ *bla*$_{OXA24-like}$, *bla*$_{OXA51-like}$ and *bla*$_{OXA58-like}$; lane 3-5, *bla*$_{OXA23-like}$ and *bla*$_{OXA51-like}$; lane 6, *bla*$_{OXA51-like}$

Table 15. OXA-type carbapenemase genes in the 453 *A. baumannii* isolates.

Carbapenemase activity	*bla*$_{\text{OXA-like}}$	No. of isolates (%)	Imipenem MIC (mg/L)			Meropenem MIC (mg/L)		
			MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
Negative	*bla*$_{\text{OXA51-like}}$,	51(11.3)	1	4	0.5-4	0.5	1	0.25-2
Positive	*bla*$_{\text{OXA51-like}}$, *bla*$_{\text{OXA23-like}}$	397(87.6)	128	256	32->256	64	128	16-256
Positive	*bla*$_{\text{OXA51-like}}$, *bla*$_{\text{OXA24-like}}$	1(0.2%)	-	-	>256	-	-	256
Positive	*bla*$_{\text{OXA51-like}}$, *bla*$_{\text{OXA23-like}}$, *bla*$_{\text{OXA58-like}}$	4(1%)	64	64	64-64	16	32	16-32

Table 16. OXA-type carbapenemase genes among 402 carbapenem-resistant *A. baumannii* isolates.

Gene	Number of isolates (%)
bla$_{\text{OXA23-like}}$	401 (99.8%)
bla$_{\text{OXA24-like}}$	1 (0.2%)
bla$_{\text{OXA51-like}}$	402 (100%)
bla$_{\text{OXA58-like}}$	4 (1%)

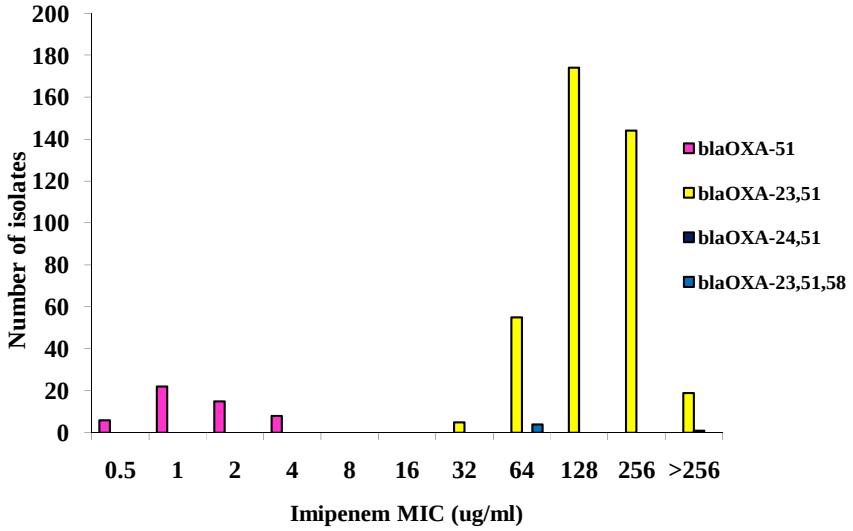


Figure 18. Distribution of genes encoding OXA-type carbapenemases and imipenem MICs

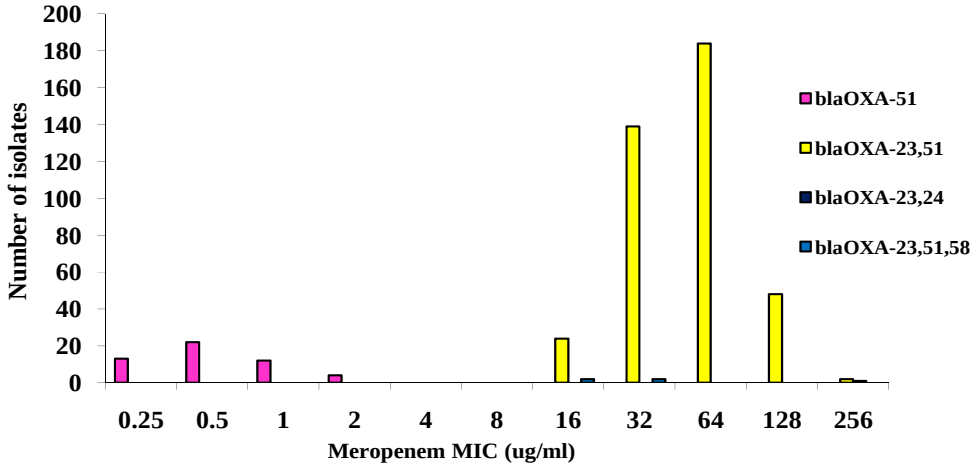


Figure 19. Distribution of genes encoding OXA-type carbapenemases and meropenem MICs

PART VII : SCREENING FOR THE PRESENCE OF IS*ABA* IN *A. BAUMANNII*

A total of 49 *A. baumannii* isolates with different MIC level (MIC range 0.25 to >256), were screened for the presence of IS*Aba1*, IS*Aba2*, IS*Aba3* and IS*Aba4* with primers specific for each type by PCR. The expected sizes of PCR products of IS*Aba1*, IS*Aba2*, IS*Aba3* and IS*Aba4* were 549 bp, 268 bp, 403 bp and 612 bp, respectively. IS*Aba1* was detected in 43 (87.8%) isolates. IS*Aba2* was detected in 7 (14.3%) isolates. IS*Aba3* was present in 8 (16.3%) isolates. IS*Aba4* was not detected in any isolates.

PART VIII : SCREENING AND SEQUENCING OF IS*ABA* UPSTREAM *BLA*$_{OXA\text{-}LIKE}$ GENES

1. IS*Aba1* upstream *bla*$_{OXA\text{-}like}$

A total of 43 isolates carrying IS*Aba1* were screened for the presence of IS*Aba1* upstream the *bla*$_{OXA\text{-}like}$ genes. The results are summarized in Table 17 and appendix E.

1.1 IS*Aba1* upstream *bla*$_{OXA\text{-}23\text{-}like}$

There were 38 *A. baumannii* isolates carrying both IS*Aba1* and *bla*$_{OXA\text{-}23\text{-}like.}$ The MIC of imipenem and meropenem ranged from 32 to >256 and 16 to 128 µg/ml, respectively. MIC$_{50}$ of imipenem and meropenem were 256 and 64 µg/ml, respectively. MIC$_{90}$ were 256 and 64 µg/ml, respectively. PCR using primer IS*Aba1*-F and *bla*$_{OXA\text{-}23\text{-}like}$- R amplified the specific DNA fragment of 1405 bp. DNA sequencing analysis of 5 representative isolates (A251, A252, A253, A254 and A285) revealed that IS*Aba1* was located upstream of *bla*$_{OXA\text{-}23}$. The schematic representation of IS*Aba1* upstream *bla*$_{OXA\text{-}23\text{-}like}$ is shown in Figure 20. Nucleotide sequence alignment of PCR fragment of the IS*Aba1*-*bla*$_{OXA\text{-}23\text{-}like}$ of the representative isolates (A285) and the sequences in Genbank accession no.EF127491 showed that both IS*Aba1*-*bla*$_{OXA\text{-}23\text{-}like}$ fragments were identical (figure 22). The -35 (TTAGAA) sequences and -10 (TTATTT) sequences separated by 16 bp performed as promoter sequences. Nucleotide sequence alignment of our 5 representative *A. baumannii* isolates and sequence accession no.EF127491 are shown in appendix F.

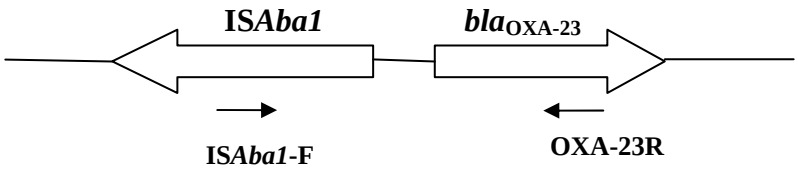


Figure 20. Schematic representation of IS*Aba1* upstream *bla*$_{OXA\text{-}23}$

1.2 IS*Aba1* upstream $bla_{\text{OXA-51-like}}$

All 43 IS*Aba1* isolates carried both IS*Aba1* and $bla_{\text{OXA-51-like}}$. However, IS*Aba1* was not identified in the upstream region of $bla_{\text{OXA-51-like}}$ in any isolates by PCR.

1.3 IS*Aba1* upstream $bla_{\text{OXA-58-like}}$

Four isolates including A98, A200, A232 and A341 carried both IS*Aba1* and $bla_{\text{OXA-58-like,}}$. The presence of IS*Aba1* upstream $bla_{\text{OXA-58-like}}$ was not detected in any isolates by PCR.

2. IS*Aba2* upstream $bla_{\text{OXA-like}}$

A total of 7 *A. baumannii* isolates including Ab1, A260, A264, A270, A282, A290 and A293 carrying IS*Aba2* were screening for the presence of IS*Aba2* upstream region of $bla_{\text{OXA-like}}$ genes, including $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-51-like}}$. IS*Aba2* upstream $bla_{\text{OXA-like}}$ genes were not present in any isolates. The results are summarized in Table 17 and appendix E.

3. IS*Aba3* upstream $bla_{\text{OXA-like}}$

A total of 8 *A. baumannii* isolates including A98, A200, A232, A255, A256 and A341 carrying IS*Aba3* were screened for the presence of IS*Aba3* upstream $bla_{\text{OXA-like}}$ genes including $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-58-like}}$. The results are summarized in Table 17 and appendix E.

3.1 IS*Aba3* upstream $bla_{\text{OXA-58-like}}$

There were 4 *A. baumannii* isolates carrying both IS*Aba3* and $bla_{\text{OXA-58-like}}$. The imipenem MIC were 64 µg/ml and meropenem MIC ranged from 16 to 32 µg/ml. MIC_{50} of imipenem and meropenem were 64 and 16 µg/ml, respectively. MIC_{90} were 64 and 32 µg/ml, respectively. PCR using primer IS*Aba3*-F and $bla_{\text{OXA-58-like}}$-R amplified the specific DNA fragment of 1046 bp. DNA sequencing analysis revealed that all 4 isolates had IS*Aba*3

upstream bla_{OXA-58}. The schematic representation of IS*Aba3* upstream $bla_{OXA-58-like}$ is shown in Figure 21. In A200, IS*Aba3* was located 17 bp upstream of bla_{OXA-58}. The -35 (TTTATC) sequences and -10 (TTTCTT) sequences separated by 16 bp performed as promoter sequences. Nucleotide sequence alignment of PCR fragment of the IS*Aba*3-$bla_{OXA-58-like}$ of A200 and the sequences in Genbank accession no.DQ987830 showed that both IS*Aba*3-$bla_{OXA-58-like}$ fragment were identical (Figure 23). Nucleotide sequence alignment of all 4 *A. baumannii* isolates and sequence accession no. DQ987830 are shown in appendix F.

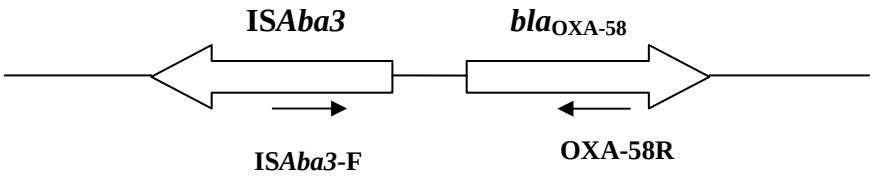


Figure 21. Schematic representation of IS*Aba3* upstream bla_{OXA-58}

Table 17. The correlation of the aquisition of IS*Aba* upstream $bla_{OXA-like}$ with MIC in carbapenem-resistant *A. baumannii*.

IS*Aba*-$bla_{OXA-like}$	No. of isolates	IPM MIC (µg/ml)			MEM MIC (µg/ml)		
		MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range
IS*Aba1*- $bla_{OXA-23-like}$	38	256	256	32->256	64	64	16-128
IS*Aba3*- $bla_{OXA-58-like}$	4	64	64	64-64	16	32	16-32

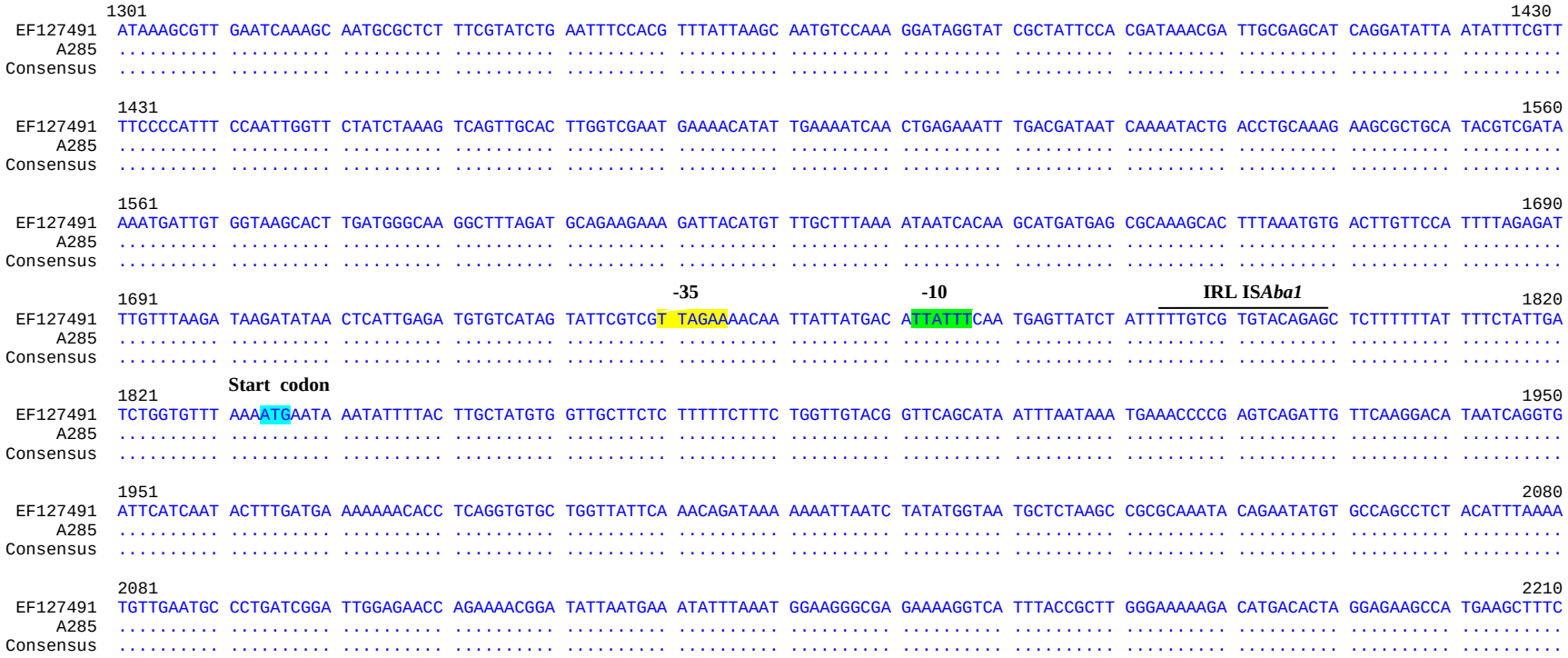


Figure 22. Comparison of IS*Aba1* upstream region of *bla*$_{\text{OXA-23}}$ gene of A285 and sequences in GenBank accession no.EF127491

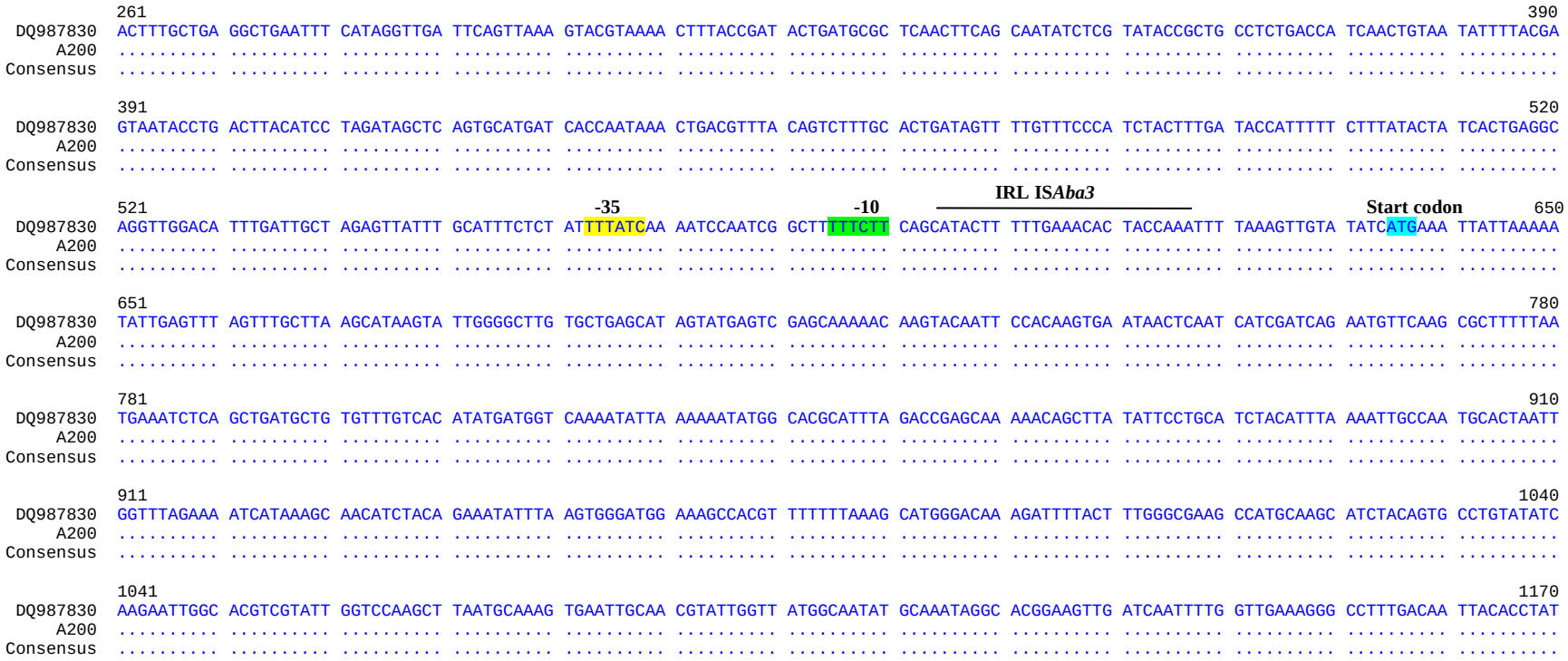


Figure 23. Comparison of IS*Aba3* upstream region of *bla*$_{\text{OXA-58}}$ gene of A200 and sequences in GenBank accession no.DQ987830

PART IX: DNA SEQUENCING ANALYSIS OF ENTIRE *BLA* GENES

1. DNA SEQUENCING ANALYSIS OF ENTIRE *BLA* GENES

The entire $bla_{\text{OXA23-like}}$, $bla_{\text{OXA51-like}}$ and $bla_{\text{OXA58-like}}$ genes were amplified by PCR and DNA sequencing. Fifteen, 12 and 4 isolates with different MIC level and $bla_{\text{OXA-like}}$ genes were selected for sequenced $bla_{\text{OXA23-like}}$, $bla_{\text{OXA51-like}}$ and $bla_{\text{OXA58-like}}$. The results of DNA sequencing are summarized in Table 18.

