ฤทธิ์ต้านเชื้อในหลอดทดลองของเมอโรพีเนม โคลิสทินและซัลแบกแทม เมื่อให้ร่วมกันสองและสามชนิดต่อ *ACINETOBACTER BAUMANNII* ที่ดื้อต่อยาหลายชนิด

นางสาวสุภรักษ์ อมรนพรัตนกุล

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IN VITRO ANTIMICROBIAL ACTIVITIES OF MEROPENEM, COLISTIN, AND SULBACTAM IN DOUBLE AND TRIPLE COMBINATIONS AGAINST MULTIDRUG- RESISTANT *ACINETOBACTER BAUMANNII*

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สุภรักษ์ อมรนพรัตนกุล : ฤทธิ์ต้านเชื้อในหลอดทดลองของเมอโรพีเนม โคลิสตินและซัลแบกแทม เมื่อให้ร่วมกันสองและสามชนิดต่อ *ACINETOBACTER BAUMANNII* ที่ดื้อต่อยาหลายชนิด. (*IN VITRO* ANTIMICROBIAL ACTIVITIES OF MEROPENEM, COLISTIN AND SULBACTAM IN DOUBLE AND TRIPLE COMBINATIONS AGAINST MULTIDRUG- RESISTANT *ACINETOBACTER BAUMANNII*) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.ศิริภรณ์ ฟุ้งวิทยา, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ. ดร.พิณทิพย์ พงษ์เพียร, 201 หน้า.

Acinetobacter baumannii เป็นสาเหตุหลักของการติดเชื้อในร.พ.โดยเฉพาะในผู้ป่วยในหน่วยอภิบาลผู้ป่วย (ICU) ปัจจุบันพบเชื้อ *A. baumannii* ที่มีการดื้อต่อยาต้านจุลชีพหลายชนิด (multidrug-resistant; MDR) รวมถึงยาต้านจุลชีพที่มีใช้อยู่ทั่วไป ด้วยเหตุนี้ จึงได้มีความสนใจในการศึกษายาซึ่งโดยปกติไม่ได้ใช้ร่วมกันในการรักษาการติดเชื้อจาก *A. baumannii* ได้แก่ colistin และ sulbactam อย่างไรก็ตาม ยังไม่มีการศึกษาถึงฤทธิ์ในการต้านเชื้อของ meropenem, colistin และ sulbactam เมื่อให้ร่วมกันสองและสามชนิดต่อ *A. baumannii* ที่ดื้อต่อยาหลายชนิดที่เก็บตัวอย่างจากในประเทศไทย ดังนั้นจึงเป็นที่มาของการศึกษาในครั้งนี้ โดยมีวัตถุประสงค์ในการศึกษาฤทธิ์ต้านเชื้อในหลอดทดลองของ meropenem, colistin และ sulbactam เมื่อให้ร่วมกันสองและสามชนิดต่อ *A. baumannii* ที่ดื้อต่อยาหลายชนิดจำนวน 30 ไอโซเลท

เชื้อทั้ง 30 ไอโซเลทจัดเป็นเชื้อ *A. baumannii* ที่ดื้อต่อยาหลายชนิด เนื่องจากมีการดื้อต่อยาต้านจุลชีพที่ออกฤทธิ์กว้างตั้งแต่ 3 ถึง 5 ชนิดที่ทำการทดสอบด้วยวิธี disk diffusion เมื่อทดสอบด้วยวิธี agar dilution พบว่าทุกไอโซเลทดื้อต่อ meropenem (MIC= 64–256 µg/ml) ในขณะที่ทุกไอโซเลทไวต่อ colistin (MIC= 0.5-2 µg/ml) และค่า MIC ของ sulbactam อยู่ในช่วง 4-64 µg/ml จากการศึกษาผลของการให้ยาร่วมกันด้วยวิธี checkerboard พบว่าเมื่อให้ meropenem ร่วมกับ sulbactam เกิดการเสริมฤทธิ์กันต่อเชื้อ 21 ไอโซเลท (70%), เมื่อให้ meropenem ร่วมกับ colistin เกิดการเสริมฤทธิ์กันต่อเชื้อ 22 ไอโซเลท (73.33%) และเมื่อให้ sulbactam ร่วมกับ colistin เกิดการเสริมฤทธิ์กันต่อเชื้อจำนวน 16 ไอโซเลท (53.33%) ในขณะที่เมื่อให้ยาทั้งสามชนิดร่วมกันจะเกิดการเสริมฤทธิ์ต่อเชื้อ 29 ไอโซเลท คิดเป็น 96.67% เมื่อนำเชื้อที่มีความไวต่อยาในระดับต่างๆกันจำนวน 10 ไอโซเลท มาศึกษาถึงฤทธิ์ของยาในการฆ่าเชื้อ (bactericidal activity) โดยวิธี time-kill พบว่า เมื่อให้ meropenem 50 µg/ml ไม่พบฤทธิ์ในการฆ่าเชื้อตลอดช่วงระยะเวลาที่ทำการศึกษา เมื่อให้ sulbactam 30 µg/ml พบฤทธิ์ในการฆ่าเชื้อ 2 ไอโซเลท ที่เวลา 8 ชั่วโมงหลังได้รับยา ในขณะที่เมื่อให้ colistin 0.5 µg/ml พบฤทธิ์ในการฆ่าเชื้อได้ที่เวลา 2 ถึง 8 ชั่วโมง อย่างไรก็ตามพบการเจริญกลับของเชื้อได้ที่ 24 ชั่วโมง ในเชื้อที่ให้ยาเดี่ยวทั้ง 3 ชนิด เมื่อใช้ meropenem ร่วมกับ sulbactam พบฤทธิ์ในการฆ่าเชื้อได้ที่เวลา 4 ถึง 24 ชั่วโมง แต่พบการกลับเจริญขึ้นได้อีกที่เวลา 24 ชั่วโมงในเชื้อจำนวน 8 ไอโซเลท (80%) จำนวนเชื้อที่ถูกฆ่าภายใน 24 ชั่วโมง (BA_{24}) แตกต่างจากการให้ meropenem เดี่ยวอย่างมีนัยสำคัญทางสถิติ แต่ไม่แตกต่างจากการให้ sulbactam เดี่ยว เมื่อให้ meropenem ร่วมกับ colistin พบฤทธิ์ในการฆ่าเชื้อได้ที่เวลา 2 ถึง 24 ชั่วโมง และค่า BA_{24} แตกต่างจากการให้ meropenem อย่างมีนัยสำคัญทางสถิติ แต่ไม่แตกต่างจากการให้ colistin เช่นเดียวกับเมื่อให้ sulbactam ร่วมกับ colistin ที่พบฤทธิ์ในการฆ่าเชื้อได้ที่เวลา 2 ถึง 24 ชั่วโมงแต่ค่า BA_{24} ไม่แตกต่างจากการให้ sulbactam และ colistin เดี่ยว และการเจริญกลับของเชื้อที่เวลา 24 ชั่วโมง ของการให้ sulbactam ร่วมกับ colistin มีจำนวนมากกว่า คือ 6 ไอโซเลท เมื่อเทียบกับ 4 ไอโซเลทของการให้ meropenem ร่วมกับ colistin ในขณะที่เมื่อให้ยาทั้งสามชนิดร่วมกัน พบฤทธิ์ในการฆ่าเชื้อได้ที่เวลา 2 ถึง 24 ชั่วโมง และพบการเจริญกลับของเชื้อที่เวลา 24 ชั่วโมงเพียง 1 ไอโซเลท ค่า BA_{24} แตกต่างจากการให้ meropenem และ sulbactam เดี่ยวอย่างมีนัยสำคัญทางสถิติ แต่ไม่แตกต่างจากการให้ colistin เดี่ยว

จากข้อมูลค่า BA_{24} พบว่าไม่มีความแตกต่างในทางสถิติระหว่างการให้ยาร่วมสองชนิดและการให้ยาร่วมสามชนิด และการให้ meropenem ร่วมกับ colistin ให้ผลใกล้เคียงกับการให้ meropenem, colistin และ sulbactam ร่วมกัน อย่างไรก็ตาม การให้ยาทั้งสามชนิดร่วมกันนั้นมีการเจริญกลับของเชื้อที่เวลา 24 ชั่วโมงน้อยกว่า ประกอบกับมีการเสริมฤทธิ์กันมากกว่าการให้ยาร่วมสองชนิดข้างต้น ดังนั้น การให้ meropenem, colistin และ sulbactam ร่วมกันจึงน่าจะเป็นทางเลือกที่ดีในการรักษาการติดเชื้อที่เกิดจาก *A. baumannii* ที่ดื้อต่อยาหลายชนิดได้

ภาควิชา...เภสัชวิทยาและสรีรวิทยา... ลายมือชื่อนิสิต.....

สาขาวิชา...เภสัชวิทยา... ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา...2552... ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

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KEYWORDS : *ACINETOBACTER BAUMANNII* / MEROPENEM / COLISTIN / SULBACTAM / COMBINATION

SUPARAK AMORNNOPPARATTANAKUL: *IN VITRO* ANTIMICROBIAL ACTIVITIES OF MEROPENEM, COLISTIN, AND SULBACTAM IN DOUBLE AND TRIPLE COMBINATIONS AGAINST MULTIDRUG-RESISTANT *ACINETOBACTER BAUMANNII*. THESIS ADVISOR : ASSOC. PROF. SIRIPORN FUNGWITTHAYA, M.Sc., THESIS CO-ADVISOR : ASSOC. PROF. PINTIP PONGPECH, Ph.D., 201 pp.

Acinetobacter baumannii has emerged as a major cause of nosocomial opportunistic infections in immunocompromised patients, particularly in the intensive care unit (ICU). It is well known as a multidrug-resistant (MDR) pathogen which is resistant to most commonly available antimicrobial agents. Non-traditional antimicrobial agents such as colistin and sulbactam have been studied for the treatment of infections caused by MDR *A. baumannii*. However, there is no data available on the activity of meropenem, colistin, and sulbactam in double and triple combinations against clinical isolates of MDR *A. baumannii* collected in Thailand. Therefore, the objective of this study is to determine the *in vitro* antimicrobial activities of meropenem, colistin, and sulbactam in double and triple combinations against 30 clinical isolates of MDR *A. baumannii*.

All isolates were considered to be MDR isolates due to the resistance of 3 to 5 broad-spectrum agents from the disk diffusion test. From the agar dilution method, all isolates were resistant to meropenem (MICs range = 64–256 µg/ml) but were susceptible to colistin (MICs range = 0.5-2 µg/ml) while the MICs of sulbactam range from 4-64 µg/ml. From the results of combination effects studied by checkerboard method, the synergistic effects of the double combination of meropenem with sulbactam, meropenem with colistin, and sulbactam with colistin were observed in 70%, 73.33%, and 53.33% of all isolates of MDR *A. baumannii*, respectively, whereas the triple combination showed synergistic effects against 96.67% of the isolates. In the time kill study, 10 MDR *A. baumannii* isolates were tested. After given meropenem 50 µg/ml, the bactericidal activity (99.9% killing or ≥3 log CFU/ml decreased) was not observed at any time. For 30 µg/ml sulbactam, 99.9% killing was observed in 2 isolates at the 8th hour. Whereas, in colistin 0.5 µg/ml, 99.9% killing was observed at the 2nd to 8th hour. However, the regrowth was shown at the 24th hour when the agent was given alone. In the combination of meropenem and sulbactam, bactericidal was observed at the 4th to 24th hour but the regrowth was also found in 8 isolates (80%) at 24th hour, bacteriolytic area for 24 hours (BA_{24}) was significantly different from meropenem alone but not different from sulbactam alone. When meropenem combined with colistin, 99.9% killing could be observed since the 2nd to 24th hour, and BA_{24} was significantly different from meropenem alone but not different from colistin alone. Similarly with the combination of sulbactam and colistin that 99.9% killing could be observed since the 2nd to 24th hour but BA_{24} was not different from sulbactam and colistin alone and the regrowth at 24th hour was shown more in the latter combination (4 and 6 isolates, respectively). In the triple combination of meropenem, sulbactam, and colistin, 99.9% killing could also be observed since the 2nd to 24th hour. The regrowth was found at 24th hour in only 1 isolate. The BA_{24} of the triple combination was significantly different from meropenem and sulbactam alone but not different from colistin alone.

There were no statistical difference in BA_{24} between the double combinations and the triple combination and the combination of meropenem and colistin was as effective as the triple combination. However, the regrowth at 24th hour occurred less in the triple combination accompanied with the synergistic effects shown more in the triple combination. Therefore, the triple combination of meropenem, colistin, and sulbactam could be the promising alternative for the treatment of infections due to MDR *A. baumannii*.

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LIST OF ABBREVIATIONS

β	=	beta
°C	=	degree Celsius
μ	=	micro
μg	=	microgram
μl	=	microlitre
μm	=	micrometer
%	=	percent
A. baumannii	=	*Acinetobacter baumannii*
AMI	=	amikacin
ARI-1	=	*Acinetobacter* resistant to imipenem
ATCC	=	American Type Culture Collection
AUBKC	=	area under the bacterial killing and regrowth curves
BA_{24}	=	bacteriolytic area of 24 hours
BSI	=	bloodstream infection
CAZ	=	ceftazidime
CIP	=	ciprofloxacin
CFU	=	colony forming unit
CHDLs	=	carbapenem-hydrolyzing activities
CLSI	=	Clinical and Laboratory Standards Institute
DHP-1	=	dehydropeptidase-1
E. coli	=	*Escherichia coli*
et al.	=	et alii (and other peoples)

FIC	=	fractional inhibitory concentration
FICI	=	fractional inhibitory concentration index
FEP	=	cefepime
GEN	=	gentamicin
HMP-AB	=	heat-modifiable protein in *Acinetobacter baumannii*
hr	=	hour
ICU	=	intensive care unit
ICUSS	=	Intensive Care Unit Surveillance System
IMP	=	imipenem
kDa	=	kiloDalton
L	=	liter
Log	=	decimal logarithm
LPS	=	lipopolysaccharides
LVX	=	levofloxacin
MBL	=	metallo-β-lactamase
Mg	=	milligram
MDR	=	multidrug-resistant
MEM	=	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
NCCLS	=	The National Committee for Clinical Laboratory Standards
NNIS	=	National Nosocomial Infections Surveillance
No.	=	number
NSS	=	Normal saline solution
OMP	=	outer membrane protein
OXA	=	Oxacillin-hydrolyzing β-lactamase
P. aeruginosa	=	*Pseudomonas aeruginosa*
PBP	=	penicillin-binding protein
RND	=	resistance-nodulation-division
SAM	=	ampicillin-sulbactam
SCOPE	=	Surveillance and Control of Pathogens of Epidemiological Importance
SENTRY	=	SENTRY Antimicrobial Surveillance Program;
SSTI	=	skin and soft tissue infection
SXT	=	trimetropim-sulfamethoxazole
TSA	=	tryptic soy agar
TSAR	=	Taiwan Surveillance of Antimicrobial Resistance
TSN	=	The Surveillance Network
TZP	=	piperacillin-tazobactam
UTI	=	urinary tract infection
VAP	=	ventilator-associated pneumonia

CHAPTER I

INTRODUCTION

Acinetobacter baumannii has emerged as a major cause of nosocomial opportunistic infections in immunocompromised patients, particularly in the intensive care unit (ICU). The associated risk factors of *Acinetobacter* nosocomial infections have been identified, including advanced age, severity of illness, immunosuppression, surgery, burns, prolonged mechanical ventilation, prior treatment with broad-spectrum antimicrobials, prior colonization with *Acinetobacter* spp., and prolonged hospital or ICU stays (Jain and Danziger, 2004; Rungruanghiranya, Somboonwit, and Kanchanapoom, 2005).

Acinetobacter spp. can cause the infections including bacteremia, endocarditis, meningitis, urinary tract infections, skin and wound infection, and pneumonia, particularly ventilator-associated pneumonia (VAP) (Bergogne-Berezin and Towner, 1996). The crude mortality rate associated with bacteremia is approximately 52% and that associated with pneumonia ranges from 23% to 73% (Jain and Danziger, 2004).

This organism was responsible for 6.9% of pneumonia, 2.4% of bloodstream infections, 2.1% of surgical site infections and 1.6% of urinary tract infections in intensive care units across USA in 2003 (NNIS, 2004). From the SENTRY antimicrobial surveillance program between 1997 and 1999, the prevalence of *Acinetobacter* spp. recovered from respiratory tract infections ranged from 2.0% in Canada, 2.5% in the United States, and up to 9.7% in Latin America which was 2-fold more frequently found from wounds (Gales et al., 2001). These correspond with the study in 155 patients who were admitted to Siriraj Hospital, Thailand in 2005. It was shown that the lower respiratory tract was the most common site of MDR *A. baumannii* nosocomial infection (74.8%) followed by urinary tract (11%), surgical site infection (4.5%) and systemic infection (4.5%) (Surasarang et al., 2007).

The widespread of multidrug-resistant (MDR) *A. baumannii* nosocomial infections in recent years was contributed, at least in part, to the ability to endure for prolonged

periods throughout the wide range of environment and the tendency to acquire diverse mechanisms of resistance to antimicrobials (Bergogne-Berezin and Towner, 1996; Maragakis and Perl, 2008). Nowadays, although definitions of multidrug-resistance (MDR) vary in the literature (Falagas, Koletsi, and Bliziotis, 2006), MDR *A. baumannii* have become resistant to almost all antimicrobial agents that are currently available, including broad-spectrum cephalosporins (ceftazidime or cefepime), carbapenems (imipenem or meropenem), ampicillin-sulbactam, fluoroquinolones (ciprofloxacin or levofloxacin), and aminoglycosides (gentamicin, tobramycin, or amikacin) (Van Looveren, Goossens, and ARPAC Steering Group., 2004). Moreover, the strains resistant to all known antimicrobials have now been reported, including in Thailand (Chaiwarith et al., 2005; Keerasuntonpong et al., 2006; Peleg, Seifert, and Paterson, 2008).

