ฤทธิ์ต้านจุลินทรีย์ในหลอดทดลองของโคลิสตินร่วมกับไรแฟมพิซินต่อเชื้อ *ACINETOBACTER BAUMANNII* ที่ดื้อต่อคาร์บาพิเนมส์

นางสาวกิตติ์สุมน คงเสน่ห์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาจุลชีววิทยา ภาควิชาชีวเคมีและจุลชีววิทยา
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
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ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย
บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)
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IN VITRO ANTIMICROBIAL ACTIVITY OF COLISTIN IN COMBINATION WITH RIFAMPICIN AGAINST CARBAPENEMS-RESISTANT *ACINETOBACTER BAUMANNII*

Miss Kitsumon Kongsanae

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Microbiology
Department of Biochemistry and Microbiology
Faculty of Pharmaceutical Sciences
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Thesis Title	*IN VITRO* ANTIMICROBIAL ACTIVITY OF COLISTIN IN COMBINATION WITH RIFAMPICIN AGAINST CARBAPENEMS-RESISTANT *ACINETOBACTER BAUMANNII*
By	Miss Kitsumon Kongsanae
Field of Study	Microbiology
Thesis Advisor	Associate Professor Pintip Pongpech, Ph.D.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

…… Dean of the Faculty of Pharmaceutical Sciences
(Associate Professor Pintip Pongpech, Ph.D.)

THESIS COMMITTEE

…….. Chairman
(Associate Professor Nongluksna Sriubolmas, Ph.D.)

…….……. Thesis Advisor
(Associate Professor Pintip Pongpech, Ph.D.)

…….. Examiner
(Assistant Professor Penphun Naenna)

…….. External Examiner
(Associate Professor Chertsak Dhiraputra, M.D.)

กิตติ์สุมน คงเสน่ห์: ฤทธิ์ต้านจุลินทรีย์ในหลอดทดลองของโคลิสตินร่วมกับไรแฟมพิซินต่อเชื้อ *Acinetobacter baumannii* ที่ดื้อต่อคาร์บาพิเนมส์. (*IN VITRO* ANTIMICROBIAL ACTIVITY OF COLISTIN IN COMBINATION WITH RIFAMPICIN AGAINST CARBAPENEMS-RESISTANT *ACINETOBACTER BAUMANNII)* อ. ที่ปรึกษาวิทยานิพนธ์หลัก : *รศ.ภญ.ดร.พิณทิพย์ พงษ์เพ็ชร*, 112 หน้า.

วัตถุประสงค์ : เพื่อศึกษาฤทธิ์ร่วมระหว่างยาโคลิสตินกับยาไรแฟมพิซินในการต้านเชื้อ *A. baumannii* ที่ดื้อต่อยาคาร์บาร์พิเนมส์

วิธีทดลอง : เชื้อที่ใช้ทดสอบในครั้งนี้ทั้ง 30 ไอโซเลต แยกได้จากผู้ป่วยที่โรงพยาบาลบำราศนราดูร ระหว่างเดือนเมษายน ปี 2007 ถึง พฤษภาคม ปี 2009 โดยทำการทดสอบความไวรับของเชื้อ *A. baumannii* ด้วยวิธี Kirby-Bauer disk diffusion และ Agar dilution จากนั้นทำการศึกษาผลการเสริมฤทธิ์ของการใช้ยาร่วมระหว่างยาโคลิสตินกับยาไรแฟมพิซินโดยวิธี checkerboard นำเชื้อที่ผลเสริมฤทธิ์กัน ไปศึกษาฤทธิ์ฆ่าเชื้อของการใช้ยาเดี่ยวและยาร่วมระหว่างยาโคลิสตินกับยาไรแฟมพิซิน ที่ความเข้มข้น 0.5×MIC และ 1×MIC โดยวิธี Time kill และประเมินการเปลี่ยนแปลงสัณฐานวิทยาของเชื้อแบคทีเรียหลังได้รับยา ด้วยภาพจากกล้องจุลทัศน์อิเล็คตรอน

ผลการทดลอง : จากการทดสอบความไวรับของเชื้อที่มีต่อยาทั้ง 12 ชนิด พบว่าทุกไอโซเลตดื้อต่อยาเมอโรพีเนม, อิมิพีเนม และไรแฟมพิซิน ขณะเดียวกัน 96.7% ของเชื้อที่ทดสอบดื้อต่อยาเซฟีพิม, แซฟตาซิดิม, พิเพอราซิลิน/ทาโซแบกแตม และซิโพรฟล๊อกซาซิน 90% ของเชื้อที่ทดสอบดื้อต่อยาอะมิกาซิน, เจนตามัยซิน, เนติลมิซิน และโตบรามัยซิน แต่ 80% ของเชื้อที่ทดสอบไวต่อยาโคลิสติน ยาโคลิสติน (MIC อยู่ระหว่าง 1-4 ไมโครกรัมต่อมิลลิลิตร) มีค่า MIC_{50}, MIC_{90} เท่ากับ 1, 2 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ ขณะที่ยาไรแฟมพิซิน (MIC อยู่ระหว่าง 8-16 ไมโครกรัมต่อมิลลิลิตร) มีค่า MIC_{50}, MIC_{90} เท่ากับ 8 ไมโครกรัมต่อมิลลิลิตร การทดสอบด้วยวิธี checkerboard พบว่า 26.7% ของเชื้อที่ทดสอบ ให้ผลการเสริมฤทธิ์กันบางส่วน (partial synergy) และจากการศึกษาด้วยวิธี time kill พบว่าการใช้ยาร่วมกันสามารถฆ่าเชื้อได้ตลอดช่วงเวลาที่ทำการทดสอบ นอกจากนี้ภาพจากกล้องจุลทัศน์อิเลคตรอน แสดงให้เห็นว่าเมื่อเชื้อสัมผัสยาทั้ง 2 ชนิดพร้อมกัน ผนังเซลล์ของเชื้อจะถูกทำลายอย่างชัดเจน เมื่อเปรียบเทียบกับเซลล์ที่สัมผัสกับยาเดี่ยวแต่ละตัว

สรุปผลการทดลอง : การใช้ยาร่วมกันระหว่างยาโคลิสตินและยาไรแฟมพิซิน มีฤทธิ์ฆ่าเชื้อที่เหนือกว่าการใช้ยาเดี่ยว ดังนั้นการใช้ยาร่วมกันจะเป็นทางเลือกหนึ่งในการรักษาโรคติดเชื้อที่มีสาเหตุจาก *A. baumannii* ที่ดื้อต่อคาร์บารพิเนมส์ได้

ภาควิชา.....ชีวเคมีและจุลชีววิทยา…… ลายมือชื่อนิสิต.....

สาขาวิชา.....จุลชีววิทยา..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา.....2554.....

527 65539 33 : MAJOR MICROBIOLOGY
KEYWORDS : *ACINETOBACTER BAUMANNII* / COLISTIN / RIFAMPICIN / ANTIMICROBIAL COMBINATION / CARBAPENEMS RESISTANT

KITSUMON KONGSANAE : *IN VITRO* ANTIMICROBIAL ACTIVITY OF COLISTIN IN COMBINATION WITH RIFAMPICIN AGAINST CARBAPENEMS-RESISTANT *ACINETOBACTER BAUMANNII*.
ADVISOR : ASSOC. PROF. PINTIP PONGPECH, Ph.D., 112 pp.

Objectives: The aim of this study was to determine the antimicrobial activities of the combination between colistin and rifampicin against carbapenems-resistant *Acinetobacter baumannii.*

Methods: Thirty carbapenems-resistant *A. baumannii* isolates from Bumratnaradoon Hospital between April 2007 and May 2009 were included in this study. Antimicrobial susceptibility tests were performed by both Kirby-Bauer disk diffusion and Agar dilution methods. Synergy effect of the combination of colistin and rifampicin was determined by checkerboard method. In addition, the time-kill study was used to determine the bactericidal activity at 0.5×MIC and 1×MIC of either colistin or rifampicin alone and the combination of both agents. Morphological cell changes of the bacteria after growth in the media plus antimicrobials were observed by using scanning electron microscopy.

Results: Susceptibility testing of 12 antimicrobial agents against carbapenems-resistant *A. baumannii* showed that all 30 isolates were resistant to meropenem, imipenem and rifampicin, while 96.7% of these isolates were resistant to cefepime, ceftazidime, piperacillin/tazobactam and ciprofloxacin. Ninety percent of tested isolates were resistant to amikacin, gentamicin, netilmicin and tobramycin, but 80% were still susceptible to colistin. The MIC ranges for colistin and rifampicin were 1-4 µg/ml and 8-16 µg/ml, respectively. MIC_{50} and MIC_{90} of colistin were 1, 2 µg/ml, respectively and MIC_{50} and MIC_{90} of rifampicin were 8 µg/ml. The synergy study showed the partial synergy effect of colistin when combined with rifampicin in 26.7% of the isolates. Bactericidal activity of the combination was observed at all incubation times by time-kill study. The more damaging effect of the bacterial cell lysis was clearly observed when the bacteria were grown in the combined antimicrobials as compared with the growth in each antimicrobial agent.

Conclusion: The *in vitro* bactericidal activity of the combination between colistin and rifampicin was superior to the single agent. The combination could be a promising alternative for the treatment of infections due to carbapenems-resistant *A. baumannii*.

Department : Biochemistry and Microbiology Student's Signature .....
Field of Study : Microbiology Advisor's Signature .....
Academic Year : 2011

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LIST OF ABBREVIATIONS

β	=	beta
%	=	percent
°C	=	degree Celsius
µg	=	microgram
µl	=	microlitre
A. baumannii	=	*Acinetobacter baumannii*
AMK	=	amikacin
ATCC	=	American Type Culture Collection
AUBKC	=	area under the bacterial killing and regrowth curves
BA_{24}	=	bacteriolytic area of 24 hours
CAZ	=	ceftazidime
CFU	=	colony forming unit
CIP	=	ciprofloxacin
CLSI	=	Clinical and Laboratory Standards Institute
COL	=	colistin
E. coli	=	*Escherichia coli*
EUCAST	=	The European Committee on Antimicrobial Susceptibility Testing
et al	=	et alii (and other peoples)
FEP	=	cefepime
FIC	=	fractional inhibitory concentration
FICI	=	fractional inhibitory concentration index
Fig	=	Figure
GEN	=	gentamicin
hr	=	hour
ICU	=	intensive care unit
IMP	=	imipenem

Log_{10}	=	decimal logarithm
MBL	=	metallo-beta-lactamase
mg	=	milligram
MER	=	meropenem
MHA	=	Meuller-Hinton Agar
MHB	=	Meuller-Hinton Broth
MIC	=	minimum inhibitory concentration
ml	=	milliliter
mm	=	millimeter
MYSTIC	=	Meropenem Yearly Susceptibility Test Information Collection
NARST	=	National Antimicrobial Resistance Surveillance Center Thailand
NET	=	netilmicin
NNIS	=	National Nosocomial Infections Surveillance
No.	=	number
OMPs	=	Outer membrane proteins
OXA	=	Oxacillin-hydrolyzing-beta-lactamase
P. aeruginosa	=	*Pseudomonas aeruginosa*
PBP	=	penicillin-binding protein
RIF	=	rifampicin
SENTRY	=	SENTRY Antimicrobial Surveillance Program
SSTI	=	skin and soft tissue infection
TOB	=	tobramycin
TSA	=	typtic soy agar
TZP	=	penicillin/tazobactam
UTI	=	urinary tract infection
VAP	=	ventrilator-associated pneumonia

CHAPTER I

INTRODUCTION

The prevalence rate of nosocomial infection in Thailand has increased steadily from 4.9% in the year 2001 to 7.6% in 2006. The highest infection rate was discovered in the intensive care units (ICUs) of tertiary hospitals. Most of the nosocomial infections were caused by gram-negative pathogens (70.2%) particular *A. baumannii* (Danchaivijitr et al., 2007).

Acinetobacter baumannii (A. baumannii) is gram-negative coccobacilli, a most common pathogen of nosocomial infections in ICUs. This organism can survive for a long period of time in a hospital environment and usually can be spread on the skin of hospital personnel and contaminated medical equipment (Fridkin et al., 2001).

A. baumannii causes various nosocomial infections including bloodstream infections, peritoneal infections, urinary tract infections, surgical wound infections, central nervous system infections, and skin and eye infections. It is especially a lower respiratory tract infection with ventilator-associated pneumonia (VAP) occurring predominantly in ICU patients, which has the highest mortality rate (Rungruanghiranya et al., 2005).

In addition, the risk factors for infection with *A. baumannii* are age, burns, surgery, use of medical devices, prolonged length of ICU and in hospital stays, the frequent and prolonged use of antimicrobials due to selective antimicrobial pressure and multiple mechanisms of antimicrobials resistance. Therefore, the development of resistance to all available antimicrobial classes is promoted leading to multidrug-resistance (MDR) of the pathogens (Bergogone-Berezin and Towner, 1996; Munoz-Price and Weinstein, 2008; Sunenshine et al., 2007). *A. baumannii* has increasingly emerged as the leading multidrug-resistant mechanism in Thailand and worldwide

causing problems in the treatment of all infectious diseases caused by this pathogen (Surasarang et al., 2007). Moreover, the study of 208 patients who were admitted to Siriraj Hospital, Thailand in 2002 showed that 57% of *A. baumannii* isolates were resistant to all available antimicrobials. The susceptibility to carbapenems, aminoglycoside, beta-lactam/ beta-lactamase inhibitors, co-trimoxazole, fluoroquinolone, 4th generation cephalosporins and 3rd generation cephalosporins were 32%, 16%, 12%, 9%, 7%, 4% and 3%, respectively. Hence, the mortality rate was as high as 54.7%. The most common infection was lower respiratory tract infections with ventilator-associated pneumonia (VAP) (Keerasuntonpong et al., 2006).

At present, carbapenems are first-line agents in the treatment of serious infections caused by *A. baumannii*, but the occurrence of carbapenem-resistant *A. baumannii* has been increasingly reported. In Thailand, the carbapenem-resistance recovery rate has been increasing from 2.1% in 2000 to 46.7% in 2005 (Apisarnthanarak et al., 2009). Thus, the limitation of therapeutic options used is the major cause of the prolong-stays in the hospital and the high mortality rate. However, developing new drugs to fight these organisms requires a long period of time and resources. This leads to the revival of older antimicrobials that are also active against *A. baumannii* and may be an alternative treatment for patients infected with MDR pathogens (Falagas and Kopterides, 2007).

A combination therapy has been selected for the treatment of multidrug-resistant pathogens in order to enhance the synergy or additive effect of each antimicrobial agent and to decrease the emergence of resistant bacteria. In addition, the combination with a lower dose of each antimicrobial agent could reduce the drug toxicity (Rahal, 2006).

Colistin is an old antimicrobial in the polymyxins group. It is still active against *A. buamannii* and it is an inexpensive agent. Even though the combination of colistin and rifampicin has been shown to have a synergistic effect *in vitro*, colistin alone as a treatment dose is usually toxic to kidneys. The combination of colistin with rifampicin is expected to reduce such side effects by decreasing the dosage and may

be an alternative treatment for severe infections caused by carbapenems-resistant *A. baumannii* (Garnacho-Montero and Amaya-Villar, 2010). In addition, the cost of colistin is about 10-20 times cheaper than other antimicrobials for the treatment of multidrug-resistant *A. baumannii* and *P. aeruginosa*, such as carbapenems, cefoperazone/sulbactam, cephalosporins and aminoglycosides (Koomanachai et al., 2007).

The hypothesis of this study is to demonstrate that the combination of colistin and rifampicin could generate the synergistic antimicrobial effects against carbapenems-resistant *A. baumannii.*

Therefore, this study will demonstrate the *in vitro* antibacterial activities of a combination of colistin and rifampicin against Thai carbapenems-resistant *A. baumannii* isolates in order to obtain the informative conclusions on this aspect. The experimental studies are designed to determine:

1. The antimicrobial susceptibility of clinical isolated *A. baumannii*

2. The combined effects of colistin and rifampicin against carbapenems-resistant *A. baumannii* by the checkerboard method.

3. The bactericidal activity of the combination of colistin and rifampicin against carbapenems-resistant *A. baumannii* by the time kill method.

4. The morphological changes of carbapenems-resistant *A. baumannii* in the combination of colistin and rifampicin by the electron microscopy.

CHAPTER II

LITERATURE REVIEWS

1. *Acinetobacter baumannii*

Microbiology

Acinetobacter baumannii is a gram-negative coccobacilli, nonmotile, nonfermentative, strictly aerobic, catalase-positive, oxidase-negative (Chastre and Trouillet, 2000). The colonies are 1 to 2 mm, dome-shaped, non-pigmented and have smooth surfaces. In addition, the characteristics of the bacterial cells are rod-shaped during the growth phase and form coccobacilli during the stationary phase (Peleg et al., 2008; Alsan and Klompas, 2010).

History of organism

In 1911, genus *Acinetobacter* was first described as *Micrococcus calcoaceticus* (Peleg et al., 2008) and became known as *Acinetobacter* in the 1950s (Munoz-price and Weinstein, 2008). Members of the genus *Acinetobacter* became significant nosocomial pathogens during the early 1970s and it has been classified under at least 15 different genera and species (Bergogne-Berezin and Tower, 1996). Only three species are often clinically related including *Acinetobacter* genomic species 2 (*Acinetobacter baumannii*), *Acinetobacter* genomic species 3 and *Acinetobacter* genomic species 13TU (Zarrilli et al., 2009).These species are of the greatest clinical importance in nosocomial outbreaks of infection and are closely related and difficult to distinguish for routine diagnostic laboratories. Therefore, the clinical laboratories have divided the genus by the term *A. baumannii-A. colcoaceticus* complex (ABC). Whereas the genomic species 1 (*Acinetobacter calcoaceticus*) is frequently found in soil and water, but that has never been involved in severe clinical diseases (Peleg et al., 2008; Munoz-Price and Weinstein, 2008).

Habitats

In general, members of the genus *Acinetobacter* are ubiquitous in the various environments. The natural habitats are commonly found in soil, water and vegetables (Bergogne-Berezin and Tower, 1996; Villegas and Hartstein, 2003). Moreover, they have been isolated from the hospital environment including sinks, mops, pillows, keyboards and medical equipment. These organisms colonized the skin of health care personnel and hospitalized patients (Villegas and Hartstein, 2003; Jain and Danziger, 2004; Maragakis and Perl, 2008) and are particularly the cause of severe infections such as bacteremia or pneumonia in ICU patients (Fournier and Richet, 2006).

2. Clinical manifestations of *A. baumannii* infections

A. baumannii is usually considered to be opportunistic nosocomial pathogens. This infection can cause diseases in patients with immune deficiency. This organism can survive for a long period of time in a hospital environment. It is the major pathogen of the nosocomial infections in the intensive care units (ICU), including surgical site infections, peritoneal infections, central nervous system infections, skin and eye infections (Rungruanghiranya et al., 2005) and the most frequent clinical manifestations for *A. baumannii* infections are ventilator-associated pneumonia (VAP), bloodstream infections and urinary tract infections (Rungruanghiranya et al., 2005; Alsan and Klomas, 2010).

2.1 Pneumonia

Pneumonia is commonly found in ICU patients with the highest mortality rate in patients with severe underlying diseases who have prolonged use of invasive devices such as mechanical ventilators. According to the data from the National Nosocomial Infections Surveillance (NNIS) system, the proportion of *Acinetobacter* species associated with ICU pneumonia increased from 4% in 1986 to 7% in 2003 (Gaynes and Edwards, 2005)

2.2 Bloodstream infection

Bacteremia is often associated with catheter-related infections and respiratory infections (Bergogne-Berezin and Tower, 1996). Nosocomial bacteremia occur in the highest rate in the patients who have a length of hospital stay for 4.5-32 days (Pittet et al., 1994). The data from Surveillance and Control of Pathogens of Epidemiological Importance (SCOPE) reported that the occurrence of bloodstream infections by *A. baumannii* in an ICU was higher than that found in a non-ICU ward (1.6% versus 0.9% of bloodstream infections, respectively) while the crude mortality from *A. baumannii* bloodstream infections was 43.4% in the ICU and 16.3% in the non-ICU (Wispinlinghoff et al., 2004).

2.3 Urinary tract infection

Urinary tract infections are commonly found in patients with an indwelling urinary catheter and who have prolonged length of ICU stays (Laupland et al., 2002). This infection has been reported to have increasingly occurred from 0.6% in the year 1975 to 1.6% in 2003 for patients with associated UTI caused by *A. baumannii* in ICU (Gaynes and Edwards, 2005).

2.4 Skin and soft tissue infection

Skin and soft tissue infection (SSTI) caused by *A. baumannii* was found to be low frequent according to the data from the National Nosocomial Infections Surveillance System in 2003 which reported 2.1% with SSTI in ICUs (Gaynes and Edwards, 2005).

2.5 Meningitis

Nosocomial meningitis caused by carbapenem-resistant *A. baumannii* is increasing, particularly in patients with ventriculography, myelography or neurosurgical procedures. The mortality rate reported was as high as 70%, but it is not common (Bergogne-Berezin and Tower, 1996; Metan et al., 2007).

3. Carbapenem resistance mechanism in *A. baumannii*

Currently, carbapenem resistant of *A. baumannii* is increasing worldwide and is usually resistant to all antimicrobials (Peleg et al., 2008; Poirel and Nordmann, 2006). This organism is resistant with a variety of mechanisms, including (1) antimicrobial-altering enzyme (β-lactamases, cabapenemases (2) decreased permeability with porin protein, efflux pump and (3) alterations in antimicrobial targets (penicillin-binding proteins (PBP) in Fig 2-1.

