การศึกษาฤทธิ์ต้านเชื้อของอิมิพีเน็มร่วมกับอะมิคาซินหรืออิมิพีเน็มร่วมกับซิโปรฟลอกซาซินในหลอดทดลองต่อ *Acinetobacter baumannii* ที่ดื้อต่อยาอิมิพีเน็ม

นางสาว สราญจิตร ดวงสีใส

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชวิทยา ภาควิชาเภสัชวิทยา
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IN VITRO ANTIBACTERIAL ACTIVITY OF IMIPENEM IN COMBINATION WITH AMIKACIN OR WITH CIPROFLOXACIN AGAINST IMIPENEM RESISTANT- *ACINETOBACTER BAUMANNII*

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สราญจิตร ดวงสีใส : การศึกษาฤทธิ์ต้านเชื้อของอิมิพีเน็มร่วมกับอะมิคาซินหรืออิมิพีเน็มร่วมกับซิโปรฟลอกซาซินในหลอดทดลองต่อ *Acinetobacter baumannii* ที่ดื้อต่อยาอิมิพีเน็ม. (*IN VITRO* ANTIBACTERIAL ACTIVITY OF IMIPENEM IN COMBINATION WITH AMIKACIN OR WITH CIPROFLOXACIN AGAINST IMIPENEM RESISTANT-*ACINETOBACTER BAUMANNII*) อ.ที่ปรึกษา : รศ.ศิริภรณ์ ฟุ้งวิทยา, อ.ที่ปรึกษาร่วม : รศ.ดร.พิณทิพย์ พงษ์เพ็ชร, 148 หน้า. ISBN 974-17-4171-5.

Acinetobacter baumannii (*A. baumannii*) เป็นเชื้อแบคทีเรียกรัมลบรูปแท่งที่สำคัญเพราะเป็นเชื้อที่ก่อให้เกิดโรคติดเชื้อในโรงพยาบาลที่พบบ่อยโดยเฉพาะในแผนกคนไข้วิกฤตในช่วงทศวรรษที่ผ่านมา *A. baumannii* มีการดื้อยาปฏิชีวนะสูงขึ้นและเป็นการดื้อยาหลายชนิดร่วมกันทั้ง broad spectrum β-lactams, aminoglycosides, fluoroquinolones และ imipenem ทำให้มีความยากลำบากในการเลือกใช้ยาปฏิชีวนะเพียงขนานเดียวในการรักษา การใช้ยาปฏิชีวนะร่วมกันเพื่อหวังผลเสริมฤทธิ์กันและลดอุบัติการณ์เชื้อดื้อยาจึงเป็นทางเลือกที่ดีอีกทางหนึ่งในการรักษาภาวะการติดเชื้อ *A. baumannii* การวิจัยครั้งนี้ต้องการศึกษาฤทธิ์ต้านเชื้อของการใช้ imipenem ร่วมกับ amikacin หรือร่วมกับ ciprofloxacin ต่อ *A. baumannii* ที่ดื้อต่อยา imipenem 30 สายพันธุ์ด้วยวิธี time kill ความเข้มข้นของยาที่ใช้เป็นความเข้มข้นเฉลี่ยของยาในซีรัมเมื่อให้ยาในขณะรักษา และตรวจหายีนที่เกี่ยวข้องกับการดื้อยาโดยการใช้เทคนิค Polymerase Chain Reaction (PCR) จากการทดลองพบว่า *A. baumannii* ทั้ง 30 สายพันธุ์ดื้อต่อ ciprofloxacin และ imipenem ค่า MIC ของ imipenem อยู่ในช่วง 8-32 มคก/มล ซึ่งแสดงการดื้อยาในระดับต่ำและมี 13 สายพันธุ์(43.33%) ของเชื้อที่ยังไวต่อ amikacin เมื่อตรวจหายีนที่เกี่ยวข้องกับการดื้อยาพบว่าเชื้อ10 สายพันธุ์ (33.33%) มียีน *integrase*, 2 สายพันธุ์ (6.67%) มียีน bla_{VEB-1} และ 11 สายพันธุ์ (36.67%) มียีน bla_{OXA-23} ส่วนยีน bla_{OXA-1}, bla_{OXA-2}, bla_{OXA-10}, bla_{IMP} and *aac*(6') ตรวจไม่พบในทุกสายพันธุ์ เมื่อตรวจหาฤทธิ์ในการทำลายยาคาร์บาพีเน็มพบว่าเชื้อ 27 สายพันธุ์ (90%) มีฤทธิ์ดังกล่าวและในทุกสายพันธุ์ที่มียีน bla_{OXA-23} จะตรวจพบว่ามีฤทธิ์ทำลายคาร์บาพีเน็มด้วย เมื่อให้ imipenem ร่วมกับ amikacin สามารถแสดงผลเสริมฤทธิ์กันใน 3 สายพันธุ์(10%) และมีผลต้านฤทธิ์กันใน 1 สายพันธุ์(3.33%) ส่วนเมื่อให้ imipenem ร่วมกับ ciprofloxacin แสดงผลเสริมฤทธิ์กันใน 1 สายพันธุ์ (3.33%) และมีผลต้านฤทธิ์กันใน 2 สายพันธุ์ (6.67%) การให้ยา imipenem เดี่ยวๆสามารถฆ่าเชื้อได้ 1 สายพันธุ์ (3.33%) ที่เวลา 6 ชั่วโมงหลังจากได้รับยา และฆ่าเชื้อได้ 16 สายพันธุ์ (53.33%) ที่เวลา 24 ชั่วโมงหลังจากได้รับยา [จำนวนเชื้อที่ถูกฆ่าภายใน 24 ชั่วโมง = 116.39 log CFU/ml·h] การให้ amikacin เดี่ยวๆ สามารถฆ่าเชื้อได้เพียง 2 สายพันธุ์ (6.67%) ในระหว่างการศึกษา ส่วน ciprofloxacin ไม่มีฤทธิ์ฆ่าเชื้อในทุกสายพันธุ์ที่ทดสอบ สำหรับการให้ imipenem ร่วมกับ amikacin สามารถฆ่าเชื้อได้ 2 สายพันธุ์ (6.67%) ที่เวลา 2 ชั่วโมง และฆ่าเชื้อได้ 28 สายพันธุ์ (93.33%) ที่เวลา 24 ชั่วโมงหลังจากได้รับยา [จำนวนเชื้อที่ถูกฆ่าภายใน 24 ชั่วโมง = 138.83 log CFU/ml·h] นอกจากนี้การให้ imipenem ร่วมกับ ciprofloxacin สามารถฆ่าเชื้อได้ 2 สายพันธุ์ (6.67%) ที่เวลา 6 ชั่วโมงหลังจากได้รับยา และฆ่าเชื้อได้ 22 สายพันธุ์ (73.33%) ที่เวลา 24 ชั่วโมงหลังจากได้รับยา [จำนวนเชื้อที่ถูกฆ่าภายใน 24 ชั่วโมง = 123.50 log CFU/ml·h] จากผลการทดลองแสดงให้เห็นว่าการให้ยา imipenem ร่วมกับ amikacin หรือร่วมกับ ciprofloxacin สามารถแสดงฤทธิ์ในการฆ่าเชื้อได้ดีกว่าการให้ยาเดี่ยวๆ เมื่อเปรียบเทียบในด้านจำนวนเชื้อที่ถูกฆ่า, จำนวนสายพันธุ์ที่ถูกฆ่าและความเร็วในการฆ่าเชื้อ ดังนั้นสามารถนำมาใช้เป็นอีกทางเลือกหนึ่งในการรักษาภาวะติดเชื้อ *A. baumannii* ที่ดื้อต่อยา imipenem ได้

ภาควิชา เภสัชวิทยา ..... ลายมือชื่อนิสิต .....สราญจิตร ดวงสีใส.....

สาขาวิชา เภสัชวิทยา ..... ลายมือชื่ออาจารย์ที่ปรึกษา .....

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KEY WORD: *Acinetobacter baumannii* / COMBINATION / IMIPENEM / AMIKACIN / CIPROFLOXACIN

SARANJIT DUANGSEESAI : *IN VITRO* ANTIBACTERIAL ACTIVITY OF IMIPENEM IN COMBINATION WITH AMIKACIN OR WITH CIPROFLOXACIN AGAINST IMIPENEM RESISTANT-*ACINETOBACTER BAUMANNII*. THESIS ADVISOR : ASSOC. PROF. SIRIPORN FUNGWITTHAYA, THESIS COADVISOR : ASSOC. PROF. PINTIP PONGPECH, 148 pp. ISBN 974-17-4171-5.

Acinetobacter baumannii (*A. baumannii*) is gram-negative, non-fermentative, non-spore forming, strictly aerobic, oxidase-negative coccobacillary. It has recently become important pathogen causing nosocomial infections, especially in intensive care unit (ICU). There are limited therapeutic alternatives against that species particularly after the evolution of resistance for antimicrobial agents like broad-spectrum β-lactams, aminoglycosides, fluoroquinolones and imipenem over the last decade. Combination therapy is the other choice in treatment *A. baumannii* with the aim of decreasing emergence of resistance strains and increasing bacterial killing. The purpose of the present study is to determine the antibacterial activity of the combination of imipenem plus amikacin or plus ciprofloxacin against 30 strains of imipenem resistant-*A. baumannii* by time kill method. The concentrations of drugs used is the mean serum concentration at therapeutic dose. Another aim is to detect antibiotic resistant genes in imipenem resistant- *A. baumannii* by Polymerase Chain Reaction (PCR) technique. All strains were resistant to ciprofloxacin and imipenem. MIC of imipenem ranged from 8-32 μg/ml, which were low level of resistance. Thirteen isolates (43.33%) were susceptible to amikacin. It was showed that *integrase* gene were positive in 33.33% of isolates, bla_{VEB-1} positive in 6.67% and bla_{OXA-23} in 36.67% as tested by PCR. The bla_{OXA-1}, bla_{OXA-2}, bla_{OXA-10}, bla_{IMP} and *aac*(6') genes could not be detected in all isolates. The carbapenemase activity could be detected in 27 strains (90%) of *A. baumannii* isolates. All of the bla_{OXA-23} gene positive had carbapenemase activity. The antibacterial activity of the combination of imipenem plus amikacin showed the synergistic effect in 3 strains (10%) and the antagonist effect in 1 strain (3.33%). When combined imipenem with ciprofloxacin the synergistic effect could be observed in 1 strain (3.33%) and the antagonist effect in 2 strains (6.67%). Imipenem alone showed bactericidal activity against 1 strain (3.33%) at 6 hour of growth and against 16 strains (53.33%) at 24 hour of growth [BA_{24} = 116.39 log CFU/ml·h]. Amikacin alone showed activity against only 2 strains (6.67%) during the time of study. Ciprofloxacin alone showed no antibactericidal activity against all stains tested. The combination of imipenem plus amikacin showed bactericidal activity against 2 strains (6.67%) at 2 hour of growth and against 28 strains (93.33%) at 24 hour of growth [BA_{24} = 138.83 log CFU/ml·h]. The combination of imipenem plus ciprofloxacin showed bactericidal activity against 2 strains (6.67%) at 6 hour of growth and against 22 strains (73.33%) at 24 hour of growth [BA_{24} = 123.50 log CFU/ml·h]. The results obtained suggested that antibacterial activity of the combination of imipenem plus amikacin or plus ciprofloxacin were higher than antibacterial activity of single drug alone. It is concluded that the combination of imipenem plus amikacin or plus ciprofloxacin could be promising alternatives for the treatment of infection due to imipenem resistant-*A. baumannii*.

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LIST OF ABBREVIATIONS

°C	= degree Celsius
A. baumannii	= *Acinetobacter baumannii*
AAC	= aminoglycoside acetyltransferases
ANT	= aminoglycoside nucleotidyltransferases
APH	= aminoglycoside phosphotransferases
AUBKC	= Area under the bacterial killing and regrowth curves
AUC	= Area under the curve
BA24	= Bacteriolytic area of 24 hours
bla	= beta lactamase gene
CFU	= Colony forming unit
E.coli	= *Escherichia coli*
e.g.	= exempli gratia (for example)
enz.	= enzyme
ESBL	= extended-spectrum β-lactamase
et al.	= et alii (and other peoples)
etc.	= et cetera (and other similar things)
Fig	= Figure
g	= gram
hr	= hour
K. pneumoniae	= *Klebsiella pneumoniae*
L	= Liter
log	= decimal logarithm
MBC	= Minimum bactericidal concentration
MHA	= Meuller-Hinton agar
MHB	= Meuller-Hinton broth
MIC	= Minimum inhibitory concentration
min	= minute
ml	= milliliter
mm	= millimeter

mol	= mole
NCCLS	= The National Committee for Clinical Laboratory Standards
nm	= nanometer
NSS	= Normal saline solution
OXA	= oxacillin-hydrolyzing β-lactamase
P. aeruginosa	= *Pseudomonas aeruginosa*
PCR	= Polymerase chain reaction
PBP	= Penicillin binding protein
TSA	= Tryptic Soy Agar
μg	= microgram
μl	= microliter
VEB	= vietnamese-extended spectrum β-lactamase

CHAPTER I

INTRODUCTION

Acinetobacter baumannii (*A. baumannii*) is a gram-negative, non-fermentative, non-spore forming, strictly aerobic, oxidase-negative coccobacillary organisms (Jain and Danziger, 2004). The organism has emerged as a significant nosocomial pathogen causing nosocomial infections, including urinary and lower respiratory tract infections as well as septicemia. There is a high risk of infections for debilitated patients in intensive care units. Frequent sources of this pathogen are intravenous and urinary catheters or endotracheal tubes. There is an increasing occurrence of *A. baumannii* in hemoculture and it may represent the most frequent pathogen isolated from blood (Hejnar et al., 1999).

Historically, *A. baumannii* was susceptible to many penicillins, cephalosporins, aminoglycosides and quinolones but, more recently, resistance to multiple antibiotics has been increasingly recognized. Imipenem and meropenem have retained in-vitro activities that are superior to those of other antimicobials and, in many centres, they are the drugs of choice for patients infected by *A. baumannii*. In recent years, Afzal-Shan and Livermore have actively sought carbapenem-resistant *A. baumannii* worldwide (Afzal-Shan and Livermore, 1998). In Thailand, an increase in the prevalence of imipenem-resistant *A. baumannii* has been reported (http://narst.dmsc.moph.go.th). It was shown that the percent susceptibility of imipenem in year 2002 (79%) has actively decreased as compared with that in year 2001 (92%). For amikacin and ciprofloxacin, the percent susceptibility have been constanly about 40%. Most *A. baumannii*, which were resistant to imipenem, may also be resistant to other antimicrobial agents (Jain and Danziger, 2004).

Antibiotic resistance is a major problem in patients, which are infected with *A. baumannii*, affects the selection of appropriate antibiotic for the treatment. Combination therapy is the other choice in the treatment *A. baumannii* aiming at decreasing emergence of resistant strains and increasing bacterial killing (Bergogne-Berezin and Towner, 1996). Several previous studies have demonstrated the synergistic activity of β-

lactams in combination with other antimicrobial agents against resistant strains of *Pseudomonas aeruginosa* (Song et al., 2003). In addition, it has been reported that the combination of imipenem plus amikacin showed synergistic activity against 2 strains of pandrug resistant *A. baumannii* (Hsueh et al., 2002). Therefore, the purpose of the present study is to determine the antibacterial activity of the combination of imipenem plus amikacin or plus ciprofloxacin against imipenem resistant-*A. baumannii* by time kill method. The concentration of drugs used in this study is the mean serum concentration at therapeutic dose. Another aim of this study is to detect the antibiotic resistant genes strains by Polymerase Chain Reaction (PCR) technique and to detect carbapenemase activity by spectrophotrometric assay in imipenem resistant-*A. baumannii*. In addition, the relationship between antibacterial activity and antibiotic resistant genes in imipenem resistant-*A. baumannii* were investigated.

CHAPTER II

LITERATURE REVIEWS

I. *Acinetobacter baumannii*

Acinetobacter baumannii (*A. baumannii*) is a gram negative, non fermentative, non-spore formimg, strictly aerobic, oxidase-negative coccobacillary organisms. It is widely found in nature, mostly in water and soil. The organism has also been isolated from skin, throat, and various other sites in healthy people, and has emerged as a significant nosocomial pathogen in hospitalized patient worldwide. The multitude of *A. baumannii* infections including pneumonia, bacteremia, meningitis, urinary tract infections, peritonitis, and skin and soft tissue infections have been reported. It is generally considered as a low virulence organism except for the isolate from the critically ill or immonocompromised patients (Borgogne-Berezin and Towner, 1996). It is most often associated with nosocomial rather than community-acquired infections. Outbreaks in hospitals have been associated with contaminated ventilator equipment, mattresses, pillows, humidifier, and overuse of specific antimicrobial. Often, these outbreaks have exhibited pattern of multidrug resistance (MDR), making them difficult to eradicate from both the patients and the environment. Resistance patterns vary from region to region, with some areas reporting only susceptible to carbapenems, where others report resistance to all commercially available antimicrobials. In recent years, there has been a worldwide increased incidence of carbapenem-resistant *A. baumannii* (Hsueh et al., 2002). In Thailand, an increase in the prevalence of imipenem-resistant *A. baumannii* has been observed as shown in Table 2-1 (http://narst.dmsc.moph.go.th/).

Biochemical and genetic mechanisms of antibiotic resistance

A. baumannii appears to have a propensity to develop antibiotic resistance extremely rapidly, perhaps as a consequence of its long-term evolutionary exposure to antibiotic-producing organisms in a soil environment. This is in contrast to more “traditional” clinical bacteria, which seem to require more times to acquire highly effective resistance mechanisms in response to the introduction of modern radical therapeutic strategies; indeed, it may be its ability to respond rapidly to challenge with

antibiotics, coupled with widespread use of antibiotics in the hospital environment, that is responsible for the recent success of *A. baumannii* as nosocomial pathogen. However, althougth all three of the major modes of chromosomal gene transfer have been demonstrated in *A. baumannii*, only conjugation has so far been shown to play a significant role in the transfer of antibiotic resistance between member of this genus. Plasmid and transposons play an importance role in *A. baumannii*. Several studies have reported that >80% of *A. baumannii* isolates carry multiple indigenous plasmids of variable molecular size, although other investigator reported the problem in the isolate on of plasmid DNA from *A. baumannii*, often because of unappreciated difficulties in lysing the cell wall of the organism (Borgogne-Berezin and Towner, 1996). Transposons probably play an important role in conjunction with integrons (Hall and Colis, 1993), in ensuring that particular novel genes can become established in a new gene pool, even if the plasmid vectors that transferred them are unstable, and there have been several reports of chromosomally located transposons carrying multiple antibiotic resistance genes in clinical isolates of *A. baumannii* (Hall and Colis, 1993).

Table 2-1 Antibiogram Year 1998-2003 from 32 Hospitals in Thailand (http://narst.dmsc.moph.go.th/)

Year	Percentage of susceptible *Acinetobacter calcoaceticus-baumannii*										
	AZM	CAZ	CPZ/SUL	IPM	PIP	CIP	NOR	AMK	GEN	NET	SXT
1998	5	40	72	98	15	45	38	48	34	68	31
1999	6	41	80	94	16	49	40	47	34	70	30
2000	3	35	81	95	18	41	35	44	34	62	29
2001	5	35	79	92	33	42	37	42	35	56	30
2002	2	38	78	79	35	40	31	42	36	56	32
2003	1	33	69	65	34	34	26	38	34	60	29

AZM = aztreonam; CAZ = ceftazidime; CPZ/SUL = cefoperazone/sulbactam; IPM = imipenem; PIP = piperacillin; CIP = ciprofloxacin; NOR = norfloxacin; AMK = amikacin; GEN = gentamicin; NET = netilmicin; SXT = trimethoprim/sulfamethoxazole

A. baumannii has a tendency to develop resistance to antimicrobials rapidly. The mechanisms of resistance include altered penicillin-binding proteins, low permeability of the outer membrane, target site mutations, and inactivation via modifying enzymes (Bou et al., 2000). Because *A. baumannii* is a gram-negative organism, it possesses an additional outer membrane that acts as a permeation barrier. Transport across the outer membrane is mediated by porins that produce water-filled channels for diffusion of hydrophilic molecules (e.g. β-lactams, carbapenems). One report

suggested that reduced expression or mutation in porins may be associated with carbapenems resistance (Clark, 1996).

The mechanisms of resistance to β-lactams involve production of β-lactamase encoded by chromosomes or plasmids, alterations of penicillin-binding proteins and the decrease in the permeability of the outer membrane to β-lactams. Major mechanism of resistance is the production β-lactamases, including the widely distributed TEM-1 and TEM-2 enzymes. Four enzymes, designated ACE-1 to ACE-4 were identified as cephalosporinase, which possessed a little activity against penicillins and no detectable hydrolyzing activity against aztreonam or the broad spectrum cephalosporins, ceftazidime and cefotaxime. A particularly worrying development is the identification of a novel β-lactamase, designated ARI-1, in an imipenem- resistant strain of *A. baumannii* isolated from a blood culture in 1985. The β-lactamases known to exist in *A. baumannii* is summarized in Table 2-2 (Bergogne-Berezin and Towner, 1996).

Table 2-2 β-lactamase describes in *A. baumannii*

Enzyme or strain	Location of gene	Predominant substrates	Molecular size (kDa)
TEM-1	Plasmid	penicillin	29
TEM-2	Plasmid	penicillin	29
CARB-5	Plasmid	penicillin	29
AmpC	Chromosome	cephaloridine	Unknown
ACE-1	Chromosome	cephalosporin	~500
ACE-2	Chromosome	cephalosporin	60.5
ACE-3	Chromosome	cephalosporin	32.5
ACE-4	Chromosome	cephalosporin	>1,000
NCTC7844	Chromosome	cephalosporin	30
ML4961	Chromosome	cephalosporin	38
ARI-1 (OXA-23)	Plasmid	carbapenem	23
OXA-24	Chromosome	penicillin, carbapenem	Unknown
OXA-25	Chromosome	oxacillin,penicillin, cephaloridine	Unknown
OXA-26	Chromosome	oxacillin, penicillin	Unknown
OXA-27	Chromosome	oxacillin, cephalothin	Unknown
PER-1-like	Chromosome	Unknown	Unknown
SHV-1	Unknown	Unknown	Unknown

Resistance to aminoglycosides is mediated by 3 mechanisms: alteration of the ribosomal target, reduction of uptake, and enzymatic modification of the drug (Towner, 1997). Alteration of the ribosomal target is not significant because it only affects streptomycin. The second mechanism is fairly common in *A. baumannii*, but it is the third mechanism that accounts for the majority of the resistant isolates. The modifying enzymes, *O*-phosphotransferase, *O*-nucleotidyltransferases and *N*-acetyltransferases are mediated primarily by plasmids or transposons and therefore can play a role in the spread of resistance. These enzymes may also be located on chromosome. Each enzyme has a different substrate that causes the specific resistance profile for the bacteria (Towner, 1997). All three types of aminoglycoside-modifying enzymes have been identified within clinical *Acinetobacter* strains (Table 2-3), but geographic variations in the incidence of particular genes has been observed (Bergogne-Berezin and Towner, 1996).

Table 2-3 Aminoglycoside-modifying enzymes identified in *Acinetobacter* spp.

Enzymes		
Acetylating	Adenylating	Phosphorylating
AAC(6')	ANT(3")I	APH(3')I
AAC(2')	AAD(3")(9)[a]	APH(3')II
AAC(3)I	ANT(2")I	APH(3')III
AAC(3)II	AAD(2")[a]	APH(3')VI
AAC(3)V[a]		APH(3")I
AAC(3)IV		

[a]Enzymatic activity detectable only *in vitro*.

Quinolones have good activity against *A. baumannii*, but resistance is increasing (Gales et al., 2001). The mechanisms of resistance are via chromosomal mutations that alter DNA gyrase and topoisomerase IV, the targets for quinolones (Seward and Towner, 1998). DNA gyrase is composed of 2 subunits, encoded by *gyr*A and *gyr*B genes. Topoisomerase IV is structurally similar to DNA gyrase, but is a secondary target for quinolones. Two subunits of topoisomerase IV are encoded by *par*C and *par*E genes. Resistance in *A. baumannii* is mediated by mutation in *gyr*A and *par*C genes (Seward and Towner, 1998). Other mechanisms of resistance have been described, such as

those in the efflux and influx systems, but the role of these mechanisms remains to be determined (Ruiz, 2003).

Therapy of *A. baumannii* infections

Very few of the major antibiotics are now reliably effective for the treatment of severe nosocomial *A. baumannii* infections, particularly in patients confined to ICUs. β-lactam antibiotics should be used only after extensive *in vitro* susceptibility testing has been performed. Ticarcillin, often combined with sulbactam, ceftazidime, or imipenem may be useful. Aminoglycosides can sometimes be used successfully in combination with an effective β-lactam, and other combination of a β-lactam with a fluoroquinolone or rifampin have also been proposed (Borgogne-Berezin and Towner, 1996). In general, the recommended drugs in most recent studies have been either extended-spectum penicillins, broad spectrum cephalosporins, or imipenem combined with an aminoglycosides (Borgogne-Berezin and Towner, 1996).

II. Imipenem

Imipenem is a semisynthetic carbapenem antibiotic. Carbapenems are differentiated from penicillins and cephalosporins by a methylene for sulfur in the five-membered α-ring structure, which as in the cephalosporins, also contains a double bond. They are derivatives of thienamycin, a compound produced by *Streptomyces cattleya* (Chamber, 2000). Imipenem differs from the conventional β-lactams in the nature and conformation of its side chain. All conventional penicillins and cephalosporins contain an acylamino side chain, whereas imipenem has a hydroxyethyl side chain. Furthermore, the side chain in the conventional β-lactams is in a *cis* configuration, whereas the hydroxyethyl side chain of imipenem is in a *trans* configuration. It is this *trans* conformation that is responsible for the β-lactamase stability of imipenem (Figure 2-1).

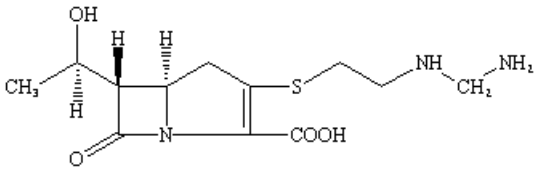


Figure 2-1 Structure of Imipenem

Imipenem is hydrolyzed by the renal peptidase dehydropeptidase-1, located on the brush border of the proximal renal tubules. To overcome the problem of the destruction of imipenem in urine, cilastatin, a dehydropeptidase inhibitor, is administered in equal amount of imipenem. Cilastain has no antibacterial activity nor does it alter the antibacterial activity of imipenem (Chamber, 2000).

The mode of action of imipenem is similar to that of other β-lactams. The β-lactams antimicrobiotics interfere primarily with the transpeptidasation reaction that seal the peptide crosslinks between glycan chains. The activity is probably due to stereochemical similarity to the D-alanyl-D-alanine end of pentapeptide. Thus, β-lactams prevent the completion synthesis in the bacterial cell wall.

The target enzymes of the β-lactams are on the cytoplasmic membrane. They are described as penicillin-binding protein (PBPs), because they were first detected with penicillin, although many of them bind other β-lactam antibiotics avidly. Several distinct PBPs found in any bacterium, are usually species specific, and vary in their abilities to react with different β-lactam antibiotics. There are also functional differences between PBPs. Some appear to be responsible for forming the peptidoglycan linkages that gives an organisms its shape, and other are particularly involved in synthesizing the cross-wall that separate newly formed cells. A β-lactam active primarily on a PBP that determines shape causes susceptible organisms to round up and swell before lysis occurs. One that acts primarily on cell separation and division can produce long cells. Properties of PBPs and theirs responses with β-lactam antibiotics were shown in Table 2-4.

Table 2-4 Properties of the PBPs from *E.coli* and theirs response with β-lactam antibiotics

PBP	Molecular weight	Molecules/cell	Morphological changes after occupied by β-lactams
1A	91	230	Spheroplasting cells
1B	86.5	81.5	Spheroplasting cells
2	66	20	Ovoidal cells
3	60	50	Filamentous cells
4	49	110	-
5	42	1800	-
6	40	5700	-

(Modified from Spratt, 1975; Hayes and Ward, 1986)

Imipenem has an affinity for and binds to most PBPs of susceptible organism, including PBPs 1a, 1b, 2, 4, 5 and 6 of *Escherichia coli* ; PBPs 1a, 1b, 2, 4, and 5 of *Pseudomonas aeruginosa*. In susceptible gram-negative bacteria, imipenem has the greater affinity to PBP 2 and the lowest to PBP 3 (McEvoy, 2001). These results in the formation of spheroplasts or ellipsoidal cells without filament formation. Because imipenem also has a high affinity for PBPs 1a and 1b of these organisms, the spheroplasts lyse rapidly. Imipenem is able to penetrate the outer membrane of most gram-negative bacteria and gain access to the PBPs more readily than many other currently available β-lactam antibiotics.

Imipenem has very wide spectrum of activity. Imipenem is active *in vitro* against most gram positive and gram negative aerobiac bacteria including *Haemophilus influenza*e, *Neisseria meningitidis*, *Pseudomonas aeruginosa*, *Acinetobacter spp*. and Enterobacteriaceae.

The mechanisms underlying resistance to carbapenems in gram negative bacteria an production of carbapenem-hydrolysing β-lactamase (carbapenemase), decreased outer membrane permeability due to the loss or reduced expression of porins, overexpression of multidrug efflux pumps and alterations in PBPs (Fernandez-Cuenca, 2003).

The dosage can be varied widely, according to the nature and severity of the infection. For the treatment of severe and/or life threatening due to less susceptible *A. baumannii* infection, the drug has been given in dosage of 1 g every 6 or 8 hours. The pharmacokinetic achievable concentration; C_{max} $C_{average}$ and C_{min} are 67.3, 36, and 3.1 μg/ml, respectively (AHFS Drugs, 2001).

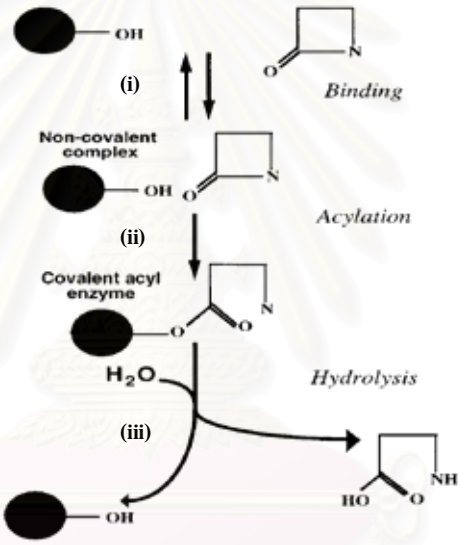
The combination of imipenem plus an aminoglycoside is generally neither synergistic nor antagonistic *in vitro* against most strains of *P. aeruginosa* and Enterobacteriaceae (AHFS Drugs, 2001). In one study, the combination of imipenem plus amikacin showed synergistic effect against pandrug resistant *A. baumannii* (Hsueh et al., 2002).

III. β- lactamases and resistance to β-lactam antibiotics

1.Basic Science of β-lactamases

β-lactamase is an enzyme discovered in most types of gram-negative bacteria. The well-known function of this enzyme is destroying β-lactam antibiotic by a serine ester hydrolysis mechanism (Figure 2-2) and a few use zinc ion to attack the β-lactam ring.

Phases of the reaction of catalyzing the β-lactam antibiotics by serine β-lactamase include (i) reversible non-covalent binding of the β-lactamase and the β-lactam ring, (ii) rupture of the β-lactam ring, which becomes covalently acylated on to the active site serine. (iii) hydrolysis of the acyl enzyme to reactivate the β-lactamase, splitting the amide bond, and liberate the inactivated drug molecule. As a result, the antibiotics can no longer inhibit bacterial cell wall synthesis.



(Modified from Livermore, 1995)

Figure 2-2 Action of a serine β-lactamase to β-lactam antibiotic

2. Classification of β-lactamases

Although all β-lactamases catalyse the same reaction, a number of different types of these enzymes have been isolated and characterized. They have been classified according to several schemes based on :

2.1 The location of genes encoding β-lactamases

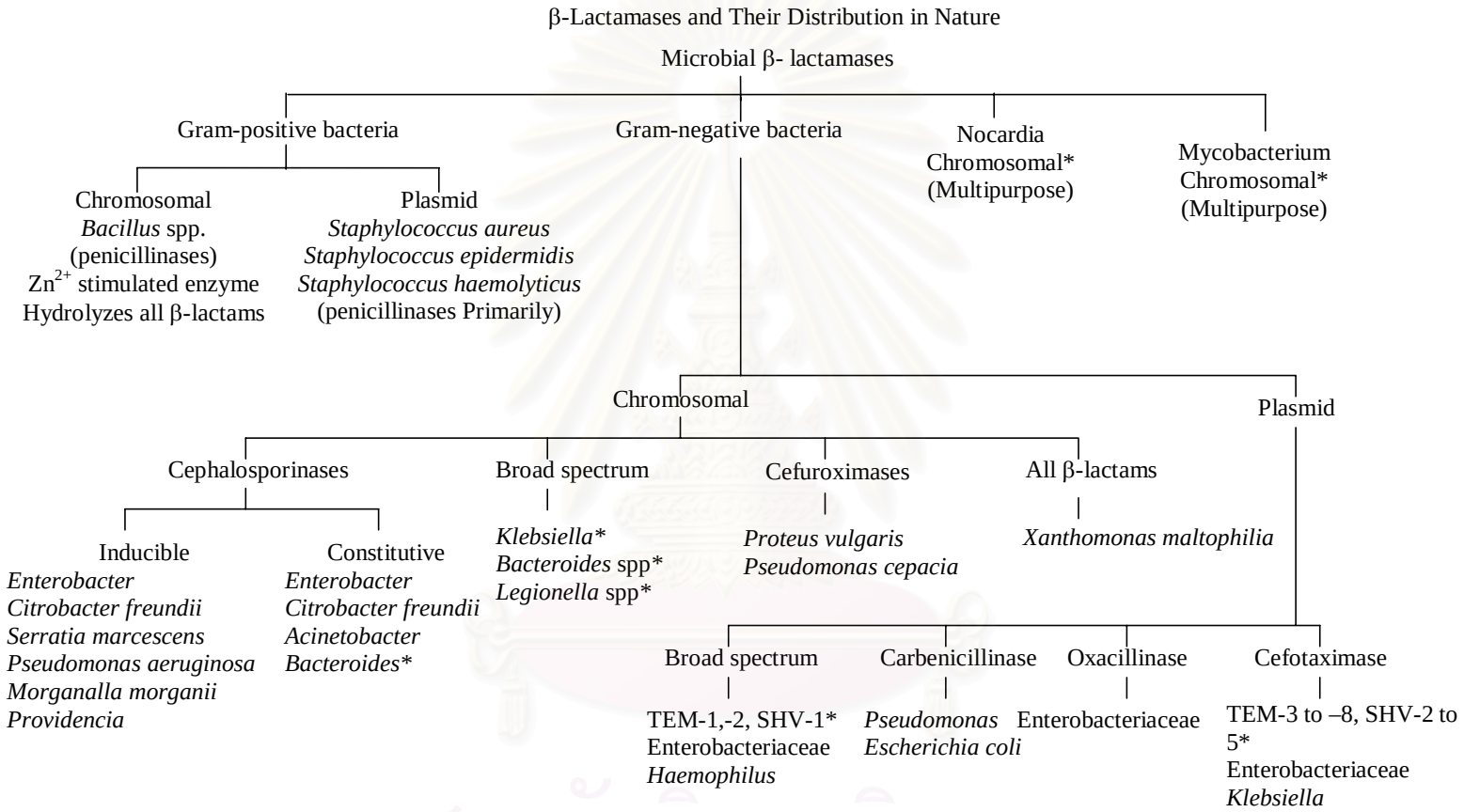
The location of genes encoding β-lactamases may be an innate part of the chromosome or on plasmids. Chromosomal β-lactamases are universal in a specific bacterial species, whereas the presence of those encoded by plasmids are variable,

and they are transferable between bacterial species. Further genetic mobility may be provided by transposons, which can carry β-lactamases genes into plasmids. This mobility is important, since it allows the possibility of spreading resistant genes through several bacterial communities. Gram-negative bacteria produce the β-lactamases either constitutively or induciby by β-lactam antibiotic or other substance, such as D-amino acids. Some β-lactamases in species such as *Acinetobacter* are constitutive chromosomal β-lactamases (Figure 2-3)

2.2 The biochemical characteristic of β-lactamase

Various classification schemes have been proposed for β-lactamase based on the biochemical characteristic such as molecular weight or substrate specificities. A commonly use classification scheme is that devised by Bush *et al*. (Table 2-5). This devides the enzymes into four groups according to substrates and inhibitor susceptibilities. Group 1 consists of cephalosporinases, which are not inhibited by clavulanic acid. Group 2 consists of penicillinases, including broad-spectrum penicillinases that are generally inhibited by the active site directed β-lactamase inhibitors. Subgroups of enzymes, namely, 2a, 2b, 2be, 2br, 2c, 2d, 2e and 2f were defined based on the rate of hydrolysis of carbenicillin, cloxacillin, extended-spectrum β-lactams such as ceftazidime, cefotaxime, or aztreonam and of inhibition profile by clavulanate, respectively. Enzymes that are inhibited by the metal chelating agent ethylenediamine tetraacetic acid (EDTA) are classified as group 3. Group 4 consists of β-lactamases that are not inhibited by clavulanic acid.

However, the classification scheme proposed by Ambler is also commonly used. This classification arranges the β-lactamases into four groups according to β-lactamases sequences. Class A, C and D comprise evolutionarily distinct groups of serine enzymes and class B enzymes utilize a zinc ion to attack the β-lactam ring. Class A β-lactamases prefer penicillins as substrates, whereas class C enzymes turn over cephalosporins better. Class B enzymes can hydrolyze a broad range of substrates including carbapenems, which resist hydrolysis by most of the other class of enzymes. Class D β-lactamases, on the other hand, hydrolyze oxacillin type β-lactams efficiently. Class A and C of β-lactamases are the most common and the second most common enzymes, respectively.



* = inhibited by clavulanic acid, sulbactam and tazobactam

(Modified from Chambers, 2000)

Figure 2-3 Diagrammatic representation of β-lactamases (Modified from Chambers, 2000)

Table 2-5 Classification schemes for bacterial β-lactamases

Structural class (Ambler)	Functional group (Bush)	Preferred substrates	Inhibition by clavulanate	Representative enzyme
Serine β-lactamase				
A	2a	Penicillins	+ +	Penicillinases from gram-positive bacteria
	2b	Penicillins, cephalosporins	+ +	TEM-1, TEM-2, SHV-1
	2be	Penicillins, narrow-spectrum and extended-spectrum cephalosporins, monobactams	+ +	TEM-3 to TEM-26, SHV-2 to SHV-6, *Klebsiella oxytoca* K1
	2br	Penicillins	-	TEM-30 to TEM-36, TRC-1
	2c	Penicillins, carbenicillin	+	PSE-1, PSE-3, PSE-4
	2e	Cephalosporins	+ +	Inducible cephalosporinases from *Proteus vulgaris*
	2f	Penicillins, cephalosporins, carbapenems	+	NMC-A from *Enterobacter cloacae*, Sme-1 from *Serratia marcescens*
C	1	Cephalosporins	-	AmpC enzymes from gram-negative bacteria; MIR-1
D	2d	Penicillins, cloxacillin	±	OXA-1 to OXA-11, PSE-2 (OXA-10)
Undetermined	4	Penicillins	-	Penicillinase from *Pseudomonas cepacia*
Zincβ-lactamase				
B	3	Most β-lactams, including carbapenems	-	L1 from *Xanthomonas maltophilia*, CcrA from *Bacteroides fragilis* IMP family

+ +, Strong inhibitor of all members of class, +, moderate inhibition, ±, inhibition varies within the class,

- , negligible inhibition

(Modified from Bush et al., 1995)

The New β-Lactamases

Agents that shared the property of resistance to the then-common β-lactamases were introduced about 20 years ago. They included cephamycins, cephalosporins with an oxyimino side chain, carbapenems, and the monobactam aztreonam. Bacteria responsed with a plethora of "new" β-lactamases including 1.extended-spectrum β-lactamases (ESBLs), 2.plasmid-mediated AmpC enzymes, and 3.carbapenem-hydrolyzing β-lactamases (carbapenemase) that with variable success can confer resistance to the latest β-lactam antibiotic (Table 2-6) (Jacoby and Munoz-Price, 2005).

1. ESBLs

The ESBLs enzymes are plasmid- mediated enzyme. ESBLs producing organism are frequently resistant to many drug classes of antibiotics, including aminoglycosides and fluoroquinolones (Nathisuwan et al., 2001).

1.1 TEM-type ESBLs (Class A)

Amino acid substitutions at many sites in TEM-1 β-lactamases can be created in the laboratory without loss of activity (Jacoby and Munoz-Price, 2005). TEM-1 is the most commonly encountered β-lactamase in gram-negative bacteria (Bradford, 2001). Those responsible for the ESBL phenotype change the configuration of the enzyme, allowing access to oxyimino-β-lactams. Opening the active site to β-lactam substrates also typically enhances the susceptibility of the enzyme to β-lactamase inhibitors, such as clavulanic acid. Amino acid substitutions distinct from those leading to the ESBL phenotype can confer resistance to inhibitors, but the combination of inhibitor resistance and an extended spectrum of activity seem to be, with rare exception, incompatible. More than 130 TEM enzymes are currently recognized, and their varieties provide a useful way to follow the spread of individual resistance genes (Jacoby and Munoz-Price, 2005).

1.2 SHV-type ESBLs (Class A)

SHV-1 shares 68 percent of its amino acids with TEM-1 and has a similar overall structure. As with TEM, SHV-type ESBLs have one or more amino acid substitutions around the active site. The SHV-1 β-lactamase is most commonly found in *Klebsiella pneumoniae* and is responsible for up to 20% of the plasmid-mediated ampicillin resistance in this species. More than 50 varieties of SHV are currently recognized on the

basis of unique combinations of amino acid replacements (Jacoby and Munoz-Price, 2005).

1.3 CTX-M type ESBLs (Class A)

The most common group of ESBLs not belonging to the TEM or SHV families is termed CTX-M that preferentially hydrolyzes cefotaxime. These enzymes are not very closely related to TEM or SHV β-lactamases showing only approximately 40% identity with these two commonly isolated β-lactamases. More than 40 CTX-M enzymes are currently known (Jacoby and Munoz-Price, 2005).

1.4 Other class A ESBLs

Other class A ESBLs are uncommon and have been found mainly in *Pseudomonas aeruginosa* and at a limited number of geographic sites: PER-1 in isolates in Turkey, France, and Italy; VEB-1 (Vietnamese extended spectrum β-lactamase) and VEB-2 in strains from South East Asia; and GES-1, GES-2, and IBC-2 in isolates from South Africa, France, and Greece. PER-1 is also common in multiresistant *Acinetobacter* species in Korea and Turkey. Some of these enzymes are found in Enterobacteriaceae as well, whereas other uncommon ESBLs (such as BES-1, IBC-1, SFO-1, and TLA-1) have been found only in Enterobacteriaceae. These enzymes all confer resistantce to oxyimino-cephalosporins, especially ceftazidime, and aztreonam (Jacoby and Munoz-Price, 2005).

1.5 OXA-type ESBLs (Class D)

The OXA (oxacillin-hydrolyzing β-lactamase) type enzymes differ from the TEM and SHV enzymes in they belong to molecule class D and functional group 2d. The OXA-type β-lactamases confer resistance to ampicillin and cephalothin and are characterized by their high hydrolytic activity against oxacillin and cloxacillin and the fact that they are poorly inhibited by clavulanic acid (Bradford, 2001). Twelve ESBLs derived from OXA-10, OXA-1, and OXA-2 by amino acid substitutions are currently known (Jacoby and Munoz-Price, 2005).