From the increasing occurrence of MDR *A. baumannii* over recent years accompany with the deficiency of new antimicrobials discovery caused limited therapeutic options globally. Consequently, colistin that was abandoned in most parts of the world in the early 1980s because of the reported high incidence of nephrotoxicity and neurotoxicity has been revived (Falagas and Kasiakou, 2005; Li, Nation, et al., 2006).

Furthermore, the non-traditional antimicrobial agents such as rifampicin and sulbactam/ampicillin have been studied for the treatment of severe infections caused by MDR *A. baumannii* (Tripodi et al., 2007). However, the emergence of rifampicin resistance during imipenem–rifampicin combination therapy when treating carbapenem-resistant *A. baumannii* infections is an important limitation for its clinical use (Saballs et al., 2003).

Sulbactam, the only one from three available β-lactamase inhibitors that has the intrinsic antimicrobial activity against *Acinetobacter* spp. (Higgins et al., 2004), is the promising choice to treat MDR *A. baumannii* infections. Nonetheless, the resistance of sulbactam is common in certain geographic areas, and this will be undoubtedly increased over time (Peleg et al., 2008).

In addition to the usage of the above-mentioned antimicrobial alone, the clinical use of antimicrobials combination therapy to treat MDR *A. baumannii* infections has become generally admitted. This strategy not only enhanced the efficacy by synergistic effect and allowed lower doses to reduce toxicity, but also prevented the emergence of resistance (Rahal, 2006). However, from the previous reviews, the study on the combination of meropenem, colistin, and sulbactam, especially in Thai patients have not been performed.

The hypothesis to be tested in this study is that the combination of meropenem, colistin, and sulbactam provide the synergistic antimicrobial activities against MDR *A. baumannii*.

Therefore, this study will emphasize the *in vitro* activities of meropenem, colistin, and sulbactam alone and in combinations against MDR *A. baumannii* from clinical isolates. In order to obtain the informative conclusions on this aspect, the experimental studies were designed to determine:

1. The antimicrobial susceptibility of clinical isolated *A. baumannii* and screening for multidrug-resistant (MDR) *A. baumannii.*

2. The double and triple combination effects of meropenem, colistin, and sulbactam against the multidrug-resistant *A. baumannii* by checkerboard method.

3. The bactericidal activity of meropenem, colistin, and sulbactam alone and in combinations against the multidrug-resistant *A. baumannii* by time-kill method.

4. The morphological changes of multidrug-resistant *A. baumannii* exposed to meropenem, colistin, and sulbactam alone and in combinations by scanning electron microscope.

CHAPTER II

LITERATURE REVIEWS

1. *Acinetobacter baumannii*

1.1 Microbiology

Acinetobacter spp. is a gram-negative, non-fermentative, non-spore forming, non-motile, encapsulated, strictly aerobic, oxidase-negative coccobacillus (Gerner-Smidt, 1994; Chastre and Trouillet, 2000). *Acinetobacter* spp. was first described in 1911 as *Micrococcus calco-aceticus.* Since then, it was described to at least 15 different genera and species, becoming known as *Acinetobacter* spp. in 1954 and more widely accepted in 1968 (Munoz-Price and Weinstein, 2008; Peleg et al., 2008).

Four of all identified species, i.e., *Acinetobacter calcoaceticus*, *Acinetobacter baumannii*, *Acinetobacter* genomic species 3, and *Acinetobacter* genomic species 13TU, are very closely related and difficult to distinguish from each other by phenotypic properties. Therefore, these species have been referred to as *A. calcoaceticus-A. baumannii* complex. However, only three species, i.e., *A. baumannii*, *Acinetobacter* genomic species 3, and *Acinetobacter* genomic species 13TU, are clinically relevant species, meanwhile *A. calcoaceticus*, regularly found in soil and water, has never been associated in serious clinical diseases; therefore, designation *A. calcoaceticus-A. baumannii* complex may be misleading and inappropriate if used in a clinical context. It has been suggested that since the three clinically relevant members of the *A. calcoaceticus-A. baumannii* complex cannot be separated by currently available commercial identification systems and they share important clinical and epidemiological characteristics, it seems appropriate to use the term *A. baumannii* instead of *A. calcoaceticus-A. baumannii* complex and also eliminates the confusion due to the inclusion of an environmental species, *A. calcoaceticus* (Peleg et al., 2008).

1.2 Habitats

Members of the genus *Acinetobacter* are ubiquitous in nature and usually found in water, soil and on vegetables (Lortholary et al., 1995; Bergogne-Berezin and Towner, 1996; Berlau et al., 1999), but not all species have their natural habitat in the environment (Peleg et al., 2008). They have also been isolated from skin, throat, and various other sites in healthy people (Bergogne-Berezin and Towner, 1996). In addition, it has usually been isolated from the skin of healthcare personnel, curtains, mattresses, pillows, humidifiers, ventilator equipment, wound care procedures, and other equipment in the hospital environment (Jain and Danziger, 2004; Wilks et al., 2006; Maragakis and Perl, 2008). Actually, *Acinetobacter* spp. is the most common gram-negative organism colonized on the skin of hospital personnel, including ICU nurses and respiratory therapists (Chastre, 2003).

2. Clinical Manifestations

In general, *Acinetobacter* spp. are non-virulent organisms, but in critically ill patients, immunosuppressed patients, patients with underlying diseases, and in those subjected to invasive procedures and treated with broad-spectrum antimicrobials, their pathogenic role has been well documented (Jain and Danziger, 2004; Perez, et al., 2007). The increased risk of nosocomial infections in the intensive care unit (ICU) patients was contributed to the susceptibility of infections due to underlying diseases or impaired immune functions of the critical ill patients, invasive monitoring devices, such as endotracheal tubes and central venous catheters, and the close contact between hospital staff and patients also promote opportunities of infections (Arnold, Forrest, and Messmer, 2007).

Acinetobacter spp. can cause a multitude of infections such as, bacteremia, endocarditis, meningitis, urinary tract infections, skin and wound infections, and pneumonia (Bergogne-Berezin and Towner, 1996). However, the most common clinical manifestations of *Acinetobacter* infections are ventilator-associated pneumonia (VAP),

bloodstream infections, and urinary tract infections (Rungruanghiranya et al., 2005; Munoz-Price and Weinstein, 2008).

2.1 Pneumonia

Several studies have reported that about 3 to 5% of nosocomial pneumonias are caused by *Acinetobacter* spp. (Bergogne-Berezin and Towner, 1996) and the data analyzed from the National Nosocomial Infections Surveillance (NNIS) system showed that the proportion of *Acinetobacter* species associated with ICU pneumonia was significantly higher in 2003 (7%), compared with 1986 (4%) (Gaynes and Edwards, 2005).

The mechanical ventilation has become the prominent cause of the *Acinetobacter* nosocomial pneumonias (Bergogne-Berezin and Towner, 1996; Munoz-Price and Weinstein, 2008). There have been reported the crude mortality rates of 30 to 75% for *Acinetobacter* pneumonia, with the highest rates reported in ventilator-dependent patients (Bergogne-Berezin and Towner, 1996).

2.2 Bloodstream Infection (BSI)

The most common sources of bacteremia by *A. baumannii* are intravascular and respiratory tract catheter, followed by surgical wounds, burns, and the urinary tract is less common, and rarely found from endocarditis (Cisneros and Rodriguez-Bano, 2002). The data generated by the Surveillance and Control of Pathogens of Epidemiological Importance (SCOPE) Project from 1995-2002 have shown that *A. baumannii* was the 10th most common causative agent, being responsible for 1.3% of all monomicrobial nosocomial bloodstream infections. *A. baumannii* was more likely to be isolated from patients in ICUs than patients in non-ICU ward (1.6% versus 0.9% of bloodstream infections, respectively, in those locations). The crude mortality rate of *A. baumannii* bloodstream infection was 34.0% in total, 43.4% which is the third rank in the ICU, and 16.3% in the non-ICU ward (Wisplinghoff et al., 2004; Peleg et al., 2008).

2.3 Urinary Tract Infection (UTI)

Nosocomial urinary tract infection is occasionally occurred by *A. baumannii*, being responsible for 1.6% of ICU-associated UTIs in 2003 which was significantly increased from 0.6% in 1975 (Gaynes and Edwards, 2005). It usually occurs in elderly debilitated patients, in patients with prolonged ICU stay, and in the patients with indwelling urinary catheter (Bergogne-Berezin and Towner, 1996; Rungruanghiranya et al., 2005).

2.4 Skin and Soft Tissue Infection (SSTI)

A. baumannii is fairy uncommon cause of skin and soft tissue infection. From the data of NNIS, this organism caused 2.1% of ICU-acquired SSTIs in 2003 (Gaynes and Edwards, 2005), and being responsible for 4.1% of all SSTIs from Latin America (Sebeny et al., 2008). Recently, the incidence of *A. baumannii* wound infection in combat casualties from Iraq-Kuwait region or Afghanistan has increased (Peleg et al., 2008; Sebeny et al., 2008).

3. Global Epidemiology of *A. baumannii*

The characteristics of *A. baumannii* to survive and persist for a long time in the environment, as well as to acquire diverse mechanisms of resistance to antimicrobial agents contribute to the formidable outbreaks of *A. baumannii* nosocomial infections (Bergogne-Berezin and Towner, 1996; Peleg et al., 2008). Moreover, these outbreaks have increasingly exhibited the pattern of multidrug-resistant (MDR) and pandrug-resistant (PDR) *A. baumannii* (Falagas et al., 2006). There are reports of these outbreaks from many regions of the world, including Europe, North America, Latin America and Asia-Pacific (Table 2-1).

Report from the SENTRY antimicrobial surveillance program (2001–2004) showed that the antimicrobial susceptibility of isolates of *Acinetobacter* spp. varied

Table 2-1 Global susceptibility of *A. baumannii* to selected antimicrobial agents

Geographic area	Location/study	Year	Susceptibility (%) to:										Reference
			IMP	MEM	FEP	CAZ	SAM	TZP	CIP	LVX	GEN	SXT	
North America	SENTRY	2001-2004	89	84	57	54	71		54				Gales, Jones, and Sader, 2006
	MYSTIC	2002-2004	93	91	63			69	64		63		Unal and Garcia-Rodriguez, 2005
	United States (ICUs)	2002-2004	88		37	77	59	45	35				Lockhart et al., 2007
	United States (hospital isolates)/SENTRY	1998-2003	93		63	62		63	61		64		Sader, Fritsche, and Jones, 2005
	United States (hospital isolates)/MYSTIC	2003	92	87	63	64		61	59	60	63		Rhomberg et al., 2003
	United States (ICUs)/TSN	2000-2002	87	66	44	42		54	40	44	47	51	Jones et al., 2004
	Canada (ICUs)/TSN	2000-2002	96	94	67	71		71	72	61	73	75	Jones et al., 2004
	United States (ICUs)/SENTRY	2001	81	79	51	57		59	53		53		Streit et al., 2004
	United States (non-ICUs)	2001	93	85	47	45		58	35	45	44		Karlowsky et al., 2003
	United States (ICUs)	2001	96	91	56	49			45	54	53		Karlowsky et al., 2003
	United States/ICUSS	2000	95		66	55	78	79	43				Friedland et al., 2003
Europe	SENTRY	2001-2004	74	70	44	40	48		39				Gales, Jones, and Sader, 2006
	MYSTIC	2002-2004	70	73	32			34	34		48		Unal and Garcia-Rodriguez, 2005
	United Kingdom and Ireland (bacteremia)	2001-2002	100			35		87	79		83		Reynolds et al., 2004
	Spain (hospital isolates)	2001	60	49	49	24	58	17	7	10	15	32	Picazo et al., 2004
	Italy (ICUs)/TSN	2000-2002	78	75	18	26		35	21	14	23	44	Jones et al., 2004
	France (ICUs)/TSN	2000-2002	94	68	28	35		75	38		49	45	Jones et al., 2004
	Germany (ICUs)/TSN	2000-2002	96	96	74	67		82	75	82	82	84	Jones et al., 2004
	Sweden (ICUs)	1999-2000	96					40	89			96	Hanberger et al., 2004
	Italy (respiratory isolates)	1997-1999	87	84	55	42		49	48		54	57	Fadda et al., 2004
Latin America	SENTRY	2001-2004	86	84	36	32	52		35				Gales, Jones, and Sader, 2006
	MYSTIC	2002-2004	72	71	28			31	35		48		Unal and Garcia-Rodriguez, 2005
	Brazil/SENTRY	2001	98	97	37	29		31	33	33	39	37	Jones et al., 2004
	Argentina (hospital isolates)	2001-2002	85		37	23	32	22		17			Casellas et al., 2003
Asia/ Pacific	SENTRY	2001-2004	74	84	58	58	59		55				Gales, Jones, and Sader, 2006
	Australasia/MYSTIC	2002-2004	99	94	81			80	83		82		Unal and Garcia-Rodriguez, 2005
	Korea (hospital isolates)	2003	87	73	59	45	53	58	42		36	43	Lee, et al., 2006
	China (ICUs)	2002	92	75	70	65	80	70	66				Wang and Chen, 2005
	Japan (hospital isolates)	2002	95		85	89	97						Ishii et al., 2005
	Taiwan (hospital isolates)/TSAR	2000	98		40	27		26	31		18	22	Lauderdale et al., 2004
	Thailand (hospital isolates)/NARST	2007	40	33	34	30	42	29	30		33	28	NARST, 2009

SENTRY, SENTRY Antimicrobial Surveillance Program; MYSTIC, Meropenem Yearly Susceptibility Test Information Collection; TSAR, Taiwan Surveillance of Antimicrobial Resistance; ICUSS, Intensive Care Unit Surveillance System; TSN, The Surveillance Network; NARST, National Antimicrobial Resistance Surveillance Center Thailand

IMP, imipenem; MEM, meropenem; FEP, cefepime; CAZ, ceftazidime; SAM, ampicillin-sulbactam; TZP, piperacillin-tazobactam; CIP, ciprofloxacin; LVX, levofloxacin; GEN, gentamicin; SXT, trimetropim-sulfamethoxazole

according to their geographical origin (Table 2-1). The lowest susceptibility rates to carbapenems were observed among isolates collected in Europe and the Asia-Pacific region (73.7% susceptible to imipenem), while isolates from Europe expressed the lowest susceptibility rates to ampicillin/sulbactam (48.4%), and isolates from Latin America demonstrated the lowest susceptibility rates to broad-spectrum cephalosporins (32.4% susceptible to ceftazidime, cefepime 36.4%), fluoroquinolones (ciprofloxacin 34.8%), and amikacin (40.4%) (Gales, Jones, and Sader, 2006).

The data from Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Program during 2002-2004 exhibited that in general, the susceptibility of *Acinetobacter* spp. from Australasia and North America was higher than that from Europe and South America (Table 2-1). The rank order of activity of the 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 Thailand, data from the National Antimicrobial Resistance Surveillance Center Thailand (NARST) showed that most antimicrobial susceptibility rates of *A. baumannii* decreased during 1998 and 2007 (Table 2-2). The susceptible rate of imipenem decreased from 98% to 40%, ceftazidime from 40% to 30%, amikacin from 48% to 37%, and ciprofloxacin from 45% to 30%. Corresponding with the prospective cohort study of 208 clinical isolates of *A. baumannii* recovered from the patients in Siriraj Hospital from January to December 2002, it revealed the susceptibility of *A. baumannii* to the following antimicrobial agents; carbapenems (32%), aminoglycosides (16%), β-lactam/β-lactamase inhibitors (12%), co-trimoxazole (9%), fluoroquinolones (7%), 4th generation cephalosporins (4%) and 3rd generation cephalosporins (3%). Fifty-seven percent of *A. baumannii* isolates were resistant to all antimicrobials currently available in Thailand (Keerasuntonpong et al., 2006).

Table 2-2 Percentage of susceptible *A. baumannii* in Thailand, during 1998-2007

Year	Susceptibility (%) to:					
	IMP	CAZ	TZP	CIP	AMI	GEN
1998	98	40	15	45	48	34
1999	94	16	16	49	47	34
2000	95	35	18	41	44	34
2001	92	36	33	42	42	35
2002	79	38	35	40	42	36
2003	65	33	34	34	38	34
2004	55	35	34	35	39	33
2005	27	30	18	31	38	30
2006	43	30	29	29	36	32
2007	40	30	29	30	37	33

IMP, imipenem; CAZ, ceftazidime; TZP, piperacillin-tazobactam; CIP, ciprofloxacin; AMI, amikacin; GEN, gentamicin

4. Antimicrobial Therapy and Mechanism of Resistance

Before 1970s, it was possible to treat nosocomial *Acinetobacter* infections with aminoglycosides, β-lactams, and tetracyclines, either as single agents or in combinations (Bergogne-Berezin and Towner, 1996). However, the increasing antimicrobial resistance of *A. baumannii* has now emerged, perhaps as a consequence of its ability to upregulate the innate resistance mechanisms and acquisition of foreign determinants, coupled with the widespread use of various antimicrobial agents in the hospital environment (Bergogne-Berezin and Towner, 1996; Peleg et al., 2008). The selection of appropriate initial empirical antibiotics is the key aspect and must rely on recent

institutional-level susceptibility data to help improve survival of the patients with *A. baumannii* infection (Peleg et al., 2008).