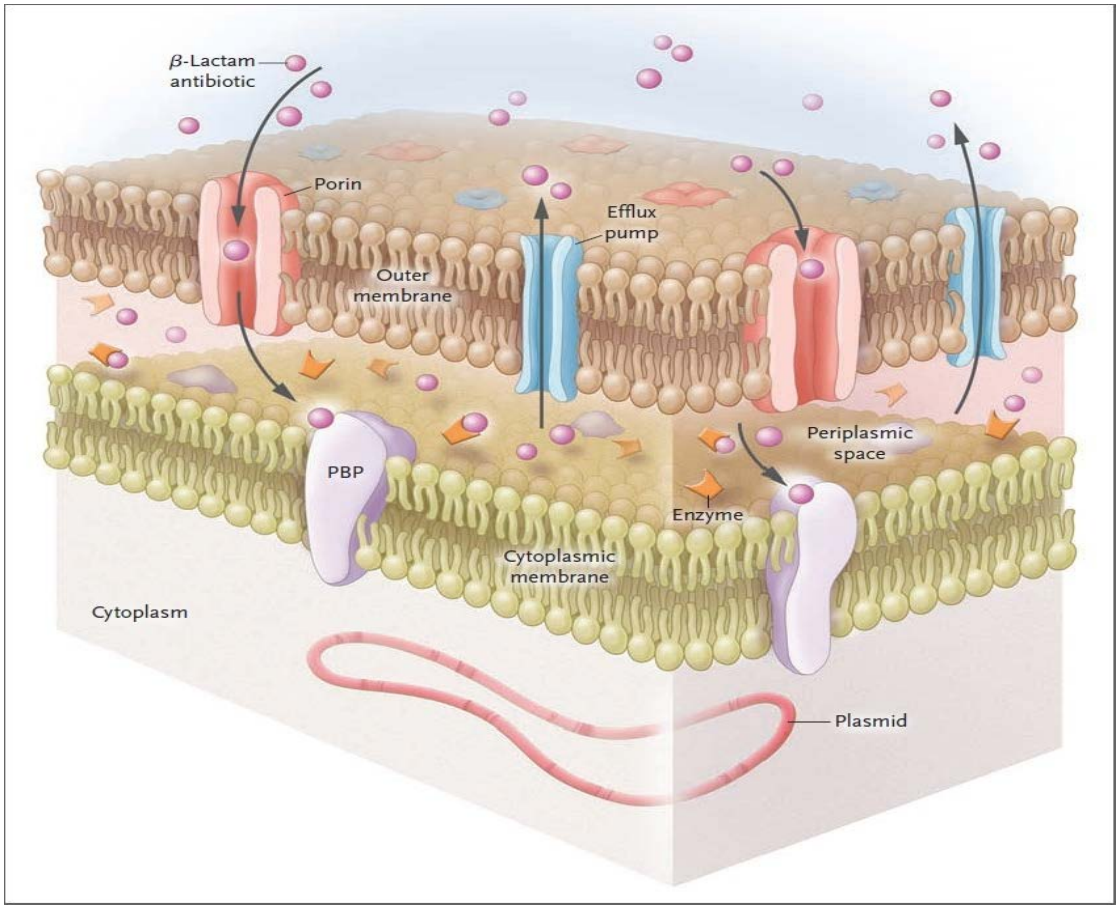

Figure 2-1 Mechanisms of resistance in *Acinetobacter* spp. (Munoz-Price and Weinstein, 2008)

The major carbapenem resistant mechanism in *A. baumannii* is β-lactamases production (Perez et al., 2007). Generally, *A. baumannii* has chromosomally encode AmpC cephalosporinases also known as *Acinetobacter*-derived cephalosporinases

(ADCs), this enzyme can hydrolyze penicillins, narrow-spectrum and extended-spectrum cephalosorins except cefepime and carbapenems (Peleg et al., 2008; Poirel and Nordmann, 2006). However, carbapenemase activity is often due to the metallo-β-lactamases (MBLs) (class B enzymes) and the serine oxacillinases (class D OXA enzymes) as described by Ambler (Poirel and Nordmann, 2006; Jacoby and Munoz-Price, 2005).

Metallo-β-lactamases (MBLs) are class B enzymes (including VIM-like, IMP-like, SIM-1, SPM-1 and GIM-1 enzymes) but only IMP, VIM and SIM enzymes have been identified in *A. baumannii* (Poirel and Nordmann, 2006). These enzymes can hydrolyze all β-lactam antibiotics except aztreonam (Urban et al., 2003). Carbapenem-resistance in *A. baumannii* has a serious concern, which is found commonly in IMP and VIP variant. There were reports on the IMP-like (IMP-1, -2, -4, -5, -6,-11) in Brazil, Australia, Italy, South Korea, Japan, Hong Kong while only the VIM-2 was found in South Korea (Lee et al., 2003; Poirel and Nordmann, 2006; Perez et al., 2007).

Moreover, class D beta-lactamase has been identified either on the chromosome or plasmids including *bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-24/40}}$ and *bla*$_{\text{OXA-58}}$ (Poirel and Nordmann, 2006). Recently, the *bla*$_{\text{OXA-23}}$ was found in *A. baumannii* isolates from Brazil, China, Iraq, Europe, Singapore, South Korea and the USA. The *bla*$_{\text{OXA-24/40}}$ was found in the United States, France, Portugal, France, Belgium, Spain (Peleg et al., 2008) whereas the *bla*$_{\text{OXA-51}}$ gene was a naturally occurring chromosomal enzyme in *A. baumannii* (Perez et al., 2007). Most frequently, the *bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-58}}$-genes have been identified in *A. baumannii*. These genes may promote carbapenem resistance in association with *bla*$_{\text{OXA-51}}$ (Turton et al., 2006).

The *bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-40}}$-genes could produce higher levels of resistance to carbapenems than *bla*$_{\text{OXA-58}}$ genes and that correlates with overexpression of AdeABC efflux pump of *A. bumannii*. These promoted the level of resistance to beta-lactam antibiotics, particular carbapenems (Heritier et al., 2005). The *bla*$_{\text{OXA-58}}$ of *A.*

baumannii has been reported in Argentina, Romania, Spain, Turkey, Belgium, France, Kuwait, the UK, Greece, Italy, Austria, and Scotland (Peleg et al., 2008) in Table 2-1.

The informations on the outer membrane proteins (OMPs) of *A. baumannii* were very limited as compared to those on the other gram-negative pathogens. The loss of a 29-kDa OMP (as known as CarO) was shown to be associated with imipenem and meropenem resistance (Limansky et al., 2002). In addition, the loss of a 33-36 kDa OMP of *A. baumannii* was associated with imipenem resistance (Clark, 1996; Poirel and Nordmann, 2006). Likewise, the isolates from Spain, the loss of 22-kDa and 33-kDa OMPs and the production of OXA-24 were shown a high resistance to carbapenems (Bou et al., 2000).

The alteration of penicillin-binding-proteins (PBPs) is also one of the resistance mechanism of carbapenem-resistance in *A. baumannii*. The mutation causing the hyper-produced a 24-kDa PBP which was a low molecular weight protein was related to the resistance to imipenem (Gehrlein et al., 1991). Moreover, another study has described 12 PBP patterns among a collection of *A. baumanii* isolates with variable beta- lactam resistance profile. In the isolates with imipenem MIC > 4mg/L, the loss of a 73.2- kDa PBP (PBP-2) was associated with resistance of *A. baumannii* to carbapenem compounds (Fernandez-Cuenca et al., 2003).

Moreover, the efflux mechanism known as AdeABC efflux belongs to the resistance-nodulation-division (RND) family type pump. AdeABC efflux pump of *A. bamannii* associated resistance to various antimicrobial agents including beta-lactams (carbapenems), aminoglycosides, erythromycin, chloramphenicol, tetracyclines, fluoroquinolones, trimethoprim and ethidium bromide (Peleg et al., 2008). Overexpression of the AdeABC efflux can increase the pump out of the antimicrobial agent from the bacteria cell (Wieczorek et al., 2008), which relates to the decrease susceptibility to other antimicrobial agents.

Table 2-1 The distribution of acquired carbapenemases identified in *A. baumannii*

β-lactamase	**Detail**	**Distribution**
IMP-1, -2, -4, -5, -6,-11	Class B metallo beta-lactamases Plasmid gene	Brazil, Australia, Italy, South Korea, Japan, Hong Kong
VIM-2	Class B metallo beta-lactamases Plasmid gene	South Korea
OXA-23	Class D beta-lactamases Plasmid	Brazil, Chaina, Iraq, Europe, Singapore, South Korea, USA
OXA-24/40	Class D beta-lactamases Chromosomal or plasmid genes	United States, France, Portugal, Spain, Belgium
OXA-58	Class D beta-lactamases Plasmid or chromosomal genes	Argentina, Romania, Spain, Turkey, Belgium, France, Kuwait, UK, Greece, Italy, Austria, Scotland,
OXA-51	Chromosomal class D beta-lactamase intrinsic to *A. baumannii*	naturally occurring

4. Global susceptibility rate of *A. baumannii*

Initial concern about carbapenem-resistant *A. baumannii* began in 1991 when the first hospital-wide outbreak occurred in New York (Urban et al., 1993). This was followed by a rapid extending of carbapenem-resistance outbreaks by *A. baumannii,* which have been increasing reported from many parts of the world, including Argentina, Belgium, Brazil, Cuba, England, France, Hong Kong, Kuwait, Singapore, Spain and Thailand (Corbella et al., 2000; Danchaivijitr et al., 2005).

The discovery rates of *A. baumannii* from natural environments and in the community are low. Whereas the carrier rate by hospitalized patients is high, which appear frequently in the ICU environment. In addition, *A. baumannii* can survive under dry conditions, but especially over moist surfaces (Dijkshoorn et al., 2007). The main transmission of *A. baumannii* is through direct contact and can be spread to patients by hospital personnel with skin colonizations and contaminated medical equipment which causes the ventilator-associated pneumonia, central-line-related bloodstream infections and catheter-related urinary tract infections (Bergogne-Berezin and Tower, 1996).

Furthermore, there are reports of a global susceptibility rate for *A. baumannii* from several areas of worldwide medical centers including, Asia/Pacific, Europe, Latin America, North America in Table 2-2.

Table 2-2 International surveillance of susceptibility rate of *A. baumannii*

Geographic area	Study/location	Year	Susceptibility (%)									Reference
			CAZ	PB	FEP	MER	IMP	TZP	CIP	LVX	GEN	
Asia/Pacific	SENTRY	2001-2004	58	-	58	73	74	-	-	-	-	Gale, Jone, and Sader, 2006
	Korea (hospital isolates)	2003	45	-	59	75	87	58	42	-	36	Lee et al., 2006
	China (ICUs)	2002	65	-	70	-	92	70	66	-	-	Wang and Chen, 2005
	Japan (hospital isolates)	2002	89	-	85	-	95	-	-	-	-	Ishii et al., 2005
Europe	SENTRY	2001-2004	40	-	44	70	74	-	39	-	-	Gale, Jone, and Sader, 2006
	France (ICUs)/TSN	2000-2002	35	-	28	68	94	75	38	-	49	Jone et al., 2004
	Italy (ICUs)/TSN	2000-2002	26	-	18	75	78	35	21	14	23	Jone et al., 2004
	Germany (ICUs)/TSN	2000-2002	67	-	74	96	96	82	75	82	82	Jone et al., 2004
	Spain (hospital isolates)	2001	24	-	49	49	60	17	7	10	15	Picazo et al., 2004
Latin America	SENTRY	2001-2004	32	-	36	84	86	-	35	-	-	Gale, Jone, and Sader, 2006
	Agentina (hospital isolates)	2001-2002	23	-	37	-	85	22	-	17	-	Casellas et al., 2003
	Brazil/SENTRY	2001	29	-	37	97	98	31	33	33	39	Jone et al., 2004
	SENTRY	1997-2001	29	96	35	87	87	27	31	32	33	Tognim et al., 2004
North america	SENTRY	2001-2004	54	-	57	84	89	-	54	-	-	Gale, Jone, and Sader, 2006
	United States (hospital isolates)/SENTRY	1998-2003	62	-	63	-	93	63	61	-	64	Sader, Fritsche, and Jones,2005
	United States (hospital isolates)/MYSTIC	2003	64	-	63	87	92	61	59	60	63	Rhomberg et al., 2004
	United States (ICUs)	2001	49	-	56	91	96	-	45	54	53	Karlowsky et al., 2003

SENTRY, SENTRY Antimicrobial Surveillance Program; MYSTIC, Meropenem Yearly Susceptibility Test Information Collection; TSN, The Surveillance Network
CAZ, ceftazidime; PB, polymyxin B; FEP, cefepime; MER, meropenem; IMP, imipenem; TZP, piperacillin-tazobactam; CIP, ciprofloxacin; LVX, levofloxacin;
GEN, gentamicin

A report from the SENTRY antimicrobial surveillance programme, 2001-2004, demonstrated that the antimicrobial susceptibility of *Acinetobacter* spp. varied according to their geographical origin (Table 2-2). Europe and the Asia-Pacific region were observed among isolates collected to have the lowest susceptibility rates to carbapenems (73.7% susceptible to imipenem). Meanwhile the isolates from Latin America exhibited the lowest susceptibility rates to broad-spectrum cephalosporins (32.4% susceptible to ceftazidime), fluoroquinolones (ciprofloxacin 34.8%) (Gales et al., 2006).

In 2002-2004, the data from Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Program showed that normally the susceptibility of Australasia and North America was higher than that from Europe and South America (Table 2-2). The result of antimicrobial agents tested against a worldwide collection of *Acinetobacter* spp. was meropenem (76.1% susceptible) followed by imipenem (74.7%), gentamicin (51.9%), ciprofloxacin (40.5%), piperacillin/tazobactam (39.8%) and ceftazidime (38.1%) (Unal and Garcia Rodriguez, 2005).

In 1997-2001 data from SENTRY antimicrobial surveillance programme, showed a good activity of polymyxin B (96.4%) against multidrug-resistant *Acinetobacter* spp. (Tognim et al., 2004). In addition, in 2001-2004 it reported that among isolates of *Acinetobacter* spp. resistant to polymyxin B, rates from 1.9% in the Asia-Pa cific region, 2.7% in Europe and 1.7% in North America and Latin America. The isolation of polymyxin B resistant *Acinetobacter* spp. had polymyxin B MIC $\geq$ 8 µg/ml (Gales et al., 2006). However, the data regarding polymyxin resistance is still limited.

Table 2-3 Percentage susceptibility of antimicrobial agent of *A. bauamnnii* in Thailand during year 1998-2007

Year	% susceptible of antimicrobial agents						
	CAZ	IMP	CIP	TZP	AMK	GEN	NET
1998	40	98	45	-	48	34	68
1999	41	94	49	-	47	34	70
2000	35	95	41	-	44	34	62
2001	36	92	42	-	42	35	56
2002	38	79	40	-	42	36	56
2003	33	65	34	34	38	34	60
2004	35	55	35	34	39	33	59
2005	30	27	31	18	38	30	57
2006	30	43	29	29	36	32	-
2007	30	43	29	29	36	32	-

CAZ, cetazidime; IMP, imipenem; CIP, Ciproflxacin; TZP, Piperacillin-tazobactam; AMK, amikacin; GEN, gentamicin, NET, netilmicin

Moreover, data from the National Antimicrobial Resistance Surveillance, Thailand (NARST) from 1998 to 2007 showed that *A. baumannii* decreased the susceptibility rate to imipenem from 98% to 43%, ceftazidime from 40% to 30%, ciprofloxacin from 45% to 29% and amikacin from 48% to 36% as shown in Table 2-3. Besides, the point prevalence survey of nosocomial infections from 42 hospitals in Thailand in 2001, showed the average of cost of antimicrobials for treatment (as 5,919 Baht/patient), a high mortality rate of 13.8% and prolonged hospital stays, all of which indicated a serious problem for health care (Danchaivijitr et al., 2005).

5. Therapy of *A. baumannii* infections

Acinetobacter baumannii are important pathogens which cause nosocomial infections and the most of this organism can develop resistance to almost all antimicrobial agents making the infections difficult to treat.

Carbapenems (including, imipenem, moropenem and doripenem) are the first choices for treating severe infections caused by multidrug-resistant *A. baumannii*. This drug has shown active activity *in vitro* and in the experimental data (Fishbain and Peleg, 2010). In the *in vivo* study by Joly-Guillou et al, (1997), imipenem showed a prolonged postantibiotic effect on a lung model with severe nosocomial pneumonia caused by *A. baumannii*. Imipenem is active *in vivo* against *A. baumannii* with low levels of resistance (MIC= 8 µg/ml), but this drug is inactive against strains with high resistance levels (Montero et al., 2004). In 2007, doripenem was approved as a new carbapenem by the FDA (the Food and Drug Adminstration). Doripenem is a broad-spectrum of carbapenem active against gram-positive and nonfermenting gram-negative pathogens including *P. aeruginosa* and *A. baumannii* (Pillar et al., 2008). This drug was used to treat for complicated urinary tract, complicated intra-abdominal infections (Sahm, 2009). In addition, it reported *in vitro* activity of doripenem was superior to impenem and meropenem against *A. baumannii* with the $bla_{\text{OXA-58}}$ gene (Marti et al., 2009).

Sulbactam is a beta-lactamases inhibitor. It exhibits bactericidal activity against *A. baumannii* (Peleg et al., 2008). The clinical practice is using sulbactam (commercially available in combination with ampicillin) for treating severe *A. baumannii* infections. In addition, Betrosian et al. (2008) reported ampicillin/sulbactam with high doses were comparably safe and efficacy for treatment in patients with VAP due to multidrug-resistant *A. baumannii* and do not suggest as superior for multidrug-resistant *A. baumannii*. However, this drug may be an alternative treatment for meningitis with *A. baumannii* resistant to imipenem and other beta-lactam (Jimenez-Mejias et al., 1997).

Aminoglycosides are remaining active against *A. bauamnnii* including, amikacin and tobramycin. However, these drugs were found to be more resistant and not used alone because of high toxicity (Fishbain and Peleg, 2010).

Recently, carbapenems and multidrug-resistant *A. baumannii* remained susceptible only to colistin and tigecycline, which showed good activity *in vitro* and as an alternative treatment for nosocomial infections (Livermore et al., 2010). Tigecycline is a new drug in the glycylcycline class, it has broadspectrum activity against gram-positive bacteria and gram-negative pathogens *in vitro* (Garnacho-Montero and Amaya-Villar, 2010). In 2005, tigecycline was approved by the FDA for treatment of complicated skin and skin structure infections and complicated intra-abdominal infections (Greer, 2006). However, tigecycline was less efficacious than imipenem in the treatment of experimental murine pneumonia caused by *A. baumannii* (Pichardo *et al*., 2010) while the data did not support enough in clinical use, particularly for bacteraemia or VAP (Karageorgopoulos et al., 2008) because the mostly resistance mechanism is efflux pump mechanism that related to reduce susceptibility to other antimicrobials agent such as aminoglycoside, fluoroquinolone, erythromycin and tetracycline (Peleg et al., 2007).

Colistin

Currently, the problems of outbreaks from severe infections in *A. bauamnnii* due to several mechanisms resistance are causes by the lack of new antimicrobial agents against *A. bauamnnii*. Recently colistin was used for the treatment of infections caused by Gram-negative pathogens including *P. aeruginosa* and *A. baumannii* which were resistant to all antimicrobial agents (Falagas et al., 2005). Moreover, colistin is not active to gram-positive bacteria and most anaerobes (Mendes and Burdmann, 2010).

Colistin is in the polymyxins family which consists of five different forms (A, B, C, D, and E). Only two major forms including, polymyxin B and polymyxin E are used clinically (Chen and Kaye, 2009). Colistin was isolated by *Bacillus colistinus*.

Colistin as known as polymyxin E was first used in Japan in 1949 and in Europe and the United States in 1950 and 1959, respectively (Reed, et al., 2001). It was used to treat common infections for 20 years until other antimicrobial agents were found with more efficacy and more safety, so it was greatly reduced around 1980, because of the adverse effects particularly neurotoxicity and nephrotoxicity (Falagas and Kasiakou, 2005). However, the prevalence of resistance to all available agents is beginning to increase. Furthermore, colistin was used for the treatment of various infection sites, including pneumonia, urinary tract infectios, central nervous system infections, otitis media, peritonitis, catheter-related infection and bacteraemias caused by multi-resistant *A. baumannii* (Levin *et al*., 1999; Markou et al., 2003).

Colistin is a cationic polypeptide antibiotic that exhibits bactericidal activity against gram-negative bacteria. The mechanism of action involves interaction with surface lipopolysaccharides and phospholipids of the outer membrane. Colistin binds to anionic lipopolysaccharide in the outer membrane by competitively displacing divalent cations (Ca^{2+} and Mg^{2+}) that stabilize the lipopolysaccharide molecule. Thus colistin penetrates between lipopolysaccharide molecules, the cause of increased permeability in the cell membranes, leads to the leak of cytoplasmic membrane and cell death (Newton, 1956; Hancock, 1997) in Figure 2-2. However, colistin has toxicity and poor pharmacokinetic if used alone. Therefore, the use of a combination of antibacterial drugs could decrease the dose and toxicity of the drugs (Rahal, 2006).

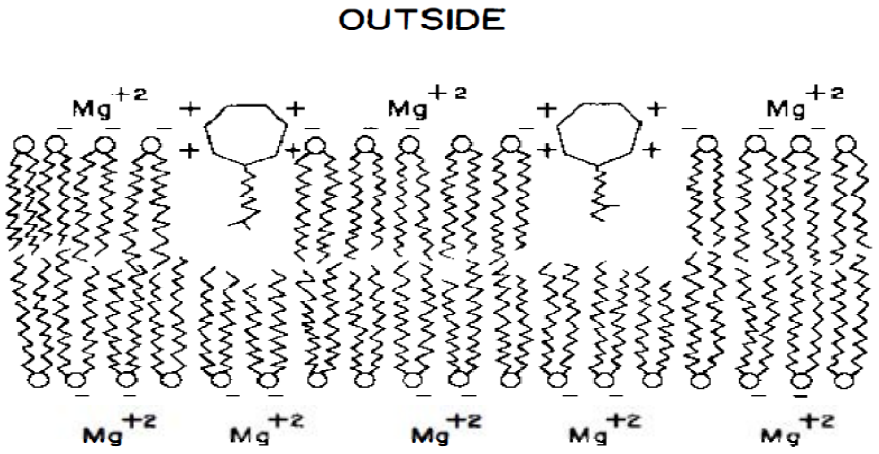

Figure 2-2 Mechanism of action of cationic polypeptide (Storm et al., 1977)

Colistin contains a cationic cyclic decapeptide linked to a fatty acid chain. The amino acid components in the molecule of colistin are D-leucine, L-threonine and L-α, γ-diaminobutyric acid in Figure 2-3. There are two forms of colistin for clinical use. The first one is colistin sulphate: which is administered topically for the treatment of skin infections and is used orally to destroy bacteria in the intestine because this form cannot be absorbed. The second form is colistimethate sodium or colistin methanesulphonate (CMS): which is administered parenterally, nebulization, intrathecally and intraventricularly. In addition, colistimethate sodium is a non-active prodrug, which is less toxic than colistin sulphate and not stable *in vivo* or *in vitro* (Falagas *et al*., 2005; Li *et al*., 2005).