Table 2-6 Select β-Lactamases of Gram-Negative Bacteria.

β-Lactamase	Examples	Substrates	Inhibition by Clavulanic acid*	Molecular class
Broad-spectrum	TEM-1, TEM-2, SHV-1	Benzylpenicillin (penicillin G), aminopenicillins (amoxicillin and ampicillin), carboxypenicillins (carbenicillin and ticarcillin), narrow spectrum cephalosporins (cefazolin, cephalothin, cefamandole, cefuroxime, and other)	+++	A
	OXA family	Substrates of the broad-spectrum group plus cloxacillin, methicillin, and oxacillin	+	D
Expanded-spectrum	TEM family and SHV family	Substrates of the broad-spectrum group plus oxyimino-cephalosporins (cefotaime, cefpodoxime, ceftazidime, and ceftriaxone) and monobactam (aztreonam)	++++	A
	Others (BES-1, GES/IBC family, PER-1, PER-2, SFO-1, TLA-1, VEB-1, AND VEB-2)	Same as for TEM family and SHV family	++++	A
	CTX-M family	Substrates of the expanded-spectrum group plus, for some enzymes, cefepime	++++	A
	OXA family	Same as for CTX-M family	+	D
AmpC	AAC-1, ACT-1, CFE-1, CMY family, DHA-1, DHA-2, FOX family, MIR-1, MOX-1, and MOX-2	Substrates of the expanded-spectrum group plus cephamycins (cefotetan, cefoxitin, and other)	0	C
Carbapenemase	IMP family, VIM family, GIM-1, and SPM-1	Substrates of the expanded-spectrum group plus cephamycins and carbapenems (ertapenem, imipenem, and meropenem)	0	B
	KPC-1, KPC-2, and KPC-3	Same as for IMP family, VIM family, GIM-1, and SPM-1	+++	A
	OXA-23, OXA-24, OXA-25, OXA-26, OXA-27, OXA-40, and OXA-48	Same as for IMP family, VIM family, GIM-1, and SPM-1	+	D

* Plus signs denote relative sensitivity to inhibition

2. Plasmid-mediated AmpC enzyme (Class C)

AmpC β-lactamases, usually inducible by β-lactams, are generally encoded by chromosomal genes in many gram negative bacilli, but now are found in plasmids. Mutations that increase their expression are responsible for ready emergence of broad-spectrum cephalosporin resistance. Characteristically, AmpC β-lactamases provide resistance to cephamycins as well as to oxyimino-β-lactams and are resistant to inhibition by clavulanic acid (Jacoby and Munoz-Price, 2005).

3. Carbapenemases (Class A, B, and D)

Carbapenemases are a diverse group of enzymes. They are currently uncommon but are a source of considerable concern, because they are active not only against oxyimino-cephalosporins and cephamycins but also against carbapenems (Jacoby and Munoz-Price, 2005).

Chromosomal carbapenemases

Several infrequent non-fermenters and aeromonads have chromosomally encoded carbapenemase belonging to molecular class B. Class B enzymes are unique among β-lactamases in having a zinc ion at their active site, and are the only β-lactamase family where carbapenemase activity is prevalent. Their catalytic activity depends on the zinc ion, and is loss if it is sequestered with EDTA. β-lactamases of the other molecular classes (A,C and D) lack zinc, have a serine-based mechanism and, with a few exceptions, lack significant carbapenemase activity (Livermore and Woodford, 2000).

Acquired carbapenemases

Bacterial strains with acquired carbapenemases remain extremely rare, but have been reported more frequently in the past two to three years, with the list of enzyme types growing sharply. This list now includes representatives of molecular class A, B and D (Table 2-7). Acquired class B carbapenemases have been found in *Acinetobacter* spp., *P. aeruginosa* and Enterobactericeae, whereas class D types have been found only in *Acinetobacter* spp. and class A types in a few Enterobacteriaceae isolates (Livermore and Woodford, 2000).

3.1 Acquired class B carbapenemases

- *IMP β-lactamases*

In 1991 Watanabe et al. reported a *P. aeruginosa* Japanese strains with a transferable class B enzyme. The authors characterized this enzyme biochemically, but did not sequence or name it; nevertheless, it almost certainly was IMP-1. An *Acinetobacter* strain from Italy that gave a PCR product with primers to bla_{IMP-1} (Cornaglia et al., 1999) was subsequently shown to have the gene encoding IMP-2. An enzyme variant with only 85% amino acid identity to IMP-1 and with a substantially different signal peptide (Riccio et al., 2000). A third variant, initially designated IMP-3, has been identified in multiple isolates of *Acinetobacter* DNA groups 2 and 3 collected from one hospital in Hong Kong between 1994 and 1998 (Chu et al., 2000). This enzyme has 95.6% amino acid homology with IMP-1 and 89.3% homology with IMP-2. All these three IMP enzymes have broad activity against β-lactams except monobactams. IMP-1 is relatively more active against carbenicillin than ampicillin. Whereas IMP-2 and IMP-3 have similar activity against both drugs (Riccio et al., 2000 and Chu et al., 2000). The bla_{IMP-1} gene often occurs as a cassette in class 1 integron (Laraki et al., 1999); bla_{IMP-2} was also found within an integron structure (Riccio et al., 2000), and a similar genetic location seems likely for bla_{IMP-3}. Integrons can be inserted into plasmids, and transferable of IMP-1 β-lactamases (Watanabe et al., 1991). Transferable of IMP-2 and IMP-3 β-lactamases has not been reported, but perhaps only because the recipients used were inappropriate for *Acinetobacter* donors. Evidence supporting this view comes from the successful *in vitro* transfer of an unsequenced bla_{IMP} variant between *A. baumannii* strains in Japan (Takahashi et al., 2000), even through no plasmids were detected in the transconjugants. The discovery of IMP-3 in multiple genospecies of *Acinetobacter* in Hong Kong also implies horizontal transfer (Chu et al., 2000). Correlation between carriage of bla_{IMP} alleles and carbapenem resistance is imperfect, and some bla_{IMP} hosts appear susceptible (Senda et al., 1996). Two explanations are possible: either bla_{IMP} is not always expressed, or substantive resistance may demand reduced uptake of the carbapenem as well as the presence of β-lactamase enzyme.

- *VIM β-lactamases*

The second family of acquired class B β-lactamases the VIM types was found reported in 1999, with the description of VIM-1 from a *P. aeruginosa* isolate collected in 1997 in Verona (Italy) (Lauretti et al., 1999). A second VIM variant, VIM-2 was described

from a single *P. aeruginosa* isolate collected in France in 1996 (Poirel et al., 2000). VIM-2 has 90% amino acid homology with VIM-1. The VIM enzymes has <40% amino acid homology with the IMP types (31% homology comparing IMP-1 and VIM-1), but both families have similar kinetic activity. Moreover, and like the IMP enzymes, VIM-1 and VIM-2 can be encoded by gene cassettes within integron.

Unsequenced metallo-β-lactamases have been reported from *Acinetobacter* spp. in Cuba (Peres et al., 1996). These may, or may not, be IMP or VIM type enzymes.

3.2 Acquired class D carbapenemases

Molecular class D β-lactamases correspond almost perfectly to the enzymes classified phenotypically as OXA types on account of their oxacillinase activity. The commonest representatives are OXA-1, -2 and -10 (PSE-2), which all lack carbapenemase activity (Livermore, 1995). OXA-2 and OXA-10 enzymes can yield mutants that attack to oxyimino-aminothiazolyl cephalosporins, but no described mutation to these enzymes confers carbapenemase activity (Nass and Nordmann, 1999). Despite the stability of carbapenems to common class D enzymes, carbapenem resistance in *Acinetobacter* isolates has repeatedly been associated with enzymes with strong oxacillinase activity. Several of these oxacillinases from carbapenem-resistant acinetobacters have now been sequenced and confirmed as class D enzymes (Table 2-5). These include OXA-23 (ARI-1) from an isolate collected in Scotland in 1985 (Donald et al., 2000), OXA-24 (Bou et al., 2000) and OXA-25 from Spain, OXA-26 from Belgium, and OXA-27 from Singapore (Afzal-Shan et al., 2001). Further, unsequenced OXA enzymes with carbapenemase activity are known from acinetobacter collected in Argentina (Afzal-Shan et al., 1999). OXA-23 and OXA-27 enzymes are distinguished by only two amino acid differences. Likewise, OXA-24, -25 and -26 are distinguished by only three or four amino acid substitutions and insertions (Afzal-Shan et al., 2001), but the OXA-23/27 and OXA-24/25/26 clusters only share 60% homology. Cephaloridine and cephalothin are substrates, but OXA carbapenemases activity against oxyimino-aminothiazolyl cephalosporins is consistently weak. Tazobactam and clavulanate are progressive inhibitors of OXA carbapenemases. Conjugative transfer of OXA-23 (ARI-1) production was reported (Scaife et al.,1995), but transfer of other types has not been

achieved, either because their genes reside on non-conjugative elements or because inappropriate recipients were used.

3.3 Acquired class A carbapenemases

The class A carbapenemases comprise Sme-1 and Sme-2, also NMC-A and IMI-1. All class A carbapenemases are penicillinase with greater activity against imipenem than meropenem, and they give resistance to penicillins and aztreonam as well as carbapenems. Oxyimino-cephalosporins are weak substrates, and the class A enzymes do not confer resistance. Clavulanate and tazobactam are inhibitors of these class A carbapenemases (Livermore and Woodford, 2000).

Table 2-7 Geographic distribution of acquired carbapenemases (Livermore and Woodford, 2000)

Enzyme	Class	Species	Countries found
IMP-1 and unsequenced homologues	B	*P. aeruginosa*, *Serratia*, *Klebsiella* (*K. pneumoniae*)	Japan, Singapore
IMP-2	B	*Acinetobacter* spp.	Italy
IMP-3	B	*Acinetobacter* spp. (multiple geno-species)	Hong Kong
VIM-1	B	*P. aeruginosa*	Italy
VIM-2	B	*P. aeruginosa*	France
VIM, unsequenced homologues	B	*P. aeruginosa*	Greece, Oman, South Korea
OXA-23/27 cluster and unsequenced homologues	D	*Acinetobacter* spp.	UK (Scotland), Singapore, Brazil
OXA-24/25/26 cluster and unsequenced homologues	D	*Acinetobacter* spp.	Spain, Belgium
Sme-1, Sme-2	A	*S.marcescens*	UK, USA
IMI-1, NMC-A	A	*E.cloacae*	France, USA

IV. Detection β-lactamases activities by Spectrophotometric Assays (Livermore & Williams, 1996)

Principle : β-lactam antibiotics generally exhibit adequate level of absorption in the UV spectrum to allow measurement of loss absorbance when the β-lactam ring is hydrolyzed. The assay is more useful for cephalosporins because of higher UV absorption than that for penicillins, but it has used for both, as well as for monobactems. The optimal wavelength absorption maxima varies with each β-lactam, as shown in Table 2-8 (Livermore & Williams, 1996).

Reagent : Use quartz cuvettes, as readings are in the UV range. β-lactam solution are prepared freshly each day. Buffer can be sample one, such as 20 mM Tris HCl pH 7.5. Recording spectrophotometer with temperature-controlled cuvette compartment. The enzyme preparation for penicillins should contain low protein concentration.

Procedure: The β-lactam antibiotics can be used over a wide substrate range and at differet pHs. Frequently used initial concentration are in the 100 to 200 μM range, with 20 mM Tris HCl pH 7.5 as buffer. The reaction should be performed at the optimal pH of the enzyme, determined by trial procedures. It is preferable to use enzyme and substrate concentration that give a linear change in absorbance for at least 5 to 10 minute. Temperature of incubation frequently used are 25, 30, or 35°C. It is preferable to carry out the reaction in recording spectrophotometer with a temperature-controlled cuvette holder. The kinetic constant K_m and V_{max} can be obtained from Lineweaver-Burk plots of at least 4 substrate concentrations.

Table 2-8 Absorption maximum for some β-lactams

β-lactams	Absorption Maximum (nm)
Cephaloridine	270
Cephalothin	270
Cefotaxime	264
Ceftriaxone	240
Cefoxitin	270
Cefuroxime	264
Cefoperazone	276
Ceftazidime	272
Cefazolin	266
Cefamandole	274
Imipenem	299
Meropenem	297
Ampicillin	235

V. Amikacin

Amikacin is a semisynthetic aminoglycoside antibiotic derived from kanamycin A. All aminoglycosides have an essential six-membered ring with amino group substituents hence the name aminocyclitol for this structure (Figure 2-4). The descriptor aminoglycoside results from the glycosidic bonds between the aminocyclitol and two or more amino containing or non-amino-containing sugars (Gilbert, 2000).

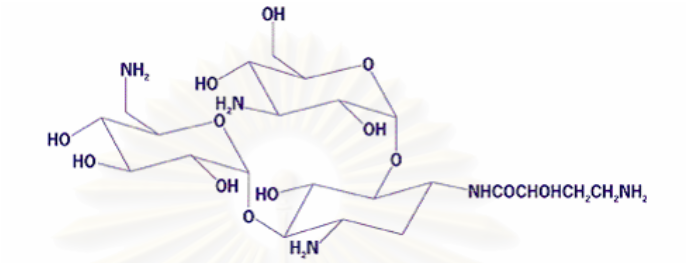


Figure 2-4 Structure of amikacin

Amikacin appears to inhibit protein synthesis in susceptible bacteria by irreversibly binding to 30S ribosomal subunits. In sufficient concentrations, aminoglycosides bind to the ribosome irreversibly, block initiation complexs, and prevent elongations of polypeptide chains, resulting in a rapid bactericidal effect. Lower concentrations lead to distortion of the site of attachment of messenger (m)RNA, misreading of the message, and failure to produce the correct proteins with dramatic effects on growth and bacterial structure.

The aminoglycosides are actively transported into the bacteria cell by a mechanism that involves oxidative phosphorylation. Thus, they have a little or no activity against strict anaerobes or facultative organisms that metabolize only fermentatively (eg. streptococci). Amikacin has a broad spectrum of bactericidal action against many aerobic and facultative gram positive and gram negative rods, including *P. aeruginosa* and *A. baumannii*.

Bacteria defend themselves against amikacin by some combination of three mechanisms: alteration in uptake, synthesis of modifying enzymes, or a change in ribosomal binding sites. Enzymatic modification is the most common mechanism (Glibert, 2000). Aminoglycoside modifying enzyme that inactivated amikacin in *A.*

baumannii were AAC(6') and APH(3')VI. Ploy et al. could detect AAC(6') in 32 % of *A. baumannii* isolates. Vila et al. could detect AAC(6') in 28 % of *A. baumannii* isolates.

The recommended dose of amikacin is 7.5 mg/kg per day, as a single daily dose or divided into two or three equal portion. The pharmacokinetic achievable concentration; C_{max}. $C_{average}$ and C_{min} are 38, 20, and 1.3 μg/ml, respectively (AHFS Drugs, 2001).

The combination of imipenem plus an aminoglycoside is generally neither synergistic nor antagonistic *in vitro* against most strains of *P. aeruginosa* and Enterobacteriaceae (AHFS Drugs, 2001). In one study, the combination of imipenem plus amikacin showed synergistic effect against pandrug resistant *A. baumannii* (Hsueh et al., 2002).

Aminoglycoside modifying enzymes

Several mechanisms have been proposed for bacterial resistance to aminoglycoside antibiotics. They include decreased antibiotic uptake and accumulation, modification of the ribosomal target, efflux of antibiotic, and enzymatic modification of aminoglycosides. The major mechanism of aminoglycoside resistance in clinical isolates of gram-negative and gram-positive bacteria is enzymatic modification of the amino or hydroxyl groups of these antibiotics (Figure 2-5). These families of enzymes that perform cofactor-dependent drug modification in the bacterial cytoplasm have been recognized; these are aminoglycoside phosphotransferases (APHs), aminoglycoside acetyltransferases (AACs), and aminoglycoside nucleotidyltransferases (ANTs). The level of resistance produced differs significantly in various microorganisms and individual strains and depends on many factors, including the amount of enzyme produced, its catalytic efficiency, and the type of aminoglycosides. Whereas many of these enzymes give sufficient activities to result in effective resistance, generally only phosphotransferases produce high level of resistance. Each of three families of enzymes is futher divided into classes, designated by the site of modification, which is indicated in parentheses. They are further subdivided into enzyme types (designated by Roman numerals) that specify unique resistance phenotypes. Finally, individual enzymes of the same class and type that produce the same phenotype but are encoded

by different genes are designated by a lowerease letter. For example, the AAC(6')-I enzymes AAC(6')-Ia, AAC(6')-Ib, AAC(6')-Ic, etc., are aminoglycoside acetyltransferases that modify the antibiotic at position 6' and produce the same phenotype (inactivation of tobramycin, amikacin, netilmicin, kanamycin, and dibekacin) but are encoded by different genes. (Vakulenko and Mobashery, 2003)

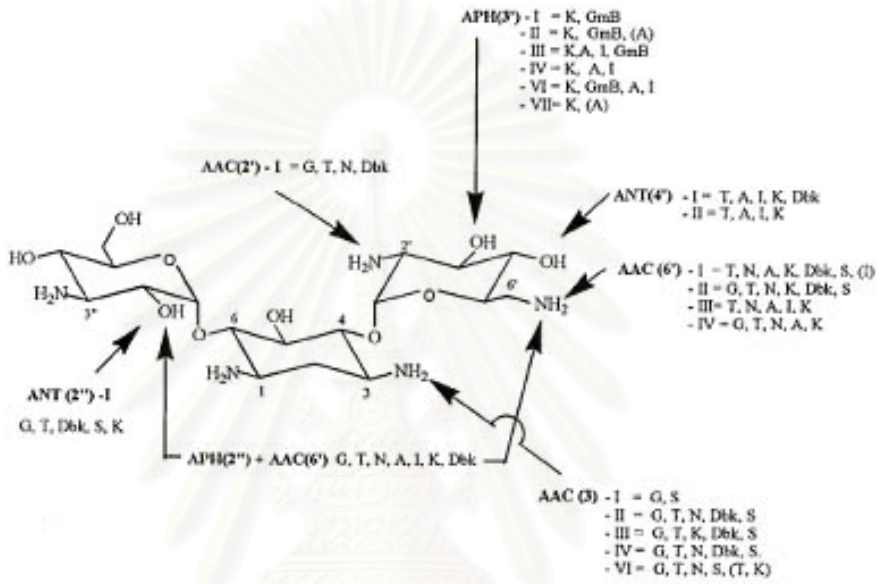


Figure 2-5 Site of modification by aminoglycoside-modifying enzymes on kanamycin B. amikacin (A), dibekacin (Dbk), gentamicin (G), gentamicin B(GmB), kanamycin A (K), isepamicin (I), netilmicin (N), sisomicin (S) and tobramycin (T)

Aminoglycoside Phosphotransferases

Aminoglycoside phosphotransferases (kinases) utilize ATP as the second substrate and are able to phosphorylate specific hydroxyl groups in all classes of aminoglycoside antibiotics. Seven classes of enzymes, APH(3'), APH(2"), APH(3"), APH(4), APH(7"), APH(6), and APH(9) have been identified in clinical isolates and aminoglycoside-producing organisms (Vakulenko and Mobashery, 2003). The largest class of aminoglycoside phosphotransferases includes enzymes that modify the hydroxyl groups of antibiotic at the 3' position. Table 2-9 summarizes the substrate profiles of the aminoglycoside phosphotransferases.

Table 2-9 Substrate profiles of aminoglycoside phosphotransferases

Phosphotransferase	Substrate(s)
APH(3')	
I	Kanamycin, neomycin, lividomycin, paromomycin, ribostamycin
II	Kanamycin, neomycin, butirosin, paromomycin, ribostamycin
III	Kanamycin, neomycin, lividomycin, paromomycin, ribostamycin, butirosin, amikacin, isepamicin
IV	Kanamycin, neomycin, butirosin, paromomycin, ribostamycin
V	Neomycin, paromomycin, ribostamycin
VI	Kanamycin, neomycin, paromomycin, ribostamycin, butirosin, amikacin, isepamicin
VII	Kanamycin, neomycin
APH(2")	
Ia (bifunctional enzyme)	Kanamycin, gentamicin, tobramycin, sisomicin, dibekacin
Ib,Id	Kanamycin, gentamicin, tobramycin, netilmicin, dibekacin
Ic	Kanamycin, gentamicin, tobramycin
APH(3")-Ia, -Ib	Streptomycin
APH(7")-Ia	Hygromycin
APH(4)-Ia, -Ib	Hygromycin
APH(6)-Ia, -Ib, -Ic, -Id	Streptomycin
APH(9)-Ia, -Ib	Spectinomycin

Aminoglycoside Acetyltransferases

Aminoglycoside acetyltransferases (AACs) comprise four classes of enzymes: AAC(1), AAC(3), AAC(2'), and AAC(6'). They utilize acetyl coenzyme A as the donor of the acetyl group in modifying aminoglycosides at position 1 and 3 of the 2-deoxystreptamine ring and position 2' and 6' of the 6-aminohexose ring. Aminoglycoside 6'-acetyltransferases are broad-spectrum enzymes capable of modifying most of the clinically important aminoglycosides. Table 2-10 summarizes the substrate profiles of the aminoglycoside acetyltransferases.

Table 2-10 Substrate profiles of aminoglycoside acetyltransferase

Acetyltransferase	Substrates(s)
AAC(6')	
I (at least 24 different enzymes)	Tobramycin, amikacin, netilmicin, dibekacin, sisomicin, kanamycin, isepamicin
II	Tobramycin, gentamicin, netilmicin, dibekacin, sisomicin, kanamycin
AAC(3)	
Ia, Ib	Gentamicin, sisomicin, fortimicin
IIa, IIb, IIc	Tobramycin, gentamicin, netilmicin, dibekacin, sisomicin
IIIa, IIIb, IIIc	Tobramycin, gentamicin, dibekacin, sisomicin, kanamycin, neomycin, paromomycin, lividomycin
IV	Tobramycin, gentamicin, netilmicin, dibekacin, sisomicin, apramycin
VII	Gentamicin
AAC(1)	Paromomycin, lividomycin, ribostamycin, apramycin
AAC(2')-Ia	Tobramycin, gentamicin, netilmicin, dibekacin, neomycin

Aminoglycoside Nucleotidyltransferases

Aminoglycoside nucleotidyltransferases (ANTs) comprise five classes, ANT(2"), ANT(3"), ANT(4'), ANT(6), and ANT(9). They utilize ATP as the second substrate and modify aminoglycoside antibiotics by transferring AMP to their hydroxyl group at position 2", 3", 4', 6, and 9, respectively. Table 2-11 summarizes the substrate profiles of the aminoglycoside nucleotidyltransferases.

Table 2-11 Substrate profiles of aminoglycoside nucleotidyltransferase

Nucleotidyltransferase	Substrate(s)
ANT(2")-I	Tobramycin, gentamicin, dibekacin, sisomicin, kanamycin
ANT(3')-I	Streptomycin, spectinomycin
ANT(4')-Ia	Tobramycin, amikacin, dibekacin, kanamycin, isepamicin
ANT(4')-IIa	Tobramycin, amikacin, kanamycin, isepamicin
ANT(6')-I	Streptomycin
ANT(9)-I	Spectinomycin

Thus, aminoglycoside modifying enzyme that can inactivate amikacin in *A. baumannii* were AAC(6') and APH(3')VI.

VI. Ciprofloxacin

Ciprofloxacin is one of the second generation of synthesis quinolone anti-infective agent. (Figure 2-6). It usually is bactericidal activity in action. Killing of bacteria by ciprofloxacin is concentration dependent.

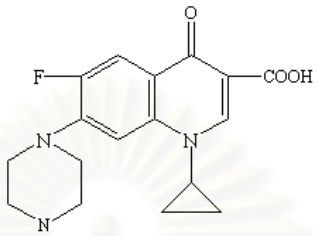


Figure 2-6 Structure of ciprofloxacin

The primary target of fluoroquinolones appears to be bacterial DNA gyrase, which is the enzyme responsible for supercoiling, nicking, and sealing bacterial DNA. Ciprofloxacin inhibit DNA topoisomerase (ATP-hydrolyzing), a type II DNA topoisomerase commonly referred to DNA gyrase, in susceptible organisms. DNA gyrase is necessary for bacterial DNA replication and some aspects of transcription, repair, recombination, and transposition. The target of ciprofloxacin appears to be the A subunit of the enzyme and interact with the B subunit of the enzyme; product of *gyrA* and *gyrB* genes. Inhibition of DNA gyrase results in inhibition of ATP-dependent negative supercoiling of DNA, inhibition of ATP-independent relaxation of supercoiling DNA, and promotion of double-stranded DNA breakage (AHFS Drugs, 2001).

Ciprofloxacin is active *in vitro* against most gram negative aerobic bacteria including Enterobacteriaceae and *P. aeruginosa*.

Bacteria acquire resistance to ciprofloxacin by spontaneously occurring mutations in chromosomal genes or alter the drug's permeation across the bacterial cell membrane (Hooper, 2000). The mechanisms of resistance are via chromosomal mutations that alter DNA gyrase and topoisomerase IV, the targets for quinolones (Seward and Towner, 1998). DNA gyrase is composed of 2 subunits, encoded by *gyr*A and *gyr*B genes. Topoisomerase IV is structurally similar to DNA gyrase, but is a secondary target for quinolones. Two subunits of topoisomerase IV are encoded by

*par*C and *par*E genes. Resistance in *A. baumannii* is mediated by mutation in *gyr*A and *par*C genes (Seward and Towner, 1998). Other mechanisms of resistance have been described, such as those in the efflux and influx systems, but the role of these mechanisms remains to be determined (Ruiz, 2003).

For the treatment of severe and/or life threatening-due to less susceptible *A. baumannii* infection, the drug has been given in dosage of 400 mg every 12 hours. The pharmacokinetic achievable concentration; C_{max}. $C_{average}$ and C_{min} are 4.6, 1, and 0.2 μg/ml, respectively (AHFS Drugs, 2001; Burgess and Nathisuwan, 2002)

The combination of β-lactam antibiotic plus ciprofloxacin displayed synergistic effect against resistant strain of *P. aeruginosa* (Song et al., 2003). In one study, the combination of meropenem plus ciprofloxacin displayed synergistic effect against *P. aeruginosa* (Willke et al., 2005).

VII. Genes cassettes and Integrons

In gram-negative bacteria and especially among *Enterobacteriaceae*, integrons are involved in antibiotic resistance (Peter et al., 2001). Integrons are gene expression elements that incorporate open reading frames (ORFs) and convert them to functional genes. All known integrons are composed of three key elements necessary for the procurement of exogenous genes: first, a gene coding for an integrase (*intI*), second, a primary recombination site (*attI*), and third, a strong promoter (Rowe-Magnus and Mazel, 1999). Integron integrases recombine discrete units of circularized DNA, known as gene cassettes, downstream of the resident promoter at the proximal *attI* site, permitting expression of their encoded proteins (Figure 2-7). Resistance cassettes normally contain a single gene associated with a specific recombination sequence, the 59 base element (59-be or *attC*).

To date four classes of integrons, each with distinct *int* genes, have been described in gram-negative bacteria isolates, with class 1 integrons being most prevalent in clinical isolates, carrying single or multiple gene cassettes. Class 2 integron is 40% identity to class 1 integron, class 3 integron is 60% identity to class 1 integron. Integron inserted gene encode for various antibiotic resistance mechanisms, including over 40 distinct genes, conferring resistance to aminoglycosides, β-lactams,

chloramphenicol, macrolides, sulphonamides, antiseptics and disinfectant. Concerning β-lactamases, integron-borne gene cassettes have been found mainly in *P. aeruginosa*, *A. baumannii* and various species of Enterobacteriaceae encompassing Ambler classes A, B and D β-lactamase enzymes, giving rise to widespread β-lactam resistance as shown in Table 2-12 to 2-14 (Weldhagen, 2004). Most integron-borne β-lactamase genes are situated on class 1 integrons, as is invident from the typical genetic structure and the co-resistance genes to quaternary ammonium compounds (*qacΔE1*) and sulphonamide (*sul1*) that classically occur at the distal 3'-end. Gene cassettes encoding aminoglycoside resistance tend to co-occur commonly with β-lactamase gene cassettes on integron structure, with *aac*-type (aminoglycoside acetyltransferase) and *aad*-type (aminoglycoside adenyltransferase) genes occurring most often.

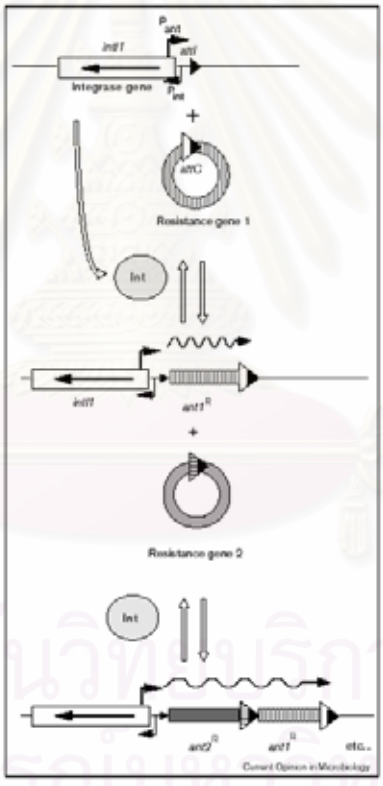


Figure 2-7 Model for cassettes exchange. Outline of the process by which circular antibiotic resistance gene cassettes (ant^R) are repeatly inserted at the specific *attI* site in a class 1 integron downstream of the strong promoter P_{ant}. *int1*, integrase encoding gene; Int, integrase IntI1; *attC*, 59-be. Wavy arrows indicate direction of transcription (Rowe-Magnus and Mazel, 1999).

Table 2-12 Ambler class A, integron-located β-lactamases reported from various gram negative bacterial species (Weldhagen, 2004)

β-lactamases	Host species	Origin
VEB-1	*Klebseilla pneumoniae*	Vietnam
	Escherichia coli	Vietnam
	Pseudomonas aeruginosa	France
	Citrobacter freundi	Thailand
VEB-1a	*Pseudomonas aeruginosa*	Kuwait
VEB-1b	*Pseudomonas aeruginosa*	Kuwait
VEB-2	*Pseudomonas aeruginosa*	Thailand
GES-1	*Klebseilla pneumoniae*	French Guiana
	Pseudomonas aeruginosa	France
	Klebseilla pneumoniae[a]	Portugal
GES-2	*Pseudomonas aeruginosa*	South Africa
IBC-1	*Enterobacter cloacae*	Greece
IBC-2	*Pseudomonas aeruginosa*	Greece
CTX-M-2	*Salmonella enterica*	Argentina
	Proteus mirabilis	Argentina
CTX-M-9	*Escherichia coli*	Spain
PSE-1	*Vibrio cholerae*	Thailand

All genes lists were found in class 1 integron structure.

[a] Class 3 integron location.

Table 2-13 Ambler class B, integron-located β-lactamases reported from various gram negative bacterial species (Weldhagen, 2004)

β-lactamases	Host species	Origin
IMP-1	*Serratia marcescens*[a]	Japan
	Pseudomonas aeruginosa	Japan
IMP-2	*Acinetobacter baumannii*	Italy
IMP-3	*Shigella flexneri*	Japan
IMP-4	*Acinetobacter baumannii*	Hong Kong
	Citrobacter youngae	China
IMP-6	*Serratia marcescens*	Japan
IMP-7	*Pseudomonas aeruginosa*	Canada
IMP-8	*Klebseilla pneumoniae*	Taiwan
IMP-12	*Pseudomonas putida*	Italy
VIM-1	*Acinetobacter baumannii*	Italy
	Pseudomonas aeruginosa	Italy
	Achromobacter xylosoxydans	Italy
	Pseudomonas aeruginosa	Greece
VIM-2	*Pseudomonas aeruginosa*	France
	Pseudomonas aeruginosa	Italy
	Pseudomonas aeruginosa	Spain

All genes lists were found in class 1 integron structure.

[a] Class 3 integron location.

Table 2-14 Ambler class D, integron-located β-lactamases reported from various gram negative bacterial species (Weldhagen, 2004)

β-lactamases	Host species	Origin
OXA-1	*Salmonella enterica*	Italy
OXA-5	*Pseudomonas aeruginosa*	South Africa
OXA-9	*Enterobacter aerogenes*	France
OXA-10	*Pseudomonas aeruginosa*	Vietnam
OXA-11	*Pseudomonas aeruginosa*	Turkey
OXA-14	*Pseudomonas aeruginosa*	Turkey
OXA-16	*Pseudomonas aeruginosa*	Turkey
OXA-19	*Pseudomonas aeruginosa*	France
OXA-20	*Pseudomonas aeruginosa*	France
OXA-28	*Pseudomonas aeruginosa*	France
OXA-30	*Escherichia coli*	France

VIII. Detection resistant gene by PCR

The polymerase chain reaction (PCR) has revolutionized molecular genetics by permitting rapid cloning and analysis of DNA (Strachan and Read, 1999). PCR is a rapid and versatile *in vitro* method for amplifying defined target DNA sequences present within a source of DNA. To permit such selective amplification, some prior DNA sequence information from target sequences is required. This information is used to design two oligonucleotide primers which are specific for the target sequence and which are often about 15-25 nucleotides long. After the primers are added to denatured template DNA, they bind specifically to complementary DNA sequences at the target site. In the presence of a suitably heat-stable DNA polymerase and the DNA precursors (the four deoxynucleoside triphosphate, dATP, dCTP, dGTP and dTTP), they initiate the synthesis of new DNA strands which are complementary to the individual (Figure 2-8).

The PCR is a chain reaction because newly synthesized DNA stands will act as templates for further DNA synthesis in subsequence cycles. After about 25-30 cycles of DNA synthesis, the production of the PCR will include, in addition to the starting DNA, about 10^5 copies of the specific target sequence, an amount which is easily visualized as a discrete band of a specific size when submitted to agarose gel electrophoresis. A heat-stable DNA polymerase is used because the reaction involves sequential cycles composed of three steps:

1.Denaturation : At 94°C, the double strand DNA melts and opens into single-stranded DNA.

2.Annealing : At 54°C, hydrogen bonds form and break between the single-stranded "primer" and the single-stranded "template." (The template provides the pattern to be copied.) The more stable bonds last longer and on that little length of double-stranded DNA (the joined primer and template), the polymerase attaches and starts copying the template.

3.Extention : At 72°C, the polymerase works best. As a result, the attraction, created by the hydrogen bonds, of the primers to the template is stronger than the forces breaking these attractions. The upshot is that bases complementary to the template are coupled to the primer.

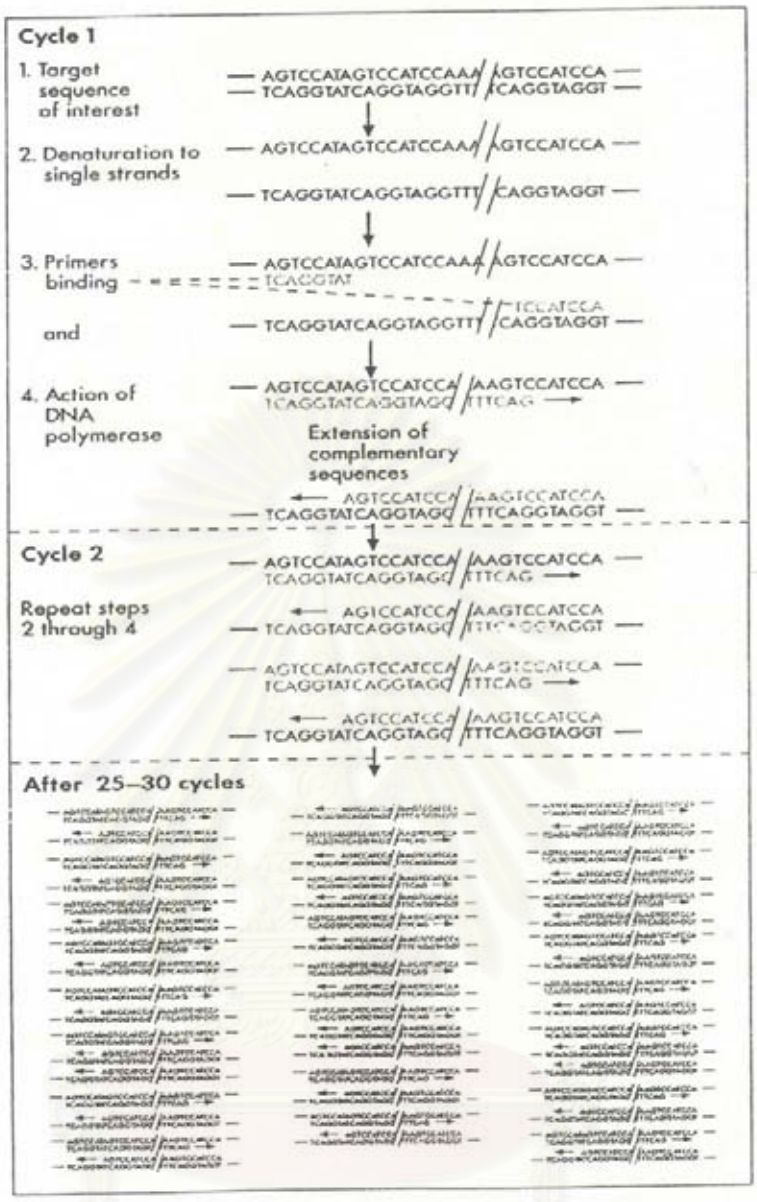


Figure 2-8 Sequence of Amplification in the PCR (Strachan and Read, 1999).

IX. Combination of antibiotics

Combinations of antimicrobial agents are often used in medical practice. They are most frequently prescribed by physicians to provide broad-spectrum coverage in patients who are seriously ill without an identifiable infecting organism (Elipoulos and Moellering, 1996). Combination therapy is used with the aim of expanding the antimicrobial spectrum, minimizing toxicity, preventing the emergence of resistants during therapy, and obtaining synergistic antimicrobial activity. Traditionally, the clinician selects the agents on the basis of the susceptibility results for the pathogen and often will choose only antimicrobial to which the organisms is interpreted as susceptible. This practice appears to assume that synergism is obtained only when the organism is susceptible to both antimicrobial agents (Song et al., 2003).

Song et al. demonstrated synergism by the chekerboard method when *P. aeruginosa* strains were resistant to β-lactam, aminoglycosides and/or fluoroquinolones. There has been a report on the synergistic activity of imipenem combined with amikacin against 2 isolates of the pandrug resistant *A. baumannii* (Hsueh et al., 2002). Another study showed that the combination of meropenem plus ciprofloxacin displayed synergistic effect against *P. aeruginosa* (Carta and Paglietti, 2005). The combination of imipenem plus an aminoglycoside is generally neither synergistic nor antagonistic *in vitro* against most strains of *P. aeruginosa* and Enterobacteriaceae (AHFS Drugs, 2001). Chang et al. reported synergistic effect in some isolates of *A. baumannii* and it reported that the combination of imipenem plus amikacin was the best choice of combination treatment in study. There has been some studies revealed that the combination of β-lactams plus amikacin possesses an enhanced killing effect against multiresistant *P. aeruginosa* (Giamarellos-Bourboulis et al., 1997 and Burgess et al., 2000). Erdem et al. reported that the combination of imipenem plus ciprofloxacin against multidrug-resistant *P. aeruginosa* strains was mostly the indifferent effect. Another study showed that the combination of β-lactams plus ciprofloxacin displayed synergistic effect against *P. aeruginosa* (Burgess and Nathisuwan, 2002).

CHAPTER III

MATERIALS & METHODS

1 Microorganisms

1.1 Clinical isolates

The bacterial strains used throughout this study were 30 strains of imipenem resistant-*Acinetobacter baumannii* which were clinically isolated from the patients at Siriraj Hospital during the year 2002-2003.

1.2 Control strains

Escherichia coli ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853

2. Chemicals

Standard powder of ciprofloxacin (potency 848.1 μg/ml) was kindly provided by Siam Bheasach, Thailand and standard powder of amikacin (potency 740 μg/ml) was purchased from Sigma (U.S.A.). Working standard solutions were prepared immediately prior to use, as specified by the manufacturers before dilute with test broth.

Working standard of imipenem was obtained by assay against standard powder of imipenem (potency 100 μg/ml). Imipenem and cilastatin for injection was assayed according to USP 24, 2000.

Antimicrobial disks were amikacin (30 μg), ceftazidime (30 μg), ciprofloxain (5 μg), and imipenem (10 μg). All of the disks, which were purchased from BBL chemicals. (U.S.A)

3. Antibiotic susceptibility test (NCCLS, 2000)

Paper disk susceptibility test was performed according to disk diffusion method by NCCLS (NCCLS, 2000). *E.coli* ATCC 25922 was also included in this study as the control strain. All isolates were tested to confirm imipenem resistant-*A. baumannii* and to determine susceptibility pattern of the organism against the other antimicrobial agents.

3.1 Preparation of Media

3.1.1 Mueller-Hinton agar (MHA) was prepared from a commercially available dehydrated base according to the manufacturer's instructions.

3.1.2 Immediately after autoclaving, allow it to cool in a 45 to 50 °C water bath.

3.1.3 Pour the freshly prepared and cooled medium into glass, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 25 ml for plates with a diameter of 100 mm.

3.1.4 The agar medium should be allowed to cool at room temperature and all prepared plates must be examined sterility by incubating at 37 °C for 24 hours.

3.1.5 Unless the plates were used the same day, stored in a refrigerator (2 to 8 °C) and should be used within 7 days after preparation.

3.2 Inoculum Preparation

3.2.1 The well-isolated colony of each 18 hours *A. baumannii* from clinical specimen and *E.coli* ATCC 25922 were selected from Tryptic soy agar (TSA) plates and transferred to a tube containing 5 ml normal saline solution (NSS).

3.2.2 The suspension was adjusted to match the tubidity of the 0.5 McFarland standard solution. This result in a suspension containing approximately 1 to 2 x 10^8 CFU/ml.

3.3 Inoculation Test Plates

3.3.1 Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.

3.3.2 The dried surface of an agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum.

3.4 Application of Disks to Inoculated Agar Plates

3.4.1 The antibiotic disks were applied to the surface of the medium with sterile forceps. Each disk must be pressed down to ensure complete contact with the agar surface. They must be distributed evenly so that they are no closer than 24 mm from center to center. Because some of the drug diffuses almost instantaneously, a disk should not be relocated once it has come into contact with the agar surface. Instead, place a new disk in another location on the agar.

3.4.2 The plates were inverted and placed in an ambient air incubator set to 37°C within 15 minutes after the disks were applied in ambient air.

3.5 Reading Plates and Interpreting Results

3.5.1 The diameter of each zone of inhibition was measured with digital sliding venier caliper.

3.5.2 The size of the inhibition zone were interpreted by referring to the NCCLS, 2000 and the organisms were reported as either susceptible, intermediate, or resistant to the agents that have been tested (Tables 3-1).

Table 3-1 Zone diameter interpretive standards breakpoints for *A. baumannii* and *E.coli* ATCC 25922 (NCCLS, 2000)

Drug	Disk content	Zone diameter (mm)			
		A. baumannii			*E.coli*
		R[a]	I[b]	S[c]	ATCC 25922
Amikacin	30 μg	≤14	15-16	≥17	19-26
Ceftazidime	30 μg	≤14	15-17	≥18	25-32
Ciprofloxacin	5 μg	≤15	16-20	≥21	30-40
Imipenem	10 μg	≤13	14-15	≥16	26-32

[a]Resistant, [b]Intermediate, [c]Susceptible

4. Agar dilution method (NCCLS, 2000)

Agar dilution method was performed according to NCCLS (NCCLS, 2000). *E.coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were also included in this test as the

control strain. All isolates were tested to determine minimum inhibitory concentration (MIC) of imipenem, amikacin and ciprofloxacin.