4.1 Carbapenems

Nowadays, carbapenems remain the treatment of choice for infections caused by *A. baumannii* if isolates retain susceptibility to this antimicrobial class (Maragakis and Perl, 2008). Carbapenems are β-lactam antimicrobial agents with an exceptionally broad spectrum of activity. They differ from penicillins (penams) in having a carbon atom replacing the sulphur at position 1 and an unsaturated bond between C2 and C3 in the five-membered ring structure (Zhanel et al., 2007) (Figure 2-1).

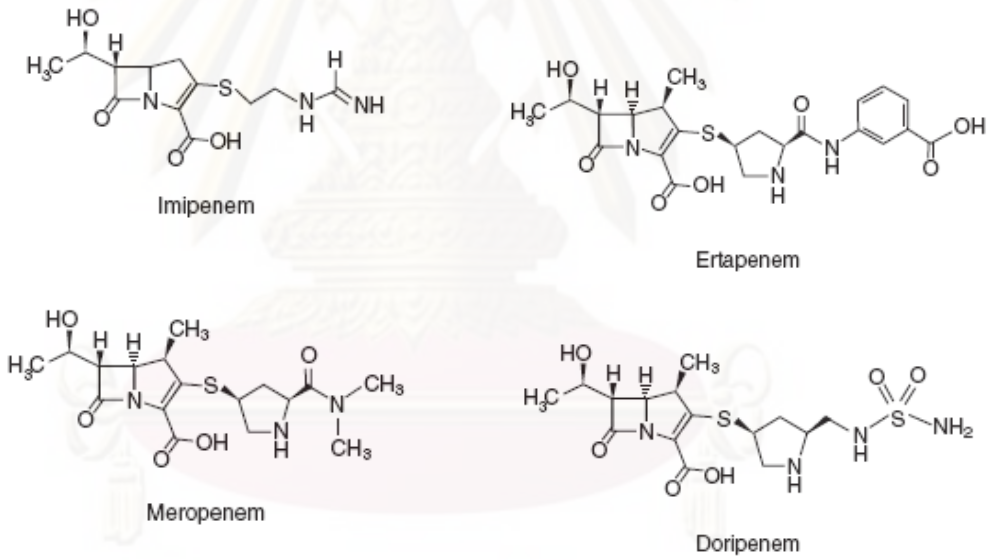

Figure 2-1 Chemical structures of carbapenems (Zhanel et al, 2007).

The older carbapenems, such as imipenem, were often susceptible to degradation by the enzyme dehydropeptidase-1 (DHP-1) located in the brush border of renal tubules and therefore required co-administration with a DHP-1 inhibitor, such as cilastatin, to prevent uptake into renal tubules. Following additions to the class, including meropenem, ertapenem and doripenem have increased stability to DHP-1 owning to the

presence of a 1-β methyl constituent on the carbapenem nucleus and can be administered without a DHP-1 inhibitor (Zhanel et al., 2007).

Meropenem differs from imipenem by having pyrrolidinyl substituent at the 2 position (Figure 2-1). This could be the reason for its superior activity against Gram-negative organisms when compared with imipenem (Zhanel et al., 2005).

The mechanism of action of carbapenems, like all β-lactam antimicrobial agents, exhibit bactericidal activity by binding to penicillin-binding proteins (PBPs) leads to preventing the cross-linking of peptidoglycan strands, thus inhibiting synthesis of the bacterial cell wall (Figure 2-2).

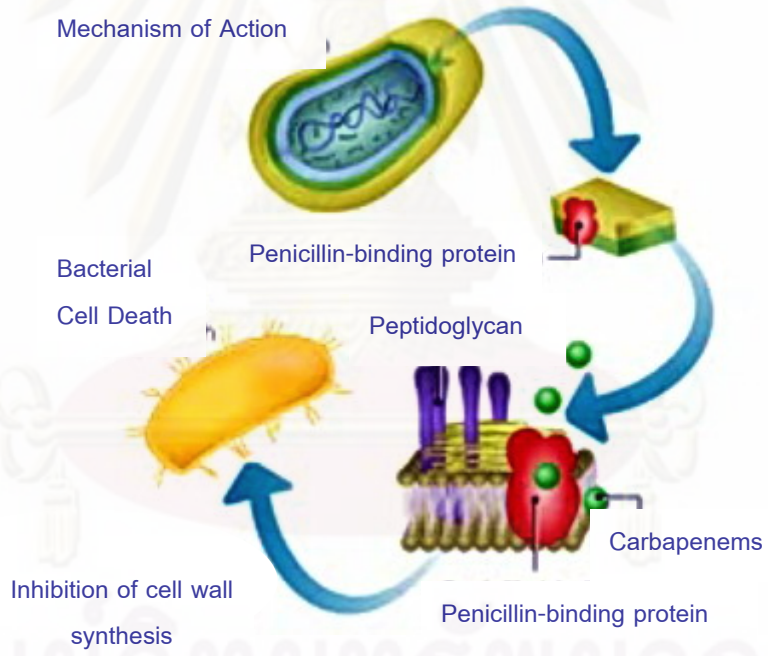

Figure 2-2 Mechanism of action of carbapenems (modified from penicillin mechanism of action)

Carbapenems are active against gram-negative bacteria that are resistant to other β-lactams, such as third-generation cephalosporins, due to the stability to almost β-lactamases including AmpC beta-lactamases and extended-spectrum beta-lactamases (ESBLs) (Boeser, 2008).

Imipenem and meropenem are the most established members of this class and are used primarily to treat moderate to severely ill patients with nosocomial infections and polymicrobial infections (Zhanel et al., 2007).

Generally, imipenem has a favor to bind to PBP2, followed by PBP1a and 1b, and has weak affinity for PBP3, while meropenem and ertapenem bind most strongly to PBP2, followed by PBP3, but also have strong affinities for PBP1a and PBP1b (Zhanel et al, 2007). Although there have been reported that imipenem is more potent than meropenem against *A. baumannii* clinical isolates in North America and Europe, meropenem is superior in activity to imipenem against *A. baumannii* isolates in Greece. Therefore, the susceptibility system should not be used either imipenem or meropenem to predict susceptibility of each other because of the variations in patterns of resistance to carbapenems in *A. baumannii* between distinct geographic regions (Ikonomidis et al., 2006).

Imipenem/cilastatin administered by intravenous infusion to healthy volunteers at either 500 mg or 1000 mg doses resulted in mean maximum plasma concentrations (C_{max}) at the end of infusion of 30–35 mg/L and 60–70 mg/L, respectively. Fairly similar to meropenem administered by intravenous infusion in 500 mg and 1000 mg doses produced C_{max} values of 26 mg/L and 50–60 mg/L, respectively (Zhanel et al., 2007).

Mechanism of Resistance

In recent years, the prevalence of carbapenem resistant *A. baumannii* appears to be increasing worldwide. The mechanisms underlying resistance to carbapenems in *A. baumannii*, like other β-lactams, can be 1) the presence of β-lactamases (carbapenemases); 2) the changes in outer membrane proteins (OMPs); 3) the alterations in the affinity or expression of penicillin-binding proteins (PBPs); and 4) overexpression of multidrug efflux pumps (Perez et al., 2007; Munoz-Price and Weinstein, 2008; Peleg et al., 2008). (Figure 2-3)

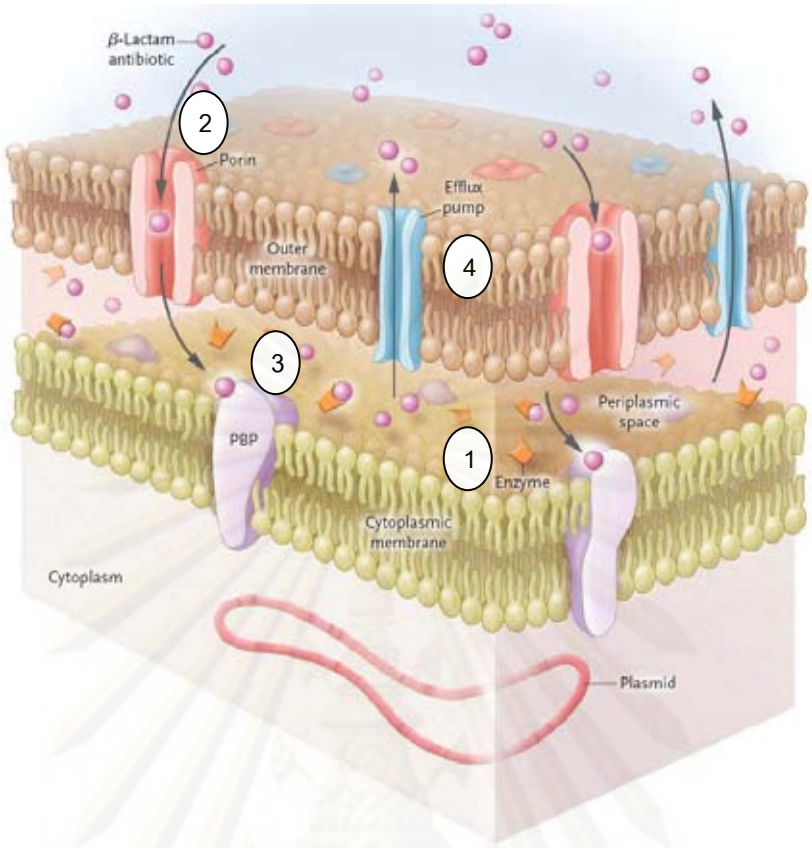

Figure 2-3 Mechanisms of carbapenem resistance in *Acinetobacter* spp. (Munoz-Price and Weinstein, 2008)

The most clinically troubling mechanism of carbapenem resistance is enzymatic degradation by β-lactamases (carbapenemases) (Table 2-3). Two intrinsic types of β-lactamase can be identified in most *A. baumanii* isolates. Firstly, the chromosomally encoded AmpC-type cephalosporinase, also known as *Acinetobacter*-derived cephalosporinases (ADCs), which are normally expressed at a basal level and not inducible with β-lactams (Poirel and Nordmann, 2006; Gootz and Marra, 2008; Peleg et al., 2008). However, the introduction of an upstream IS element known as IS*Aba1* of the bla_{AmpC} gene, resulting in increased bla_{AmpC} gene expression and resistance to ceftazidime, but not to cefepime and carbapenems. The second intrinsic β-lactamase is an oxacillinase represented by the OXA-51/69 variants, bla_{OXA-51}-like genes located in chromosome and their roles in carbapenem resistance are related to the presence of IS*Aba1* (Poirel and Nordmann, 2006; Peleg et al., 2008).

Table 2-3 Summaries of the intrinsic β-lactamase and most concerning acquired carbapenemases in *A. baumannii*

β-lactamases	Examples	Ambler Class	Encoded	Distribution
Intrinsic β-lactamases	AmpC	C	chromosomal	Global distribution due to naturally occurring in *A. baumannii*
	OXA-51/69 like (OXA-51, OXA-64, OXA-65, OXA-66, OXA-68, OXA-69, OXA-70, OXA-71, OXA-78,	D	chromosomal	
Acquired Carbapenemases	IMP family (IMP-1, IMP-2, IMP-4, IMP-5, IMP-6, IMP-11)	B	plasmid	Italy, Japan, South Korea, Hong Kong, Portugal, Brazil, Australia
	OXA-23	D	plasmid	Europe, USA, Brazil, Iraq, Singapore, South Korea, and China
	OXA-24, OXA-25, OXA-26, OXA-40		chromosomal or plasmid (OXA-40)	Spain, Belgium, France, Portugal, USA
	OXA-58		plasmid or chromosomal	France, UK, Scotland, Argentina, Spain, Turkey, Romania, Austria, Greece, Kuwait, and Italy

Besides the intrinsic β-lactamase, several acquired β-lactamases have been identified as a source of carbapenem resistance in *A. baumannii*, especially carbapenem resistance which is now rapidly increased worldwide. The carbapenemase enzymes in *A. baumannii* belong either to the class B (metallo-β-lactamases), or to the class D (oxacillinases) defined on the basis of their primary structure into 4 molecular classes by Ambler (Jacoby and Munoz-Price, 2005; Poirel and Nordmann, 2006).

Metallo-β-lactamases (MBLs) are class B β-lactamase that are able to hydrolyze all β-lactams (including carbapenems) with the exception of monobactam aztreonam (Perez et al., 2007). MBLs have less commonly been identified in *A. baumannii* than class D oxacillinases, however, their hydrolytic activities against carbapenems are

significantly more potent (100- to 1000-fold) (Peleg et al., 2008). They differ from class A and D carbapenemases by having a metal ion in the active site, usually zinc, which participates in catalysis, thus providing the Etest strips containing imipenem with or without EDTA is helpful for identification of MBL production (Poirel and Nordmann, 2006; Perez et al., 2007). Acquired MBLs have been described in 5 groups to date (IMP-like, VIM-like, SIM-1, SPM-1 and GIM-1 enzymes), but only the first three have been identified in *A. baumannii* (Poirel and Nordmann, 2006).

Oxacillinases or class D OXA-type enzymes, which have the capability to hydrolyse imipenem (but not always meropenem), are grouped in a particular subgroup of β-lactamases termed carbapenem hydrolysing oxacillinases (CHDLs). Most CHDLs are susceptible to NaCl inhibition, allowing a means of their laboratory identification (Poirel and Nordmann, 2006).

The first OXA-type enzyme with carbapenem-hydrolyzing activity (CHDLs) was identified from a clinical *A. baumannii* strain isolated in 1985 from Edinburgh, Scotland (Paton et al., 1993). This plasmid-encoded resistance determinant, originally named ARI-1 (*Acinetobacter* resistant to imipenem), was found to be transferable and was renamed OXA-23 following its genetic and biochemical characterization. OXA-23 is a representative of a CHDL subgroup that also includes OXA-27 and OXA-49 which are closely related enzymes. This enzyme type now contributes to carbapenem resistance in *A. baumannii* globally, including UK, France, Romania, United States, Brazil, Iraq, Polynesia, Singapore, South Korea, and China (Poirel and Nordmann, 2006; Perez et al., 2007; Peleg et al., 2008).

A second group of CHDLs, OXA-24 (comprising OXA-24, OXA-25, OXA-26 and OXA-40), the OXA-24 and OXA-25 variants were identified in the carbapenem resistant *A. baumannii* isolates recovered from Spain, whereas OXA-26 was identified in an isolate from Belgium. In France, *A. baumannii* producing OXA-40 was recovered from a Portuguese patient (Poirel and Nordmann, 2006). The recent discovery of the crystal structure of OXA-24 provides the important catalytic role for future drug development toward this emerging class of carbapenemases (Santillana et al., 2007).

A third group of CHDLs contains OXA-58, which was identified originally in a carbapenem resistant *A. baumannii* isolate recovered from Toulouse, France in 2003 (Poirel et al., 2005). The bla_{OXA-58}, similar to bla_{OXA-23}, is often plasmid mediated, which may explain its widespread distribution. The bla_{OXA-58} was found in France, UK, Scotland, Argentina, Spain, Turkey, Romania, Austria, Greece, Kuwait, and Italy (Perez et al., 2007; Peleg et al., 2008).

Although the understanding of the outer membrane proteins (OMPs) of *A. baumannii* is very little compare with the other gram-negative pathogens. The loss of a 29-kDa OMP, also known as CarO, was shown to be associated with imipenem and meropenem resistance. Moreover, the carbapenem resistant isolates with reduced expression of 37-, 44-, and 47-kDa OMPs were presented in MDR *A. baumannii* strains endemic to New York City, similarly, in isolates from Madrid, Spain, the reduced expression of 22- and 33- kDa OMPs in association with the production of OXA-24 resulted in resistance to carbapenems (Perez et al., 2007; Peleg et al., 2008).

Recently, the heat-modifiable protein in *Acinetobacter baumannii* (HMP-AB), which is homologous to OmpA of *Enterobacteriaceae* and OmpF of *P. aeruginosa*; a 33- to 36-kDa protein; a 43-kDa protein which shows significant homology to OprD from *P. aeruginosa*; and OmpW, which is homologous to OmpW proteins found in *E. coli* and *P. aeruginosa* were identified. However, the further studies are still needed to clarify the importance of these porins (Peleg et al., 2008).

The alteration in the affinity or expression of penicillin-binding proteins (PBPs) as a source of carbapenem resistance in *A. baumannii* has been rarely investigated. The hyperproduction of the low molecular weight protein of 24-kDa PBP has been shown (Gehrlein et al., 1991). Another study has described the presence of 12 PBP patterns among a collection of *A. baumannii* isolates with variable β-lactam resistance profiles. In isolates with imipenem MICs > 4 mg/L, the absence of a 73.2-kDa PBP, corresponding to PBP2, was associated with resistance in conjunction with production of carbapenemases (Fernandez-Cuenca et al., 2003).

The other mechanism of resistance in *A. baumannii* is multidrug efflux pump. The resistance-nodulation-division (RND) family-type pump AdeABC is known to accommodate a broad range of structurally unrelated molecules that includes β-lactams (including carbapenems), aminoglycosides, erythromycin, chloramphenicol, tetracyclines, fluoroquinolones, trimethoprim, and ethidium bromide. AdeABC is chromosomally encoded and is normally regulated by a two-component system with a sensor kinase (AdeS) and its associated response regulator (AdeR) and other RND-type pumps have been described for different *Acinetobacter* genomic species (Peleg et al., 2008).

4.2 Sulbactam

The non-traditional antimicrobial agent such as sulbactam has also been tested clinically for the treatment of severe infections caused by MDR *A. baumannii.* Sulbactam is the β-lactamase inhibitor that has the intrinsic antimicrobial activity against *Acinetobacter* spp. It is superior to the other two commercially available β-lactamase inhibitors, clavulanic acid and tazobactam (Higgins et al., 2004). This intrinsic activity is a result of its binding to penicillin-binding protein 2 (PBP2) (Peleg et al., 2008).