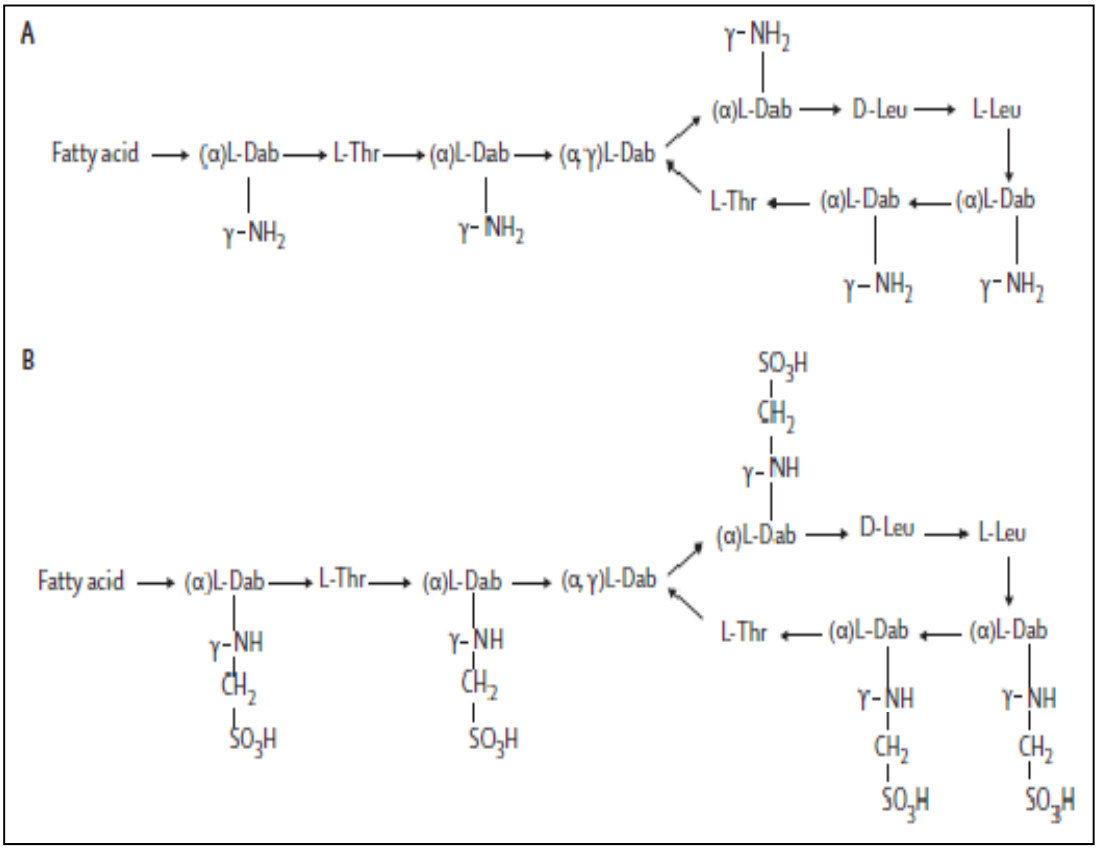

Figure 2-3 Chemical structure of (A) Colistin, (B) colistimethate sodium; Leu=leucine; Thr=threonine; Dab=α, γ-diaminobutyric acid. α and γ indicate the respective amino group involved in the peptide linkage (Li *et al*., 2006)

Clinical uses

The intravenous of colistimethate sodium recommended for patients with normal renal functions with weights less than 60 kg is 2.5-5 mg/kg (31,250-62,500 IU/mg) per day divided into 2-4 doses in the United States, while in the United Kingdom that dosage is 4-6 mg/kg (50,000-75,000 IU/kg) per day, divided into three doses and 80-160 mg (1-2 million IU) three times a day for patients weighing more than 60 kg (Falagas and Kasiakou, 2005).

There were reports of colistimethate sodium that showed good outcomes in patients with multidrug-resistant *A. baumannii* particularly pneumonia (Fishbian and Peleg, 2010). However, many authors reported the dosages of colistin remain confusing because the formulations differ between countries (Falagas and Kasiakou, 2005). In addition, the most common adverse effects of colistin are nephrotoxicity and neurotoxicity. In particular, the nephrotoxic effect is associated with acute tubular necrosis as it decreased the creatinine clearance and increased the creatinine level, serum urea (Reed et al., 2001). Both adverse effects were also correlated to high concentrations of colistin. Therefore, there should be concern about the appropriate dosing for treatment in patients with renal failure. Moreover, it was reported that aerosolized colistimethate sodium has been used as supplementary therapy with conventional intravenous antibiotic in the patients with pneumonia due to multi-resistant gram-negative including *P. aeroginosa* and *A. baumannii* in the critical care setting (Kwa et al., 2005). In addition, it was recommended in the inhalation dosage form: colistimethate sodium (CMS) 40 mg every 12 hours for patients with body weights less than 40 kg while the patients with body weights greater than 40 kg for 80 mg every 12 hours (Falagas and Kasiakou, 2005). However, the administration by inhalation colistin sodium showed bronchoconstriction in patients with cystic fibrosis (Reed et al., 2001). Recently, colistimethate sodium (5 mg/kg/day in 2 divided doses) was demonstrated for treatment in Thai patients with infections of multi-drug resistant *A. baumannii* and *P. aeruginosa* at Siriraj Hospital between January 2005 and April 2006, the results showed a good clinical outcome, including reduced mortality and low cost (Koomanachai et al., 2007).

Mechanisms of resistances

The mechanism of resistance to colistin is not yet clear. It might be either decreased permeability by efflux pump or decreased concentration of Mg^{2+} and Ca^{2+} in the outer membrane, but not occurring enzyme production (Falagas and Kasiakou, 2005; Moore et al., 1984). However, the recently viewed data of development resistance to colistin is rare (Bonomo and Szabo, 2006).

Rifampicin

Rifampicin was first introduced in 1965, a semi-synthetic derivative of rifampicin B, which was produced by *Streptomyces mediterranei*. This drug has a broad spectrum against gram-positive bacteria, gram-negative and mycobacteria, particularly *Mycobacterium tuberculosis* (Thornsberry et al., 1983). Rifampicin is orange-brown to red – brown and is soluble in organic solvents (Kenny and Strates, 1981). The chemical structure of rifampicin is $C_{43}H_{58}N_4O_{12}$ in Figure 2-4.

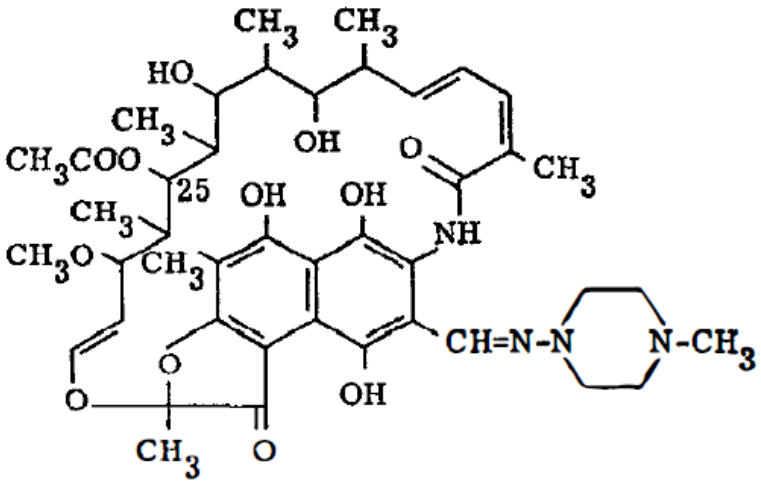

Figure 2-4 Chemical structure of rifampicin (Lester, 1972)

In 1968, rifampicin was the most used drug for the treatment of tuberculosis. It is combined with other drugs including isoniazid, ethambutol, pyrazinamide and streptomycin because rifampicin can diffuse freely into cells and is active against intracellular mycobacteria (Chen and Kaye, 2009).

The mechanism of action is to inhibit DNA dependent RNA polymerase and interfere with the transcription of mRNA transfer bacteria to make proteins (Wehrli et al., 1968). Rifampicin is considered to be a bactericidal antimicrobial, but it may be bacteriostatic depending on the concentration of the drug and the organism (Thornsberry et al., 1983; Chen and Kaye, 2009). In addition, the monotherapy of rifampicin is occurs rapidly resistance both *in vitro* and *in vivo*. There have been reports that the combination of rifampicin with other antimicrobial agents, which have shown enhanced activity against multidrug-resistant strains in order to avoid the development of drug resistance during treatment (Garnacho-Montero and Amaya-Villar, 2010).

Rifampicin is currently available in two dosage forms, including capsules (150, 300) mg and powder for injection 600 mg/vial. In addition, rifampicin (orally) is rapidly absorbed from the intestine and widely distributed into many tissues and fluids of the human body (Kenny and Strates, 1981). It has less toxicity and excretes less in urine (Titarenko et al., 1983).

Mechanism of resistance

Generally, rifampicin has been resistant to various mechanisms (1) mutation in the gene (*rpoB*) encoding the beta subunit of DNA-dependent RNA polymerase, (2) decrease permeability in the outer membrane (Wehrli *et al*., 1983).

6. Combinations of antibiotics

The combination has been demonstrated *in vitro*, *in vivo* and clinical trials on the use of new drugs or old drugs.There are advantages for the decreasing emergence of resistance and reduced toxicity between each agent by using low doses. The previous studies reported both *in vitro* and animal studies which support the combined therapy.

In addition, several non-traditional antimicrobials were combined between those antimicrobials, particularly in combination with polymyxin B or colistin for alternatives against multidrug resistant strains. In 1998, the activities of the two-drug combination of rifampicin, polymyxin B and ampicillin/sulbactam against 5 isolates of multidrug-resistant *A. baumannii* showed the synergy between the combination of rifampicin plus polymyxin B (3 out of 5 isolates) and rifampicin plus ampicillin/sulbactam (2 out of 5 isolates) while the combination of polymyxin B plus ampicillin/sulbactam showed indifference (Tascini *et al*., 1998).

The study by Hogg et al. (1998) demonstrated the *in vitro* combination activities of colistin plus rifampicin against 13 isolates of multidrug-resistant *A. baumannii* by the checkerboard method, there were 11 out of 13 isolates which showed synergic effect (2 out of 13 isolates showed indifference and non antagonist). Corresponding with Giamarellos-Bourboulis et al. (2001) who reported the interactive activities of 1× MIC colistin or 4× MIC colistin with rifampicin on a large number of strains (39 isolates of multidrug-resistant *A. baumannii* were tested) by the time kill studies, the activity was increased when in the presence of rifampicin.

Yoon et al. (2004) demonstrated the activities of double and triple combinations of imipenem, polymyxin B and rifampicin against 8 isolates of multidrug-resistant *A. baumannii* by the checkerboard and time kill studies. The double combinations of polymyxin B + imipenem and polymyxin B + rifampicin showed the bactericidal activity against 7 out of 8 isolates while the triple combinations showed the bactericidal activity against all isolates at 24 hours of

incubation. This was similar to Tripodi et al. (2007) which reported the double combinations of colistin with rifampicin or imipenem or sulbactam/ampicillin, imipenem with rifampicin and the triple combinations of imipenem + colistin + rifampicin and sulbactam/ampicillin +colistin+rifampicin. The double and triple combinations showed good bactericidal activities against *A. baumannii* which produced OXA-58 carbapenemases. However, Wareham et al. (2006) reported that no synergistic effect was observed of from the *in vitro* combination activities of polymyxin B with imipenem, rifampicin or azithromyxin against multidrug-resistant *A. baumannii* which produced OXA-23 carbapenemases. Thus, they suggested that such combinations should not be used for empirical treatment.

Furthermore, the combined effect of non-traditional antimicrobials included the combination of colistin with azithromycin or doxycycline or rifampicin against multidrug-resistant *A. buamannii* were determined by the checkerboard method. The *in vitro* synergistic activity of colistin with rifampicin against multidrug-resistance pathogens were observed by Timurkaynak et al. (2006). In accordance with the study by Song et al. (2007) which showed that the combined effect of colistin with rifampicin was higher than the activity of the combination between imipenem with sulbctam against 43 carbapenem-resistant *A. bauamannii* isolates

Rifampicin showed good efficacy in various combinations with other antimicrobials in animal models (Pachon-Ibanez et al., 2006; Saballs et al., 2006). The studies of the combination of rifampicin with tobramycin, aminoglycoside or colistin in the mouse with *A. baumannii* pneumonia model, may be advantageous even if this organism exhibited moderate resistance to rifampicin (Montero et al., 2004). In general, rifampicin should not be use as monotherapy because of the development of resistance during treatment (Hogg *et al*., 1998).

Recently, Pongpech et al. (2010) evaluated the *in vitro* activity of double and triple combinations against multidrug-resistant *A. baumannii* in Thailand which showed that imipenem combined with colistin was superior over a single agent.

Moreover, the triple combination of meropenem+colistin+sulbactam showed more effective than the double combination.

The combination therapy appears to be an alternative treatment to fight the resistance of *A.baumannii*. The previous study by Lee et al. (2005) demonstrated the role of sulbactam in the treatment of 2 groups of patients with infections caused by pan-resistant *A. bauamnnii*. The first group was treated by the combination of sulbactam plus carbapenem and the second group was treated by second or third generation cephalosporins, antipseudomonas penicillins or fluorquinolones plus aminoglycosides. The outcomes of the 2 groups were not significantly different but the combination of sulbactam and carbapenem showed low MICs for pan-resistant *A. baumannii*. In addition, it reported that a retrospective study involving 55 patients with bacteraemia due to multidrug-resistant *A. baumannii* were treated by the combination of carbapenem plus ampicillin-sulbactam that showed the outcome (mortality rate 30.8%) better than carbapenem plus amikacin (mortality rate 50%) or carbapenem alone (mortality rate 58.3%) (Kuo et al., 2007).

Moreover, Saballs et al. (2006) studied the effect of the combination between imipenem and rifampicin in 10 patients with carbapenem-resistant *A. baumannii* infections. The results did not suggest the benefit of this therapy regimen. At the same time, the study by Falagas et al. evaluated the efficacy and nephrotoxicity of colistin monotherapy compared with the combination therapy of colistin and meropenem in patients with multidrug-resistant Gram-negative bacteria, the outcome of monotherapy was not significantly different from the combination therapy.

The first study for human data in Morocco reported the combined effect between colistin and rifampicin against *A. baumannii* isolated from critically ill patients with multidrug-resistant infection. The combination therapy showed favourable outcomes for all patients, including patients with nosocomial pneumonia, meningitis and bacteraemia, but with a limited number of patients and the lack of a control group (Motaouakkil et al., 2006). Similarly, the study of Bassetti et al. (2008) on 29 patients with bacteraemia and pneumonia due to resistance to all antibiotics

(except colistin) in intensive care units, the patients were treated with intravenous colistin sulphomethate sodium (2 million IU, 3 times/day) plus intravenous rifampicin (10 mg/kg every 12 hours), the results of treatment showed mortality as 21% and 10% of the patients had nephotoxicity development during treatment. At present, the appropriate dosing regimen for the treatment is still unknown.

CHAPTER III

MATERIALS & METHODS

MATERIALS

1. Microorganism

The 30 carbapenems-resistants *A.baumannii* isolates used throughout this study were clinically isolated from different patients in Bumratnaradoon Hospital, Thailand during April 2007- May 2009. *Escherichia coli* ATCC 25922 was used as the control strain. All isolates were stored at -20°C in tryptic soy broth: glycerin (85:15). Each isolate was subcultured twice before testing in order to ensure active growing culture on the tryptic soy agar plates.

2. Chemicals

- Standard powders

Standard powder of rifampicin (potency 958 µg/mg) was kindly provided by Siam Bheasach, Thailand and standard powder of colistin (potency 656.53 µg/mg) was purchased from Sigma (U.S.A.). The working standard solutions were prepared immediately prior to use, as specified by the manufacturers.

- Susceptibility disks

The twelve antimicrobial disks used to determine the susceptibility pattern of the bacterial strains were rifampicin (5 µg/disk), meropenem (10 µg/disk), ceftazidime (30µg/disk) and cefepime (30µg/disk) from BBL chemicals (Benex Limited,USA), imipenem (10 µg/disk) and ciprofloxacin (5µg/disk) from BBL chemicals (Becton Dickinson, USA), amikacin (30µg/disk), piperacillin/tazobactam (100/10 µg/disk), tobramycin (10 µg/disk), gentamicin (10 µg/disk), netilmicin (30 µg/disk) and colistin (10 µg/disk) from Oxiod Ltd., England.

METHODS

1. Kirby-Bauer disk diffusion method

The Kirby-Bauer disk diffusion method was performed according to the disk diffusion method by Clinical and Laboratory Standards Institute (CLSI, 2010). *E. coli* ATCC 25922 was used as the control strain in this study. All isolates were tested to determine their susceptibility to 12 antimicrobial agents.

1.1 Preparation of media

Mueller-Hinton agar (MHA) was prepared from a commercially available dehydrated medium according to the manufacturer's instructions. Immediately after autoclaving, it was allowed to cool at 50°C in a water bath. Twenty-five ml of sterile prepared medium was pipetted into the petri dishes (with a diameter of 90 mm). This corresponded on a level surface to the medium depth of 4 mm. The agar medium plates were allowed to solidify at room temperature. Unless the plates were used the same day, the plates should be stored in a refrigerator at 4°C and should be used within 7 days after preparation.

1.2 Preparation of inoculums

Three to five well-isolated colonies of *A. baumannii* from clinical specimen and *E. coli* ATCC 25922 were selected from tryptic soy agar (TSA) plates and transferred to a tube containing 7 ml of sterile normal saline solution. The suspension was adjusted to match the turbidity of a 0.5 McFarland standard. This resulted in a bacterial suspension containing approximately 1 to 2×10^8 CFU/ml.

1.3 Inoculation of the test plates

A sterile cotton swab was dipped into the inoculum suspension within 15 minutes after adjusting the turbidity of the inoculum suspension. The excess inoculum was removed by pressing the swab against the wall of the test tube. The dried surface of an agar plate was inoculated by streaking the swab over the entire sterile agar surface. This process was repeated three times, rotating the plate approximately 60°C each time to ensure an even distribution of the inoculum.

1.4 Application of disks to inoculated agar plates

The antibiotic disks were placed onto the surface of the medium by using a sterile forcep. Each disk was pressed down firmly to ensure complete contact with the agar surface. The disks were placed evenly, not closer than 24 mm apart from one another. The plates were inverted and incubated in an incubator at 37°C for 24 hours before measuring the zones of inhibition.

1.5 Reading plates and interpreting results

After 24 hours of incubation, each plate was examined. The diameters of each zone of inhibition were measured by an automatic digital caliper vernier. The size of the inhibition zone was interpreted by the CLSI (2010) in order to make an interpretation of susceptible, intermediate, or resistant of each agent that have been tested (Tables 3-1).

Table 3-1 Zone diameter interpretive standards breakpoints for *A. baumannii* and *E. coli* ATCC 25922 to 12 antimicrobial agents (CLSI, 2010).

Antimicrobial Agents	Disk Content (µg/ml)	Zone Diameter (mm)			
		A.baumannii			*E. coli* ATCC 25922
		R[a]	I[b]	S[c]	
Amikacin	30	≤14	15-16	≥17	19-26
Cefepime	30	≤14	15-17	≥18	31-37
Ceftazidime	30	≤14	15-17	≥18	25-32
Ciprofloxacin	5	≤15	16-20	≥21	30-40
Colistin	10	≤12	13	≥14	11-17
Gentamicin	10	≤12	13-14	≥15	19-26
Imipenem	10	≤13	14-15	≥16	26-32
Meropenem	10	≤13	14-15	≥16	28-34
Netilmicin*	30	≤15	-	≥15	22-30
Piperacillin/tazobactam	100/10	≤17	18-20	≥21	24-30
Rifampicin**	5	≤16	17-19	≥20	8-10
Tobramycin	10	≤12	13-14	≥15	18-26

R[a] = Resistant, I[b] = intermediate, S[c] = susceptible

*The inhibition zone from netilmicin was interpreted based on the EUCAST guideline (2010).

**The inhibition zone from rifampicin was interpreted based upon the recommendation for *Staphylococcus* spp. (inhibition zone < 20 mm, resistant) following CLSI guideline (2010).

2. Agar dilution method

The Agar dilution method was performed according to CLSI (2010) in order to determine the minimal inhibitory concentration (MIC) of colistin and rifampicin against all 30 isolates.

2.1 Preparation of agar dilution plates

The two-fold dilution of colistin solution (0.03-256 µg/ml), and rifampicin solution (0.03-256 µg/ml) were prepared because the final volume in each plate consisted of 2.5 ml of each dilution antimicrobial agent and 22.5 ml of MHA. Thus, the antimicrobial concentrations used in the initial (stock) solutions were prepared ten-fold greater than the desired final concentrations.

The MHA was prepared from a commercially dehydrated medium according to the manufacturer's instructions. Immediately after autoclaving, the melted agar was allowed to cool to 55°C in a water bath and then 6 ml of each antimicrobial dilution was pipetted into MHA 54 ml. The agar and antimicrobial agent solution were mixed thoroughly and then 25 ml was pipetted into each plate. The agar dilution plates were allowed to solidify at room temperature, and used immediately.