A total of 15 representative *A. baumannii* isolates habouring $bla_{\text{OXA23-like}}$ were selected for DNA sequencing, including A98, A200, A232, A251, A252, A253, A254, A258, A259, A292, A294, A297, A299, A300 and A341. The isolates haboured various types of $bla_{\text{OXA-like}}$ as shown in table 18. The MIC range of imipenem and meropenem were 32 to >256 µg/ml and 16 to 128 µg/ml, respectively. DNA sequence analysis of the 1,065-bp fragments of 15 representative *A. baumannii* revealed an open reading frame of 822 bp, encoding a 271 amino acids. They shared 100% nucleotide sequences and amino acid sequences identity to $bla_{\text{OXA-23}}$ and OXA-23 enzyme, respectively (GenBank accession no.EU022368 and ABU24822). Nucleotide sequence alignment of these *A. baumannii* and sequence accession no.EU022368 are shown in appendix F.

A total of 12 representative *A. baumannii* isolates habouring $bla_{\text{OXA51-like}}$ were selected for DNA sequencing, including A98, A200, A232, A251, A252, A253, A254, A258, A259, A299, A300 and A341. The isolates haboured various types of $bla_{\text{OXA-like}}$ as shown in table 18. The MIC range of imipenem and meropenem were 32 to >256 µg/ml and 16 to 128 µg/ml, respectively. The OXA-51 group was intrinsic enzyme in *A. baumannii* and showed the low level of carbapenem hydrolytic activity. In our study, these isolates had co-existence of $bla_{\text{OXA51-like}}$ and other $bla_{\text{OXA-like}}$ which caused high level of imipenem and meropenem MIC. DNA sequence analysis of the 1,416-bp fragments in 12 representative *A. baumannii* revealed an open reading frame of 825 bp, encoding a 274 amino acids. Of 12 isolates, 7 isolates showed 100% nucleotide sequences and amino acid sequences identity to $bla_{\text{OXA-66}}$ and OXA-66

enzyme, respectively (GenBank accession no.FJ360530 and ACP34154). One isolate shared 100% nucleotide sequence and amino acid sequence identity to *bla*$_{\text{OXA-69}}$ and OXA-69 enzyme, respectively (GenBank accession no.CU459141 and CAM86994). Four isolates shared 99% nucleotide sequences and amino acid sequences identity to *bla*$_{\text{OXA-65}}$ and OXA-65 (GenBank accession no.AY750908 and AAW81337), respectively. Nucleotide sequence alignment of these *A. baumannii* and sequence accession no. FJ360530, no.CU459141 and no.AY750908 are shown in appendix F.

A total of 4 *A. baumannii* isolates habouring *bla*$_{\text{OXA58-like}}$ were performed DNA sequencing included A98, A200, A232 and A341. The MIC of imipenem was 64 µg/ml and meropenem were 16 to 32 µg/ml as shown in table 18. DNA sequence analysis of the 933-bp fragments in 4 *A. baumannii* revealed an open reading frame of 841 bp, encoding a 280 amino acids. They shared 100% nucleotide sequences and amino acid sequences identity to *bla*$_{\text{OXA-58}}$ and OXA-58 enzyme, respectively (GenBank accession no.HQ219687 and ADQ27319). Nucleotide sequence alignment of all 4 *A. baumannii* isolates and sequence accession no.HQ219687 are shown in appendix F.

Table 18. Sequencing analysis of entire *bla*$_{\text{OXA-like}}$

Isolates	MIC (µg/ml)		Presence of *bla* genes				Type of *bla* genes
	Imipenem	Meropenem	*bla*$_{\text{OXA-23-like}}$	*bla*$_{\text{OXA-24-like}}$	*bla*$_{\text{OXA-51-like}}$	*bla*$_{\text{OXA-58-like}}$	
A98	64	32	+	-	+	+	*bla*$_{\text{OXA-23,}}$ *bla*$_{\text{OXA-65}}$ and *bla*$_{\text{OXA-58}}$
A200	64	16	+	-	+	+	*bla*$_{\text{OXA-23,}}$ *bla*$_{\text{OXA-65}}$ and *bla*$_{\text{OXA-58}}$
A232	64	16	+	-	+	+	*bla*$_{\text{OXA-23,}}$ *bla*$_{\text{OXA-65}}$ and *bla*$_{\text{OXA-58}}$
A341	64	32	+	-	+	+	*bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-65}}$ and *bla*$_{\text{OXA-58}}$
A253	128	32	+	-	+	-	*bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-66}}$
A254	128	32	+	-	+	-	*bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-66}}$
A252	256	64	+	-	+	-	*bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-66}}$
A258	256	64	+	-	+	-	*bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-66}}$
A259	256	64	+	-	+	-	*bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-66}}$
A300	256	64	+	-	+	-	*bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-66}}$
A251	>256	128	+	-	+	-	*bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-66}}$
A299	32	16	+	-	+	-	*bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-69}}$
A292	128	64	+	-	+	-	*bla*$_{\text{OXA-23}}$
A294	256	64	+	-	+	-	*bla*$_{\text{OXA-23}}$
A297	256	32	+	-	+	-	*bla*$_{\text{OXA-23}}$

PART X: DETERMINATION OF CARBAPENEM EFFLUX PUMP IN *A. BAUMANNII*

The efflux pump activity was detected by agar dilution with or without carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), the proton pump inhibitor. The decrease of carbapenem MIC equal or greater than 4-fold is the criteria for efflux pump positive. There were 453 isolates of *A. baumannii* tested for the presence of efflux pump. The samples were selected by type of bla_{OXA} and level of MIC for imipenem and meropenem. The effect of CCCP on 453 *A. baumannii* isolates are shown in Appendix H.

In the presence of CCCP, imipenem and meropenem MICs were decreased for 2-fold in 63 (15.7%) isolates and 97 (24.1%) isolates, respectively. There were 339 (84.1%) isolates and 305 (75.7%) isolates which had no change in imipenem and meropenem MICs in the presence of CCCP. The results are summarized in table 19. The susceptible isolates had no change in imipenem and meropenem MICs. The efflux pump mechanism was not found in our carbapenem-resistant isolates.

Table 19. The effect of CCCP among 402 carbapenem-resistant *A. baumannii* isolates.

Antimicrobial agents	No. of fold decreased	No. of isolates (%)	MIC range (ug/ml)	
			no CCCP	CCCP
Imipenem	2	63(15.7%)	64-512	32-256
	-	339(84.1%)	32-512	32-512
Meropenem	2	97(24.1%)	32-128	16-64
	-	305(75.7%)	16-256	16-256

- = no change in MIC

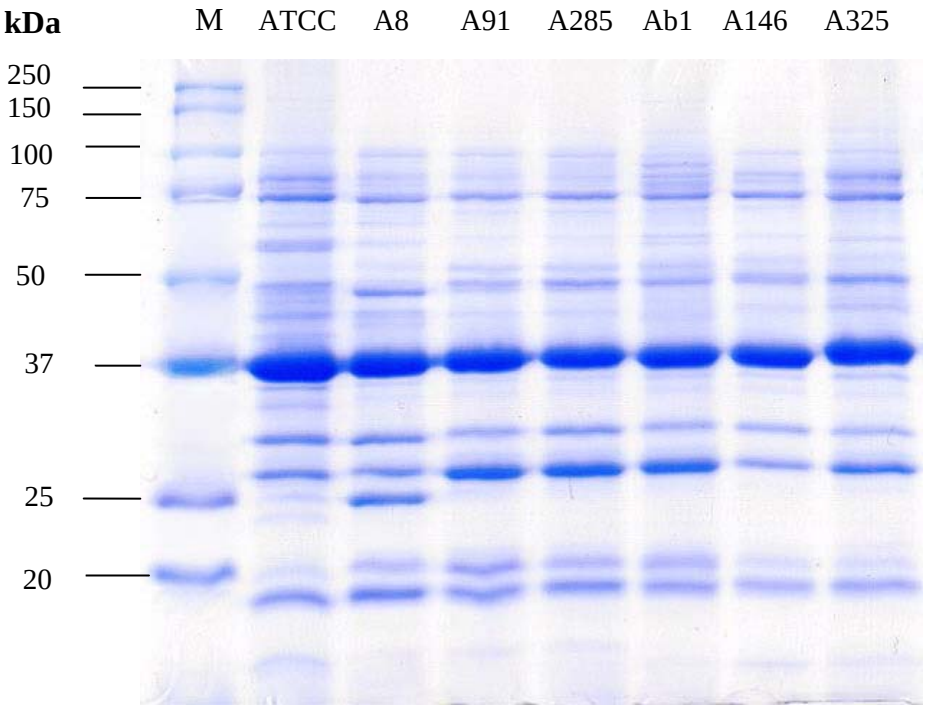
PART XI : DETERMINATION OF OUTER MEMBRANE PROTEIN IN CARBAPENEM-RESISTANT *A. BAUMANNII*

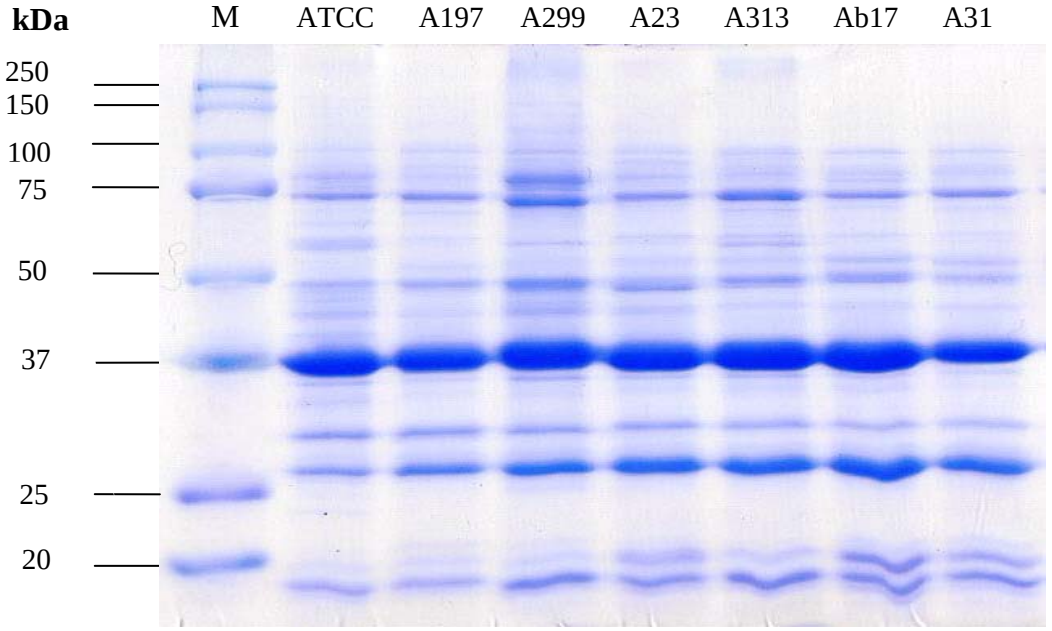
Outer membrane proteins were analyzed by SDS-PAGE in 23 selected isolates. The isolates were selected by different types of bla_{OXA} and level of MIC for imipenem and meropenem. Among 23 *A. baumannii* isolates, 21 were resistant to imipenem and meropenem and 2 isolates were susceptible to these antimicrobial agents. The MICs for imipenem-resistant isolates ranged from 32 to > 256 µg/ml, whereas the MIC of meropenem-resistant isolates ranged from 16 to 256 µg/ml.

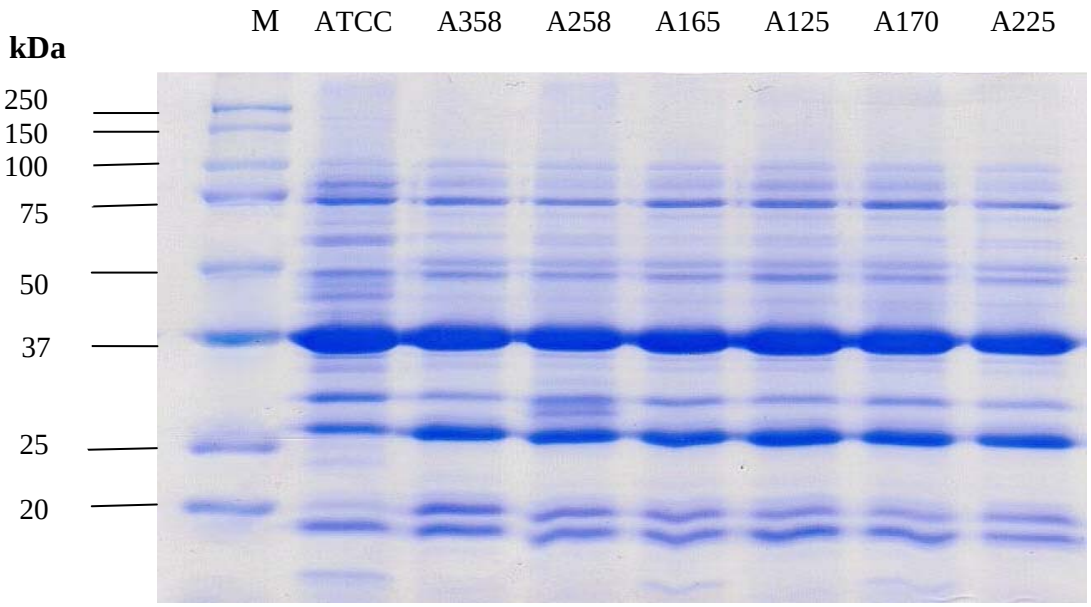
The OMP profiles were devided into 4 patterns in SDS-PAGE gels. OMP profile A was observed in 19 isolates. These isolates contained 8 major OMP bands of 19 kDa, 20 kDa, 26 kDa, 29 kDa, 37 kDa, 43 kDa, 48 kDa and 72 kDa. The OMP profile B was found in 2 isolates which showed 9 major bands. This profile differed from profile A in the presence of the 81 kDa OMP. OMP profile C differed from OMP pattern A by containing an additional band of the 28 kDa OMP. There is only 1 isolate in this profile. OMP profile D had 1 member differed from profile A in the presence of 25 kDa and 47 kDa OMP and in the absence of 48 kDa. The results are summarized in Figure 24.

OMP profile A was found in 19 isolates of *A. baumannii* (A313, A53, A146, A197, A98, A341, A125, A358, A200, A232, A91, A225, Ab17, A31, A165, A285, A170, A265, Ab1). Eighteen isolates were resistant to carbapenems and 1 isolate was susceptible to carbapenems. The MIC range of imipenem and meropenem of this profile ranged from 1 to >256 µg/ml and 0.5 to 256 µg/ml, respectively. OMP profile B (A299 and A325) was found in 2 isolates and both isolates were resistant to carbapenems. The MICs of imipenem and meropenem were both 32 µg/ml and 16 µg/ml, respectively. The OMP profile C (A258) was found in only 1 isolate which was resistant to carbapenems. The MICs of imipenem and meropenem were 256 µg/ml and 64 µg/ml, respectively. The fourth profile was profile D (A8) which was observed in only one susceptible isolate. The MICs of imipenem and meropenem were 2 µg/ml and 1 µg/ml, respectively. Five carbapenem-resistant *A. baumannii* isolates had profile A (A53, A91, A197 and A265) and C (A258) showed the reduction of 43 kDa OMP. The MICs of imipenem and meropenem in 5 isolates

which reduced 43 kDa OMP ranged from 64 µg/ml to 256 µg/ml and 32 to 64 µg/ml, respectively. The results are summarized in Table 20.