4.1 Preparation of agar dilution plates

4.1.1 The two-fold dilution of imipenem solution (0.03-256 μg/ml), amikacin and ciprofloxacin solution (0.125-256 μg/ml) were prepared. Because final volume in each plate consisted of 2.5 ml of each dilution antimicrobial agents and 22.5 ml of MHA. Thus antimicrobial concentrations used in initial (stock) solutions should be prepared ten-fold in greater than the desired final concentration.

4.1.2 MHA was prepared from a commercially available dehydrated base according to the manufacturer's instructions.

4.1.3 Immediately after autoclaving, allow it to cool in a 56 °C water bath and then pipetted 2.5 ml of each dilution into MHA 22.5 ml.

4.1.4 The agar and antimicrobial agent solution were mixed thoroughly and then pour into plates.

4.1.5 The agar dilution plates are allowed to solidity at room temperature, and used immediately.

4.2 Inoculum preparation

4.2.1 The well-isolated colony of each 18 hours *A. baumannii* from clinical specimen, *E.coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were selected from Tryptic soy agar (TSA) plates and transferred to a tube containing 5 ml normal saline solution (NSS).

4.2.2 The suspension was adjusted to match the tubidity of the 0.5 McFarland standard solution. This result in a suspension containing approximately 1 to 2 x 10^8 CFU/ml.

4.2.3 The 200 μl-inoculum suspension was pipitted into inoculumn replicators.

4.3 Inoculating agar dilution plates

4.3.1 The agar plates are marked for orientation of the inoculum spots.

4.3.2 A 1 μl of each inoculum was applied to the agar surface by the use of an inocula-replicating device. The final inoculumn on the agar will then be approximately 10^4 CFU per spot.

4.3.3 A growth-control plate (no antimicrobial agent) was inoculated first and then, starting the lowest concentration, the plates containing the different concentrations were inoculated.

4.4 Incubating agar dilution plates

The inoculated plates were allowed to stand at room temperature until the moisture in the inoculumn spots has been absorbed into the agar until the spots are dry, but no more than 30 minutes. The plates were inverted and incubated at 37 °C for 24 hours.

4.5 Determining agar dilution end points

4.5.1 The MICs were recorded as the lowest concentration of antimicrobial agent that completely inhibits growth, disregarding a single colony or a faint haze caused by the inoculum.

4.5.2 The MICs were interpreted by referring to the NCCLS, 2000 and the organisms were reported as either susceptible, intermediate, or resistant to the agents that have been tested (Tables 3-2)

Table 3-2 MICs interpretive standards breakpoints (μg/ml) (NCCLS, 2000)

Drug	Minimum Inhibitory Concentrations[MICs] (μg/ml)				
	A. baumannii			*E.coli*	*P. aeruginosa*
	S[a]	I[b]	R[c]	ATCC 25922	ATCC 27853
Imipenem	≤ 4	8	≥ 16	0.06-0.25	1-4
Amikacin	≤ 16	32	≥ 64	0.5-4	1-4
Ciprofloxacin	≤ 1	2	≥ 4	0.001-0.016	0.25-1

[a]Susceptible, [b]Intermediate, [c]Resistant

5. Detection *integrase* gene and antibiotic resistant genes by Polymerase Chain Reaction (PCR) technique (Sambrook and Russel, 2001)

5.1 DNA extraction

DNA extraction was performed according to the method described by MO BIO Laboratory, Inc briefly as followed:

5.1.1 A single colony of *A. baumannii* from 24 hours on TSA agar plate was inoculated into 5 ml Luria-Bertani (LB) broth. The culture media were incubated under aerobic condition for 24 hours at 37°C.

5.1.2 The 1.8 ml-bacterial culture was added to a 1.9 ml microcentrifuge tube then it was centrifuged at 10,000 x *g* for 30 seconds. The supernatant was decanted to remove the media supernatant completely with a pipet tip.

5.1.3 The cell pellet was resuspended in 300 μl of MicroBead Solution and gently vortex to mix. The resuspended cells were transfered to MicroBead tube, added 50 μl of Solution MD1 to the MicroBead tube and heat at 65°C for 10 minutes.

5.1.4 Bead tubes were secured horizontally using Vortex at maximum speed for 10 minutes and then were centrifuged at 10,000 x *g* for 30 seconds.

5.1.5 The supernatant was transferred to a clean microcentrifuge tube, then added 100 μl of Solution MD2 to the supernatant, vortex 5 seconds and incubated at 4 °C for 5 min.

5.1.6 The tubes were centrifuged for 1 minute at 10,000 x *g* before the entire volume of supernatant was transferred to a clean 1.9 ml tube, then added 900 μl of Solution MD3 to the supernatant and vortex 5 seconds.

5.1.7 The supernatant was loaded about 700 μl into spin filter, centrifuged at 10,000 x *g* for 30 second, discarded the flow through, added the remaining supernatant to spin filter, and centrifuged at 10,000 x *g* for 30 seconds.

5.1.8 Then added 300 μl of Solution MD4 and centrifuge for 30 seconds at 10,000 x *g*, discarded the flow through and centrifuged again for 1 minute.

5.1.9 Spin filter was placed in a new 1.9 ml tube, added 50 μl of Solution MD5 to the center of the white filter membrane and centrifuged 30 seconds. Exacted DNA was stored at -20°C.

5.2 PCR amplification to detect *integrase* gene and antibiotic resistant genes

PCR amplification of *bla*$_{OXA-23}$ and *bla*$_{IMP}$ were performed due to they are carbapenem-hydrolyzing β-lactamases (carbapenemase) which have been detected in *A. baumannii*. The *bla*$_{OXA-1}$, *bla*$_{OXA-2}$ and *bla*$_{OXA-10}$ which are the most common representatives of OXA type β-lactamases were also detected. PCR amplification of *bla*$_{VEB-1}$ due to it is common ESBL types in Southeast Asia. In addition, *aac*(6') was amplified due to it is the most common aminoglycoside modifying enzymes that can inactivate amikacin in *A. baumannii*. Besides we studied PCR amplification of *integrase* due to a site-specific recombination enzyme which allows insertion of antibiotic resistant genes cassettes called that integrons.

5.2.1 PCR assay was done in a total volume of 50 μl containing

Distilled water	38 μl
dNTP	5 μl
Buffer	5 μl
Primer A	0.5 μl
Primer B	0.5 μl
Taq DNA polymerase	0.5 μl
DNA template	0.5 μl

The sequences of the oligonucleotide primers for PCR amplification used in this study were list in Table 3-3.

5.2.2 The thermal profile for the 30 cycles of PCR was used in this experiment included a denaturing step at 94°C for 60 seconds followed by annealing of the primer at 55°C for 60 seconds with an DNA extension at 72°C for 60 seconds.

5.3 Amplification products were analyzed by running Agarose Gel Electrophoresis

5.3.1 2% agarose gel was mixed in 0.5x TBE buffer 40 ml, then heated in a microwave oven until completely melted.

5.3.2 After cooling to about 60°C, it was poured into a casting tray containing a sample comb and allowed to solidify at room temperature.

5.3.3 After the gel had solidified, the comb was removed, using care not to rip the bottom of the wells. The gel, still in its plastic tray, was inserted

horizontally into the electrophoresis chamber and just covered with buffer.

5.3.4 The 7 μl-DNA was mixed with loading dye 3 μl then pipetted into the sample well. DNA markers included in all gels.

5.3.5 Gels were run in 0.5xTBE buffer at a constant 100 volts for 60 minutes.

Table 3-3 Sequences of the PCR primers

PCR product	Primer name	Sequence	PCR product
integrase	INT1F INT1R	5´-AAGGATCGGGCCTTGATGTT-3´ 5´-CAGCGCATCAAGCGGTGAGC-3´	472 bp
bla_{VEB-1}	VEB1F VEB1R	5´-CGACTTCCATTTCCCGATGC-3´ 5´-GGACTCTGCAACAAATACGC-3´	643 bp
bla_{OXA-1}	OXA1F OXA1R	5´-TTTTCTGTTGTTTGGGTTTT-3´ 5´-TTTCTTGGCTTTTATGCTTG-3´	427 bp
bla_{OXA-2}	OXA2F OXA2R	5´-AAGAAACGCTACTCGCCTGC-3´ 5´-CCACTCAACCCATAATACCC-3´	478 bp
bla_{OXA-10}	OXA10F OXA10R	5´-TCAACAAATCGCCAGAGAAG-3´ 5´-TCCCACACCAGAAAAACCAG-3´	187 bp
bla_{OXA-23}	OXA23F OXA23R	5´-GATGTGTCATAGTATTCGTCG-3´ 5´-TCACAACAACTAAAAGCACTG-3´	1065 bp
bla_{IMP}	IMP-F IMP-R	5´-CTTTGCCAGATTTAAAAAT-3´ 5´-ACCAGTTTTGCCTTACCATA-3´	587 bp
aac(6´)	aac6-F aac6-R	5´-GTTAGAAGGCCCAGGCTATG-3´ 5´-CGCTGGAATGAAGGGTTAGA-3´	472 bp

5.4 Gels were stained in an ethidium bromide for 20 minutes and then destained in ultrapure water for 40 minutes.

5.5 To visualized DNA, the gel was placed on ultraviolet transilluminator and then photographed under UV light.

6. Detection carbapenemase activity by spectrophotometric assay (Livermore & Williams, 1996)

6.1 *A. baumannii* was inoculated into MHB 5 ml and then incubated overnight at 37°C.

6.2 Bacterial cultures were centrifuged for 15 minutes at 10,000 x g and then were decanted the supernatant.

6.3 The cells were washed with 0.1 M phosphate buffer pH 7.0 1 ml and were disrupted by sonication used as the crude extract enzyme.

6.4 Carbapenemase activity was determined by spectrophotometric assay to measured level of absorption of meropenem at 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 and 120 seconds, respectively.

6.4.1 Quartz cuvette control contained 20mM Tris HCl pH 7.5 100 μl used as buffer of enzyme and zero baseline.

6.4.2 Quartz cuvette substrate contained 20 mM Tris HCl pH 7.5 92 μl, meropenem 5,000 μM 4 μl and crude extract enzyme 4 μl (absorption maximum of meropenem was 295 nM).

6.4.3 Measured level of absorption of control compared with substrate, if the crude extract produced carbapenemase level of absorption was deceased.

7. Bactericidal activity test by time kill method (Elipoulos and Moellering, 1996)

The antibacterial activity of the combination was performed according to time kill method by Elipoulos and Moellering, 1996. All isolates were tested to detemined the antibacterial activity of the combination of imipenem plus amikacin or plus ciprofloxacin against imipenem resistant-*A. baumannii*.

7.1 Imipenem concentration was prepared to 36 μg/ml (AHFS Drugs, 2001), amikacin concentration to 20 μg/ml (AHFS Drugs, 2001) and ciprofloxacin concentration to 1 μg/ml (Burgess and Nathisuwan, 2002) which referred to the mean serum concentrations of the drug at therapeutic dose. Antimicrobial concentrations used in initial (stock) solutions were prepared ten fold greater than desired final concentration.

7.2 A 1 ml of each drug was pipetted into Mueller Hinton broth (MHB) for prepared working media before adding the standardized inoculum (Final volume of working media = 9 ml). As the result, there had been 6 groups were control (no antimicrobial agents), imipenem alone, amikacin alone, ciprofloxacin alone, imipenem combined with amikacin and imipenem combined with ciprofloxacin.

7.3 Inoculum was adjusted to match the tubidity of the 0.5 McFarland standard solution containing approximately 1 to 2 $x10^8$ CFU/ml then, it was diluted ten fold to obtaine bacterial quantity 1 to 2 $x10^7$CFU/ml.

7.4 A 1 ml of inoculum was pipetted to working media and incubated at 37°C in a shaking water bath.

7.5 The samples were collected to detect for colony forming unit at the time 0,2,4,6,8,10 and 24 hours after microorganism exposed to drugs in each group including the control group. A 0.5 ml was diluted ten fold in NSS and dropped 20 μl of each dilution to TSA plates.

7.6 The inoculum was incubated at 37°C for 16-18 hours.

7.7 The quantity of survival bacteria in each group was calculated to obtain the killing curves data.

7.8 Killing curves were constructed by Microsoft Excel 97. At each time intervals the $\log_{10}$ change of the viable cell counts compared to the starting inoculum was determined. The criteria to define the bactericidal property was the decreasing in colony forming unit from the origin point $\geq$ 3 log CFU/ml at 24 hours of exposure. The regrowth was defined as an increase of $\geq$ 2 log CFU/ml after $\geq$ 6 hours. (Amsterdam, 1996; Pankuch, Jacobs and Appelbaum, 1994; Satta, et al., 1995). Synergy was defined by a $\geq$2 $\log_{10}$ reduction in CFU/ml relative to that of the more active antimicrobial agent alone. An additive effect was defined as a reduction in CFUs by $\geq$1 but <2 $\log_{10}$ CFU/ml relative to the more active antimicrobial agent alone, and indifference was defined by either no reduction or a reduction of <1 $\log_{10}$ CFU/ml relative to the more active antimicrobial agent alone. Antagonism was defined by a $\geq$2 $\log_{10}$ increase in CFU/ml relative to that of the more active antimicrobial agent alone (Pohlman, 1996). The quantitative

evaluation of antimicrobial effect was calculated as in the published article (Firsov, et al.,1997).

The Quantitative Evaluation of Antimicrobial Effect

1. The following parameters were estimated by extrapolation of the killing curves as shown in Figure 3-1.

$T_{99.9\%}$ = The time to reduce the initial inoculum 1000 fold

T_{min} = The time to reach the minimum number of bacteria resulting from exposure to antibiotic

$T_{eradication}$ = The time to reach limit of detection

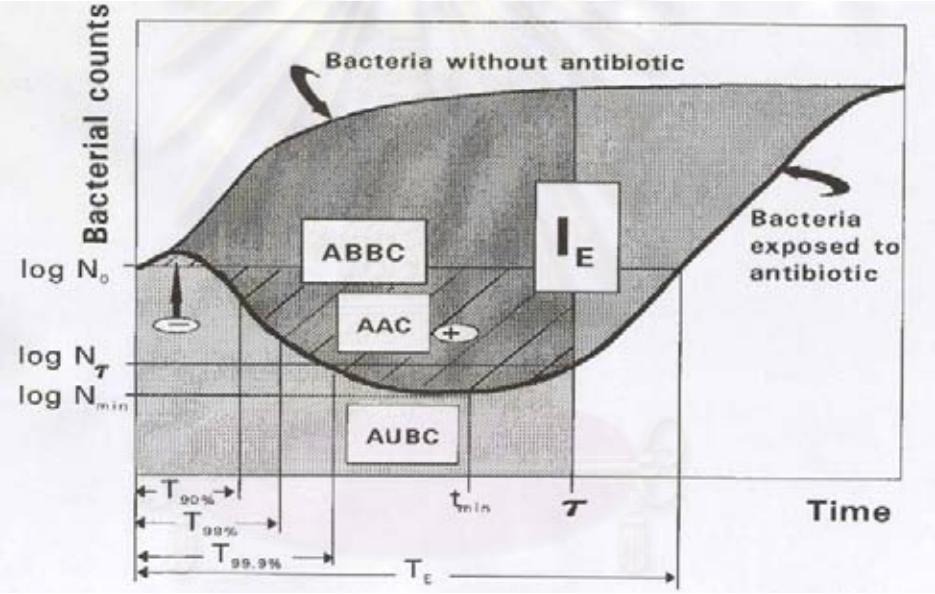


Figure 3-1 Parameters for quantifying bacterial killing and regrowth curve and the antimicrobial effect.

(Modified from Firsov, et al., 1997)

2. The following data were computed from the difference of viable counts in various times.

ΔlogCFU 6 hours = The difference between the number of viable counts at time zero versus the number of viable counts after exposed to antimicrobial for 6 hours

ΔlogCFU 24 hours = The difference between the number of viable counts at time zero versus the number of viable counts after exposed to antimicrobial for 24 hours

Δmax = The difference between the number of viable counts at time zero versus the minimum number of viable counts after exposed to antimicrobial

3. The following parameters were calculated by various methodologies as followed:

$AUBKC_{0-24}$ = Area under the bacterial killing and regrowth curves that calculated by the trapezoidal rule for 24 hours

Bacteriolytic area for 24 hours (ABBC, BA24) = The area between control growth curve and the bacterial killing and regrowth curves ($AUBKC_{0-24}$ of the control growth curve substracted by $AUBKC_{0-24}$ of the bacterial killing and regrowth curves)

7.9 Statistical analysis

Log_{10} change of viable cell counts, $AUBKC_{0-24}$ and BA24 were expressed their mean value ($\pm$ SD) values. Comparison changes were performed by analysis of variance (ANOVA), Post hoc comparison of Scheffe´. Any value of P below 0.05 was considered as significant.

CHAPTER IV

RESULT

1. Susceptibility testing

From disk diffusion test according to NCCLS, all *A. baumannii* isolates were resistant to imipenem and ciprofloxacin. For amikacin and ceftazidime, 30% of the tested organisms were susceptible to amikacin and 6.67% to ceftazidime as shown in Table 4-1.

The range of the MICs observed, as well as the MIC_{50}, MIC_{90} and percentage of susceptible strains of imipenem, amikacin and ciprofloxacin among the 30 isolates were summarized in Table 4-2. Imipenem had no activity against all strains tested. MICs of imipenem ranged from 8 to 32 μg/ml which were the low levels of resistance (susceptibility breakpoint $\leq$ 4 μg/ml). The MIC_{50} and MIC_{90} of imipenem were 16 and 32 μg/ml, respectively. Slightly less than one half (43.33%) of the strains were susceptible to amikacin (susceptibility breakpoint $\leq$ 16 μg/ml). The MIC_{50} and MIC_{90} of amikacin were 32 and >256 μg/ml, respectively. MICs of amikacin against 13 amikacin-susceptible *A. baumannii* strains and 17-amikacin resistant *A. baumannii* strains were ranged from 1 to 16 and 32 to >256 μg/ml, respectively. All strains were resistant to ciprofloxacin, both of MIC_{50} and MIC_{90} were 64 μg/ml (susceptibility breakpoint $\leq$ 1 μg/ml). The MICs of ciprofloxacin ranged from 4 to 128 μg/ml. The percent susceptibility of the tested organisms to amikacin was slightly different when the results from disk diffusion method (Table 4-1) was compared with those from agar dilution method (Table 4-2) [Zone diameter interpretive standards breakpoints of amikacin are shown as followed: resistance $\leq$14 mm; intermediate 15-16 mm and susceptible $\geq$17 mm]. One strain was resistant to amikacin as tested by disk diffusion method (zone size = 13.47 mm) but was susceptible to amikacin as tested by agar dilution method (MIC = 16 μg/ml). Three strains were intermediate resistance to amikacin as tested by disk diffusion method (zone size = 14.79 mm, 15.46 mm and 15.76 mm, respectively) but were susceptible to amikacin as tested by agar dilution method (MIC = 16 μg/ml in all 3 strains). (Raw data of susceptibility testing by disk diffusion method and agar dilution

method were shown in Table A-1 in Appendices.) Figure 4-1 to 4-3 showed assessment MICs against *A. baumannii* by agar dilution method of imipenem, amikacin and ciprofloxacin, respectively.

Table 4-1 *In vitro* activity of imipenem, amikacin, ciprofloxacin and ceftazidime against 30 strains of *A. baumannii* as tested by disk diffusion method.

Drug	No. of isolates (% susceptibility)		
	Resistant	Intermediate	Susceptible
Imipenem	30 (100)	0	0
Amikacin	18 (60)	3 (10)	9 (30)
Ciprofloxacin	28 (93.33)	2 (6.67)	0
Ceftazidime	27 (90)	1 (3.33)	2 (6.67)

Table 4-2 *In vitro* activity of imipenem, amikacin and ciprofloxacin against 30 strains of *A. baumannii* as tested by agar dilution method.

Drug	MICs (μg/ml)			No. of isolates (% susceptibility)		
	Range	MIC_{50}	MIC_{90}	Resistant	Intermediate	Susceptible
Imipenem	8-32	16	32	23 (76.67)	7 (23.33)	0
Amikacin	1- >256	32	>256	9 (30)	8 (26.67)	13 (43.33)
Ciprofloxacin	4-128	64	64	30 (100)	0	0

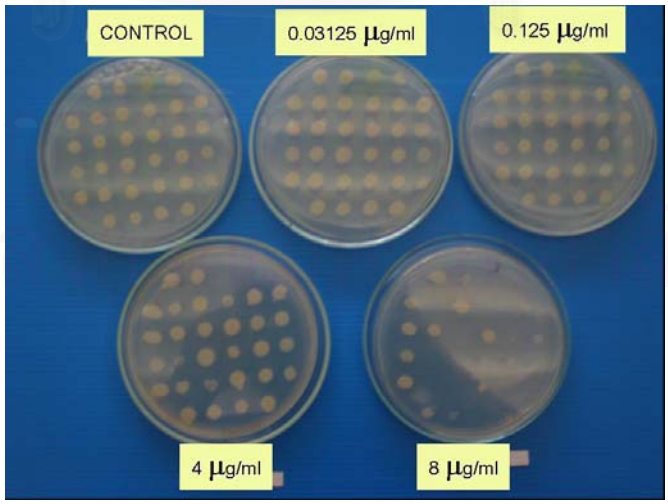


Figure 4-1 Assessment MICs of imipenem against 30 isolates of *A. baumannii* by agar dilution method

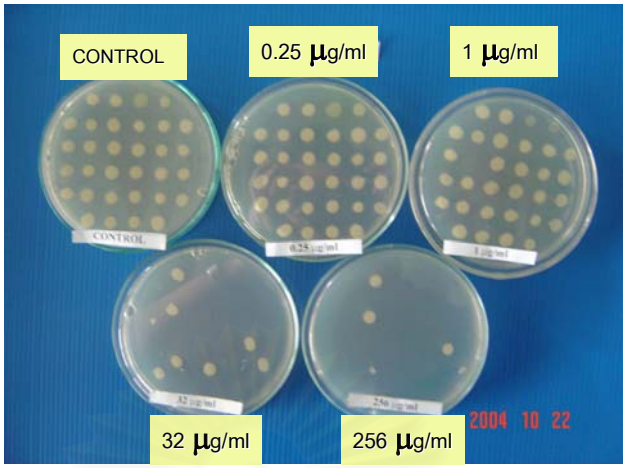


Figure 4-2 Assessment MICs of amikacin against 30 isolates of *A. baumannii* by agar dilution method

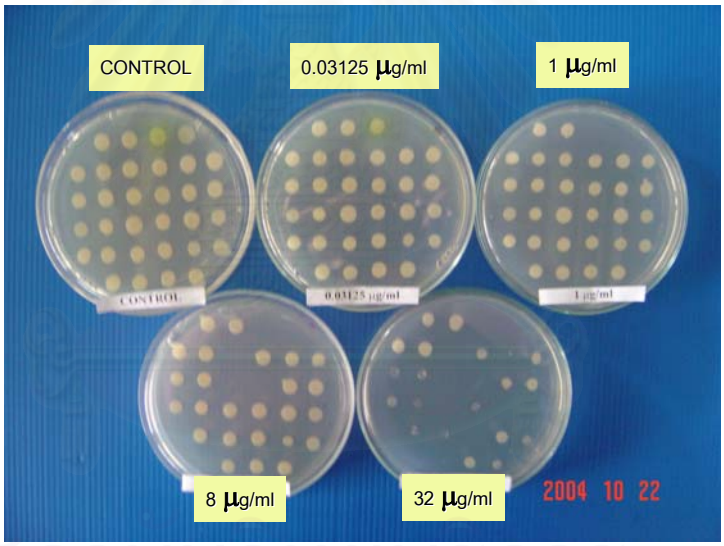


Figure 4-3 Assessment MICs of ciprofloxacin against 30 isolates of *A. baumannii* by agar dilution method

2. <u>Detection antibiotic resistant gene by Polymerase Chain Reaction (PCR) and carbepenemase activity detection by spectrophotometer assay</u>

It was showed that *integrase* gene were positive in 33.33% of isolates, bla_{VEB-1} positive in 6.67% and bla_{OXA-23} in 36.67%. The other antibiotic resistant genes, bla_{OXA-1}, bla_{OXA-2}, bla_{OXA-10}, bla_{IMP} and *aac*(6') could not be detected in all isolates as shown in Table 4-3. The PCR product of *integrase*, bla_{VEB-1} and bla_{OXA-23} genes from *A. baumannii* were shown in Figure 4-4 to 4-6, respectively. Two strains of *A. baumanniii* had both bla_{VEB-1} and bla_{OXA-23} genes. In addition, both *integrase* and bla_{OXA-23} genes were detected in 3 strains (Table 4-4 and 4-5).

The carbapenemase activity could be detected in 27 strains (90%) of *A. baumannii* by measuring the percent absorption of meropenem at various times. All of the bla_{OXA-23} gene positive had carbapenemase activity as shown in Table A-2 in Appendices. The percent absorption at wavelength 297 nm measured from *A. baumannii* with negative and positive carbapenemase activity were shown in Figure 4-7 and 4-8, respectively. (Raw data on the detection carbapenemase activity were shown in Table A-3 and Figure A-1 to A-30 in Appendices.)

Among 13 amikacin susceptible *A. baumannii* strains, *integrase* gene were positive in 6 strains (46.15%) of isolates, bla_{VEB-1} positive in 1 strain (7.69%) and bla_{OXA-23} in 4 strains (30.76%). All isolates in this group had carbapenemase activity positive. Among 17 amikacin resistant *A. baumannii* strains, *integrase* gene were positive in 4 strains (23.52%) of isolates, bla_{VEB-1} positive in 1 strain (5.88%) and bla_{OXA-23} in 7 strains (41.18%). Most of the tested organisms in this group (82.65%) had carbapenemase activity positive. Characteristics of *A. baumannii* in amikacin susceptible strains and amikacin resistant strains were shown in Table 4-4 and 4-5.

Table 4-3 Percent of *A. baumannii* with positive resistant genes and carbapenemase activity

	No.of isolates (%)
integrase	10 (33.33)
bla_{VEB-1}	2 (6.67)
bla_{OXA-1}	0
bla_{OXA-2}	0
bla_{OXA-10}	0
bla_{OXA-23}	11(36.67)
bla_{IMP}	0
aac(6´)	0
carbapenemase activity	27 (90)

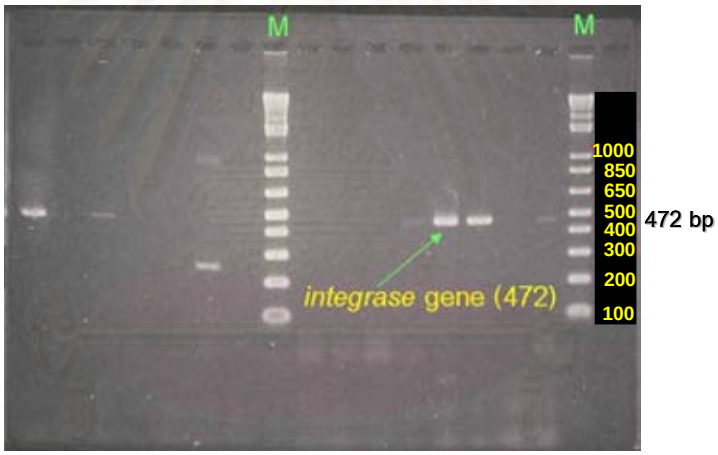


Figure 4-4 PCR product of *integrase* gene in *A. baumannii*. M = Marker

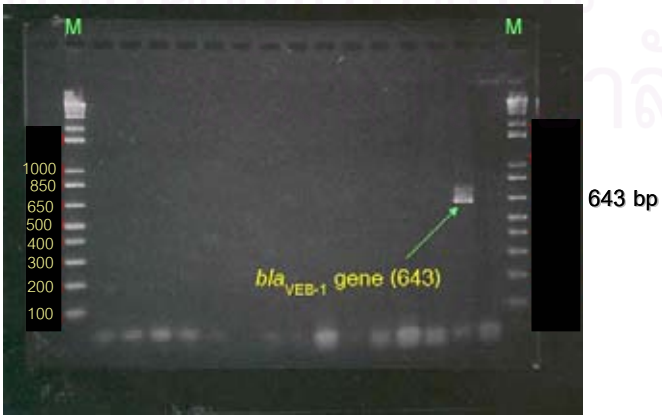


Figure 4-5 PCR product of bla_{VEB-1} gene in *A. baumannii*. M = Marker

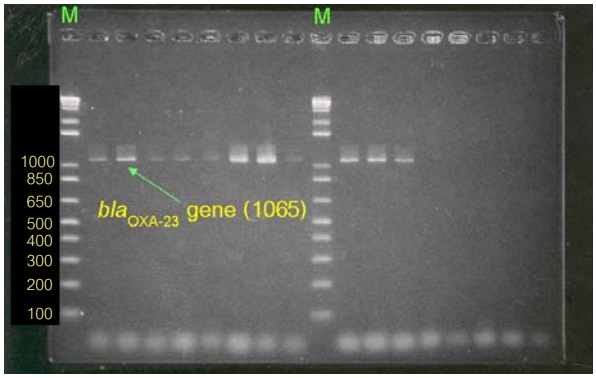


Figure 4-6 PCR product of *bla*$_{OXA-23}$ gene in *A. baumannii*. M = Marker

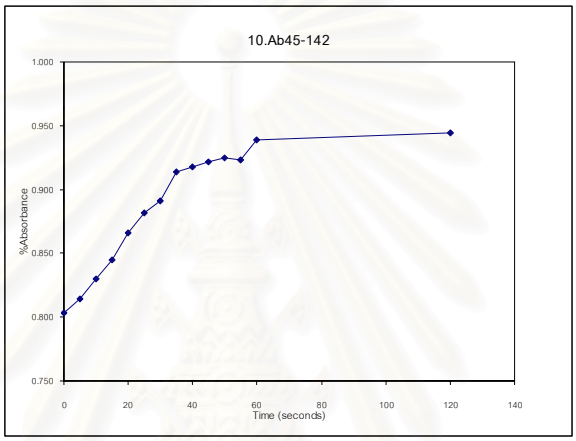


Figure 4-7 Percent absorption at various times of *A. baumannii* strain no. Ab45-142 with negative carbapenemase activity. (the increase in the percent absorbance at various time)

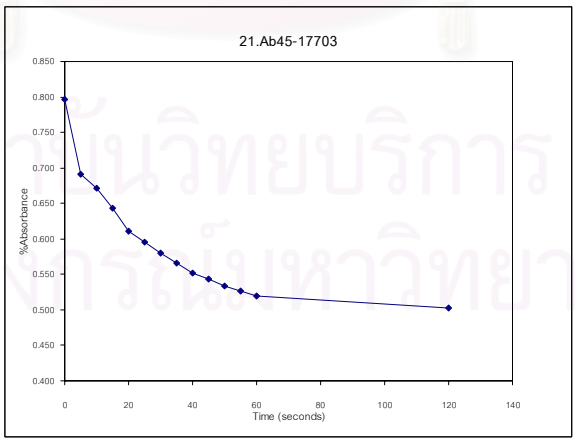


Figure 4-8 Percent absorption at various times of *A. baumannii* strain no. Ab45-17703 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

Table 4-4 Characteristics of imipenem resistant-*A. baumannii* which were susceptible to amikacin

No.	*A. baumannii* strains no.	MICs (μg/ml)			gene detection	Carbapenemase activity
		Imipenem[a]	Amikacin[b]	Ciprofloxacin[c]		
1	Ab45-51	16	16	128	*integrase*	+
2	Ab45-63	16	16	64	-	+
3	Ab45-64	16	16	64	bla_{OXA-23}	+
4	Ab45-17703	16	16	32	bla_{VEB-1}, bla_{OXA-23}	+
5	Ab45-162	16	8	64	*integrase*	+
6	Ab45-164	16	8	32	bla_{OXA-23}	+
7	Ab46-17	32	16	64	-	+
8	Ab45-163	16	4	32	*integrase*	+
9	Ab45-148	16	1	64	-	+
10	Ab46-54	16	8	64	*integrase*	+
11	Ab45-17706	8	4	64	*integrase*,bla_{OXA-23}	+
12	Ab46-28	8	8	64	-	+
13	Ab46-29	8	4	64	*integrase*	+

[a]Susceptibility break point concentration of imipenem = 4 μg/ml

[b]Susceptibility break point concentration of amikacin = 16 μg/ml

[c]Susceptibility break point concentration of ciprofloxacin = 1 μg/ml

Table 4-5 Characteristics of imipenem resistant-*A. baumannii* which were resistant to amikacin

No.	*A. baumannii* strains no.	MICs (μg/ml)			gene detection	Carbapenemase activity
		Imipenem[a]	Amikacin[b]	Ciprofloxacin[c]		
1	Ab45-52	32	>256	64	-	-
2	Ab45-75	16	256	64	*integrase*	+
3	Ab45-122	16	256	64	-	+
4	Ab45-127	16	>256	64	-	+
5	Ab46-31	16	>256	32	*integrase*, bla_{OXA-23}	+
6	Ab46-33	16	64	64	bla_{OXA-23}	+
7	Ab181	16	32	32	bla_{OXA-23}	+
8	Ab45-85	32	32	4	-	+
9	Ab45-117	16	32	64	bla_{VEB-1}, bla_{OXA-23}	+
10	Ab45-128	32	32	4	-	+
11	Ab45-170	16	32	64	*integrase*, bla_{OXA-23}	+
12	Ab46-47	32	32	8	-	+
13	Ab182	16	32	8	bla_{OXA-23}	+
14	Ab45-111	8	>256	64	bla_{OXA-23}	+
15	Ab46-69	8	64	64	-	+
16	Ab45-142	8	32	8	-	-
17	Ab46-32	8	32	64	*integrase*	-

[a]Susceptibility break point concentration of imipenem = 4 μg/ml

[b]Susceptibility break point concentration of amikacin = 16 μg/ml

[c]Susceptibility break point concentration of ciprofloxacin = 1 μg/ml

3. Time kill studies (Raw data were shown in Appendices Table A-4, A-5, A-6 and Figure A-31 to A-60)

The antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against 30 strains of imipenem resistant-*A. baumannii* tested by time kill method were shown in Table 4-6. The combination of imipenem plus amikacin showed the synergistic effect in 3 strains (10%), the additional effect in 8 strains (26.67%), the indifference effect in 18 strains (60%) and the antagonist effect in 1 strain (3.33%). When combined imipenem with ciprofloxacin, the synergistic effect could be observed in 1 strain (3.33%), the additional effect in 6 strains (20%), the indifference effect in 21 strains (70%) and the antagonist effect in 2 strains (6.67%). The percentage of strains showing synergistic and additional effects of imipenem plus amikacin were higher than of imipenem plus ciprofloxacin (36.67% and 23.33%, respectively). Most strains showed indifference effect when tested with any of the two combinations.

Table 4-6 Effect of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against 30 strains of imipenem resistant-*A. baumannii*

Effect	Combination [number (%) of isolates]	
	Imipenem-amikacin combination	Imipenem-ciprofloxacin combination
Synergism	3 (10)	1 (3.33)
Addition	8 (26.67)	6 (20)
Indifference	18 (60)	21 (70)
Antagonism	1 (3.33)	2 (6.67)

The mean $\log_{10}$ decrease of viable cell counts and bacteriolytic area for 24 hours (BA_{24}) by the combination of imipenem plus amikacin and imipenem plus ciprofloxacin were shown in Figure 4-9 and Table 4-7. Imipenem alone was showed to have bacteriostatic activity during the time of study. The combination of imipenem plus amikacin could reduce $\geq$ 3 log CFU/ml during the first 8 hour of growth. The combination of imipenem plus ciprofloxacin could reduce $\geq$ 3 log CFU/ml within 24 hour of growth.

Number of the strains killed at various time intervals and the amount of bacteria killed were shown in Table 4-8. The amount of bacteria killed (BA_{24}) by the combination of imipenem plus amikacin were significantly higher than those killed by single drug alone ($p<0.05$). However, the number of bacteria killed by the combination of imipenem plus amikacin were not significantly different from those killed by the combination of imipenem plus ciprofloxacin ($p>0.05$). Imipenem alone showed bactericidal activity (could reduce $\geq$ 3 log CFU/ml) against 1 strain (3.33%) at 6 hour of growth and against 16 strains (53.33%) at 24 hour of growth. Amikacin alone showed bactericidal activity against only 2 strains during the time of study. Ciprofloxacin alone showed no antibacterial activity against all strains tested. The combination of imipenem plus amikacin showed bactericidal activity against 2 strains (6.67%) at 2 hour of growth and against 28 strains (93.33%) at 24 hour of growth. The combination of imipenem plus ciprofloxacin showed bactericidal activity against 2 strains (6.67%) at 6 hour of growth and against 22 strains (73.33%) at 24 hour of growth.

Antibacterial activities were observed from the time kill study. The comparative activities between imipenem alone and the combination of imipenem plus amikacin, imipenem alone and the combination of imipenem plus ciprofloxacin and the combination of imipenem plus amikacin and the combination of imipenem plus ciprofloxacin could be summarized as followed:

1. <u>Imipenem alone versus the combination of imipenem plus amikacin</u>

The number of bacteria killed by the combination of imipenem plus amikacin [BA_{24} = 138.83 log CFU/ml·h] were significantly higher than the number killed by imipenem alone [BA_{24} = 116.38 log CFU/ml·h] ($p<0.05$). In addition, the number of strains killed to the level of $\geq$ 3 log CFU/ml at 24 hour by the combination of imipenem

plus amikacin (93.33%) were higher than those killed by imipenem alone (53.33%). Time that cells were reduced to the level of $\geq$ 3 log CFU/ml by the combination of imipenem plus amikacin (2 strains, at 2 hours) were faster than the killing time by imipenem alone (1 strain, at 6 hours). [Table 4-8]

2. Imipenem alone versus the combination of imipenem plus ciprofloxacin

The number of bacteria killed by the combination of imipenem plus ciprofloxacin [BA_{24} = 123.50 log CFU/ml•h] were not significantly higher than those killed by imipenem alone [BA_{24} = 116.38 log CFU/ml•h] ($p>0.05$). However, the number of strains killed to the level of $\geq$ 3 log CFU/ml at 24 hour by the combination of imipenem plus ciprofloxacin (73.33%) were higher than those killed by imipenem alone (53.33%). Time that cells were reduced to the level of $\geq$ 3 log CFU/ml by the combination of imipenem plus ciprofloxacin (2 strains, at 6 hours) were similar to the killing time by imipenem alone (1 strain, at 6 hours). [Table 4-8]

3. The combination of imipenem plus amikacin versus imipenem plus ciprofloxacin combination

The number of bacteria killed by the combination of imipenem plus amikacin [BA_{24} = 138.83 log CFU/ml•h] were not significantly higher than those killed by the combination of imipenem plus ciprofloxacin [BA_{24} = 123.50 log CFU/ml•h] ($p>0.05$).However, the number of strains killed to the level of $\geq$ 3 log CFU/ml at 24 hour by the combination of imipenem plus amikacin (93.33%) were higher than those killed by the combination of imipenem plus ciprofloxacin (73.33%). Time that cells were reduced to the level of $\geq$ 3 log CFU/ml by the combination of imipenem plus amikacin (2 strains, at 2 hours) were faster than the killing time by the combination of imipenem plus ciprofloxacin (2 strains, at 6 hours). [Table 4-8]

Table 4-7 Mean log change viable cell counts at various time intervals, $AUBKC_{0-24}$ and BA_{24} in 30 isolates of *A. baumannii*

Drug	Mean (±SD) log change viable cell counts						Mean (±SD) $AUBKC_{0-24}$	Mean (±SD) BA_{24}
	Δ_2	Δ_4	Δ_6	Δ_8	Δ_{10}	Δ_{24}		
control	0.9652 ± 0.1697	1.8423 ± 0.1956	2.1246 ± 0.1754	2.2788 ± 0.2013	2.4361 ± 0.2454	4.4210 ± 1.3195	210.4687 ± 10.5592	-
imipenem	-1.0182 ± 0.2837	-1.5863 ± 0.4385	-2.0235 ± 0.5160	-2.3326 ± 0.7224	-2.5797 ± 1.0110	-2.4211 ± 2.2211	94.0831 ± 23.5284	116.3856 ± 23.4308
amikacin	-0.4795 ± 1.2101	-0.5973 ± 1.6397	-0.5946 ± 1.8651	-0.2092 ± 1.9342	0.4174 ± 1.9357	3.2070 ± 2.3235	167.0613 ± 39.5486	43.4073 ± 42.7406
ciprofloxacin	0.8175 ± 0.3756	1.5859 ± 0.5356	1.9325 ± 0.4859	2.0738 ± 0.4487	2.1924 ± 0.5398	4.0091 ± 1.5085	204.1613 ± 15.7125	6.3073 ± 14.2215
imipenem+amikacin	-1.3835 ± 0.9104 [a]	-2.0082 ± 0.8204 [a]	-2.6106 ± 0.7390 [a]	-3.0838 ± 0.6871 [a]	-3.4457 ± 0.7147 [a]	-3.9661 ± 1.2245 [a]	71.6370 ± 17.1619 [*]	138.8316 ± 20.0747[*]
imipenem+ciprofloxacin	-1.0517 ± 0.2817	-1.6259 ± 0.4444 [a]	-2.0994 ± 0.5708 [a]	-2.4210 ± 0.6802 [a]	-2.7416 ± 0.9938 [a]	-3.1751 ± 1.9038 [a]	86.9666 ± 21.9477 [a]	123.5021 ± 19.5738 [a]

[a] P >0.05 compared to the activity of imipenem ; P < 0.05 compared to the activity of amikacin alone and ciprofloxacin alone.

[*] P< 0.05 compared to the activity of single drug alone; P not significant compared to activity of the combination of imipenem plus ciprofloxacin.

Δ = Mean log change viable cell counts at 2,4,6,8,10 and 24 hours, respectively.

$AUBKC_{0-24}$ = Area under bacterial killing and regrowth curves for 24 hours

BA_{24} = Bacterolytic area for 24 hours

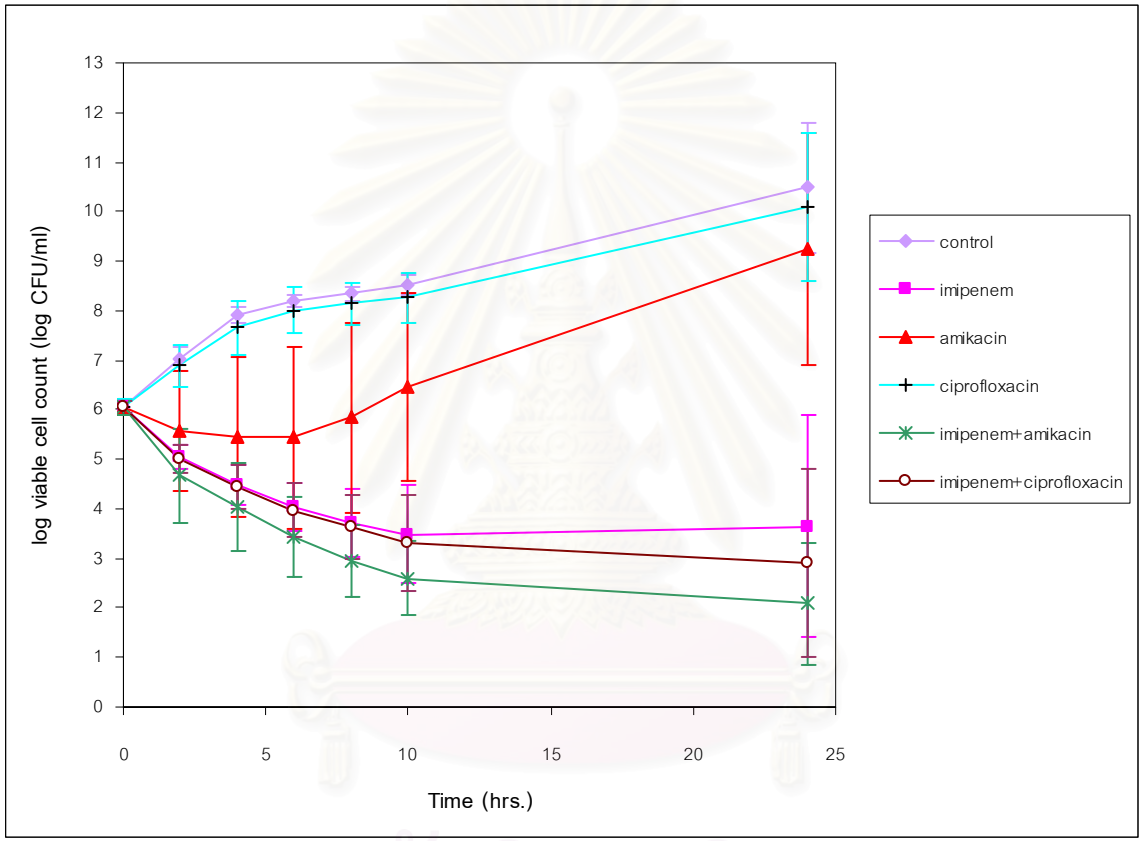


Figure 4-9 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against all 30 strains *A. baumannii*.