2.2 Preparation of inoculums

Three to five well-isolated colonies of *A. baumannii* from clinical specimens and *E. coli* ATCC 25922 were selected from Tryptic Soy Aagar (TSA) plates and transferred to a tube containing 7 of ml sterile normal saline solution. The suspension was adjusted to match the turbidity of a 0.5 McFarland standard. This resulted in a bacterial suspension contained approximately 1 to 2×10^8 CFU/ml. The 200 µl-inoculum suspension was transferred into inoculum replicators.

2.3 Inoculation agar dilution plates

The agar plates were marked for orientation of the inoculum spots. One µl of each inoculum was applied to the agar surface by use of an inocula-replicating device. The final inoculum on the agar was approximately 10^4 CFU per spot. A control plate (no antimicrobial agent) was inoculated at the start of each agar dilution run and at the end in order to evaluate an organism's ability to grow on the agar plate. The agar dilution plates were inoculated from the lowest concentration to the highest concentration.

2.4 Incubation of agar dilution plates

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

2.5 Determination of minimum inhibitory concentrations

The MICs were recorded at the lowest concentration of antimicrobial agent that completely inhibited the growth, a faint haze or a single colony of possible growth was generally disregarded. The MICs were interpreted by the CLSI (2010) in order to make an interpretation of susceptible, intermediate, or resistant for each agent that had been tested (Tables 3-2).

Table 3-2 MIC interpretive standard (µg/ml) for breakpoint by agar dilution method for *A. baumannii* (CLSI 2010).

Antimicrobial Agents	Minimum Inhibitory Concentrations (MICs) (µg/ml)		
	A. baumannii		*E. coli* ATCC 25922
	S	R	
Colistin	≤2	≥4	0.5-2
Rifampicin	≤1	≥4	4-16

S = Susceptible

R = Resistant interpreted based upon the recommendation for *Staphylococcus* spp. because the recommendation for Gram-negative bacteria could not be observed.

3. Checkerboard method

The Checkerboard method was performed according to Pillai, Moellering and Eliopoulos (2005). All isolates were determined for the combined effects of colistin and rifampicin. The concentrations tested for colistin were 0.03, 0.06, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16 and 32 µg/ml while the concentrations of rifampicin were 0.5, 1, 2, 4, 8, 16 and 32 µg/ml, respectively.

3.1 Preparation of test broth

Mueller-Hinton broth (MHB) was prepared from a commercially dehydrated medium according to the manufacturer's instructions. The medium concentrations used in the initial solutions were four-fold greater than the desired final concentrations.

3.2 Preparation of antimicrobial solution

The two-fold dilutions of antimicrobial agents were prepared volumetrically in the broth. The concentrations of colistin and rifampicin used in the initial solution were four-fold greater than the desired final concentrations. The concentrations tested for each antimicrobial agent ranged from four to five dilutions lower than the MIC and at least two dilutions higher than the MIC.

3.3 Broth dilution test

A standardized inoculum for the microdilution broth method was prepared by suspending colonies of the tested isolates directly into sterile normal saline solution adjusted to match the turbidity of a 0.5 McFarland standard. The adjusted inoculums suspension was diluted in broth within 15 minutes after the inoculation, each well contained approximately 5×10^5 CFU/ml.

The final volume of 200 µl in each well consisted of 50 µl of MHB, 50 µl of colistin, 50 µl of rifampicin and 50 µl of bacterial suspension. The series of antimicrobials containing four times the desired final concentrations were taken to produce the desired range of drug concentration by adding an aliquot of those solutions to each well in the appropriate row or column as shown in Figure 3-1.

Rifampicin (µg/ml)

32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure 3-1 checkerboard technique, serial dilution of colistin and rifampicin are performed using drugs proportional to MICs of the drug being tested (Modified from Pillai, Moellering and Eliopoulos, 2005).

3.4 Interpretation of results

After 24 hours, each well was examined to determine the MIC; the MIC is the lowest concentration of antimicrobial agent that completely inhibits growth of the organism in the wells as detected by the unaided eye. The amount of growth in the wells containing the antimicrobial agent was compared with the amount of growth in the positive-control well (no antibiotics) and the negative-control well (no organism) used in each set of tests when determining the growth end points. The interpretation of the antimicrobial combination interaction was done by reading the first clear well in each row of panels with both agents.

Based on this reading, fractional inhibitory concentrations (FICs) were calculated for each antimicrobial alone and in combination. The following formulas were used to calculate the FIC:

$$\text{FIC of colistin} = \frac{\text{MIC of colistin in combination}}{\text{MIC of colistin alone}}$$

$$\text{FIC of rifampicin} = \frac{\text{MIC of rifampicin in combination}}{\text{MIC of rifampicin alone}}$$

The fractional inhibitory concentration index (FICI) or ∑FIC for this combination was calculated according to the following formula:

$$\text{FIC index } (\sum\text{FIC}) = \text{FIC of colistin} + \text{FIC of rifampicin}$$

FIC index results for each combination were defined as:

Synergist:	$\sum$FIC $\leq$ 0.5
Partial synergist:	$\sum$FIC > 0.5 and < 1.0
Additive:	$\sum$FIC = 1.0
Indifferent:	$\sum$FIC > 1 and < 4
Antagonist:	$\sum$FIC $\geq$ 4

The FICI value was used to prove the antimicrobial combination interaction for each specific isolate. The results were expressed as percentages of isolates with synergist, partial synergist, additive, indifferent and antagonist.

4. Time kill method

The antibacterial activity of the combination against the eight isolates of carbapenems-resistant *A. baumannii* was performed according to the time kill method (Pillai, Moellering and Eliopoulos, 2005) including isolate no. 3, 4, 5, 8, 10, 11, 22, and 45. All 8 isolates were selected according to the partial synergistic effect of the combination against such isolates observed in the previous study. The characteristics of all 8 selected isolates were shown in table 3-3. The time kill study was performed to determine the bactericidal activity of colistin, rifampicin alone and the combination of colistin with rifampicin. The concentrations of colistin and rifampicin chosen were shown in Table 3-4.

Table 3-3 Characteristics of the selected isolates in time kill study

Isolate No.	[a]MICs (µg/ml)		[b]∑FIC from checkerboard method
	COL	RIF	
3	1	4	0.625
4	1	4	0.75
5	2	8	0.75
8	4	8	0.562
10	1	8	0.625
11	1	4	0.625
22	1	8	0.562
45	1	4	0.625

[a] MICs from the checkerboard method

[b] ∑FIC > 0.5 and < 1.0 (Partial synergist)

COL, colistin; RIF, rifampicin

Table 3-4 The concentration of colistin and rifampicin chosen for the assessment of bactericidal activity by time kill method

Isolates No.	Combination therapy			
	Colistin(µg/ml)		Rifampicin(µg/ml)	
	0.5×MIC	1×MIC	0.5×MIC	1×MIC
3	0.5	1	2	4
4	0.5	1	2	4
5	1	2	4	8
8	2	4	4	8
10	0.5	1	4	8
11	0.5	1	2	4
22	0.5	1	4	8
45	0.5	1	2	4

4.1 Determination of bactericidal activity of colistin, rifampicin alone and in combination.

Colistin concentrations were prepared to 0.5×MIC and 1×MIC and rifampicin concentrations were prepared to 0.5×MIC and 1×MIC. Antimicrobial concentrations used in the initial (stock) solutions were ten-fold greater than the desired final concentrations. One ml of each drug was pipetted into the Mueller Hinton broth (MHB) for the preparation of the working media before adding the standardized inoculums (final volume of working media = 9 ml). There were 9 groups including control (no antimicrobial agents), 0.5×MIC of colistin, 1×MIC of colistin, 0.5×MIC of rifampicin, 1×MIC of rifampicin, 0.5×MIC of colistin with 0.5×MIC rifampicin, 0.5×MIC of colistin with 1×MIC rifampicin, 1×MIC of colistin with 0.5×MIC rifampicin and 1×MIC of colistin with 1×MIC rifampicin

The inoculums which were adjusted to match the turbidity of a 0.5 McFarland standard, contained approximately 1 to 2 $\times 10^8$ CFU/ml which was then diluted ten-fold to make 1 to 2×10^7 CFU/ml of the bacterial inoculums. One ml of

inoculums was pipetted into the working media 9 ml which was incubated at 37°C in a shaking water bath.

The samples were collected for cultures at 0, 2, 4, 6, 8 and 24 hours after the microorganisms were exposed to each group of the antimicrobials and the control group. Then 0.5 ml of the collected sample was diluted ten-fold in 4.5 ml of sterile normal saline solution and 20 µl of each dilution was dropped to the surface of TSA plates which were incubated at 37°C for 18 hours.

The quantity of survival bacteria in each group was calculated to obtain the killing curve data. Killing curves were constructed by Microsoft Excel 2007 at each time interval. The $\log_{10}$ change of the viable cell counts compared to the staring inoculums was determined. The results were analyzed by determining the number of strains which yield changes in the log number of CFU/ml of -1, -2 and -3 at 2, 4, 6, 8 and 24 hours compared to the counts at 0 hours. Bactericidal activity was defined as $\geq$ 3 $\log_{10}$ CFU/ml decrease in the starting inoculums and bacteriostatic activity was defined as $<$ 3 $\log_{10}$ CFU/ml decrease in the starting inoculum. The regrowth was defined as $\geq$ 2 $\log_{10}$ CFU/ml after $\geq$ 6 hours (Pankuch, Jacobs and Appelbaum, 1994).

The quantitative evaluation of antimicrobial effect was calculates as in the published article (Firsov *et al*., 1997).

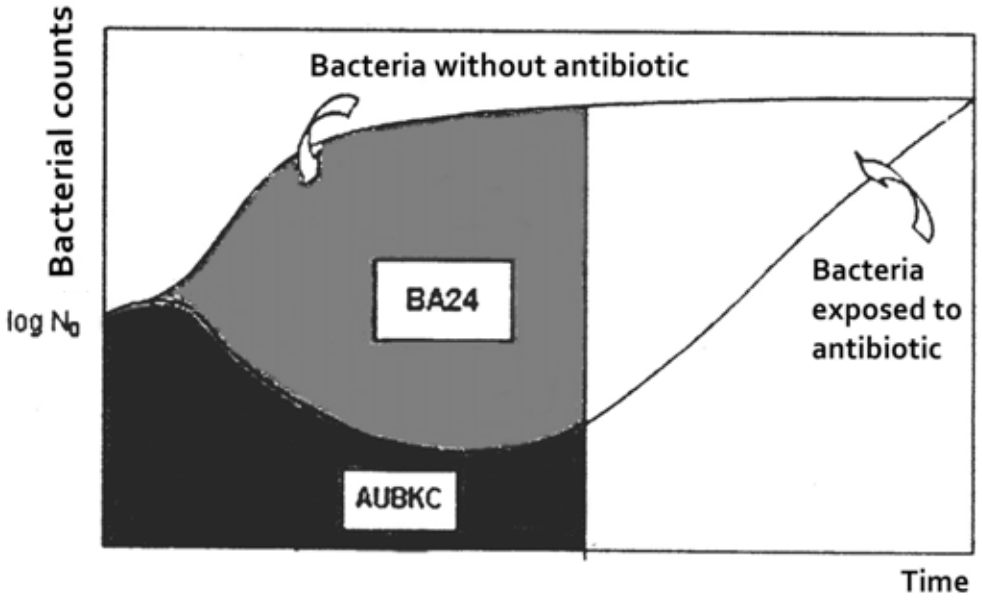

Figure 3-2 Parameters for quantifying bacterial killing, regrowth curve and the antimicrobial effect (Modified from Firsov *et al*., 1997)

The following parameters were calculated by various methodologies as followed:

$AUBK_{0\text{-}24}$ = Area under the bacterial killing and regrowth curves that were calculated by the trapezoidal rule for 24 hours.

Bacteriolytic area for 24 hours (BA_{24}) = the area between the control growth curve and the bacterial killing and regrowth curves ($AUBKC_{0\text{-}24}$ of the control growth curve subtracted by $AUBK_{0\text{-}24}$ of the bacterial killing and regrowth curves)

Statistic analysis

One-way ANOVA was used to compare the BA_{24}, which were expressed in their mean value (± SD) values. Any value of $p < 0.05$ was defined as a significant difference.

5. Scanning electron microscopy of *A. baumannii*

The scanning electron microscopy was chosen to examine the morphological change in *A. baumannii* when exposed to colistin, rifampicin alone and the combination after 4 and 6 hours. The selected concentration of drugs and bacterial strains in this study were correlated to those in the time kill study.

Colistin and rifampicin concentrations were prepared to 0.5×MIC and 1×MIC. Antimicrobial concentrations used in initial (stock) solutions were ten-fold greater than the desired final concentrations. One ml of each drug was pipetted into the Mueller Hinton broth (MHB) for the working media preparation before adding the standardized inoculums (final volume of working media = 9 ml). There were 9 groups including control (no antimicrobial agents), 0.5×MIC of colistin, 1×MIC of colistin, 0.5×MIC of rifampicin, 1×MIC of rifampicin, 0.5×MIC of colistin with 0.5×MIC of rifampicin, 0.5×MIC of colistin with 1×MIC of rifampicin, 1×MIC of colistin with 0.5×MIC of rifampicin and 1×MIC of colistin with 1×MIC of rifampicin.

The inoculum was adjusted to match the turbidity of a 0.5 McFarland standard containing approximately 1 to 2× 10^8 CFU/ml that was then diluted ten-fold to make 1 to 2 × 10^7 CFU/ml of the bacterial inoculum. One ml of inoculum was pipetted to the working media which was incubated at 37°C in a shaking water bath.

The samples were collected after 4 and 6 hours of exposure in order to detect the morphological changes. The samples were fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer pH 7.2 for 2 hours then they were rinsed twice in phosphate buffer for 10 min/each and once in distilled water for 10 minutes. After that, the samples were dehydrated with a graded series of ethanol (30%, 50%, 70%, 90% 5 min/each and absolute ethanol 3 times, 10 minutes/time). The samples were then critical point dried (Critical Point Dryer, Balzer model CPD 020), mounted and coated with gold (Sputter Coater, Balzers model SCD 040). The samples were observed under a scanning electron microscopy (JEOL, model JSM-5410LV).

CHAPTER IV

RESULTS

1. Disk diffusion test

The antimicrobial susceptibility of 12 antimicrobial agents against 30 isolates of carbapenems-resistant *A. baumannii* which were isolated from patients at Bumratnaradoon Hospital in Nonthaburi, Thailand between April 2007 and May 2009 as shown in Table 4-1.

Table 4-1 Antimicrobial activity of 12 antimicrobial agents against 30 *A. baumannii* isolates

Antimicrobial agents	No. of isolates (%)		
	Resistant	Intermediate	Susceptible
Amikacin	27(90%)	1(3.3%)	2(6.7%)
Cefepime	29(96.7%)	-	1(3.3%)
Ceftazidime	29(96.7%)	-	1(3.3%)
Ciprofloxacin	29(96.7%)	-	1(3.3%)
Colistin	-	6(20%)	24(80%)
Gentamicin	27(90%)	2(6.7%)	1(3.3%)
Imipenem	30(100%)	-	0(0%)
Meropenem	30(100%)	-	0(0%)
Netilmicin*	27(90%)	-	3(10%)
Piperacillin/tazobactam	29(96.7%)	1(3.3%)	-
Rifampicin**	30(100%)	-	-
Tobramycin	27(90%)	-	3(10%)

*The inhibition zone from netilmicin was interpreted based on the EUCAST guideline (2010)

**The inhibition zone from rifampicin was interpreted based upon the recommendation for *Staphylococcus* spp. (inhibition zone < 20 mm, resistant) following CLSI guideline

All isolates were resistant to meropenem, imipenem and rifampicin. Among these isolates, 33% of the 30 isolates were high resistant to rifampicin that the inhibition zone of each drug was less than 10 mm (Thapa *et al*, 2009). The 96.7% of all isolates were resistant to cefepime, ceftazidime, piperacillin/tazobactam and ciprofloxacin, Ninety percent of tested isolates were resistant to amikacin, gentamicin, netilmicin and tobramycin, while 80% were still susceptible to colistin (24 isolates susceptible and 6 isolates intermediate susceptible).

2. Minimum inhibitory concentrations (MICs) of colistin, rifampicin against 30 *A. baumannii* isolates as determined by agar dilution method

MICs of colistin and rifampicin were determined by agar dilution method. The MICs ranges and MIC_{50}, MIC_{90} against all 30 isolates of *A. baumannii* were shown in Table 4-2. The 96.7% of the tested organism were susceptible to colistin (MIC range from 1-4 µg/ml; susceptible breakpoint ≤ 2 µg/ml), MIC_{50} and MIC_{90} of colistin were 1, 2 µg/ml, respectively. All isolates were resistant to rifampicin (MIC range from 8-16 µg/ml; susceptible breakpoint ≤1 µg/ml), MIC_{50} and MIC_{90} of rifampicin were 8 µg/ml, respectively.

Table 4-2 Suceptibilities of 30 carbapenems-resistant *A. baumannii* isolates

Antimicrobial Agents	No. of isolates (%)			Range (µg/ml)	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)
	Suceptible	Intermediate	Resistant			
Colistin[a]	29(96.7%)	-	1(3.3%)	1-4	1	2
Rifampicin[b]	0(0%)	-	30(100%)	8-16	8	8

[a]susceptible, ≤ 2µg/ml; resistant, ≥ 4 µg/ml interpreted by CLSI guideline

[b]suceptible, ≤ 1µg/ml; resistant, ≥ 4 µg/ml interpreted based upon the recommendation for *Staphylococcus* spp. by CLSI guideline

3. Synergy study

The synergistic effect of the combination of colistin and rifampicin against 30 isolates of *A. baumannii* were shown in Table 4-3, 4-4. The combination effect was evaluated from the fractional inhibitory concentration (FIC) index.

Table 4-3 MICs of colistin, rifampicin and FIC index were determined by checkerboard method

Isolates No.	Colistin		Rifampicin		FIC index	Interpreted[b]
	MIC[a]	Interpreted	MIC[a]	Interpreted		
1	1	S	8	R	1	A
2	1	S	8	R	1	A
3	1	S	8	R	0.625	P
4	1	S	8	R	0.75	P
5	1	S	8	R	0.75	P
6	1	S	8	R	2	I
7	2	S	8	R	2	I
8	2	S	8	R	0.562	P
9	1	S	8	R	1	A
10	1	S	16	R	0.625	P
11	1	S	8	R	0.625	P
12	1	S	8	R	2	I
13	2	S	8	R	2	I
14	2	S	8	R	1	A
15	2	S	8	R	2	I
16	1	S	8	R	1	A
17	1	S	8	R	1	A
18	2	S	8	R	2	I
20	2	S	8	R	1	A
21	1	S	8	R	2	I
22	1	S	8	R	0.562	P
23	1	S	8	R	2	I
26	4	R	8	R	2	I
27	2	S	8	R	2	I
28	2	S	8	R	2	I
29	1	S	8	R	2	I
31	1	S	8	R	2	I
45	1	S	8	R	0.625	P
46	1	S	16	R	2	I
47	2	S	8	R	2	I

[a]MICs from agar dilution method ; [b]P = partial synergist, A= additive, I= Indifferent
R= resistant; S= susceptible

Table 4-4 Combination effect of colistin and rifampicin against 30 isolates of *A. baumannii*

	Number of isolates	% of isolates
Synergist	0	0
Partial synergist	8	26.7
Additive	7	23.3
Indifferent	15	50
Antagonist	0	0

The combination effects were partial synergist against 8 isolates (26.7%) (Isolates no. 3, 4, 5, 8, 10, 11, 22 and 45) with FIC index from 0.562-0.75, additive effect against 7 isolates (23.3%) (Isolates no. 1, 2, 9, 14, 16, 17 and 20) with FIC index as 1. Indifferent in 15 isolates (50%) (Isolates no. 6, 7, 12, 13, 15, 18, 21, 23, 26, 27, 28, 29, 31, 46 and 47) with FIC index as 2 was also observed. The results of synergist and antagonist were not observed.

4. Time kill study

The bactericidal activities of colistin alone, rifampicin alone and of the combination between colistin and rifampicin against 8 isolates of *A. baumannii* were performed by time kill method, including isolate no. 3, 4, 5, 8, 10, 11, 22 and 45.

The extent of bacterial killing was evaluated by the number of isolates which were killed at various time intervals. The data were shown in Table 4-5.

Table 4-5 Reduction of viable cell counts of *A. baumannii* (8 isolates) at various time intervals.

Antimicrobial agent and concentration (μg/ml)	No. of isolates killed* at each time point																
	2hours			4hours			6hours			8hours				24hours			
	-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3	R**	-1	-2	-3	R**
COL 0.5×MIC	2	-	-	6	2	-	4	4	-	3	3	1	-	-	1	1	5
COL 1×MIC	5	-	-	5	3	-	2	2	4	1	3	4	-	2	-	2	3
RIF 0.5× MIC	-	-	-	1	-	-	1	-	-	-	-	-	2	-	-	-	8
RIF 1×MIC	2	-	-	3	2	-	4	1	-	3	-	-	2	-	-	-	5
COL 0.5× MIC +RIF 0.5×MIC	3	2	1	2	4	2	2	1	5	2	1	5	-	2	1	3	2
COL 0.5×MIC +RIF 1×MIC	1	4	1	2	4	2	2	1	5	1	-	7	-	1	1	5	1
COL 1×MIC +RIF 0.5× MIC	2	3	3	2	3	3	-	2	6	-	1	7	-	1	1	5	1
COL 1×MIC +RIF 1×MIC	2	2	4	1	2	5	-	1	7	-	-	8	-	2	-	6	1

*-1= 90% of viable reduction versus intial inoculums; -2= 99% of viable reduction versus intial inoculums; -3 = 99.9% of viable reduction versus initial inoculums

**R= regrowth

COL, colistin; RIF, rifampicin

One isolate (isolate No. 8) (12.5%) was killed by 0.5×MIC of colistin alone at 8th and 24th hour and the regrowth was observed in 5 isolates (isolate No. 4, 10, 11, 22, 45) (62.5%) at 24th hour. The increase in concentration of colistin to 1×MIC showed

bactericidal activity against 4 isolates (isolate No. 5, 8, 11, 45) (50%) at 6th, 8th hour and 2 isolates (isolate No. 5, 8) (25%) at 24th hour, respectively. However, regrowth of 3 isolates (isolate No. 4, 22, 45) (37.5%) were observed at 24th hour.