M
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A358
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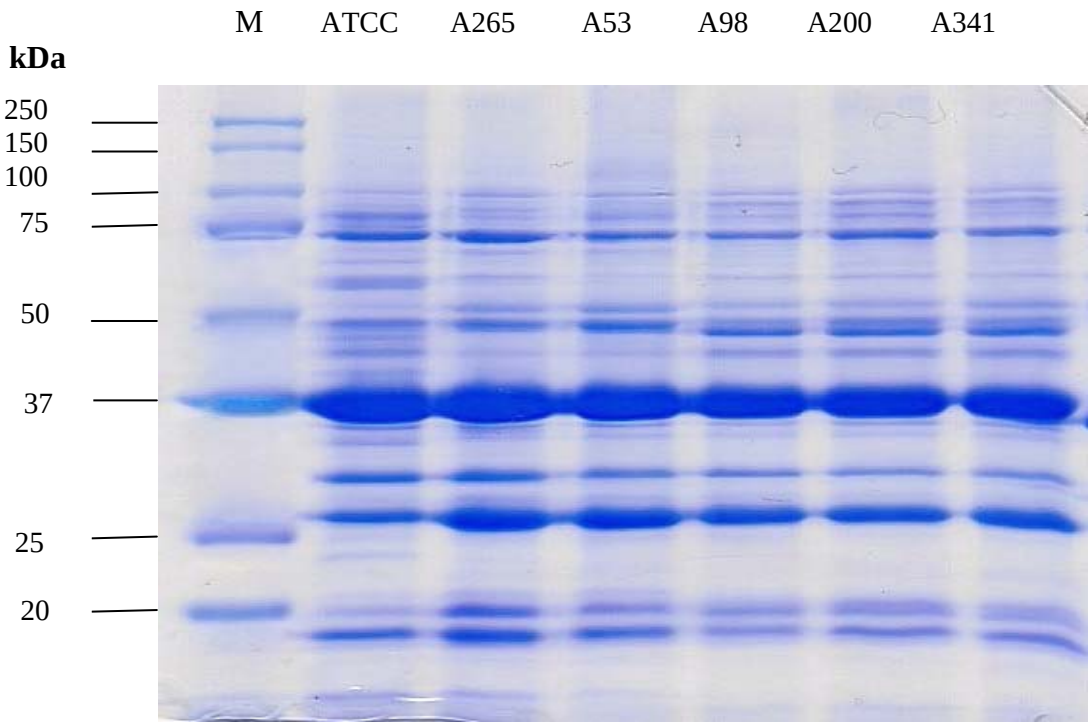
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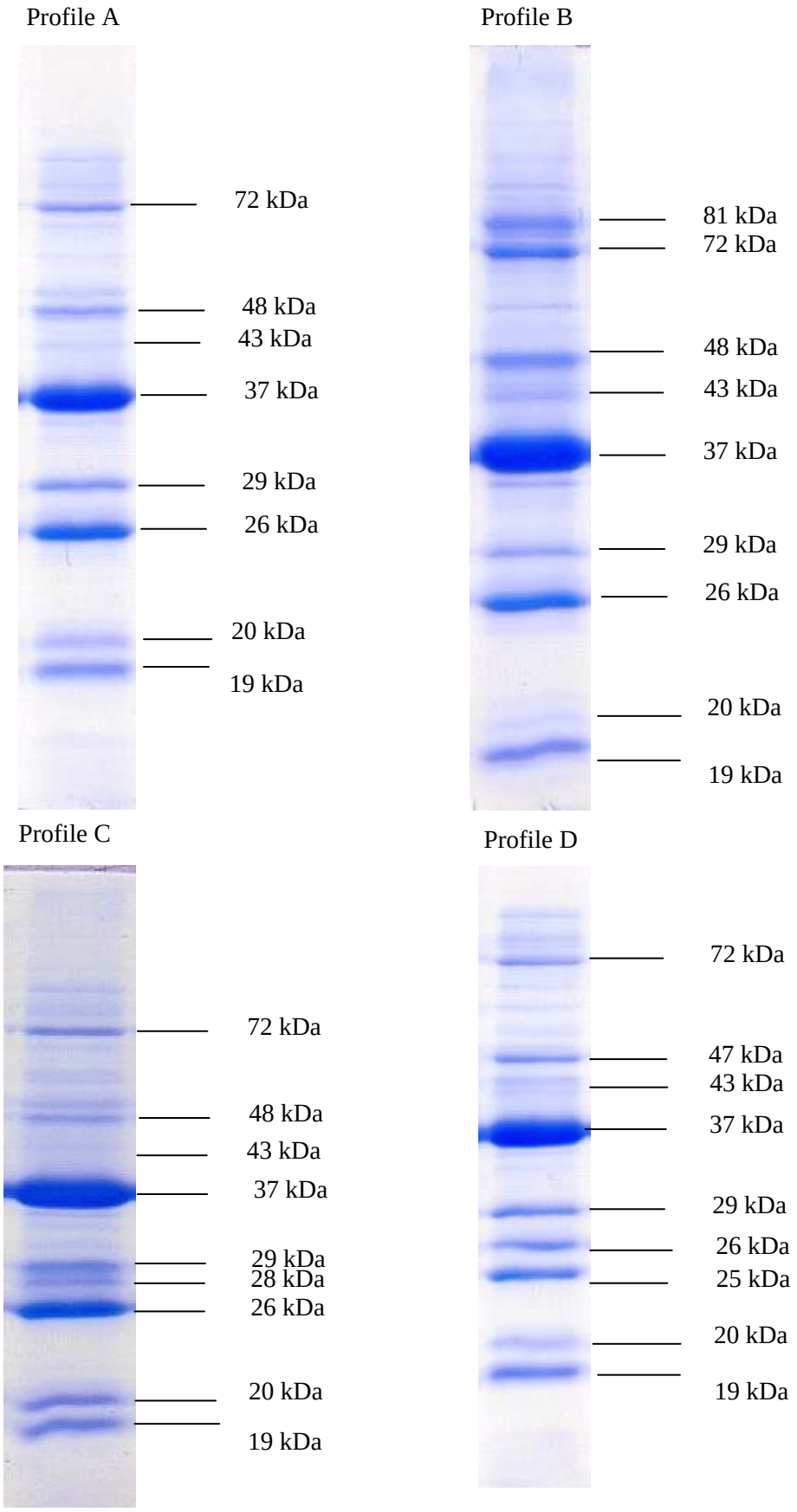

Figure 24. Outer membrane protein profile in 23 *A. baumannii* isolates

Table 20. The outer membrane profiles of 23 representative *A. baumannii*

Isolates	Imipenem MIC (µg/ml)	Meropenem MIC (µg/ml)	*bla*$_{OXA\text{-}like}$				OMP profile	Reduced OMPs
			bla$_{OXA\text{-}23}$	*bla*$_{OXA\text{-}24}$	*bla*$_{OXA\text{-}51}$	*bla*$_{OXA\text{-}58}$		
A313	1	0.5	-	-	+	-	A	Negative
A8	2	1	-	-	+	-	D	Negative
A299	32	16	+	-	+	-	B	Negative
A325	32	16	+	-	+	-	B	Negative
A53	64	32	+	-	+	-	A	Positive
A146	64	32	+	-	+	-	A	Negative
A197	64	32	+	-	+	-	A	Positive
A98	64	32	+	-	+	+	A	Negative
A341	64	32	+	-	+	+	A	Negative
A125	64	32	+	-	+	-	A	Negative
A358	64	16	+	-	+	-	A	Negative
A200	64	16	+	-	+	+	A	Negative
A232	64	16	+	-	+	+	A	Negative
A91	128	64	+	-	+	-	A	Positive
A225	128	64	+	-	+	-	A	Negative
Ab17	128	32	+	-	+	-	A	Negative
A31	128	32	+	-	+	-	A	Negative
A165	128	32	+	-	+	-	A	Negative
A258	256	64	+	-	+	-	C	Positive
A285	256	64	+	-	+	-	A	Negative
A170	256	64	+	-	+	-	A	Negative
A265	256	64	+	-	+	-	A	Positive
Ab1	>256	256	-	+	+	-	A	Negative

CHAPTER VI

DISCUSSION

Acinetobacter baumannii is a major opportunistic nosocomial pathogen. Carbapenems are the very broad spectrum antibiotics used to treat *A. baumannii* in serious infection. Unfortunately, carbapenem-resistant *A. baumannii* has been increasingly reported worldwide. The MYSTIC (Meropenem Year Susceptibility Test Information Collection) programme reported imipenem and meropenem resistance rate of *A. baumannii* in European countries including Belgium, Croatia, Czech Republic, Finland, Germany, Greece, Poland, Russia, Spain, Sweden, Turkey and United Kingdom increased from 16% and 18%, respectively in 1997-2000 (123) to 42.5% and 43.4%, respectively in 2006 (124). In 2003, prevalence of imipenem resistance in *A. baumannii* isolated from Spain was 40% (122). Carbapenem resistance in *A. baumannii* isolated from Iran was 50% in 2008 (12) and high rate of resistance was reported to be 70.1% in Greece (128). During 2006-2009, meropenem resistance rate of *A. baumannii* in Mexico was 59% (11). In Thailand, the study from Police General Hospital showed that imipenem resistance rate of *A. baumannii* was 61% in 2003 (46). Carbapenems resistance rate of *A. baumannii* was 60% in 2005 at Maharaj Nakorn Chiang Mai Hospital (47) and 68% in 2006 at Siriraj Hospital (13). In 2007, imipenem resistantce rate of *A. baumannii* isolated from Songklanagarind Hospital was 34.7% (48). In this study, all 453 *A. baumannii* isolates were collected from King Chulalongkorn Memorial Hospital, Bangkok, Thailand between March 2008 and April 2010. The resistant rates of imipenem and meropenem was 88.7%. The results showed high prevalence of carbapenem resistance. This is similar to the larger Tigecycline Evaluation and Surveillance Trial (TEST) programme which reported imipenem resistance in 32 countries to be 91.5% in 2010.

OXA-23 carbapenemase enzyme was the first OXA-type characterized from Scotland in 1985. It was first described as ARI-1 from imipenem-resistant *A. baumannii*. Although $bla_{\text{OXA-23-like}}$ was first reported from plasmid but it was also found on chromosome. Villgas *et al*. reported that 3 of 4 clones of carbapenem-resistant *A. baumannii* isolates had the $bla_{\text{OXA-23}}$ gene on the chromosome and one

clone on plasmid (23). Mugnier *et al*. demonstrated that 10 of 20 isolates carried *bla*$_{\text{OXA-23}}$ gene on the chromosome, 9 isolates had *bla*$_{\text{OXA-23}}$ gene on plasmid and 1 isolates haboured *bla*$_{\text{OXA-23}}$ gene on both the chromosome and plasmid (22). Our study showed that *bla*$_{\text{OXA-23-like}}$ was present in 88.5% of all 453 *A. baumannii* isolates and was found in 99.8% of imipenem- and meropenem-resistant isolates. Many outbreaks of *bla*$_{\text{OXA-23-like}}$ genes in *A. baumannii* isolates were reported in many countries. The *bla*$_{\text{OXA-23-like}}$ was identified in all carbapenem-resistant *A. baumannii* isolates from Bulgaria (26) and China in 2009 (139). Carvalho *et al.* reported that prevalence of *bla*$_{\text{OXA-23-like}}$ was 87.3% in Brazil (50). In Thailand, Nuimsup *et al*. (140) and Thapa *et al*. (131) showed high prevalence of *bla*$_{\text{OXA-23-like}}$ (100%), similar to our study. This suggests that OXA-23 is disseminated in Thailand and worldwide. OXA-23 group comprises of OXA-23, OXA-27 and OXA-49. In our study, we detected *bla*$_{\text{OXA-23}}$ in all fifteen representative isolates by sequencing entire *bla*$_{\text{OXA-23-like}}$. Corvec *et al*. investigated the genetic structure surrounding of the *bla*$_{\text{OXA-23}}$ and showed that IS*Aba1* and IS*Aba4* upstream *bla*$_{\text{OXA-23}}$ were on Tn*2006* and Tn*2007*, respectively. IS*Aba1* was located 34 bp upstream of the start codon of *bla*$_{\text{OXA-23}}$ and the +1 transcription start was located inside IS*Aba1* and upstream *bla*$_{\text{OXA-23}}$ for 60 bp. The -35 sequence (TTAGAA) and -10 sequence (TTATTT) were separated by 16 bp (112). In our study, IS*Aba1* was found upstream of *bla*$_{\text{OXA-23}}$ in all representative *A. baumannii* isolates and the genetic structure of IS*Aba1* and *bla*$_{\text{OXA-23}}$ were at the same location as that of Corvec *et al*..

OXA-24 group comprises of OXA-24, OXA-25, OXA-26 and OXA-72 enzymes. These enzyme was first identified as chromosomally encoded in carbapenem-resistant *A. baumannii* isolated from Spain (108). The *bla*$_{\text{OXA24-like}}$ gene was reported to be on both plasmid and chromosome (31). The high prevalence of carbapenem-resistant *A. baumannii* habouring *bla*$_{\text{OXA-24-like}}$ was observed in Spain and Portugal (10, 29). The *bla*$_{\text{OXA-24-like}}$ was also reported in USA, Czech Republic and Belgium (30-32). In Asia, high prevalence of *bla*$_{\text{OXA24-like}}$ in carbapenem-resistant *A. baumannii* isolates was found in Taiwan (33) and was also detected in Thailand, Indonesia and Iran (12, 28). In this study, *bla*$_{\text{OXA24-like}}$ was found in only 1 isolate with the MIC of imipenem and meropenem of > 256 µg/ml and 256 µg/ml, respectively. The study of Afzal-Shah *el al.* showed that carbapenemase activity of OXA-25 and

OXA-26 enzymes was higher than OXA-27, the member of OXA-23 group. The *bla*$_{\text{OXA-24-like}}$ gene was not found to be located in mobile genetic elements such as insertion sequence, transposon and integron. The mobilization of *bla*$_{\text{OXA-24-like}}$ was associated with site-specific recombination binding site including XerC and XerD binding sites (141-143).

The first report of OXA-51 group was from Argentina in 2004 (144). These enzymes had weakly hydrolytic activity. The *bla*$_{\text{OXA-51-like}}$ was an intrinsic gene in *A. baumannii*. Several studies showed that all strains of *A. baumannii* carried this gene. In this study, all of our *A. baumannii* isolates haboured *bla*$_{\text{OXA-51-like}}$. This result is similar to the studies of Turton *et al*. in 2006 (145), Merkier *et al.* in 2006 (146) and Wang *et al.* in 2007 (147). However, Koh *et al.* reported from Singapore in 2007 that not all *A. baumannii* isolates (77.3%) had *bla*$_{\text{OXA-51-like}}$ (148). In this study, sequencing analysis of entire showed that *bla*$_{\text{OXA-51-like}}$ genes were the *bla*$_{\text{OXA-66}}$ (7 isolates, 58.3%), *bla*$_{\text{OXA-65}}$ (4 isolates, 33.3%) and *bla*$_{\text{OXA-69}}$ (1 isolate, 8.4%). The *bla*$_{\text{OXA-66}}$ was reported from Greece, China, Taiwan and Spain (149-152). The *bla*$_{\text{OXA-65}}$ was identified from Argentina (153). The *bla*$_{\text{OXA-69}}$ was detected from Greece, Brazil and Norway (37, 154, 155). The *bla*$_{\text{OXA-51-like}}$ has been associated with IS*Aba1*. The IS*Aba1* element upstream *bla*$_{\text{OXA-51-like}}$ provides promoter for enhancing of *bla*$_{\text{OXA-51-like}}$ expression (29).

OXA-58 was first described by Poirel *et al.* in 2003 from France. The members of this group were OXA-58, OXA-96 and OXA-97. (37). In our study, *bla*$_{\text{OXA-58-like}}$ was found together with *bla*$_{\text{OXA-23-like}}$ and *bla*$_{\text{OXA-51-like}}$ in 4 carbapenem-resistant isolates. Sequencing analysis revealed that all 4 isolates were *bla*$_{\text{OXA-58}}$. Ruiz *et al*. in 2007 reported that 20% of 83 imipenem-resistant *A. baumannii* isolates contained *bla*$_{\text{OXA-58-like}}$ (29). The study of Giannouli *et al.*of multiple Mediterranean hospitals including Greece, Italy, Lebanon, and Turkey reported that all 21 *A. baumannii* isolates from ICU had *bla*$_{\text{OXA-58-like}}$ (156). Tsakris *et al.* from Greece reported that *bla*$_{\text{OXA-58-like}}$ was found in 26 of 31 imipenem-resistant *A. baumannii* isolates (149). Pournaras *et al.* demonstrated that *bla*$_{\text{OXA-58-like}}$ was detected in 14 of 17 of imipenem-resistant *A. baumannii* isolates in the outbreak in ICU from Greece (37). In our study, all 4 *A. baumannii* isolates showed high level resistance. However,

we could not exclude the activity of OXA-23 in these isolates. However, Tsakris *et al.* reported widespread *bla*$_{\text{OXA-58-like}}$ which exhibited weak carbapenemase activity (157). The *bla*$_{\text{OXA-58-like}}$ was also reported from Italy (34), Iran (12) and China (158). In 2007, *bla*$_{\text{OXA-96}}$ was found in one of 44 imipenem-resistant *A. baumannii* isolated from Singapore (148). The *bla*$_{\text{OXA-97}}$ was detected in 19 in 39 carbapenem-resistant *A. baumannii* isolated from Tunesia (38). The *bla*$_{\text{OXA-58-like}}$ has been involved in a variety of genetic structure. IS*Aba1*, IS*Aba2*, IS*Aba3* and IS18 upstream *bla*$_{\text{OXA-58}}$ were described to provide the promoter sequence enhancing the expression of *bla*$_{\text{OXA-58}}$ (113).

The first report of IS*Aba1* was from France in 2003. Corvec *et al.* described the presence of insertion sequence upstream of *bla*$_{ampC}$ gene in ceftazidime-resistant *A. baumannii* isolates (159). Turton *et al.* reported that IS*Aba1* upstream *bla*$_{\text{OXA-51-like}}$ and *bla*$_{\text{OXA-23-like}}$ was found in carbapenem non-susceptible *A. baumannii* isolates (160). Corvec *et al.* demonstrated that -35 and -10 sequences located in IS*Aba1* provided strong promoter to *bla*$_{\text{OXA-23-like}}$ in imipenem-resistant isolates (112). In 2006, Poirel *et al.* studied genetic structure surrounding *bla*$_{\text{OXA-58-like}}$ and found that IS*Aba1* was detected upstream of *bla*$_{\text{OXA-58-like}}$ and IS*Aba2*- IS*Aba3-like* (113). IS*Aba4* was located in the upstream region of *bla*$_{\text{OXA-23-like}}$ on the Tn*2007* (112). In this study, IS*Aba1* was found upstream of *bla*$_{\text{OXA-23-like}}$ in all representative isolates but it was not present upstream of *bla*$_{\text{OXA-51-like}}$. This result is similar to that of Stoeva *et al*. from Bulgaria in 2008 which reported that all 29 carbapenem-resistant *A. baumannii* had IS*Aba1* in the upstream region of *bla*$_{\text{OXA-23-like}}$ but IS*Aba1* was not detected upstream of *bla*$_{\text{OXA-51-like}}$ (26). The results are in agreement with Jubelle *et al*. in 2007 from Australia (161). Thapa *et al.* also reported that all 37 carbapenem-resistant *A. baumannii* isolated from Thailand had IS*Aba1* upstream *bla*$_{\text{OXA-23-like}}$ (131). This is in contrast to the study of Ruiz *et al*. from Spain which showed that five representative isolates had IS*Aba1* located upstream of *bla*$_{\text{OXA-51-like}}$ (29). In this study, the -35 and -10 sequence located in IS*Aba1* upstream *bla*$_{\text{OXA-23-like}}$ was identical to that of Genbank accession No.EF127491 (112). The MIC of imipenem and merpenem of 38 representative isolates showed high level resistance, suggesting that IS*Aba1* may provide the strong promoter for *bla*$_{\text{OXA-23-like}}$. IS*Aba3* which was in the upstream region of *bla*$_{\text{OXA-58-like}}$ was found in all of our isolates, similar to the

study by Giannouli *et al* (162). The -35 and -10 sequence was identical to those of Genbank accession No.DQ987830 in the study of Poirel *et al*. (113).