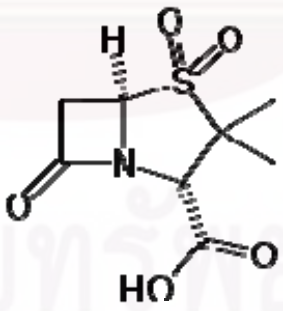

Figure 2-4 Chemical structure of sulbactam

There are two sulbactam combinations available in clinical use: ampicillin-sulbactam which is used both parenteral (intravenous or intramuscular) and oral formulations (as the mutual prodrug sultamicillin) and cefoperazone–sulbactam which is

used as a parenteral (intravenous or intramuscular) formulation only. Sulbactam is also available as a single agent in France, Germany, and Spain (Levin, 2002; Peleg et al., 2008).

Mean peak serum levels for sulbactam range from 48-88 μg/ml and 21-40 μg/ml after administered of 2000 mg ampicillin plus 1000 mg sulbactam and 1000 mg ampicillin plus 500 mg sulbactam, respectively.

Although there were the studies showed the successful use of ampicillin-sulbactam monotherapy in the treatment of MDR *A. baumannii* VAP in similar outcome with imipenem-cilastatin patients (Wood et al., 2002) and the cure rate of 67% using ampicillin-sulbactam to treat carbapenem-resistant *Acinetobacter* infection (Levin et al., 2003), the limitations of these studies must be considered, including its retrospective design, the small number of patients, and the lower severity of illness.

4.3 Colistin

The worldwide increasing prevalence of nosocomial infections caused by MDR *A. baumannii* and the shortage of novel antimicrobial agents discovery have led to the resurgence of polymyxins, such as polymyxin B and polymyxin E (colistin), which were gradually abandoned in most parts of the world around 1980s because of the reported high incidence of nephrotoxicity and neurotoxicity (Falagas and Kasiakou, 2005; Li, Nation, et al., 2006).

Polymyxins, a group of polypeptide antibiotics, were first discovered in the late 1940s and classified as polymyxin A-E but only polymyxin B and E (colistin) have been used in clinical practice (Arnold et al., 2007). There are two forms of commercially available colistin, colistin (colistin sulfate) and colistimethate (also called colistimethate sodium, colistin methanesulfate, pentasodium colistimethanesulfate, and colistin sulfonyl methate). Colistin consists of a cationic cyclic decapeptide linked to a fatty acid chain through an α-amide linkage and colistimethate is prepared from colistin by reaction of the free γ-amino groups of the five α, γ-diaminobutyric acid residues with formaldehyde

followed by sodium bisulphate (Falagas and Kasiakou, 2005; Li, Nation, et al., 2006) (Figure 2-4).

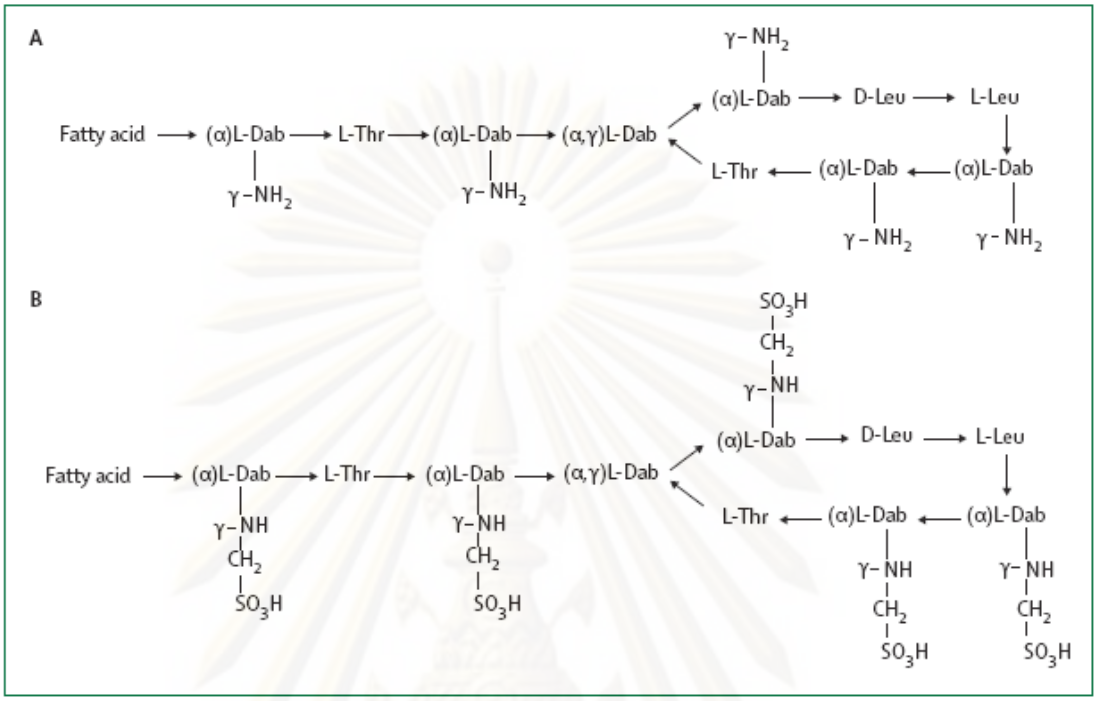

Figure 2-5 Chemical structures of colistin (A) Structures of colistin; (B) structures of colistimethate; Thr=threonine; Leu=leucine; Dab=α, γ-diaminobutyric acid. α and γ indicate the respective amino group involved in the peptide linkage (Li, Nation, et al., 2006).

Colistin can be administered orally (tablets or syrup) for bowel decontamination and topically as a powder for the treatment of bacterial skin infections. Whereas colistimethate in parenteral formulations can be administered intravenously, intramuscularly, or by nebulization since is less potent and less toxic than colistin, it is not stable *in vitro* or *in vivo* and is hydrolysed to a series of methanesulphonated derivatives plus colistin (Falagas and Kasiakou, 2005; Li, Nation, et al., 2006). The term “colistin” for parenteral administration throughout this review refers to the formulation of colistimethate sodium.

Colistin appears to have a surface detergent effect on the gram-negative bacterial cell membranes. The detergent effect occurs first when the polycationic peptide ring of colistin binds to the anionic lipopolysaccharides (LPS) in the outer membrane of the -

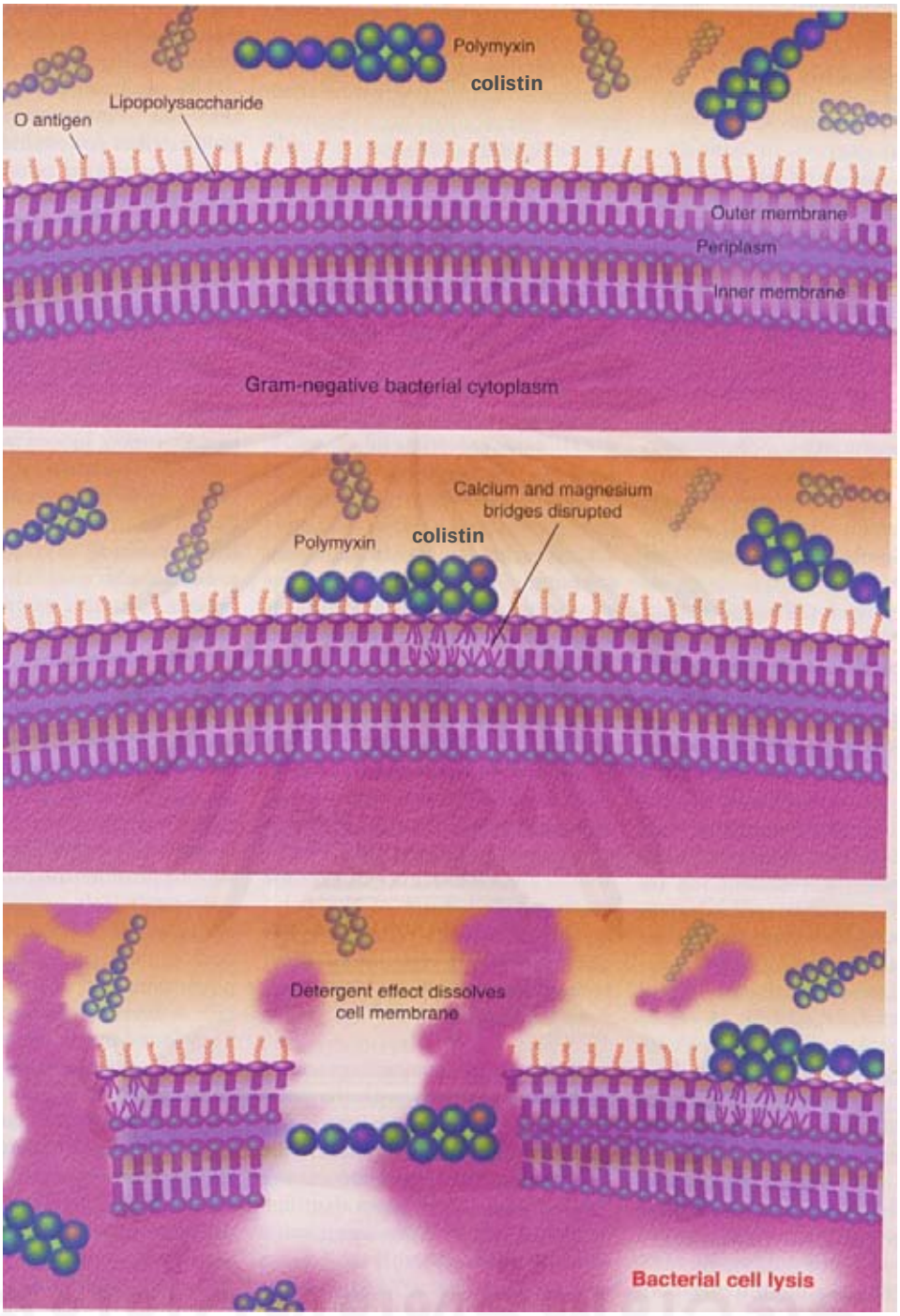

Figure 2-6 Mechanism of action of colistin. In the top panel, colistin approaches the gram-negative bacterial cell, which has outer membranes with phospholipid bilayers stabilized by calcium and magnesium bridges. I the middle panel, the colistin's cationic peptide ring binds to the organism and begins disrupting the calcium and magnesium bridges. The drug's fatty acid side chain also interacts with the LPS of the outer membrane. In the bottom panel, colistin has become inserted into the outer membrane, resulting in increased permeability of the cell membrane, leakage of cell contents, and cell death (Arnold et al., 2007).

organism, leading to disrupting the calcium (Ca^{2+}) and magnesium (Mg^{2+}) bridges that stabilize the LPS molecules. Then the LPS molecules and the fatty acid side chain of the colistin interact until colistin is inserted into the outer membrane, resulting in increased permeability of cell membrane, leakage of cell contents and subsequently, cell death (Figure 2-5) (Falagas and Kasiakou, 2005; Arnold et al., 2007).

The most common potential toxicities of colistin are nephrotoxicity and neurotoxicity. Renal toxicity mainly includes acute tubular necrosis. The neurotoxic effects include perioral paresthesias, ataxia, vertigo, visual disturbances, confusion, vasomotor instability, and neuromuscular blockade which can lead to respiratory failure (Jain and Danziger, 2004; Falagas and Kasiakou, 2005). However, the toxicity observed in prior clinical studies, mainly nephrotoxicity, may be less prominent than previously thought. According to the improvement in supportive treatment offered to critically ill patients, the close monitoring of renal function and of factors that affect it when colistin is administered, and the avoidance of coadministration of other agents with known nephrotoxicity (Falagas and Kasiakou, 2005). Moreover, the alternative routes of administration have been explored, including the inhaled and intrathecal/intraventricular routes. Inhaled colistin has been used in cystic fibrosis to decrease colonization with MDR gram-negative organisms. Intrathecal/intraventricular colistimethate sodium has been successfully used in the treatment of ventriculitis due to carbapenem-resistant *A. baumannii* (Jain and Danziger, 2004).

Mechanism of Resistance

Polymyxin B and polymyxin E (colistin, intravenous colistimethate sodium) have been increasingly used in the treatment of *A. baumannii* infections. Unfortunately, polymyxins resistance in *A. baumannii* has been identified (Urban et al., 2001; Li, Rayner, et al., 2006). The mechanism of resistance remains not exactly known, maybe asscociated with the modifications in the lipopolysaccharide of *A. baumannii* (acidification, acylation, or presence of antigens that interfere with binding of the antibiotic to the cell membrane) (Perez et al., 2007).

4.4 Combination Therapy

Combination therapy is the strategy often used in the treatment of MDR *A. baumannii* infections. The rationale of this strategy is not only strengthen the efficacy of treatment itself and allowed lower dose of either agent to reduce toxicity, but also reduce the emergence of resistant isolates (Rahal, 2006).

The combination of imipenem plus amikacin has demonstrated *in vitro* synergism, partial synergism, and additive against *A. baumannii* 36%, 50%, and 14%, respectively (Chang et al., 2005). However, the contrast results obtained *in vivo*, the combination of imipenem and amikacin showed no advantages of combination therapy over imipenem monotherapy (Rodriguez-Hernandez et al., 2000; Bernabeu-Wittel et al., 2005).

The *in vitro* studies using checkerboard method demonstrated moderate synergy when sulbactam is combined with cefepime (Tong et al., 2006) or amikacin (Marques et al., 1997). Synergistic activities of carbapenems such as imipenem with sulbactam against *A. baumannii* using time-kill method have been reported, including two imipenem-resistant *A. baumannii* strains (Choi et al., 2004), correspond with Song et al. (2007) that have reported the synergistic activity of combination of imipenem and sulbactam, both at a concentration of 1xMIC, against most of the carbapenem-resistant strains after 4 hours and the bactericidal effect after 8 hours after inoculation.

Ko et al. (2004) reported that meropenem 0.5xMIC combined with sulbactam 1xMIC exhibited more potent antimicrobial activity against MDR *A. baumannii* than did meropenem or sulbactam alone. Kiffer et al. (2005) have also shown the synergism and partial synergism in the majority of *A. baumannii* isolates with the combination of meropenem and sulbactam.

In a mouse model, the survival rate of mice treated with meropenem in combination with sulbactam was significantly higher than those treated with meropenem or sulbactam alone (Ko et al., 2004).

For human data, a retrospective study involving 55 patients with *A. baumannii* bloodstream infection showed better outcomes in those given a combination of a carbapenem and ampicillin/sulbactam compared to those given a carbapenem and amikacin or a carbapenem alone (mortality rate 30.8% versus 50.0% and 58.3%, respectively) (Kuo et al., 2007). Correspond to the favorable clinical outcomes of four patients with MDR *A. baumannii* infections that were treated with the combination of imipenem or meropenem and sulbactam (Lee et al., 2007).

Despite the limitations of appropriately designed controls clinical trials and random allocation of treatments, there have been suggested that sulbactam should be considered as a therapeutic option for mild to severe *A. baumannii* infections caused by sulbactam-susceptible organisms (Perez et al., 2007; Peleg et al., 2008).

Previously reported *in vitro* and animal studies support the role of combination therapy with polymyxin B or colistin, particularly when combined with a carbapenem and/or rifampicin seems most promising (Peleg et al., 2008). Yoon et al. (2004) performed checkerboard and time-kill studies showing the synergy in the combination of polymyxin B, imipenem, and rifampicin. Double combination of polymyxin B and imipenem and of polymyxin B and rifampin were bactericidal for seven of eight isolates, and triple combinations were bactericidal for all isolates within 24 hours. Also, the synergy was found with the combination of colistin and rifampicin in carbapenem-resistant *A. baumannii* (Song et al., 2007). The combination of colistin and rifampin, meropenem, azithromycin, or doxycycline demonstrated synergy with all except partial synergy with doxycycline (Timurkaynak et al. 2006).

The studies of colistin in combination with rifampin were performed in mouse pneumonia model showing synergy compared to colistin but not difference from rifampicin alone (Montero et al., 2004) and the neutropenic rat thigh infection model resulting in improvement in 6-day survival with combinations (Pantopoulou et al., 2007).

Unfortunately, much of the clinical data on colistin efficacy comes from uncontrolled or retrospective case series, it is difficult to make conclusions about the potential benefits of combination therapy (Peleg et al., 2008). However, in several

retrospective and prospective case series across arrange of infection type, the curative responses ranged from 55% to 77% (Levin et al., 1999; Garnacho-Montero et al., 2005; Holloway et al., 2006; Kallel et al., 2006). Most recently in Thailand, a further prospective cohort study reported a statistically significant improvement in outcome for patients receiving colistin (80.8%) compared with those receiving other antibiotics (clinical response 80.8% versus 26.7%, respectively) (Koomanachai et al., 2007).

The lower rates of nephrotoxicity and neurotoxicity than previously reported become the most amazing feature of the colistin revival, and seem to be reversible with cessation of the drugs. Nevertheless, adjusted dosing should be done in the patients with preexisting renal impairment, those given concomitant nephrotoxin, and the elderly (Peleg et al., 2008).

CHAPTER III

MATERIAL & METHODS

MATERIALS

1. Microorganisms

The bacterial isolates used throughout this study were 30 isolates of *Acinetobacter baumannii* which were clinically isolated from patients in Siriraj Hospital during the year 2006-2007. (All isolates were kindly provided from Assistant Professor Chanwit Tribuddharat, M.D., Ph.D., Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as the control strain. All isolates were stored at -20º C in Tryptic Soy Broth: glycerin (85:15) and subcultured twice before use.