Rifampicin alone at 0.5×MIC and 1×MIC did not show any bactericidal activity at any time during the time of study. Only 1 isolate (isolate No. 22) was killed by 0.5×MIC of rifampicin at level of 90% killing (≥1 log CFU/ml decreased) at 4th and 6th hour but the regrowth could be observed in 2 isolates (isolate No. 22,45) (25%) and regrowth of all isolates (100%) could be observed at 8th and 24th hour, respectively. For 1×MIC of rifampicin, the 90% killing of 2 isolates (isolate No. 4, 8) at 2th hour, 3 isolates (isolate No. 5, 8, 45) at 4th hour, 4 isolates (isolate No. 4, 5, 8, 45) at 6th hour and 3 isolates (isolate No. 5, 8, 10) at 8th hour, respectively were observed. The 99% killing (≥ 2 log CFU/ml decreased) was observed in 2 isolates (isolate No. 4, 22) at 4th hour and 1 isolate (isolate No. 22) at 6th hour. The regrowth in rifampicin 1×MIC was observed in 2 isolates (isolate No. 22, 45) (25%) at 8th hour and 5 isolates (isolate No. 4, 5, 8, 22, 45) (62.5%) at 24th hour, respectively.

The combination between 0.5×MIC colistin and either 0.5×MIC or 1×MIC rifampicin showed 99.9% killing of 1 isolate at 2nd hour and the number of the isolates killed were increased as the incubation time was increased. However, the regrowth in the combination of 0.5×MIC colistin with 0.5×MIC rifampicin was observed in 2 isolates (isolate No. 4, 22) (25%) at 24th hour. For 0.5×MIC colistin combined with 1×MIC rifampicin, 99.9% killing was observed in 7 isolates (87.5%) at 8th hour and 5 isolates (62.5%) at 24th hour, respectively. However, the regrowth was found at 24th hour in 1 isolate (12.5%) (isolate No. 4).

In addition, the combination of 1×MIC colistin with either 0.5×MIC or 1×MIC rifampicin exhibited 99.9% killing of at least 3 isolates at 2nd hour to 8 isolates at 8th hour. In particular, colistin 1×MIC combined with rifampicin 1×MIC exhitbited bactericidal activity in all isolates at 8th hour and 6 isolates (75%) at 24th hour, respectively. However, the regrowth was observed in both the combinations of

1×MIC colistin with either 0.5×MIC or 1×MIC rifampicin at 24th hour in 1 isolate (12.5%) (isolate No. 4).

The mean $\log_{10}$ change of viable cell count and bacteriolytic area for 24 hours (BA_{24}) in 8 isolates of *A. baumannii* were shown in Table 4-6. The average time kill curves for the antimicrobial activity of the combinations of colistin with rifampicn were shown in Fig 4-1.

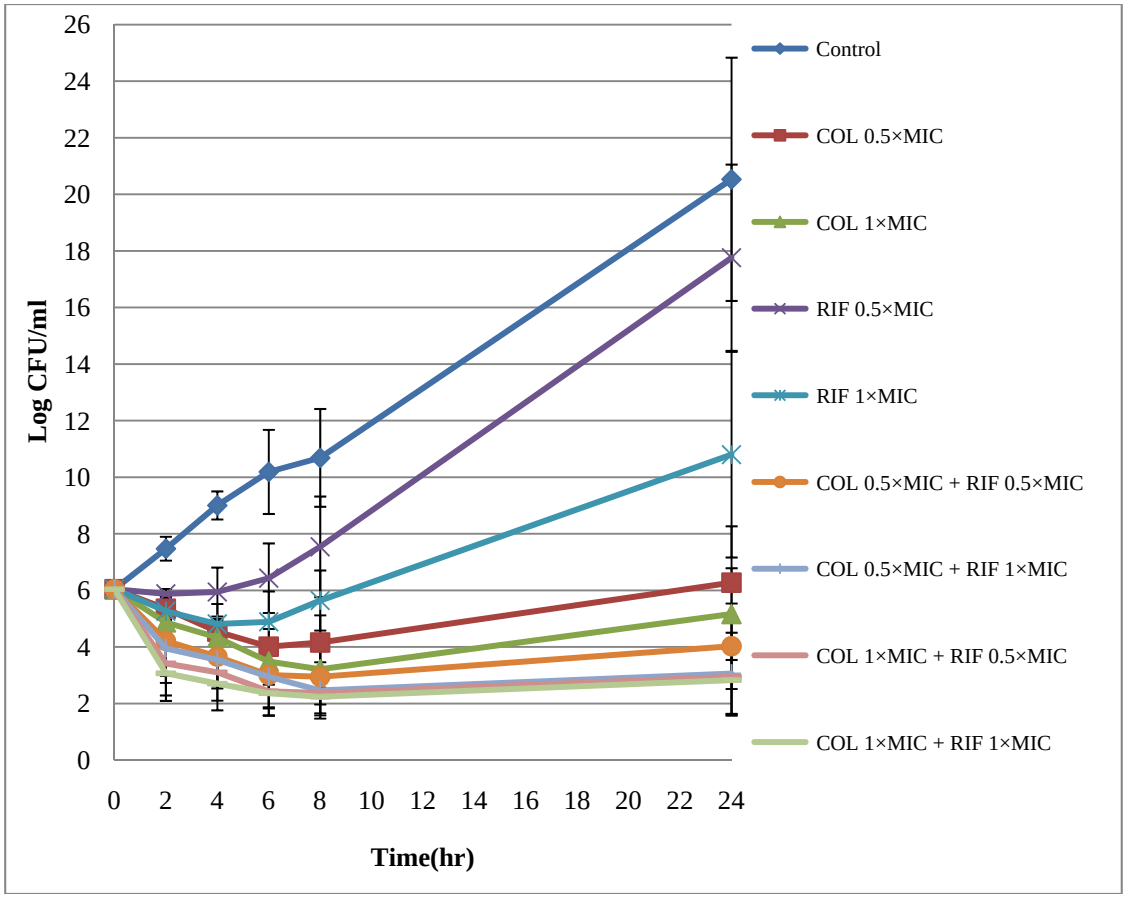

Figure 4-1 Average time-kill curve showing the antibacterial activity of colistin and rifampicin alone and in combinations against 8 isolates of *A. baumannii*. Data mean ± SD (error bars). COL, colistin; RIF, rifampicin

Table 4-6 Mean±SD of log change viable cell counts at various time intervals, $AUBKC_{0-24}$ and BA_{24} in 8 isolates of *A. baumannii*

Condition	Change in viable cell count (log CFU/ml)					Mean (±SD) $AUBKC_{0-24}$	Mean (±SD) BA_{24}
	Δ2	Δ4	Δ6	Δ8	Δ24		
Control	1.43±0.40	2.96±0.48	4.15±1.51	4.60±1.72	14.49±4.30	319.76±42.96	-
COL 0.5×MIC	-0.69±0.44	-1.49±0.43	-2.03±0.61	-1.89±0.95	0.23±2.13	121.40±25.00	198.36±56.37
COL 1×MIC	-1.17±0.32	-1.71±0.56	-2.56±0.78	-2.83±0.64	-0.90±1.75	101.58±19.09	218.18±46.16
RIF 0.5×MIC	-0.20±0.18	-0.10±0.89	0.40±1.27	1.50±1.81	11.72±3.29	252.45±26.06	67.31±30.35[a]
RIF 1×MIC	-0.77±0.29	-1.23±0.73	-1.15±1.10	-0.40±1.09	4.76±3.63	173.10±30.86	146.66±41.87[b]
COL 0.5×MIC+ RIF 0.5×MIC	-1.83±1.24	-2.38±1.02	-3.03±1.15	-3.10±0.98	-2.02±1.50	90.18±28.52	229.59±47.67[b,c,d]
COL 0.5×MIC+ RIF 1×MIC	-2.10±1.21	-2.50±1.01	-3.10±1.13	-3.58±0.99	-2.98±1.43	73.54±23.56	246.22±48.99[b,c,d]
COL 1×MIC+ RIF 0.5×MIC	-2.62±1.14	-2.94±1.02	-3.61±0.85	-3.67±0.78	-3.09±1.30	68.87±20.40	250.89±48.13[b,c,d]
COL 1×MIC+ RIF 1×MIC	-2.97±0.97	-3.34±0.95	-3.68±0.76	-3.80±0.56	-3.21±1.24	65.05±16.82	254.72±45.07[b,c,d]

[a]= $p < 0.05$ compared to activity of colistin 0.5×MIC and 1×MIC alone

[b]= $p < 0.05$ compared to activity of rifampicin 0.5×MIC alone

[c]= $p < 0.05$ compared to activity of rifampicin 1×MIC alone

[d]= $p > 0.05$ compared to activity of colistin 0.5×MIC and 1×MIC alone

Δ = Mean log change viable cell count at 2, 4, 6, 8 and 24 hours, respectively; $AUBKC_{0-24}$ = Area under bacterial killing and regrowth curves for 24 hours;

BA_{24} = Bacteriolytic area for 24 hours; COL, colistin; RIF, rifampicin

The bactericidal activity (≥3 log CFU/ml decreased or 99.9% killing) could not be observed in 0.5×MIC or 1×MIC colistin alone at any time during the time of study. In addition, BA_{24} of colistin 0.5×MIC (198.36±56.37 log CFU/ml.h) was no significantly different when compared to BA_{24} of colistin 1×MIC (218.18±46.16 log CFU/ml.h). However, the bacterial regrowth were found in 0.5×MIC of colistin at 24th hour.

The bacterial killing effects from rifampicin 0.5×MIC and 1×MIC alone were less than 90% killing as shown throughout the time of this study. BA_{24} of rifampicin 0.5×MIC (67.31±30.35 log CFU/ml.h) was significantly different from that of colistin alone (0.5×MIC and 1×MIC) while which was different from that of rifampicin of 1×MIC (146.66±41.87 log CFU/ml.h). The bacterial regrowth were found in both concentrations of rifampicin at 24th hour.

The combinations between colistin 0.5×MIC and rifampicin 0.5×MIC, exhibited 99.9% killing at 6th and 8th hour without bacterial regrowth during the time of study. BA_{24} of this combination was 229.99±47.67 log CFU/ml.h, which was significantly different from the BA_{24} of rifampicin 0.5×MIC and 1×MIC alone. However, the combination of colistin 0.5×MIC and rifampicin 0.5×MIC was not significantly difference ($p>0.05$) from BA_{24} of colistin 0.5×MIC and 1×MIC alone.

Colistin 0.5×MIC when was combined with rifampicin 1×MIC, 99% killing was observed at 2nd and 4th hour, and 99.9% killing was observed at 6th to 8th hour without any bacterial regrowth during the time of study. BA_{24} of colistin 0.5×MIC combined with rifampicin 1×MIC was 246.22±48.99 log CFU/ml.h. For the combination of colistin 1×MIC with rifampicin 0.5×MIC 99.9% killing at 6th to 24th hour was observed and BA_{24} of this combination was 250.89±48.13 log CFU/ml.h. BA_{24} of both combinations were significantly difference ($p<0.05$) from BA_{24} of rifampicin 0.5×MIC or 1×MIC alone but not significantly difference ($p>0.05$) from those of colistin 0.5×MIC and 1×MIC.

Bactericidal activity of the combination between colistin 1×MIC and rifampicin 1×MIC was observed at 4^{th} to 24^{th} hour without any bacterial regrowth during the time of study. Although, BA_{24} of this combination was 254.72±45.07 log CFU/ml.h which was higher than colistin alone (0.5×MIC and 1×MIC), rifampicin alone (0.5×MIC and 1×MIC). However, BA_{24} of the combination between colistin 1×MIC and rifampicin 1×MIC was no significantly difference ($p>0.05$) from those in the different concentrations of the combinations.

5. Study on the bacterial cell morphology change by scanning electron microscopy

Carbapenems-resistant *A. baumannii* code no. 22 which was killed by the combination of coistin and rifampicin in the time kill study started at 4^{th} hour and 6^{th} hour was selected to be the tested isolate in this study. The morphological cell structure of the tested isolate was observed by scanning electron microscope, after it was exposed to 0.5×MIC colistin (0.5µg/ml), 1×MIC colistin (1µg/ml), 0.5×MIC rifampicin (4µg/ml) and 1×MIC (8µg/ml) rifampicin alone and in the combinations for 4, 6 hours.

Morphological structure of bacteria cell had a smooth surface as control cell (Figure A; no antimicrobial agent). Colistin alone (0.5 and 1×MIC) produced minor protrusion on the surface of cell (Figure B, C), rifampicin alone (0.5×MIC) did not cause the cell destruction while 1×MIC rifampicin alone caused less abnormal forms and less surface protrusion than colistin alone (Figure D, E). The combination of two agents caused more damage of bacteria cell wall leading to cell lysis. The lower number of bacteria was observed in the combinations when compared to those in each antimicrobial agent alone (Figure F to J).

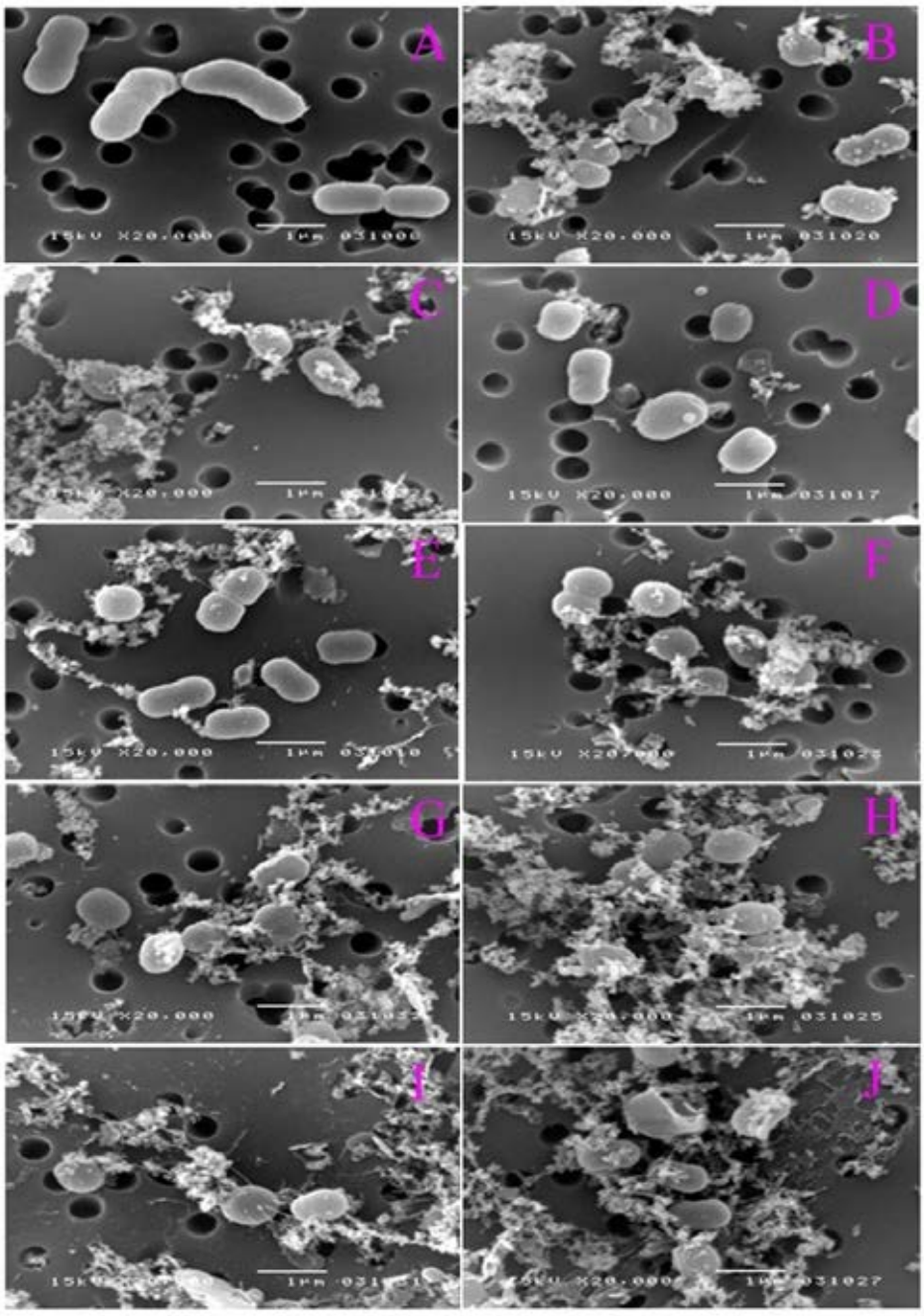

Figure 4-2 Scanning electron micrographs of *A. baumannii* No. 22 after 6 hours exposed to (A) no antimicrobial agent, (B) Colistin 0.5×MIC (0.5 μg/ml), (C) Colistin 1×MIC (1μg/ml), (D) Rifampicin 0.5×MIC (4μg/ml), (E) Rifampicin 1×MIC (8μg/ml), (F) Colistin 0.5×MIC (0.5μg/ml)+Rifampicin 0.5×MIC (4μg/ml), (G) Colistin 0.5×MIC (0.5μg/ml)+Rifampicin 1×MIC (8μg/ml), (H) Colistin 1×MIC (1μg/ml)+Rifampicin 0.5×MIC (4μg/ml), (I) Colistin 1×MIC (1μg/ml)+Rifampicin 1×MIC (8μg/ml) and (J) Colistin 1×MIC (1μg/ml)+Rifampicin 1×MIC (8μg/ml) after 4 hours

CHAPTER V

DISCUSSION AND CONCLUSION

A. baumannii infection is a major cause of nosocomial infections in intensive care units (ICUs), especially critical ill patients. The risk factor for infections may be contaminated with medical-devices such as ventilator associated pneumonia (VAP) infection and blood stream infection (Slama, 2008). These factors are difficult to improve because patients are severely ill and they required these devices to maintain the situations.

Most of antibiotics for infections caused by *A. baumannii* are mainly carbapenems. However, the incidence of carbapenems resistance has raised (Corbella et al., 2000; Poirel and Nordmann, 2006). There are several mechanisms of carbapenem resistance including beta-lactamase production, penicillin-binding protein changes and reduction of porin protein. Therefore, treatment for *A. bauamnnii* infection is difficult to manage and control (Perez et al., 2007; Munoz-Price and Weinstein, 2008).

According to the present study, among all 30 carbapenems-resistant isolates, 96.7% of them were also resistant to cefepime, ceftazidime, piperacillin/tazobactam and ciprofloxacin and 90% were resistant to amikacin, gentamicin, netilmicin and tobramycin. However, the result on the susceptibility to colistin from the disk diffusion method was slightly different from that from the agar dilution method. Only 24 isolates (80%) were shown to be susceptible to colistin while the other 6 isolates were intermediate susceptible, in contrast 97% (29 isolates) exhibited susceptible to colistin (susceptible breakpoint $\leq$ 2 µg/ml) and 1 isolate exhibited resistance as confirmed by agar dilution method. This could be explained in part that colistin was the large molecular drug which could diffuse through the agar with some difficulties. In addition, the MIC of colistin against 1 isolate that was shown to resist to the drug was 4 µg/ml which was the borderline value to classify as resistant strain (Galani et al., 2008).

Thapa et al., 2009 demonstrated the susceptibility of rifampicin determined by disk diffusion test against 111 clinical isolates of carbapenems-resistant *A. baumannii* from Siriraj Hospital, Thailand. The result showed that all isolates were resistant to rifampicin. The inhibition zone of <10 mm that indicated the high resistance to rifampicin were observed in 35 resistant strains (31.5%). Similar to the results from the present study that all carbepenem resistant strains were also resistant to rifampicin which about 33.3% of the isolates tested exhibited the high resistance to rifampicin (inhibition zone <10 mm).

Nowadays, non-traditional antimicrobial agents (such as polymyxin B and colistin) were considered as the potential drug in the treatment of *A. baumannii* (Gupta et al., 2009). Colistin acts mainly on the cell wall of Gram-negative bacteria and shows the high bactericidal activity but it also has nephrotoxicity and poor pharmacokinetics. For these reasons, colistin was substituted in the 1980s by other antimicrobials that were less toxic (Falagas and Kasiakou, 2005). Recently, colistin has been used as salvage therapy for severe infections in critically ill patients caused by multidrug-resistant *A. baumannii*. It has been commonly used in the combination therapy in order to increase the bactericidal activity and also to reduce the adverse effects (Li et al., 2006; Rahal, 2006).

Previously, Giamarellos-Bourboulis et al. (2001) have reported the susceptibility of colistin and rifampicin against 39 isolates multidrug-resistant *A. baumannii*. The MIC_{50} and MIC_{90} of colistin were 0.12 and 1 µg/ml, respectively while MIC_{50} and MIC_{90} of rifampicin were 4 and 64 µg/ml, respectively. In the study by Song et al. (2007), the susceptibility of 43 carbapenem-resistant *A. baumannii* isolates were shown. The MIC_{50} and MIC_{90} of colistin were 1 µg/ml and rifampicin of MIC_{50} and MIC_{90} were 4 µg/ml. As compare to the results from this study, the MICs at which 50 and 90% of tested isolates were inhibited for colistin and rifampicin were 1, 2 µg/ml and 8, 8 µg/ml, respectively. The susceptibilities to colistin and rifampicin observed in this study were higher than those reported by the previous studies. The isolates tested in this study were all intermediate resistant to rifampicin with the decrease in colistin susceptibility.