Metallo-β-lactamases in *A. baumannii* was less common than OXA-type carbapenemases. Carbapenem resistance level of MBLs is 100-1000 fold more than OXA-type carbapenemases (100). Metallo-β-lactamase genes in *A. baumannii* included $bla_{\text{IMP-like}}$, $bla_{\text{VIM-like}}$ and $bla_{\text{SIM-like}}$. In our study, all 453 *A. baumannii* isolates had no metallo-β-lactamases. Our results are similar to the study of Zhou *et al.* from China in 2007 which showed that the majority of imipenem-resistant isolates had $bla_{\text{OXA-23-like}}$ and $bla_{\text{IMP-like}}$, $bla_{\text{VIM-like}}$ and $bla_{\text{SIM-like}}$ were not detected (27). Metallo-β-lactamase genes were not found in all 92 isolates of meropenem-resistant *A. baumannii* isolates but $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-58-like}}$ were found in 64.1%, 1.1% and 2.2%, respectively (33). Papa *et al.* also reported 0% of $bla_{\text{IMP-like}}$ and $bla_{\text{VIM-like}}$ in 164 carbapenem-resistant *A. baumannii* isolated from Greek hospital (163). In contrast to the study of Sung *et al*. which reported that 31 carbapenem-resistant *A. baumannii* isolates had $bla_{\text{IMP-1}}$(15 isolates) and $bla_{\text{VIM-2}}$ (1 isolate), whereas, the $bla_{\text{OXA-23}}$ was found in 7 isolates. Tsakris *et al.* reported the high prevalence of $bla_{\text{VIM-1}}$, $bla_{\text{VIM-4}}$ and $bla_{\text{OXA-58}}$ in 31 imipenem-resistant *A. baumannii* isolates from Greece in 2008 (149).

AdeABC are efflux pumps belonging to resistance-nodulation-division (RND) family. The wide range of antibiotics such as aminoglicosides, fluoroquinolones, erythromycin, tetracyclines, trimethoprim, chloramphenicol, ethidium bromide, β-lactams and tigecycline are substrates of these pumps (92, 93, 116, 164). Overexpression of AdeABC efflux pump conferred carbapenem resistance in *A. baumannii*. In our study, the MIC of imipenem and meropenem in carbapenem-resistant isolates were not detected by CCCP. Our results demonstrated that the mechanism of efflux pump was not involved in carbapenem resistance in our *A. baumannii* isolates. Several studies reported the absence of efflux pump mechanism in carbapenem-resistant *A. baumannii*. Quale *et al.* studied meropenem efflux pump in 2003 from New York and showed that no isolate had this resistance mechanism (165). Similarly, Pournaras *et al*. from Greece in 2006, reported that no isolate were significantly decreased in MIC of imipenem in the presence of CCCP

(37). Jeong *et al*. reported from Korea in 2009 that 1 of 17 imipenem-resistant *A. baumannii* isolates had decreased level of MIC in the presence of CCCP (39). In 2010, Huang *et al*. showed that MIC of imipenem was decreased 4-fold in 1 of 22 isolates of imipenem-resistant *A. baumannii* from China (40). However, the study of Huang *et al*. reported from China in 2008 that MIC of meropenem in several isolates was decreased at least 4-fold after CCCP added (41). Although AdeABC system can efflux wide range of antibiotics but it predominated in aminoglycosides and quinolones. Overexpression of efflux pump can combine with other resistance mechanisms such as OXA-type carbapenemases which cause high level of carbapenem resistance (166).

The modification of outer membrane permeability was another mechanism of carbapenem resistance in *A. baumannii*. In 1996, Clark reported that imipenem-resistant *A. baumannii* isolates reduced expression of a 33-36 kDa outer membrane protein (44). Limansky *et al*. reported from Argentina in 2002 that imipenem resistance in *A. baumannii* caused by the loss of a 29 kDa outer membrane protein (43). In 2005, Dupont reported loss of 29 kDa and 43 kDa outer membrane protein in imipenem-resistant *A. baumannii* isolate from France (42). Analysis of 43 kDa outer membrane protein by MALDI-MS showed that this outer membrane protein was homologous to OprD in *P. aeruginosa* (42). Similar to our study, the 43 kDa OMP was found to decrease in 5 isolates from 21 carbapenem-resistant *A. baumannii* isolates. In contrast to the study from Zarrilli *et al* in 2008 from Labanese hospital, the decrease in outer membrane protein was not found in any isolate of imipenem-resistant *A. baumannii* (167).

Several studies demonstrated the combination of decreased OMP with other resistance mechanisms. The study of Bou *et al*. in 2000 showed the presence of $bla_{\text{OXA-24}}$ and the decrease of 22 kDa and 33 kDa outer membrane proteins (168). Costa *et al*. from Brazil in 2000 reported that a loss of 31-36 kDa and production of β-lactamase caused imipenem resistance in *A. baumannii* isolates (45). The study of Fernandez-Cuenca *et al*. in 2003 showed that the loss of 22.5 kDa OMP, the absence of PBP_2 and the production of β-lactamases were the causes of imipenem resistance in *A. baumannii*

CHAPTER VII

CONCLUSION

Increasing number of multidrug-resistant *A. baumannii* has been reported worldwide. Carbapenems are the drug of choices for the treatment of multidrug-resistant *A. baumannii* for many years. Unfortunately, carbapenem-resistant *A. baumannii* has been global increasingly reported. In this study, a total of 453 *A. baumannii* isolates obtained from patients in King Chulalongkorn Memorial Hospital between March 2008 and April 2010 were studied. The prevalence of imipenem and meropenem resistance were both 88.7%. MIC_{50} and MIC_{90} of imipenem were 128 µg/ml and 256 µg/ml, respectively. MIC_{50} and MIC_{90} of meropenem were 64 µg/ml and 128 µg/ml, respectively. High resistance rates of imipenem and meropenem was found in both ICU and non-ICU isolates.

Of 453 *A. baumannii* isolates, 11.3% were susceptible to imipenem and meropenem and were negative for carbapenemase activity. These isolates had only the $bla_{\text{OXA-51-like}}$. All *A. baumannii* isolates with carbapenemase activity (88.7%) were resistant to both imipenem and meropenem. The co-existence of $bla_{\text{OXA-51-like}}$ and other $bla_{\text{OXA-like}}$ including $bla_{\text{OXA-51-like}}$ together with $bla_{\text{OXA-23-like}}$ (99.8%), $bla_{\text{OXA-51-like}}$ together with $bla_{\text{OXA-24-like}}$ (0.2%) and $bla_{\text{OXA-51-like}}$ together with $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-58-like}}$ (4%) was found in these isolates. Insertion sequence, IS*Aba1*, was found in the upstream region of $bla_{\text{OXA-23-like}}$ in all 38 representative isolates. DNA sequences upstream $bla_{\text{OXA-23-like}}$ gene showed IS*Aba1*-mediated -35 and -10 promoter sequences. IS*Aba3* was present upstream $bla_{\text{OXA-58-like}}$ in all 4 isolates and also showed the -35 and -10 promoter sequences. The results suggest that IS*Aba1* and IS*Aba3* play an important role in the expression of the bla_{OXA} genes in our isolates.

DNA sequencing analysis of 15 entire $bla_{\text{OXA-23-like}}$ and 4 entire $bla_{\text{OXA-58-like}}$ genes revealed that $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-58}}$ were identified in 100% of $bla_{\text{OXA-23-like}}$

and *bla*$_{\text{OXA-58-like}}$. Twelve entire *bla*$_{\text{OXA-51-like}}$ sequences were *bla*$_{\text{OXA-66}}$ (58.3%), *bla*$_{\text{OXA-65}}$ (33.3%) and *bla*$_{\text{OXA-69}}$ (8.4%).

In the study of efflux pump mechanism, the exposure of effux pump inhibitor, CCCP, could not reduce the MIC of imipenem and meropenem equal or more than 4-fold. The result showed no efflux pump mechanism in all of the 159 representative isolates. The reduction of 43 kDa OMP was detected in 5 of the 21 representative carbapenem-resistant *A. baumannii* isolates but 29 kDa and 33-36 kDa were not lost or reduced in this study. Our results showed high rate of carbapenem resistance in *A. baumannii* and the main mechanism of carbapenem resistance was due to the production of OXA-23-like carbapenemases.

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APPENDICES

APPENDIX A

REAGENTS, MATERIALS AND INSTRUMENTS

A. REAGENTS

Absolute ethanol	(Merck, Germany)
Ammonium persulfate (APS)	(Amresco, USA)
β-Mercapto Ethanol	(Merck, Germany)
Boric acid	(Sigma, USA)
Bromophenol Blue	(BIO-RAD, USA)
CCCP	(Sigma, USA)
Coomassie Brilliant Blue R-250	(BIO-RAD, USA)
dNTPs	(Promega, USA)
EDTA	(Amresco, USA)
Ethidium bromide	(Amresco, USA)
Glacial acetic acid	(Merck, Germany)
HCl	(Merck, Germany)
LB broth	(Pronadisa, Spain)
MacConkey agar	(Oxoid, England)
Methanol	(Merck, Germany)
Methylene bis Acrylamide	Methylene bis Acrylamide
Muller-Hinton II agar	(BBL, USA)
NaCl	(Merck, Germany)
NaOH	(Sigma, USA)
Taq DNA Polymerase	(Fermentas, USA)
TEMED	TEMED
Tris	(Amresco, USA)
Trytic soy agar	(BBL, USA)
Trytic soy broth	(BBL, USA)
100 bp DNA ladder	(Fermentas, USA)
100 bp plus DNA ladder	(Fermentas, USA)

Sodium lauroyl sarcosinate	(Sigma, USA)
SDS	(Merck, Germany)

B. METERIALS

-

C. INSTRUMENTS

Automatic pipette	(Gilson, Lyon, France)
Beckman Optima^{TM} TLX Ultracentrifuge	(Beckman, USA)
Camera Gel Doc^{TM} 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)
High intensity ultrasonic processor	(Sonics, USA)
Beckman Avantis^{TM} J-25I Centrifuge	(Beckman, USA)
Hettich zentrifuge	(Hettich, Germany)

APPENDIX B

MEDIA AND ANTIBIOTIC SOLUTION PREPARATION

1. MacConkey agar (Oxoid, England)

Suspend 51.5 grams in 1,000 ml of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121^{o}C for 15 minutes. Once the medium is prepared, store at 4^{o}C.

2. 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^{o}C (15 lbs. sp) for 15 minutes. Store medium at 4^{o}C.

3. Muller-Hinton II agar (BBL, USA)

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

4. Tryptic soy broth (BBL, USA)

Suspend 30 grams in 1,000 ml of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121^{o}C for 15 minutes. Once the medium is prepared, store at 4^{o}C.

5. Trytic soy agar (BBL, USA)

Suspend 40 grams in 1,000 ml of distilled water. Bring to the boil to dissolve completely. Sterilize at 121^{o}C for 15 minutes by autoclaving. Once the medium is prepared, store at 4^{o}C.

6. 0.85% NaCl (Merck, Germany)

Suspend 8.5 grams in 1,000 ml of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121^{o}C for 15 minutes. Once the medium is prepared, store at room temperature.

7. Triple Sugar Iron agar (Oxoid, England)

Suspend 65 grams in 1,000 ml of distilled water. Bring to the boil to dissolve completely. Mix well and distribute into containers. Sterilize by autoclaving at 121^{o}C for 15 minutes. Allow to set as slopes with 2.5 cm butts. Once the medium is prepared, store at 4^{o}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 by autoclaving at 121^{o}C (15 lbs. sp) for 15 minutes. Once the medium is prepared, store at 4^{o}C.

9. 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 and sterilize by filtration (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 th agar is dissolved. Sterilize by autoclaving at 121^{o}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 by incubate tubes at 37^{o}C for 24 hours. Store tubes in refrigerator at 4^{o}C until used.

10. Motility

Suspend 8.5 grams in 1,000 ml of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121^{o}C for 15 minutes. Once the medium is prepared, store at room temperature.

11. Simmon citrate

Suspend 24.2 grams in 1,000 ml of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121^{o}C for 15 minutes. Once the medium is prepared, store at room temperature.

Antibiotic solution preparation

Imipenem, stock concentration 5120 µg/ml

- Prepare a stock solution; dissolve 0.0512 g in 0.1 N NaOH and 4 ml distilled water

Meropenem, stock concentration 5120 µg/ml

- Prepare a stock solution; dissolve 0.0256 g in 5 ml distilled water.

Netilmycin, stock concentration 5120 µg/ml

- Prepare a stock solution; dissolve 256 µl in 4744 µl distilled water.

Piperacillin/tazobactam, stock concentration 5120 µg/ml

- Prepare a stock solution; dissolve 0.0256 g in 5 ml distilled water.

Colistin, stock concentration 5120 µg/ml

- Prepare a stock solution; dissolve 0.0256 g in 5 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 1,000 ml with distilled water. The solution was mixed and sterilized by autoclaving at 121^{o}C for 15 minutes.

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 1,000 ml. 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^{o}C for 15 minutes.

4. 1 % Agarose gel

Agarose	1	g
0.5X TBE	100	ml

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

5. 6X 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 and aliquot into 1.5 microtubes. Store at 4^{o}C.

7. 10 mM Tris HCl pH 8.0

Tris	1.21	g
Distilled water	1	L

Adjust to pH 8.0 by adding conc. HCl. Adjust volume to 1,000 ml and sterilized by autoclaving at 121^{o}C for 15 minutes.

8. SDS-PAGE Staining solution

Coomassie Brilliant Blue R-250	2.5	g
Methanol	500	ml
Glacial acetic acid	100	ml
Distilled water	400	ml

Keep flask on dark at room temperature.

9. SDS-PAGE Destaining solution

10% acetic acid

10. SDS-PAGE Sample buffer (6X)

Glycerol	3	ml
SDS	1	g
β-Mercapto Ethanol	0.5	ml
0.5 mM Tris-HCl (pH 6.8)	6	ml
Brom phenol blue	0.0012	g

aliquots of 1 ml, keep at -20 C

11. SDS-PAGE Running buffer (5X)

Tris	15.1	g
Glycine	72	g
SDS	5	g
Distilled water	1	L

Keep at RT

12. 12% polyacrylamide gel (separating gel)

DDW	4.4	ml
40% Acrylamide /Bis	3	ml
1.5 M Tris-HCl pH 8.8	2.5	ml
10% SDS	0.1	ml
10% APS	50	µl
TEMED	5	µl

Add TEMED and APS at the end

13. 4% polyacrylamide gel (stacking gel)

DDW	6.7	ml
40% Acrylamide /Bis	0.97	ml
0.5 M Tris-HCl pH 6.8	2.5	ml
10% SDS	0.1	ml
10% APS	50	µl
TEMED	10	µl

Add TEMED and APS at the end.

15. Isopropanol fixing solution (500ml)

100% acetic acid	5	ml
100% isopropanol	31.25	ml
DDW	463.75	ml

16. Equilibrating solution (500ml)

Glycerol	20	ml
70% ethanol	71.25	ml
DDW	408.75	ml

17. 10% APS

Ammonium persulfate	100	mg
DDW	1	ml

18. Comassie blue stain

Acetic acid 10% (v/v)

Comassie blue dye 0.006% (w/v)

ddH_20 90%

19. Isopropanol fixing solution

Acetic acid 10% (v/v)	30	ml
Isopropanol 25% (v/v)	75	ml
ddH_20 65%	195	ml

20. 1.5M Tris-HCl, pH 8.8

Tris base	27.23	g
DDW	80	ml

Adjust to pH 8.0 by adding conc. HCl. Adjust volume to 150 ml and sterilized by autoclaving at 121^{o}C for 15 minutes.

21. 0.5 M Tris-HCl, pH 6.8

Tris base	6	g
DDW	60	ml

Adjust to pH 6.8 by adding conc. HCl. Adjust volume to 100 ml and sterilized by autoclaving at 121^{o}C for 15 minutes.

APPENDIX D

THE RESULTS OF ALL TESTS IN THIS STUDY

Results of imipenem and meropenem susceptibility, carbapenemase phenotype, MBLs phenotype and the presence of *bla* genes of 453 A. *baumannii* isolates