Table 4-8 Reduction of *A. baumannii* viable cell counts at various time intervals and BA_{24} in 30 strains of *A. baumannii*

Antimicrobial agents	No. of strains to be killed at time point																		BA_{24} (log CFU/ml•h)
	2 hours			4 hours			6 hours			8 hours			10 hours			24 hours			
	-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3	
imipenem	13	0	0	23	6	0	16	12	1	8	13	8	5	13	10	1	5	16	116.3856 ± 23.4308
amikacin	6	0	2	12	0	2	16	1	2	6	3	2	2	2	2	0	0	2	43.4073 ± 42.7406
ciprofloxacin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.3073 ± 14.2215
imipenem+amikacin	15	2	2	22	3	5	7	17	6	2	10	18	0	8	22	0	1	28	138.8316 ± 20.0747[b,c]
imipenem+ciprofloxacin	17	0	0	22	7	0	16	12	2	12	10	8	2	13	13	2	3	22	123.5021 ± 19.5738 [a,b]

(-1 : 90 % of viable reduction versus initial inoculum; -2 : 99 % of viable reduction versus initial inoculum; -3 : 99.9 % of viable reduction versus initial inoculum)

[a]P>0.05 if compare with imipenem alone; [b] P > 0.05 if compare with other combination; [c]P <0.05 if compare with single drug alone; [d]<0.05 if compare with other combination

BA_{24} = Bacteriolytic area for 24 hours

Among 13 amikacin susceptible *A. baumannii* strains, the synergistic effect of imipenem plus amikacin could be observed in 2 strains (15.38%), the additional effect in 2 strains (15.38%), and the indifference effect in 9 strains (69.24%). No antagonistic effect could be observed in all strains tested as shown in Table A-7 in Appendices. Mean log viable cell counts were shown in Figure 4-10 and raw data were shown in Table A-9 in Appendices. Imipenem alone showed bacteriostatic activity during the time of study. Amikacin showed the bacteriostatic activity at 2,4,6,8 and 10 hour of growth, however from 8 hours to 24 hour of growth, there had been an increase in the number of viable cell counts. The antibacterial activity of ciprofloxacin could not be observed during the time of study. The bactericidal activity of imipenem plus amikacin could be observed at 8, 10 and 24 hour of growth, accordingly while only bacteriostatic activity of imipenem plus ciprofloxacin could be obsevred during the time of study. In addition, the bacterial regrowth in the presence of imipenem plus ciprofloxacin could be observed after 24 hour of growth.

Number of the strains killed at various time intervals and the amount of bacteria killed were shown in Table 4-9. The amount of bacteria killed (BA_{24}) by the combination of imipenem plus amikacin were not significantly higher than that killed by imipenem alone or by the combination of imipenem plus ciprofloxacin ($p>0.05$). Imipenem alone showed bactericidal activity against 1 strain (7.69%) at 6 hour of growth and against 8 strains (61.54%) at 24 hour of growth. Amikacin alone showed bactericidal activity against only 2 strains (15.38%) during the time of study. Ciprofloxacin showed no antibacterial activity in all strains tested. The combination of imipenem plus amikacin showed bactericidal activity against 2 strains (15.38%) at 2 hour of growth and against 13 strains (100%) at 24 hour of growth. The combination of imipenem plus ciprofloxacin showed bactericidal activity against 2 strains (15.38%) at 6 hour of growth and against 9 strains (69.23%) at 24 hour of growth. Antibacterial activities were observed from the time kill study. The comparative activities between imipenem alone and the combination of imipenem plus amikacin, imipenem alone and the combination of imipenem plus ciprofloxacin and the combination of imipenem plus amikacin and the combination of imipenem plus ciprofloxacin could be summarized as followed:

1. <u>Imipenem alone versus the combination of imipenem plus amikacin</u>

The number of bacteria killed by the combination of imipenem plus amikacin [BA_{24} = 145.38 log CFU/ml·h] were not significantly higher than those killed by imipenem alone [BA_{24} = 120.63 log CFU/ml·h] ($p>0.05$). The number of strains killed to the level of $\geq$ 3 log CFU/ml at 24 hour by the combination of imipenem plus amikacin (100%) were higher than those killed by imipenem alone (61.54%). The combination of imipenem plus amikacin killed all the stains in this group. Time that cells were reduced to the level of $\geq$ 3 log CFU/ml by the combination of imipenem plus amikacin (2 strains, at 2 hours) were faster than the killing time by imipenem alone (1 strain, at 6 hours). [Table 4-9]

2. Imipenem alone versus the combination of imipenem plus ciprofloxacin

The number of bacteria killed by the combination of imipenem plus ciprofloxacin [BA_{24} = 125.95 log CFU/ml·h] were not significantly higher than those killed by imipenem alone [BA_{24} = 120.63 log CFU/ml·h] ($p>0.05$). The number of strains killed to the level of $\geq$ 3 log CFU/ml at 24 hour by the combination of imipenem plus ciprofloxacin (69.23%) were higher than those killed by imipenem alone (61.54%). Time that cells were reduced to the level of $\geq$ 3 log CFU/ml by the combination of imipenem plus ciprofloxacin (2 strains, at 6 hours) were similar to the killing time by imipenem alone (1 strain, at 6 hours). [Table 4-9]

3. The combination of imipenem plus amikacin versus imipenem plus ciprofloxacin combination

The number of bacteria killed by the combination of imipenem plus amikacin [BA_{24} = 145.38 log CFU/ml·h] were not significantly higher than those killed by the combination of imipenem plus ciprofloxacin [BA_{24} = 125.95 log CFU/ml·h] ($p>0.05$). The number of strains killed to the level of $\geq$ 3 log CFU/ml at 24 hour by the combination of imipenem plus amikacin (100%) were higher than the number of strains killed by the combination of imipenem plus ciprofloxacin (69.23%). The combination of imipenem plus amikacin killed all the stains in this group. Time that cells were reduced to the level of $\geq$ 3 log CFU/ml by the combination of imipenem plus amikacin (2 strains, at 2 hours) were faster than time killing time the combination of imipenem plus ciprofloxacin (2 strains, at 6 hours). [Table 4-9]

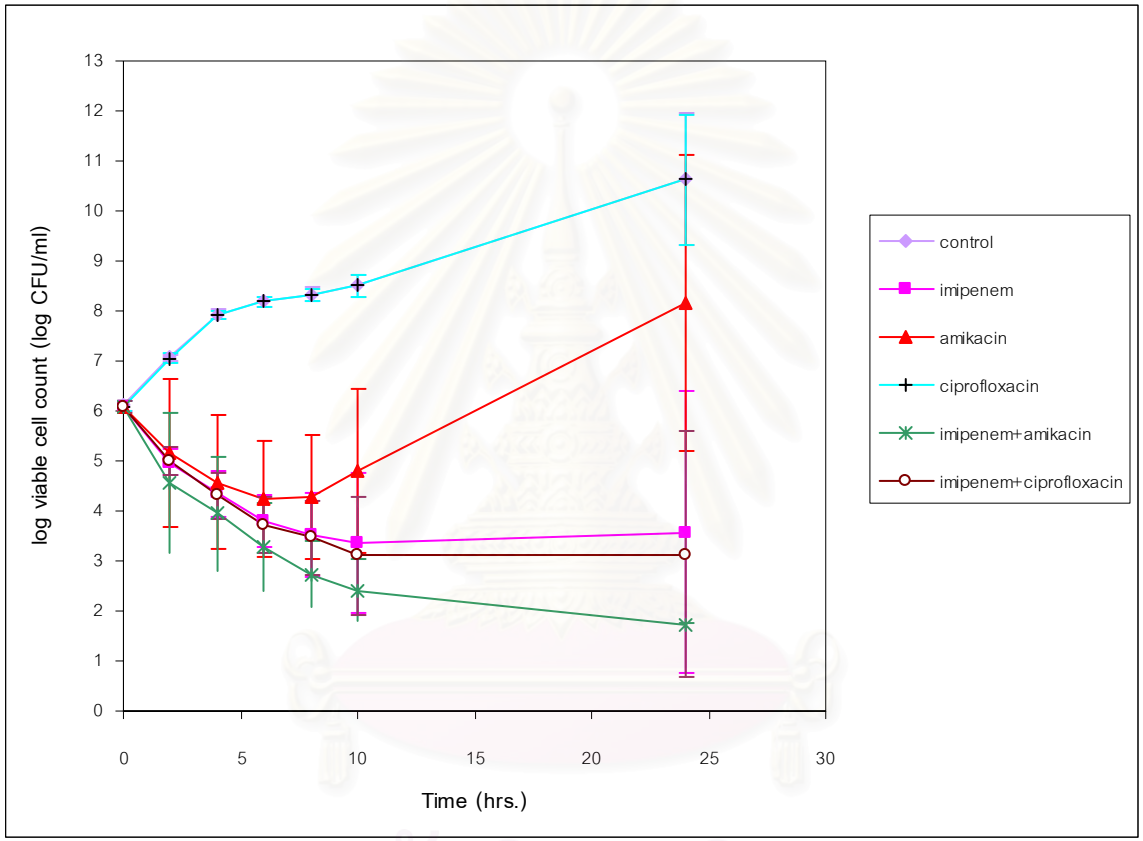


Figure 4-10 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against 13 strains of amikacin susceptible-*A. baumannii*.

Table 4-9 Reduction of *A. baumannii* viable cell counts at various time intervals and BA_{24} in 13 amikacin susceptible *A. baumannii* strains

Antimicrobial agents	No. of strains to be killed at time point																		BA_{24} (log CFU/ml•h)
	2 hours			4 hours			6 hours			8 hours			10 hours			24 hours			
	-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3	
imipenem	8	0	0	9	4	0	6	6	1	1	4	7	0	4	7	1	2	8	120.6325±31.2359
amikacin	1	0	2	5	0	2	9	1	2	5	3	2	2	2	2	0	0	2	73.6322±45.8808
ciprofloxacin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1931±0.7051
imipenem+amikacin	4	1	2	9	1	3	1	9	3	0	4	9	0	1	12	0	0	13	145.3762±17.2994[a,b]
imipenem+ciprofloxacin	8	0	0	9	4	0	4	7	2	4	3	6	0	4	8	0	2	9	125.9526±23.4684[a,b]

(-1 : 90 % of viable reduction versus initial inoculum; -2 : 99 % of viable reduction versus initial inoculum; -3 : 99.9 % of viable reduction versus initial inoculum)

[a]P>0.05 if compare with imipenem alone; [b] P > 0.05 if compare with other combination; [c]P <0.05 if compare with single drug alone; [d]<0.05 if compare with other combination

BA_{24} = Bacteriolytic area for 24 hours

Among 17 amikacin resistant *A. baumannii* strains, synergistic effect of imipenem plus amikacin could be observed in 1 strain (5.88%), the additional effect in 6 strains (35.29%), the indifference effect in 9 strains (52.95%), while the antagonistic effect in 1 strain (5.88%) as shown in Table A-8 in Appendices. Mean log viable cell counts were shown in Figure 4-11 and raw data were shown in Table A-10 in Appendices. Imipenem alone showed the bacteriostatic activity during the time of study. Amikacin showed the bacteriostatic activity at 2 hour of growth, however from 4 hour to 24 hour of growth there had been an increase in the number of viable cell counts. The antibacterial activity of ciprofloxacin could not be observed during the time of study. While such activity of imipenem plus amikacin was observed after the 10^{th} hour of growth. The combination of imipenem plus ciprofloxacin showed the bacteriostatic activity throughout the time of study until at the 24^{th} hour of growth bactericidal activity could be observed.

Number of the strains killed at various time intervals and the amount of bacteria killed were shown in Table 4-10. The amount of bacteria killed (BA_{24}) by the combination of imipenem plus amikacin were significantly higher than the number killed by single drug ($p<0.05$). However, the number of bacteria killed by the combination of imipenem plus amikacin were not significantly different from those killed by the combination of imipenem plus ciprofloxacin ($p>0.05$). Imipenem alone showed bactericidal activity against 1 strain (5.88%) at 8 hour of growth and against 8 strains (47.06%) at 24 hour of growth. Amikacin and ciprofloxacin alone showed no antibacterial activity against all strains tested. Imipenem combined with amikacin showed bactericidal activity against 2 strains (11.76%) at 4 hour of growth and against 15 strains (88.24%) at 24 hour of growth. The combination of imipenem plus ciprofloxacin showed bactericidal activity against 2 strains (11.76%) at 8 hour of growth and against 13 strains (76.48%) at 24 hour of growth.

Antibacterial activities were observed from the time kill study. The comparative activities between imipenem alone and the combination of imipenem plus amikacin, imipenem alone and the combination of imipenem plus ciprofloxacin and the combination of imipenem plus amikacin and the combination of imipenem plus ciprofloxacin could be summarized as followed:

1. Imipenem alone versus the combination of imipenem plus amikacin

The number of bacteria killed by the combination of imipenem plus amikacin [BA_{24} = 133.83 log CFU/ml•h] were significantly higher than those killed by imipenem alone [BA_{24} = 113.14 log CFU/ml•h] ($p<0.05$). The number of strains killed to the level of $\geq$ 3 log CFU/ml at 24 hour by the combination of imipenem plus amikacin (88.24%) were higher than those killed by imipenem alone (47.06%). Time that cells were reduced to the level of $\geq$ 3 log CFU/ml by the combination of imipenem plus amikacin (2 strains, at 4 hours) were faster than the killing time by imipenem alone (1 strain, at 8 hours). [Table 4-10]

2. Imipenem alone versus the combination of imipenem plus ciprofloxacin

The number of bacteria killed by the combination of imipenem plus ciprofloxacin [BA_{24} = 121.63 log CFU/ml•h] were not significantly higher than those killed by imipenem alone [BA_{24} = 113.14 log CFU/ml•h] ($p>0.05$). The number of strains killed to the level of $\geq$ 3 log CFU/ml at 24 hour by the combination of imipenem plus ciprofloxacin (76.48%) were higher than those killed by imipenem alone (47.06%). Time that cells were reduced to the level of $\geq$ 3 log CFU/ml by the combination of imipenem plus ciprofloxacin (2 strains, at 8 hours) were similar to the killing time by imipenem alone (1 strain, at 8 hours). [Table 4-10]

3. The combination of imipenem plus amikacin versus imipenem plus ciprofloxacin combination

The number of bacteria killed by the combination of imipenem plus amikacin [BA_{24} = 133.83 log CFU/ml•h] were not significantly higher than those killed by the combination of imipenem plus ciprofloxacin [BA_{24} = 121.63 log CFU/ml•h] ($p>0.05$). The number of strains killed to the level of $\geq$ 3 log CFU/ml at 24 hour by the combination of imipenem plus amikacin (88.24%) were higher than those killed by the combination of imipenem plus ciprofloxacin (76.48%). Time that cells were reduced to the level of $\geq$ 3 log CFU/ml by the combination of imipenem plus amikacin (2 strains, at 4 hours) were faster than killing time by the combination of imipenem plus ciprofloxacin (2 strains, at 8 hours). [Table 4-10]

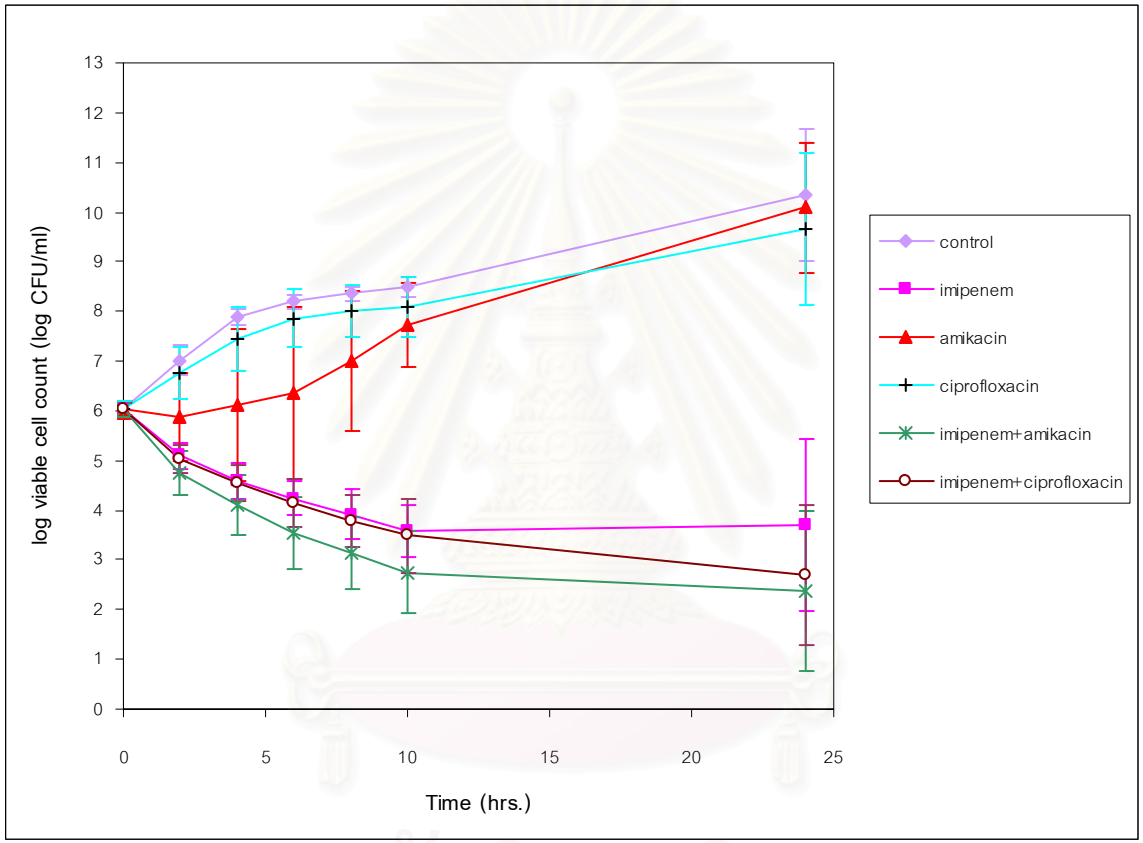


Figure 4-11 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against against 17 strains of amikacin resistant-*A. baumannii*.

Table 4-10 Reduction of *A. baumannii* viable cell counts at various time intervals and BA_{24} in 17 amikacin resistant *A. baumannii* strains

Antimicrobial agents	No. of strains to be killed at time point																		BA_{24} (log CFU/ml•h)
	2 hours			4 hours			6 hours			8 hours			10 hours			24 hours			
	-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3	
imipenem	5	0	0	14	2	0	10	6	0	7	9	1	5	9	3	0	3	8	113.1379±15.4093
amikacin	5	0	0	7	0	0	7	0	0	1	0	0	0	0	0	0	0	0	20.2942±20.5511
ciprofloxacin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10.9830±17.6806
imipenem+amikacin	11	1	0	13	2	2	6	8	3	2	6	9	0	7	10	0	1	15	133.8270±21.0845[b,c]
imipenem+ciprofloxacin	9	0	0	13	3	0	12	5	0	8	7	2	2	9	5	2	1	13	121.6282±16.8298[a,b]

(-1 : 90 % of viable reduction versus initial inoculum; -2 : 99 % of viable reduction versus initial inoculum; -3 : 99.9 % of viable reduction versus initial inoculum)

[a]P > 0.05 if compare with imipenem alone; [b] P > 0.05 if compare with other combination; [c]P <0.05 if compare with single drug alone; [d]<0.05 if compare with other combination

BA_{24} = Bacteriolytic area for 24 hours

Among 16 strains which were killed to the level of ≥3 log CFU/ml at 24 hours by imipenem alone, *integrase* gene were positive in 37.5% of isolates, bla_{VEB-1} positive in 6.25% and bla_{OXA-23} positive in 31.25% (Table 4-11). Among 14 strains which were not killed to the level of ≥3 log CFU/ml at 24 hours by imipenem alone, *integrase* gene were positive in 28.57% of isolates, bla_{VEB-1} positive in 7.14% and bla_{OXA-23} positive in 51.02% (Table 4-12). Among 2 strains which were not killed to the level of ≥3 log CFU/ml at 24 hours by the combination of imipenem plus amikacin, *integrase* gene were positive in 50% of isolates and bla_{OXA-23} positive in 100% (Table 4-13).

Among 22 strains which were killed to the level of ≥3 log CFU/ml at 24 hours by the combination of imipenem plus ciprofloxacin, *integrase* gene were positive in 31.82% of isolates, bla_{VEB-1} positive in 9.09% and bla_{OXA-23} positive in 36.36% (Table 4-14). Among 8 strains which were not killed to the level of ≥3 log CFU/ml at 24 hours by the combination of imipenem plus ciprofloxacin, *integrase* gene were positive in 37.5% of isolates and bla_{OXA-23} positive in 37.5% (Table 4-15).

Table 4-11 Characteristic of imipenem resistant-*A. baumannii* which were killed to the level of ≥3 log CFU/ml at 24 hours by imipenem alone

No.	*A.baumannii* strains no.	MICs (μg/ml)			gene detection	Carbapenemase activity
		Imipenem[a]	Amikacin[b]	Ciprofloxacin[c]		
1	Ab45-51	16	16	128	*integrase*	+
2	Ab45-63	16	16	64	-	+
3	Ab45-64	16	16	64	bla_{OXA-23}	+
4	Ab45-163	16	4	32	*integrase*	+
5	Ab45-148	16	1	64	-	+
6	Ab45-17706	8	4	64	*integrase*,bla_{OXA-23}	+
7	Ab46-28	8	8	64	-	+
8	Ab46-29	8	4	64	*integrase*	+
9	Ab45-52	32	>256	64	-	-
10	Ab45-75	16	256	64	*integrase*	+
11	Ab45-122	16	256	64	-	+
12	Ab46-31	16	>256	32	*integrase*, bla_{OXA-23}	+
13	Ab45-117	16	32	64	bla_{VEB-1}, bla_{OXA-23}	+
14	Ab46-47	32	32	8	-	+
15	Ab182	16	32	8	bla_{OXA-23}	+
16	Ab45-142	8	32	8	-	-

[a]Susceptibility break point concentration of imipenem = 4 μg/ml

[b]Susceptibility break point concentration of amikacin = 16 μg/ml

[c]Susceptibility break point concentration of ciprofloxacin = 1 μg/ml

Table 4-12 Characteristic of imipenem resistant-*A. baumannii* which were not killed to the level of ≥3 log CFU/ml at 24 hours by imipenem alone

No.	*A.baumannii* strains no.	MICs (μg/ml)			gene detection	Carbapenemase activity
		Imipenem[a]	Amikacin[b]	Ciprofloxacin[c]		
1	Ab45-17703	16	16	32	bla_{VEB-1}, bla_{OXA-23}	+
2	Ab45-162	16	8	64	*integrase*	+
3	Ab45-164	16	8	32	bla_{OXA-23}	+
4	Ab46-17	32	16	64	-	+
5	Ab46-54	16	8	64	*integrase*	+
6	Ab45-127	16	>256	64	-	+
7	Ab46-33	16	64	64	bla_{OXA-23}	+
8	Ab181	16	32	32	bla_{OXA-23}	+
9	Ab45-85	32	32	4	-	+
10	Ab45-128	32	32	4	-	+
11	Ab45-170	16	32	64	*integrase*, bla_{OXA-23}	+
12	Ab45-111	8	>256	64	bla_{OXA-23}	+
13	Ab46-69	8	64	64	-	+
14	Ab46-32	8	32	64	*integrase*	-

[a]Susceptibility break point concentration of imipenem = 4 μg/ml

[b]Susceptibility break point concentration of amikacin = 16 μg/ml

[c]Susceptibility break point concentration of ciprofloxacin = 1 μg/ml

Table 4-13 Characteristic of imipenem resistant-*A. baumannii* which were killed to the level of ≥3 log CFU/ml at 24 hours by the combination of imipenem plus amikacin

No.	*A.baumannii* strains no.	MICs (μg/ml)			gene detection	Carbapenemase activity
		Imipenem[a]	Amikacin[b]	Ciprofloxacin[c]		
1	Ab181	16	32	32	bla_{OXA-23}	+
2	Ab45-170	16	32	64	*integrase*, bla_{OXA-23}	+

[a]Susceptibility break point concentration of imipenem = 4 μg/ml

[b]Susceptibility break point concentration of amikacin = 16 μg/ml

[c]Susceptibility break point concentration of ciprofloxacin = 1 μg/ml

Table 4-14 Characteristic of imipenem resistant-*A. baumannii* which were killed to the level of ≥3 log CFU/ml at 24 hours by the combination of imipenem plus ciprofloxacin

No.	*A.baumannii* strains no.	MICs (μg/ml)			gene detection	Carbapenemase activity
		Imipenem[a]	Amikacin[b]	Ciprofloxacin[c]		
1	Ab45-51	16	16	128	*integrase*	+
2	Ab45-63	16	16	64	-	+
3	Ab45-17703	16	16	32	bla_{VEB-1}, bla_{OXA-23}	+
4	Ab45-164	16	8	32	bla_{OXA-23}	+
5	Ab45-163	16	4	32	*integrase*	+
6	Ab45-148	16	1	64	-	+
7	Ab45-17706	8	4	64	*integrase*,bla_{OXA-23}	+
8	Ab46-28	8	8	64	-	+
9	Ab46-29	8	4	64	*integrase*	+
10	Ab45-52	32	>256	64	-	-
11	Ab45-75	16	256	64	*integrase*	+
12	Ab45-122	16	256	64	-	+
13	Ab45-127	16	>256	64	-	+
14	Ab46-31	16	>256	32	*integrase*, bla_{OXA-23}	+
15	Ab46-33	16	64	64	bla_{OXA-23}	+
16	Ab45-85	32	32	4	-	+
17	Ab45-117	16	32	64	bla_{VEB-1}, bla_{OXA-23}	+
18	Ab45-128	32	32	4	-	+
19	Ab182	16	32	8	bla_{OXA-23}	+
20	Ab45-111	8	>256	64	bla_{OXA-23}	+
21	Ab46-69	8	64	64	-	+
22	Ab46-32	8	32	64	*integrase*	-

[a]Susceptibility break point concentration of imipenem = 4 μg/ml

[b]Susceptibility break point concentration of amikacin = 16 μg/ml

[c]Susceptibility break point concentration of ciprofloxacin = 1 μg/ml

Table 4-15 Characteristic of imipenem resistant-*A. baumannii* which were not killed to the level of ≥3 log CFU/ml at 24 hours by the combination of imipenem plus ciprofloxacin

No.	*A.baumannii* strains no.	MICs (μg/ml)			gene detection	Carbapenemase activity
		Imipenem[a]	Amikacin[b]	Ciprofloxacin[c]		
1	Ab45-64	16	16	64	bla_{OXA-23}	+
2	Ab45-162	16	8	64	*integrase*	+
3	Ab46-17	32	16	64	-	+
4	Ab46-54	16	8	64	*integrase*	+
5	Ab181	16	32	32	bla_{OXA-23}	+
6	Ab45-170	16	32	64	*integrase*, bla_{OXA-23}	+
7	Ab46-47	32	32	8	-	+
8	Ab45-142	8	32	8	-	-

[a]Susceptibility break point concentration of imipenem = 4 μg/ml

[b]Susceptibility break point concentration of amikacin = 16 μg/ml

[c]Susceptibility break point concentration of ciprofloxacin = 1 μg/ml

CHAPTER V

DISCUSSION

In recent years, the incidence of imipenem resistant-*A. baumannii* has been increasing. The organism has also been resistant to almost all commercially available antibiotics including all cephalosporins, aztreonam, aminoglycosides, and ciprofloxacin. However, it has been reported that imipenem-resistant *A. baumannii* strains were still susceptible to amikacin and to ciprofloxacin (Silva, 1999). In contrary, the result from this part of the study demonstrated that most imipenem resistant-*A. baumannii* were also resistant to amikacin, ciprofloxacin and ceftazidime. Our result showed that there was a correlation between imipenem resistant *A. baumannii* and the susceptibility to other antimicrobial agents. Most imipenem-resistant *A. baumannii* strains were resistant to amikacin (56.67%) and ceftazidime (93.33%). In addition, all strains were resistant to ciprofloxacin. Boe et al. reported that high-level carbapenem resistance in *A. baumannii* was due to more than one resistant mechanism (Bou et al., 2000). Thus, the mechanism of imipenem resistance in *A. baumannii* strains in our study might be from only one mechanism because of the low level of MICs.

Regarding to the detection of the antibiotic resistance genes and carbapenemase activity, it has been demonstrated that most imipenem-resistant *A. baumannii* strains produced carbapenemase enzyme possibly to destroy imipenem which showed that this finding was similar to the result by the study of Fernandez-cuenca et al. However, some strains may use the other resistant mechanisms against imipenem such as porins reduction, alternation of PBPs, and the presence of specific efflux pump (Bou et al. 2000). Furthermore, *A. baumannii* which produced carbapenemase enzyme might also produce other OXA-type β-lactamase beside metallo-β-lactamase because bla_{IMP} gene could not be detected in all strains tested.

For the detection of *aac*(6') gene which encoded for the major aminoglycoside modifying enzymes, Ploy et al. could detect *aac*(6') gene in 32% of *A. baumannii* isolates. In our study, *aac*(6') gene could not be detected in all amikacin resistant-*A. baumannii* strains indicating that these isolates might use the other aminoglycoside

modifying enzymes such as APH(3')VI which is the new type of 3'-o-phosphotransferase that inactivates amikacin or used the other resistant mechanisms such as efflux pump (Towner, 1997).

Among 28 isolates which were resistant to ceftazidime, only 2 isolates were bla_{VEB-1} gene positive indicating that these isolates might use other β-lactamases to inactivate ceftazidime. Poirel et al. identified bla_{VEB-1} gene positive in 56.33% of isolates which was higher than bla_{VEB-1} gene positive (6.67%) in our study.

For the detection of *integrase* gene, which carried multiple resistant genes cassette in most gram negative bacteria, *integrase* gene could be detected in 33.33% of the isolates which was lower than the result from previous study done by Gombac et al. who detected integron in 44 % of *A. baumannii* isolates. Integron inserted genes encode for various antibiotic resistance mechanisms, conferring resistance to aminoglycosides, β-lactams, chloramphenicol, macrolides, sulphonamides, antiseptics and disinfectant (Weldhagen, 2004). Thus, *A.baumannii* which was *integrase* gene positive might be multidrug resistant (MDR) strains.

The antibacterial activity of imipenem was studied by time kill method. It was demonstrated that imipenem alone still has antibacterial activity against some imipenem resistant-*A. baumannii* strains. It might be because the concentration of imipenem used in this study was higher than MICs of imipenem in all strains tested. This concentration was equal to the mean serum level of imipenem at therapeutic dose. In addition, imipenem has time–dependent bactericidal activity.

The antibacterial activity of ciprofloxacin was studied by time kill method. It was demonstrated that ciprofloxacin alone has no antibacterial activity in all strains tested. This could be explained that ciprofloxacin has the concentration dependent bactericidal activity and the concentration of ciprofloxacin used in this study was lower than MICs of ciprofloxacin in all strains tested. This concentration was equal to the mean serum level of ciprofloxacin at therapeutic dose.

Despite the fact that 13 strains of *A. baumannii* were susceptible to amikacin, the bactericidal activity of amikacin was observed in only two strains, one with the MIC of amikacin = 1 μg/ml and another strain with MIC of amikacin = 8 μg/ml. This result indicated that there was no correlation between MICs value and bactericidal activity.

Other amikacin susceptible strains might use the other regulation resistant mechanisms such as the production of the other aminoglycoside modifying enzymes, the alteration of targer site or the presence of the efflux pump. Therefore, this outcome suggested that we could not use amikacin alone at therapeutic dose for the treatment of infectious disease caused by imipenem resistant-*A. baumannii* which were either susceptible or resistant to amikacin except strains with the low level of MICs (1 μg/ml).

The combination of imipenem plus amikacin displayed the indifference effect in most strains. It might be because the concentration of imipenem used in this study was higher than MICs of imipenem in all strains tested. Thus, it is impossible to separate the synergistic effect from an indifference effect (Elipoulos and Moellering, 1996). The combination of imipenem plus amikacin showed the better bactericidal activity against these isolates than the activity of the other antimicrobial agents when compared the number of cells killed, the number of strains killed and the time that bacteria were killed. In amikacin susceptible strains, the combination of imipenem plus amikacin could kill all strains tested. This could be explained that imipenem might alter the permeability of bacterial cell wall and enhancing the entry of amikacin while amikacin inhibited the β-lactamases that inactivated imipenem (Elipoulos and Moellering, 1996). Hsueh et al. showed that there has been a synergistic effect in imipenem-amikacin combination in pandrug resistant-*A. baumannii*. Thus, it is similar to the result obtained in our study. The combination of imipenem plus amikacin was shown to be the best regiment in our study. This finding was similar to the result from the study of Chang et al. who reported that the combination of imipenem plus amikacin was the best results. Therefore, this outcome suggested that we could possibly use the combination of imipenem plus amikacin in the treatment of infectious disease caused by imipenem resistant-*A. baumannii* strains. It also suggested that the combination of imipenem plus amikacin could be used in the treatment of all imipenem resistant-*A. baumannii* strains which were still susceptible to amikacin. In addition, most imipenem resistant-*A. baumannii* strains which were resistant to amikacin could be killed by the combination of impenem plus amikacin, while only 2 amikacin resistant strains were the failure in the treatment. However, our results need to be examined further by the *in vivo* studies in order to obtain more conclusive evidence.

The combination of imipenem plus ciprofloxacin displayed the indifference effect in most strains. This finding was similar to the result from the study of Erdem et al. who reported that the combination of imipenem plus ciprofloxacin showed the indifference effect in most strains of multidrug-resistant *P. aeruginosa*. However, the combination of imipenem plus ciprofloxacin showed the better bactericidal activity against these isolates than the activity of the single antimicrobial agents when the number of cells killed, the number of strains killed and time that bacteria were killed were determined. Imipenem may alter the permeability of bacterial cell wall and enhancing the entry of ciprofloxacin (Elipoulos and Moellering, 1996). Therefore, this outcome suggested that we could possibly use the combination of imipenem plus ciprofloxacin in the treatment of infectious disease caused by imipenem resistant-*A. baumannii* strains. However, the combination of imipenem plus ciprofloxacin was not the best result in this study.

This study displayed no correlation between antibacterial activity of imipenem alone / the combination of imipenem plus ciprofloxacin and antibiotic resistant genes identified. The antibiotic resistant genes included in this study (bla_{VEB-1}, bla_{OXA-1}, bla_{OXA-2}, bla_{OXA-10}, bla_{OXA-23}, bla_{IMP} and *aac*(6') gene) are the most common antibiotic resistant genes identified in *A. baumannii*. To date, several antibiotic resistant genes in *A. baumannii* besides these genes were identified in this study. Thus, the characteristics of the genes identified in imipenem resistant-*A. baumannii* that were no correlation with antibacterial activity. While the combination of imipenem plus amikacin could kill all bla_{OXA-23} negative strains. Some strains carried bla_{OXA-23} gene could be killed by the combination of imipenem plus amikacin, while only 2 strains which carried bla_{OXA-23} gene were the failure in the treatment. From this result indicated that imipenem resistant-*A. baumannii* might produce the other carbapenemase enzymes such as OXA-24/25-/26/-27 (Bergogne-Berezin and Towner, 1996) that inactivated imipenem or use the other resistant mechanisms beside produced OXA-23 β-lactamase.

Benefit of this study is that the characteristics of the antibiotic resistant genes and integrase gene identified in imipenem resistant-*A. baumannii* in Siriraj Hospital have been demonstrated. The preliminary study on the use of the combination of imipenem plus amikacin or plus ciprofloxacin in the treatment of infections due to imipenem

resistant-*A. baumannii* was performed. Further animal studies and clinical trials are needed to confirm the relevance of these combinations.

CONCLUSION

This study suggested that infections due to imipenem resistant-*A. baumannii* strains, might be treated with a combination of antimicrobials. The combination of imipenem plus amikacin or plus ciprofloxacin could be promising alternatives for the treatment of infection due to imipenem resistant-*A. baumannii* strains. Especially among amikacin susceptible strains, the combination of imipenem plus amikacin could show the bactericidal activity against all strains tested. However, *in vitro* data must be validated by assessing the clinical performance of combinations of antimicrobial agents before specific recommendations to modify existing treatment.

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APPENDICES

Table A-1 Raw data of susceptibility testing by disk diffusion method and by agar dilution method.

No.	*A. baumannii* strains No.	Imipenem				Amikacin				Ciprofloxacin				Ceftazidime	
		Disk diffusion method		Agar dilution method		Disk diffusion method		Agar dilution method		Disk diffusion method		Agar dilution method		Disk diffusion method	
		zone diameter (mm)	Interpretion	MICs (μg/ml)	Interpretion	zone diameter (mm)	Interpretion	MICs (μg/ml)	Interpretion	zone diameter (mm)	Interpretion	MICs (μg/ml)	Interpretion	zone diameter (mm)	Interpretion
1	Ab45-51	9.56	R	16	R	13.47	R	16	S	5.11	R	128	R	5.12	R
2	Ab45-52	7.75	R	32	R	NZ	R	>256	R	NZ	R	64	R	NZ	R
3	Ab45-75	10.56	R	16	R	NZ	R	256	R	NZ	R	64	R	NZ	R
4	Ab45-85	5.94	R	32	R	13.03	R	32	I	14.35	R	4	R	5.94	R
5	Ab45-111	11.92	R	8	I	NZ	R	>256	R	NZ	R	64	R	NZ	R
6	Ab45-117	9.58	R	16	R	12.08	R	32	I	NZ	R	64	R	NZ	R
7	Ab45-122	8.03	R	16	R	NZ	R	256	R	NZ	R	64	R	NZ	R
8	Ab45-127	10.38	R	16	R	NZ	R	>256	R	NZ	R	64	R	NZ	R
9	Ab45-128	6.23	R	32	R	11.79	R	32	I	14.85	R	4	R	7.41	R
10	Ab45-142	10.57	R	8	I	11.92	R	32	I	11.79	R	8	R	5.87	R
11	Ab45-170	10.41	R	16	R	11.84	R	32	I	NZ	R	64	R	7.92	R
12	Ab46-31	8.18	R	16	R	NZ	R	>256	R	NZ	R	32	R	9.35	R
13	Ab46-32	12.89	R	8	I	13.91	R	32	I	NZ	R	64	R	NZ	R
14	Ab46-33	10.26	R	16	R	7.85	R	64	R	10.89	R	64	R	9.09	R
15	Ab46-69	11.94	R	8	I	10.58	R	64	R	NZ	R	64	R	NZ	R

R = resistant, I = Intermediate, S = Susceptible, NZ = no inhibition zone

Table A-1 (continue) Raw data of susceptibility testing by disk diffusion method and by agar dilution method.