2. Chemicals

- Standard powders

Three standard powders were used: meropenem (potency = 717.3 µg/mg) and sulbactam (potency = 891.5 µg/mg) were kindly provided from Siam Bheasach Co., Ltd, Thailand and colistin (potency =656.53 µg/mg) (Sigma, St. Louis, Mo.). The working standard solutions were prepared immediately prior to use, as specified by the manufacturers.

- Susceptibility disks

Five antimicrobial disks which were used to determine susceptibility pattern of bacterial strains were amikacin (30 µg), ciprofloxacin (5 µg) from BBL chemicals (Beckton Dickinson, USA), cefepime (30 µg) and meropenem (10 µg) from BBL chemicals (Benex Limited, USA) and piperacillin/tazobactam (100/10 µg) from Oxoid Ltd., England.

3. Media and Reagents

- Muller-Hinton Agar (MHA) and Muller-Hinton Broth (MHB) (BBL chemicals, USA) were used as the test medium for all bacterial strains.

- Tryptic Soy Agar (TSA) (BBL chemicals, USA) was used as the culture media for *A. baumannii* and control strains (*E. coli* ATCC25922 and *P. aeruginosa* ATCC 27853).

- Sterile water was used as the solvent for the chemical powders

- Sterile normal saline solution (NSS) was chosen as the diluent of the inoculum in turbidity adjusting process to quantity the precise numbers of bacteria. This NSS also applied as the diluents of specimens in colony counting procedures of time kill method.

4. Laboratory equipment and instruments

- Cotton swabs were used to take and streak standard inoculum on to the solid media before impregnated the disk as performed in the disk susceptibility and E-test method.

- Petri dishes were practiced as agar containing plate for culture microorganism in the whole processes such as subculture, susceptibility testing and colony counting.

- Erlenmeyer flasks were used for the media preparation, sterile water, and sterile NSS before autoclaving.

- Cylinders were picked to measure the gross quantity of water and liquid media in preparing procedures.

- Glass tubes were used throughout the experiments such as in the preparation of the standard solution, dilute inoculum, specimen, etc.

- Pipettes, used in experiment divided into 2 types;

1. Glass pipettes were chosen to measure media, inoculum, drugs, and solvent as general equipment processes.

2. Micropipettes were used for calibrate specimens in colony counting procedures from time kill method.

- Sterile loop was selected for streaking bacteria in general procedures such as subculture, inoculum preparation, etc.

- Digital vernier-caliper was chosen for measuring the clear zone in disk susceptibility method.

- Tube racks were used as shelf to hold a large number of tubes, both in broth macro dilution procedures and time kill procedures.

- Inocula-replicating device

- 96-well microtitre plates were used to perform the checkerboard method.

- Vortex mixer was used to mix media, inoculum, drugs, and solvent.

- Colorimeter

- Autoclave was used to sterilize equipment, media, diluents, inoculum, and the others throughout the experiment for sterile condition in the research.

- Refrigerator was used to maintain bacteriostatic condition between research process and also preserved media before using in all experiments.

- Incubator was used to provide the appropriated environmental condition for bacterial growth throughout the procedures such as subculture, disk susceptibility process, inoculum preparation, etc.

- Water bath shaker was chosen to apply appropriated bacterial growth condition of liquid media that simulate human body temperature in the time kill method.

- Hot air oven was used to keep drying and sterilize all glass equipment before using.

METHODS

1. Kirby-Bauer disk diffusion method was performed to determine inhibition zones of 5 broad-spectrum antimicrobial agents and screening for MDR strains of *A. baumannii*.

2. Agar dilution method was performed to determine the minimum inhibitory concentration (MIC) of the test agents.

3. Checkerboard method was done to determine double and triple combination effects of meropenem, colistin, and sulbactam.

4. Time kill method was done to determine the bactericidal activity of meropenem, colistin, and sulbactam alone and in combinations.

5. Scanning electron microscope was used to examine the morphological cell changes of *A. baumannii* exposed to meropenem, colistin, and sulbactam alone and in combinations.

1. Kirby-Bauer disk diffusion method

Kirby-Bauer disk diffusion method was performed according to the disk diffusion method by Clinical and Laboratory Standards Institute (CLSI, 2006b). All isolates including the control strain were tested to determine inhibition zone of 5 broad-spectrum antimicrobial agents and screening for multidrug-resistant (MDR) strains of *A. baumannii*.

1.1 Preparation of media

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

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

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

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

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

1.2 Preparation of inoculum

1.2.1 The well-isolates colony of each 18 hours *A. baumannii* from clinical specimen and *E. coli* ATCC 25922 were selected from tryptic soy agar (TSA) plates and transferred to a tube containing 7 ml normal saline solution (NSS).

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

1.3 Inoculation of the test plates

1.3.1 Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several time and pressed firmly on the inside wall of the tube above the fluid level in order to remove excess inoculum from the swab.

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

1.3.3 The lid may be left agar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the antibiotic disks.

1.4 Application of antibiotic disks to the inoculated agar plates

1.4.1 The antibiotic disks were applied to the surface of the medium with sterile forceps. Each disk was pressed down to ensure complete contact with the agar surface. They were distributed evenly so that they were not closer than 24 mm. from center to center.

1.4.2 The plates were inverted and incubated at 37^{o}C for 24 hours before measuring the zones of inhibition.

1.5 Reading plates and interpreting results

1.5.1 After 24 hours of incubation, each plate was examined. The resulting zones of inhibition were uniformly circular and there was a confluent lawn

of growth. The diameters of zones of inhibition, including the diameter of the disk were measured with digital sliding vernier caliper.

1.5.2 The sizes of the inhibition zone were interpreted by referring to the CLSI (2007) and the organisms were reported as either susceptible, intermediate, or resistant to the agents that have been tested (Tables 3-1).

Table 3-1 Zone diameter interpretive standards breakpoints for *A. baumannii*, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 (CLSI, 2007)

Antimicrobial Agents	Disk Content (µg)	Zone Diameter (mm)				
		A. baumannii			*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
		R[a]	I[b]	S[c]		
amikacin	30	≤14	15-16	≥17	19-26	18-26
cefepime	30	≤14	15-17	≥18	31-37	24-30
ciprofloxacin	5	≤15	16-20	≥21	30-40	25-33
meropenem	10	≤13	14-15	≥16	28-34	27-33
piperacillin/tazobactam	100/10	≤17	18-20	≥21	24-30	25-33

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

1.6 Screening for multidrug-resistant strains

Multidrug-resistant strain was defined as resistant to three or more of the following broad-spectrum agents: amikacin, cefepime, ciprofloxacin, meropenem, and piperacillin/tazobactam (Timurkarynak et al., 2006).

2. Agar dilution method

Agar dilution method was performed according to CLSI (2006a) in order to determine minimal inhibitory concentration (MIC) of meropenem, colistin, and sulbactam against all tested isolates.

2.1 Preparation of agar dilution plates

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

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

2.1.3 Immediately after autoclaving, was allowed it to cool in a 55°C water bath and then 6 ml of each dilution was pipetted into MHA 54 ml.

2.1.4 The agar and antimicrobial agent solution were mixed thoroughly and then 25 ml was pipetted into each plate.

2.1.5 The agar dilution plates were allowed to solidity at room temperature, and used immediately.

2.2 Preparation of inoculum

2.2.1 The well-isolates colony of each 18 hours *A. baumannii* from clinical specimen and *E. coli* ATCC 25922 were selected from Tryptic Soy Agar (TSA) plates and transferred to a tube containing 7 ml normal saline solution (NSS)

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

2.2.3 The 200 µl-inoculum suspension was pipetted into inoculum replicators.

2.3 Inoculating agar dilution plates

2.3.1 The agar plates were marked for orientation of the inoculum spots.

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

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

2.4 Incubating agar dilution plates

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

2.5 Determining agar dilution end points

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

2.5.2 The MICs were interpreted by referring to the CLSI (2007) and the organisms were reported as either susceptible, intermediate, or resistant to the agents that have been tested (Table 3-2).

Table 3-2 MIC interpretive standard (μg/ml) for breakpoints by agar dilution method for *A. baumannii* (CLSI, 2007)

Antimicrobial Agent	MIC (μg/ml) Interpretive Standard				
	A. baumannii			*E. coli* ATCC	*P. aeruginosa*
	S[a]	I[b]	R[c]	25922	ATCC 27853
Meropenem	≤4	8	≥16	0.008-0.06	0.25-1
Colistin	≤2	-	≥4	0.25-1	0.25-2

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

3. Checkerboard method

Checkerboard method was modified from Eliopoulos and Moellering (1996) and Yoon et al. (2004) to determine double and triple combination effect of meropenem, colistin, and sulbactam. The concentrations tested for each antimicrobial agent was ranging from four dilutions below the MIC to twice the MIC.

3.1 Preparing test broth

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

3.1.2 The medium concentrations used in the initial solutions were prepared four-fold in greater than the desired final concentration.

3.2 Preparing diluted antimicrobial agents

3.2.1 The two-fold dilutions of antimicrobial agents were prepared volumetrically in the broth.

3.2.2 The concentrations of meropenem and sulbactam used in the initial solutions were prepared in eight-fold greater than the desired final concentration and colistin was prepared in four-fold greater than the

desired final concentration. The concentrations tested for each antimicrobial agent typically ranged from four dilutions below the MIC to twice the MIC.

3.3 Broth dilution testing

A standardized inoculum for the microdilution broth method was prepared by suspending the colonies of the tested isolates directly to NSS to obtain the turbidity of the 0.5 McFarland standard.

3.3.1 Optimally, within 15 minutes the adjusted inoculum suspension was diluted in the broth so that after inoculation, each tube contained approximately $5x10^5$ CFU/ml.

3.3.2 The final volume of 200 µl in each well consisted of 50 µl of MHB, 50 µl of broth for colistin, 25 µl of broth for meropenem, 25 µl of broth for sulbactam and 50 µl of broth containing a suspension of the organism was obtained.

3.3.3 The first microtitre plate contained no colistin, increasing concentrations of meropenem on the *x* axis and increasing concentrations of sulbactam on the *y* axis. Each of the subsequent seven plates contained a fixed concentration of colistin ranging from four dilutions below the MIC to twice the MIC.

3.4 Reading plates and interpreting results

3.4.1 After 24 hours, each tube was examined to determine MIC (the MIC is the lowest concentration of antimicrobial agent that completely inhibits growth of the organism in the tubes as detected by the unaided eye.) The amount of growth in the tubes containing the antimicrobial agent was compared with the amount of growth in the positive-control well (no antimicrobial agent) and the negative-control well (no organism) used in each set of tests when determining the growth end points.

3.4.2 The interpretation of the antimicrobial combination interaction was done by reading the first clear well in each row of panel with both agents.

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

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

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

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

3.4.4 The fractional inhibitory concentration index (FICI) or ΣFIC for the combination was calculated according to the following formula.

FIC index (ΣFIC) = Sum of the FICs of each antimicrobial agent

3.4.5 FIC index results for each combination were defined as:

FICI < 1.0, synergy

FICI = 1.0, additive

FICI > 1.0, antagonism

The smallest FIC value was used to establish the antimicrobial combination interaction for each specific isolate. Results were expressed as percentage of isolates with synergy, additive and antagonist.

4. Time kill method

The antibacterial activity of the combinations was performed according to the time kill method by National Committee for Clinical Laboratory Standards (NCCLS, 1999). Ten multidrug-resistant *A. baumannii* isolates at various antimicrobial susceptibilities were tested to determine the bactericidal activity of meropenem, colistin, and sulbactam alone and the combination of meropenem with sulbactam, meropenem with colistin, sulbactam with colistin, and meropenem, colistin, and sulbactam. The concentration of each agent used in the time-kill study was selected based on the average achievable serum concentration in human with standard dosing: meropenem 50 μg/ml and sulbactam 30 μg/ml. While using colistin 0.5 μg/ml which is 0.5x$MIC_{50,90}$ of colistin from the checkerboard microdilution method in this study.

4.1 Determination of bactericidal activity of meropenem, colistin, and sulbactam alone and in combinations.

4.1.1 The antimicrobial agents concentrations used in this method; meropenem 50 μg/ml, colistin 0.5 μg/ml, and sulbactam 30 μg/ml. Initial (stock) solutions were prepared ten fold greater than desired final concentration.

4.1.2 A1 ml of each agent was pipetted into 6 ml Mueller Hinton broth (MHB) for the preparation of working media before adding the standardized inoculum (final volume of working media = 9). By doing so, there were 8 groups including

1) control (no antimicrobial agents)
2) meropenem 50 μg/ml
3) colistin 0.5 μg/ml
4) sulbactam 30 μg/ml
5) meropenem 50 μg/ml with sulbactam 30 μg/ml
6) meropenem 50 μg/ml with colistin 0.5 μg/ml

7) sulbactam 30 µg/ml with colistin 0.5 µg/ml

8) meropenem 50 µg/ml, sulbactam 30 µg/ml, and colistin 0.5 µg/ml.

4.1.3 Inoculum which was adjusted to match the turbidity of the 0.5 McFarland standard solution, contained approximately 1 to 2 x 10^8 CFU/ml was then diluted ten fold to make 1 to 2 x 10^7 CFU/ml of the bacterial inoculum.

4.1.4 A 1 ml of inoculum was pipetted to working media 9 ml and incubated at 37°C in a shaking water bath.

4.1.5 The samples were collected for culture at the time 0, 2, 4, 6, 8, and 24 hours after the microorganisms were exposed to each group of the antimicrobials including the control group.

4.1.6 A 0.5 ml of the collected sample was diluted ten fold in 4.5 ml NSS and 20 µl of each dilution was dropped to the surface of TSA plates which were then incubated at 37°C for 18 hours.

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

4.1.8 Killing curves were constructed by Microsoft Office Excel 2003 at each time interval. The $\log_{10}$ change of the viable cell counts compared to the starting inoculum was determined.

4.1.8.1 The results were analyzed by determining the number of strains which yield changes in the $\log_{10}$ 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 $\log_{10}$ decrease in the initial inoculum in CFU/ml (Montero et al., 2004) or bacteriostatic if between 0-3 $\log_{10}$ decrease in the initial inoculum in CFU/ml. The regrowth was defined as an increase of $\geq$ 2 $\log_{10}$ CFU/ml after $\geq$ 6 hours (Amsterdam, 1996; Pankuch, Jacobs and Appelbaum, 1994).

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

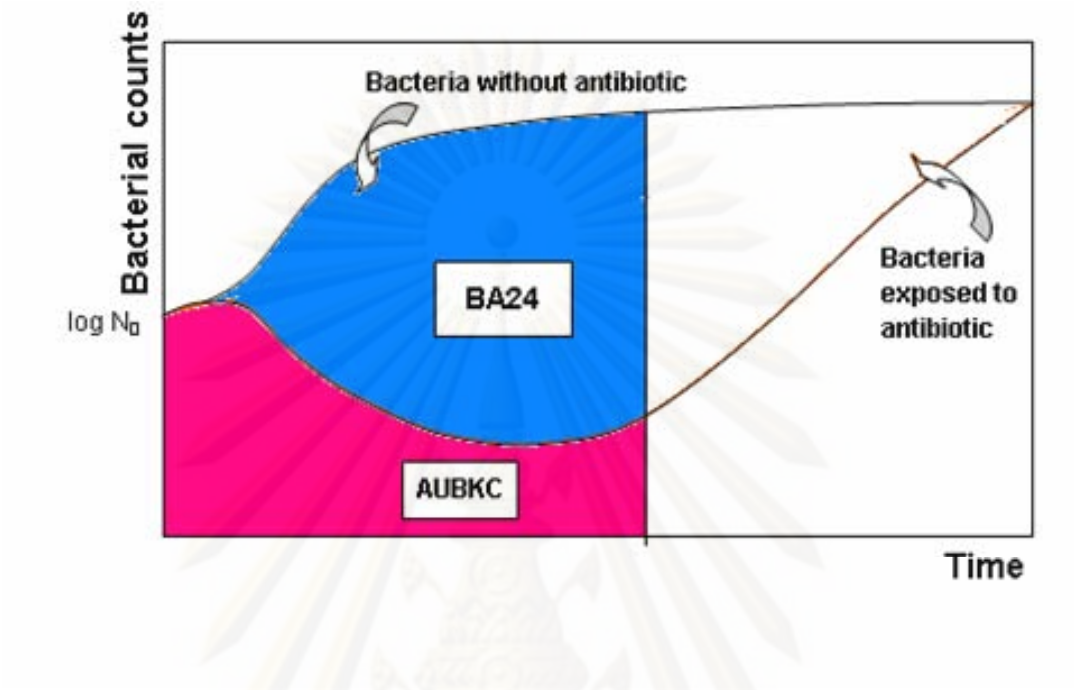

Figure 3-1 Parameters for quantifying bacterial killing, regrowth curve and the antimicrobial effect (Modified from Firsov et al., 1997)

The following parameters were calculated by various methodologies as followed:

$AUBKC_{0-24}$ = 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 control growth curve and the bacterial killing and regrowth curves ($AUBKC_{0-24}$ of the control growth curve subtracted by $AUBKC_{0-24}$ 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 considered as significant difference.

5. Scanning electron microscope of *A. baumannii*

The scanning electron microscopy was chosen to examine the morphological changes in *A. baumannii* when exposed to meropenem, colistin, and sulbactam alone and in combinations after 2 hours. The selected concentration of agents and bacterial strains in the study were correlated to those in time kill study.

5.1 Meropenem, colistin, and sulbactam concentrations were prepared 50 μg/ml, 0.5 μg/ml, and 30 μg/ml, respectively. Antimicrobial concentrations used in initial (stock) solutions were prepared ten fold greater than the desired final concentration.