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			*bla*$_{\text{OXA23-like}}$	*bla*$_{\text{OXA24-like}}$	*bla*$_{\text{OXA51-like}}$	*bla*$_{\text{OXA58-like}}$	*bla*$_{\text{IMP-like}}$	*bla*$_{\text{VIM-like}}$	*bla*$_{\text{SIM-1}}$	*bla*$_{\text{SPM-1}}$	*bla*$_{\text{GIM-1}}$
1	Ab1	>256	256	+	-	-	+	+	-	-	-	-	-	-
2	Ab2	256	128	+	-	+	-	+	-	-	-	-	-	-
3	Ab3	256	128	+	-	+	-	+	-	-	-	-	-	-
4	Ab4	256	64	+	-	+	-	+	-	-	-	-	-	-
5	Ab6	256	32	+	-	+	-	+	-	-	-	-	-	-
6	Ab7	1	1	-	-	-	-	+	-	-	-	-	-	-
7	Ab8	128	64	+	-	+	-	+	-	-	-	-	-	-
8	Ab9	128	64	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			$bla_{\text{OXA23-like}}$	$bla_{\text{OXA24-like}}$	$bla_{\text{OXA51-like}}$	$bla_{\text{OXA58-like}}$	$bla_{\text{IMP-like}}$	$bla_{\text{VIM-like}}$	$bla_{\text{SIM-1}}$	$bla_{\text{SPM-1}}$	$bla_{\text{GIM-1}}$
9	Ab10	128	64	+	-	+	-	+	-	-	-	-	-	-
10	Ab11	>256	128	+	-	+	-	+	-	-	-	-	-	-
11	Ab13	128	128	+	-	+	-	+	-	-	-	-	-	-
12	Ab14	256	64	+	-	+	-	+	-	-	-	-	-	-
13	Ab16	>256	128	+	-	+	-	+	-	-	-	-	-	-
14	Ab17	128	32	+	-	+	-	+	-	-	-	-	-	-
15	Ab18	2	1	-	-	-	-	+	-	-	-	-	-	-
16	Ab19	1	0.25	-	-	-	-	+	-	-	-	-	-	-
17	Ab20	256	64	+	-	+	-	+	-	-	-	-	-	-
18	Ab21	256	64	+	-	+	-	+	-	-	-	-	-	-
19	Ab22	256	64	+	-	+	-	+	-	-	-	-	-	-
20	Ab23	128	32	+	-	+	-	+	-	-	-	-	-	-
21	Ab24	2	1	-	-	-	-	+	-	-	-	-	-	-
22	Ab26	128	64	+	-	+	-	+	-	-	-	-	-	-
23	Ab27	256	64	+	-	+	-	+	-	-	-	-	-	-
24	Ab28	4	2	-	-	-	-	+	-	-	-	-	-	-
25	Ab29	1	0.5	-	-	-	-	+	-	-	-	-	-	-
26	Ab30	256	128	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			$bla_{\text{OXA23-like}}$	$bla_{\text{OXA24-like}}$	$bla_{\text{OXA51-like}}$	$bla_{\text{OXA58-like}}$	$bla_{\text{IMP-like}}$	$bla_{\text{VIM-like}}$	$bla_{\text{SIM-1}}$	$bla_{\text{SPM-1}}$	$bla_{\text{GIM-1}}$
27	Ab31	128	64	+	-	+	-	+	-	-	-	-	-	-
28	Ab32	128	64	+	-	+	-	+	-	-	-	-	-	-
29	Ab33	4	2	-	-	-	-	+	-	-	-	-	-	-
30	Ab34	1	0.5	-	-	-	-	+	-	-	-	-	-	-
31	Ab35	64	32	+	-	+	-	+	-	-	-	-	-	-
32	Ab36	128	32	+	-	+	-	+	-	-	-	-	-	-
33	Ab37	256	128	+	-	+	-	+	-	-	-	-	-	-
34	Ab38	256	128	+	-	+	-	+	-	-	-	-	-	-
35	Ab39	1	1	-	-	-	-	+	-	-	-	-	-	-
36	Ab40	256	64	+	-	+	-	+	-	-	-	-	-	-
37	Ab41	128	32	+	-	+	-	+	-	-	-	-	-	-
38	Ab42	1	0.5	-	-	-	-	+	-	-	-	-	-	-
39	Ab43	128	32	+	-	+	-	+	-	-	-	-	-	-
40	Ab44	128	32	+	-	+	-	+	-	-	-	-	-	-
41	Ab45	128	32	+	-	+	-	+	-	-	-	-	-	-
42	Ab46	128	32	+	-	+	-	+	-	-	-	-	-	-
43	Ab47	256	128	+	-	+	-	+	-	-	-	-	-	-
44	Ab48	256	32	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			$bla_{\text{OXA23-like}}$	$bla_{\text{OXA24-like}}$	$bla_{\text{OXA51-like}}$	$bla_{\text{OXA58-like}}$	$bla_{\text{IMP-like}}$	$bla_{\text{VIM-like}}$	$bla_{\text{SIM-1}}$	$bla_{\text{SPM-1}}$	$bla_{\text{GIM-1}}$
45	Ab49	256	64	+	-	+	-	+	-	-	-	-	-	-
46	Ab50	256	64	+	-	+	-	+	-	-	-	-	-	-
47	Ab52	256	64	+	-	+	-	+	-	-	-	-	-	-
48	Ab53	256	64	+	-	+	-	+	-	-	-	-	-	-
49	Ab54	128	32	+	-	+	-	+	-	-	-	-	-	-
50	Ab55	256	64	+	-	+	-	+	-	-	-	-	-	-
51	Ab56	128	64	+	-	+	-	+	-	-	-	-	-	-
52	Ab57	2	0.5	-	-	-	-	+	-	-	-	-	-	-
53	Ab58	256	32	+	-	+	-	+	-	-	-	-	-	-
54	Ab59	>256	64	+	-	+	-	+	-	-	-	-	-	-
55	Ab60	64	32	+	-	+	-	+	-	-	-	-	-	-
56	Ab61	128	32	+	-	+	-	+	-	-	-	-	-	-
57	Ab62	256	64	+	-	+	-	+	-	-	-	-	-	-
58	Ab63	256	64	+	-	+	-	+	-	-	-	-	-	-
59	Ab64	128	32	+	-	+	-	+	-	-	-	-	-	-
60	Ab65	1	0.25	-	-	-	-	+	-	-	-	-	-	-
61	Ab66	128	32	+	-	+	-	+	-	-	-	-	-	-
62	Ab67	128	32	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			$bla_{\text{OXA23-like}}$	$bla_{\text{OXA24-like}}$	$bla_{\text{OXA51-like}}$	$bla_{\text{OXA58-like}}$	$bla_{\text{IMP-like}}$	$bla_{\text{VIM-like}}$	$bla_{\text{SIM-1}}$	$bla_{\text{SPM-1}}$	$bla_{\text{GIM-1}}$
63	Ab68	256	64	+	-	+	-	+	-	-	-	-	-	-
64	Ab69	128	32	+	-	+	-	+	-	-	-	-	-	-
65	Ab70	>256	32	+	-	+	-	+	-	-	-	-	-	-
66	Ab72	1	0.25	-	-	-	-	+	-	-	-	-	-	-
67	Ab73	1	0.5	-	-	-	-	+	-	-	-	-	-	-
68	Ab74	256	64	+	-	+	-	+	-	-	-	-	-	-
69	Ab75	>256	64	+	-	+	-	+	-	-	-	-	-	-
70	Ab76	256	64	+	-	+	-	+	-	-	-	-	-	-
71	Ab78	256	32	+	-	+	-	+	-	-	-	-	-	-
72	Ab79	128	32	+	-	+	-	+	-	-	-	-	-	-
73	Ab81	128	32	+	-	+	-	+	-	-	-	-	-	-
74	Ab82	64	16	+	-	+	-	+	-	-	-	-	-	-
75	Ab83	256	64	+	-	+	-	+	-	-	-	-	-	-
76	Ab84	128	64	+	-	+	-	+	-	-	-	-	-	-
77	Ab85	4	1	-	-	-	-	+	-	-	-	-	-	-
78	Ab86	256	64	+	-	+	-	+	-	-	-	-	-	-
79	Ab87	256	64	+	-	+	-	+	-	-	-	-	-	-
80	Ab88	256	64	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			*bla*$_{\text{OXA23-like}}$	*bla*$_{\text{OXA24-like}}$	*bla*$_{\text{OXA51-like}}$	*bla*$_{\text{OXA58-like}}$	*bla*$_{\text{IMP-like}}$	*bla*$_{\text{VIM-like}}$	*bla*$_{\text{SIM-1}}$	*bla*$_{\text{SPM-1}}$	*bla*$_{\text{GIM-1}}$
81	Ab89	1	1	-	-	-	-	+	-	-	-	-	-	-
82	Ab90	64	16	+	-	+	-	+	-	-	-	-	-	-
83	Ab91	>256	256	+	-	+	-	+	-	-	-	-	-	-
84	Ab92	1	0.5	-	-	-	-	+	-	-	-	-	-	-
85	Ab93	64	16	+	-	+	-	+	-	-	-	-	-	-
86	Ab94	256	64	+	-	+	-	+	-	-	-	-	-	-
87	Ab95	256	64	+	-	+	-	+	-	-	-	-	-	-
88	Ab96	128	32	+	-	+	-	+	-	-	-	-	-	-
89	Ab97	128	16	+	-	+	-	+	-	-	-	-	-	-
90	Ab98	64	16	+	-	+	-	+	-	-	-	-	-	-
91	Ab100	128	64	+	-	+	-	+	-	-	-	-	-	-
92	A2	128	32	+	-	+	-	+	-	-	-	-	-	-
93	A3	>256	128	+	-	+	-	+	-	-	-	-	-	-
94	A5	256	64	+	-	+	-	+	-	-	-	-	-	-
95	A6	128	32	+	-	+	-	+	-	-	-	-	-	-
96	A7	128	32	+	-	+	-	+	-	-	-	-	-	-
97	A8	2	1	-	-	-	-	+	-	-	-	-	-	-
98	A9	128	64	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			$bla_{\text{OXA23-like}}$	$bla_{\text{OXA24-like}}$	$bla_{\text{OXA51-like}}$	$bla_{\text{OXA58-like}}$	$bla_{\text{IMP-like}}$	$bla_{\text{VIM-like}}$	$bla_{\text{SIM-1}}$	$bla_{\text{SPM-1}}$	$bla_{\text{GIM-1}}$
99	A10	128	64	+	-	+	-	+	-	-	-	-	-	-
100	A11	128	32	+	-	+	-	+	-	-	-	-	-	-
101	A12	4	2	-	-	-	-	+	-	-	-	-	-	-
102	A13	256	64	+	-	+	-	+	-	-	-	-	-	-
103	A15	256	64	+	-	+	-	+	-	-	-	-	-	-
104	A16	256	64	+	-	+	-	+	-	-	-	-	-	-
105	A17	256	64	+	-	+	-	+	-	-	-	-	-	-
106	A18	64	32	+	-	+	-	+	-	-	-	-	-	-
107	A19	1	0.25	-	-	-	-	+	-	-	-	-	-	-
108	A20	128	32	+	-	+	-	+	-	-	-	-	-	-
109	A21	2	0.5	-	-	-	-	+	-	-	-	-	-	-
110	A22	256	64	+	-	+	-	+	-	-	-	-	-	-
111	A24	256	64	+	-	+	-	+	-	-	-	-	-	-
112	A26	256	64	+	-	+	-	+	-	-	-	-	-	-
113	A27	256	64	+	-	+	-	+	-	-	-	-	-	-
114	A28	1	0.5	-	-	-	-	+	-	-	-	-	-	-
115	A29	64	16	+	-	+	-	+	-	-	-	-	-	-
116	A30	256	64	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			$bla_{\text{OXA23-like}}$	$bla_{\text{OXA24-like}}$	$bla_{\text{OXA51-like}}$	$bla_{\text{OXA58-like}}$	$bla_{\text{IMP-like}}$	$bla_{\text{VIM-like}}$	$bla_{\text{SIM-1}}$	$bla_{\text{SPM-1}}$	$bla_{\text{GIM-1}}$
117	A31	128	32	+	-	+	-	+	-	-	-	-	-	-
118	A32	256	64	+	-	+	-	+	-	-	-	-	-	-
119	A33	128	32	+	-	+	-	+	-	-	-	-	-	-
120	A34	256	128	+	-	+	-	+	-	-	-	-	-	-
121	A35	256	128	+	-	+	-	+	-	-	-	-	-	-
122	A36	256	128	+	-	+	-	+	-	-	-	-	-	-
123	A38	128	32	+	-	+	-	+	-	-	-	-	-	-
124	A39	128	32	+	-	+	-	+	-	-	-	-	-	-
125	A40	64	32	+	-	+	-	+	-	-	-	-	-	-
126	A42	64	32	+	-	+	-	+	-	-	-	-	-	-
127	A43	128	64	+	-	+	-	+	-	-	-	-	-	-
128	A44	>256	128	+	-	+	-	+	-	-	-	-	-	-
129	A45	256	64	+	-	+	-	+	-	-	-	-	-	-
130	A46	128	32	+	-	+	-	+	-	-	-	-	-	-
131	A47	128	32	+	-	+	-	+	-	-	-	-	-	-
132	A48	256	64	+	-	+	-	+	-	-	-	-	-	-
133	A50	128	32	+	-	+	-	+	-	-	-	-	-	-
134	A51	256	64	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			$bla_{\text{OXA23-like}}$	$bla_{\text{OXA24-like}}$	$bla_{\text{OXA51-like}}$	$bla_{\text{OXA58-like}}$	$bla_{\text{IMP-like}}$	$bla_{\text{VIM-like}}$	$bla_{\text{SIM-1}}$	$bla_{\text{SPM-1}}$	$bla_{\text{GIM-1}}$
135	A52	128	128	+	-	+	-	+	-	-	-	-	-	-
136	A53	64	32	+	-	+	-	+	-	-	-	-	-	-
137	A54	128	128	+	-	+	-	+	-	-	-	-	-	-
138	A55	>256	64	+	-	+	-	+	-	-	-	-	-	-
139	A56	256	64	+	-	+	-	+	-	-	-	-	-	-
140	A57	128	64	+	-	+	-	+	-	-	-	-	-	-
141	A58	128	64	+	-	+	-	+	-	-	-	-	-	-
142	A59	64	32	+	-	+	-	+	-	-	-	-	-	-
143	A60	256	64	+	-	+	-	+	-	-	-	-	-	-
144	A61	256	128	+	-	+	-	+	-	-	-	-	-	-
145	A63	256	128	+	-	+	-	+	-	-	-	-	-	-
146	A64	256	64	+	-	+	-	+	-	-	-	-	-	-
147	A65	256	128	+	-	+	-	+	-	-	-	-	-	-
148	A66	256	64	+	-	+	-	+	-	-	-	-	-	-
149	A67	256	64	+	-	+	-	+	-	-	-	-	-	-
150	A68	256	64	+	-	+	-	+	-	-	-	-	-	-
151	A69	128	64	+	-	+	-	+	-	-	-	-	-	-
152	A71	128	64	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			*bla*$_{\text{OXA23-like}}$	*bla*$_{\text{OXA24-like}}$	*bla*$_{\text{OXA51-like}}$	*bla*$_{\text{OXA58-like}}$	*bla*$_{\text{IMP-like}}$	*bla*$_{\text{VIM-like}}$	*bla*$_{\text{SIM-1}}$	*bla*$_{\text{SPM-1}}$	*bla*$_{\text{GIM-1}}$
153	A72	>256	64	+	-	+	-	+	-	-	-	-	-	-
154	A73	256	64	+	-	+	-	+	-	-	-	-	-	-
155	A74	256	64	+	-	+	-	+	-	-	-	-	-	-
156	A75	2	0.5	-	-	-	-	+	-	-	-	-	-	-
157	A76	128	32	+	-	+	-	+	-	-	-	-	-	-
158	A78	256	64	+	-	+	-	+	-	-	-	-	-	-
159	A79	128	64	+	-	+	-	+	-	-	-	-	-	-
160	A80	128	64	+	-	+	-	+	-	-	-	-	-	-
161	A81	256	128	+	-	+	-	+	-	-	-	-	-	-
162	A82	256	128	+	-	+	-	+	-	-	-	-	-	-
163	A83	128	64	+	-	+	-	+	-	-	-	-	-	-
164	A84	128	64	+	-	+	-	+	-	-	-	-	-	-
165	A85	128	64	+	-	+	-	+	-	-	-	-	-	-
166	A86	128	64	+	-	+	-	+	-	-	-	-	-	-
167	A87	64	32	+	-	+	-	+	-	-	-	-	-	-
168	A88	64	32	+	-	+	-	+	-	-	-	-	-	-
169	A89	64	32	+	-	+	-	+	-	-	-	-	-	-
170	A90	128	128	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			*bla*$_{\text{OXA23-like}}$	*bla*$_{\text{OXA24-like}}$	*bla*$_{\text{OXA51-like}}$	*bla*$_{\text{OXA58-like}}$	*bla*$_{\text{IMP-like}}$	*bla*$_{\text{VIM-like}}$	*bla*$_{\text{SIM-1}}$	*bla*$_{\text{SPM-1}}$	*bla*$_{\text{GIM-1}}$
171	A91	128	64	+	-	+	-	+	-	-	-	-	-	-
172	A92	128	64	+	-	+	-	+	-	-	-	-	-	-
173	A94	128	32	+	-	+	-	+	-	-	-	-	-	-
174	A95	256	32	+	-	+	-	+	-	-	-	-	-	-
175	A96	2	2	-	-	-	-	+	-	-	-	-	-	-
176	A97	256	128	+	-	+	-	+	-	-	-	-	-	-
177	A98	64	32	+	-	+	-	+	+	-	-	-	-	-
178	A100	256	128	+	-	+	-	+	-	-	-	-	-	-
179	A101	64	32	+	-	+	-	+	-	-	-	-	-	-
180	A102	256	128	+	-	+	-	+	-	-	-	-	-	-
181	A103	64	32	+	-	+	-	+	-	-	-	-	-	-
182	A104	64	32	+	-	+	-	+	-	-	-	-	-	-
183	A105	128	32	+	-	+	-	+	-	-	-	-	-	-
184	A106	256	64	+	-	+	-	+	-	-	-	-	-	-
185	A107	256	128	+	-	+	-	+	-	-	-	-	-	-
186	A110	256	64	+	-	+	-	+	-	-	-	-	-	-
187	A111	128	64	+	-	+	-	+	-	-	-	-	-	-
188	A112	128	64	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			$bla_{\text{OXA23-like}}$	$bla_{\text{OXA24-like}}$	$bla_{\text{OXA51-like}}$	$bla_{\text{OXA58-like}}$	$bla_{\text{IMP-like}}$	$bla_{\text{VIM-like}}$	$bla_{\text{SIM-1}}$	$bla_{\text{SPM-1}}$	$bla_{\text{GIM-1}}$
189	A113	0.5	0.25	-	-	-	-	+	-	-	-	-	-	-
190	A114	128	64	+	-	+	-	+	-	-	-	-	-	-
191	A115	128	64	+	-	+	-	+	-	-	-	-	-	-
192	A116	128	64	+	-	+	-	+	-	-	-	-	-	-
193	A117	0.5	0.5	-	-	-	-	+	-	-	-	-	-	-
194	A118	128	64	+	-	+	-	+	-	-	-	-	-	-
195	A119	>256	64	+	-	+	-	+	-	-	-	-	-	-
196	A121	256	128	+	-	+	-	+	-	-	-	-	-	-
197	A122	256	128	+	-	+	-	+	-	-	-	-	-	-
198	A123	>256	128	+	-	+	-	+	-	-	-	-	-	-
199	A124	>256	128	+	-	+	-	+	-	-	-	-	-	-
200	A125	64	32	+	-	+	-	+	-	-	-	-	-	-
201	A126	128	64	+	-	+	-	+	-	-	-	-	-	-
202	A127	128	64	+	-	+	-	+	-	-	-	-	-	-
203	A128	128	64	+	-	+	-	+	-	-	-	-	-	-
204	A129	64	32	+	-	+	-	+	-	-	-	-	-	-
205	A130	128	64	+	-	+	-	+	-	-	-	-	-	-
206	A132	128	64	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			$bla_{\text{OXA23-like}}$	$bla_{\text{OXA24-like}}$	$bla_{\text{OXA51-like}}$	$bla_{\text{OXA58-like}}$	$bla_{\text{IMP-like}}$	$bla_{\text{VIM-like}}$	$bla_{\text{SIM-1}}$	$bla_{\text{SPM-1}}$	$bla_{\text{GIM-1}}$
207	A133	128	64	+	-	+	-	+	-	-	-	-	-	-
208	A134	128	64	+	-	+	-	+	-	-	-	-	-	-
209	A135	128	64	+	-	+	-	+	-	-	-	-	-	-
210	A137	128	64	+	-	+	-	+	-	-	-	-	-	-
211	A138	128	64	+	-	+	-	+	-	-	-	-	-	-
212	A139	128	32	+	-	+	-	+	-	-	-	-	-	-
213	A140	64	32	+	-	+	-	+	-	-	-	-	-	-
214	A141	64	32	+	-	+	-	+	-	-	-	-	-	-
215	A142	128	64	+	-	+	-	+	-	-	-	-	-	-
216	A143	128	64	+	-	+	-	+	-	-	-	-	-	-
217	A144	>256	256	+	-	+	-	+	-	-	-	-	-	-
218	A145	256	128	+	-	+	-	+	-	-	-	-	-	-
219	A146	64	32	+	-	+	-	+	-	-	-	-	-	-
220	A147	64	32	+	-	+	-	+	-	-	-	-	-	-
221	A148	32	16	+	-	+	-	+	-	-	-	-	-	-
222	A149	128	32	+	-	+	-	+	-	-	-	-	-	-
223	A150	128	64	+	-	+	-	+	-	-	-	-	-	-
224	A151	64	32	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			$bla_{\text{OXA23-like}}$	$bla_{\text{OXA24-like}}$	$bla_{\text{OXA51-like}}$	$bla_{\text{OXA58-like}}$	$bla_{\text{IMP-like}}$	$bla_{\text{VIM-like}}$	$bla_{\text{SIM-1}}$	$bla_{\text{SPM-1}}$	$bla_{\text{GIM-1}}$
225	A152	64	32	+	-	+	-	+	-	-	-	-	-	-
226	A153	64	32	+	-	+	-	+	-	-	-	-	-	-
227	A154	64	32	+	-	+	-	+	-	-	-	-	-	-
228	A155	128	64	+	-	+	-	+	-	-	-	-	-	-
229	A156	128	64	+	-	+	-	+	-	-	-	-	-	-
230	A157	256	64	+	-	+	-	+	-	-	-	-	-	-
231	A159	128	64	+	-	+	-	+	-	-	-	-	-	-
232	A162	128	64	+	-	+	-	+	-	-	-	-	-	-
233	A163	256	128	+	-	+	-	+	-	-	-	-	-	-
234	A164	256	128	+	-	+	-	+	-	-	-	-	-	-
235	A165	128	32	+	-	+	-	+	-	-	-	-	-	-
236	A166	128	64	+	-	+	-	+	-	-	-	-	-	-
237	A167	64	32	+	-	+	-	+	-	-	-	-	-	-
238	A168	64	16	+	-	+	-	+	-	-	-	-	-	-
239	A169	128	32	+	-	+	-	+	-	-	-	-	-	-
240	A170	128	64	+	-	+	-	+	-	-	-	-	-	-
241	A171	128	64	+	-	+	-	+	-	-	-	-	-	-
242	A173	128	64	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			$bla_{\text{OXA23-like}}$	$bla_{\text{OXA24-like}}$	$bla_{\text{OXA51-like}}$	$bla_{\text{OXA58-like}}$	$bla_{\text{IMP-like}}$	$bla_{\text{VIM-like}}$	$bla_{\text{SIM-1}}$	$bla_{\text{SPM-1}}$	$bla_{\text{GIM-1}}$
243	A174	128	64	+	-	+	-	+	-	-	-	-	-	-
244	A175	256	128	+	-	+	-	+	-	-	-	-	-	-
245	A176	256	64	+	-	+	-	+	-	-	-	-	-	-
246	A177	128	32	+	-	+	-	+	-	-	-	-	-	-
247	A178	256	128	+	-	+	-	+	-	-	-	-	-	-
248	A179	256	128	+	-	+	-	+	-	-	-	-	-	-
249	A180	128	16	+	-	+	-	+	-	-	-	-	-	-
250	A182	256	64	+	-	+	-	+	-	-	-	-	-	-
251	A183	256	64	+	-	+	-	+	-	-	-	-	-	-
252	A184	256	128	+	-	+	-	+	-	-	-	-	-	-
253	A185	256	64	+	-	+	-	+	-	-	-	-	-	-
254	A186	>256	64	+	-	+	-	+	-	-	-	-	-	-
255	A187	256	128	+	-	+	-	+	-	-	-	-	-	-
256	A188	256	64	+	-	+	-	+	-	-	-	-	-	-
257	A189	256	128	+	-	+	-	+	-	-	-	-	-	-
258	A190	256	64	+	-	+	-	+	-	-	-	-	-	-
259	A191	64	16	+	-	+	-	+	-	-	-	-	-	-
260	A192	256	128	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			$bla_{\text{OXA23-like}}$	$bla_{\text{OXA24-like}}$	$bla_{\text{OXA51-like}}$	$bla_{\text{OXA58-like}}$	$bla_{\text{IMP-like}}$	$bla_{\text{VIM-like}}$	$bla_{\text{SIM-1}}$	$bla_{\text{SPM-1}}$	$bla_{\text{GIM-1}}$
261	A193	256	128	+	-	+	-	+	-	-	-	-	-	-
262	A195	64	32	+	-	+	-	+	-	-	-	-	-	-
263	A196	256	64	+	-	+	-	+	-	-	-	-	-	-
264	A197	64	32	+	-	+	-	+	-	-	-	-	-	-
265	A198	64	16	+	-	+	-	+	-	-	-	-	-	-
266	A199	1	0.5	-	-	-	-	+	-	-	-	-	-	-
267	A200	64	16	+	-	+	-	+	+	-	-	-	-	-
268	A201	128	64	+	-	+	-	+	-	-	-	-	-	-
269	A202	128	64	+	-	+	-	+	-	-	-	-	-	-
270	A203	64	32	+	-	+	-	+	-	-	-	-	-	-
271	A204	4	0.25	-	-	-	-	+	-	-	-	-	-	-
272	A205	128	64	+	-	+	-	+	-	-	-	-	-	-
273	A206	128	64	+	-	+	-	+	-	-	-	-	-	-
274	A207	64	32	+	-	+	-	+	-	-	-	-	-	-
275	A208	64	16	+	-	+	-	+	-	-	-	-	-	-
276	A209	0.5	0.25	-	-	-	-	+	-	-	-	-	-	-
277	A211	128	64	+	-	+	-	+	-	-	-	-	-	-
278	A212	128	32	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			*bla*$_{\text{OXA23-like}}$	*bla*$_{\text{OXA24-like}}$	*bla*$_{\text{OXA51-like}}$	*bla*$_{\text{OXA58-like}}$	*bla*$_{\text{IMP-like}}$	*bla*$_{\text{VIM-like}}$	*bla*$_{\text{SIM-1}}$	*bla*$_{\text{SPM-1}}$	*bla*$_{\text{GIM-1}}$
279	A213	1	0.5	-	-	-	-	+	-	-	-	-	-	-
280	A214	128	64	+	-	+	-	+	-	-	-	-	-	-
281	A215	64	32	+	-	+	-	+	-	-	-	-	-	-
282	A216	1	0.5	-	-	-	-	+	-	-	-	-	-	-
283	A217	128	64	+	-	+	-	+	-	-	-	-	-	-
284	A218	64	32	+	-	+	-	+	-	-	-	-	-	-
285	A219	128	64	+	-	+	-	+	-	-	-	-	-	-
286	A220	128	64	+	-	+	-	+	-	-	-	-	-	-
287	A221	64	64	+	-	+	-	+	-	-	-	-	-	-
288	A222	256	64	+	-	+	-	+	-	-	-	-	-	-
289	A223	128	64	+	-	+	-	+	-	-	-	-	-	-
290	A224	128	32	+	-	+	-	+	-	-	-	-	-	-
291	A225	128	64	+	-	+	-	+	-	-	-	-	-	-
292	A226	1	0.5	-	-	-	-	+	-	-	-	-	-	-
293	A227	128	64	+	-	+	-	+	-	-	-	-	-	-
294	A228	128	64	+	-	+	-	+	-	-	-	-	-	-
295	A229	256	64	+	-	+	-	+	-	-	-	-	-	-
296	A230	128	64	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			$bla_{\text{OXA23-like}}$	$bla_{\text{OXA24-like}}$	$bla_{\text{OXA51-like}}$	$bla_{\text{OXA58-like}}$	$bla_{\text{IMP-like}}$	$bla_{\text{VIM-like}}$	$bla_{\text{SIM-1}}$	$bla_{\text{SPM-1}}$	$bla_{\text{GIM-1}}$
297	A231	0.5	0.25	-	-	-	-	+	-	-	-	-	-	-
298	A232	64	16	+	-	+	-	+	+	-	-	-	-	-
299	A233	128	32	+	-	+	-	+	-	-	-	-	-	-
300	A234	128	64	+	-	+	-	+	-	-	-	-	-	-
301	A235	128	32	+	-	+	-	+	-	-	-	-	-	-
302	A237	128	64	+	-	+	-	+	-	-	-	-	-	-
303	A238	64	32	+	-	+	-	+	-	-	-	-	-	-
304	A239	128	32	+	-	+	-	+	-	-	-	-	-	-
305	A240	256	64	+	-	+	-	+	-	-	-	-	-	-
306	A241	128	64	+	-	+	-	+	-	-	-	-	-	-
307	A242	128	32	+	-	+	-	+	-	-	-	-	-	-
308	A243	128	64	+	-	+	-	+	-	-	-	-	-	-
309	A245	128	32	+	-	+	-	+	-	-	-	-	-	-
310	A246	128	64	+	-	+	-	+	-	-	-	-	-	-
311	A247	128	64	+	-	+	-	+	-	-	-	-	-	-
312	A248	128	32	+	-	+	-	+	-	-	-	-	-	-
313	A249	128	64	+	-	+	-	+	-	-	-	-	-	-
314	A250	128	64	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			$bla_{\text{OXA23-like}}$	$bla_{\text{OXA24-like}}$	$bla_{\text{OXA51-like}}$	$bla_{\text{OXA58-like}}$	$bla_{\text{IMP-like}}$	$bla_{\text{VIM-like}}$	$bla_{\text{SIM-1}}$	$bla_{\text{SPM-1}}$	$bla_{\text{GIM-1}}$
315	A251	>256	128	+	-	+	-	+	-	-	-	-	-	-
316	A252	256	64	+	-	+	-	+	-	-	-	-	-	-
317	A253	128	32	+	-	+	-	+	-	-	-	-	-	-
318	A254	128	32	+	-	+	-	+	-	-	-	-	-	-
319	A255	128	32	+	-	+	-	+	-	-	-	-	-	-
320	A256	64	32	+	-	+	-	+	-	-	-	-	-	-
321	A257	128	32	+	-	+	-	+	-	-	-	-	-	-
322	A258	256	64	+	-	+	-	+	-	-	-	-	-	-
323	A259	256	64	+	-	+	-	+	-	-	-	-	-	-
324	A260	1	0.25	-	-	-	-	+	-	-	-	-	-	-
325	A261	2	0.25	-	-	-	-	+	-	-	-	-	-	-
326	A262	256	128	+	-	+	-	+	-	-	-	-	-	-
327	A263	256	64	+	-	+	-	+	-	-	-	-	-	-
328	A264	2	1	-	-	-	-	+	-	-	-	-	-	-
329	A265	256	64	+	-	+	-	+	-	-	-	-	-	-
330	A266	128	32	+	-	+	-	+	-	-	-	-	-	-
331	A268	128	32	+	-	+	-	+	-	-	-	-	-	-
332	A270	2	0.5	-	-	-	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			*bla*$_{\text{OXA23-like}}$	*bla*$_{\text{OXA24-like}}$	*bla*$_{\text{OXA51-like}}$	*bla*$_{\text{OXA58-like}}$	*bla*$_{\text{IMP-like}}$	*bla*$_{\text{VIM-like}}$	*bla*$_{\text{SIM-1}}$	*bla*$_{\text{SPM-1}}$	*bla*$_{\text{GIM-1}}$
333	A272	128	32	+	-	+	-	+	-	-	-	-	-	-
334	A274	256	64	+	-	+	-	+	-	-	-	-	-	-
335	A275	256	64	+	-	+	-	+	-	-	-	-	-	-
336	A276	256	64	+	-	+	-	+	-	-	-	-	-	-
337	A277	256	64	+	-	+	-	+	-	-	-	-	-	-
338	A279	256	64	+	-	+	-	+	-	-	-	-	-	-
339	A280	128	16	+	-	+	-	+	-	-	-	-	-	-
340	A281	4	1	-	-	-	-	+	-	-	-	-	-	-
341	A282	2	0.25	-	-	-	-	+	-	-	-	-	-	-
342	A283	4	0.5	-	-	-	-	+	-	-	-	-	-	-
343	A284	128	32	+	-	+	-	+	-	-	-	-	-	-
344	A285	256	64	+	-	+	-	+	-	-	-	-	-	-
345	A286	128	32	+	-	+	-	+	-	-	-	-	-	-
346	A287	256	64	+	-	+	-	+	-	-	-	-	-	-
347	A288	128	32	+	-	+	-	+	-	-	-	-	-	-
348	A289	2	1	-	-	-	-	+	-	-	-	-	-	-
349	A290	64	16	+	-	+	-	+	-	-	-	-	-	-
350	A291	256	64	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			*bla*$_{\text{OXA23-like}}$	*bla*$_{\text{OXA24-like}}$	*bla*$_{\text{OXA51-like}}$	*bla*$_{\text{OXA58-like}}$	*bla*$_{\text{IMP-like}}$	*bla*$_{\text{VIM-like}}$	*bla*$_{\text{SIM-1}}$	*bla*$_{\text{SPM-1}}$	*bla*$_{\text{GIM-1}}$
351	A292	128	64	+	-	+	-	+	-	-	-	-	-	-
352	A293	2	0.25	-	-	-	-	+	-	-	-	-	-	-
353	A294	256	64	+	-	+	-	+	-	-	-	-	-	-
354	A295	4	1	-	-	-	-	+	-	-	-	-	-	-
355	A296	256	32	+	-	+	-	+	-	-	-	-	-	-
356	A297	256	32	+	-	+	-	+	-	-	-	-	-	-
357	A299	32	16	+	-	+	-	+	-	-	-	-	-	-
358	A300	256	64	+	-	+	-	+	-	-	-	-	-	-
359	A301	32	16	+	-	+	-	+	-	-	-	-	-	-
360	A302	0.5	0.5	-	-	-	-	+	-	-	-	-	-	-
361	A303	128	64	+	-	+	-	+	-	-	-	-	-	-
362	A304	128	64	+	-	+	-	+	-	-	-	-	-	-
363	A305	128	32	+	-	+	-	+	-	-	-	-	-	-
364	A306	2	1	-	-	-	-	+	-	-	-	-	-	-
365	A310	128	32	+	-	+	-	+	-	-	-	-	-	-
366	A311	1	0.5	-	-	-	-	+	-	-	-	-	-	-
367	A312	128	32	+	-	+	-	+	-	-	-	-	-	-
368	A313	1	0.5	-	-	-	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			$bla_{\text{OXA23-like}}$	$bla_{\text{OXA24-like}}$	$bla_{\text{OXA51-like}}$	$bla_{\text{OXA58-like}}$	$bla_{\text{IMP-like}}$	$bla_{\text{VIM-like}}$	$bla_{\text{SIM-1}}$	$bla_{\text{SPM-1}}$	$bla_{\text{GIM-1}}$
369	A314	128	64	+	-	+	-	+	-	-	-	-	-	-
370	A315	64	16	+	-	+	-	+	-	-	-	-	-	-
371	A317	2	0.5	-	-	-	-	+	-	-	-	-	-	-
372	A318	32	16	+	-	+	-	+	-	-	-	-	-	-
373	A319	0.5	0.5	-	-	-	-	+	-	-	-	-	-	-
374	A320	128	32	+	-	+	-	+	-	-	-	-	-	-
375	A321	64	32	+	-	+	-	+	-	-	-	-	-	-
376	A322	64	16	+	-	+	-	+	-	-	-	-	-	-
377	A323	128	64	+	-	+	-	+	-	-	-	-	-	-
378	A324	128	64	+	-	+	-	+	-	-	-	-	-	-
379	A325	32	16	+	-	+	-	+	-	-	-	-	-	-
380	A326	128	32	+	-	+	-	+	-	-	-	-	-	-
381	A327	128	32	+	-	+	-	+	-	-	-	-	-	-
382	A328	128	32	+	-	+	-	+	-	-	-	-	-	-
383	A329	64	16	+	-	+	-	+	-	-	-	-	-	-
384	A330	128	64	+	-	+	-	+	-	-	-	-	-	-
385	A331	128	32	+	-	+	-	+	-	-	-	-	-	-
386	A332	128	32	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			*bla*$_{\text{OXA23-like}}$	*bla*$_{\text{OXA24-like}}$	*bla*$_{\text{OXA51-like}}$	*bla*$_{\text{OXA58-like}}$	*bla*$_{\text{IMP-like}}$	*bla*$_{\text{VIM-like}}$	*bla*$_{\text{SIM-1}}$	*bla*$_{\text{SPM-1}}$	*bla*$_{\text{GIM-1}}$
387	A333	128	32	+	-	+	-	+	-	-	-	-	-	-
388	A334	>256	64	+	-	+	-	+	-	-	-	-	-	-
389	A336	256	64	+	-	+	-	+	-	-	-	-	-	-
390	A337	128	32	+	-	+	-	+	-	-	-	-	-	-
391	A338	256	64	+	-	+	-	+	-	-	-	-	-	-
392	A339	128	32	+	-	+	-	+	-	-	-	-	-	-
393	A340	128	32	+	-	+	-	+	-	-	-	-	-	-
394	A341	64	32	+	-	+	-	+	+	-	-	-	-	-
395	A342	256	64	+	-	+	-	+	-	-	-	-	-	-
396	A343	256	64	+	-	+	-	+	-	-	-	-	-	-
397	A344	128	32	+	-	+	-	+	-	-	-	-	-	-
398	A345	256	32	+	-	+	-	+	-	-	-	-	-	-
399	A346	128	32	+	-	+	-	+	-	-	-	-	-	-
400	A347	128	32	+	-	+	-	+	-	-	-	-	-	-
401	A348	256	64	+	-	+	-	+	-	-	-	-	-	-
402	A349	128	32	+	-	+	-	+	-	-	-	-	-	-
403	A350	256	64	+	-	+	-	+	-	-	-	-	-	-
404	A351	256	64	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			*bla*$_{\text{OXA23-like}}$	*bla*$_{\text{OXA24-like}}$	*bla*$_{\text{OXA51-like}}$	*bla*$_{\text{OXA58-like}}$	*bla*$_{\text{IMP-like}}$	*bla*$_{\text{VIM-like}}$	*bla*$_{\text{SIM-1}}$	*bla*$_{\text{SPM-1}}$	*bla*$_{\text{GIM-1}}$
405	A352	256	64	+	-	+	-	+	-	-	-	-	-	-
406	A353	1	0.25	-	-	-	-	+	-	-	-	-	-	-
407	A354	256	32	+	-	+	-	+	-	-	-	-	-	-
408	A355	64	32	+	-	+	-	+	-	-	-	-	-	-
409	A356	128	32	+	-	+	-	+	-	-	-	-	-	-
410	A357	256	64	+	-	+	-	+	-	-	-	-	-	-
411	A358	256	64	+	-	+	-	+	-	-	-	-	-	-
412	A359	>256	128	+	-	+	-	+	-	-	-	-	-	-
413	A360	256	64	+	-	+	-	+	-	-	-	-	-	-
414	A361	64	32	+	-	+	-	+	-	-	-	-	-	-
415	A362	>256	128	+	-	+	-	+	-	-	-	-	-	-
416	A364	128	32	+	-	+	-	+	-	-	-	-	-	-
417	A365	256	32	+	-	+	-	+	-	-	-	-	-	-
418	A366	256	32	+	-	+	-	+	-	-	-	-	-	-
419	A367	256	32	+	-	+	-	+	-	-	-	-	-	-
420	A368	256	32	+	-	+	-	+	-	-	-	-	-	-
421	A369	256	32	+	-	+	-	+	-	-	-	-	-	-
422	A370	64	16	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (μg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			$bla_{\text{OXA23-like}}$	$bla_{\text{OXA24-like}}$	$bla_{\text{OXA51-like}}$	$bla_{\text{OXA58-like}}$	$bla_{\text{IMP-like}}$	$bla_{\text{VIM-like}}$	$bla_{\text{SIM-1}}$	$bla_{\text{SPM-1}}$	$bla_{\text{GIM-1}}$
423	A371	128	32	+	-	+	-	+	-	-	-	-	-	-
424	A372	128	32	+	-	+	-	+	-	-	-	-	-	-
425	A373	256	128	+	-	+	-	+	-	-	-	-	-	-
426	A374	256	128	+	-	+	-	+	-	-	-	-	-	-
427	A375	256	32	+	-	+	-	+	-	-	-	-	-	-
428	A376	64	16	+	-	+	-	+	-	-	-	-	-	-
429	A377	256	128	+	-	+	-	+	-	-	-	-	-	-
430	A378	1	0.5	-	-	-	-	+	-	-	-	-	-	-
431	A379	256	64	+	-	+	-	+	-	-	-	-	-	-
432	A380	256	64	+	-	+	-	+	-	-	-	-	-	-
433	A381	256	64	+	-	+	-	+	-	-	-	-	-	-
434	A382	256	64	+	-	+	-	+	-	-	-	-	-	-
435	A383	128	32	+	-	+	-	+	-	-	-	-	-	-
436	A384	128	32	+	-	+	-	+	-	-	-	-	-	-
437	A385	128	32	+	-	+	-	+	-	-	-	-	-	-
438	A386	128	32	+	-	+	-	+	-	-	-	-	-	-
439	A388	128	32	+	-	+	-	+	-	-	-	-	-	-
440	A389	256	64	+	-	+	-	+	-	-	-	-	-	-