No.	*A. baumannii* strains No.	Imipenem				Amikacin				Ciprofloxacin				Ceftazidime	
		Disk diffusion method		Agar dilution method		Disk diffusion method		Agar dilution method		Disk diffusion method		Agar dilution method		Disk diffusion method	
		zone diameter (mm)	Interpretion	MICs (μg/ml)	Interpretion	zone diameter (mm)	Interpretion	MICs (μg/ml)	Interpretion	zone diameter (mm)	Interpretion	MICs (μg/ml)	Interpretion	zone diameter (mm)	Interpretion
16	Ab181	9.6	R	16	R	NZ	R	>256	R	6.74	R	32	R	11.56	R
17	Ab46-47	6.48	R	32	R	10.65	R	32	I	15.98	I	8	R	8.54	R
18	Ab182	10.34	R	16	R	11.33	R	32	I	17.95	I	8	R	10.29	R
19	Ab45-63	12.8	R	16	R	14.79	I	16	S	NZ	R	64	R	8.13	R
20	Ab45-64	9.19	R	16	R	15.46	I	16	S	6.1	R	64	R	6.1	R
21	Ab45-17703	7.52	R	16	R	15.76	I	16	S	6.32	R	32	R	NZ	R
22	Ab45-162	7.37	R	16	R	21.74	S	8	S	NZ	R	64	R	NZ	R
23	Ab45-164	7.73	R	16	R	19.74	S	8	S	NZ	R	32	R	NZ	R
24	Ab45-17706	12.93	R	8	I	19.52	S	4	S	NZ	R	64	R	NZ	R
25	Ab46-17	7.85	R	32	R	19.04	S	16	S	NZ	R	64	R	NZ	R
26	Ab46-28	8.57	R	8	I	18.14	S	8	S	NZ	R	64	R	NZ	R
27	Ab46-29	9.38	R	8	I	22.48	S	4	S	NZ	R	64	R	NZ	R
28	Ab45-163	7.56	R	16	R	21.12	S	4	S	5.52	R	32	R	16.12	I
29	Ab45-148	7.56	R	16	R	22.16	S	1	S	NZ	R	64	R	18.21	S
30	Ab46-54	9.09	R	16	R	20.15	S	8	S	NZ	R	64	R	20.22	S

R = resistant, I = Intermediate, S = Susceptible, NZ = no inhibition zone

Table A-2 Antibiotic resistant gene detection and carbapenemase activity in 30 strains of *A. baumannii*

No.	*A. baumannii* strains No.	*integrase*	bla_{VEB-1}	bla_{OXA-1}	bla_{OXA-2}	bla_{OXA-10}	bla_{OXA-23}	bla_{IMP}	*aac(6´)*	carbapenemase activity
1	Ab45-51	+	-	-	-	-	-	-	-	+
2	Ab45-52	-	-	-	-	-	-	-	-	-
3	Ab45-75	+	-	-	-	-	-	-	-	+
4	Ab45-85	-	-	-	-	-	-	-	-	+
5	Ab45-111	-	-	-	-	-	+	-	-	+
6	Ab45-117	-	+	-	-	-	+	-	-	+
7	Ab45-122	-	-	-	-	-	-	-	-	+
8	Ab45-127	-	-	-	-	-	-	-	-	+
9	Ab45-128	-	-	-	-	-	-	-	-	+
10	Ab45-142	-	-	-	-	-	-	-	-	-
11	Ab45-170	+	-	-	-	-	+	-	-	+
12	Ab46-31	+	-	-	-	-	+	-	-	+
13	Ab46-32	+	-	-	-	-	-	-	-	-
14	Ab46-33	-	-	-	-	-	+	-	-	+
15	Ab46-69	-	-	-	-	-	-	-	-	+

+ = positive; - = negative

Table A-2 (continue) Antibiotic resistant gene detection and carbapenemase activity in 30 strains of *A. baumannii*

No.	*A. baumannii* strains No.	*integrase*	bla_{VEB-1}	bla_{OXA-1}	bla_{OXA-2}	bla_{OXA-10}	bla_{OXA-23}	bla_{IMP}	*aac(6´)*	carbapenemase activity
16	Ab181	-	-	-	-	-	+	-	-	+
17	Ab46-47	-	-	-	-	-	-	-	-	+
18	Ab182	-	-	-	-	-	+	-	-	+
19	Ab45-63	-	-	-	-	-	-	-	-	+
20	Ab45-64	-	-	-	-	-	+	-	-	+
21	Ab45-17703	-	+	-	-	-	+	-	-	+
22	Ab45-162	+	-	-	-	-	-	-	-	+
23	Ab45-164	-	-	-	-	-	+	-	-	+
24	Ab45-17706	+	-	-	-	-	+	-	-	+
25	Ab46-17	-	-	-	-	-	-	-	-	+
26	Ab46-28	-	-	-	-	-	-	-	-	+
27	Ab46-29	+	-	-	-	-	-	-	-	+
28	Ab45-163	+	-	-	-	-	-	-	-	+
29	Ab45-148	-	-	-	-	-	-	-	-	+
30	Ab46-54	+	-	-	-	-	-	-	-	+

+ = positive; - = negative

Table A-3 Raw data of percent absorption at various times to detect carbapenemase activity in 30 strains of *A. baumannii*

No.	*A. baumannii* strains no.	Time (seconds)													
		0	5	10	15	20	25	30	35	40	45	50	55	60	120
1	Ab45-51	0.885	0.877	0.870	0.870	0.864	0.856	0.856	0.850	0.849	0.847	0.848	0.845	0.844	0.840
2	Ab45-52	1.053	1.012	0.992	1.040	1.042	1.031	1.039	1.063	1.043	1.061	1.057	1.069	1.062	1.074
3	Ab45-75	1.954	1.924	1.873	1.856	1.863	1.858	1.789	1.819	1.839	1.830	1.860	1.862	1.797	1.769
4	Ab45-85	0.902	0.889	0.875	0.871	0.854	0.843	0.848	0.847	0.841	0.844	0.845	0.844	0.843	0.835
5	Ab45-111	1.101	1.062	1.050	1.043	1.046	1.034	1.032	1.044	1.035	1.029	1.026	1.021	1.024	1.014
6	Ab45-117	0.668	0.245	0.189	0.139	0.114	0.113	0.119	0.115	0.122	0.128	0.128	0.130	0.130	0.149
7	Ab45-122	0.993	0.988	0.986	0.978	0.973	0.988	0.978	0.967	0.971	0.974	0.979	0.972	0.988	0.988
8	Ab45-127	2.159	1.904	1.805	1.795	1.731	1.678	1.706	1.672	1.669	1.637	1.701	1.633	1.571	1.543
9	Ab45-128	0.694	0.677	0.662	0.661	0.655	0.650	0.647	0.646	0.637	0.637	0.635	0.637	0.634	0.632
10	Ab45-142	0.803	0.814	0.830	0.845	0.866	0.882	0.891	0.914	0.918	0.922	0.925	0.923	0.939	0.944
11	Ab45-170	0.919	0.905	0.887	0.878	0.871	0.854	0.849	0.852	0.848	0.844	0.841	0.839	0.842	0.841
12	Ab46-31	1.783	1.642	1.451	1.257	1.162	1.113	1.076	1.023	0.994	0.966	0.927	0.906	0.897	0.840
13	Ab46-32	1.127	1.121	1.159	1.211	1.237	1.232	1.256	1.262	1.266	1.293	1.298	1.298	1.292	1.326
14	Ab46-33	1.092	0.873	0.795	0.731	0.711	0.686	0.683	0.675	0.673	0.668	0.670	0.673	0.666	0.653
15	Ab46-69	1.137	1.120	1.071	1.042	0.992	0.959	0.943	0.931	0.910	0.904	0.901	0.886	0.886	0.810
16	Ab181	1.556	1.241	1.137	0.968	0.819	0.703	0.651	0.607	0.572	0.558	0.544	0.526	0.514	0.484

Table A-3 (continue) Raw data of percent absorption at various times to detect carbapenemase activity in 30 strains of *A. baumannii*

No.	*A. baumannii* strains no.	Time (seconds)													
		0	5	10	15	20	25	30	35	40	45	50	55	60	120
17	Ab46-47	0.546	0.474	0.457	0.428	0.409	0.397	0.388	0.380	0.375	0.370	0.364	0.359	0.356	0.321
18	Ab182	0.679	0.681	0.700	0.696	0.695	0.683	0.677	0.679	0.676	0.679	0.679	0.673	0.673	0.662
19	Ab45-63	0.988	1.020	0.991	0.941	0.902	0.877	0.854	0.842	0.842	0.835	0.829	0.830	0.837	0.867
20	Ab45-64	0.514	0.441	0.400	0.362	0.337	0.320	0.311	0.297	0.297	0.296	0.295	0.297	0.297	0.316
21	Ab45-17703	0.796	0.691	0.671	0.643	0.611	0.596	0.580	0.566	0.552	0.543	0.534	0.527	0.520	0.503
22	Ab45-162	0.723	0.480	0.434	0.405	0.368	0.341	0.322	0.302	0.293	0.286	0.284	0.281	0.279	0.290
23	Ab45-164	0.944	0.916	0.896	0.870	0.854	0.831	0.820	0.808	0.802	0.782	0.767	0.749	0.743	0.674
24	Ab45-17706	0.604	0.601	0.592	0.590	0.582	0.577	0.570	0.564	0.558	0.558	0.550	0.544	0.541	0.519
25	Ab46-17	0.556	0.536	0.483	0.443	0.409	0.397	0.384	0.371	0.356	0.351	0.343	0.334	0.327	0.289
26	Ab46-28	0.612	0.588	0.599	0.545	0.534	0.504	0.498	0.485	0.477	0.463	0.464	0.464	0.462	0.468
27	Ab46-29	1.173	1.151	1.143	1.144	1.129	1.124	1.127	1.134	1.135	1.118	1.127	1.115	1.113	1.084
28	Ab45-163	0.713	0.655	0.599	0.551	0.523	0.509	0.508	0.502	0.498	0.491	0.494	0.489	0.491	0.505
29	Ab45-148	1.278	1.264	1.268	1.258	1.234	1.232	1.224	1.228	1.214	1.057	1.063	1.047	1.057	1.011
30	Ab46-54	0.949	0.890	0.846	0.827	0.813	0.806	0.795	0.793	0.789	0.787	0.786	0.781	0.773	0.780
31	Control	0.646	0.654	0.657	0.660	0.653	0.649	0.644	0.638	0.642	0.643	0.643	0.640	0.643	0.683

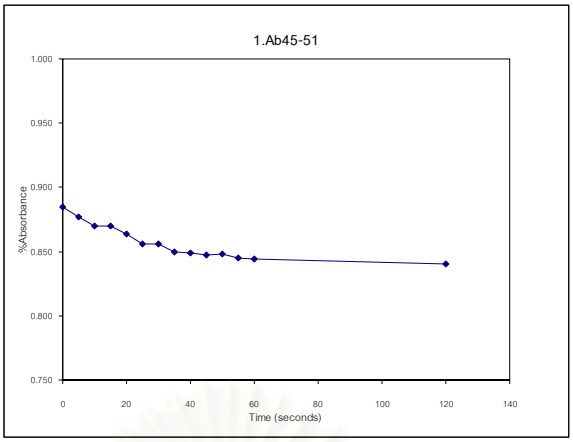

Figure A-1 Percent absorption at various times of *A. baumannii* strain no Ab45-51 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

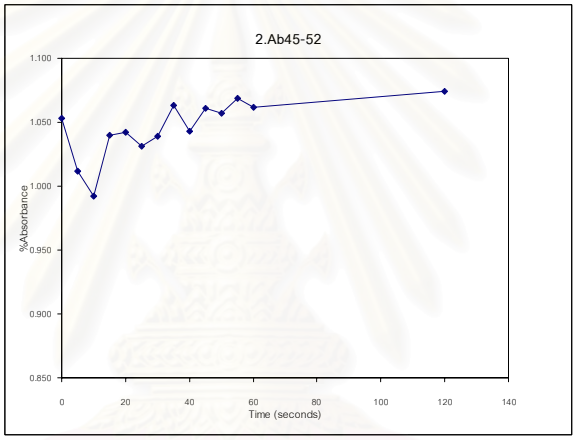

Figure A-2 Percent absorption at various times of *A. baumannii* strain no Ab45-52 with negative carbapenemase activity. (the increase in the percent absorbance at various time)

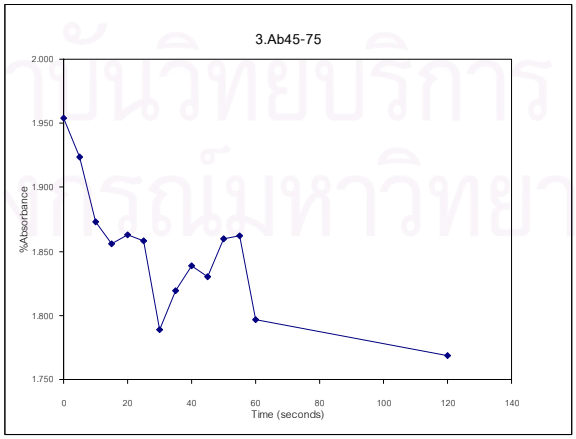

Figure A-3 Percent absorption at various times of *A. baumannii* strain no Ab45-75 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

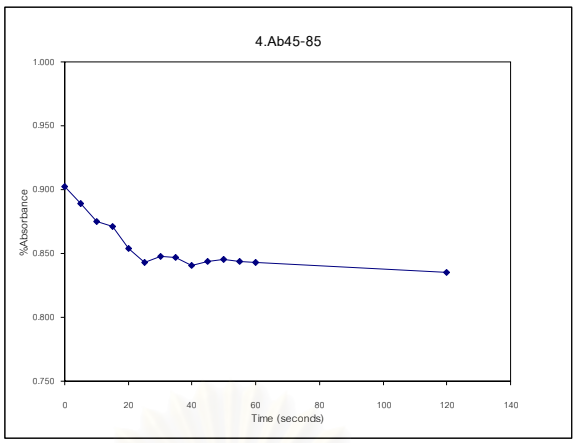

Figure A-4 Percent absorption at various times of *A. baumannii* strain no Ab45-85 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

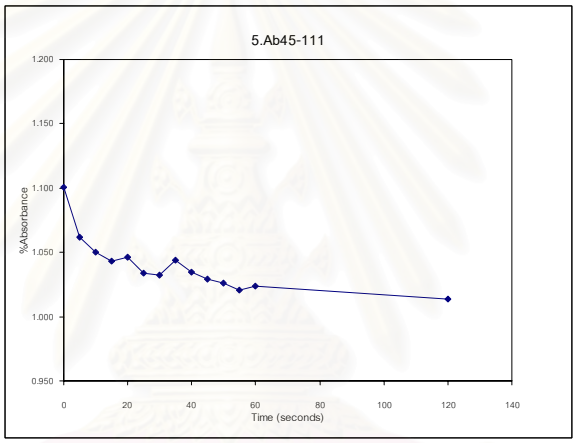

Figure A-5 Percent absorption at various times of *A. baumannii* strain no Ab45-111 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

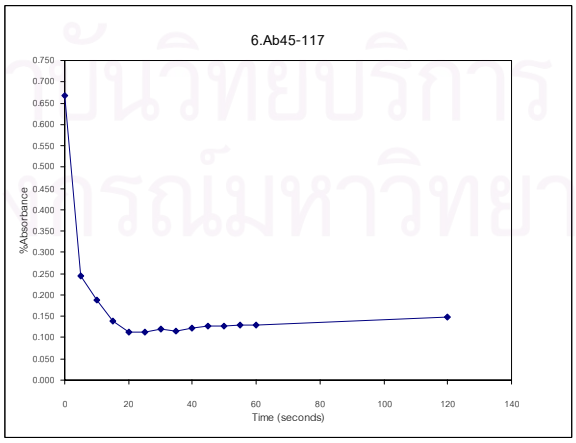

Figure A-6 Percent absorption at various times of *A. baumannii* strain no Ab45-117 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

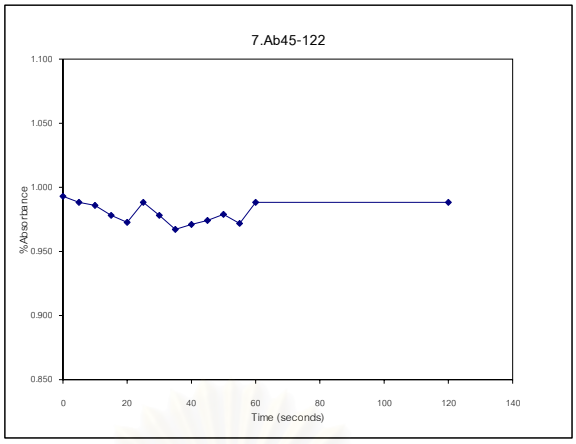

Figure A-7 Percent absorption at various times of *A. baumannii* strain no Ab45-122 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

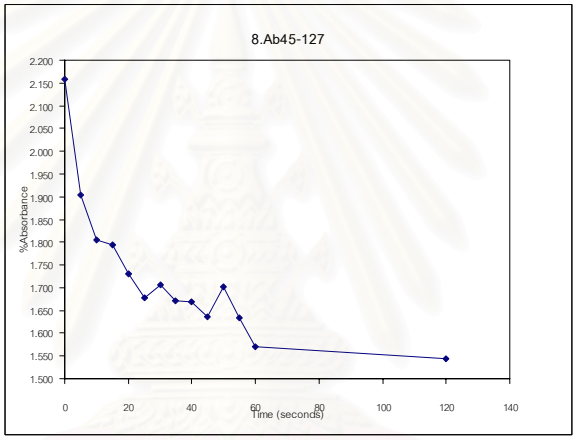

Figure A-8 Percent absorption at various times of *A. baumannii* strain no Ab45-127 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

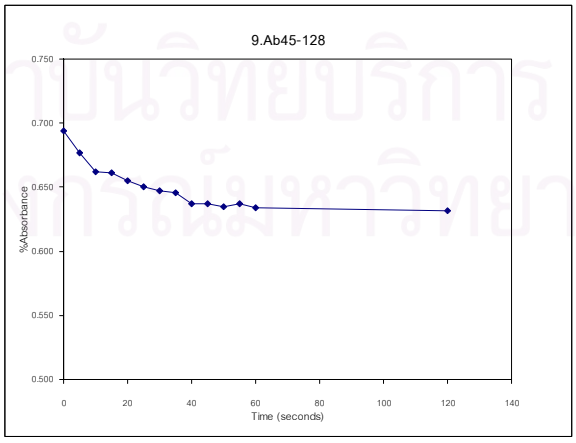

Figure A-9 Percent absorption at various times of *A. baumannii* strain no Ab45-128 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

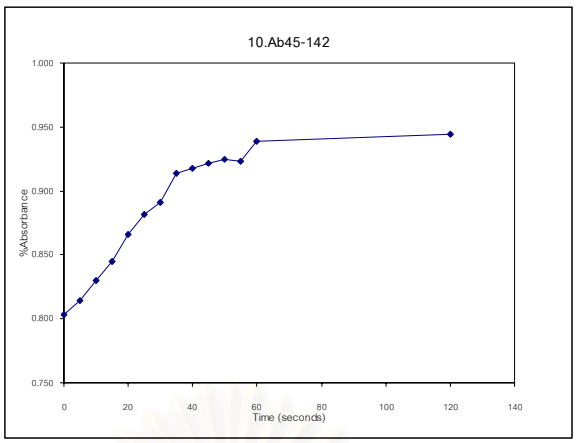

Figure A-10 Percent absorption at various times of *A. baumannii* strain no Ab45-142 with negative carbapenemase activity. (the increase in the percent absorbance at various time)

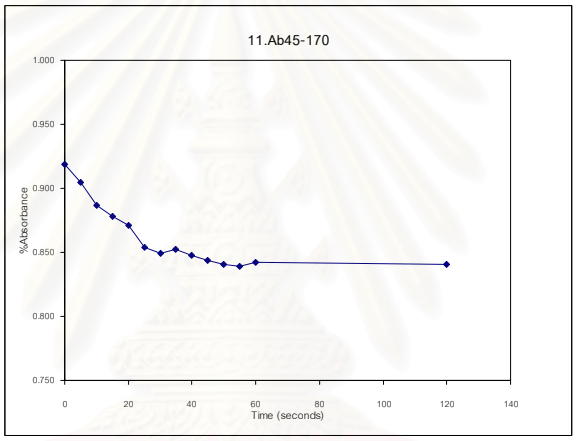

Figure A-11 Percent absorption at various times of *A. baumannii* strain no Ab45-170 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

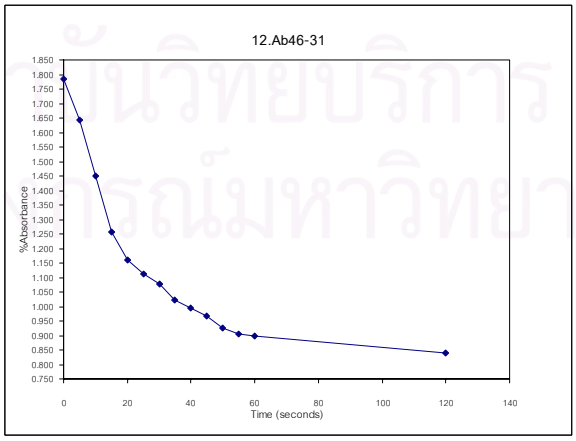

Figure A-12 Percent absorption at various times of *A. baumannii* strain no Ab46-31 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

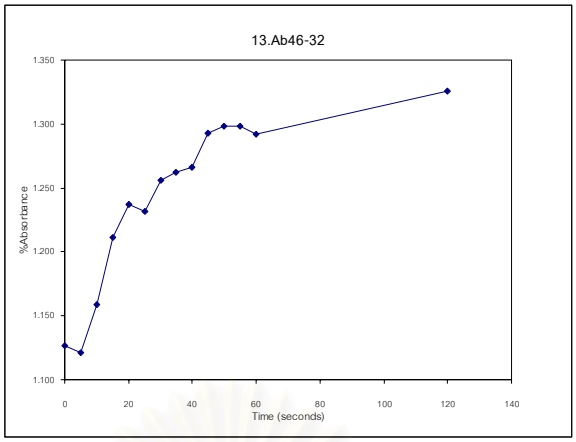

Figure A-13 Percent absorption at various times of *A. baumannii* strain no Ab46-32 with negative carbapenemase activity. (the increase in the percent absorbance at various time)

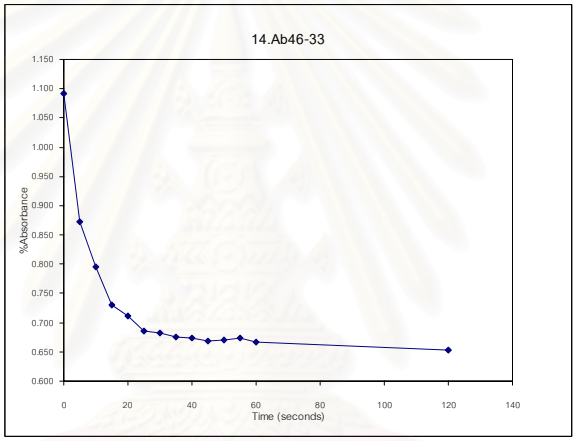

Figure A-14 Percent absorption at various times of *A. baumannii* strain no Ab46-33 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

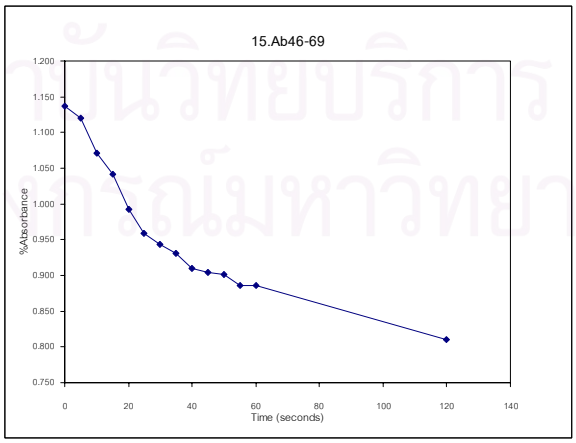

Figure A-15 Percent absorption at various times of *A. baumannii* strain no Ab46-69 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

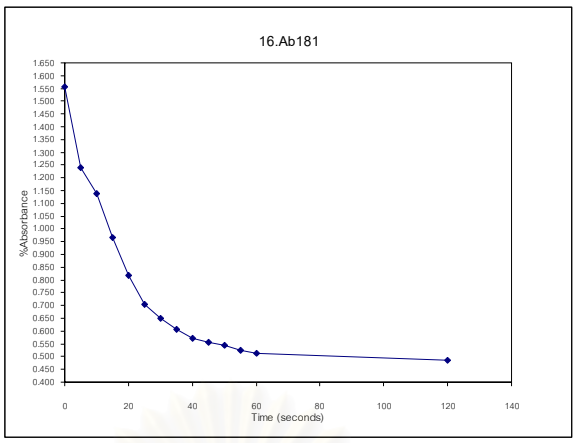

Figure A-16 Percent absorption at various times of *A. baumannii* strain no Ab181 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

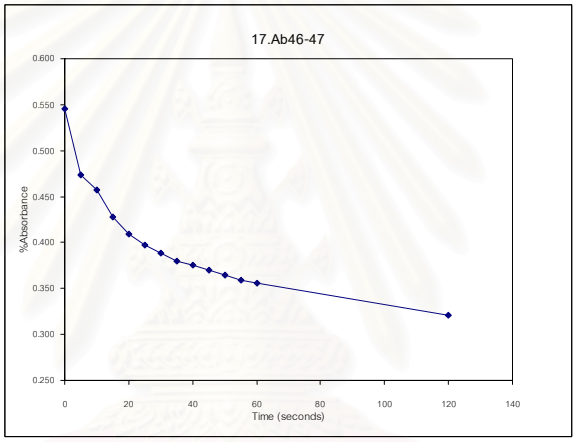

Figure A-17 Percent absorption at various times of *A. baumannii* strain no Ab46-47 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

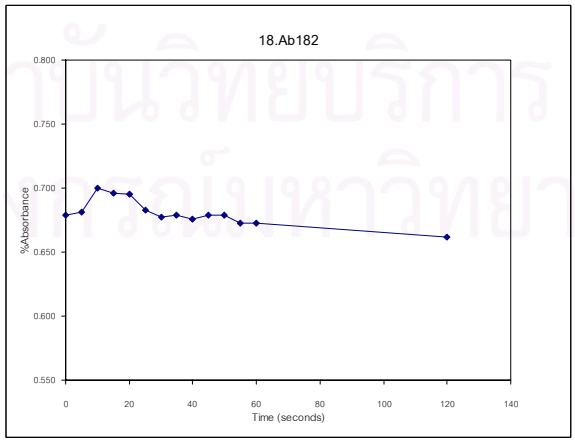

Figure A-18 Percent absorption at various times of *A. baumannii* strain no Ab182 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

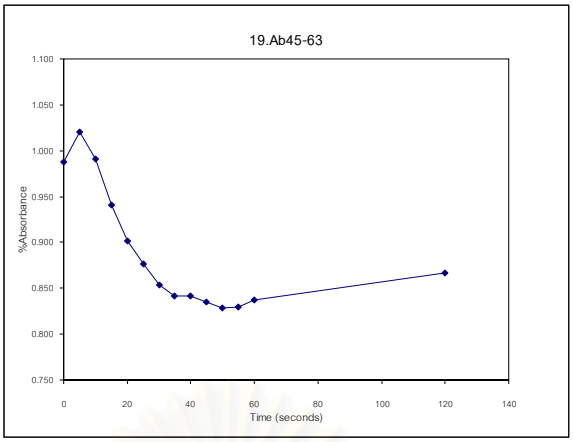

Figure A-19 Percent absorption at various times of *A. baumannii* strain no Ab45-63 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

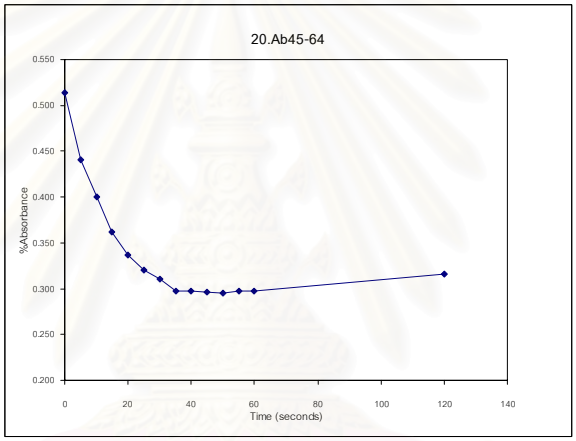

Figure A-20 Percent absorption at various times of *A. baumannii* strain no Ab45-64 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

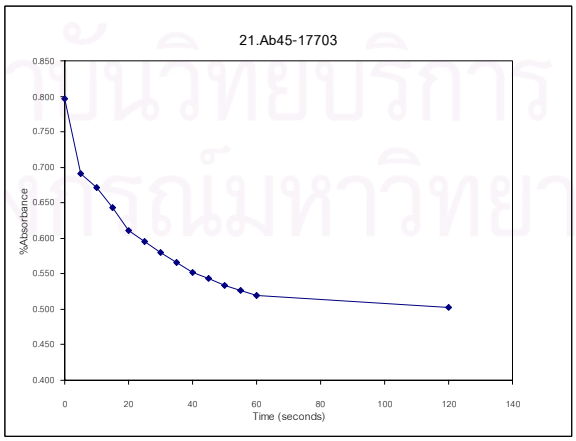

Figure A-21 Percent absorption at various times of *A. baumannii* strain no Ab45-17703 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

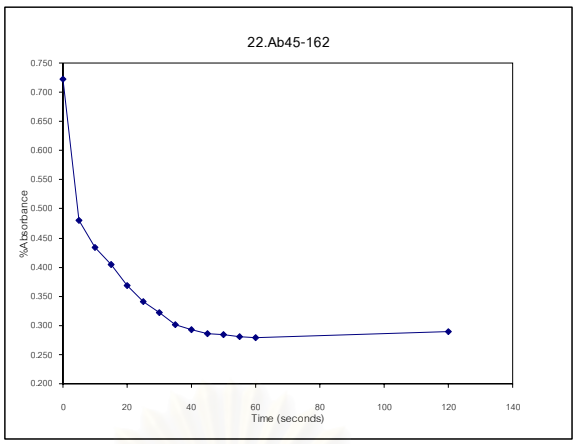

Figure A-22 Percent absorption at various times of *A. baumannii* strain no Ab45-162 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

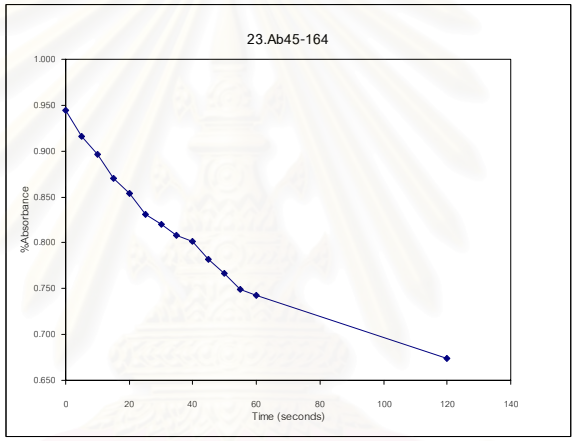

Figure A-23 Percent absorption at various times of *A. baumannii* strain no Ab45-164 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

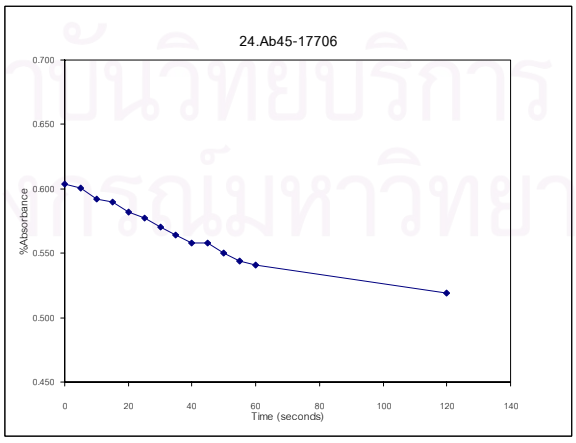

Figure A-24 Percent absorption at various times of *A. baumannii* strain no Ab45-17706 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

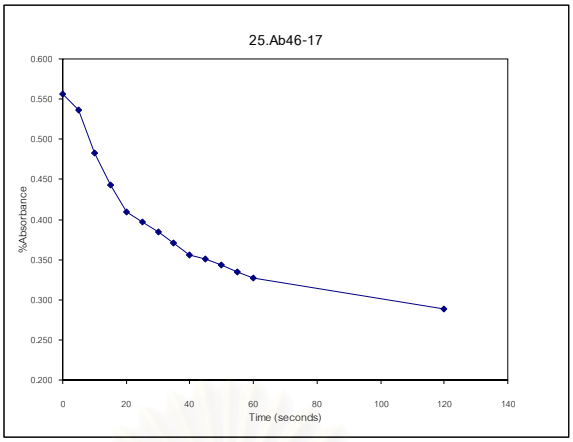

Figure A-25 Percent absorption at various times of *A. baumannii* strain no Ab46-17 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

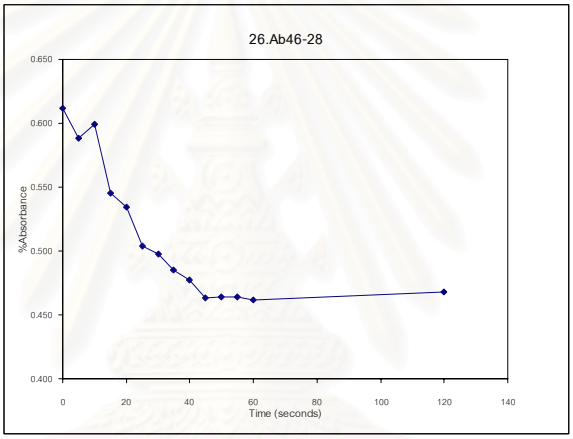

Figure A-26 Percent absorption at various times of *A. baumannii* strain no Ab46-28 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

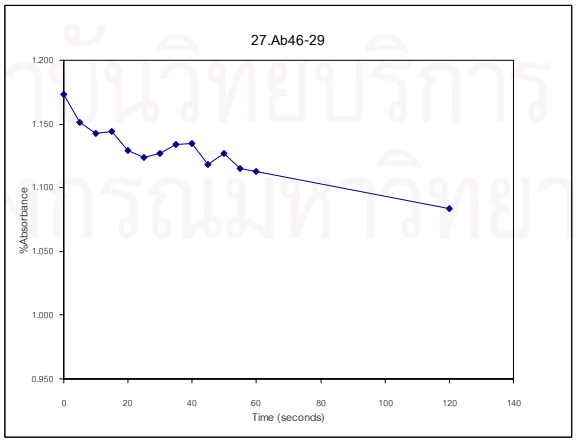

Figure A-27 Percent absorption at various times of *A. baumannii* strain no Ab46-29 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

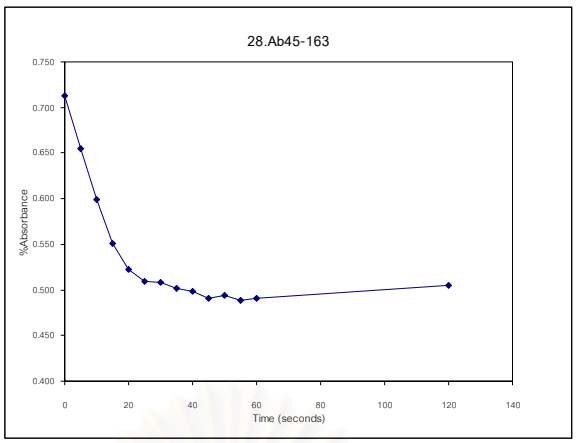

Figure A-28 Percent absorption at various times of *A. baumannii* strain no Ab45-163 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

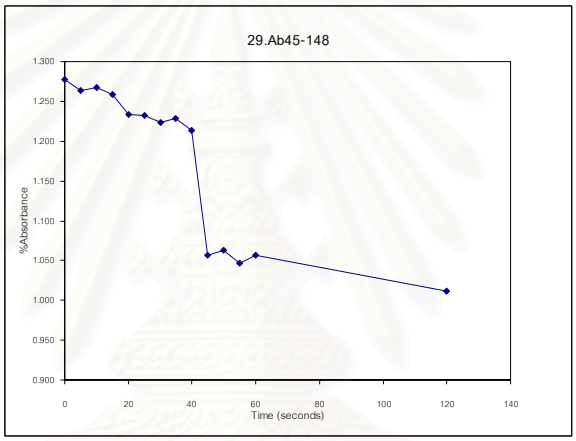

Figure A-29 Percent absorption at various times of *A. baumannii* strain no Ab45-148 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

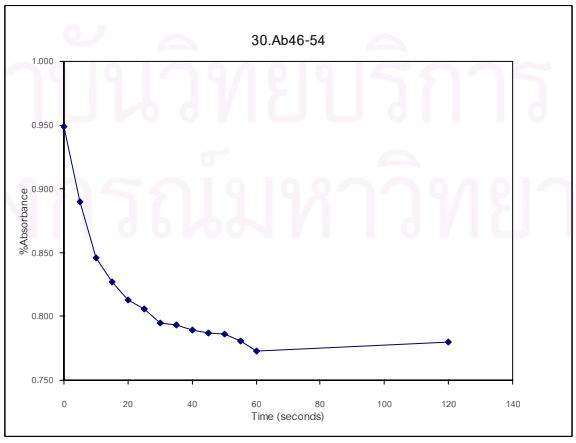

Figure A-30 Percent absorption at various times of *A. baumannii* strain no Ab46-54 with positive carbapenemase activity. (the decrease in the percent absorbance at various time)

Table A-4 Viable cell counts and log viable cell counts at times point in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable count (CFU/ml) at time point							log viable count (log CFU/ml) at time point						
		0	2	4	6	8	10	24	0	2	4	6	8	10	24
1.Ab45-51	1.control	1.02E+06	1.00E+07	6.17E+07	1.22E+08	2.37E+08	2.80E+08	3.80E+11	6.0086	7.0000	7.7903	8.0864	8.3747	8.4472	11.5798
	2.imipenem (MIC = 16 ; R)	1.18E+06	3.38E+04	3.45E+03	1.60E+03	6.00E+02	2.00E+02	1.50E+02	6.0719	4.5289	3.5378	3.2041	2.7782	2.3010	2.1761
	3.amikacin (MIC = 16 ; S)	1.22E+06	1.12E+05	2.22E+04	1.18E+04	8.17E+03	2.48E+03	2.38E+08	6.0864	5.0492	4.3464	4.0719	3.9122	3.3945	8.3766
	4.ciprofloxacin (MIC = 128 ; R)	9.17E+05	1.18E+07	8.00E+07	1.78E+08	2.43E+08	2.82E+08	3.63E+11	5.9624	7.0719	7.9031	8.2504	8.3856	8.4502	11.5599
	5.imipenem+amikacin	9.67E+05	2.35E+03	5.67E+02	2.67E+02	6.67E+01	5.00E+01	5.00E+01	5.9854	3.3711	2.7536	2.4265	1.8241	1.6990	1.6990
	6.imipenem+ciprofloxacin	1.07E+06	3.93E+04	3.02E+03	1.40E+03	6.33E+02	2.50E+02	6.67E+01	6.0294	4.5944	3.4800	3.1461	2.8014	2.3979	1.8241
2.Ab45-52	1.control	9.83E+05	9.83E+06	6.83E+07	1.68E+08	4.67E+08	4.83E+08	9.50E+08	5.9926	6.9926	7.8344	8.2253	8.6693	8.6839	8.9777
	2.imipenem (MIC = 32 ; R)	1.33E+06	1.47E+05	3.05E+04	1.37E+04	1.03E+04	5.83E+03	1.33E+02	6.1239	5.1673	4.4843	4.1367	4.0128	3.7657	2.1239
	3.amikacin (MIC > 256 ; R)	9.67E+05	1.23E+07	8.50E+07	1.12E+08	5.00E+08	6.83E+08	9.50E+08	5.9854	7.0899	7.9294	8.0492	8.6990	8.8344	8.9777
	4.ciprofloxacin (MIC = 64 ; R)	1.15E+06	1.17E+07	1.07E+08	2.03E+08	3.17E+08	4.67E+08	1.78E+09	6.0607	7.0682	8.0294	8.3075	8.5011	8.6693	9.2504
	5.imipenem+amikacin	1.08E+06	9.17E+04	1.52E+04	2.32E+03	9.67E+02	2.83E+02	5.00E+01	6.0334	4.9624	4.1818	3.3655	2.9854	2.4518	1.6990
	6.imipenem+ciprofloxacin	1.20E+06	9.17E+04	1.15E+04	1.97E+03	9.83E+02	4.50E+02	5.00E+01	6.0792	4.9624	4.0607	3.2945	2.9926	2.6532	1.6990
3.Ab45-75	1.control	8.17E+05	1.68E+06	6.50E+07	9.33E+07	1.75E+08	2.00E+08	4.50E+08	5.9122	6.2253	7.8129	7.9699	8.2430	8.3010	8.6532
	2.imipenem (MIC = 16 ; R)	5.33E+05	1.38E+05	1.80E+04	6.17E+03	1.17E+03	4.25E+02	5.00E+01	5.7267	5.1399	4.2553	3.7903	3.0682	2.6284	1.6990
	3.amikacin (MIC = 256 ; R)	4.00E+05	2.33E+06	4.67E+07	1.17E+08	1.37E+08	1.82E+08	7.50E+08	5.6021	6.3674	7.6693	8.0682	8.1367	8.2601	8.8751
	4.ciprofloxacin (MIC = 64 ; R)	6.33E+05	2.25E+06	6.50E+07	1.18E+08	1.40E+08	1.83E+08	1.20E+09	5.8014	6.3522	7.8129	8.0719	8.1461	8.2625	9.0792
	5.imipenem+amikacin	5.17E+05	7.83E+04	8.00E+03	1.10E+03	3.67E+02	7.50E+01	5.00E+01	5.7135	4.8938	3.9031	3.0414	2.5647	1.8751	1.6990
	6.imipenem+ciprofloxacin	7.33E+05	9.50E+04	1.75E+04	2.40E+03	1.05E+03	3.75E+02	5.00E+01	5.8651	4.9777	4.2430	3.3802	3.0212	2.5740	1.6990

Table A-4 (continue) Viable cell counts and log viable cell counts at times point in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable count (CFU/ml) at time point							log viable count (log CFU/ml) at time point						
		0	2	4	6	8	10	24	0	2	4	6	8	10	24
4.Ab45-85	1.control	1.53E+06	1.80E+07	8.83E+07	1.65E+08	2.60E+08	3.50E+08	2.95E+10	6.1847	7.2553	7.9460	8.2175	8.4150	8.5441	10.4698
	2.imipenem (MIC = 32 ; R)	1.40E+06	1.65E+05	8.17E+04	2.67E+04	1.30E+04	2.85E+03	2.38E+06	6.1461	5.2175	4.9122	4.4265	4.1139	3.4548	6.3766
	3.amikacin (MIC = 32 ; I)	1.40E+06	1.27E+05	4.83E+04	3.43E+04	9.17E+05	3.12E+07	1.37E+09	6.1461	5.1038	4.6839	4.5353	5.9624	7.4942	9.1367
	4.ciprofloxacin (MIC = 4 ; R)	1.63E+06	5.67E+06	1.32E+07	1.78E+07	2.30E+07	1.35E+07	9.33E+06	6.2122	6.7536	7.1206	7.2504	7.3617	7.1303	6.9699
	5.imipenem+amikacin	1.35E+06	1.02E+05	2.15E+04	1.30E+03	4.83E+02	5.00E+01	5.00E+01	6.1303	5.0086	4.3324	3.1139	2.6839	1.6990	1.6990
	6.imipenem+ciprofloxacin	1.63E+06	1.52E+05	1.05E+05	3.50E+04	1.60E+04	2.67E+03	2.83E+02	6.2122	5.1818	5.0212	4.5441	4.2041	3.4265	2.4518
5.Ab45-111	1.control	1.25E+06	1.30E+07	1.18E+08	2.38E+08	2.90E+08	4.22E+08	3.76E+12	6.0969	7.1139	8.0719	8.3766	8.4624	8.6253	12.5752
	2.imipenem (MIC = 8 ; I)	1.27E+06	9.67E+04	2.82E+04	1.23E+04	3.30E+03	2.18E+03	1.37E+03	6.1038	4.9854	4.4502	4.0899	3.5185	3.3385	3.1367
	3.amikacin (MIC > 256 ; R)	1.30E+06	8.67E+06	1.10E+08	1.92E+08	2.73E+08	3.23E+08	3.17E+12	6.1139	6.9380	8.0414	8.2833	8.4362	8.5092	12.5011
	4.ciprofloxacin (MIC = 64 ; R)	1.30E+06	1.27E+07	9.50E+07	2.03E+08	2.87E+08	3.35E+08	3.35E+12	6.1139	7.1038	7.9777	8.3075	8.4579	8.5250	12.5250
	5.imipenem+amikacin	1.08E+06	7.50E+04	1.98E+04	8.67E+03	2.23E+03	1.63E+03	1.03E+03	6.0334	4.8751	4.2967	3.9380	3.3483	3.2122	3.0128
	6.imipenem+ciprofloxacin	1.17E+06	8.83E+04	2.47E+04	1.30E+04	3.52E+03	1.95E+03	8.33E+01	6.0682	4.9460	4.3927	4.1139	3.5465	3.2900	1.9206
6.Ab45-117	1.control	1.73E+06	9.67E+06	6.50E+07	1.37E+08	1.62E+08	2.52E+08	6.00E+08	6.2380	6.9854	7.8129	8.1367	8.2095	8.4014	8.7782
	2.imipenem (MIC = 16 ; R)	1.72E+06	1.90E+05	3.22E+04	1.32E+04	2.68E+03	1.32E+03	2.00E+02	6.2355	5.2788	4.5079	4.1206	3.4281	3.1206	2.3010
	3.amikacin (MIC = 32 ; I)	1.63E+06	1.95E+06	1.38E+06	1.27E+07	2.82E+07	8.83E+07	2.97E+08	6.2122	6.2900	6.1399	7.1038	7.4502	7.9460	8.4728
	4.ciprofloxacin (MIC = 64 ; R)	1.72E+06	1.22E+07	8.50E+07	1.47E+08	1.62E+08	1.93E+08	5.17E+08	6.2355	7.0864	7.9294	8.1673	8.2095	8.2856	8.7135
	5.imipenem+amikacin	1.65E+06	9.33E+04	2.08E+04	5.67E+03	1.57E+03	4.00E+02	5.00E+01	6.2175	4.9699	4.3181	3.7536	3.1959	2.6021	1.6990
	6.imipenem+ciprofloxacin	1.65E+06	1.52E+05	3.42E+04	1.37E+04	2.38E+03	9.83E+02	6.67E+01	6.2175	5.1818	4.5340	4.1367	3.3766	2.9926	1.8241