Several reports have demonstrated the combined effect of colistin with several other antimicrobial agents, in particular the combination with rifampicin against multidrug-resistant *A. buamannii*. Most of these combinations showed synergistic effect (Hogg et al., 1998; Giamarellos-Bourboulis et al., 2001; Timurkaynak et al., 2006; Song et al., 2007; Tripodi et al., 2007). With aspect to synergy of combination between colistin plus rifampicin, colistin causes rapid permeabilization of cell membrane, which enhance the penetration for rifampicin in the combination. Action of rifampicin is on the enzyme RNA polymerase subunit and interferring the transcription of mRNA leading to the failure in protein synthesis. The two agents have different mechanisms of action and different excretion (Titarenko et al., 1983). Therefore, the combinated therapy using colistin and rifampicin has advantages for dose reduction and decrease in toxicity from each antimicrobial agent. In addition, the development of drug resistance in multidrug-resistant pathogens could be prevented (Rahal, 2006).

The combination effect was studied by checkerboard method, which showed that only partial synergy effect aginst 8 isolates (26.7%) (FICs, 0.562-0.75), additive against 7 isolates (23.3%) (FICs, 1), indifferent against 15 isolates (50%) (FICs, 2), and antagonist was not observed. This was similar to the result from the previous study by Hogg et al. (1998) who demonstrated that the combination of colistin and rifampicin exhibited the synergistic effect against 11 isolates multidrug-resistant *A. baumannii* while indifferent effect were observed in two isolates. In addition, no antagonism was observed.

The partial synergistic effect against all 8 isolates at the concentration of colistin ranged from 1 to 4 µg/ml which was within therapeutic plasma level of colistimethate sodium when administrated by intramuscular (IM) or intravenous (IV) in 2.5 mg/kg dose (peak serum level of 6 or 20 µg/ml, respectively). The concentration of rifampicin ranged from 4 to 8 µg/ml which was the concentration within therapeutic level (peak serum concentrations for rifampicin are 7- 10 µg/ml after administrated 600 mg orally) (Chen and Kaye, 2009).

In the time kill study, after the isolates were exposed to colistin alone (at 0.5×MIC and 1×MIC), the bactericidal activity (99.9% killing or ≥3log CFU/ml decreased) was observed at 6^{th} to 24^{th} hour. However, the regrowth was also found in both concentrations at the 24^{th} hour in some isolates. In addition, rifampicin alone (at 0.5×MIC and 1×MIC) could not show the bactericidal activity at any time during the time of study. The regrowth of both concentrations in particular of rifampicin 0.5×MIC was observed in all isolates (100%) at the 24^{th} hour. Thus, the result indicated the disadvantage of monotherapy according to the bacteria regrowth. The result agreed with the recent study by Pachon-Ibanez et al. (2010) who suggested that rifampicin monotherapy should not be appropriate while the combination of rifampicin with colistin showed good efficacy in experiment models of infection caused by imipenem-resistant *A. baumannii* in order reduce the development bacterial regrowth in rifampicin monotherapy.

Even though, high concentration of colistin shows good bactericidal effect but the serious adverse effect of colistin is nephrotoxicity, which correlates with high concentration of colistin. In the previous study by Giamarellos-Bourboulis et al. (2001) who demonstrated the bactericidal activity of the combination between colistin (1×MIC or 4×MIC) and rifampicin (2 µg/ml) against 39 multidrug-resistant *A. baumannii* isolates *in vitro* by time kill method, the combination of 1×MIC of colistin with rifampicin (2 µg/ml) was showed to have bactericidal effect at 2^{nd} to 6^{th} hour while the combination of 4×MIC of colistin with rifampicin (2 µg/ml) was showed to have bactericidal effect at all time study. Song et al. (2007) reported that the combined effect of colistin and rifampicin at 1×MIC against 8 carbapenem-resistant *A. baumannii* isolates was all synergy and bactericidal effect of the combined could be observed after 8 hour which was the same as the effect from 4×MIC or 8×MIC of colistin alone. Thus, the observed in 1×MIC of colistin alone could not bactericidal effect.

In this study, the combination of colistin and rifampicin showed bactericidal activity at all time study. In addition, the bacterial regrowth could not be observed, in particular 100% bacterial killing could be observed in the combination of colistin at

1×MIC with rifampicin 1×MIC at 8th hour, which corresponded with the result from the previous study which reported that the administration of colistin commonly in clinical practice every 8 hour was the most effective dose interval in minimizing resistance (Falagas and Kasiakou, 2005). In similar, Bergen et al. (2008) reported that the administration of colistimethate at different dose interval from 8, 12 and 24 hour did not cause different in the antibactericidal effect against *P. aeruginosa* but the least emergence of drug resistance was found when using the 8 hour as dose interval.

The bacteriolytic area for 24 hours (BA_{24}) was used to evaluate the quantitative total antibacterial effect during 24 hour in time kill study. Although, the combinations of colistin and rifampicin did not statistically significant different in BA_{24} as compared to colistin alone. However, the combinations of colistin and rifampicin showed bactericidal activity at all time intervals and these combinations could reduce the amount of bacterial regrowth after 24 hr.

In addition, all the combination did not show significantly difference (p>0.05) among the BA_{24} from the different concentrations of the combinations. The combination of 1×MIC colistin with either 0.5×MIC or 1×MIC of rifampicin showed 99.9% killing of at least 3 isolates at 2nd hour to 8 isolates at 8th hour particular, All isolates (8 isolates) at 8th hour were killed by the combination of 1×MIC colistin with 1×MIC rifampicin and which showed the best bactericidal activity in this study. These combinations could decrease a mean of viable cell count >3 log CFU/ml at 24th hour.

The combination of 0.5×MIC colistin and 1×MIC rifampicin was shown to have bactericidal activity aginst 7 isolates at 8th hour and 5 isolates at 24th hour similar to the result of the combination between 1×MIC colistin and 0.5×MIC of rifampicin. At 24th hour, the bacterial regrowth was observed in only one isolate. For the combination of 0.5×MIC colistin and 0.5×MIC rifampicin, the bactericidal activity aginst 5 isolates at 8th hour could be observed but the number of the isolates were decreased to 3 isolates at 24th hour with the bacterial regrowth in 2 isolates.

Because colistin is mainly nephrotoxicity and neurotoxicity. Therefore, this study suggested that the combination of 0.5×MIC colistin and 0.5×MIC or 1×MIC rifampicin may be alternative treatment in patients with renal failure caused by carbapenem-resistant *A. baumannii* infections in order to reduce toxicity of colistin. Therefore, clinicians should consider all of these primary informations and adjust the dose of each antimicrobial in the combination.

The morphology of cell changed after the exposure to the combination of colistin and rifampicin was observed under scanning electron microscopy. Bacterial cells were in the uncommon forms with roughly spherical surface and numerous protrusion on the cell surface. The osmotic barrier of the bacterial cells were almost completely damaged which leading to the leakage of cytoplasmic membrane and cell lysis by colistin. In addition, bacterial cells showed slightly abnormal forms after it was exposed to rifampicin, which might due to the action of rifampicin that inhibited protein synthesis. The combined effects of colistin caused the increased in the permeability of cell membrane and facilitated the entry of rifampicin into bacterial cells. This may result in synergistic killing between colistin and rifampicin. As a result, the lower number of bacteria was observed in the combinations when compared to those in each antimicrobial agent. Thereofore, the combination therapy could enhance activity with each agent, these agent have different mechanism of action.

However, colistin poorly penetrates that limit the drug distribution in patients with osteomyelitis, endocarditis, biliary tract disease, central nervous system infection (CNS) and respiratory infection in the lung (Hancock and Chapple, 1999), but rifampicin can penenetrate very well from the serum in to the various tissues and body fluids of the human body because the drug could exhibit high solubility in lipid (Kenny and States, 1981). The combination of colistin with rifampicin may be useful to increase the efficacy of the treatment of infection in cerebrospinal fluid. Recently, several studies evaluated the efficacy of aerosolized or intravenous colistin combined intravenous rifampicin for the treatment in patients with nosocomial infection due to multidrug-resistant *A. baumannii*. The results were favourable for all patients

including ventrilator-associated pneumonia (VAP) and menigitis (Motaouakkil et al., 2006; Bassetti et al., 2008). However, these studies have the limited number of patients and without a control group.

In conclusion, this study showed that the combinations of colistin and rifampicin has bactericidal activity superior than that of each agent. Moreover, the combinations prevented regrowth occuring which occurred in the single agent after 24^{th} hour. The decrease of each drug concentration in combination therapy may reduce the toxicity and the development of resistant pathogens during treatment. Because colistin has been abandoned since 1980s and it was substituted to less toxicity antimicrobial agents, the informations of colistin on the pharmacokinetic and pharmacodynamic *in vitro* were very limited. The results from this study should be primary informations in order to support the future study on the combined effect of colistin and rifampicin therapy in human as the alternative treatment of infections caused by carbapenem-resistant *A. baumannii* isolates in Thailand.

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APPENDICES

APPENDIX A

Antimicrobial susceptibility

Table A-1 Susceptibilities of 30 clinical isolates of *A. baumannii* to 12 antimicrobial agents by disk diffusion method

Isolate no.	Zone Diameter (mm)/ Interpretation											
	MER	IMP	TZP	GEN	NET	TOB	AMK	FEP	CAZ	CIP	COL	RIF
1	NZ R	7.27 R	7.21 R	NZ R	15.17 S	NZ R	9.85 R	10.19 R	NZ R	NZ R	14.65 S	11.13 R
2	7.47 R	10.89 R	9.53 R	NZ R	15.48 S	NZ R	9.10 R	12.52 R	NZ R	NZ R	15.33 S	10.59 R
3	NZ R	8.84 R	NZ R	NZ R	NZ R	NZ R	NZ R	8.32 R	NZ R	NZ R	14.98 S	11.16 R
4	NZ R	9.45 R	19.02 I	21.78 S	23.31 S	20.95 S	20.28 S	24.11 S	22.19 S	27.89 S	13.99 S	12.22 R
5	NZ R	9.42 R	NZ R	NZ R	NZ R	NZ R	NZ R	7.91 R	NZ R	NZ R	14.63 S	10.09 R
6	NZ R	8.73 R	7.39 R	NZ R	13.05 R	NZ R	9.06 R	11.39 R	NZ R	NZ R	14.44 S	11.61 R
7	7.30 R	11.32 R	9.75 R	11.35 R	NZ R	7.22 R	9.56 R	12.50 R	NZ R	NZ R	14.41 S	11.88 R
8	NZ R	9.35 R	7.48 R	NZ R	NZ R	NZ R	NZ R	6.56 R	NZ R	NZ R	15.25 S	10.97 R
9	NZ R	8.38 R	7.27 R	NZ R	14.56 R	NZ R	8.26 R	11.82 R	NZ R	NZ R	14.87 S	11.28 R
10	NZ R	8.65 R	8.03 R	13.75 I	11.04 R	16.58 S	15.79 I	8.04 R	NZ R	NZ R	13.97 I	9.63 R
11	NZ R	9.41 R	7.50 R	13.30 I	10.98 R	17.13 S	11.17 R	8.02 R	NZ R	NZ R	14.17 S	10.43 R
12	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	7.03 R	NZ R	NZ R	14.44 S	9.56 R
13	NZ R	10.25 R	9.48 R	NZ R	NZ R	NZ R	NZ R	8.95 R	NZ R	NZ R	15.29 S	9.04 R
14	NZ R	9.55 R	7.96 R	NZ R	NZ R	NZ R	NZ R	7.18 R	NZ R	NZ R	13.71 I	10.62 R
15	NZ R	8.79 R	NZ R	NZ R	NZ R	NZ R	NZ R	13.10 R	NZ R	NZ R	14.04 S	11.62 R
16	NZ R	10.15 R	6.59 R	NZ R	NZ R	NZ R	NZ R	7.70 R	NZ R	NZ R	14.50 S	10.79 R

MER, meropenem; IMP, imipenem; TZP, penicillin/tazobactam; GEN, gentamicin; NET, netilmicin; TOB, tobramycin; AMK, amikacin; FEB, cefepime; CAZ, ceftazidime; CIP,ciprofloxacin; COL,colistin; RIF,rifampicin

R= resistant; I= intermediate; S= susceptible; NZ= no inhibition zone

Table A-1 (continued) Susceptibilities of 30 clinical isolates of *A. baumannii* to 12 antimicrobial agents by disk diffusion method.

Isolate no.	Zone Diameter (mm)/ Interpretation											
	MER	IMP	TZP	GEN	NET	TOB	AMK	FEP	CAZ	CIP	COL	RIF
17	NZ R	8.95 R	7.67 R	NZ R	NZ R	NZ R	NZ R	7.27 R	NZ R	NZ R	13.51 I	9.91 R
18	NZ R	9.75 R	7.03 R	NZ R	NZ R	NZ R	NZ R	9.16 R	NZ R	NZ R	14.31 S	10.42 R
20	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	7.05 R	NZ R	NZ R	14.59 S	NZ R
21	NZ R	8.24 R	7.92 R	NZ R	NZ R	NZ R	NZ R	8.03 R	NZ R	NZ R	14.18 S	15.73 R
22	NZ R	7.13 R	NZ R	NZ R	14.20 R	NZ R	NZ R	NZ R	NZ R	NZ R	14.51 S	10.98 R
23	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	13.83 I	10.44 R
26	NZ R	9.56 R	8.30 R	NZ R	13.98 R	NZ R	7.04 R	12.19 R	NZ R	NZ R	14.99 S	10.30 R
27	NZ R	8.76 R	7.65 R	NZ R	14.19 R	NZ R	19.39 S	10.01 R	NZ R	NZ R	14.52 S	9.98 R
28	NZ R	8.30 R	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	13.64 I	11.80 R
29	NZ R	8.28 R	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	14.25 S	10.63 R
31	NZ R	8.90 R	7.09 R	NZ R	NZ R	NZ R	NZ R	8.46 R	NZ R	NZ R	14.64 S	10.29 R
45	7.30 R	NZ R	NZ R	NZ R	NZ R	8.28 R	NZ R	NZ R	NZ R	NZ R	14.03 S	9.53 R
46	NZ R	NZ R	7.04 R	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	14.10 S	9.44 R
47	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	NZ R	13.01 I	9.07 R

MER, meropenem; IMP, imipenem; TZP, penicillin/tazobactam; GEN, gentamicin; NET, netilmicin; TOB, tobramycin; AMK, amikacin; FEB, cefepime; CAZ, ceftazidime; CIP,ciprofloxacin; COL,colistin; RIF,rifampicin

R= resistant; I= intermediate; S= susceptible; NZ= no inhibition zone

Table A-2 The minimum inhibitory concentrations (MICs; μg/ml) of colistin and rifampicin by agar dilution method, checkerboard method and FIC index

Isolated no.	Colistin		Rifampicin		FIC index	Interpreted
	Agar dilution	Checkerboard	Agar dilution	Checkerboard		
1	1	1	8	8	1	Additive
2	1	1	8	4	1	Additive
3	1	1	8	4	0.625	Partial synergist
4	1	1	8	4	0.75	Partial synergist
5	1	2	8	8	0.75	Partial synergist
6	1	2	8	4	2	Indifferent
7	2	2	8	8	2	Indifferent
8	2	4	8	8	0.562	Partial synergist
9	1	1	8	8	1	Additive
10	1	1	16	8	0.625	Partial synergist
11	1	1	8	4	0.625	Partial synergist
12	1	1	8	8	2	Indifferent
13	2	4	8	8	2	Indifferent
14	2	4	8	8	1	Additive
15	2	2	8	4	2	Indifferent

Table A-2 (continued) The minimum inhibitory concentrations (MICs; µg/ml) of colistin and rifampicin by agar dilution method, checkerboard method and FIC index

Isolated no.	Colistin		Rifampicin		FIC index	Interpreted
	Agar dilution	Checkerboard	Agar dilution	Checkerboard		
16	1	1	8	8	1	Additive
17	1	1	8	8	1	Additive
18	2	1	8	4	2	Indifferent
20	2	4	8	8	1	Additive
21	1	1	8	4	2	Indifferent
22	1	1	8	8	0.562	Partial synergist
23	1	1	8	8	2	Indifferent
26	4	1	8	8	2	Indifferent
27	2	1	8	8	2	Indifferent
28	2	1	8	8	2	Indifferent
29	1	1	8	4	2	Indifferent
31	1	1	8	4	2	Indifferent
45	1	1	8	4	0.625	Partial synergist
46	1	1	16	4	2	Indifferent
47	2	2	8	8	2	Indifferent

Table A-3 MIC disribution and MIC_{50} and MIC_{90} of colistin and rifampicin by agar dilution method

Colistin		Rifampicin	
MIC range	%(No.)	MIC range	%(No.)
0.03	0	0.03	0
0.06	0	0.06	0
0.12	0	0.12	0
0.25	0	0.25	0
0.5	0	0.5	0
1	19(63.3%)	1	0
2	10 (33.3%)	2	0
4	1(3.3%)	4	0
8	0	8	28(93.3%)
16	0	16	2(6.7%)
32	0	32	0
64	0	64	0
128	0	128	0
256	0	256	0
$MIC_{50=}$ 1		$MIC_{50=}$ 8	8
$MIC_{90=}$ 2		$MIC_{90=}$ 8	8

APPENDIX B

Viable count, Killing rate, Change in viable cell count, and kinetic parameters of 8 *A. baumannii* isolates

Table B-1 Viable cell count (log CFU/ml) at the following time of *A. baumannii* (8 isolates)

Antimicrobial agents	Viable cell count (log CFU/ml) at point time (hr)					
	0	2	4	6	8	24
A 3						
Control	6.021	7.352	8.829	9.512	10.138	20.860
COL 0.5×MIC	6.021	5.031	4.477	4.720	4.916	5.512
COL 1×MIC	6.021	4.875	4.929	3.860	3.574	5.301
RIF 0.5×MIC	6.021	6.097	7.301	8.301	8.628	17.138
RIF 1×MIC	6.021	5.301	5.477	5.829	6.000	7.097
COL 0.5×MIC+RIF 0.5×MIC	6.021	5.398	4.813	4.720	4.602	5.966
COL 0.5×MIC+RIF 1×MIC	6.021	5.243	4.740	4.778	4.512	5.000
COL 1×MIC+RIF 0.5×MIC	6.021	4.778	4.628	3.398	3.778	5.161
COL 1×MIC+RIF 1×MIC	6.021	4.070	4.176	3.000	3.000	4.903
A 4						
Control	6.061	6.875	9.942	11.954	11.966	18.845
COL 0.5×MIC	6.061	5.813	4.845	4.628	5.699	7.051
COL 1×MIC	6.061	5.114	3.875	3.398	3.653	6.070
RIF 0.5×MIC	6.061	5.903	5.176	6.097	8.031	16.845
RIF 1×MIC	6.061	4.966	3.699	4.439	5.352	12.114
COL 0.5×MIC+RIF 0.5×MIC	6.061	4.760	3.699	2.301	2.740	5.398
COL 0.5×MIC+RIF 1×MIC	6.061	4.829	3.760	2.439	1.699	5.176
COL 1×MIC+RIF 0.5×MIC	6.061	2.301	1.875	1.699	1.699	4.352
COL 1×MIC+RIF 1×MIC	6.061	2.243	1.699	1.699	1.699	4.512
A 5						
Control	6.021	7.146	8.875	10.122	9.130	24.122
COL 0.5×MIC	6.021	4.875	4.000	3.574	3.352	3.677
COL 1×MIC	6.021	4.301	3.512	2.875	3.000	3.000
RIF 0.5×MIC	6.021	5.574	5.677	5.544	5.154	17.106
RIF 1×MIC	6.021	5.051	4.860	4.512	4.829	10.041
COL 0.5×MIC+RIF 0.5×MIC	6.021	1.699	1.699	1.699	1.699	2.740
COL 0.5×MIC+RIF 1×MIC	6.021	1.699	1.699	1.699	1.699	1.699
COL 1×MIC+RIF 0.5×MIC	6.021	1.699	1.699	1.699	1.699	1.699
COL 1×MIC+RIF 1×MIC	6.021	1.699	1.699	1.699	1.699	1.699

COL,colistin; RIF, rifampicin

Table B-1 (continued) Viable cell count (log CFU/ml) at the following time of *A. baumannii* (8 isolates)

Antimicrobial agents	Viable cell count (log CFU/ml) at point time (hr)					
	0	2	4	6	8	24
A8						
Control	6.079	8.051	8.929	9.903	10.989	24.051
COL 0.5×MIC	6.079	5.243	4.000	3.352	2.699	2.699
COL 1×MIC	6.079	4.860	4.352	2.875	2.699	2.699
RIF 0.5×MIC	6.079	5.740	5.889	5.699	5.243	21.243
RIF 1×MIC	6.079	5.000	4.628	4.243	4.097	11.398
COL 0.5×MIC+RIF 0.5×MIC	6.079	3.439	3.000	2.699	2.699	2.699
COL 0.5×MIC+RIF 1×MIC	6.079	3.097	3.000	2.699	2.699	2.699
COL 1×MIC+RIF 0.5×MIC	6.079	2.699	2.699	2.699	2.699	2.699
COL 1×MIC+RIF 1×MIC	6.079	2.699	2.699	2.699	2.699	2.699
A 10						
Control	6.041	7.602	8.301	8.875	8.477	21.130
COL 0.5×MIC	6.041	5.079	5.000	4.677	4.243	8.796
COL 1×MIC	6.041	5.000	4.628	4.176	4.398	5.796
RIF 0.5×MIC	6.041	6.000	5.875	6.574	7.097	19.720
RIF 1×MIC	6.041	5.439	5.106	5.176	4.889	6.398
COL 0.5×MIC+RIF 0.5×MIC	6.041	5.398	4.860	4.653	4.106	4.796
COL 0.5×MIC+RIF 1×MIC	6.041	5.352	4.916	4.301	3.000	3.796
COL 1×MIC+RIF 0.5×MIC	6.041	4.916	4.070	3.860	3.000	3.574
COL 1×MIC+RIF 1×MIC	6.041	4.628	3.602	3.720	2.699	2.699
A 11						
Control	6.011	7.138	8.740	8.653	10.021	11.130
COL 0.5×MIC	6.011	5.845	4.978	3.243	3.875	7.097
COL 1×MIC	6.011	5.138	4.544	2.954	2.352	4.677
RIF 0.5×MIC	6.011	6.000	7.021	7.720	9.301	10.813
RIF 1×MIC	6.011	5.699	5.889	6.954	7.146	8.051
COL 0.5×MIC+RIF 0.5×MIC	6.011	4.138	3.760	2.000	2.000	1.699
COL 0.5×MIC+RIF 1×MIC	6.011	3.602	3.398	1.699	1.699	1.699
COL 1×MIC+RIF 0.5×MIC	6.011	3.574	3.243	1.699	1.699	1.699
COL 1×MIC+RIF 1×MIC	6.011	2.512	1.699	1.699	1.699	1.699