No.	Isolate	MIC (µg/ml)		Carbapenemase phenotype	MBLs phenotype	The presence of *bla* genes								
		IPM	MEM			$bla_{\text{OXA23-like}}$	$bla_{\text{OXA24-like}}$	$bla_{\text{OXA51-like}}$	$bla_{\text{OXA58-like}}$	$bla_{\text{IMP-like}}$	$bla_{\text{VIM-like}}$	$bla_{\text{SIM-1}}$	$bla_{\text{SPM-1}}$	$bla_{\text{GIM-1}}$
441	A390	128	32	+	-	+	-	+	-	-	-	-	-	-
442	A391	128	32	+	-	+	-	+	-	-	-	-	-	-
443	A393	128	32	+	-	+	-	+	-	-	-	-	-	-
444	A395	64	16	+	-	+	-	+	-	-	-	-	-	-
445	A397	64	32	+	-	+	-	+	-	-	-	-	-	-
446	A398	256	64	+	-	+	-	+	-	-	-	-	-	-
447	A399	256	64	+	-	+	-	+	-	-	-	-	-	-
448	A400	128	64	+	-	+	-	+	-	-	-	-	-	-
449	A401	256	64	+	-	+	-	+	-	-	-	-	-	-
450	A402	256	64	+	-	+	-	+	-	-	-	-	-	-
451	A403	64	32	+	-	+	-	+	-	-	-	-	-	-
452	A404	64	32	+	-	+	-	+	-	-	-	-	-	-
453	A405	128	32	+	-	+	-	+	-	-	-	-	-	-