Table A-4 (continue) Viable cell counts and log viable cell counts at times point in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable count (CFU/ml) at time point							log viable count (log CFU/ml) at time point						
		0	2	4	6	8	10	24	0	2	4	6	8	10	24
7.Ab45-122	1.control	3.00E+05	1.93E+06	7.17E+07	1.30E+08	1.85E+08	2.22E+08	1.10E+09	5.4771	6.2856	7.8555	8.1139	8.2672	8.3464	9.0414
	2.imipenem (MIC = 16 ; R)	4.67E+05	1.53E+05	9.00E+04	5.64E+04	4.17E+04	1.77E+04	5.00E+01	5.6693	5.1847	4.9542	4.7513	4.6201	4.2480	1.6990
	3.amikacin (MIC = 256 ; R)	4.00E+05	1.85E+06	5.17E+07	1.13E+08	1.80E+08	2.37E+08	1.47E+09	5.6021	6.2672	7.7135	8.0531	8.2553	8.3747	9.1673
	4.ciprofloxacin (MIC = 64 ; R)	3.83E+05	1.85E+06	2.40E+07	9.83E+07	1.47E+08	1.73E+08	6.50E+08	5.5832	6.2672	7.3802	7.9926	8.1673	8.2380	8.8129
	5.imipenem+amikacin	4.33E+05	4.33E+04	9.33E+03	1.02E+03	4.33E+02	1.83E+02	5.00E+01	5.6365	4.6365	3.9699	3.0086	2.6365	2.2625	1.6990
	6.imipenem+ciprofloxacin	4.50E+05	1.02E+05	5.83E+04	1.87E+04	1.78E+04	1.07E+04	1.00E+02	5.6532	5.0086	4.7657	4.2718	4.2504	4.0294	2.0000
8.Ab45-127	1.control	1.02E+06	1.00E+07	3.13E+07	9.67E+07	1.32E+08	1.98E+08	7.00E+08	6.0086	7.0000	7.4955	7.9854	8.1206	8.2967	8.8451
	2.imipenem (MIC = 16 ; R)	1.02E+06	1.05E+05	2.47E+04	1.02E+04	1.00E+04	9.83E+03	2.28E+06	6.0086	5.0212	4.3927	4.0086	4.0000	3.9926	6.3579
	3.amikacin (MIC > 256 ; R)	1.03E+06	9.00E+06	3.03E+07	1.20E+08	1.62E+08	2.18E+08	8.00E+08	6.0128	6.9542	7.4814	8.0792	8.2095	8.3385	8.9031
	4.ciprofloxacin (MIC = 64 ; R)	9.50E+05	7.83E+06	2.88E+07	1.08E+08	1.37E+08	2.85E+08	9.00E+08	5.9777	6.8938	7.4594	8.0334	8.1367	8.4548	8.9542
	5.imipenem+amikacin	8.33E+05	6.17E+04	1.62E+04	8.50E+03	1.95E+03	1.60E+03	6.67E+01	5.9206	4.7903	4.2095	3.9294	3.2900	3.2041	1.8241
	6.imipenem+ciprofloxacin	9.50E+05	6.50E+04	2.00E+04	1.22E+04	2.78E+03	1.52E+03	5.00E+01	5.9777	4.8129	4.3010	4.0864	3.4440	3.1818	1.6990
9.Ab45-128	1.control	1.00E+06	1.22E+07	8.50E+07	1.38E+08	2.43E+08	2.67E+08	4.12E+10	6.0000	7.0864	7.9294	8.1399	8.3856	8.4265	10.6149
	2.imipenem (MIC = 32 ; R)	1.22E+06	9.83E+04	1.60E+04	7.33E+03	2.18E+03	1.30E+03	2.02E+05	6.0864	4.9926	4.2041	3.8651	3.3385	3.1139	5.3054
	3.amikacin (MIC = 32 ; I)	1.05E+06	2.40E+04	1.10E+04	1.06E+04	1.40E+05	1.03E+07	3.30E+09	6.0212	4.3802	4.0414	4.0253	5.1461	7.0128	9.5185
	4.ciprofloxacin (MIC = 4 ; R)	1.18E+06	1.92E+06	2.60E+06	8.00E+06	1.15E+07	1.12E+07	2.48E+07	6.0719	6.2833	6.4150	6.9031	7.0607	7.0492	7.3945
	5.imipenem+amikacin	1.22E+06	1.25E+04	1.02E+03	4.00E+02	1.00E+02	6.67E+01	5.00E+01	6.0864	4.0969	3.0086	2.6021	2.0000	1.8241	1.6990
	6.imipenem+ciprofloxacin	1.05E+06	5.17E+04	1.53E+04	3.56E+03	2.02E+03	9.67E+02	1.33E+02	6.0212	4.7135	4.1847	3.5514	3.3054	2.9854	2.1239

Table A-4 (continue) Viable cell counts and log viable cell counts at times point in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable count (CFU/ml) at time point							log viable count (log CFU/ml) at time point						
		0	2	4	6	8	10	24	0	2	4	6	8	10	24
10.Ab45-142	1.control	1.40E+06	1.27E+07	8.67E+07	1.58E+08	2.50E+08	3.02E+08	3.60E+11	6.1461	7.1038	7.9380	8.1987	8.3979	8.4800	11.5563
	2.imipenem (MIC = 8 ; I)	1.15E+06	1.88E+05	8.83E+04	3.15E+04	1.60E+04	3.87E+03	4.83E+02	6.0607	5.2742	4.9460	4.4983	4.2041	3.5877	2.6839
	3.amikacin (MIC = 32 ; I)	1.30E+06	2.43E+05	1.22E+05	7.50E+04	2.02E+05	8.83E+06	3.43E+11	6.1139	5.3856	5.0864	4.8751	5.3054	6.9460	11.5353
	4.ciprofloxacin (MIC = 8 ; R)	1.40E+06	9.50E+06	3.48E+07	8.83E+07	1.02E+08	9.67E+07	1.37E+09	6.1461	6.9777	7.5416	7.9460	8.0086	7.9854	9.1367
	5.imipenem+amikacin	1.22E+06	1.30E+05	3.03E+04	2.28E+03	4.33E+02	6.67E+01	5.00E+01	6.0864	5.1139	4.4814	3.3579	2.6365	1.8241	1.6990
	6.imipenem+ciprofloxacin	1.07E+06	2.27E+05	8.83E+04	3.32E+04	1.72E+04	3.35E+03	1.60E+04	6.0294	5.3560	4.9460	4.5211	4.2355	3.5250	4.2041
11.Ab45-170	1.control	1.17E+06	1.65E+07	2.90E+07	2.81E+08	3.12E+08	3.97E+08	3.75E+11	6.0682	7.2175	7.4624	8.4487	8.4942	8.5988	11.5740
	2.imipenem (MIC = 16 ; R)	1.28E+06	1.95E+05	1.08E+05	4.02E+04	2.63E+04	1.67E+04	2.08E+05	6.1072	5.2900	5.0334	4.6042	4.4200	4.2227	5.3181
	3.amikacin (MIC = 32 ; I)	1.63E+06	1.32E+05	8.00E+04	7.33E+04	1.47E+05	2.43E+06	3.00E+11	6.2122	5.1206	4.9031	4.8651	5.1673	6.3856	11.4771
	4.ciprofloxacin (MIC = 64 ; R)	1.52E+06	2.03E+07	3.42E+07	2.60E+08	2.78E+08	3.33E+08	3.67E+11	6.1818	7.3075	7.5340	8.4150	8.4440	8.5224	11.5647
	5.imipenem+amikacin	1.35E+06	9.17E+04	8.00E+04	3.07E+04	1.32E+04	6.83E+03	2.00E+03	6.1303	4.9624	4.9031	4.4871	4.1206	3.8344	3.3010
	6.imipenem+ciprofloxacin	1.28E+06	1.97E+05	9.50E+04	3.48E+04	1.95E+04	3.33E+05	3.20E+06	6.1072	5.2945	4.9777	4.5416	4.2900	5.5224	6.5051
12.Ab46-31	1.control	1.25E+06	1.38E+07	1.02E+08	1.15E+08	1.37E+08	2.07E+08	7.50E+08	6.0969	7.1399	8.0086	8.0607	8.1367	8.3160	8.8751
	2.imipenem (MIC = 16 ; R)	1.07E+06	1.58E+05	8.33E+04	3.30E+04	1.78E+04	1.32E+04	1.17E+02	6.0294	5.1987	4.9206	4.5185	4.2504	4.1206	2.0682
	3.amikacin (MIC > 256 ; R)	1.38E+06	6.50E+06	4.33E+07	1.42E+08	2.68E+08	2.75E+08	9.33E+08	6.1399	6.8129	7.6365	8.1523	8.4281	8.4393	8.9699
	4.ciprofloxacin (MIC = 32 ; R)	1.05E+06	8.17E+06	4.83E+07	1.17E+08	1.82E+08	2.17E+08	8.50E+08	6.0212	6.9122	7.6839	8.0682	8.2601	8.3365	8.9294
	5.imipenem+amikacin	1.10E+06	1.15E+05	7.17E+04	2.85E+04	1.92E+04	1.08E+04	1.50E+02	6.0414	5.0607	4.8555	4.4548	4.2833	4.0334	2.1761
	6.imipenem+ciprofloxacin	1.33E+06	1.63E+05	8.17E+04	3.42E+04	2.55E+04	1.17E+04	3.17E+02	6.1239	5.2122	4.9122	4.5340	4.4065	4.0682	2.5011

Table A-4 (continue) Viable cell counts and log viable cell counts at times point in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable count (CFU/ml) at time point							log viable count (log CFU/ml) at time point						
		0	2	4	6	8	10	24	0	2	4	6	8	10	24
13.Ab46-32	1.control	1.02E+06	1.15E+07	1.03E+08	1.67E+08	2.47E+08	3.40E+08	2.76E+11	6.0086	7.0607	8.0128	8.2227	8.3927	8.5315	11.4409
	2.imipenem (MIC = 8 ; I)	9.17E+05	3.42E+04	8.00E+03	3.67E+03	7.33E+02	6.67E+02	1.45E+03	5.9624	4.5340	3.9031	3.5647	2.8651	2.8241	3.1614
	3.amikacin (MIC = 32 ; I)	7.33E+05	1.33E+05	1.98E+04	1.57E+04	1.80E+05	4.15E+06	2.90E+09	5.8651	5.1239	4.2967	4.1959	5.2553	6.6180	9.4624
	4.ciprofloxacin (MIC = 64 ; R)	9.17E+05	1.40E+07	7.67E+07	1.55E+08	2.72E+08	3.92E+08	3.90E+11	5.9624	7.1461	7.8848	8.1903	8.4346	8.5933	11.5911
	5.imipenem+amikacin	8.00E+05	2.95E+03	5.33E+02	6.67E+01	5.00E+01	5.00E+01	5.00E+01	5.9031	3.4698	2.7267	1.8241	1.6990	1.6990	1.6990
	6.imipenem+ciprofloxacin	1.07E+06	3.42E+04	1.05E+04	2.03E+03	8.83E+02	3.00E+02	5.00E+01	6.0294	4.5340	4.0212	3.3075	2.9460	2.4771	1.6990
14.Ab46-33	1.control	1.22E+06	2.33E+07	1.13E+08	1.75E+08	2.88E+08	1.27E+09	2.28E+09	6.0864	7.3674	8.0531	8.2430	8.4594	9.1038	9.3579
	2.imipenem (MIC = 16 ; R)	1.48E+06	3.00E+04	1.32E+04	9.67E+03	5.50E+03	1.55E+03	7.67E+05	6.1703	4.4771	4.1206	3.9854	3.7404	3.1903	5.8848
	3.amikacin (MIC = 64 ; R)	1.33E+06	2.58E+05	1.00E+06	1.27E+07	1.12E+08	2.52E+08	4.05E+10	6.1239	5.4116	6.0000	7.1038	8.0492	8.4014	10.6075
	4.ciprofloxacin (MIC = 64 ; R)	1.45E+06	1.62E+07	9.00E+07	2.40E+08	2.52E+08	2.99E+08	3.75E+10	6.1614	7.2095	7.9542	8.3802	8.4014	8.4757	10.5740
	5.imipenem+amikacin	1.30E+06	1.45E+04	2.87E+03	2.05E+03	1.55E+03	7.00E+02	1.50E+02	6.1139	4.1614	3.4579	3.3118	3.1903	2.8451	2.1761
	6.imipenem+ciprofloxacin	1.35E+06	3.23E+04	1.00E+04	5.33E+04	2.62E+03	1.83E+03	6.67E+01	6.1303	4.5092	4.0000	4.7267	3.4183	3.2625	1.8241
15.Ab46-69	1.control	1.25E+06	1.15E+07	8.50E+07	1.18E+08	1.70E+08	1.76E+08	3.63E+11	6.0969	7.0607	7.9294	8.0719	8.2304	8.2455	11.5599
	2.imipenem (MIC = 8 ; I)	1.08E+06	9.83E+04	2.50E+04	1.92E+04	9.17E+03	3.27E+03	1.10E+06	6.0334	4.9926	4.3979	4.2833	3.9624	3.5145	6.0414
	3.amikacin (MIC = 64 ; R)	1.05E+06	1.42E+06	2.52E+05	7.17E+05	1.38E+07	1.15E+08	3.20E+11	6.0212	6.1523	5.4014	5.8555	7.1399	8.0607	11.5051
	4.ciprofloxacin (MIC = 64 ; R)	1.27E+06	1.25E+07	6.17E+07	1.07E+08	1.55E+08	1.65E+08	3.37E+11	6.1038	7.0969	7.7903	8.0294	8.1903	8.2175	11.5276
	5.imipenem+amikacin	1.13E+06	1.47E+05	2.97E+04	1.73E+04	9.83E+03	2.77E+03	2.83E+02	6.0531	5.1673	4.4728	4.2380	3.9926	3.4425	2.4518
	6.imipenem+ciprofloxacin	9.33E+05	8.67E+04	2.32E+04	1.72E+04	1.06E+04	2.72E+03	1.33E+02	5.9699	4.9380	4.3655	4.2355	4.0253	3.4346	2.1239

Table A-4 (continue) Viable cell counts and log viable cell counts at times point in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable count (CFU/ml) at time point							log viable count (log CFU/ml) at time point						
		0	2	4	6	8	10	24	0	2	4	6	8	10	24
16.Ab181	1.control	1.40E+06	9.67E+06	7.33E+07	1.98E+08	2.42E+08	2.95E+08	3.98E+10	6.1461	6.9854	7.8651	8.2967	8.3838	8.4698	10.5999
	2.imipenem (MIC = 16 ; R)	1.30E+06	2.03E+05	9.83E+04	3.93E+04	2.31E+04	1.37E+04	2.12E+03	6.1139	5.3075	4.9926	4.5944	4.3636	4.1367	3.3263
	3.amikacin (MIC > 256 ; R)	1.18E+06	7.83E+06	7.33E+07	1.20E+08	1.98E+08	2.45E+08	2.23E+09	6.0719	6.8938	7.8651	8.0792	8.2967	8.3892	9.3483
	4.ciprofloxacin (MIC = 32 ; R)	1.52E+06	1.32E+07	8.83E+07	1.47E+08	2.08E+08	3.10E+08	2.95E+09	6.1818	7.1206	7.9460	8.1673	8.3181	8.4914	9.4698
	5.imipenem+amikacin	1.38E+06	1.45E+05	6.17E+04	3.10E+04	1.77E+04	1.22E+04	1.97E+08	6.1399	5.1614	4.7903	4.4914	4.2480	4.0864	8.2945
	6.imipenem+ciprofloxacin	1.27E+06	2.22E+05	8.83E+04	3.87E+04	2.03E+04	1.52E+04	1.07E+05	6.1038	5.3464	4.9460	4.5877	4.3075	4.1818	5.0294
17.Ab46-47	1.control	1.13E+06	1.27E+07	1.02E+08	1.82E+08	2.37E+08	3.90E+08	3.92E+11	6.0531	7.1038	8.0086	8.2601	8.3747	8.5911	11.5933
	2.imipenem (MIC = 32 ; R)	1.15E+06	2.30E+05	8.67E+04	3.60E+04	2.68E+04	1.55E+04	7.67E+02	6.0607	5.3617	4.9380	4.5563	4.4281	4.1903	2.8848
	3.amikacin (MIC = 32 ; I)	1.30E+06	9.00E+04	3.62E+04	2.95E+04	4.15E+05	1.75E+06	2.42E+11	6.1139	4.9542	4.5587	4.4698	5.6180	6.2430	11.3838
	4.ciprofloxacin (MIC = 8 ; R)	1.13E+06	6.67E+05	2.77E+06	1.38E+07	1.87E+07	1.90E+07	1.18E+09	6.0531	5.8241	6.4425	7.1399	7.2718	7.2788	9.0719
	5.imipenem+amikacin	1.02E+06	6.67E+04	2.63E+04	1.48E+04	2.93E+03	1.35E+03	5.00E+01	6.0086	4.8241	4.4200	4.1703	3.4669	3.1303	1.6990
	6.imipenem+ciprofloxacin	1.30E+06	1.93E+05	9.50E+04	3.33E+04	2.28E+04	1.28E+04	8.50E+03	6.1139	5.2856	4.9777	4.5224	4.3579	4.1072	3.9294
18.Ab182	1.control	1.00E+06	1.52E+07	1.18E+08	2.32E+08	2.68E+08	3.78E+08	4.10E+11	6.0000	7.1818	8.0719	8.3655	8.4281	8.5775	11.6128
	2.imipenem (MIC = 16 ; R)	1.18E+06	1.90E+05	3.67E+04	1.88E+04	1.32E+04	3.73E+03	3.67E+02	6.0719	5.2788	4.5647	4.2742	4.1206	3.5717	2.5647
	3.amikacin (MIC = 32 ; I)	9.33E+05	6.83E+04	2.67E+04	3.48E+04	4.67E+05	1.20E+07	4.05E+11	5.9699	4.8344	4.4265	4.5416	5.6693	7.0792	11.6075
	4.ciprofloxacin (MIC = 8 ; R)	1.02E+06	3.37E+05	6.83E+05	2.08E+06	7.00E+06	7.67E+06	4.08E+10	6.0086	5.5276	5.8344	6.3181	6.8451	6.8848	10.6107
	5.imipenem+amikacin	1.23E+06	2.95E+04	3.35E+03	1.78E+03	1.08E+03	5.00E+02	5.00E+01	6.0899	4.4698	3.5250	3.2504	3.0334	2.6990	1.6990
	6.imipenem+ciprofloxacin	9.83E+05	1.72E+05	3.47E+04	1.87E+04	1.23E+04	3.88E+03	4.67E+02	5.9926	5.2355	4.5403	4.2718	4.0899	3.5888	2.6693

Table A-4 (continue) Viable cell counts and log viable cell counts at times point in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable count (CFU/ml) at time point							log viable count (log CFU/ml) at time point						
		0	2	4	6	8	10	24	0	2	4	6	8	10	24
19.Ab45-63	1.control	1.03E+06	1.05E+07	1.42E+08	1.50E+08	1.58E+08	2.22E+08	3.90E+11	6.0128	7.0212	8.1523	8.1761	8.1987	8.3464	11.5911
	2.imipenem (MIC = 16 ; R)	1.25E+06	1.40E+05	1.00E+05	2.72E+04	1.53E+04	2.97E+03	9.50E+02	6.0969	5.1461	5.0000	4.4346	4.1847	3.4728	2.9777
	3.amikacin (MIC = 16 ; S)	9.33E+05	1.55E+05	1.03E+05	9.33E+04	6.17E+04	2.10E+05	2.73E+10	5.9699	5.1903	5.0128	4.9699	4.7903	5.3222	10.4362
	4.ciprofloxacin (MIC = 64 ; R)	1.17E+06	1.00E+07	8.83E+07	1.55E+08	1.58E+08	2.40E+08	3.57E+11	6.0682	7.0000	7.9460	8.1903	8.1987	8.3802	11.5527
	5.imipenem+amikacin	1.33E+06	1.03E+05	6.83E+04	1.57E+04	2.42E+03	1.23E+03	5.00E+01	6.1239	5.0128	4.8344	4.1959	3.3838	3.0899	1.6990
	6.imipenem+ciprofloxacin	1.13E+06	1.25E+05	8.83E+04	2.23E+04	1.18E+04	2.63E+03	6.33E+02	6.0531	5.0969	4.9460	4.3483	4.0719	3.4200	2.8014
20.Ab45-64	1.control	1.43E+06	1.53E+07	9.00E+07	1.40E+08	1.90E+08	2.45E+08	1.53E+10	6.1553	7.1847	7.9542	8.1461	8.2788	8.3892	10.1847
	2.imipenem (MIC = 16 ; R)	1.33E+06	8.67E+04	7.17E+03	1.45E+03	5.00E+02	2.67E+02	5.00E+01	6.1239	4.9380	3.8555	3.1614	2.6990	2.4265	1.6990
	3.amikacin (MIC = 16 ; S)	1.43E+06	7.33E+05	1.35E+05	3.45E+04	1.36E+04	1.10E+05	1.98E+08	6.1553	5.8651	5.1303	4.5378	4.1335	5.0414	8.2967
	4.ciprofloxacin (MIC = 64 ; R)	1.43E+06	1.30E+07	7.33E+07	1.75E+08	2.08E+08	2.32E+08	1.47E+10	6.1553	7.1139	7.8651	8.2430	8.3181	8.3655	10.1673
	5.imipenem+amikacin	1.32E+06	7.17E+05	7.17E+04	1.63E+03	3.50E+02	5.00E+01	5.00E+01	6.1206	5.8555	4.8555	3.2122	2.5441	1.6990	1.6990
	6.imipenem+ciprofloxacin	1.53E+06	6.33E+04	8.50E+03	9.17E+02	4.00E+02	1.33E+02	9.17E+03	6.1847	4.8014	3.9294	2.9624	2.6021	2.1239	3.9624
21.Ab45-17703	1.control	1.35E+06	1.07E+07	8.50E+07	1.15E+08	1.47E+08	1.82E+08	1.33E+09	6.1303	7.0294	7.9294	8.0607	8.1673	8.2601	9.1239
	2.imipenem (MIC = 16 ; R)	1.42E+06	1.00E+05	1.08E+04	1.10E+03	4.83E+02	1.50E+02	9.50E+03	6.1523	5.0000	4.0334	3.0414	2.6839	2.1761	3.9777
	3.amikacin (MIC = 16 ; S)	1.13E+06	7.83E+05	1.58E+05	8.67E+04	4.50E+05	1.07E+06	7.50E+08	6.0531	5.8938	5.1987	4.9380	5.6532	6.0294	8.8751
	4.ciprofloxacin (MIC = 32 ; R)	1.22E+06	8.33E+06	8.33E+07	1.22E+08	1.38E+08	1.60E+08	1.25E+09	6.0864	6.9206	7.9206	8.0864	8.1399	8.2041	9.0969
	5.imipenem+amikacin	1.23E+06	1.33E+05	1.00E+05	1.27E+03	6.67E+02	2.17E+02	5.00E+01	6.0899	5.1239	5.0000	3.1038	2.8241	2.3365	1.6990
	6.imipenem+ciprofloxacin	1.23E+06	1.15E+05	8.67E+03	8.17E+02	4.33E+02	2.17E+02	5.00E+01	6.0899	5.0607	3.9380	2.9122	2.6365	2.3365	1.6990

Table A-4 (continue) Viable cell counts and log viable cell counts at times point in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable count (CFU/ml) at time point							log viable count (log CFU/ml) at time point						
		0	2	4	6	8	10	24	0	2	4	6	8	10	24
22.Ab45-162	1.control	1.72E+06	1.45E+07	1.02E+08	1.62E+08	2.40E+08	3.40E+08	2.27E+09	6.2355	7.1614	8.0086	8.2095	8.3802	8.5315	9.3560
	2.imipenem (MIC = 16 ; R)	1.52E+06	1.63E+05	7.50E+04	2.33E+04	1.30E+04	2.10E+05	3.03E+08	6.1818	5.2122	4.8751	4.3674	4.1139	5.3222	8.4814
	3.amikacin (MIC = 8 ; S)	1.62E+06	1.02E+06	2.05E+05	8.67E+04	9.83E+04	2.08E+06	1.37E+09	6.2095	6.0086	5.3118	4.9380	4.9926	6.3181	9.1367
	4.ciprofloxacin (MIC = 64 ; R)	1.82E+06	1.76E+07	1.05E+08	1.50E+08	2.18E+08	2.93E+08	3.50E+09	6.2601	7.2455	8.0212	8.1761	8.3385	8.4669	9.5441
	5.imipenem+amikacin	1.83E+06	2.23E+05	4.67E+04	9.17E+03	1.60E+03	7.67E+02	5.00E+01	6.2625	5.3483	4.6693	3.9624	3.2041	2.8848	1.6990
	6.imipenem+ciprofloxacin	1.73E+06	2.45E+05	8.17E+04	9.67E+03	2.42E+04	8.00E+02	4.17E+03	6.2380	5.3892	4.9122	3.9854	4.3838	2.9031	3.6201
23.Ab45-164	1.control	1.42E+06	1.00E+07	8.67E+07	1.42E+08	1.65E+08	2.07E+08	1.40E+09	6.1523	7.0000	7.9380	8.1523	8.2175	8.3160	9.1461
	2.imipenem (MIC = 16 ; R)	1.25E+06	1.37E+05	2.18E+04	5.33E+03	1.17E+03	7.17E+02	1.50E+03	6.0969	5.1367	4.3385	3.7267	3.0682	2.8555	3.1761
	3.amikacin (MIC = 8 ; S)	1.12E+06	1.08E+06	6.83E+05	1.23E+05	7.17E+04	3.67E+05	9.83E+08	6.0492	6.0334	5.8344	5.0899	4.8555	5.5647	8.9926
	4.ciprofloxacin (MIC = 32 ; R)	1.22E+06	1.18E+07	8.33E+07	1.22E+08	1.68E+08	2.32E+08	1.28E+09	6.0864	7.0719	7.9206	8.0864	8.2253	8.3655	9.1072
	5.imipenem+amikacin	1.15E+06	1.32E+05	1.03E+04	1.20E+03	1.50E+02	5.00E+01	5.00E+01	6.0607	5.1206	4.0128	3.0792	2.1761	1.6990	1.6990
	6.imipenem+ciprofloxacin	1.17E+06	1.43E+05	2.06E+04	5.50E+03	1.23E+03	5.50E+02	5.00E+01	6.0682	5.1553	4.3139	3.7404	3.0899	2.7404	1.6990
24.Ab45-17706	1.control	1.68E+06	1.25E+07	8.67E+07	1.28E+08	1.85E+08	2.83E+08	7.67E+08	6.2253	7.0969	7.9380	8.1072	8.2672	8.4518	8.8848
	2.imipenem (MIC = 8 ; I)	1.73E+06	1.52E+05	2.55E+04	6.67E+03	1.33E+03	6.83E+02	5.00E+01	6.2380	5.1818	4.4065	3.8241	3.1239	2.8344	1.6990
	3.amikacin (MIC = 4 ; S)	1.80E+06	1.62E+06	2.62E+05	1.35E+05	9.33E+04	9.00E+05	6.67E+08	6.2553	6.2095	5.4183	5.1303	4.9699	5.9542	8.8241
	4.ciprofloxacin (MIC = 64 ; R)	1.80E+06	1.43E+07	7.50E+07	1.43E+08	1.90E+08	2.37E+08	7.17E+08	6.2553	7.1553	7.8751	8.1553	8.2788	8.3747	8.8555
	5.imipenem+amikacin	1.77E+06	1.65E+05	3.20E+04	1.13E+04	1.12E+03	5.50E+02	6.67E+01	6.2480	5.2175	4.5051	4.0531	3.0492	2.7404	1.8241
	6.imipenem+ciprofloxacin	1.77E+06	1.63E+05	2.58E+04	6.83E+03	1.20E+03	5.83E+02	6.67E+01	6.2480	5.2122	4.4116	3.8344	3.0792	2.7657	1.8241

Table A-4 (continue) Viable cell counts and log viable cell counts at times point in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable count (CFU/ml) at time point							log viable count (log CFU/ml) at time point						
		0	2	4	6	8	10	24	0	2	4	6	8	10	24
25.Ab46-17	1.control	9.00E+05	9.33E+06	8.67E+07	2.37E+08	3.47E+08	8.50E+08	2.73E+12	5.9542	6.9699	7.9380	8.3747	8.5403	8.9294	12.4362
	2.imipenem (MIC = 32 ; R)	1.00E+06	8.17E+04	2.13E+04	1.43E+04	4.22E+05	1.55E+07	3.82E+10	6.0000	4.9122	4.3284	4.1553	5.6253	7.1903	10.5821
	3.amikacin (MIC = 16 ; S)	1.03E+06	3.02E+05	5.83E+04	2.25E+04	1.08E+05	8.67E+05	2.60E+10	6.0128	5.4800	4.7657	4.3522	5.0334	5.9380	10.4150
	4.ciprofloxacin (MIC = 64 ; R)	9.00E+05	9.00E+06	1.03E+08	1.97E+08	3.12E+08	7.83E+08	2.40E+12	5.9542	6.9542	8.0128	8.2945	8.4942	8.8938	12.3802
	5.imipenem+amikacin	1.05E+06	1.60E+05	1.50E+04	2.27E+03	1.03E+03	3.67E+02	5.00E+01	6.0212	5.2041	4.1761	3.3560	3.0128	2.5647	1.6990
	6.imipenem+ciprofloxacin	1.06E+06	7.00E+04	1.57E+04	6.67E+03	7.33E+04	6.17E+06	3.02E+10	6.0253	4.8451	4.1959	3.8241	4.8651	6.7903	10.4800
26.Ab46-28	1.control	1.32E+06	1.10E+07	1.22E+08	2.20E+08	3.63E+08	8.33E+08	2.67E+12	6.1206	7.0414	8.0864	8.3424	8.5599	8.9206	12.4265
	2.imipenem (MIC = 8 ; I)	9.17E+05	1.00E+05	3.25E+04	1.48E+04	3.13E+03	1.02E+03	6.67E+01	5.9624	5.0000	4.5119	4.1703	3.4955	3.0086	1.8241
	3.amikacin (MIC = 8 ; S)	8.67E+05	2.23E+05	8.33E+04	3.20E+04	1.53E+05	2.60E+06	3.67E+10	5.9380	5.3483	4.9206	4.5051	5.1847	6.4150	10.5647
	4.ciprofloxacin (MIC = 64 ; R)	1.07E+06	1.07E+07	1.00E+08	2.43E+08	3.27E+08	8.17E+08	2.35E+12	6.0294	7.0294	8.0000	8.3856	8.5145	8.9122	12.3711
	5.imipenem+amikacin	1.05E+06	9.50E+04	2.83E+04	9.17E+03	1.73E+03	9.00E+02	5.00E+01	6.0212	4.9777	4.4518	3.9624	3.2380	2.9542	1.6990
	6.imipenem+ciprofloxacin	1.12E+06	9.83E+04	3.23E+04	3.82E+04	2.48E+03	1.53E+03	5.00E+01	6.0492	4.9926	4.5092	4.5821	3.3945	3.1847	1.6990
27.Ab46-29	1.control	1.55E+06	1.08E+07	6.50E+07	1.48E+08	1.95E+08	2.83E+08	3.02E+10	6.1903	7.0334	7.8129	8.1703	8.2900	8.4518	10.4800
	2.imipenem (MIC = 8 ; I)	1.62E+06	1.18E+05	2.03E+04	2.95E+03	1.28E+03	6.50E+02	5.00E+01	6.2095	5.0719	4.3075	3.4698	3.1072	2.8129	1.6990
	3.amikacin (MIC = 4 ; S)	1.43E+06	7.67E+05	1.75E+05	9.00E+04	6.33E+04	1.42E+05	2.30E+09	6.1553	5.8848	5.2430	4.9542	4.8014	5.1523	9.3617
	4.ciprofloxacin (MIC = 64 ; R)	1.62E+06	8.83E+06	7.50E+07	1.30E+08	2.08E+08	2.67E+08	2.57E+10	6.2095	6.9460	7.8751	8.1139	8.3181	8.4265	10.4099
	5.imipenem+amikacin	1.50E+06	1.50E+05	2.93E+04	1.25E+04	2.80E+03	1.12E+03	5.00E+01	6.1761	5.1761	4.4669	4.0969	3.4472	3.0492	1.6990
	6.imipenem+ciprofloxacin	1.63E+06	1.22E+05	2.32E+04	2.57E+03	1.03E+03	4.00E+02	2.33E+02	6.2122	5.0864	4.3655	3.4099	3.0128	2.6021	2.3674

Table A-4 (continue) Viable cell counts and log viable cell counts at times point in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable count (CFU/ml) at time point							log viable count (log CFU/ml)						
		0	2	4	6	8	10	24	0	2	4	6	8	10	24
28.Ab45-163	1.control	1.48E+06	1.13E+07	7.33E+07	1.48E+08	2.07E+08	2.97E+08	3.52E+10	6.1703	7.0531	7.8651	8.1703	8.3160	8.4728	10.5465
	2.imipenem (MIC = 16 ; R)	1.27E+06	2.63E+04	6.17E+03	1.48E+03	7.50E+02	2.83E+02	5.00E+01	6.1038	4.4200	3.7903	3.1703	2.8751	2.4518	1.6990
	3.amikacin (MIC = 4 ; S)	1.57E+06	1.10E+06	8.33E+04	1.72E+04	1.17E+04	9.00E+03	2.38E+09	6.1959	6.0414	4.9206	4.2355	4.0682	3.9542	9.3766
	4.ciprofloxacin (MIC = 32 ; R)	1.33E+06	1.42E+07	6.33E+07	1.25E+08	1.95E+08	2.73E+08	3.57E+10	6.1239	7.1523	7.8014	8.0969	8.2900	8.4362	10.5527
	5.imipenem+amikacin	1.45E+06	3.07E+05	1.58E+04	8.33E+03	2.70E+03	1.63E+03	5.00E+01	6.1614	5.4871	4.1987	3.9206	3.4314	3.2122	1.6990
	6.imipenem+ciprofloxacin	1.47E+06	2.35E+04	4.33E+03	1.57E+03	8.33E+02	2.33E+02	5.00E+01	6.1673	4.3711	3.6365	3.1959	2.9206	2.3674	1.6990
29.Ab45-148	1.control	1.15E+06	1.30E+07	6.17E+07	1.43E+08	1.63E+08	2.28E+08	2.20E+10	6.0607	7.1139	7.7903	8.1553	8.2122	8.3579	10.3424
	2.imipenem (MIC = 16 ; R)	1.15E+06	2.07E+05	9.67E+04	2.37E+04	9.00E+03	2.68E+03	1.17E+02	6.0607	5.3160	4.9854	4.3747	3.9542	3.4281	2.0682
	3.amikacin (MIC = 1 ; S)	1.00E+06	5.00E+01	5.00E+01	5.00E+01	5.00E+01	5.00E+01	5.00E+01	6.0000	1.6990	1.6990	1.6990	1.6990	1.6990	1.6990
	4.ciprofloxacin (MIC = 64 ; R)	1.32E+06	1.28E+07	6.50E+07	1.32E+08	1.67E+08	2.38E+08	1.92E+10	6.1206	7.1072	7.8129	8.1206	8.2227	8.3766	10.2833
	5.imipenem+amikacin	8.67E+05	5.00E+01	5.00E+01	5.00E+01	5.00E+01	5.00E+01	5.00E+01	5.9380	1.6990	1.6990	1.6990	1.6990	1.6990	1.6990
	6.imipenem+ciprofloxacin	1.07E+06	2.17E+05	8.83E+04	2.08E+04	9.67E+03	2.45E+03	1.17E+02	6.0294	5.3365	4.9460	4.3181	3.9854	3.3892	2.0682
30.Ab46-54	1.control	9.67E+05	1.25E+07	9.33E+07	1.85E+08	3.20E+08	4.83E+08	2.63E+12	5.9854	7.0969	7.9699	8.2672	8.5051	8.6839	12.4200
	2.imipenem (MIC = 16 ; R)	9.50E+05	7.50E+04	2.82E+04	1.30E+04	7.00E+03	1.97E+03	2.30E+04	5.9777	4.8751	4.4502	4.1139	3.8451	3.2945	4.3617
	3.amikacin (MIC = 8 ; S)	7.67E+05	1.33E+02	5.00E+01	5.00E+01	5.00E+01	5.00E+01	5.00E+01	5.8848	2.1239	1.6990	1.6990	1.6990	1.6990	1.6990
	4.ciprofloxacin (MIC = 64 ; R)	9.67E+05	1.00E+07	1.15E+08	1.83E+08	2.80E+08	7.83E+08	2.45E+12	5.9854	7.0000	8.0607	8.2625	8.4472	8.8938	12.3892
	5.imipenem+amikacin	7.50E+05	5.00E+01	5.00E+01	5.00E+01	5.00E+01	5.00E+01	5.00E+01	5.8751	1.6990	1.6990	1.6990	1.6990	1.6990	1.6990
	6.imipenem+ciprofloxacin	6.50E+05	8.17E+04	2.25E+04	1.08E+04	1.63E+04	2.15E+03	1.00E+05	5.8129	4.9122	4.3522	4.0334	4.2122	3.3324	5.0000

Table A-5 Log change viable cell counts at times point and kinetic parameters in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable log change							T 99.9%	T min	T eradication	AUBKC $_{0\text{-}24}$ (log CFU/ml.h)	BA_{24}
		Δ_2	Δ_4	Δ_6	Δ_8	Δ_{10}	Δ_{24}	Δ max					
1.Ab45-51	1.control	0.9914	1.7817	2.0778	2.3661	2.4386	5.5712	-	ND	ND	ND	217.1471	
	2.imipenem (MIC = 16 ; R)	-1.5430	-2.5341	-2.8678	-3.2937	-3.7709	-3.8958	-3.8958	8	24	ND	67.8108	149.3364
	3.amikacin (MIC = 16 ; S)	-1.0371	-1.7400	-2.0145	-2.1741	-2.6919	2.2902	-2.6919	ND	10	ND	126.6374	90.5098
	4.ciprofloxacin (MIC = 128 ; R)	1.1095	1.9407	2.2881	2.4232	2.4879	5.5975	-	ND	ND	ND	217.7057	-0.5586
	5.imipenem+amikacin	-2.6144	-3.2318	-3.5589	-4.1613	-4.2865	-4.2865	-4.2865	4	10	10	52.2206	164.9266
	6.imipenem+ciprofloxacin	-1.4350	-2.5494	-2.8833	-3.2280	-3.6314	-4.2053	-4.2053	8	24	ND	66.0256	151.1215
2.Ab45-52	1.control	1.0000	1.8419	2.2328	2.6768	2.6914	2.9852	-	ND	ND	ND	201.7514	
	2.imipenem (MIC = 32 ; R)	-0.9565	-1.6396	-1.9871	-2.1110	-2.3582	-4.0000	-4.0000	24	24	ND	86.7185	115.0329
	3.amikacin (MIC > 256 ; R)	1.1045	1.9440	2.0638	2.7135	2.8490	2.9923	-	ND	ND	ND	203.0399	-1.2885
	4.ciprofloxacin(MIC = 64 ; R)	1.0075	1.9687	2.2468	2.4404	2.6086	3.1897	-	ND	ND	ND	203.9804	-2.2290
	5.imipenem+amikacin	-1.0711	-1.8516	-2.6679	-3.0480	-3.5816	-4.3345	-4.3345	8	24	24	68.5308	133.2206
	6.imipenem+ciprofloxacin	-1.1168	-2.0185	-2.7847	-3.0866	-3.4260	-4.3802	-4.3802	8	24	24	69.8178	131.9336
3.Ab45-75	1.control	0.3131	1.9007	2.0577	2.3308	2.3888	2.7410	-	ND	ND	ND	193.3952	
	2.imipenem (MIC = 16 ; R)	-0.5868	-1.4715	-1.9364	-2.6585	-3.0983	-4.0278	-4.0278	10	24	24	71.1539	122.2414
	3.amikacin (MIC = 256 ; R)	0.7653	2.0673	2.4661	2.5347	2.6580	3.2730	-	ND	ND	ND	194.2912	-0.8960
	4.ciprofloxacin (MIC = 64 ; R)	0.5508	2.0115	2.2705	2.3447	2.4610	3.2778	-	ND	ND	ND	196.2215	-2.8263
	5.imipenem+amikacin	-0.8197	-1.8104	-2.6721	-3.1488	-3.8384	-4.0145	-4.0145	8	24	24	61.4126	131.9826
	6.imipenem+ciprofloxacin	-0.8874	-1.6221	-2.4849	-2.8439	-3.2911	-4.1661	-4.1661	10	24	24	69.5945	123.8008

ND =not determined

Table A-5 (continue) Log change viable cell counts at times point and kinetic parameters in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable log change							T 99.9%	T min	T eradication	AUBKC $_{0-24}$ (log CFU/ml.h)	BA_{24}
		Δ_2	Δ_4	Δ_6	Δ_8	Δ_{10}	Δ_{24}	Δ max					
4.Ab45-85	1.control	1.0706	1.7613	2.0328	2.2303	2.3594	4.2851	-	ND	ND	ND	211.4934	
	2.imipenem (MIC = 32 ; R)	-0.9286	-1.2339	-1.7196	-2.0322	-2.6913	0.2304	-2.6913	ND	10	ND	115.7612	95.7321
	3.amikacin (MIC = 32 ; I)	-1.0423	-1.4622	-1.6108	-0.1838	1.3480	2.9906	-1.6108	ND	6	ND	170.6272	40.8661
	4.ciprofloxacin (MIC = 4 ; R)	0.5414	0.9084	1.0382	1.1495	0.9181	0.7577	-	ND	ND	ND	169.0166	42.4767
	5.imipenem+amikacin	-1.1217	-1.7979	-3.0164	-3.4464	-4.4314	-4.4314	-4.4314	6	10	10	61.8927	149.6006
	6.imipenem+ciprofloxacin	-1.0303	-1.1910	-1.6681	-2.0081	-2.7857	-3.7604	-3.7604	24	24	ND	88.6892	122.8041
5.Ab45-111	1.control	1.0170	1.9750	2.2797	2.3655	2.5284	6.4783	-	ND	ND	ND	227.1753	
	2.imipenem (MIC = 8 ; I)	-1.1184	-1.6536	-2.0139	-2.5853	-2.7653	-2.9671	-2.9671	ND	24	ND	88.8567	138.3186
	3.amikacin (MIC > 256 ; R)	0.8241	1.9274	2.1694	2.3222	2.3953	6.3871	-	ND	ND	ND	225.0927	2.0826
	4.ciprofloxacin (MIC = 64 ; R)	0.9899	1.8638	2.1936	2.3439	2.4111	6.4111	-	ND	ND	ND	225.6834	1.4919
	5.imipenem+amikacin	-1.1584	-1.7368	-2.0954	-2.6851	-2.8212	-3.0206	-3.0206	24	24	ND	85.7369	141.4384
	6.imipenem+ciprofloxacin	-1.1222	-1.6755	-1.9542	-2.5216	-2.7782	-4.1475	-4.1475	24	24	ND	79.8313	147.3441
6.Ab45-117	1.control	0.7474	1.5749	1.8987	1.9715	2.1634	2.5401	-	ND	ND	ND	197.1855	
	2.imipenem (MIC = 16 ; R)	-0.9568	-1.7277	-2.1150	-2.8074	-3.1150	-3.9345	-3.9345	10	24	ND	81.9780	115.2075
	3.amikacin (MIC = 32 ; I)	0.0778	-0.0723	0.8916	1.2381	1.7338	2.2606	-0.0723	ND	4	ND	183.0571	14.1284
	4.ciprofloxacin (MIC = 64 ; R)	0.8508	1.6939	1.9318	1.9740	2.0500	2.4780	-	ND	ND	ND	196.2996	0.8858
	5.imipenem+amikacin	-1.2476	-1.8994	-2.4639	-3.0216	-3.6154	-4.5185	-4.5185	8	24	24	71.4016	125.7839
	6.imipenem+ciprofloxacin	-1.0356	-1.6835	-2.0808	-2.8409	-3.2249	-4.3934	-4.3934	10	24	ND	77.3851	119.8003