COL,colistin; RIF, rifampicin

Table B-1 (continued) Viable cell count (log CFU/ml) at the following time of *A. baumannii* (8 isolates)

Antimicrobial agents	Viable cell count (log CFU/ml) at point time (hr)					
	0	2	4	6	8	24
A22						
Control	6.130	8.011	9.477	9.574	9.978	20.079
COL 0.5×MIC	6.130	5.051	4.916	4.243	4.796	7.903
COL 1×MIC	6.130	4.574	5.114	5.041	3.176	8.088
RIF 0.5×MIC	6.130	5.942	4.699	4.512	6.860	18.352
RIF 1×MIC	6.130	5.628	4.097	3.512	5.845	14.544
COL .5×MIC+RIF 0.5×MIC	6.130	3.916	3.544	2.699	2.699	4.966
COL .5×MIC+RIF 1×MIC	6.130	3.860	3.301	2.699	2.699	2.699
COL1×MIC+RIF 0.5×MIC	6.130	3.628	3.176	2.699	2.699	2.699
COL1×MIC+RIF 1×MIC	6.130	3.512	2.875	2.699	2.699	2.699
A45						
Control	5.954	7.602	8.929	12.903	13.989	24.031
COL 0.5×MIC	5.954	5.860	4.122	3.628	3.653	7.439
COL 1×MIC	5.954	5.097	3.677	2.699	2.829	5.653
RIF 0.5×MIC	5.954	5.740	5.889	7.041	9.989	20.860
RIF 1×MIC	5.954	5.041	4.740	4.439	6.966	16.720
COL0.5×MIC+RIF 0.5×MIC	5.954	4.954	3.875	3.301	3.000	3.929
COL0.5×MIC+RIF 1×MIC	5.954	3.875	3.544	3.243	1.699	1.699
COL1 ×MIC+RIF 0.5×MIC	5.954	3.740	3.439	1.699	1.699	1.699
COL1 ×MIC+RIF 1×MIC	5.954	3.176	3.176	1.699	1.699	1.699

COL,colistin; RIF, rifampicin

Table B-1 (continued) Viable cell count (log CFU/ml) at the following time of *A. baumannii* (8 isolates)

Antimicrobial agents	Viable cell count (log CFU/ml) at point time (hr)					
	0	2	4	6	8	24
Average[a]						
Control	6.040±0.05	7.472±0.42	9.003±0.50	10.187±1.49	10.683±1.73	20.531±4.30
COL 0.5×MIC	6.040±0.05	5.350±0.42	4.542±0.45	4.008±0.63	4.154±0.96	6.272±1.99
COL 1×MIC	6.040±0.05	4.870±0.30	4.329±0.59	3.485±0.82	3.310±0.65	5.160±1.62
RIF 0.5×MIC	6.040±0.05	5.875±0.17	5.941±0.86	6.431±1.23	7.538±1.78	17.760±3.29
RIF 1×MIC	6.040±0.05	5.266±0.29	4.812±0.71	4.888±1.08	5.641±1.06	10.795±3.63
COL0.5×MIC +RIF 0.5×MIC	6.040±0.05	4.213±1.23	3.656±1.01	3.009±1.14	2.943±0.98	4.024±1.51
COL0.5×MIC +RIF 1×MIC	6.040±0.05	3.945±1.22	3.545±1.01	2.945±1.12	2.463±1.00	3.053±1.44
COL1×MIC +RIF 0.5×MIC	6.040±0.05	3.417±1.13	3.104±1.00	2.432±0.87	2.371±0.79	2.948±1.31
COL1×MIC +RIF 1×MIC	6.040±0.05	3.067±0.97	2.703±0.95	2.364±0.78	2.237±0.58	2.826±1.25

[a] = Mean±SD, COL,colistin; RIF, rifampicin

Table B-2 Change in viable cell count (log CFU/ml) at the following time and kinetic parameters in 8 isolates of *A. baumannii*

Antimicrobial agent	Change in viable cell count (log CFU/ml)					$AUBKC_{0-24}$	BA_{24}
	Δ2	Δ4	Δ6	Δ8	Δ24		
A3							
Control	1.331	2.808	3.491	4.895	14.839	322.539	
COL 0.5×MIC	-0.990	-1.544	-1.301	-1.105	-0.509	122.822	199.717
COL 1×MIC	-1.146	-1.092	-2.161	-2.447	-0.72	107.925	214.613
RIF 0.5×MIC	0.076	1.28	2.28	2.607	11.117	264.181	58.358
RIF 1×MIC	-0.72	-0.544	-0.192	-0.021	1.076	150.011	172.527
COL 0.5×MIC +RIF 0.5×MIC	-0.623	-1.208	-1.301	-1.419	-0.055	125.031	197.508
COL 0.5×MIC +RIF 1×MIC	-0.778	-1.281	-1.243	-1.509	-1.021	116.151	206.387
COL 1×MIC +RIF 0.5×MIC	-1.243	-1.393	-2.623	-2.243	-0.86	106.924	215.614
COL 1×MIC +RIF 1×MIC	-1.951	-1.845	-3.021	-3.021	-1.118	94.738	227.801
A4							
Control	0.814	3.881	5.893	5.567	12.784	322.059	
COL 0.5×MIC	-0.248	-1.216	-1.433	-0.362	0.99	144.333	177.726
COL 1×MIC	-0.947	-2.186	-2.663	-2.408	0.009	112.274	209.786
RIF 0.5×MIC	-0.158	-0.885	0.036	1.97	10.784	247.456	74.603
RIF 1×MIC	-1.095	-2.362	-1.622	-0.709	6.053	177.351	144.709
COL 0.5×MIC +RIF 0.5×MIC	-1.301	-2.362	-3.76	-3.321	-0.663	125.031	197.028
COL 0.5×MIC +RIF 1×MIC	-1.232	-2.301	-3.622	-4.362	-0.885	84.817	237.243
COL 1×MIC +RIF 0.5×MIC	-3.76	-4.216	-4.362	-4.362	-1.709	67.919	254.140
COL 1×MIC +RIF 1×MIC	-3.818	-4.362	-4.362	-4.362	-1.549	68.728	253.331
A5							
Control	1.125	2.854	4.101	3.109	18.101	333.459	
COL 0.5×MIC	-1.146	-2.000	-2.447	-2.669	-2.344	90.503	242.956
COL 1×MIC	-1.72	-2.509	-3.146	-3.021	-3.201	78.397	255.062
RIF 0.5×MIC	-0.447	-0.344	-0.477	-0.867	11.085	222.839	110.620
RIF 1×MIC	-0.97	-1.161	-1.509	-1.192	4.020	158.663	174.796
COL 0.5×MIC +RIF 0.5×MIC	-4.322	-4.322	-4.322	-4.322	-3.281	53.429	280.030
COL 0.5×MIC +RIF 1×MIC	-4.322	-4.322	-4.322	-4.322	-4.322	45.097	288.361
COL 1×MIC +RIF 0.5×MIC	-4.322	-4.322	-4.322	-4.322	-4.322	45.097	288.361
COL 1×MIC +RIF 1×MIC	-4.322	-4.322	-4.322	-4.322	-4.322	45.097	288.361

COL,colistin; RIF, rifampicin

Table B-2 (continued) Change in viable cell count (log CFU/ml) at the following time and kinetic parameters in 8 isolates of *A. baumannii*

Antimicrobial agent	Change in viable cell count (log CFU/ml)					$AUBKC_{0-24}$	BA_{24}
	Δ2	Δ4	Δ6	Δ8	Δ24		
A8							
Control	1.972	2.85	3.824	4.91	17.972	351.157	
COL 0.5×MIC	-0.836	-2.079	-2.727	-3.380	-3.380	77.152	274.005
COL 1×MIC	-1.219	-1.727	-3.204	-3.380	-3.380	76.137	275.020
RIF 0.5×MIC	-0.339	-0.190	-0.380	-0.836	15.164	257.868	93.289
RIF 1×MIC	-1.079	-1.451	-1.836	-1.982	5.319	161.878	189.279
COL 0.5×MIC +RIF 0.5×MIC	-2.640	-3.079	-3.380	-3.380	-3.380	70.238	280.918
COL 0.5×MIC +RIF 1×MIC	-2.982	-3.079	-3.380	-3.380	-3.380	69.553	281.603
COL 1×MIC +RIF 0.5×MIC	-3.380	-3.380	-3.380	-3.380	-3.380	68.155	283.001
COL 1×MIC +RIF 1×MIC	-3.380	-3.380	-3.380	-3.380	-3.380	68.155	283.001
A10							
Control	1.561	2.260	2.834	2.436	15.089	300.934	
COL 0.5×MIC	-0.962	-1.041	-1.364	-1.798	2.755	144.108	156.827
COL 1×MIC	-1.041	-1.413	-1.865	-1.643	-0.245	119.599	181.336
RIF 0.5×MIC	-0.342	-0.166	0.533	1.056	13.679	264.573	36.361
RIF 1×MIC	-0.602	-0.935	-0.865	-1.152	0.357	132.670	168.264
COL 0.5×MIC +RIF 0.5×MIC	-0.643	-1.181	-1.388	-1.935	-1.245	111.181	189.753
COL 0.5×MIC +RIF 1×MIC	-0.689	-1.125	-1.740	-3.041	-2.245	92.548	208.387
COL 1×MIC +RIF 0.5×MIC	-1.125	-1.971	-2.181	-3.041	-2.467	87.327	213.607
COL 1×MIC +RIF 1×MIC	-1.413	-2.439	-2.321	-3.342	-3.342	75.825	225.109
A11							
Control	1.127	2.729	2.642	4.010	5.119	234.308	
COL 0.5×MIC	-0.166	-1.033	-2.768	-2.136	1.086	125.793	108.515
COL 1×MIC	-0.873	-1.467	-3.057	-3.659	-1.334	89.867	144.441
RIF 0.5×MIC	-0.011	1.010	1.709	3.290	4.802	217.619	16.689
RIF 1×MIC	-0.312	-0.122	0.943	1.135	2.040	171.820	62.488
COL 0.5×MIC +RIF 0.5×MIC	-1.873	-2.251	-4.011	-4.011	-4.312	57.398	176.909
COL 0.5×MIC +RIF 1×MIC	-2.409	-2.613	-4.312	-4.312	-4.312	52.291	182.017
COL 1×MIC +RIF 0.5×MIC	-2.437	-2.768	-4.312	-4.312	-4.312	51.925	182.383
COL 1×MIC +RIF 1×MIC	-3.499	-4.312	-4.312	-4.312	-4.312	46.713	187.595

COL,colistin; RIF, rifampicin

Table B-2 (continued) Change viable cell count (log CFU/ml) at following time and kinetic parameters in 8 isolates of *A. baumannii*

Antimicrobial agent	Change in viable cell count (log CFU/ml)					$AUBKC_{0-24}$	BA_{24}
	Δ2	Δ4	Δ6	Δ8	Δ24		
A22							
Control	1.881	3.347	3.444	3.848	13.949	310.687	
COL 0.5×MIC	-1.079	-1.214	-1.887	-1.334	1.773	140.939	169.748
COL 1×MIC	-1.556	-1.016	-1.089	-2.954	1.958	128.879	181.808
RIF 0.5×MIC	-0.188	-1.431	-1.618	0.730	12.222	244.997	65.690
RIF 1×MIC	-0.502	-2.033	-2.618	-0.285	8.414	201.563	109.124
COL 0.5×MIC +RIF 0.5×MIC	-2.214	-2.586	-3.431	-3.431	-1.164	90.469	220.218
COL 0.5×MIC +RIF 1×MIC	-2.270	-2.829	-3.431	-3.431	-3.431	71.733	238.954
COL 1×MIC +RIF 0.5×MIC	-2.502	-2.954	-3.431	-3.431	-3.431	71.020	239.667
COL 1×MIC +RIF 1×MIC	-2.618	-3.255	-3.431	-3.431	-3.431	70.185	240.502
A45							
Control	1.648	2.975	6.949	8.035	18.077	382.976	
COL 0.5×MIC	-0.094	-1.832	-2.326	-2.301	1.485	125.570	257.406
COL 1×MIC	-0.857	-2.277	-3.255	-3.125	-0.301	99.589	283.387
RIF 0.5×MIC	-0.214	-0.065	1.087	4.035	14.906	300.080	82.896
RIF 1×MIC	-0.913	-1.214	-1.515	1.012	10.766	230.853	152.123
COL 0.5×MIC +RIF 0.5×MIC	-1.000	-2.079	-2.653	-2.954	-2.025	88.650	294.325
COL 0.5×MIC +RIF 1×MIC	-2.079	-2.410	-2.711	-4.255	-4.255	56.161	326.815
COL 1×MIC +RIF 0.5×MIC	-2.214	-2.515	-4.255	-4.255	-4.255	52.594	330.382
COL 1×MIC +RIF 1×MIC	-2.778	-2.778	-4.255	-4.255	-4.255	50.939	332.037

COL,colistin; RIF, rifampicin

Table B-3 Kill rate of *A. baumannii* 8 isolates by colistin, rifampicin alone and the combination of colistin and rifampicin

Isolated No.	Antimicrobial agents	Time(hr)for 3 log killing	Time(hr) for regrowth
A3			
	Control	-	24
	COL 0.5×MIC	-	-
	COL 1×MIC	-	-
	RIF 0.5×MIC	-	24
	RIF 1×MIC	-	-
	COL 0.5×MIC+RIF0.5×MIC	-	-
	COL 0.5×MIC+RIF 1×MIC	-	-
	COL 1×MIC+RIF 0.5×MIC	-	-
	COL 1×MIC+RIF 1×MIC	6	-
A4			
	Control	-	24
	COL 0.5×MIC	-	24
	COL 1×MIC	-	24
	RIF 0.5×MIC	-	24
	RIF 1×MIC	-	24
	COL 0.5×MIC+RIF0.5×MIC	6	24
	COL 0.5×MIC+RIF 1×MIC	6	24
	COL 1×MIC+RIF 0.5×MIC	2	24
	COL 1×MIC+RIF 1×MIC	2	24
A5			
	Control	-	24
	COL 0.5×MIC	-	-
	COL 1×MIC	6	-
	RIF 0.5×MIC	-	24
	RIF 1×MIC	-	24
	COL 0.5×MIC+RIF0.5×MIC	2	-
	COL 0.5×MIC+RIF 1×MIC	2	-
	COL 1×MIC+RIF 0.5×MIC	2	-
	COL 1×MIC+RIF 1×MIC	2	-

COL,colistin; RIF, rifampicin

Table B-3 (continued) Kill rate of *A. baumannii* 8 isolates by colistin, rifampicin alone and the combination of colistin and rifampicin

Isolated No.	Antimicrobial agents	Time(hr)for 3 log killing	Time(hr) for regrowth
A8			
	Control	-	24
	COL 0.5×MIC	8	-
	COL 1×MIC	8	-
	RIF 0.5×MIC	-	24
	RIF 1×MIC	-	24
	COL 0.5×MIC+RIF0.5×MIC	4	-
	COL 0.5×MIC+RIF 1×MIC	4	-
	COL 1×MIC+RIF 0.5×MIC	2	-
	COL 1×MIC+RIF 1×MIC	2	-
A10			
	Control	-	24
	COL 0.5×MIC	-	24
	COL 1×MIC	-	-
	RIF 0.5×MIC	-	24
	RIF 1×MIC	-	-
	COL 0.5×MIC+RIF 0.5×MIC	-	-
	COL 0.5×MIC+RIF 1×MIC	8	-
	COL 1×MIC+RIF 0.5×MIC	8	-
	COL 1×MIC+RIF 1×MIC	8	-
A11			
	Control	-	24
	COL 0.5×MIC	-	24
	COL 1×MIC	6	-
	RIF 0.5×MIC	-	24
	RIF 1×MIC	-	-
	COL 0.5×MIC+RIF 0.5×MIC	6	-
	COL 0.5×MIC+RIF 1×MIC	6	-
	COL 1×MIC+RIF 0.5×MIC	6	-
	COL 1×MIC+RIF 1×MIC	2	-

COL,colistin; RIF, rifampicin

Table B-3 (continued) Kill rate of *A. baumannii* 8 isolates by colistin, rifampicin alone and the combination of colistin and rifampicin

Isolated No.	Antimicrobial agents	Time(hr)for 3 log killing	Time(hr) for regrowth
A22			
	Control	-	24
	COL 0.5×MIC	-	24
	COL 1×MIC	-	24
	RIF 0.5×MIC	-	8
	RIF 1×MIC	-	8
	COL 0.5×MIC+RIF 0.5×MIC	6	24
	COL 0.5×MIC+RIF 1×MIC	6	-
	COL 1×MIC+RIF 0.5×MIC	6	-
	COL 1×MIC+RIF 1×MIC	4	-
A45			
	Control	-	24
	COL 0.5×MIC	-	24
	COL 1×MIC	6	24
	RIF 0.5×MIC	-	8
	RIF 1×MIC	-	8
	COL 0.5×MIC+RIF 0.5×MIC	-	-
	COL 0.5×MIC+RIF 1×MIC	8	-
	COL 1×MIC+RIF 0.5×MIC	6	-
	COL 1×MIC+RIF 1×MIC	6	-

COL,colistin; RIF, rifampicin

APPENDIX C

Checkerboard results of colistin and rifampicin in *A. baumannii* 30 isolates

Isolation no.1

Rifampicin (µg/ml)											
32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-1 The checkerboard data of *A. baumannii* isolate no.1 was showed FICI= 1, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.2

Rifampicin (µg/ml)											
32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-2 The checkerboard data of *A. baumannii* isolate no.2 was showed FICI= 1, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.3

Rifampicin (µg/ml)											
32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-3 The checkerboard data of *A. baumannii* isolate no.3 was showed FICI= 0.625, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.4

Rifampicin (µg/ml)

32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-4 The checkerboard data of *A. baumannii* isolate no.4 was showed FICI= 0.75, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.5

Rifampicin (µg/ml)

32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-5 The checkerboard data of *A. baumannii* isolate no.5 was showed FICI= 0.75, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.6

Rifampicin (µg/ml)

32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-6 The checkerboard data of *A. baumannii* isolate no.6 was showed FICI= 2, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.7

Rifampicin (µg/ml)

32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-7 The checkerboard data of *A. baumannii* isolate no.7 was showed FICI= 2, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.8

Rifampicin (µg/ml)

32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-8 The checkerboard data of *A. baumannii* isolate no.8 was showed FICI= 0.562, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.9

Rifampicin (µg/ml)

32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-9 The checkerboard data of *A. baumannii* isolate no.9 was showed FICI= 1, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.10

Rifampicin (µg/ml)											
32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-10 The checkerboard data of *A. baumannii* isolate no.10 was showed FICI= 0.625, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.11

Rifampicin (µg/ml)											
32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-11 The checkerboard data of *A. baumannii* isolate no.11 was showed FICI= 0.625, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.12

Rifampicin (µg/ml)											
32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-12 The checkerboard data of *A. baumannii* isolate no.12 was showed FICI= 2, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.13

Rifampicin (µg/ml)											
32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-13 The checkerboard data of *A. baumannii* isolate no.13 was showed FICI= 2, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.14

Rifampicin (µg/ml)											
32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-14 The checkerboard data of *A. baumannii* isolate no.14 was showed FICI= 1, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.15

Rifampicin (µg/ml)											
32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-15 The checkerboard data of *A. baumannii* isolate no.15 was showed FICI= 2, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.16

Rifampicin (µg/ml)

32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-16 The checkerboard data of *A. baumannii* isolate no.16 was showed FICI= 1, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.17

Rifampicin (µg/ml)

32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-17 The checkerboard data of *A. baumannii* isolate no.17was showed FICI= 1, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.18

Rifampicin (µg/ml)

32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-18 The checkerboard data of *A. baumannii* isolate no.18 was showed FICI=2, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.20

Rifampicin (µg/ml)

32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-19 The checkerboard data of *A. baumannii* isolate no.20 was showed FICI=1, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.21

Rifampicin (µg/ml)

32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-20 The checkerboard data of *A. baumannii* isolate no.21 was showed FICI=2, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.22

Rifampicin (µg/ml)

32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-21 The checkerboard data of *A. baumannii* isolate no.22 was showed FICI=0.562, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.23

Rifampicin (µg/ml)											
32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-22 The checkerboard data of *A. baumannii* isolate no.23 was showed FICI=2, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.26

Rifampicin (µg/ml)											
32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-23 The checkerboard data of *A. baumannii* isolate no.26 was showed FICI=2, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.27

Rifampicin (µg/ml)											
32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-24 The checkerboard data of *A. baumannii* isolate no.27 was showed FICI=2, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.28

Rifampicin (µg/ml)											
32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figur C-25 The checkerboard data of *A. baumannii* isolate no.28 was showed FICI=2, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.29

Rifampicin (µg/ml)											
32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-26 The checkerboard data of *A. baumannii* isolate no.29 was showed FICI=2, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.31

Rifampicin (µg/ml)											
32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-27 The checkerboard data of *A. baumannii* isolate no.31 was showed FICI=2, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.45

Rifampicin (µg/ml)

32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-28 The checkerboard data of *A. baumannii* isolate no.45 was showed FICI=0.625, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.46

Rifampicin (µg/ml)

32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-29 The checkerboard data of *A. baumannii* isolate no.46 was showed FICI=2, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

Isolation no.47

Rifampicin (µg/ml)

32/0	32/0.03	32/0.06	32/0.125	32/0.25	32/0.5	32/1	32/2	32/4	32/8	32/16	32/32
16/0	16/0.03	16/0.06	15/0.125	16/0.25	16/0.5	16/1	16/2	16/4	16/8	16/16	16/32
8/0	8/0.03	8/0.06	8/0.125	8/0.25	8/0.5	8/1	8/2	8/4	8/8	8/16	8/32
4/0	4/0.03	4/0.06	4/0.125	4/0.25	4/0.5	4/1	4/2	4/4	4/8	4/16	4/32
2/0	2/0.03	2/0.06	2/0.125	2/0.25	2/0.5	2/1	2/2	2/4	2/8	2/16	2/32
1/0	1/0.03	1/0.06	1/0.125	1/0.25	1/0.5	1/1	1/2	1/4	1/8	1/16	1/32
0.5/0	0.5/0.03	0.5/0.06	0.5/0.125	0.5/0.25	0.5/0.5	0.5/1	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32
0/0	0/0.03	0/0.06	0/0.125	0/0.25	0/0.5	0/1	0/2	0/4	0/8	0/16	0/32

Colistin (µg/ml)

Figure C-30 The checkerboard data of *A. baumannii* isolate no.47 was showed FICI=2, gray zone : visible microorganism growth, white zone : no microorganism growth was observed.