IPM, imipenem ; MEM, meropenem; +, positive ; **-,** negative

APPENDIX E

PCR screening for detection of gene encoding *bla*$_{\text{OXA-like}}$, IS*Aba* and IS*Aba* upstream *bla*$_{\text{OXA-like}}$ in 49 *A. baumannii*

Isolate	MIC (µg/ml)		*bla*$_{\text{OXA-like}}$				ISAba			ISAba1 upstream *bla*$_{\text{OXA23-like}}$	ISAba3 upstream *bla*$_{\text{OXA58-like}}$
	IPM	MEM	*bla*$_{\text{OXA23-like}}$	*bla*$_{\text{OXA24-like}}$	*bla*$_{\text{OXA51-like}}$	*bla*$_{\text{OXA58-like}}$	IS*Aba1*	IS*Aba2*	IS*Aba3*		
Ab1	>256	256	-	+	+	-	-	+	-	-	-
A98	64	32	+	-	+	+	+	-	+	+	+
A200	64	16	+	-	+	+	+	-	+	+	+
A232	64	16	+	-	+	+	+	-	+	+	+
A341	64	32	+	-	+	+	+	-	+	+	+
A251	>256	128	+	-	+	-	+	-	-	+	-
A252	256	64	+	-	+	-	+	-	-	+	-
A253	128	32	+	-	+	-	+	-	-	+	-
A254	128	32	+	-	+	-	+	-	-	+	-
A255	128	32	+	-	+	-	+	-	+	+	-

Isolate	MIC (μg/ml)		*bla*$_{OXA-like}$				ISAba			ISAba1 upstream *bla*$_{OXA23-like}$	ISAba3 upstream *bla*$_{OXA58-like}$
	IPM	MEM	*bla*$_{OXA23-like}$	*bla*$_{OXA24-like}$	*bla*$_{OXA51-like}$	*bla*$_{OXA58-like}$	IS*Aba1*	IS*Aba2*	IS*Aba3*		
A256	64	32	+	-	+	-	+	-	+	+	-
A257	128	32	+	-	+	-	+	-	-	+	-
A258	256	64	+	-	+	-	+	-	-	+	-
A259	256	64	+	-	+	-	+	-	-	+	-
A260	1	0.25	+	-	+	-	-	+	-	-	-
A261	2	0.25	+	-	+	-	-	-	-	-	-
A262	256	128	+	-	+	-	+	-	-	+	-
A263	256	64	+	-	+	-	+	-	-	+	-
A264	2	1	+	-	+	-	-	+	-	-	-
A265	256	64	+	-	+	-	+	-	-	+	-
A266	128	32	+	-	+	-	+	-	-	+	-
A268	128	32	+	-	+	-	+	-	-	+	-
A270	2	0.5	+	-	+	-	-	+	+	-	-
A272	128	32	+	-	+	-	+	-	-	+	-
A274	256	64	+	-	+	-	+	-	-	+	-

Isolate	MIC (µg/ml)		*bla*$_{OXA-like}$				ISAba			ISAba1 upstream *bla*$_{OXA23-like}$	ISAba3 upstream *bla*$_{OXA58-like}$
	IPM	MEM	*bla*$_{OXA23-like}$	*bla*$_{OXA24-like}$	*bla*$_{OXA51-like}$	*bla*$_{OXA58-like}$	IS*Aba1*	IS*Aba2*	IS*Aba3*		
A275	256	64	+	-	+	-	+	-	-	+	-
A276	256	64	+	-	+	-	+	-	-	+	-
A277	256	64	+	-	+	-	+	-	-	+	-
A279	256	64	+	-	+	-	+	-	-	+	-
A280	128	16	+	-	+	-	+	-	-	+	-
A281	4	1	+	-	+	-	+	-	+	-	-
A282	2	0.25	+	-	+	-	-	+	-	-	-
A283	4	0.5	+	-	+	-	+	-	-	-	-
A284	128	32	+	-	+	-	+	-	-	+	-
A285	256	64	+	-	+	-	+	-	-	+	-
A286	128	32	+	-	+	-	+	-	-	+	-
A287	256	64	+	-	+	-	+	-	-	+	-
A288	128	32	+	-	+	-	+	-	-	+	-
A289	2	1	+	-	+	-	+	-	-	-	-
A290	64	16	+	-	+	-	+	+	-	+	-

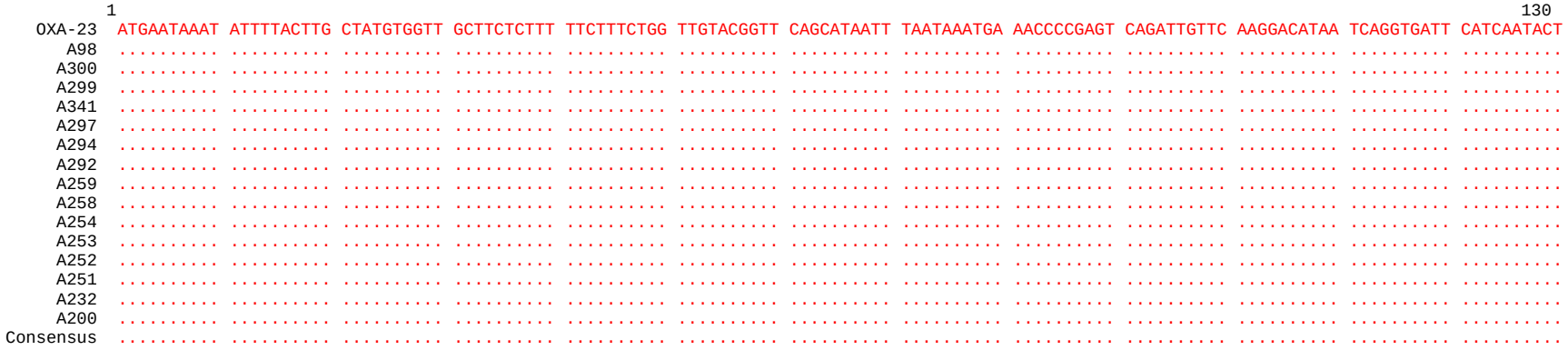
Isolate	MIC (µg/ml)		*bla*$_{OXA-like}$				ISAba			ISAba1 upstream *bla*$_{OXA23-like}$	ISAba3 upstream *bla*$_{OXA58-like}$
	IPM	MEM	*bla*$_{OXA23-like}$	*bla*$_{OXA24-like}$	*bla*$_{OXA51-like}$	*bla*$_{OXA58-like}$	IS*Aba1*	IS*Aba2*	IS*Aba3*		
A291	256	64	+	-	+	-	+	-	-	+	-
A292	128	64	+	-	+	-	+	-	-	+	-
A293	2	0.25	+	-	+	-	+	+	-	-	-
A294	256	64	+	-	+	-	+	-	-	+	-
A295	4	1	+	-	+	-	+	-	-	-	-
A296	256	32	+	-	+	-	+	-	-	+	-
A297	256	32	+	-	+	-	+	-	-	+	-
A299	32	16	+	-	+	-	+	-	-	+	-
A300	256	64	+	-	+	-	+	-	-	+	-

IPM, imipenem ; MEM, meropenem; +, positive ; **-,** negative

APPENDIX F

NUCLEOTIDE SEQUENCES ALIGNMENT OF SELECTED ISOLATES

1. 15 *A. baumannii* isolates with *bla*$_{\text{OXA-23}}$



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          131                                                
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          391                                                
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          651                                                
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2. 12 *A. baumannii* isolates with *bla*$_{\text{OXA-51}}$ group

2.1 7 *A. baumannii* isolates with *bla*$_{\text{OXA-66}}$

```
          1                                                                                                                                  130
OXA-66    ATGAACATTA AAGCACTCTT ACTTATAACA AGCGCTATTT TTATTTCAGC CTGCTCACCT TATATAGTGA CTGCTAATCC AAATCACAGC GCTTCAAAAT CTGATGTAAA AGCAGAGAAA ATTAAAAATT
A251      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A300      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A259      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A258      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A254      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A253      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A252      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          131                                                                                                                                260
OXA-66    TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG ACCTTCAAAA TGCTTAATGC
A251      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A300      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A259      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A258      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A254      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A253      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A252      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          261                                                                                                                                390
OXA-66    TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACCACAGAA GTATTTAAGT GGGATGGTAA AAAAAGGTTA TTCCCAGAAT GGGAAAAGGA CATGACCCTA GGCGATGCCA TGAAAGCTTC CGCTATTCCA
A251      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A300      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A259      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A258      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A254      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A253      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A252      .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
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          391                                                
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2.2 4 *A. baumannii* isolates with $bla_{\text{OXA-65}}$

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         1                                                                                                                                  130
OXA-65   ATGAACATTA AAGCACTCTT ACTTATAACA AGCGCTATTT TTATTTCAGC CTGCTCACCT TATATAGTGA CTGCTAATCC AAATCACAGT GCTTCAAAAT CTGATGAAAA AGCAGAGAAA ATTAAAAATT
OXA-98   .......... .......... .......... .......... .......... .......... .......... .......... .........C .......... .......... .......... ..........
A200     .......... .......... .......... .......... .......... .......... .......... .......... .........C .......... .......... .......... ..........
A341     .......... .......... .......... .......... .......... .......... .......... .......... .........C .......... .......... .......... ..........
A232     .......... .......... .......... .......... .......... .......... .......... .......... .........C .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .........c .......... .......... .......... ..........

         131                                                                                                                                260
OXA-65   TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA ACAAGGCCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG ACCTTCAAAA TGCTTAATGC
OXA-98   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A200     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A341     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A232     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

         261                                                                                                                                390
OXA-65   TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACCACAGAA GTATTTAAGT GGGATGGTAA AAAAAGGTTA TTCCCAGAAT GGGAAAAGGA CATGACCCTA GGCGATGCCA TGAAAGCTTC CGCTATTCCA
OXA-98   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A200     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A341     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A232     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

         391                                                                                                                                520
OXA-65   GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAGCTCA TGTCTAAGGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC CAAGTCGATA ATTTTTGGCT GGTGGGTCCT TTAAAAATTA
OXA-98   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A200     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A341     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
A232     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

         521                                                                                                                                650
OXA-65   CTCCTCAGCA AGAGGCACAG TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC AAAAAGTCCA AGATGAAGTG CAATCCATGC TATTCATAGA AGAAAAGAAT GGAAACAAAA TATACGCAAA
OXA-98   .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .....T.... ..........
A200     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .....T.... ..........
A341     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .....T.... ..........
A232     .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .....T.... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .....t.... ..........
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          651                                                
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2.3 1 *A. baumannii* isolates with $bla_{\text{OXA-69}}$

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           1                                                                                                                                                130
   OXA-69  ATGAACATTA AAGCACTCTT ACTTATAACA AGCGCTATTT TTATTTCAGC CTGCTCACCT TATATAGTGA CTGCTAATCC AAATCACAGT GCTTCAAAAT CTGATGACAA AGCAGAGAAA ATTAAAAATT
     A299  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           131                                                                                                                                              260
   OXA-69  TATTTAACGA AGCACACACT ACGGGTGTTT TAGTTATCCA TCAAGGTCAA ACTCAACAAA GCTATGGTAA TGATCTTGCT CGTGCTTCGA CCGAGTATGT ACCTGCTTCG ACCTTCAAAA TGCTTAATGC
     A299  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           261                                                                                                                                              390
   OXA-69  TTTGATCGGC CTTGAGCACC ATAAGGCAAC CACCACAGAA GTATTTAAAT GGGATGGGGA AAAAAGGCTA TTCCCAGAAT GGGAAAAGAA CATGACCCTA GGCGATGCTA TGAAAGCTTC CGCTATTCCG
     A299  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           391                                                                                                                                              520
   OXA-69  GTTTATCAAG ATTTAGCTCG TCGTATTGGA CTTGAGCTCA TGTCTAAGGA AGTGAAGCGT GTTGGTTATG GCAATGCAGA TATCGGTACC CAAGTCGATA ATTTTTGGCT GGTGGGTCCT CTAAAAATTA
     A299  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           521                                                                                                                                              650
   OXA-69  CTCCTCAGCA AGAGGCACAG TTTGCTTACA AGCTAGCTAA TAAAACGCTT CCATTTAGCC AAAAAGTCCA AGATGAAGTG CAATCCATGC TATTCATAGA AGAAAAGAAT GGAAATAAAA TATACGCAAA
     A299  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           651                                                                                                                                              780
   OXA-69  AAGTGGTTGG GGATGGGATG TAAACCCACA AGTAGGCTGG TTAACTGGAT GGGTTGTTCA GCCTCAAGGG AATATTGTAG CGTTCTCCCT TAACTTAGAA ATGAAAAAAG GAATACCTAG CTCTGTTCGA
     A299  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus  .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

           781                                   825
   OXA-69  AAAGAGATTA CTTATAAAAG TTTAGAACAA TTAGGTATTT TATAG
     A299  .......... .......... .......... .......... .....
Consensus  .......... .......... .......... .......... .....
```