ND =not determined

Table A-5 (continue) Log change viable cell counts at times point and kinetic parameters in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable log change							T 99.9%	T min	T eradication	AUBKC $_{0-24}$ (log CFU/ml.h)	BA_{24}
		Δ_2	Δ_4	Δ_6	Δ_8	Δ_{10}	Δ_{24}	Δ max					
7.Ab45-122	1.control	0.8084	2.3784	2.6368	2.7901	2.8692	3.5643	-	ND	ND	ND	196.5821	
	2.imipenem (MIC = 16 ; R)	-0.4846	-0.7151	-0.9180	-1.0492	-1.4213	-3.9703	-3.9703	24	24	24	90.5666	106.0155
	3.amikacin (MIC = 256 ; R)	0.6651	2.1114	2.4510	2.6532	2.7727	3.5653	-	ND	ND	ND	197.3493	-0.7672
	4.ciprofloxacin (MIC = 64 ; R)	0.6840	1.7970	2.4094	2.5841	2.6548	3.2297	-	ND	ND	ND	192.7925	3.7896
	5.imipenem+amikacin	-1.0000	-1.6666	-2.6279	-3.0000	-3.3740	-3.9375	-3.9375	8	24	24	64.1318	132.4503
	6.imipenem+ciprofloxacin	-0.6446	-0.8875	-1.3814	-1.4028	-1.6238	-3.6532	-3.6532	24	24	ND	88.4813	108.1007
8.Ab45-127	1.control	0.9914	1.4869	1.9768	2.1120	2.2881	2.8365	-	ND	ND	ND	195.5007	
	2.imipenem (MIC = 16 ; R)	-0.9874	-1.6159	-2.0000	-2.0086	-2.0160	0.3493	-2.0160	ND	10	ND	117.2995	78.2012
	3.amikacin (MIC > 256 ; R)	0.9414	1.4686	2.0663	2.1967	2.3256	2.8903	-	ND	ND	ND	196.4909	-0.9902
	4.ciprofloxacin (MIC = 64 ; R)	0.9160	1.4817	2.0557	2.1590	2.4771	2.9765	-	ND	ND	ND	197.3428	-1.8421
	5.imipenem+amikacin	-1.1304	-1.7111	-1.9912	-2.6306	-2.7165	-4.0965	-4.0965	24	24	ND	76.7610	118.7397
	6.imipenem+ciprofloxacin	-1.1648	-1.6767	-1.8914	-2.5337	-2.7959	-4.2788	-4.2788	24	24	24	76.6140	118.8867
9.Ab45-128	1.control	1.0864	1.9294	2.1399	2.3856	2.4265	4.6149	-	ND	ND	ND	210.7989	
	2.imipenem (MIC = 32 ; R)	-1.0938	-1.8822	-2.2213	-2.7479	-2.9724	-0.7810	-2.9724	ND	10	ND	100.9358	109.8631
	3.amikacin (MIC = 32 ; I)	-1.6410	-1.9798	-1.9959	-0.8751	0.9916	3.4973	-1.9959	ND	6	ND	163.9396	46.8593
	4.ciprofloxacin (MIC = 4 ; R)	0.2114	0.3431	0.8312	0.9888	0.9773	1.3226	-	ND	ND	ND	167.5509	43.2480
	5.imipenem+amikacin	-1.9894	-3.0778	-3.4843	-4.0864	-4.2622	-4.3874	-4.3874	4	24	24	55.9873	154.8116
	6.imipenem+ciprofloxacin	-1.3077	-1.8365	-2.4697	-2.7158	-3.0358	-3.8973	-3.8973	10	24	ND	76.2815	134.5174

ND =not determined

Table A-5 (continue) Log change viable cell counts at times point and kinetic parameters in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable log change							T 99.9%	T min	T eradication	AUBKC $_{0-24}$ (log CFU/ml.h)	BA_{24}
		Δ_2	Δ_4	Δ_6	Δ_8	Δ_{10}	Δ_{24}	Δ max					
10.Ab45-142	1.control	0.9577	1.7919	2.0525	2.2518	2.3339	5.4102	-	ND	ND	ND	218.1571	
	2.imipenem (MIC = 8 ; I)	-0.7865	-1.1147	-1.5624	-1.8566	-2.4730	-3.3768	-3.3768	24	24	ND	91.3951	126.7620
	3.amikacin (MIC = 32 ; I)	-0.7283	-1.0276	-1.2389	-0.8086	0.8320	5.4214	-1.2389	ND	6	ND	183.7334	34.4237
	4.ciprofloxacin (MIC = 8 ; R)	0.8316	1.3955	1.7998	1.8625	1.8393	2.9906	-	ND	ND	ND	194.9343	23.2228
	5.imipenem+amikacin	-0.9724	-1.6049	-2.7284	-3.4499	-4.2622	-4.3874	-4.3874	8	24	24	63.7518	154.4054
	6.imipenem+ciprofloxacin	-0.6734	-1.0834	-1.5082	-1.7939	-2.5043	-1.8253	-2.5043	ND	10	ND	101.7759	116.3813
11.Ab45-170	1.control	1.1493	1.3942	2.3805	2.4260	2.5306	5.5058	-	ND	ND	ND	219.1222	
	2.imipenem (MIC = 16 ; R)	-0.8172	-1.0738	-1.5030	-1.6873	-1.8845	-0.7891	-1.8845	ND	10	ND	115.8107	103.3115
	3.amikacin (MIC = 32 ; I)	-1.0916	-1.3091	-1.3471	-1.0449	0.1734	5.2649	-1.3471	ND	6	ND	177.7491	41.3732
	4.ciprofloxacin (MIC = 64 ; R)	1.1257	1.3522	2.2331	2.2622	2.3406	5.3828	-	ND	ND	ND	218.7151	0.4071
	5.imipenem+amikacin	-1.1680	-1.2272	-1.6432	-2.0098	-2.2959	-2.8293	-2.8293	ND	24	ND	96.8593	122.2630
	6.imipenem+ciprofloxacin	-0.8127	-1.1295	-1.5656	-1.8172	-0.5848	0.3979	-1.8172	ND	8	ND	134.0304	85.0918
12.Ab46-31	1.control	1.0430	1.9117	1.9638	2.0398	2.2191	2.7782	-	ND	ND	ND	197.4419	
	2.imipenem (MIC = 16 ; R)	-0.8307	-1.1087	-1.5109	-1.7790	-1.9088	-3.9612	-3.9612	24	24	ND	91.2477	106.1941
	3.amikacin (MIC > 256 ; R)	0.6730	1.4966	2.0124	2.2883	2.2995	2.8300	-	ND	ND	ND	198.5034	-1.0615
	4.ciprofloxacin (MIC = 32 ; R)	0.8910	1.6628	2.0470	2.2389	2.3153	2.9082	-	ND	ND	ND	197.0677	0.3742
	5.imipenem+amikacin	-0.9807	-1.1859	-1.5865	-1.7581	-2.0080	-3.8653	-3.8653	24	24	ND	90.8501	106.5917
	6.imipenem+ciprofloxacin	-0.9117	-1.2116	-1.5898	-1.7173	-2.0557	-3.6228	-3.6228	24	24	ND	94.3067	103.1352

ND =not determined

Table A-5 (continue) Log change viable cell counts at times point and kinetic parameters in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable log change							T 99.9%	T min	T eradication	AUBKC $_{0-24}$ (log CFU/ml.h)	BA_{24}
		Δ_2	Δ_4	Δ_6	Δ_8	Δ_{10}	Δ_{24}	Δ max					
13.Ab46-32	1.control	1.0521	2.0042	2.2141	2.3841	2.5229	5.4323	-	ND	ND	ND	217.7247	
	2.imipenem (MIC = 8 ; I)	-1.4283	-2.0593	-2.3977	-3.0973	-3.1382	-2.8010	-3.1382	8	10	ND	80.4187	137.3060
	3.amikacin (MIC = 32 ; I)	-0.7413	-1.5684	-1.6692	-0.6098	0.7529	3.5973	-1.6692	ND	6	ND	162.7897	54.9350
	4.ciprofloxacin (MIC = 64 ; R)	1.1838	1.9224	2.2280	2.4722	2.6309	5.6287	-	ND	ND	ND	219.1578	-1.4331
	5.imipenem+amikacin	-2.4333	-3.1764	-4.0790	-4.2041	-4.2041	-4.2041	-4.2041	4	8	8	50.8269	166.8978
	6.imipenem+ciprofloxacin	-1.4954	-2.0082	-2.7219	-3.0834	-3.5523	-4.3304	-4.3304	8	24	24	67.3565	150.3682
14.Ab46-33	1.control	1.2810	1.9667	2.1567	2.3730	3.0174	3.2716	-	ND	ND	ND	208.6681	
	2.imipenem (MIC = 16 ; R)	-1.6931	-2.0497	-2.1848	-2.4299	-2.9799	-0.2855	-2.9799	ND	10	ND	105.5335	103.1346
	3.amikacin (MIC = 64 ; R)	-0.7122	-0.1239	0.9800	1.9254	2.2775	4.4836	-0.1239	ND	2	ND	200.7165	7.9515
	4.ciprofloxacin (MIC = 64 ; R)	1.0481	1.7929	2.2188	2.2400	2.3143	4.4127	-	ND	ND	ND	211.8757	-3.2076
	5.imipenem+amikacin	-1.9526	-2.6561	-2.8022	-2.9236	-3.2688	-3.9379	-3.9379	10	24	ND	72.3500	136.3180
	6.imipenem+ciprofloxacin	-1.6211	-2.1303	-1.4036	-2.7120	-2.8679	-4.3062	-4.3062	24	24	ND	78.3073	130.3608
15.Ab46-69	1.control	0.9638	1.8325	1.9750	2.1335	2.1486	5.4630	-	ND	ND	ND	215.5653	
	2.imipenem (MIC = 8 ; I)	-1.0409	-1.6355	-1.7501	-2.0711	-2.5189	0.0080	-2.5189	ND	10	ND	111.7119	103.8534
	3.amikacin (MIC = 64 ; R)	0.1311	-0.6198	-0.1657	1.1187	2.0395	5.4840	-0.6198	ND	ND	ND	200.1410	15.4243
	4.ciprofloxacin (MIC = 64 ; R)	0.9931	1.6865	1.9256	2.0865	2.1137	5.4238	-	ND	ND	ND	214.7509	0.8143
	5.imipenem+amikacin	-0.8858	-1.5803	-1.8150	-2.0605	-2.6106	-3.6013	-3.6013	24	24	ND	86.4968	129.0685
	6.imipenem+ciprofloxacin	-1.0319	-1.6044	-1.7344	-1.9446	-2.5353	-3.8460	-3.8460	24	24	ND	83.4421	132.1232

ND =not determined

Table A-5 (continue) Log change viable cell counts at times point and kinetic parameters in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable log change							T 99.9%	T min	T eradication	AUBKC $_{0\text{-}24}$ (log CFU/ml.h)	BA_{24}
		Δ_2	Δ_4	Δ_6	Δ_8	Δ_{10}	Δ_{24}	Δ max					
16.Ab181	1.control	0.8393	1.7190	2.1505	2.2377	2.3237	4.4538	-	ND	ND	ND	211.1659	
	2.imipenem (MIC = 16 ; R)	-0.8064	-1.1214	-1.5196	-1.7503	-1.9772	-2.7876	-2.7876	ND	24	ND	101.0082	110.1577
	3.amikacin (MIC > 256 ; R)	0.8219	1.7932	2.0073	2.2248	2.3173	3.2764	-	ND	ND	ND	200.8928	10.2731
	4.ciprofloxacin (MIC = 32 ; R)	0.9387	1.7641	1.9855	2.1362	2.3095	3.2880	-	ND	ND	ND	203.5053	7.6606
	5.imipenem+amikacin	-0.9785	-1.3496	-1.6485	-1.8919	-2.0535	2.1546	-2.0535	ND	10	ND	134.2740	76.8919
	6.imipenem+ciprofloxacin	-0.7575	-1.1578	-1.5161	-1.7963	-1.9220	-1.0744	-1.9220	ND	10	ND	113.1393	98.0266
17.Ab46-47	1.control	1.0507	1.9555	2.2070	2.3217	2.5380	5.5402	-	ND	ND	ND	219.4290	
	2.imipenem (MIC = 32 ; R)	-0.6990	-1.1227	-1.5044	-1.6326	-1.8704	-3.1759	-3.1759	24	24	ND	98.3453	121.0838
	3.amikacin (MIC = 32 ; I)	-1.1597	-1.5552	-1.6441	-0.4959	0.1291	5.2699	-1.6441	ND	6	ND	174.9466	44.4824
	4.ciprofloxacin (MIC = 8 ; R)	-0.2290	0.3894	1.0868	1.2188	1.2257	3.0188	-0.2290	ND	2	ND	181.1429	38.2861
	5.imipenem+amikacin	-1.1845	-1.5886	-1.8383	-2.5417	-2.8783	-4.3096	-4.3096	24	24	24	76.7065	142.7226
	6.imipenem+ciprofloxacin	-0.8284	-1.1362	-1.5915	-1.7560	-2.0067	-2.1845	-2.1845	ND	24	ND	104.7649	114.6642
18.Ab182	1.control	1.1818	2.0719	2.3655	2.4281	2.5775	5.6128	-	ND	ND	ND	220.0041	
	2.imipenem (MIC = 16 ; R)	-0.7931	-1.5072	-1.7977	-1.9513	-2.5002	-3.5072	-3.5072	24	24	ND	89.0745	130.9296
	3.amikacin (MIC = 32 ; I)	-1.1355	-1.5434	-1.4283	-0.3006	1.1093	5.6376	-1.5434	ND	4	ND	182.7992	37.2049
	4.ciprofloxacin (MIC = 8 ; R)	-0.4810	-0.1742	0.3095	0.8365	0.8762	4.6021	-0.4810	ND	2	ND	184.4120	35.5921
	5.imipenem+amikacin	-1.6201	-2.5649	-2.8395	-3.0565	-3.3909	-4.3909	-4.3909	8	24	24	68.1319	151.8722
	6.imipenem+ciprofloxacin	-0.7570	-1.4522	-1.7207	-1.9026	-2.4037	-3.3232	-3.3232	24	24	ND	89.6636	130.3405

ND =not determined

Table A-5 (continue) Log change viable cell counts at times point and kinetic parameters in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable log change							T 99.9%	T min	T eradication	AUBKC $_{0\text{-}24}$ (log CFU/ml.h)	BA_{24}
		Δ_2	Δ_4	Δ_6	Δ_8	Δ_{10}	Δ_{24}	Δ max					
19.Ab45-63	1.control	1.0084	2.1395	2.1633	2.1858	2.3335	5.5782	-	ND	ND	ND	217.0176	
	2.imipenem (MIC = 16 ; R)	-0.9508	-1.0969	-1.6623	-1.9122	-2.6242	-3.1192	-3.1192	24	24	ND	92.2538	124.7638
	3.amikacin (MIC = 16 ; S)	-0.7795	-0.9570	-1.0000	-1.1796	-0.6477	4.4663	-1.1796	ND	8	ND	161.5274	55.4901
	4.ciprofloxacin (MIC = 64 ; R)	0.9318	1.8778	2.1221	2.1305	2.3120	5.4845	-	ND	ND	ND	216.6485	0.3691
	5.imipenem+amikacin	-1.1110	-1.2894	-1.9280	-2.7400	-3.0339	-4.4249	-4.4249	10	24	24	77.5898	139.4277
	6.imipenem+ciprofloxacin	-0.9562	-1.1071	-1.7048	-1.9812	-2.6331	-3.2517	-3.2517	24	24	ND	89.9487	127.0689
20.Ab45-64	1.control	1.0294	1.7989	1.9908	2.1234	2.2338	4.0294	-	ND	ND	ND	207.6891	
	2.imipenem (MIC = 16 ; R)	-1.1858	-2.2683	-2.9625	-3.4249	-3.6973	-4.4249	-4.4249	8	24	24	66.7365	140.9527
	3.amikacin (MIC = 16 ; S)	-0.2902	-1.0250	-1.6175	-2.0218	-1.1139	2.1413	-2.0218	ND	8	ND	143.8967	63.7924
	4.ciprofloxacin (MIC = 64 ; R)	0.9586	1.7098	2.0877	2.1627	2.2102	4.0120	-	ND	ND	ND	207.3308	0.3584
	5.imipenem+amikacin	-0.2651	-1.2651	-2.9084	-3.5765	-4.4216	-4.4216	-4.4216	8	10	10	64.5397	143.1494
	6.imipenem+ciprofloxacin	-1.3833	-2.2553	-3.2223	-3.5826	-4.0608	-2.2223	-4.0608	6	10	ND	79.5026	128.1865
21.Ab45-17703	1.control	0.8991	1.7991	1.9304	2.0370	2.1297	2.9935	-	ND	ND	ND	198.4515	
	2.imipenem (MIC = 16 ; R)	-1.1523	-2.1189	-3.1109	-3.4683	-3.9762	-2.1746	-3.9762	6	10	ND	80.9226	117.5289
	3.amikacin (MIC = 16 ; S)	-0.1593	-0.8544	-1.1151	-0.3999	-0.0237	2.8220	-1.1151	ND	6	ND	159.7809	38.6706
	4.ciprofloxacin (MIC = 32 ; R)	0.8343	1.8343	2.0000	2.0535	2.1178	3.0106	-	ND	ND	ND	197.5327	0.9188
	5.imipenem+amikacin	-0.9661	-1.0899	-2.9861	-3.2658	-3.7534	-4.3909	-4.3909	8	24	24	68.7779	129.6736
	6.imipenem+ciprofloxacin	-1.0292	-2.1519	-3.1777	-3.4534	-3.7534	-4.3909	-4.3909	6	24	24	65.7692	132.6823

ND =not determined

Table A-5 (continue) Log change viable cell counts at times point and kinetic parameters in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable log change							T 99.9%	T min	T eradication	AUBKC $_{0\text{-}24}$ (log CFU/ml.h)	BA_{24}
		Δ_2	Δ_4	Δ_6	Δ_8	Δ_{10}	Δ_{24}	Δ max					
22.Ab45-162	1.control	0.9258	1.7731	1.9740	2.1447	2.2960	3.1205	-	ND	ND	ND	203.4989	
	2.imipenem (MIC = 16 ; R)	-0.9697	-1.3068	-1.8145	-2.0679	-0.8596	2.2996	-2.0679	ND	8	ND	145.2668	58.2321
	3.amikacin (MIC = 8 ; S)	-0.2009	-0.8978	-1.2715	-1.2170	0.1085	2.9272	-1.2715	ND	6	ND	163.2129	40.2860
	4.ciprofloxacin (MIC = 64 ; R)	0.9854	1.7611	1.9160	2.0784	2.2068	3.2840	-	ND	ND	ND	204.3660	-0.8671
	5.imipenem+amikacin	-0.9141	-1.5931	-2.3001	-3.0583	-3.3777	-4.5635	-4.5635	8	24	24	75.6018	127.8971
	6.imipenem+ciprofloxacin	-0.8489	-1.3258	-2.2526	-1.8542	-3.3350	-2.6179	-3.3350	10	10	ND	92.1450	111.3540
23.Ab45-164	1.control	0.8477	1.7857	2.0000	2.0652	2.1637	2.9938	-	ND	ND	ND	199.3185	
	2.imipenem (MIC = 16 ; R)	-0.9602	-1.7585	-2.3702	-3.0287	-3.2414	-2.9208	-3.2414	8	10	ND	83.7139	115.6046
	3.amikacin (MIC = 8 ; S)	-0.0158	-0.2148	-0.9593	-1.1937	-0.4846	2.9433	-1.1937	ND	8	ND	157.1410	42.1776
	4.ciprofloxacin (MIC = 32 ; R)	0.9855	1.8343	2.0000	2.1389	2.2791	3.0209	-	ND	ND	ND	199.3691	-0.0506
	5.imipenem+amikacin	-0.9401	-2.0479	-2.9815	-3.8846	-4.3617	-4.3617	-4.3617	8	10	10	60.3226	138.9959
	6.imipenem+ciprofloxacin	-0.9128	-1.7543	-2.3278	-2.9783	-3.3278	-4.3692	-4.3692	10	24	24	72.4828	126.8357
24.Ab45-17706	1.control	0.8716	1.7127	1.8819	2.0419	2.2265	2.6595	-	ND	ND	ND	198.8518	
	2.imipenem (MIC = 8 ; I)	-1.0562	-1.8315	-2.4139	-3.1142	-3.4036	-4.5391	-4.5391	8	24	24	73.8789	124.9729
	3.amikacin (MIC = 4 ; S)	-0.0458	-0.8370	-1.1249	-1.2854	-0.3010	2.5689	-1.2854	ND	8	ND	159.1142	39.7376
	4.ciprofloxacin (MIC = 64 ; R)	0.9001	1.6198	1.9001	2.0235	2.1195	2.6002	-	ND	ND	ND	198.1709	0.6809
	5.imipenem+amikacin	-1.0305	-1.7428	-2.1949	-3.1988	-3.5076	-4.4238	-4.4238	8	24	ND	74.5896	124.2622
	6.imipenem+ciprofloxacin	-1.0358	-1.8364	-2.4136	-3.1688	-3.4823	-4.4238	-4.4238	8	24	ND	74.2170	124.6348

ND =not determined

Table A-5 (continue) Log change viable cell counts at times point and kinetic parameters in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable log change							T 99.9%	T min	T eradication	AUBKC $_{0\text{-}24}$ (log CFU/ml.h)	BA_{24}
		Δ_2	Δ_4	Δ_6	Δ_8	Δ_{10}	Δ_{24}	Δ max					
25.Ab46-17	1.control	1.0156	1.9838	2.4205	2.5861	2.9752	6.4819	-	ND	ND	ND	228.0887	
	2.imipenem (MIC = 32 ; R)	-1.0878	-1.6716	-1.8447	-0.3747	1.1903	4.5821	-1.8447	ND	6	ND	175.6396	52.4491
	3.amikacin (MIC = 16 ; S)	-0.5328	-1.2472	-1.6607	-0.9794	-0.0748	4.4021	-1.6607	ND	6	ND	165.6844	62.4043
	4.ciprofloxacin (MIC = 64 ; R)	1.0000	2.0586	2.3402	2.5399	2.9395	6.4260	-	ND	ND	ND	227.2772	0.8115
	5.imipenem+amikacin	-0.8171	-1.8451	-2.6652	-3.0084	-3.4565	-4.3222	-4.3222	8	24	24	69.9295	158.1592
	6.imipenem+ciprofloxacin	-1.1802	-1.8294	-2.2012	-1.1602	0.7650	4.4547	-2.2012	ND	6	ND	169.1681	58.9206
26.Ab46-28	1.control	0.9208	1.9658	2.2218	2.4393	2.8001	6.3059	-	ND	ND	ND	228.5315	
	2.imipenem (MIC = 8 ; I)	-0.9624	-1.4505	-1.7921	-2.4668	-2.9538	-4.1382	-4.1382	24	24	ND	77.1554	151.3760
	3.amikacin (MIC = 8 ; S)	-0.5897	-1.0174	-1.4329	-0.7533	0.4770	4.6266	-1.4329	ND	6	ND	171.1281	57.4034
	4.ciprofloxacin (MIC = 64 ; R)	1.0000	1.9706	2.3562	2.4852	2.8828	6.3417	-	ND	ND	ND	227.7837	0.7478
	5.imipenem+amikacin	-1.0435	-1.5694	-2.0588	-2.7831	-3.0669	-4.3222	-4.3222	10	24	24	74.8078	153.7237
	6.imipenem+ciprofloxacin	-1.0567	-1.5400	-1.4672	-2.6548	-2.8645	-4.3502	-4.3502	24	24	24	78.3761	150.1554
27.Ab46-29	1.control	0.8431	1.6226	1.9799	2.0997	2.2615	4.2897	-	ND	ND	ND	209.7779	
	2.imipenem (MIC = 8 ; I)	-1.1376	-1.9020	-2.7397	-3.1023	-3.3966	-4.5105	-4.5105	8	24	24	72.5184	137.2595
	3.amikacin (MIC = 4 ; S)	-0.2705	-0.9123	-1.2011	-1.3539	-1.0030	3.2064	-1.3539	ND	8	ND	154.6727	55.1052
	4.ciprofloxacin (MIC = 64 ; R)	0.7364	1.6655	1.9044	2.1085	2.2170	4.2004	-	ND	ND	ND	208.9972	0.7807
	5.imipenem+amikacin	-1.0000	-1.7092	-2.0792	-2.7289	-3.1269	-4.4771	-4.4771	10	24	24	76.8367	132.9413
	6.imipenem+ciprofloxacin	-1.1258	-1.8467	-2.8023	-3.1994	-3.6101	-3.8448	-3.8448	8	24	24	75.3494	134.4285

ND =not determined

Table A-5 (continue) Log change viable cell counts at times point and kinetic parameters in 30 strains of *A. baumannii*

Strain No.	Antimicrobial agents	viable log change							T 99.9%	T min	T eradication	AUBKC $_{0-24}$ (log CFU/ml.h)	BA_{24}
		Δ_2	Δ_4	Δ_6	Δ_8	Δ_{10}	Δ_{24}	Δ max					
28.Ab45-163	1.control	0.8828	1.6948	2.0000	2.1457	2.3025	4.3763	-	ND	ND	ND	210.5869	
	2.imipenem (MIC = 16 ; R)	-1.6838	-2.3135	-2.9335	-3.2287	-3.6520	-4.4048	-4.4048	8	24	24	66.1220	144.4649
	3.amikacin (MIC = 4 ; S)	-0.1545	-1.2753	-1.9604	-2.1277	-2.2417	3.1807	-2.2417	ND	10	ND	141.9974	68.5896
	4.ciprofloxacin (MIC = 32 ; R)	1.0284	1.6776	1.9731	2.1662	2.3123	4.4288	-	ND	ND	ND	210.1631	0.4238
	5.imipenem+amikacin	-0.6742	-1.9627	-2.2407	-2.7300	-2.9492	-4.4624	-4.4624	24	24	24	77.8273	132.7597
	6.imipenem+ciprofloxacin	-1.7962	-2.5308	-2.9714	-3.2467	-3.8000	-4.4683	-4.4683	8	24	24	65.2472	145.3398
29.Ab45-148	1.control	1.0532	1.7296	2.0946	2.1515	2.2972	4.2817	-	ND	ND	ND	207.8646	
	2.imipenem (MIC = 16 ; R)	-0.7447	-1.0753	-1.6859	-2.1065	-2.6326	-3.9925	-3.9925	24	24	ND	85.2239	122.6408
	3.amikacin (MIC = 1 ; S)	-4.3010	-4.3010	-4.3010	-4.3010	-4.3010	-4.3010	-4.3010	2	2	2	45.0763	162.7883
	4.ciprofloxacin (MIC = 64 ; R)	0.9866	1.6923	2.0000	2.1021	2.2560	4.1627	-	ND	ND	ND	207.6431	0.2215
	5.imipenem+amikacin	-4.2390	-4.2390	-4.2390	-4.2390	-4.2390	-4.2390	-4.2390	2	2	2	45.0143	162.8503
	6.imipenem+ciprofloxacin	-0.6929	-1.0834	-1.7113	-2.0440	-2.6402	-3.9612	-3.9612	24	24	ND	84.7918	123.0728
30.Ab46-54	1.control	1.1115	1.9845	2.2817	2.5197	2.6985	6.4345	-	ND	ND	ND	226.0749	
	2.imipenem (MIC = 16 ; R)	-1.1027	-1.5275	-1.8638	-2.1326	-2.6833	-1.6160	-2.6833	ND	10	ND	97.4343	128.6407
	3.amikacin (MIC = 8 ; S)	-3.7609	-4.1858	-4.1858	-4.1858	-4.1858	-4.1858	-4.1858	2	4	4	45.8109	180.2641
	4.ciprofloxacin (MIC = 64 ; R)	1.0146	2.0753	2.2770	2.4617	2.9083	6.4037	-	ND	ND	ND	227.4003	-1.3254
	5.imipenem+amikacin	-4.1761	-4.1761	-4.1761	-4.1761	-4.1761	-4.1761	-4.1761	2	2	2	44.9514	181.1235
	6.imipenem+ciprofloxacin	-0.9007	-1.4607	-1.7795	-1.6007	-2.4805	-0.8129	-2.4805	ND	10	ND	102.4925	123.5825

ND =not determine

Table A-6 Combination activity in 30 strains of *A. baumannii*

No.	*A. baumannii* strains No.	Activity of Combination	
		Imipenem combination with amikacin	Imipenem combination with ciprofloxacin
1	Ab45-51	Indifference	Indifference
2	Ab45-52	Indifference	Indifference
3	Ab45-75	Indifference	Indifference
4	Ab45-85	Addition	Addition
5	Ab45-111	Indifference	Addition
6	Ab45-117	Indifference	Indifference
7	Ab45-122	Indifference	Indifference
8	Ab45-127	Synergy	Synergy
9	Ab45-128	Addition	Indifference
10	Ab45-142	Indifference	Indifference
11	Ab45-170	Indifference	Antagonism
12	Ab46-31	Indifference	Indifference
13	Ab46-32	Addition	Addition
14	Ab46-33	Addition	Addition
15	Ab46-69	Addition	Addition
16	Ab181	Antagonism	Indifference
17	Ab46-47	Addition	Indifference
18	Ab182	Indifference	Indifference
19	Ab45-63	Addition	Indifference
20	Ab45-64	Indifference	Antagonism
21	Ab45-17703	Indifference	Indifference
22	Ab45-162	Synergy	Indifference
23	Ab45-164	Addition	Addition
24	Ab45-17706	Indifference	Indifference
25	Ab46-17	Synergy	Indifference
26	Ab46-28	Indifference	Indifference
27	Ab46-29	Indifference	Indifference
28	Ab45-163	Indifference	Indifference
29	Ab45-148	Indifference	Indifference
30	Ab46-54	Indifference	Indifference

Table A-7 Combination activity of the combination of imipenem plus amikacin in imipenem resistant-*A. baumannii* which were susceptible to amikacin

No.	*A. baumannii* strains no.	Activity of combination
1	Ab45-51	Indifference
2	Ab45-63	Addition
3	Ab45-64	Indifference
4	Ab45-17703	Indifference
5	Ab45-162	Synergy
6	Ab45-164	Addition
7	Ab46-17	Synergy
8	Ab45-163	Indifference
9	Ab45-148	Indifference
10	Ab46-54	Indifference
11	Ab45-17706	Indifference
12	Ab46-28	Indifference
13	Ab46-29	Indifference

Table A-8 Combination activity of the combination of imipenem plus amikacin in imipenem resistant-*A. baumannii* which were resistant to amikacin

No.	*A. baumannii* strains no.	Activity of combination
1	Ab45-52	Indifference
2	Ab45-75	Indifference
3	Ab45-122	Indifference
4	Ab45-127	Synergy
5	Ab46-31	Indifference
6	Ab46-33	Addition
7	Ab181	Antagonism
8	Ab45-85	Addition
9	Ab45-117	Indifference
10	Ab45-128	Addition
11	Ab45-170	Indifference
12	Ab46-47	Addition
13	Ab182	Indifference
14	Ab45-111	Indifference
15	Ab46-69	Addition
16	Ab45-142	Indifference
17	Ab46-32	Addition

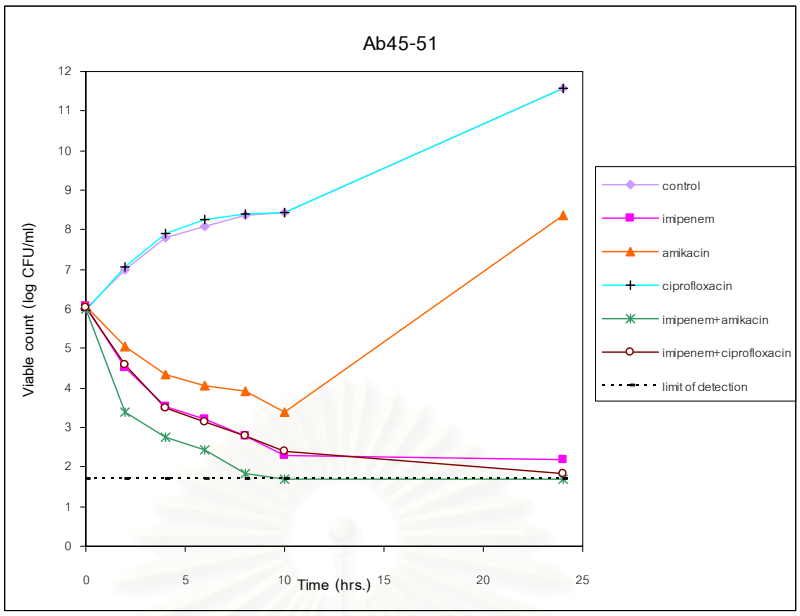

Figure A-31 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab45-51.

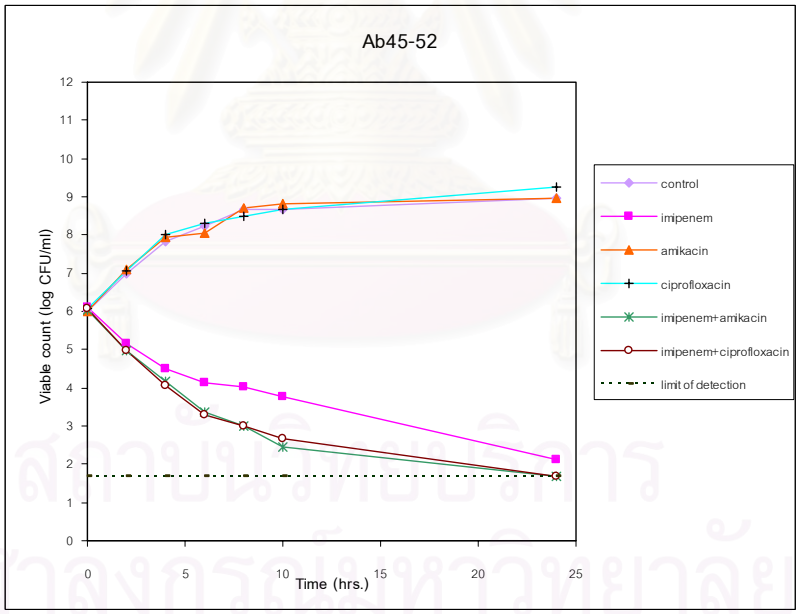

Figure A-32 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab45-52.

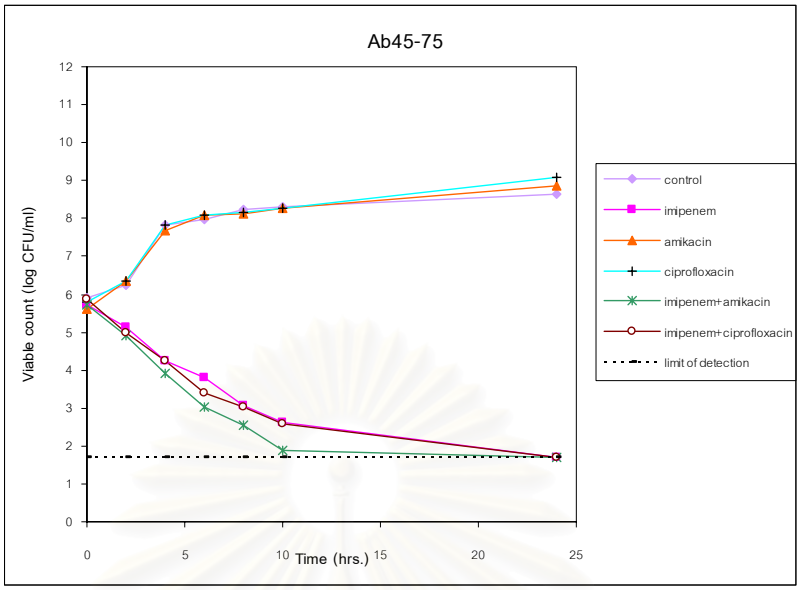

Figure A-33 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab45-75.

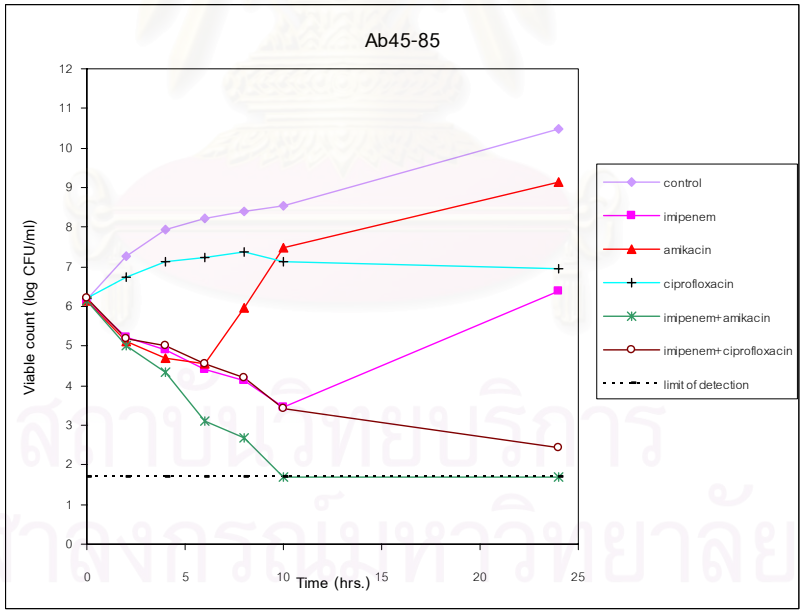

Figure A-34 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab45-85.

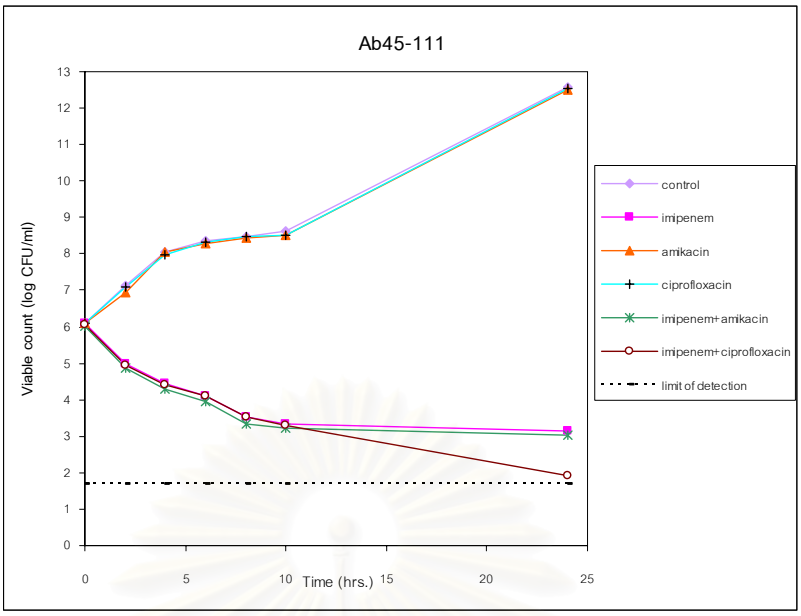

Figure A-35 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab45-111.

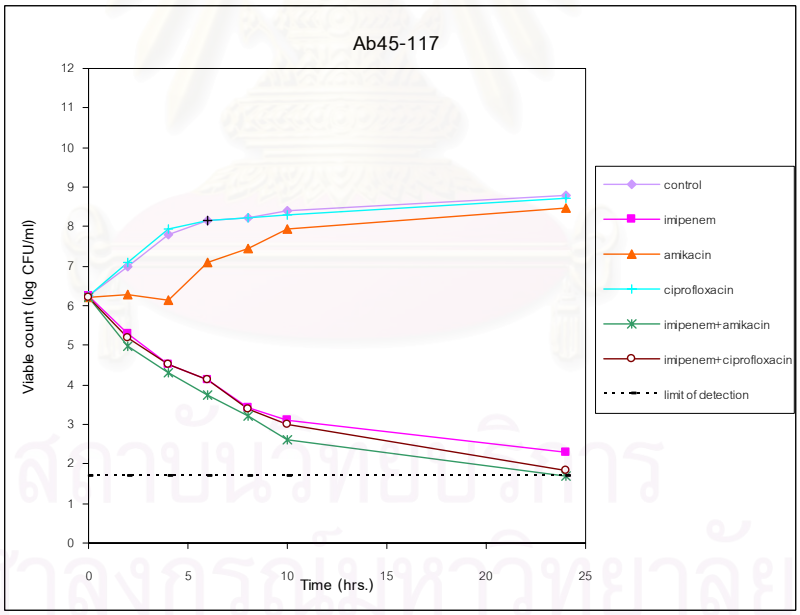

Figure A-36 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab45-117.

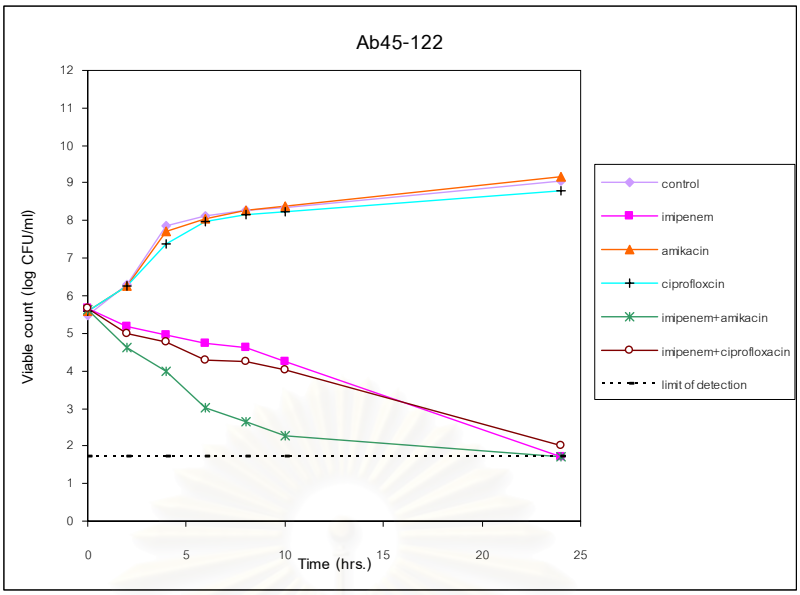

Figure A-37 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab45-122.

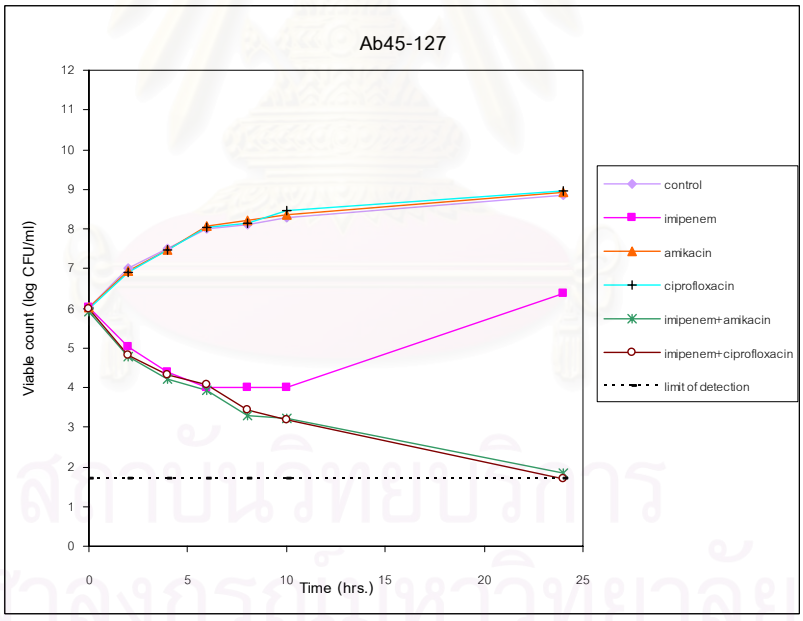

Figure A-38 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab45-127.