5.2 One ml of each agent was pipetted into 6 ml of Mueller Hinton broth (MHB) for the working media preparation before adding the standardized inoculum (final volume of working media = 9 ml). By doing so, there were 8 groups including

1) control (no antimicrobial agents)
2) meropenem 50 μg/ml
3) colistin 0.5 μg/ml
4) sulbactam 30 μg/ml
5) meropenem 50 μg/ml with sulbactam 30 μg/ml
6) meropenem 50 μg/ml with colistin 0.5 μg/ml
7) sulbactam 30 μg/ml with colistin 0.5 μg/ml
8) meropenem 50 μg/ml, sulbactam 30 μg/ml, and colistin 0.5 μg/ml.

5.3 Inoculum was adjusted to match the turbidity of the 0.5 McFarland standard solution, contained approximately 1 to 2 x 10^8 CFU/ml was then diluted ten fold to make 1 to 2 x 10^7 CFU/ml of the bacterial inoculum.

5.4 One ml of inoculum was pipetted to the working media and was then incubated at 37°C in a shaking water bath.

5.5 The specimens were collected after 2 hours of exposure in order to detect the morphological changes.

5.6 The specimens was 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 minutes/each and once in distilled water for 10 minutes.

5.7 After that the specimens were dehydrated with a graded series of ethanol (30%, 50%, 70%, 90% 5 minutes/each and absolute ethanol 3 times, 10 minutes/time).

5.8 The specimens were then critical point dried (Critical point dryer, Balzer model CPD 020), mounted and coated with gold (Sputter coater, Balzers model SCD 040).

5.10 The cell morphology of *A. baumannii* was observed under a scanning electron microscope (JEOL, model JSM-5410LV).

CHAPTER IV

RESULTS

1. Disk diffusion test

From Kirby-Bauer disk diffusion method (Figure 4-1) according to CLSI (2006), the antimicrobial susceptibility of 30 isolates of *Acinetobacter baumannii* (*A. baumannii*), which were isolated from Siriraj Hospital during 2006-2007, against 5 broad spectrum antimicrobial agents were determined as shown in Table 4.1. All *A. baumannii* were resistant to ciprofloxacin, meropenem, and piperacillin/tazobactam, while 80% were resistant to amikacin and 73.33% were resistant to cefepime.

Table 4-1 *In vitro* activity of amikacin, cefepime, ciprofloxacin, meropenem, and piperacillin/tazobactam against 30 isolates of *A. baumannii* as tested by disk diffusion method.

Antimicrobial Agents	No. of isolates (%)		
	Resistant	Intermediate	Susceptible
amikacin	24 (80)	4 (13.33)	2 (6.67)
cefepime	22 (73.33)	7 (23.33)	1 (3.33)
ciprofloxacin	30 (100)	0	0
meropenem	30 (100)	0	0
piperacillin/tazobactam	30 (100)	0	0

All isolates were resistant to three to five of the broad-spectrum agents in the disk diffusion test. Thus, all isolates were considered to be multidrug-resistant (MDR) isolates. The distribution of multidrug-resistant isolates of *A. baumannii* according to the number of antimicrobial agents to which they showed resistance is shown in Figure 4-2.

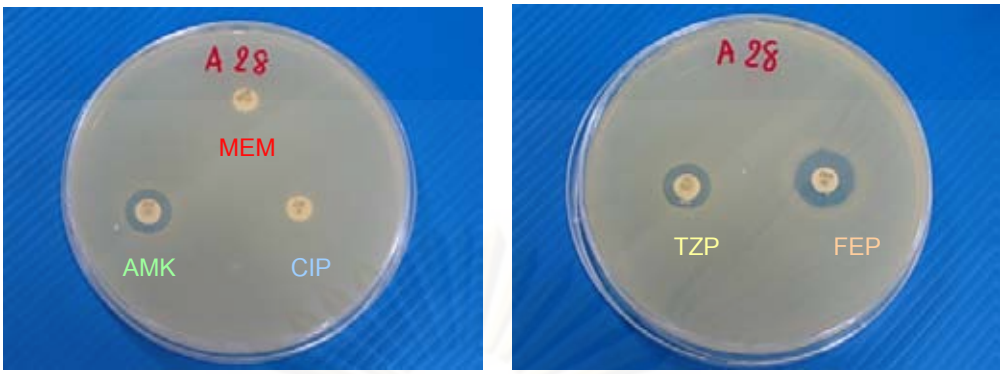

Figure 4-1 Example of the inhibition zones of 5 broad spectrum antimicrobial agents of *A. baumannii* determined by Kirby-Bauer disk diffusion method (AMK, amikacin; CIP, ciprofloxacin; FEP, cefepime; MEM, meropenem; TZP, piperacillin/tazobactam).

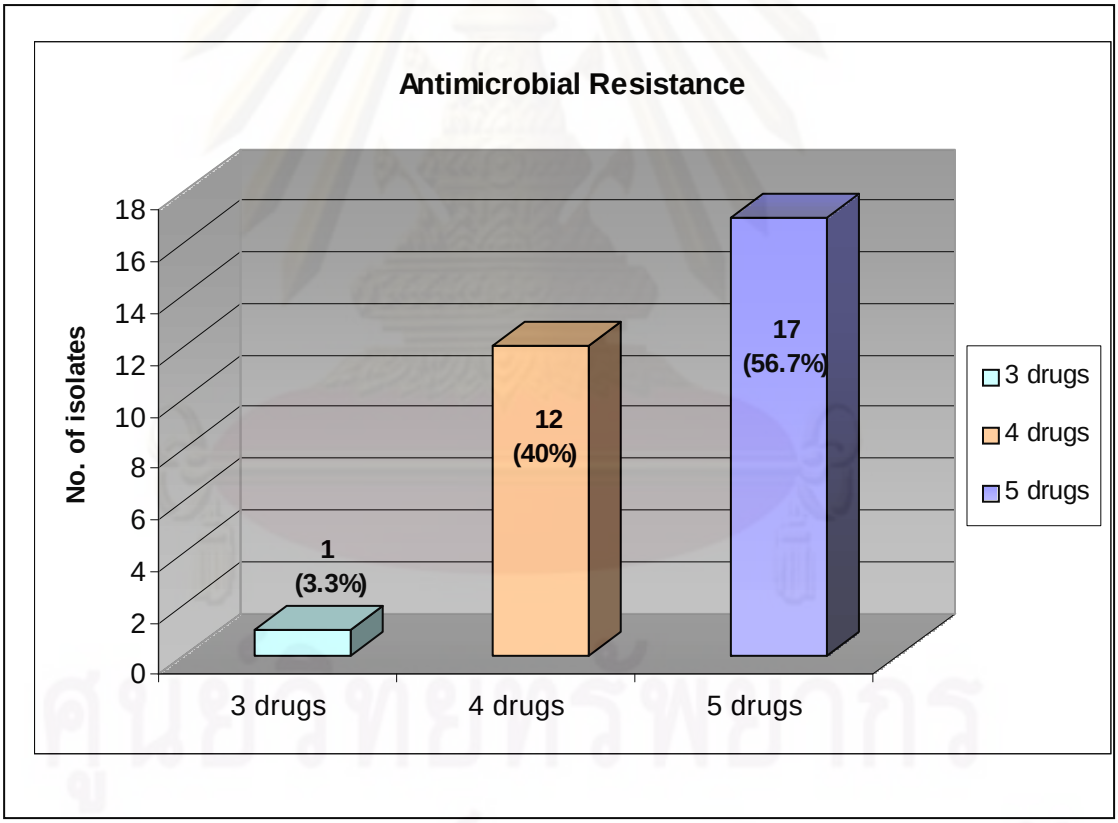

Figure 4-2 Distribution of multidrug-resistant (MDR) isolates of *A. baumannii* according to the number of antimicrobial agents to which they showed resistance.

2. Minimum inhibitory concentrations (MICs) for meropenem, sulbactam, and colistin determined by agar dilution method

The MICs ranges, as well as the MIC_{50}, MIC_{90}, and percentage of susceptible isolates of MDR *A. baumannii* to meropenem, colistin, and sulbactam 30 were shown in Table 4-2. Meropenem had no activity against all isolates tested. All isolates were resistant to meropenem (MICs range from 64–256 µg/ml; susceptibility breakpoint $\leq$ 4 µg/ml, while all isolates were susceptible to colistin (MICs range from 0.5-2 µg/ml; susceptibility breakpoint $\leq$ 2 µg/ml). The MIC_{50} and MIC_{90} of meropenem, colistin, and sulbactam were 128, 1, and 32 µg/ml, respectively, and the MICs of sulbactam ranged from 4-64 µg/ml.

Table 4-2 Susceptibilities of 30 MDR *A. baumannii* isolates

Antimicrobial	No. of isolates (%)			Range	MIC_{50}	MIC_{90}
Agents	Susceptible	Intermediate	Resistant	(µg/ml)	(µg/ml)	(µg/ml)
Meropenem[a]	0 (0%)	0 (0%)	30(100%)	64-256	128	128
Colistin[b]	30(100%)	-	0 (0%)	0.5-2	1	1
Sulbactam	-	-	-	4-64	32	32

[a]susceptible, $\leq$4 µg/ml; intermediate, 8 µg/ml; resistant, $\geq$16 µg/ml

[b]susceptible, $\leq$2 µg/ml; resistant, $\geq$4 µg/ml

3. Double and triple combination effects of meropenem, colistin, and sulbactam determined by the three-dimensional checkerboard microdilution method

The combination effects of double and triple combination of meropenem, colistin, and sulbactam against 30 isolates of MDR *A. baumannii* determined by the three-dimensional checkerboard microdilution method are shown in Table 4-3 and Table 4-4. The combination effects were evaluated for the fractional inhibitory concentration (FIC) index.

Table 4-3 Combination effects of double and triple combination of meropenem, colistin, and sulbactam against 30 isolates of MDR *A. baumannii*

Antimicrobial combinations	Synergy (ΣFIC < 1)	Additive (ΣFIC = 1)	Antagonism (ΣFIC > 1)	FICI range
MEM + SUL	21 (70%)	9 (30%)	0 (0%)	0.313-1
MEM + COL	22 (73.33%)	6 (20%)	2 (6.67%)	0.37-1.125
SUL + COL	16 (53.33%)	10 (33.33%)	4 (13.33%)	0.37-1.125
MEM+ SUL+ COL	29 (96.67%)	1 (3.33%)	0 (0%)	0.405-1

MEM, meropenem; SUL, sulbactam; COL, colistin

The synergistic effects of the double combination of meropenem with sulbactam, meropenem with colistin, and sulbactam with colistin were observed in 70%, 73.33%, and 53.33% of 30 isolates of MDR *A. baumannii*, respectively. Whereas the triple combination of meropenem, sulbactam, and colistin showed synergistic effects against 96.67% of MDR *A. baumannii*.

Table 4-4 Individual MICs, FICs and FIC index of meropenem (MEM), sulbactam (SUL), and colistin (COL) alone and in combinations against 30 isolates of *A. baumannii*

Isolate No.	MIC (μg/ml)																				
	Alone[a]		Combined			Alone[a]		Combined			Alone[a]		Combined			Alone[a]			Combined			
	MEM	SUL	MEM	SUL	∑FIC[b]	MEM	COL	MEM	COL	∑FIC[b]	SUL	COL	SUL	COL	∑FIC[b]	MEM	SUL	COL	MEM	SUL	COL	∑FIC[b]
1	64	32	32	8	0.75	64	1	32	0.5	1	32	1	2	1	1.063	64	32	1	32	8	0.12	0.87
2	32	32	16	16	1	32	0.5	4	0.25	0.625	32	0.5	4	0.25	0.625	32	32	0.5	16	2	0.06	0.683
3	256	32	32	16	0.675	128	1	64	0.5	1	16	1	8	0.5	1	128	16	1	64	2	0.03	0.655
			128	4	0.675																	
4	256	32	8	16	0.313	128	1	16	0.5	0.563	32	1	8	0.5	0.75	128	32	1	32	8	0.06	0.56
5	64	32	32	16	1	64	1	16	0.5	0.75	32	1	8	0.5	0.75	64	32	1	32	2	0.06	0.623
6	32	32	16	16	1	64	1	32	0.5	1	32	1	16	0.5	1	64	32	1	16	8	0.03	0.53
7	128	32	64	2	0.563	64	1	8	0.5	0.625	16	1	8	0.03	0.53	64	16	1	8	8	0.03	0.655
																			32	2	0.03	0.655
8	128	16	32	4	0.5	128	2	4	1	0.531	4	2	0.5	1	0.625	128	4	2	32	0.5	0.5	0.625
9	64	32	32	16	1	64	2	32	0.5	0.75	32	2	16	0.5	0.75	64	32	2	8	8	0.5	0.625
10	128	32	64	2	0.56	128	2	16	0.5	0.375	32	2	4	1	0.625	128	32	2	16	4	0.5	0.5

a: MIC from checkerboard ; Meropenem ≤ 4 μg/ml (Susceptible), = 8 μg/ml (Intermediate), ≥ 16 μg/ml (Resistant); Colistin ≤ 2 μg/ml (Susceptible), ≥ 4 μg/ml (Resistant)

b: ∑FIC < 1 (Synergy), ∑FIC =1 (Additive), ∑FIC > 1 (Antagonist); Highlighted zone, the combination which synergistic effect was shown

Table 4-4(continued) Individual MICs, FICs and FIC index of meropenem (MEM), sulbactam (SUL), and colistin (COL) alone and in combinations against 30 isolates of *A. baumannii*

Isolate No.	MIC (μg/ml)																				
	Alone		Combined			Alone		Combined			Alone		Combined			Alone			Combined			
	MEM	SUL	MEM	SUL	∑FIC[b]	MEM	COL	MEM	COL	∑FIC[b]	SUL	COL	SUL	COL	∑FIC[b]	MEM	SUL	COL	MEM	SUL	COL	∑FIC[b]
11	32	32	16	2	0.56	32	1	16	0.06	0.56	16	1	8	0.06	0.56	32	16	1	8	1	0.25	0.563
								2	0.5	0.56												
12	64	32	16	16	0.75	64	2	4	1	0.563	32	2	4	1	0.625	64	32	2	16	4	0.5	0.625
			32	8	0.75																	
13	64	64	4	32	0.563	32	0.5	4	0.5	1.125	16	0.5	4	0.5	1.25	32	16	0.5	16	4	0.03	0.81
14	64	32	16	8	0.5	64	1	32	0.03	0.53	16	1	8	0.5	1	64	16	1	16	2	0.03	0.405
15	32	32	16	8	0.75	32	0.5	16	0.25	1	16	0.5	2	0.5	1.125	32	16	0.5	8	8	0.06	0.87
			8	16	0.75																	
16	64	16	32	1	0.563	32	1	8	0.5	0.75	16	1	8	0.5	1	32	16	1	8	8	0.03	0.78
																			16	4	0.03	0.78
17	64	32	16	32	1	64	1	32	0.5	1	32	1	16	0.5	1	64	32	1	32	2	0.06	0.62
																			16	8	0.12	0.62
18	32	8	8	4	0.75	32	1	8	0.12	0.37	8	1	2	0.12	0.37	32	8	1	2	2	0.12	0.433
													1	0.25	0.37							
19	32	16	16	4	0.75	32	2	2	1	0.563	8	2	4	0.12	0.56	32	8	2	16	1	0.03	0.64
20	64	16	32	8	1	64	2	16	1	0.75	16	2	1	1	0.563	64	16	2	4	2	1	0.688

a: MIC from checkerboard ; Meropenem ≤ 4 μg/ml (Susceptible), = 8 μg/ml (Intermediate), ≥ 16 μg/ml (Resistant); Colistin ≤ 2 μg/ml (Susceptible), ≥ 4 μg/ml (Resistant)

b: ∑FIC < 1 (Synergy), ∑FIC =1 (Additive), ∑FIC > 1 (Antagonist); Highlighted zone, the combination which synergistic effect was shown

Table 4-4 (continued) Individual MICs, FICs and FIC index of meropenem (MEM), sulbactam (SUL), and colistin (COL) alone and in combinations against 30 isolates of *A. baumannii*

Isolate No.	MIC (μg/ml)																				
	Alone		Combined			Alone		Combined			Alone		Combined			Alone			Combined			
	MEM	SUL	MEM	SUL	∑FIC[b]	MEM	COL	MEM	COL	∑FIC[b]	SUL	COL	SUL	COL	∑FIC[b]	MEM	SUL	COL	MEM	SUL	COL	∑FIC[b]
21	16	8	8	4	1	16	1	8	0.5	1	8	1	4	0.5	1	16	8	1	4	4	0.12	0.87
22	64	32	32	4	0.625	64	1	8	1	1.125	32	1	2	1	1.063	64	32	1	16	16	0.25	1
																			32	8	0.25	1
23	128	4	16	2	0.625	128	1	8	0.5	0.563	8	1	4	0.5	1	128	8	1	64	0.5	0.06	0.593
			64	0.5	0.625																	
24	128	32	64	4	0.625	128	1	64	0.25	0.75	32	1	16	0.5	1	128	32	1	64	2	0.12	0.683
25	64	32	16	8	0.5	64	1	32	0.25	0.75	32	1	16	0.25	0.75	64	32	1	4	16	0.06	0.623
								16	0.5	0.75			8	0.5	0.75							
26	64	32	32	16	1	32	2	16	0.25	0.625	32	2	2	1	0.563	64	32	2	16	4	0.03	0.64
								4	1	0.625												
27	16	16	8	8	1	16	1	2	0.5	0.625	16	1	1	0.5	0.625	16	16	1	2	1	0.5	0.688
28	128	8	64	0.5	0.563	128	1	8	0.5	0.563	8	1	4	0.5	1	128	8	1	64	0.5	0.12	0.683
29	32	16	16	4	0.75	32	2	4	1	0.625	16	2	8	0.12	0.56	32	16	2	16	2	0.03	0.64
30	64	64	32	16	0.75	32	1	16	0.25	0.75	32	1	16	0.5	1	32	32	1	4	16	0.12	0.745
								8	0.5	0.75									16	4	0.12	0.745

a: MIC from checkerboard ; Meropenem ≤ 4 μg/ml (Susceptible), = 8 μg/ml (Intermediate), ≥ 16 μg/ml (Resistant); Colistin ≤ 2 μg/ml (Susceptible), ≥ 4 μg/ml (Resistant)

b: ∑FIC < 1 (Synergy), ∑FIC =1 (Additive), ∑FIC > 1 (Antagonist); Highlighted zone, the combination which synergistic effect was show

Table 4-5 Synergy range and FICI range of double and triple combination of meropenem, colistin, and sulbactam against 30 isolates of MDR *A. baumannii*

Antimicrobial combinations	Synergy Range[a] (μg/ml)			FICI range[b]
	MEM	SUL	COL	
MEM + SUL	4-128	0.5-32	-	0.313-0.75
MEM + COL	4-64	-	0.5-1	0.37-0.75
SUL + COL	-	0.5-16	0.03-1	0.37-0.75
MEM + SUL + COL	2-64	0.5-16	0.03-1	0.405-0.87

[a]drug concentration range over which synergistic effect was observed for individual antimicrobials in combinations.