APPENDIX D

Time kill curve of 8 isolates of *A. baumannii*

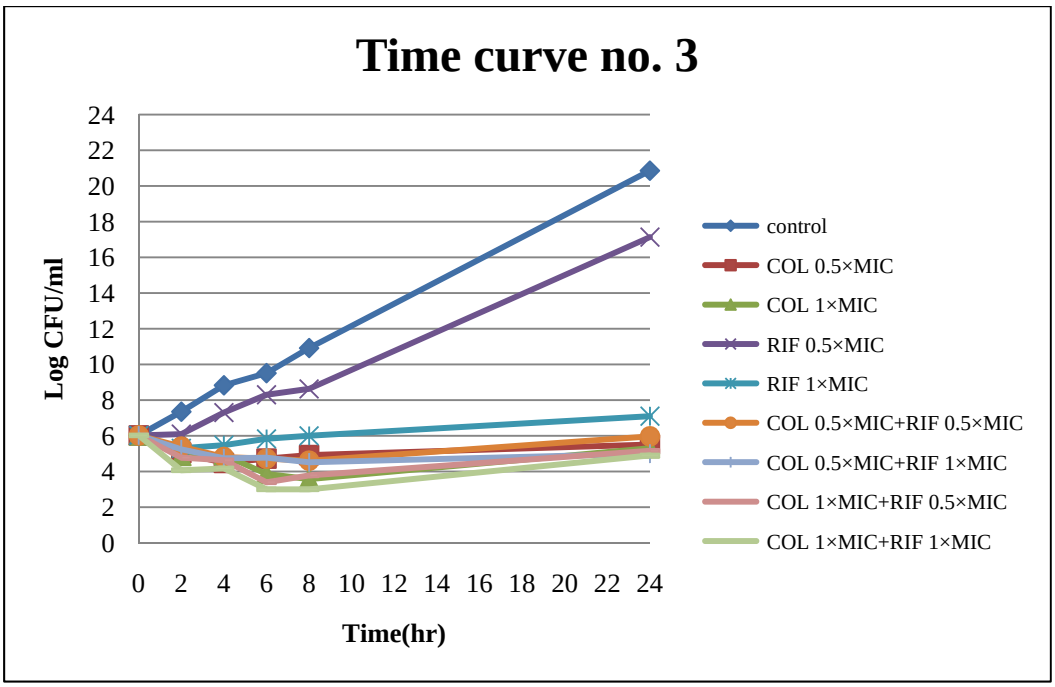

Figure D-1 Time kill curve of colistin 0.5×MIC, 1×MIC and rifampicin 0.5 ×MIC, 1×MIC alone, in combinations against *A. baumannii* no.3

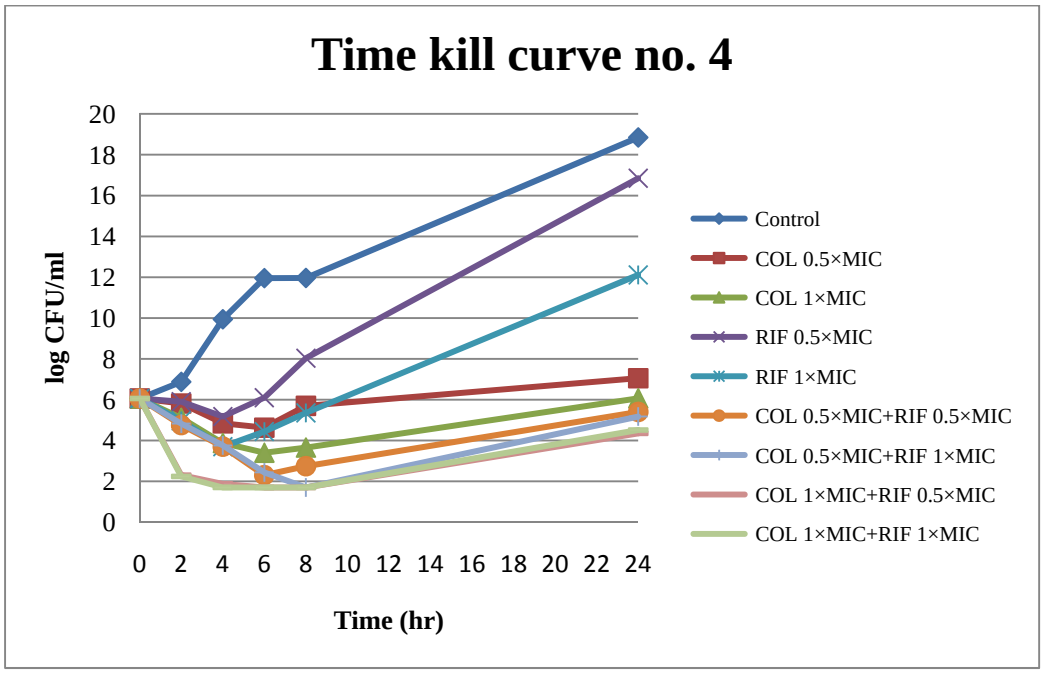

Figure D-2 Time kill curve of colistin 0.5×MIC, 1×MIC and rifampicin 0.5 ×MIC, 1×MIC alone, in combinations against *A. baumannii* no.4

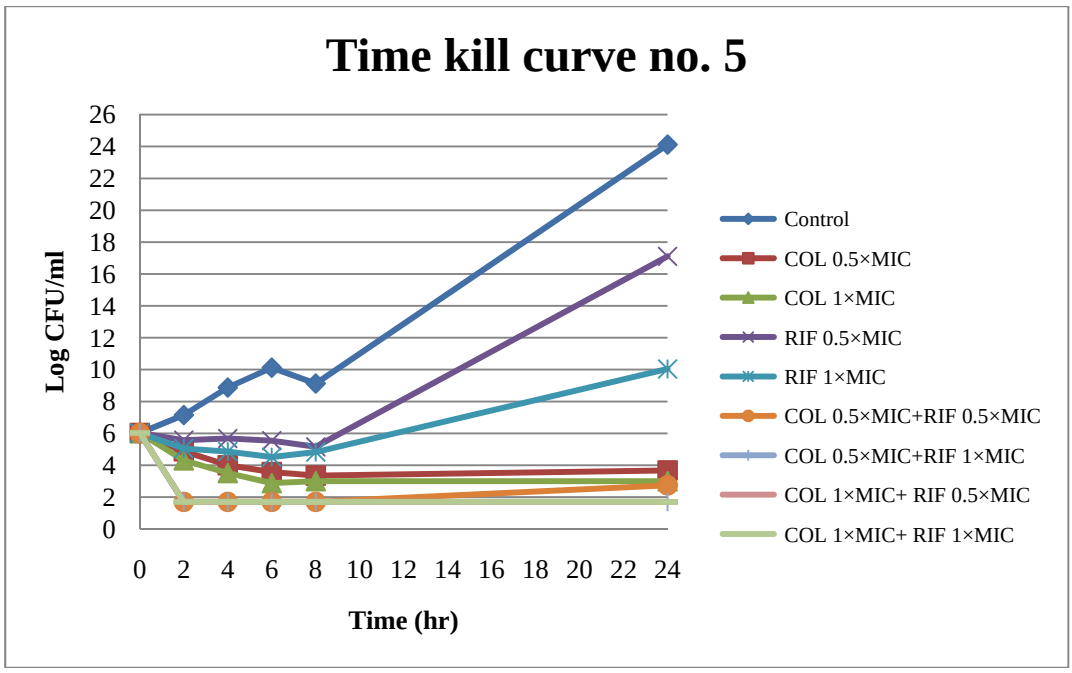

Figure D-3 Time kill curve of colistin 0.5×MIC, 1×MIC and rifampicin 0.5 ×MIC, 1×MIC alone, in combinations against *A. baumannii* no.5

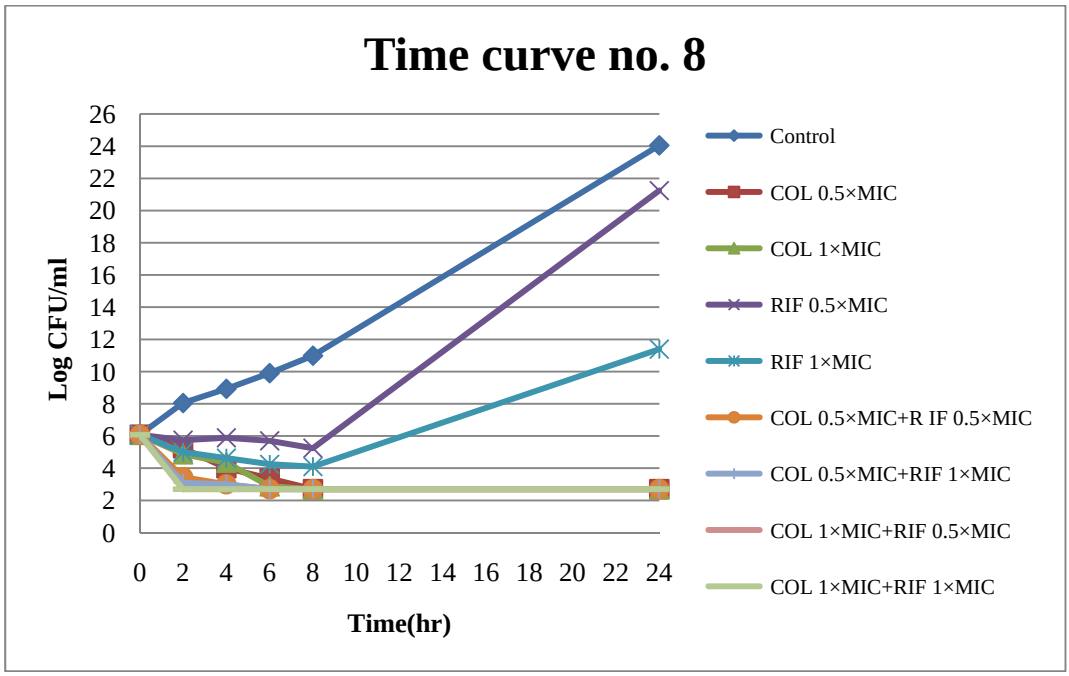

Figure D-4 Time kill curve showing the antibacterial activity of colistin 0.5×MIC, 1×MIC and rifampicin 0.5 ×MIC, 1×MIC alone, in combinations against *A. baumannii* no.8

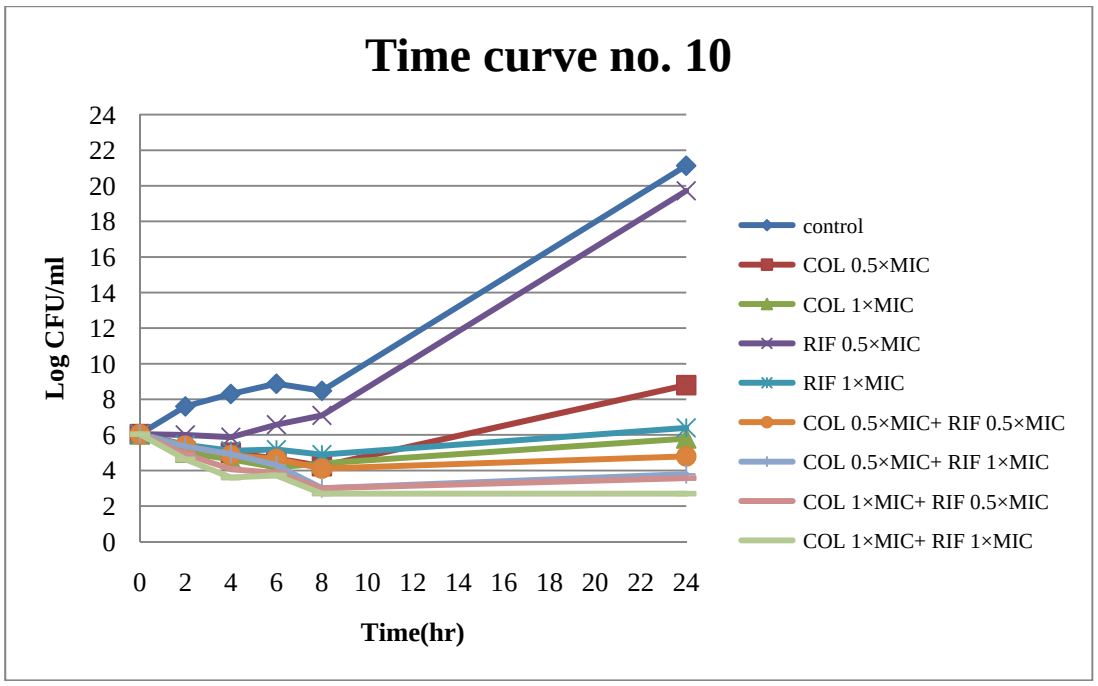

Figure D-5 Time kill curve of colistin 0.5×MIC, 1×MIC and rifampicin 0.5 ×MIC, 1×MIC alone, in combinations against *A. baumannii* no.10

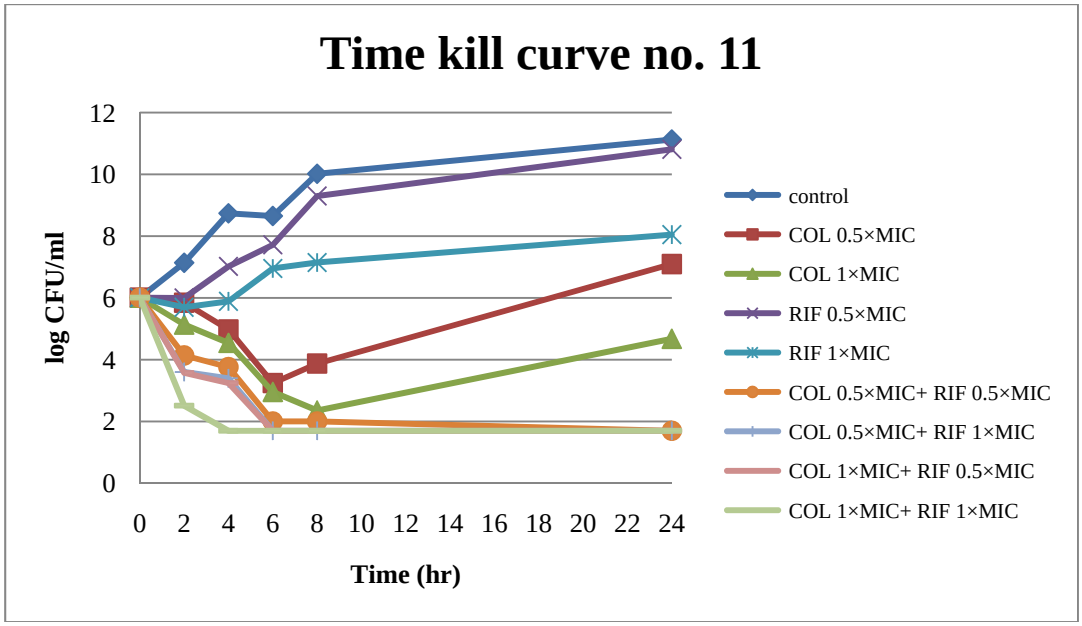

Figure D-6 Time kill curve of colistin 0.5×MIC, 1×MIC and rifampicin 0.5 ×MIC, 1×MIC alone, in combinations against *A. baumannii* no.11

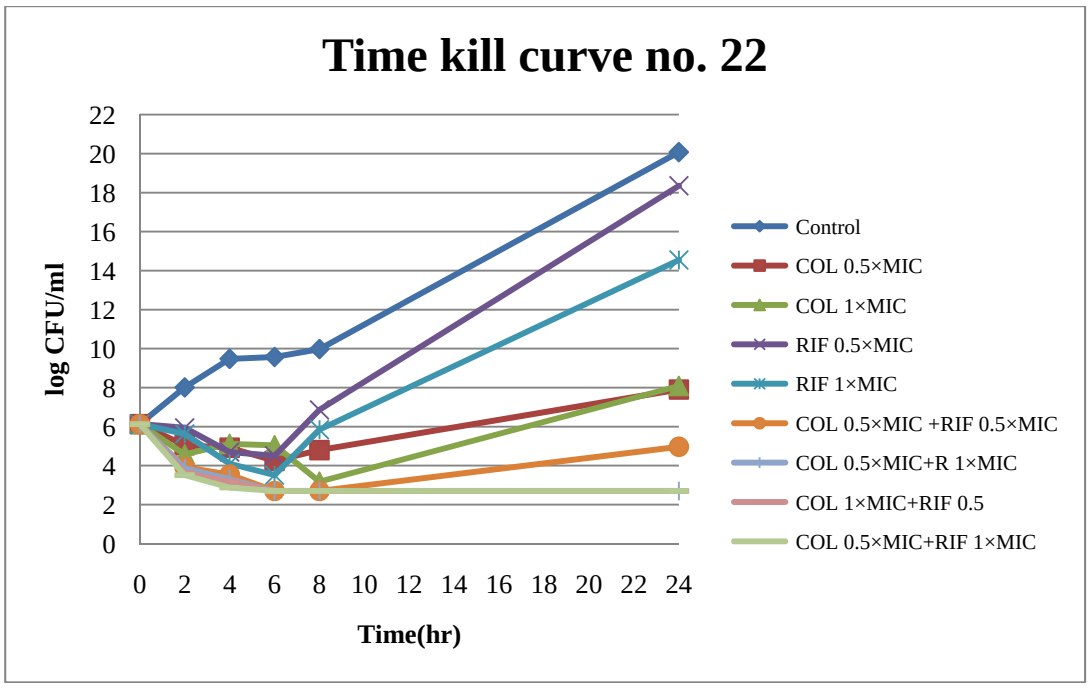

Figure D-7 Time kill curve of colistin 0.5×MIC, 1×MIC and rifampicin 0.5 ×MIC, 1×MIC alone, in combinations against *A. baumannii* no.22

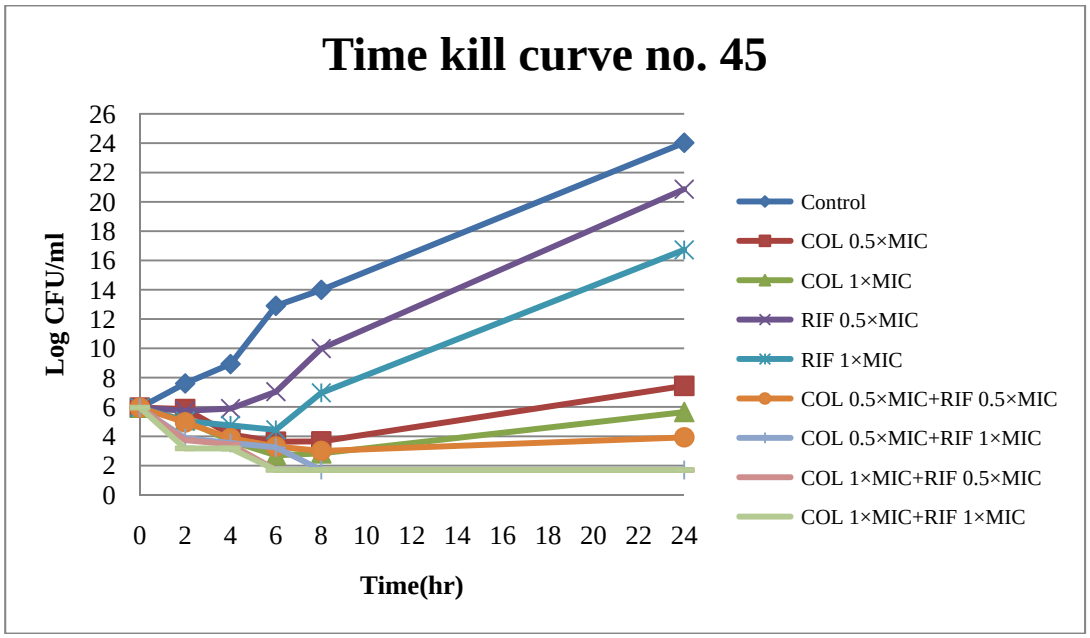

Figure D-8 Time kill curve of colistin 0.5×MIC, 1×MIC and rifampicin 0.5 ×MIC, 1×MIC alone, in combinations against *A. baumannii* no.45

BIOGRAPHY

My name is Kitsumon Kongsanae, I was born in 20 February 1981 at Suratthani. I graduated the bachelor degree in Pharmacy from Rangsit University since 2004. I started to work as a pharmacist in Bureau of drug and narcotic, Department of medical sciences, Ministry of Public Health, Nonthaburi in 2006. Then, I have enrolled for the master's degree in Microbiology at the department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences Chulalongkorn University since June 2009.