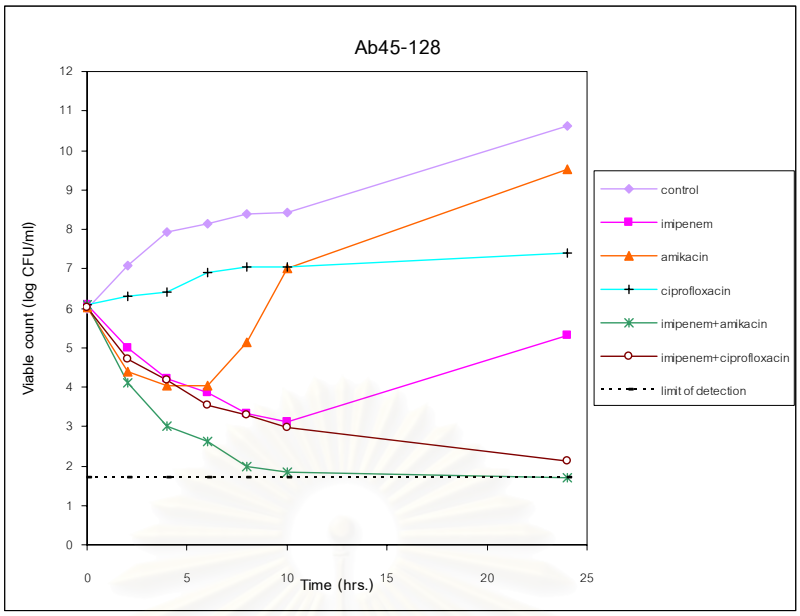

Figure A-39 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab45-128.

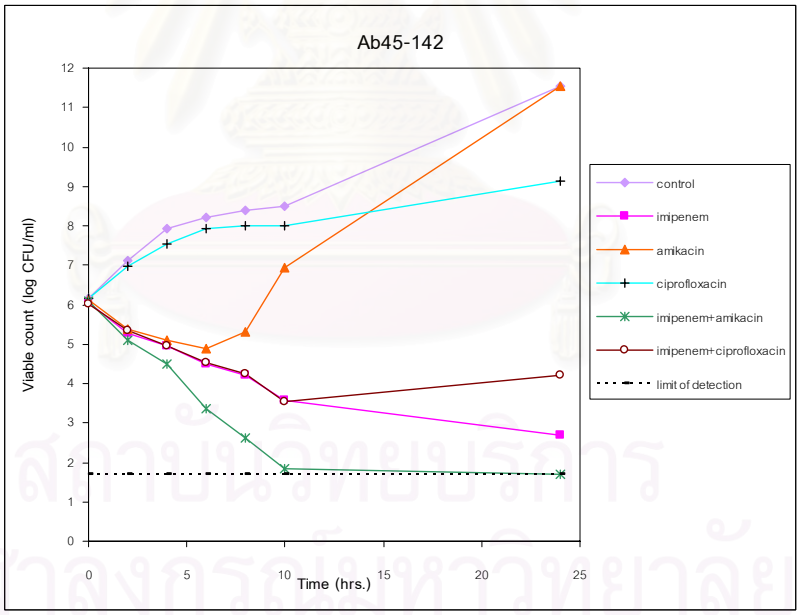

Figure A-40 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab45-142.

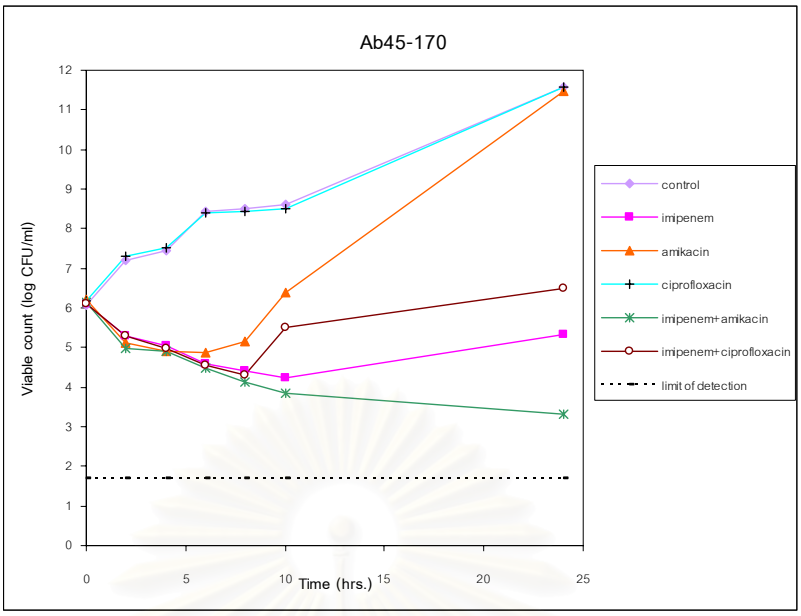

Figure A-41 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab45-170.

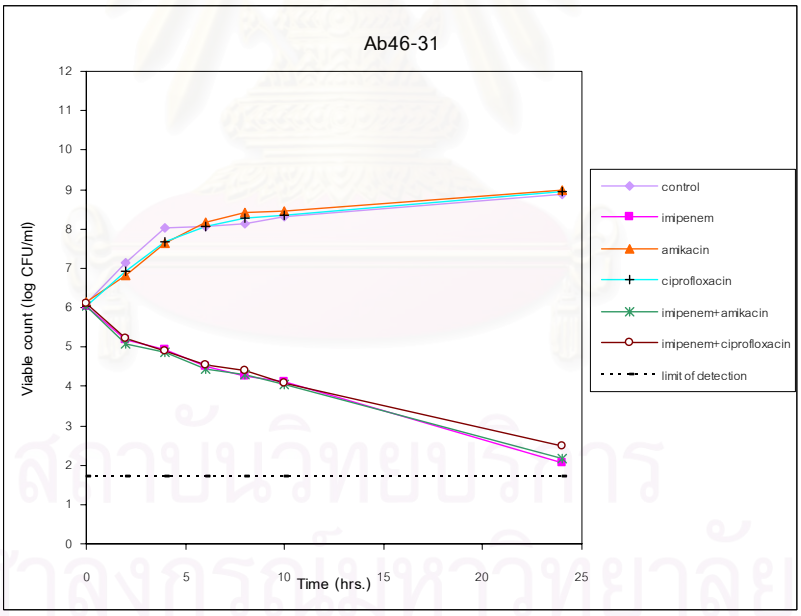

Figure A-42 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab46-31.

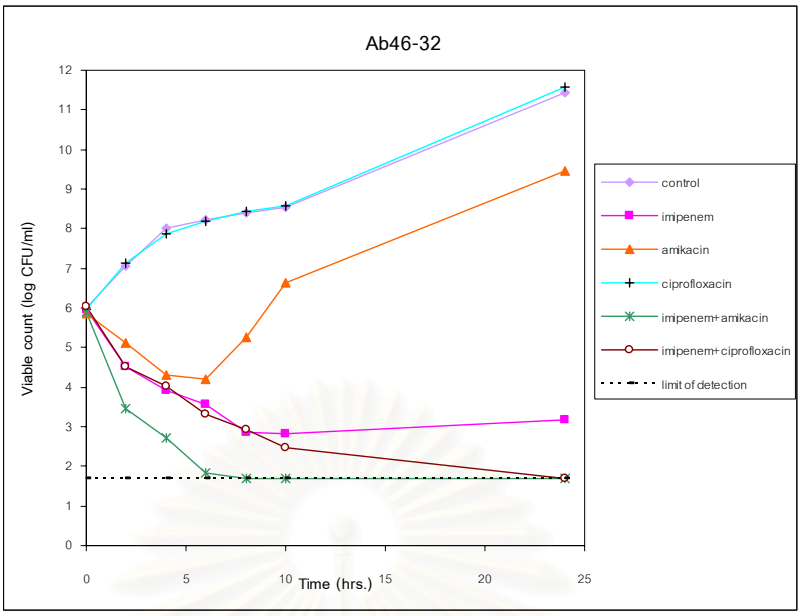

Figure A-43 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab46-32.

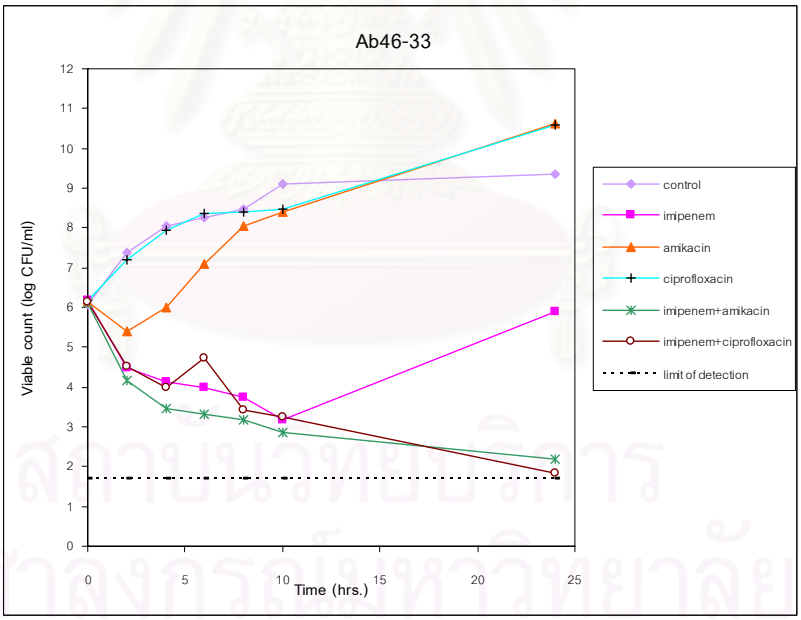

Figure A-44 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab46-33.

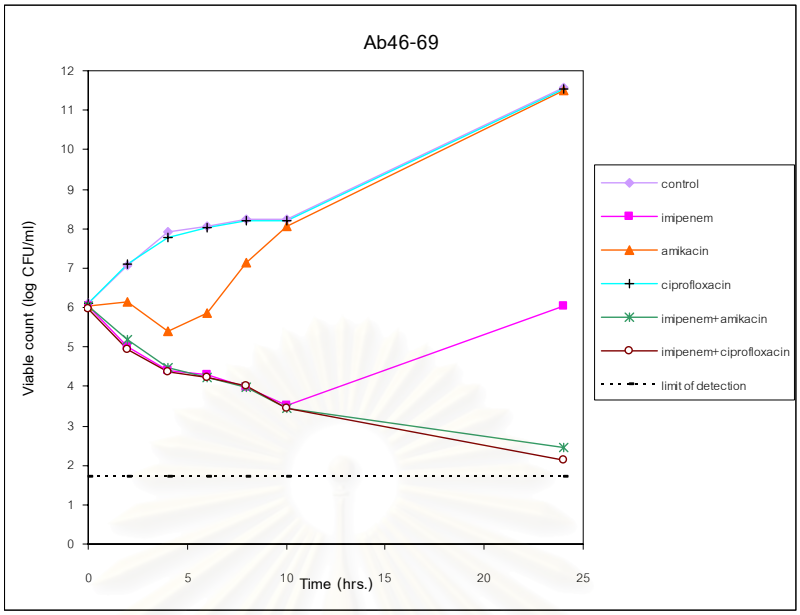

Figure A-45 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab46-69.

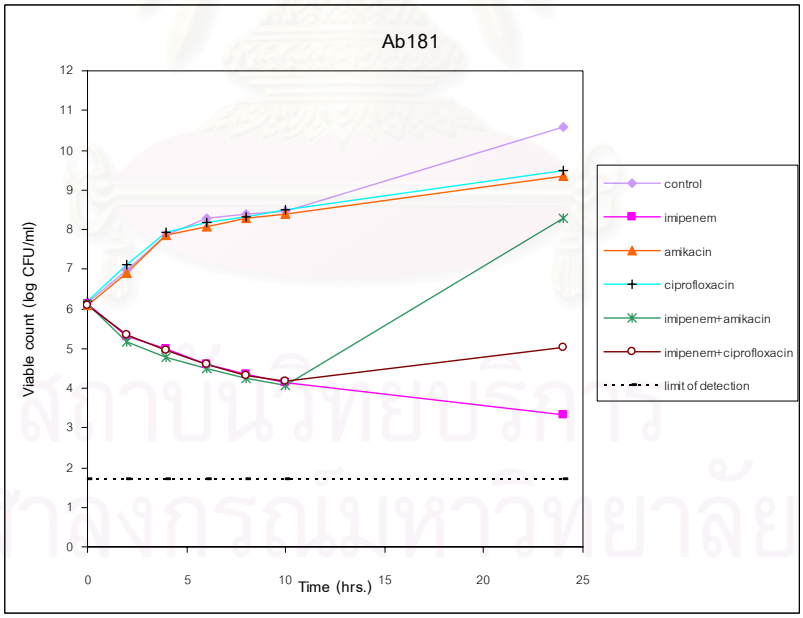

Figure A-46 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab181.

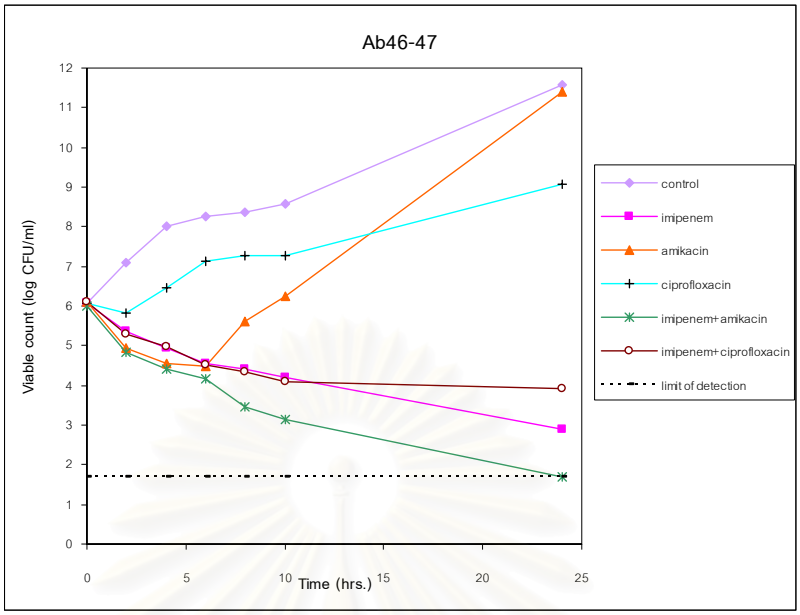

Figure A-47 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab46-47.

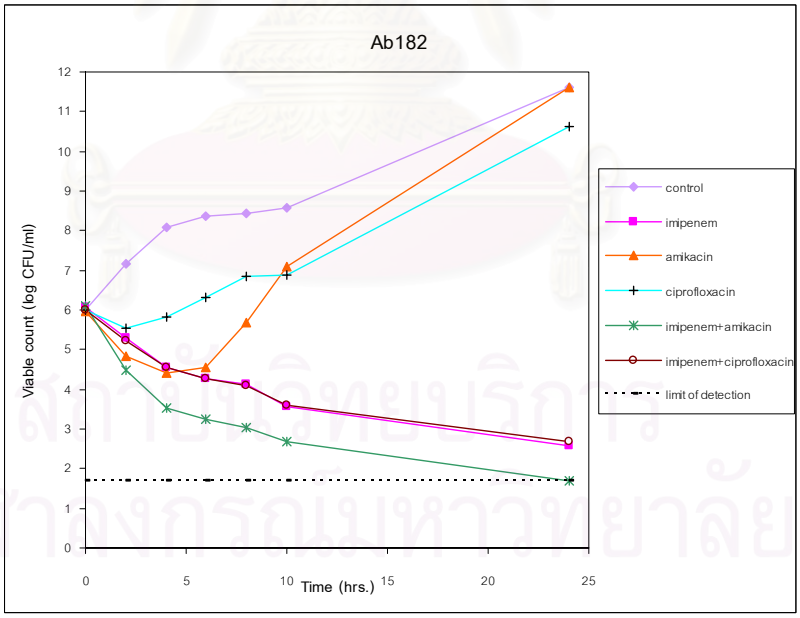

Figure A-48 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab182.

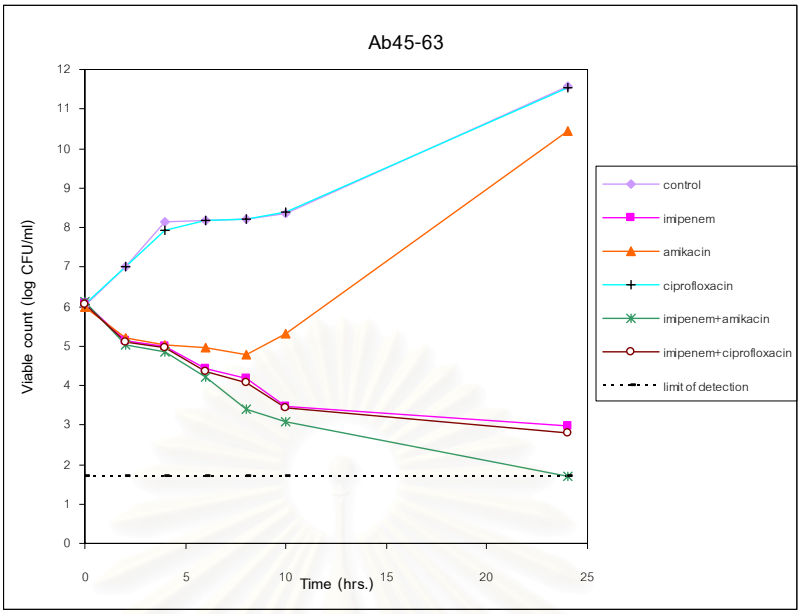

Figure A-49 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab45-63.

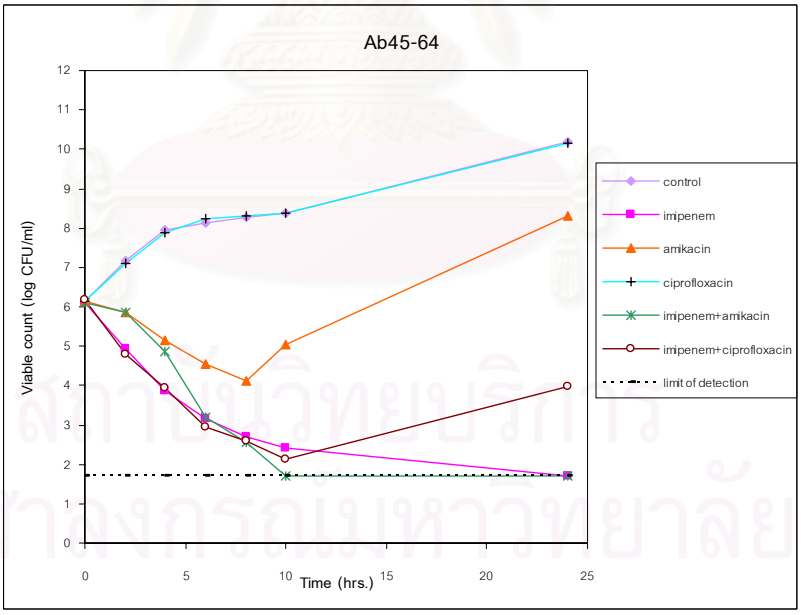

Figure A-50 Time kill curves of showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab45-64.

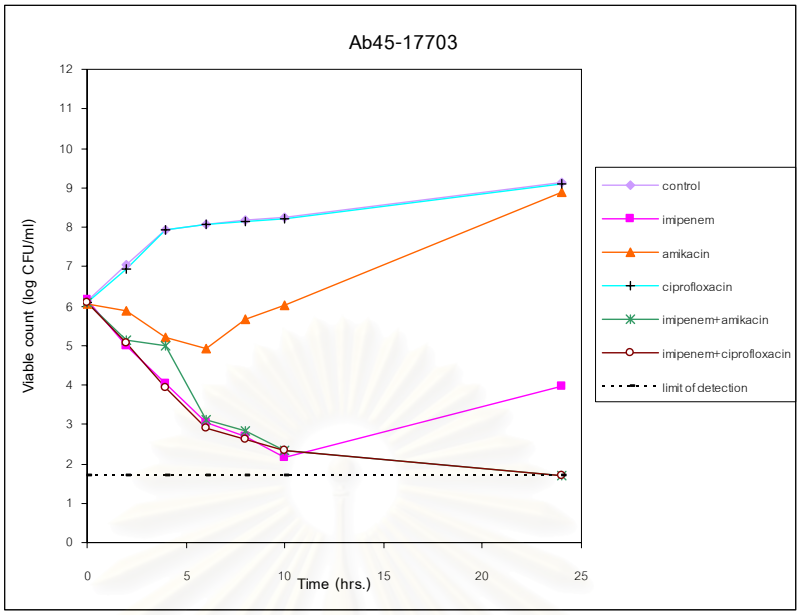

Figure A-51 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab45-17703.

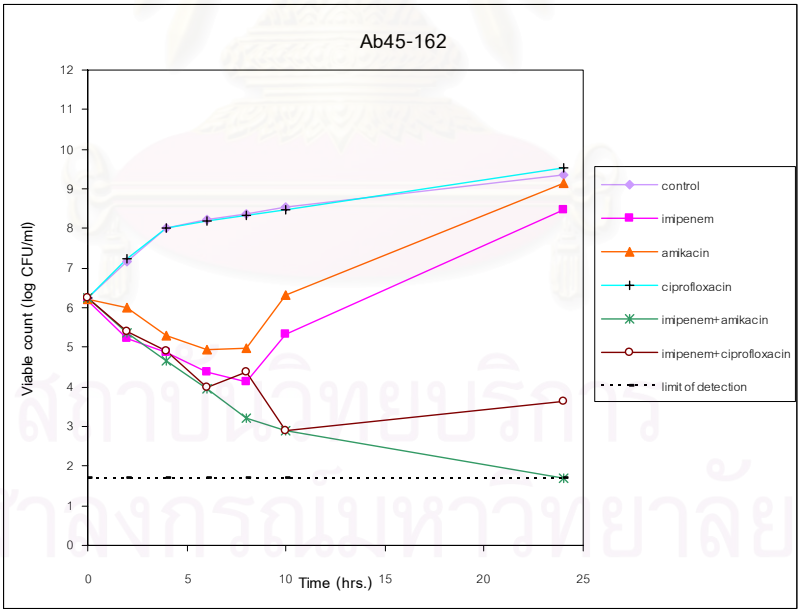

Figure A-52 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab45-162.

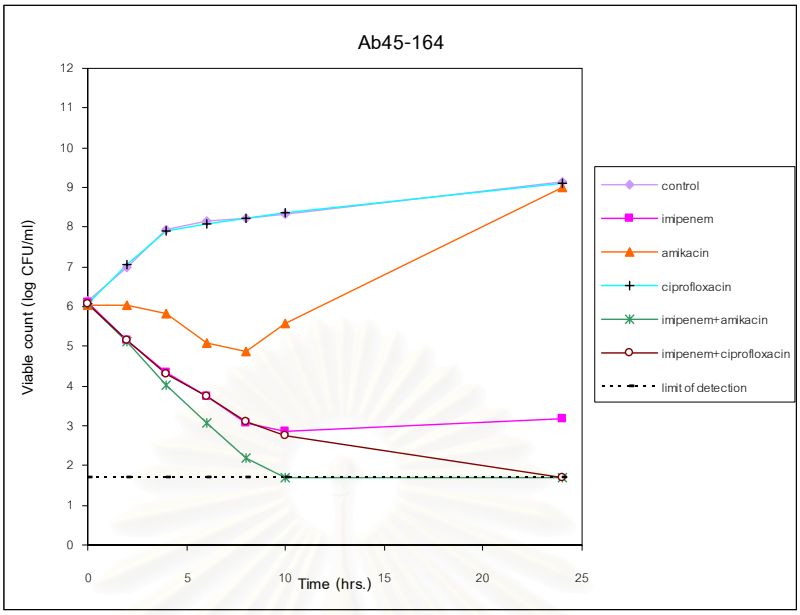

Figure A-53 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab45-164.

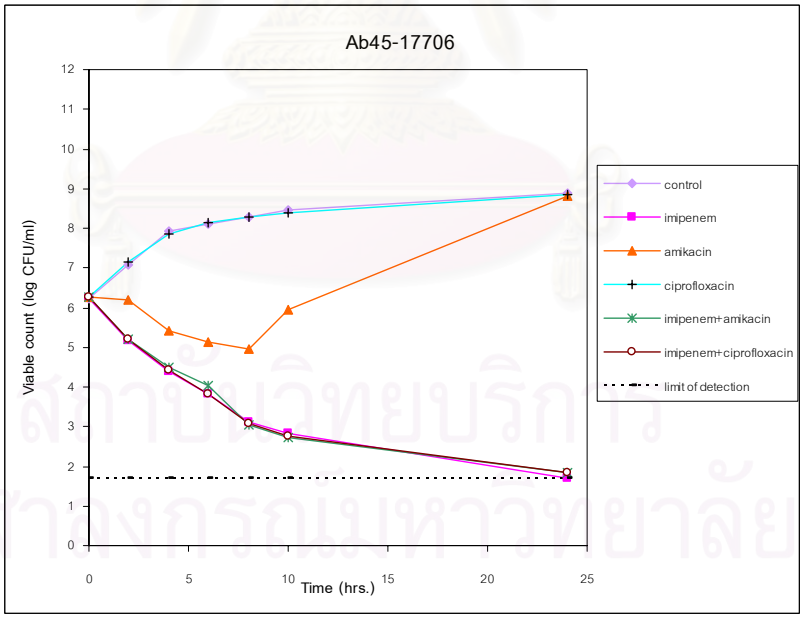

Figure A-54 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab45-17706.

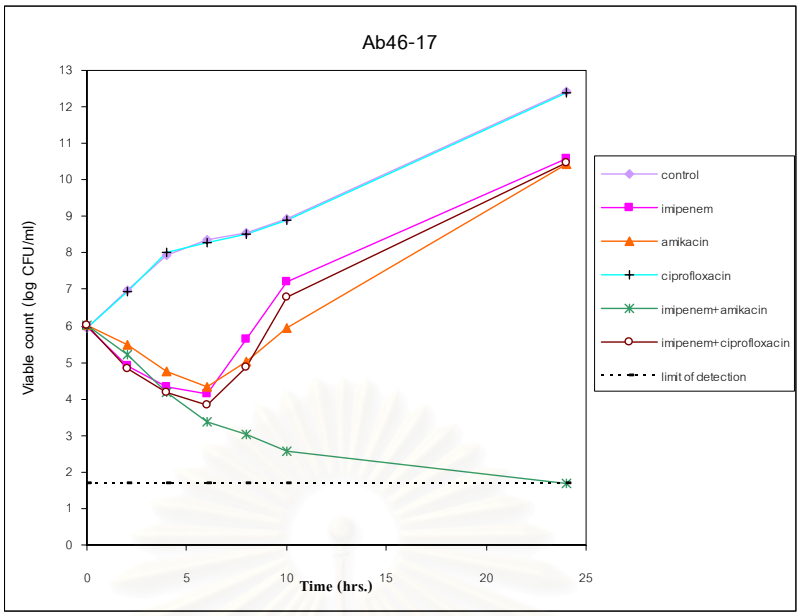

Figure A-55 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab46-17.

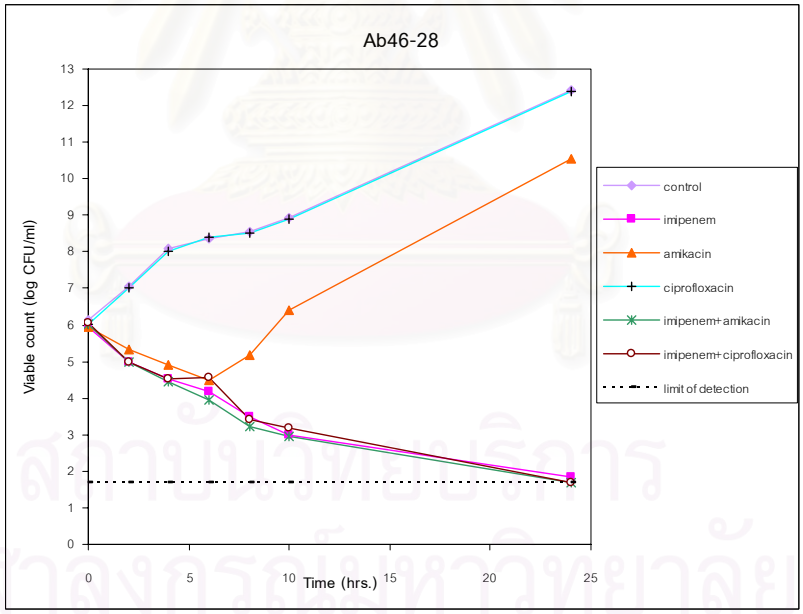

Figure A-56 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab46-28

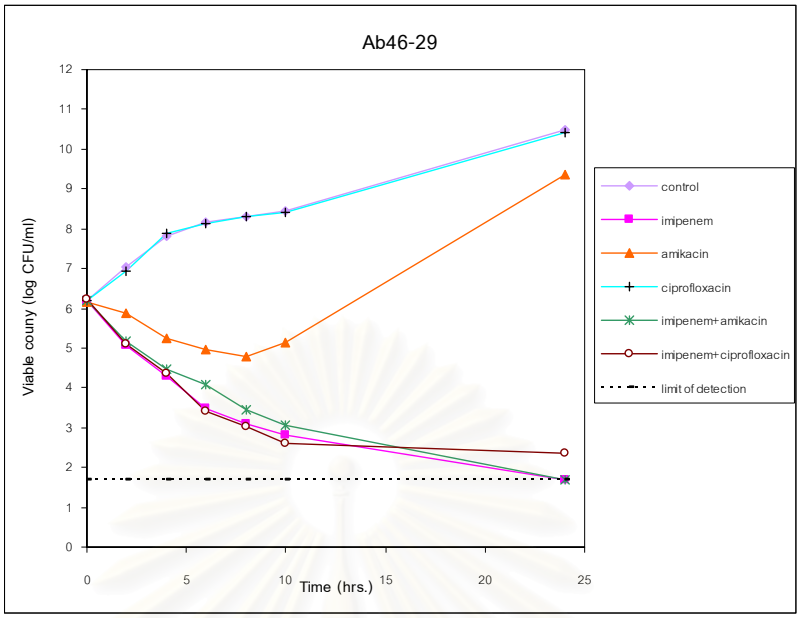

Figure A-57 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab46-29.

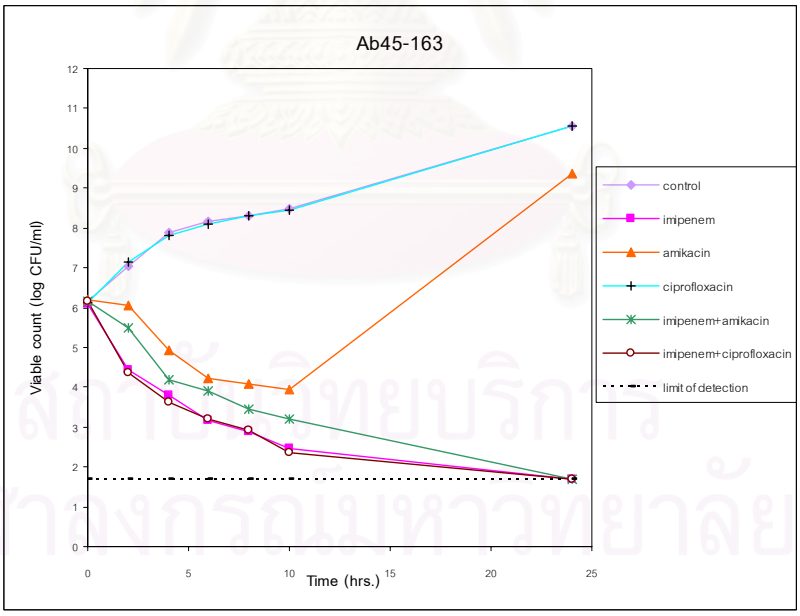

Figure A-58 Time kill curves showing the antibacterial activity of the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab45-163.

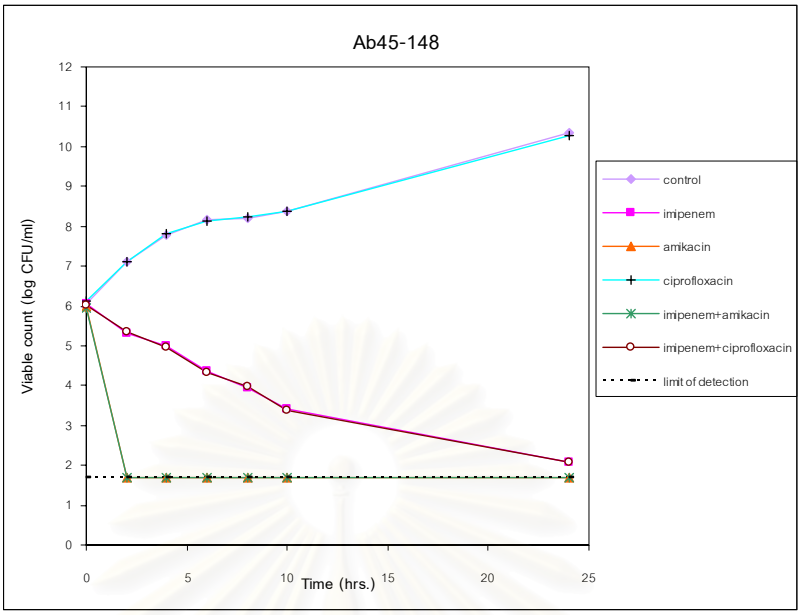

Figure A-59 Time kill curves showing the antibacterial activity the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab45-148.

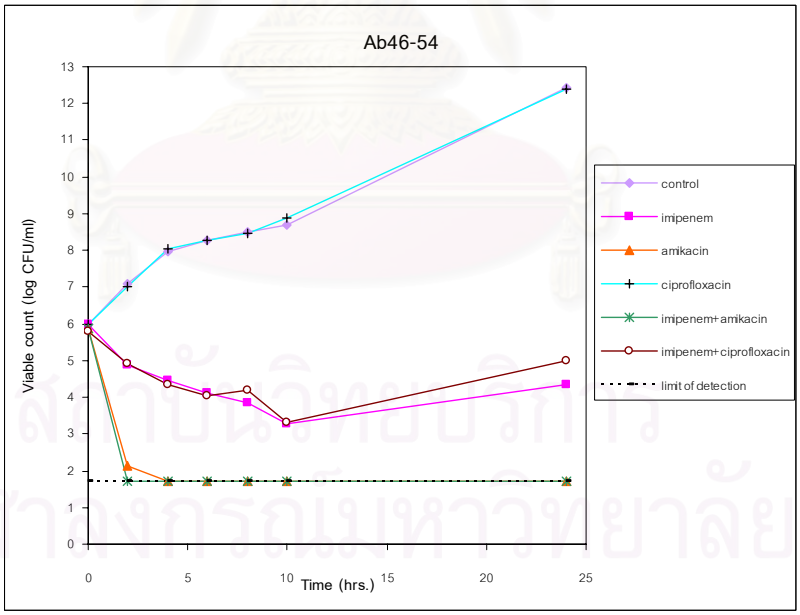

Figure A-60 Time kill curves of showing the antibacterial activity the combination of imipenem plus amikacin and imipenem plus ciprofloxacin against *A. baumannii* strain no Ab46-54

Table A-9 Mean log viable cell counts at time point in 13 strains of amikacin susceptible-*A. baumannii*

Antimicrobial agents	Mean log viable cell counts at time point						
	0 hour	2 hour	4 hour	6 hour	8 hour	10 hour	24 hour
control	6.1078±0.0937	7.0617±0.0647	7.9364±0.1076	8.1860±0.0928	8.3314±0.1326	8.5045±0.2144	10.6552±1.3216
imipenem	6.0981±0.0853	4.9799±0.2588	4.3400±0.4528	3.7857±0.5215	3.5042±0.8361	3.3519±1.4058	3.5709±2.8261
amikacin	6.0743±0.1136	5.1406±1.4795	4.5770±1.3243	4.2401±1.1778	4.2918±1.2464	4.8063±1.6334	8.1580±2.9598
ciprofloxacin	6.0998±0.1023	7.0591±0.0954	7.9242±0.0808	8.1894±0.0927	8.3209±0.1145	8.5036±0.2349	10.6361±1.3003
imipenem+amikacin	6.0834±0.1152	4.5610±1.3899	3.9479±1.1450	3.2898±0.8782	2.7333±0.6705	2.4097±0.6260	1.7086±0.0347
imipenem+ciprofloxacin	6.0929±0.1185	4.9888±0.2864	4.3028±0.4712	3.7148±0.5489	3.4658±0.7455	3.1041±1.1888	3.1341±2.4473

Table A-10 Mean log viable cell counts at time point in 17 strains of amikacin resistant-*A. baumannii*

Antimicrobial agents	Mean log viable cell counts at time point						
	0 hour	2 hour	4 hour	6 hour	8 hour	10 hour	24 hour
control	6.0360±0.1650	7.0097±0.3020	7.8887±0.1767	8.1961±0.1338	8.3571±0.1402	8.5023±0.2032	10.3603±1.3279
imipenem	6.0418±0.1445	5.1001±0.2537	4.5869±0.3552	4.2393±0.3323	3.9091±0.5056	3.5895±0.5107	3.7019±1.7415
amikacin	6.0193±0.1805	5.8871±0.5902	6.1103±1.5247	6.3727±1.7248	7.0132±1.4096	7.7254±0.8404	10.0852±1.3013
ciprofloxacin	6.0516±0.1620	6.7606±0.5204	7.4551±0.6452	7.8640±0.5933	8.0127±0.5289	8.0824±0.5969	9.6574±1.5200
imipenem+amikacin	6.0199±0.1514	4.7426±0.4558	4.1090±0.6181	3.5493±0.7337	3.1397±0.7441	2.7485±0.8179	2.3662±1.6059
imipenem+ciprofloxacin	6.0409±0.1334	5.0292±0.2682	4.5406±0.3748	4.1546±0.4803	3.7775±0.5366	3.4883±0.7453	2.7002±1.3938

Imipenem and Cilastatin for Injection (USP,2004)

Imipenem and Cilastatin for Injection is a sterile mixture of Imipenem, Cilastatin Sodium and Sodium Bicarbonate. It contains not less than 90.0 percent and not more than 115.0 percent of the labeled amounts of imipenem ($C_{12}H_{17}N_3O_4S$) and cilastatin ($C_{16}H_{26}N_2O_5S$).

Assay

pH 6.8 buffer – Dissolve 0.54 g of monobasic potassium phoaphate in 3600 ml of water, adjust with 0.5 *N* sodium hydroxide or 0.5 M phosphoric acid to a pH of 6.8±0.1, dilute with water to make 4000 ml of solution, and mix. Filter this solution through a filter of 0.5 μm or finer porosity.

Mobile phase – Dissolve 2.0 g of sodium 1-hexanesulfonate in 800 ml of *pH 6.8 buffer*, adjust with 0.5 *N* sodium hydroxide or 0.5 M phosphoric acid to a pH of 6.8±0.1, and dilute with *pH 6.8 buffer* to make 1000 ml of solution. Filter this solution through a filter of 0.5 μm or finer porosity, and degas.

Imipenem standard preparation – Transfer about 13 mg of USP Imipenem Monohydrate RS, accurately weighed, to a 25-ml volumetric flask. Add 5 ml of saline TS, 0.5 ml of *pH 6.8 buffer*, and dissolve by shaking and sonicating. [NOTE – The duration of sonication should not exceed 1 minute.] Dilute with *pH 6.8 buffer* to volume, and mix. This solution contains the equivalent of about 500 μg of anhydrous imipenem per ml. Use this solution immediately.

Assay preparation – Constitute Imipenem and Cilastatin for Injection in a volume of saline TS, accurately measured, corresponding to the volume of solvent specified in the labeling. Quantitatively transfer this suspension to a 100-ml volumetric flask with the aid of *pH 6.8 buffer*, dilute with *pH 6.8 buffer* to volume, and mix. Dilute an accurately measured volume of this solution quantitatively with *pH 6.8 buffer* to obtain an *Assay preparation* having a concentration of about 500 μg of imipenem per ml.

Chromatographic system – The liquid chromatography is equipped with a 254-nm detector and a 4.6-mm X 30-cm column that contains packing L1, and it maintained at a temperature of 50±1.0°. The flow rate is about 2 ml per minute. Chromatograph the *Imipenem standard preparation*, and record the peak responses as directed under

Procedure : the column efficiency determined from the imipenem peak is not less than 600 therretical plates when calculated by the formula:

$$5.545(t/W_{h/2})^2,$$

in which t is the retention time measured from time of injection to time of elution of peak maximum, and $W_{h/2}$ is the width of peak at half height.

the tailing factor for the imipenem peak is not more than 1.5 when calculated by the formula:

$$W_{0.1}/2f,$$

where $W_{0.1}$ is the width of the peak at 10% height, and the relative standard deviation for replicate injections is not more than 2.0%, and *f* is the distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5% of the peak height from the baseline.

Procedure – Separately inject equal volumes (about 10 μl) of the *Imipenem standard preparation* and the *Assay preparation* into the chromatograph, recordthe chromatograms, and measure the responses for the major peaks. Calculate the quantities, in mg of anhydrous imipenem ($C_{12}H_{17}N_3O_4S$) in the container, taken by the same formula:

$$(CPL/D)(r_u/r_s),$$

in which C is the concentration, in mg per ml, of USP Imipenem MonohydrateRS in the *Imipenem Standard preparation*, P is the content, in μg per mg of anhydrous imipenem ($C_{12}H_{17}N_3O_4S$) in the relevant Reference Standard, L is labeled quantity, in mg, of imipenem in the container, D is the concentration, in μg per ml of imipenem in the *Assay preparation* based on the labeled quantity in the container and the extend of dilution, and r_u and r_s are the peak responses of the corresponding analyte obtained from the *Assay preparation* and the *Imipenem standard preparation*, respectively.

Table A-11 Result of assay imipenem and cilastatin for injection

	Imipenem standard preparation	Assay preparation
Peak response(area) 1	57088	57427
Peak response(area) 2	56441	57113
Peak response(area) 3	57164	58592
Mean peak response ± S.D.	56897.67±397.30	57710.67±779.24
Relative standard deviation (%)	0.698	1.35

Calculate the quantities, in mg, of anhydrous imipenem ($C_{12}H_{17}N_3O_4S$) in the container, taken by the same formula:

$$(CPL/D)(r_u/r_s),$$

C = 0.548 mg/ml r_u = 57710.67

P = 943.21 μg/mg r_s = 56897.67

L = 500 mg

D = 500 μg/ml

quantity = (0.548 mg/ml * 943.21 μg/mg * 500 mg/500 μg/ml) * (57710.67/56897.67)

= 524.26 mg

BIOGRAPHY

My name is Saranjit Duangseesai, I was born in 18 April 1978 at Nakhonpratom. I have graduated the bachelor degree in Pharmacy from Chulalonkorn University since 2000. I started to work as a pharmacist in Ratchaburi Hospital until 2003. Consequently, I have enrolled for the master's degree in Pharmacology at the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Chulalongkorn University since June 2003.