3. 4 *A. baumannii* isolates with $bla_{\text{OXA-58}}$

```
         1                                                                                                                                  130
  OXA-58 ATGAAATTAT TAAAAATATT GAGTTTAGTT TGCTTAAGCA TAAGTATTGG GGCTTGTGCT GAGCATAGTA TGAGTCGAGC AAAAACAAGT ACAATTCCAC AAGTGAATAA CTCAATCATC GATCAGAATG
   OXA-98 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
 0XA-341 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
 0XA-232 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
  OXA-200 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

         131                                                                                                                                260
  OXA-58 TTCAAGCGCT TTTTAATGAA ATCTCAGCTG ATGCTGTGTT TGTCACATAT GATGGTCAAA ATATTAAAAA ATATGGCACG CATTTAGACC GAGCAAAAAC AGCTTATATT CCTGCATCTA CATTTAAAAT
   OXA-98 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
 0XA-341 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
 0XA-232 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
  OXA-200 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

         261                                                                                                                                390
  OXA-58 TGCCAATGCA CTAATTGGTT TAGAAAATCA TAAAGCAACA TCTACAGAAA TATTTAAGTG GGATGGAAAG CCACGTTTTT TTAAAGCATG GGACAAAGAT TTTACTTTGG GCGAAGCCAT GCAAGCATCT
   OXA-98 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
 0XA-341 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
 0XA-232 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
  OXA-200 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

         391                                                                                                                                520
  OXA-58 ACAGTGCCTG TATATCAAGA ATTGGCACGT CGTATTGGTC CAAGCTTAAT GCAAAGTGAA TTGCAACGTA TTGGTTATGG CAATATGCAA ATAGGCACGG AAGTTGATCA ATTTTGGTTG AAAGGGCCTT
   OXA-98 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
 0XA-341 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
 0XA-232 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
  OXA-200 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

         521                                                                                                                                650
  OXA-58 TGACAATTAC ACCTATACAA GAAGTAAAGT TTGTGTATGA TTTAGCCCAA GGGCAATTGC CTTTTAAACC TGAAGTTCAG CAACAAGTGA AAGAGATGTT GTATGTAGAG CGCAGAGGGG AGAATCGTCT
   OXA-98 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
 0XA-341 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
 0XA-232 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
  OXA-200 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
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          651                                                
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5. 5 *A. baumannii* isolates with IS*Aba1* upstream region of *bla*$_{\text{OXA-23}}$ gene

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          1691                                                                                                                1820
EF127491  TTGTTTAAGA TAAGATATAA CTCATTGAGA TGTGTCATAG TATTCGTCGT TAGAAAACAA TTATTATGAC ATTATTTCAA TGAGTTATCT ATTTTTGTCG TGTACAGAGC TCTTTTTTAT TTTCTATTGA
     A252 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     A254 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     A253 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     A251 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     A285 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          1821                                                                                                                1950
EF127491  TCTGGTGTTT AAAATGAATA AATATTTTAC TTGCTATGTG GTTGCTTCTC TTTTTCTTTC TGGTTGTACG GTTCAGCATA ATTTAATAAA TGAAACCCCG AGTCAGATTG TTCAAGGACA TAATCAGGTG
     A252 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     A254 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     A253 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     A251 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     A285 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          1951                                                                                                                2080
EF127491  ATTCATCAAT ACTTTGATGA AAAAAACACC TCAGGTGTGC TGGTTATTCA AACAGATAAA AAAATTAATC TATATGGTAA TGCTCTAAGC CGCGCAAATA CAGAATATGT GCCAGCCTCT ACATTTAAAA
     A252 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     A254 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     A253 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     A251 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     A285 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

          2081                                                                                                                2210
EF127491  TGTTGAATGC CCTGATCGGA TTGGAGAACC AGAAAACGGA TATTAATGAA ATATTTAAAT GGAAGGGCGA GAAAAGGTCA TTTACCGCTT GGGAAAAAGA CATGACACTA GGAGAAGCCA TGAAGCTTTC
     A252 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     A254 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     A253 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     A251 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
     A285 .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
Consensus .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
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6. 4 *A. baumannii* isolates with IS*Aba3* upstream region of $bla_{\text{OXA-58}}$ gene

APPENDIX G

DNA CODON

One- and Three-Letter symbols for the amino acids

A	Ala	Alanine
B	Asx	Asparagine or aspartic acid
C	Cys	Cysteine
D	Asp	Aspartic acid
E	Glu	Glutamic acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Trytophan
Y	Tyr	Tyrosine
Z	Glx	Gln or Glu

The standard genetic code

First position (5'end)	Second position				Third position (3' end)
	U	**C**	**A**	**G**	
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUU Phe	UCU Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met[a]	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

[a]AUG forms part of the initiation signal as well as coding for internal Met residues

APPENDIX H

Results of MIC with CCCP inhibited AdeABC efflux pump of 453 *A. baumannii* isolates

Isolate	MIC (µg/ml)		MIC (µg/ml)	
	IPM	IPM +10 µM CCCP	MEM	MEM+10 µMCCCP
Ab1	512	512	256	256
Ab2	256	256	128	128
Ab3	256	256	64	32
Ab4	256	256	64	64
Ab6	256	128	32	32
Ab7	1	1	1	1
Ab8	256	256	64	32
Ab9	128	128	64	32
Ab10	256	128	64	32
Ab11	256	256	128	64
Ab13	256	256	128	64
Ab14	256	256	64	64
Ab16	256	256	64	32
Ab17	128	128	32	32
Ab18	2	2	1	1
Ab19	2	2	0.25	0.25
Ab20	256	256	32	32
Ab21	256	256	64	64
Ab22	256	256	64	64
Ab23	256	256	128	128
Ab24	2	2	1	1
Ab26	128	128	32	16
Ab27	256	256	64	64
Ab28	4	4	2	2
Ab29	1	1	0.5	0.5
Ab30	256	256	64	64
Ab31	128	128	64	64
Ab32	128	128	64	64
Ab33	4	4	2	2
Ab34	1	1	0.5	0.5
Ab35	64	64	32	32
Ab36	128	128	16	16
Ab37	256	256	64	64
Ab38	256	256	64	64
Ab39	1	1	1	1

Isolate	MIC (µg/ml)		MIC (µg/ml)	
	IPM	IPM +10 µM CCCP	MEM	MEM+10 µMCCCP
Ab40	256	256	64	64
Ab41	128	128	32	32
Ab42	1	1	0.5	0.5
Ab43	256	256	32	32
Ab44	128	128	32	32
Ab45	256	256	64	64
Ab46	256	256	64	64
Ab47	256	256	128	64
Ab48	256	256	64	64
Ab49	256	256	64	64
Ab50	256	256	128	64
Ab52	256	256	32	32
Ab53	256	256	64	64
Ab54	256	128	64	32
Ab55	256	256	64	64
Ab56	256	256	64	32
Ab57	1	1	0.5	0.5
Ab58	256	256	64	32
Ab59	512	512	128	128
Ab60	128	128	32	16
Ab61	128	128	32	16
Ab62	256	256	64	64
Ab63	128	128	32	32
Ab64	64	64	32	32
Ab65	1	1	0.25	0.25
Ab66	64	64	32	32
Ab67	64	64	32	16
Ab68	128	128	64	64
Ab69	64	64	32	32
Ab70	64	64	16	16
Ab72	1	1	0.25	0.25
Ab73	1	1	0.5	0.5
Ab74	128	128	64	64
Ab75	256	256	128	64
Ab76	128	128	32	32
Ab78	128	128	64	32
Ab79	128	64	32	32
Ab81	64	64	32	16
Ab82	64	64	32	32
Ab83	256	256	64	64
Ab84	64	64	32	32
Ab85	4	4	1	1
Ab86	128	128	64	64
Ab87	128	128	64	64

Isolate	MIC (µg/ml)		MIC (µg/ml)	
	IPM	IPM +10 µM CCCP	MEM	MEM+10 µMCCCP
Ab88	128	128	32	32
Ab89	1	1	1	1
Ab90	32	32	16	16
Ab91	256	256	256	256
Ab92	1	1	0.5	0.5
Ab93	64	64	16	16
Ab94	128	128	64	64
Ab95	128	64	64	32
Ab96	128	64	64	32
Ab97	64	32	32	16
Ab98	32	32	16	16
Ab100	64	64	32	32
A2	64	64	32	32
A3	128	128	32	32
A5	128	128	64	32
A6	128	128	64	32
A7	64	64	32	32
A8	2	2	1	1
A9	256	256	64	64
A10	128	128	32	32
A11	64	64	32	32
A12	4	4	2	2
A13	128	128	64	32
A15	128	128	64	64
A16	128	128	64	64
A17	256	256	64	64
A18	64	64	32	32
A19	1	1	0.25	0.25
A20	128	64	32	32
A21	2	2	0.5	0.5
A22	256	128	64	64
A24	128	64	64	32
A26	128	128	64	32
A27	128	128	32	32
A28	1	1	0.5	0.5
A29	64	64	16	16
A30	64	64	32	32
A31	32	32	16	16
A32	128	128	64	32
A33	64	64	16	16
A34	128	128	64	64
A35	128	128	64	64
A36	128	128	64	64
A38	64	64	32	32
A39	64	64	64	32

Isolate	MIC (µg/ml)		MIC (µg/ml)	
	IPM	IPM +10 µM CCCP	MEM	MEM+10 µMCCCP
A40	64	64	32	32
A42	64	64	32	16
A43	64	64	32	32
A44	256	256	64	64
A45	128	128	64	64
A46	64	64	32	32
A47	64	64	32	32
A48	128	128	64	32
A50	64	32	32	32
A51	128	128	32	32
A52	128	64	64	64
A53	64	32	16	16
A54	64	64	32	32
A55	64	32	32	16
A56	128	128	32	32
A57	64	64	32	32
A58	64	64	32	16
A59	64	32	16	16
A60	128	64	32	32
A61	256	256	64	64
A63	128	128	64	64
A64	128	128	32	32
A65	128	64	64	64
A66	256	128	32	32
A67	64	64	32	32
A68	64	64	16	16
A69	256	256	256	256
A71	256	256	256	256
A72	256	256	64	64
A73	128	128	64	64
A74	128	128	64	64
A75	1	1	0.5	0.5
A76	256	256	16	16
A78	128	128	64	64
A79	128	64	32	32
A80	128	128	64	32
A81	256	256	64	64
A82	128	128	64	64
A83	64	64	32	32
A84	128	128	32	32
A85	128	128	64	32
A86	128	128	32	32
A87	64	64	32	32
A88	64	32	16	16

Isolate	MIC (µg/ml)		MIC (µg/ml)	
	IPM	IPM +10 µM CCCP	MEM	MEM+10 µMCCCP
A89	32	32	16	16
A90	128	128	64	64
A91	64	32	32	32
A92	64	64	32	32
A94	64	32	16	16
A95	128	128	64	64
A96	2	2	2	2
A97	128	128	64	64
A98	64	64	16	16
A100	128	128	64	64
A101	64	64	16	16
A102	256	256	128	64
A103	128	128	32	16
A104	64	64	32	32
A105	128	64	32	16
A106	256	128	128	64
A107	256	256	128	128
A110	256	128	128	64
A111	128	64	32	32
A112	128	128	32	32
A113	0.5	0.5	0.25	0.25
A114	128	128	64	32
A115	128	64	32	32
A116	128	64	32	32
A117	0.5	0.5	0.5	0.5
A118	128	64	32	32
A119	256	256	128	128
A121	256	128	128	64
A122	256	256	64	64
A123	256	128	128	128
A124	256	256	128	128
A125	64	64	16	16
A126	128	128	64	64
A127	128	128	64	64
A128	128	128	64	64
A129	128	128	64	32
A130	128	128	64	64
A132	128	128	64	64
A133	128	128	64	64
A134	128	128	64	64
A135	128	128	64	64
A137	256	256	64	64
A138	256	128	32	32
A139	128	64	32	32

Isolate	MIC (µg/ml)		MIC (µg/ml)	
	IPM	IPM +10 µM CCCP	MEM	MEM+10 µMCCCP
A140	128	64	32	32
A141	128	64	32	32
A142	128	128	64	64
A143	256	128	64	64
A144	256	256	128	128
A145	256	256	64	64
A146	64	64	32	32
A147	128	64	32	32
A148	128	128	32	32
A149	128	128	32	32
A150	128	128	64	32
A151	128	128	32	32
A152	64	64	16	16
A153	64	64	32	16
A154	64	64	16	16
A155	256	256	128	64
A156	256	128	32	32
A157	256	256	64	64
A159	256	256	64	64
A162	256	256	64	64
A163	256	128	128	64
A164	256	128	64	64
A165	64	64	32	32
A166	128	128	64	64
A167	32	32	16	16
A168	64	32	32	32
A169	128	128	32	16
A170	256	256	64	64
A171	256	256	128	128
A173	64	64	32	32
A174	256	128	64	64
A175	256	256	64	64
A176	128	128	64	64
A177	64	64	32	32
A178	256	256	64	64
A179	256	256	64	64
A180	64	32	16	16
A182	128	128	64	64
A183	128	128	64	64
A184	256	256	128	128
A185	128	128	64	64
A186	256	256	64	64
A187	256	256	128	128
A188	128	128	64	64

Isolate	MIC (µg/ml)		MIC (µg/ml)	
	IPM	IPM +10 µM CCCP	MEM	MEM+10 µMCCCP
A189	256	256	128	128
A190	128	128	64	64
A191	128	128	32	32
A192	256	128	128	128
A193	256	256	64	64
A195	32	32	16	16
A196	128	128	64	64
A197	128	128	64	64
A198	64	64	16	16
A199	1	1	0.5	0.5
A200	64	64	16	16
A201	256	256	64	64
A202	256	128	64	64
A203	64	64	16	16
A204	4	4	0.25	0.25
A205	128	128	64	64
A206	128	128	64	32
A207	128	128	64	32
A208	32	32	16	16
A209	0.5	0.5	0.25	0.25
A211	128	128	64	64
A212	128	128	32	32
A213	1	1	0.5	0.5
A214	256	256	64	64
A215	256	256	32	32
A216	1	1	0.5	0.5
A217	128	128	64	64
A218	128	128	64	32
A219	256	256	128	128
A220	128	128	64	64
A221	128	64	32	32
A222	128	128	64	64
A223	128	128	64	32
A224	256	128	64	64
A225	128	128	64	32
A226	1	1	0.5	0.5
A227	128	128	64	32
A228	128	128	64	64
A229	256	256	64	64
A230	128	128	64	32
A231	0.5	0.5	0.25	0.25
A232	64	64	16	16
A233	64	64	32	32
A234	128	128	64	64

Isolate	MIC (µg/ml)		MIC (µg/ml)	
	IPM	IPM +10 µM CCCP	MEM	MEM+10 µMCCCP
A235	64	64	32	32
A237	128	128	64	64
A238	64	64	32	32
A239	64	64	32	32
A240	128	128	64	64
A241	256	128	64	32
A242	64	64	32	32
A243	128	128	64	64
A245	64	64	32	32
A246	128	128	64	64
A247	256	256	64	32
A248	64	64	32	32
A249	128	128	64	32
A250	128	128	64	64
A251	256	256	128	128
A252	128	128	64	64
A253	64	64	32	16
A254	128	128	64	64
A255	64	64	32	16
A256	64	64	32	16
A257	64	32	32	16
A258	128	128	64	64
A259	128	128	64	64
A260	0.5	0.5	0.5	0.25
A261	1	1	0.25	0.25
A262	256	128	64	64
A263	128	128	64	64
A264	1	1	0.5	0.5
A265	128	128	64	64
A266	64	64	32	16
A268	128	64	32	16
A270	2	2	0.5	0.5
A272	64	64	32	32
A274	128	128	64	64
A275	128	128	32	32
A276	128	128	64	64
A277	128	128	64	32
A279	128	64	64	64
A280	128	64	16	16
A281	4	4	1	1
A282	2	2	0.5	0.5
A283	4	4	0.5	0.5
A284	128	128	64	64
A285	128	64	64	32

Isolate	MIC (µg/ml)		MIC (µg/ml)	
	IPM	IPM +10 µM CCCP	MEM	MEM+10 µMCCCP
A286	64	64	16	16
A287	128	128	64	32
A288	64	64	16	16
A289	2	2	1	1
A290	32	32	16	16
A291	128	128	64	32
A292	128	128	64	32
A293	2	2	0.5	0.5
A294	128	128	64	32
A295	4	4	1	1
A296	64	64	16	16
A297	64	64	32	32
A299	16	16	16	16
A300	256	128	64	64
A301	64	64	32	16
A302	0.5	0.5	0.5	0.5
A303	256	256	128	64
A304	128	128	32	32
A305	128	128	32	32
A306	2	2	1	1
A310	128	64	32	32
A311	1	1	0.5	0.5
A312	128	128	64	64
A313	1	1	0.5	0.5
A314	128	128	64	64
A315	64	64	16	16
A317	2	2	0.5	0.5
A318	64	64	16	16
A319	0.5	0.5	0.5	0.5
A320	128	128	32	32
A321	128	64	64	64
A322	64	64	32	32
A323	128	128	64	32
A324	128	128	64	64
A325	64	64	16	16
A326	128	128	32	32
A327	128	128	32	32
A328	64	64	32	32
A329	256	256	64	32
A330	128	128	64	64
A331	128	64	32	32
A332	128	128	32	32
A333	64	64	32	32
A334	128	128	64	64

Isolate	MIC (µg/ml)		MIC (µg/ml)	
	IPM	IPM +10 µM CCCP	MEM	MEM+10 µMCCCP
A336	128	128	64	64
A337	64	64	32	32
A338	128	128	64	64
A339	64	64	32	16
A340	128	64	32	32
A341	64	64	16	16
A342	128	128	64	64
A343	128	128	64	64
A344	128	128	32	32
A345	64	64	32	32
A346	64	64	32	32
A347	128	128	64	32
A348	128	64	64	64
A349	64	64	32	32
A350	128	128	64	64
A351	256	256	64	64
A352	256	256	64	32
A353	1	1	0.25	0.25
A354	256	256	32	32
A355	64	64	32	16
A356	64	64	32	16
A357	256	256	64	32
A358	256	256	64	32
A359	512	256	128	64
A360	256	256	64	32
A361	64	64	16	16
A362	256	256	128	128
A364	128	128	32	16
A365	128	128	32	32
A366	128	128	32	16
A367	128	128	32	16
A368	128	128	32	16
A369	256	256	32	16
A370	64	64	32	16
A371	64	64	32	32
A372	64	64	32	16
A373	256	256	64	32
A374	256	256	64	64
A375	256	128	32	16
A376	64	64	16	16
A377	256	256	64	64
A378	1	0.5	0.25	0.25
A379	128	128	32	32
A380	256	256	64	64

Isolate	MIC (µg/ml)		MIC (µg/ml)	
	IPM	IPM +10 µM CCCP	MEM	MEM+10 µMCCCP
A381	256	256	64	64
A382	128	128	64	64
A383	128	64	32	32
A384	64	64	32	32
A385	128	128	32	16
A386	128	128	64	32
A388	128	128	32	32
A389	256	256	64	64
A390	128	128	32	32
A391	64	64	64	32
A393	128	128	32	32
A395	64	64	16	16
A397	64	64	32	32
A398	256	256	64	64
A399	128	128	32	32
A400	64	64	32	16
A401	128	64	64	64
A402	128	128	64	64
A403	64	64	32	32
A404	64	64	32	16
A405	128	128	32	32

IPM, imipenem ; MEM, meropenem;

CCCP, carbonyl cyanide m-chlorophenylhydrazon

BIOGRAPHY

Miss Pantipa Samarnthai was born on September 5, 1976 in Petchaboon province, Thailand. She graduated with a Bachelor degree of Science (Medical Technology) from the Faculty of Allied Health Sciences, Chulalongkorn University in 1997. She has studied in the Master degree in the Inter-Department of Medical Microbiology, Faculty of Graduate School, Chulalongkorn University since 2007.