[b]FIC index range of the combinations that showed synergistic effect

MEM, meropenem; SUL, sulbactam; COL, colstin

$\sum$FIC < 1 (Synergy), $\sum$FIC =1 (Additive), $\sum$FIC > 1 (Antagonist)

Using three-dimensional checkerboard microdilution with meropenem, colistin, and sulbactam, the combined concentrations of each agent showing synergy and their $\sum$FICs of less than 1.0 against 30 isolates of *A. baumannii* were presented as the highlighted zones in Table 4-4 and summarized in Table 4-5.

The synergistic effect of the combination of meropenem (4-128 μg/ml) and sulbactam (0.5-32 μg/ml) was found in 21 isolates (70%) (Isolate No.1, 3, 4, 7, 8, 10, 11, 12, 13, 14, 15, 16, 18, 19, 22, 23, 24, 25, 28, 29, and 30) with FIC index ranged from 0.313-0.75. The MICs of meropenem and sulbactam alone ranged from 16-256 μg/ml and 4-64 μg/ml, respectively.

Double combination of meropenem (4-64 μg/ml) and colistin (0.5-1 μg/ml) were synergistic against 22 isolates (73.33%) (Isolate No. 2, 4, 5, 7, 8, 9, 10, 11, 12, 14, 16, 18, 19, 20, 23, 24, 25, 26, 27, 28, 29, and 30) with FIC index ranged from 0.37-0.75. The MICs of meropenem and colistin alone ranged from 16-128 μg/ml and 0.5-2 μg/ml, respectively.

Synergy was observed with a combination of sulbactam and colistin in 16 isolates (53.33%) (Isolate No. 2, 4, 5, 7, 8, 9, 10, 11, 12, 18, 19, 20, 25, 26, 27, and 29) at concentrations of 0.5-16 μg/ml of sulbactam and 0.03-1 μg/ml of colistin. The FIC index ranged from 0.37-0.75 and the MICs of sulbactam and colistin alone ranged from 4-32 μg/ml and 0.5-2 μg/ml, respectively.

Triple combination of meropenem (2-64 μg/ml), sulbactam (0.5-16 μg/ml), and colistin (0.03-1 μg/ml) were also synergistic against almost all of the tested isolates (96.67%) except isolate No. 22 which was additive. The FIC index ranged from 0.405-0.87 and the MICs of meropenem, sulbactam, and colistin alone ranged from 16-128 μg/ml, 4-32 μg/ml, and 0.5-2 μg/ml, respectively.

4. Time kill study

The bactericidal activities of meropenem, colistin, and sulbactam alone and in combinations against 10 isolates of MDR *A. baumannii* were performed by time kill method. Ten isolates of MDR *A. baumannii* tested in this study, including isolate No. 1, 2, 8, 11, 18, 19, 20, 24, 25, and 28, were chosen from the isolates that showed synergistic effects in checkerboard method with various susceptibilities to each agent (Table 4-6). The concentration of each agent was selected based on the average achievable serum concentration in human with standard dosing: meropenem 50 μg/ml and sulbactam 30 μg/ml. While using colistin 0.5 μg/ml which was 0.5x$MIC_{50,90}$ of colistin from the checkerboard microdilution method in this study.

Table 4-6 Characteristics of the chosen isolates in time kill study

Isolate No.	MICs[a] (μg/ml)			∑FIC from checkerboard method[b]			
	MEM	SUL	COL	MEM+ SUL	MEM+COL	SUL+COL	MEM+SUL+COL
1	64	32	1	0.75	1	1.063	0.87
2	32	32	0.5	1	0.625	0.625	0.683
8	128	4	2	0.5	0.532	0.625	0.625
11	32	16	1	0.56	0.56	0.56	0.563
18	32	8	1	0.75	0.37	0.37	0.433
19	32	8	2	0.75	0.56	0.56	0.64
20	64	16	2	1	0.75	0.563	0.688
24	128	32	1	0.625	0.75	1	0.683
25	64	32	1	0.5	0.75	0.75	0.623
28	128	8	1	0.563	0.563	1	0.683

[a] MICs from the checkerboard microdilution method

[b] ∑FIC < 1 (Synergy), ∑FIC =1 (Additive), ∑FIC > 1 (Antagonist); Highlighted zone, the combination which synergistic effect was shown; MEM, meropenem; SUL, sulbactam; COL, colistin

The extent of bacterial killing was estimated by the number of isolates which were killed at various time intervals. The data were shown in Table 4-7.

Table 4-7 Reduction of viable cell counts of MDR *A. baumannii* (10 isolates) at various time intervals

Antimicrobial agents	No. of isolates to be killed at time point																
	2 h			4 h			6 h			8 h				24 h			
	-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3	R	-1	-2	-3	R
MEM 50 µg/ml	3	-	-	2	-	-	1	-	-	-	-	-	-	-	-	-	5
SUL 30 µg/ml	-	-	-	4	3	-	2	4	-	-	2	2	-	-	-	-	9
COL 0.5 µg/ml	3	1	3	3	1	4	2	2	2	3	3	1	-	-	-	-	8
MEM 50 + SUL30	4	2	-	-	5	3	-	4	4	1	2	4	-	-	-	2	8
MEM 50 + COL 0.5	-	4	6	-	1	9	1	-	9	1	-	8	1	1	1	5	4
SUL 30 + COL 0.5	2	3	5	2	2	6	1	1	8	-	-	9	-	1	1	3	6
MEM50+SUL30+ COL0.5	-	3	7	-	2	8	-	2	8	1	1	8	-	1	3	4	1

-1 = 90% of viable reduction versus initial inoculums, -2 = 99% of viable reduction versus initial inoculums, -3 = 99.9 % of viable reduction versus initial inoculums, R= regrowth);

MEM, meropenem; SUL, sulbactam; COL, colistin

After given 50 µg/ml meropenem, the bactericidal activity (99.9% killing or ≥3 log CFU/ml decreased) could not be observed at any time during the time of study. Only 3 isolates (30%) (isolate No. 2, 11, and 25) were killed at the level of 90% killing (≥1 log CFU/ml decreased) at 2nd hour and decreased to 2 (isolate No. 11 and 20) and 1 isolate (isolate No. 20) at 4th and 6th hour, respectively. The regrowth could be observed in 5 isolates (50%) at 24th hour (isolate No. 1, 19, 20, 24, and 25). For 30 µg/ml sulbactam, 99.9% killing could be observed in 2 isolates at 8th hour (isolate No. 8 and 18). Isolate No. 8 was killed at the level of 90% killing (≥1 log CFU/ml decreased) at 4th hour, increased to 99% killing (≥2 log CFU/ml decreased) at 6th hour and finally increased to

99.9% killing at 8th hour. Isolate No. 18 was kill at the level of 99% killing during the 4th to 6th hour and increased to 99.9% killing in 8th hour. Nine isolates (90%) were regrowth at 24th hour, except for 1 isolate (No. 18). With colistin 0.5 µg/ml, 99.9% killing could be observed at 2nd to 8th hour. However, at 24th hour, 8 isolates (80%) were regrowth.

In double combinations, the combination of 50 µg/ml meropenem with 30 µg/ml sulbactam exhibited 99.9% killing at 4th to 24th hour. At 24th hour, only 2 isolates (isolate No. 2, 8) were killed while the others (8 isolates; 80%) were regrowth. When meropenem 50 µg/ml combined with colistin 0.5 µg/ml, 99.9% killing could be observed since the 2nd to 24th hour. The regrowth could be observed in 1 isolate at 8th hour and 4 isolates at 24th hour. Similarly with the combination of sulbactam 30 µg/ml and colistin 0.5 µg/ml that 99.9% killing could be observed since the 2nd to 24th hour but the regrowth at 24th hour was shown more in the latter combination (4 and 6 isolates, respectively).

In the triple combination of 50 µg/ml meropenem, 30 µg/ml sulbactam, and 0.5 µg/ml colistin, 99.9% killing could be also observed since the 2nd to 24th hour. The regrowth was found at 24th hour in 1 isolate (isolate No.11), which was the same isolate in the combination of meropenem with sulbactam and sulbactam with colistin. In addition, two isolates (isolate No. 24 and 25) were regrowth in both single agent and all double combinations given except in this triple combination.

The mean $\log_{10}$ change of viable cell count and bacteriolytic area for 24 hours (BA_{24}) in 10 isolates of MDR *A. baumannii* were shown in Table 4-8. The average time-kill curves for the antibacterial activity of the combination of meropenem, colistin, and sulbactam were shown in Fig 4-3.

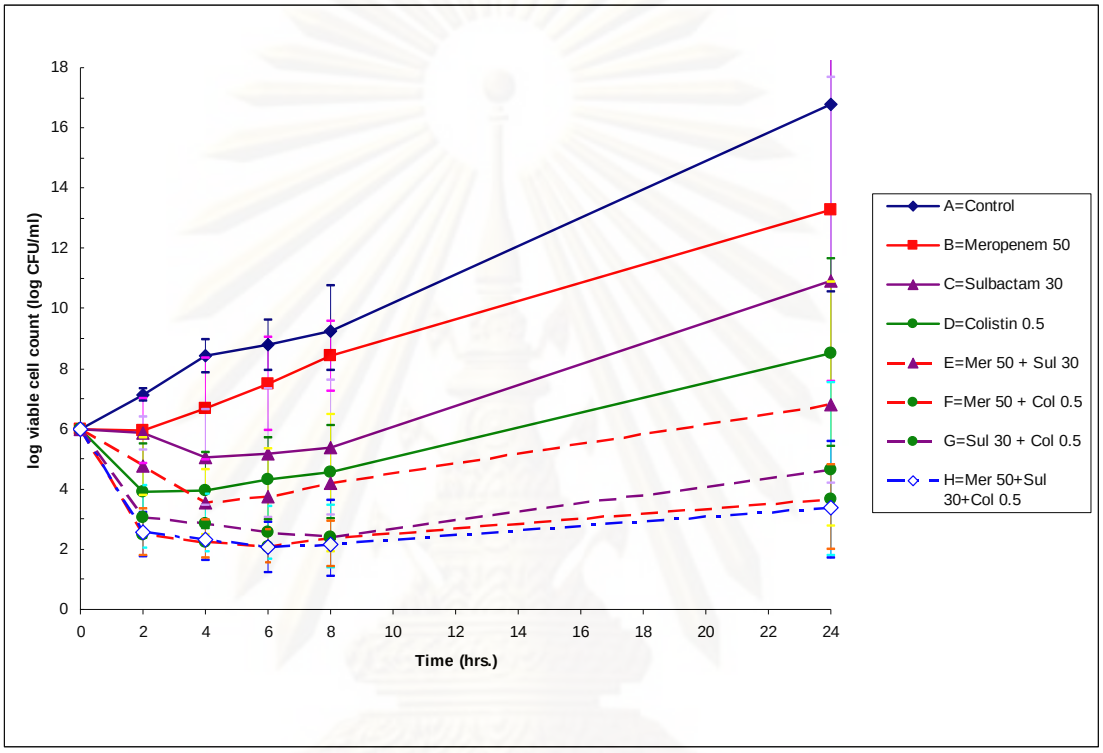

Figure 4-3 Average time-kill curve showing the antibacterial activity of meropenem, colistin, and sulbactam alone and in combinations against 10 isolates of MDR *A. baumannii*. Data are means ± SD (error bars). MEM, meropenem; SUL, sulbactam; COL; colistin

Table 4-8 Mean log change viable cell counts at various time intervals, $AUBKC_{0-24}$ and BA_{24} in 10 isolates of MDR *A. baumannii*

	Antimicrobial agents	Change in viable cell count (log CFU/ml)					Mean ±SD	
		Δ_2	Δ_4	Δ_6	Δ_8	Δ_{24}	$AUBKC_{0-24}$	BA_{24}
Average	A=Control	1.14 ±0.35	2.43 ±0.65	2.80 ±0.91	3.25 ±1.49	10.73 ±6.32	271.36 ±59.67	-
	B=MEM 50 µg/ml	-0.05 ±1.18	0.69 ±1.76	1.52 ±1.61	2.43 ±1.22	7.29 ±5.72	228.03 ±56.94	44.92 ±42.00
	C=SUL 30 µg/ml	-0.13 ±0.58	-0.93 ±1.63	-0.93 ±1.63	-0.61 ±2.26	4.94 ±6.76	173.85 ±79.62	99.11 ±65.05
	D=COL 0.5 µg/ml	-2.06 ±1.45	-2.04 ±1.19	-2.04 ±1.19	-1.42 ±1.44	2.55 ±3.12	139.70 ±38.08	133.26 ±73.73
	E=MEM 50 + SUL 30	-1.23 ±1.00	-2.42 ±1.16	-2.42 ±1.16	-1.80 ±2.34	0.84 ±4.10	122.31 ±57.19	150.65 ±64.76[a,b]
	F=MEM 50 + COL 0.5	-3.48 ±0.69	-3.74 ±0.62	-3.74 ±0.62	-3.61 ±1.31	-2.32 ±2.05	70.19 ±26.73	202.76 ±53.48[a,d]
	G=SUL 30 + COL 0.5	-2.91 ±0.96	-3.11 ±0.90	-3.11 ±0.90	-3.56 ±1.11	-1.33 ±2.88	81.99 ±35.15	190.97 ±55.68[b,d]
	H=MEM50+SUL30+COL0.5	-3.41 ±0.73	-3.64 ±0.63	-3.64 ±0.63	-3.81 ±0.84	-2.59 ±1.51	66.68 ±17.67	206.28 ±58.38[a,c,d]

[a] = $p < 0.05$ compared to activity of meropenem alone, [b] = $p > 0.05$ compared to activity of sulbactam alone, [c] = $p < 0.05$ compared to activity of sulbactam alone,

[d] = $p > 0.05$ compared to activity of colistin alone

Δ = Mean log change viable cell counts 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; MEM, meropenem; SUL, sulbactam; COL; colistin

From the mean log change viable cell counts at various time intervals; in meropenem 50 µg/ml, the bacterial killing effects were less than 90% killing and shown throughout the time of this study. BA_{24} of 50 µg/ml meropenem was 44.92 ±42.00 log CFU/ml.h. Similar result was observed with 30 µg/ml sulbactam that showed bacterial killing effects that was less than 90% killing throughout the time of this study, but BA_{24} of sulbactam was 99.11 ±65.05 log CFU/ml.h. For colistin 0.5 µg/ml, 99% killing was observed during the 2nd to 6th hour and 90% killing was observed at the 8th hour. BA_{24} of colistin was 133.26 ±73.73 log CFU/ml.h. The bacterial regrowth at 24th hour were found in all agents when given alone.

In double combinations, the combination of meropenem 50 µg/ml with sulbactam 30 µg/ml exhibited 90% killing at 2nd and 8th hour, and 99% killing was also observed at 4th and 6th hour. The bacterial regrowth could be found at the 24th hour. BA_{24} of this combination was 150.65 ±64.76 log CFU/ml.h, which was significantly different from that of meropenem alone but not statistically different from sulbactam alone.

When meropenem 50 µg/ml combined with colistin 0.5 µg/ml, bactericidal activity could be observed since the 2nd hour to 8th hour without any regrowth during the time of study. BA_{24} of this combination was 202.76 ±53.48 log CFU/ml.h, which was significantly different from that of meropenem alone but not statistically different from colistin alone.

Bactericidal activity of sulbactam 30 µg/ml combined with colistin 0.5 µg/ml was found since the 4th hour to 8th hour without any regrowth during the time of this study. Even though the BA_{24} of this combination (190.97 ±55.68 log CFU/ml.h) was higher than BA_{24} of sulbactam (99.11 ±65.05 log CFU/ml.h) and colistin (133.26 ±73.73 log CFU/ml.h) alone, no statistically significant difference was observed between them.

In triple combination, bactericidal activity could be observed since the 2nd to 8th hour without any bacterial regrowth during the time of this study. BA_{24} of this combination was 206.28 ±58.38 log CFU/ml.h, which was significantly different from meropenem and

sulbactam alone but not statistically different from colistin alone. In addition, there was not statistically different between the triple combination and all double combinations.

5. Determination of the morphological cell structure change of *A. baumannii* by scanning electron microscope

MDR *A. baumannii* isolate no. 18 (MIC of meropenem, sulbactam, and colistin were 32, 8, and 1 μg/ml, respectively) was chosen to determine the morphological cell structure change by scanning electron microscope. The morphological changes in MDR *A.baumannii* isolate no. 18 after exposed to 50 μg/ml meropenem, 30 μg/ml sulbactam and 0.5 μg/ml colistin alone and in combinations for 2 hours are shown in Figure 4-4 to 4-6.

Control cell (Figure 4-4A; without antimicrobial agent) had a smooth surface. While 50 μg/ml meropenem alone (Figure 4-4B) caused conversion of the bacterial cell to the spheroplast and produced some protrusions correspondingly with 30 μg/ml sulbactam alone (Figure 4-4C) but less protrusion was observed. The 0.5 μg/ml colistin alone (Figure 4-4D) exhibited modulated morphological alternation and more protrusions were observed. The combination of two agents (Figure 4-4E-G), including meropenem with sulbactam, meropenem with colistin and sulbactam with colistin, and the triple combination (Figure 4-4H) caused lower number of the bacterial cell as compared to those in the single agent. Also, the collapses of bacterial cell walls and cell lysis were observed especially in the combination of meropenem with colistin (Figure 4-5) and the triple combination of meropenem, colistin, and sulbactam (Figure 4-6).

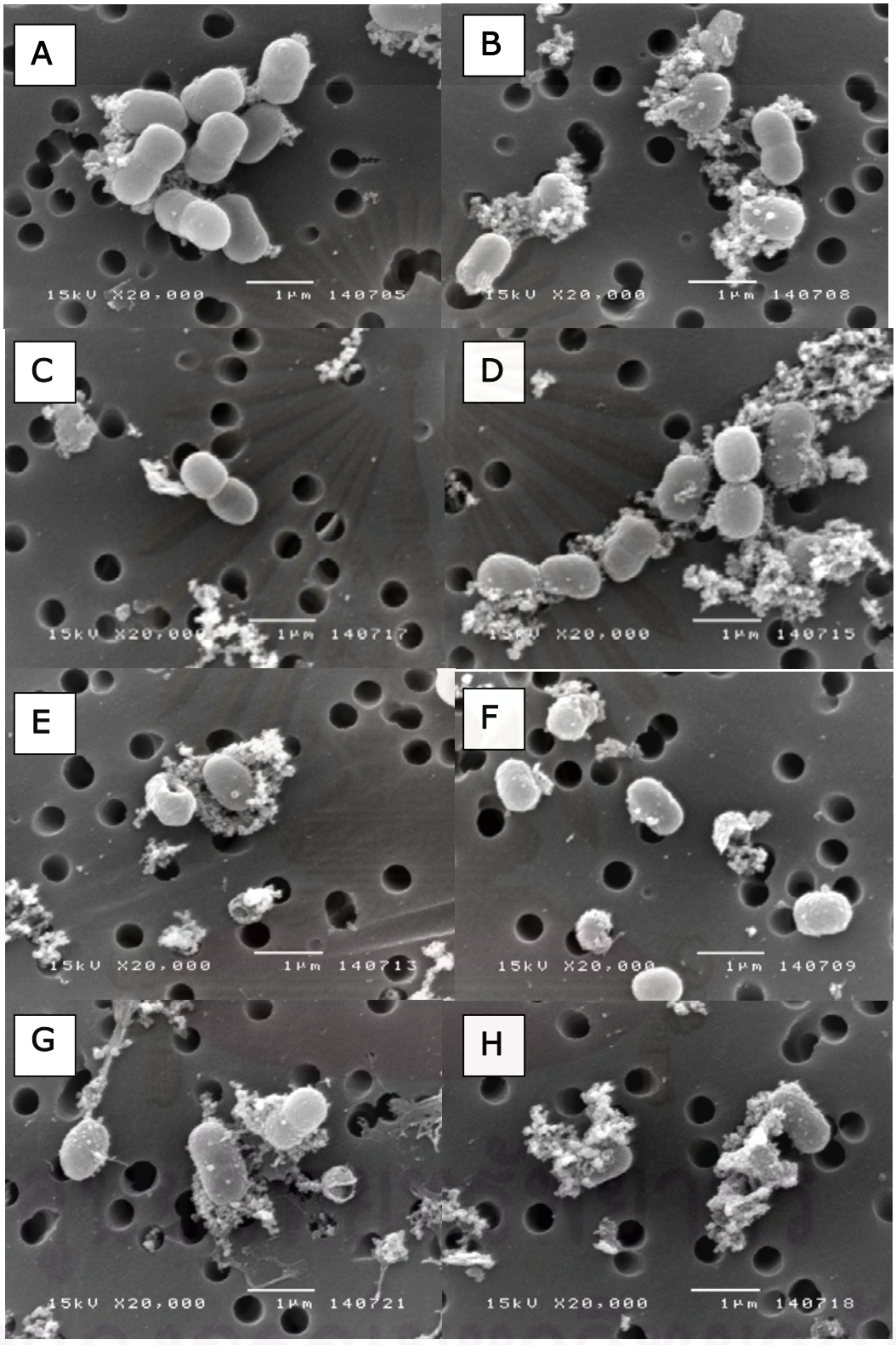

Figure 4-4 Scanning electron micrographs of *A. baumannii* isolate no.18 after 2 hours exposed to (A) no antimicrobial agent, (B) 50 µg/ml meropenem, (C) 30 µg/ml sulbactam, (D) 0.5 µg/ml colistin, (E) 50 µg/ml meropenem + 30 µg/ml sulbactam, (F) 50 µg/ml meropenem + 0.5 µg/ml colistin, (G) 30 µg/ml sulbactam + 0.5 µg/ml colistin, and (H) triple combination of 50 µg/ml meropenem, 30 µg/ml sulbactam, and 0.5 µg/ml colistin. Each bar indicates 1 µm.

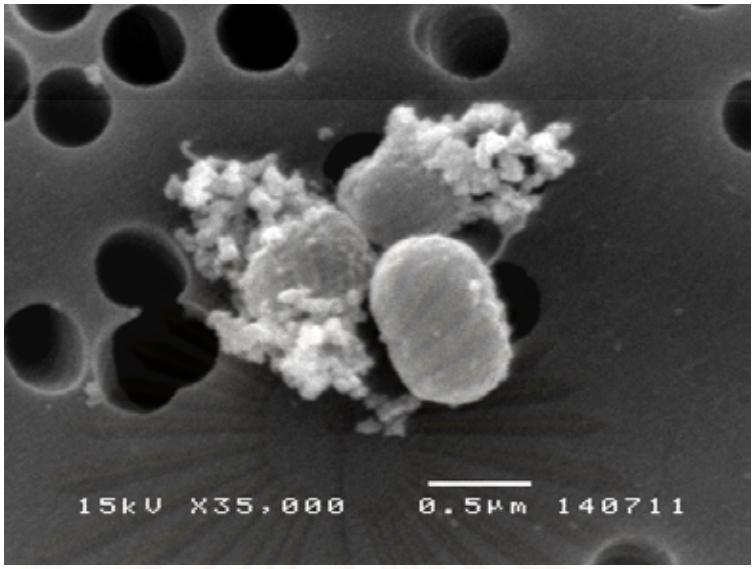

Figure 4-5 Scanning electron micrographs of *A. baumannii* isolate no.18 after 2 hours exposed to the combination of 50 µg/ml meropenem and 0.5 µg/ml colistin. Each bar indicates 0.5 µm.

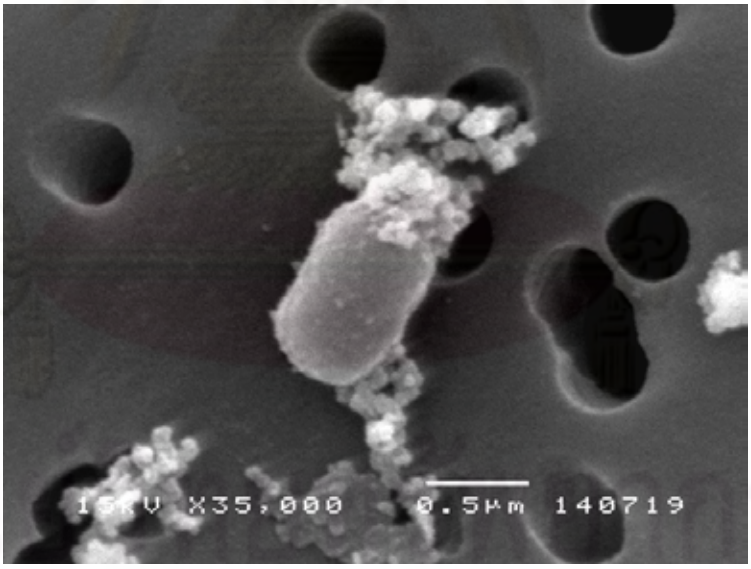

Figure 4-6 Scanning electron micrographs of *A. baumannii* isolate no.18 after 2 hours exposed to the combination of 50 µg/ml meropenem, 30 µg/ml sulbactam, and 0.5 µg/ml colistin. Each bar indicates 0.5 µm.

CHAPTER V

DISCUSSION AND CONCLUSION

Acinetobacter baumannii is well known as a multidrug-resistant (MDR) nosocomial pathogen. It is resistant to most commonly available antimicrobial agents, including penicillins, cephalosporins, monobactams, aminoglycosides, and fluoroquinolones worldwide (Bergogne-Berezin and Towner, 1996; Perez et al., 2007; Peleg et al., 2008), also as our endemic areas (Tribuddharat et al. 2003; Chaiwarith et al., 2006; Keerasuntonpong et al., 2006; Surasarang et al., 2007; Saelao and Utiswannakul, 2008).

Carbapenems are usually recommended as the drugs of choice for treating *A. baumannii* infections, but the carbapenem resistance is also increasingly, recognized as a threat to the effective treatment of these infections (Afzal-Shah and Livermore, 1998; Da Silva, Leitao, and Peixe, 1999; Corbella et al., 2000; Poirel and Nordmann, 2006). The mechanisms of carbapenem resistance is often attributed to the presence of β-lactamases (carbapenemases), the changes in outer membrane proteins (OMPs), the alterations in the affinity or expression of penicillin-binding proteins (PBPs) and the overexpression of multidrug efflux pumps (Perez et al., 2007; Munoz-Price and Weinstein, 2008; Peleg et al., 2008). The emergence of multidrug-resistance including carbapenem resistance as described above has led to the deficiency for the treatment of *A. baumannii* infections.

Corresponding with the present study, all 30 isolates of *A. baumannii* were defined as multidrug-resistant (MDR) on account of the resistance to 3 to 5 of broad-spectrum antimicrobial agents, such as amikacin, cefepime, ciprofloxacin, meropenem, and piperacillin/tazobactam. Most of isolates (56.7%) were resistant to all 5 broad-spectrum antimicrobial agents; in addition, all isolates were resistant to meropenem, ciprofloxacin, and piperacillin/tazobactam. This may be explained by the collaboration of various mechanisms of resistance such as enzymatic inactivation (β-lactamases, aminoglycosides-modifying enzymes), target modification (alteration of the ribosomal

target, modifications in the structure of DNA gyrase or topoisomerase IV, the alteration of PBPs), and decrease in intracellular concentration (porin mutation, multidrug efflux pump).

The worldwide prevalence of nosocomial infections caused by MDR *A. baumannii* and the lack of new antimicrobial agents discovery have led to the use of non-traditional antimicrobial agents such as sulbactam, the β-lactamase inhibitor that has the intrinsic antimicrobial activity against *Acinetobacter* spp. (Higgins et al., 2004), and polymyxins (polymyxin B and colistin), the abandoned agents, owing to their nephrotoxicity and neurotoxicity (Falagas and Kasiakou, 2005; Li, Nation, et al., 2006). In this study, the MIC of sulbactam ranged from 4-64 μg/ml, and all isolates were still susceptible to colistin (MICs ranging from 0.5-2 μg/ml, MIC_{50} and MIC_{90} were 1 μg/ml). Corresponding with the previous study of Tribuddharat et al. (2003) which showed that all 100 isolates of MDR *A. baumannii* from Thai patients were susceptible to polymyxin B and colistin.

The combination antimicrobial therapy is usually used to enhance the efficacy by synergistic effect, to allow lower doses to reduce toxicity and also to prevent the emergence of resistance (Rahal, 2006).The present study is partially supported by other findings (Marques et al., 1997; Choi et al., 2004; Ko et al., 2004; Yoon et al. 2004; Kiffer et al., 2005; Timurkaynak et al. 2006; Tong et al., 2006; Song et al., 2007). Choi et al. (2004) have demonstrated synergistic activities of imipenem with sulbactam against *A. baumannii*, including two imipenem-resistant *A. baumannii* strains, corresponded with Song et al. (2007). Ko et al. (2004) have shown synergism of meropenem and sulbactam against a specific *A. baumannii* clone, which was similar to the results from the study of Kiffer et al. (2005). In addition, Yoon et al. (2004) and Timurkaya et al. (2006) have shown that polymyxin B or colistin played important roles, particularly when combined with carbapenems and/or rifampicin.

From the results of combination effects studied by checkerboard method, both double and triple combinations of meropenem, colistin, and sulbactam showed synergy.

Meropenem and sulbactam showed synergistic activity in 21 isolates (70%) with synergy range of meropenem at 4-128 μg/ml. However, the therapeutic plasma concentrations should be concerned. There were only 17 out of 21 isolates that had synergistic effects at the concentration of meropenem within therapeutic plasma level (meropenem administered by intravenous infusion in 500 mg and 1000 mg doses produced C_{max} values of 26 μg/ml and 50–60 μg/ml, respectively). Compared with the synergy range of meropenem 4-64 μg/ml in the combination of meropenem and colistin which showed synergy in 22 isolates (73.33%) and only 1 out of 22 isolates was shown synergistic effect at the concentration of meropenem higher than therapeutic plasma level.

The combination of sulbactam and colistin showed synergistic effect in 16 isolates (53.33%) with the synergy range of sulbactam 0.5-16 μg/ml which was the concentration within therapeutic level (mean peak serum levels for sulbactam range from 48-88 μg/ml and 21-40 μg/ml after administered of 2000 mg ampicillin plus 1000 mg sulbactam and 1000 mg ampicillin plus 500 mg sulbactam, respectively). Synergy range of colistin in this combination was only 0.03-1 μg/ml, while the 0.5-1 μg/ml of colistin was found in the combination of meropenem and colistin. This may be explained by the capability of colistin that increases bacterial membrane permeability, allowing enhanced penetration and activity of both meropenem and sulbactam.

The triple combination was superior to the others by showing synergy against nearly all of 30 MDR *A. baumannii* isolates (29 out of 30 isolates; 96.67%) and no antagonism was observed. There were 25 out of 29 isolates that had synergistic effects at the concentration of meropenem within therapeutic level. The synergy range of sulbactam was 0.5-16 μg/ml which was also the concentration within therapeutic level and the synergy range of colistin in this combination was only 0.03-1 μg/ml.

Antagonism (FIC index >1) was demonstrated in the combinations of meropenem with colistin and sulbactam with colistin (2 and 4 isolates, respectively).

However, the MIC values were not increased in the combinations when tested with each agent alone and in combinations.

Synergism and bactericidal activities have also exhibited in the time kill study. The antimicrobial agent concentrations used were the concentrations that were achievable in human serum during treatment: 50 μg/ml meropenem and 30 μg/ml sulbactam, while 0.5 μg/ml colistin, which was 0.5x$MIC_{50,90}$ of colistin in this study, was used in the purpose of the reduction of the toxicity and prevention of the resistance that may be occurred. The bacteriolytic area for 24 hours (BA_{24}) was used to evaluate the quantitative of total antibacterial effect during 24 hours in time kill study. In the present study, the bactericidal activity (99.9% killing or $\geq$3 log CFU/ml decreased) could not be observed at any time during the time intervals of this study after given meropenem 50 μg/ml alone. This may be due to the high level of meropenem resistance of 10 isolates in this study (MICs from checkerboard method ranging from 32-128 μg/ml, MICs from agar dilution method ranging from 64-128 μg/ml). In addition, for the clinical application, the time that the plasma drug concentration is maintained above the MIC (T>MIC) is an important factor determining the bactericidal effects of β-lactam antimicrobial agents. For carbapenems, a T>MIC of ~20% is required for bacteriostatic effects while T>MIC of ~40% achieves bactericidal effects (Zhanel et al., 2007).

There are many previous reports on the use of ampicillin/sulbactam in the treatment of pneumonia caused by imipenem-resistant *Acinetobacter* spp., Urban et al. (1993) reported the case series that showed clinical improvement in 8 of 8 patients with pneumonia or tracheobronchitis. Similarly, Jimenez-Mejias et al. (1997) reported that ampicillin/sulbactam was used successfully in the treatment of 6 of 8 cases of meningitis caused by imipenem-resistant isolates. However, it should be noted that the susceptibility testing methods used by these investigators may falsely report imipenem resistance. Likewise, Cisneros et al. (1996) and Wood et al. (2002) showed that ampicillin-sulbactam had an efficacy similar to that of imipenem-cilastatin in patients with *Acinetobacter* bacteremia and VAP, respectively. In this study, 30 μg/ml sulbactam alone showed bactericidal effect in 2 isolates at the 8^{th} hour. However, MICs of

sulbactam for these studied isolates were relative low level (4-32 μg/ml), especially in the isolates that 99.9% killing was shown (MICs of sulbactam were 4 and 8 μg/ml) and the regrowth was found in 9 isolates (90%) at 24th hour.

Synergistic activity of imipenem and sulbactam against *A. baumannii* has been previously reported, including the imipenem-resistant strains (Choi et al., 2004; Song et al., 2007). Likewise, Ko et al. (2004) and Kiffer et al. (2005) have shown synergism of meropenem and sulbactam against *A. baumannii*. In our study, when combined meropenem 50 μg/ml with sulbactam 30 μg/ml, bactericidal was observed at 4th to 24th hour but the regrowth was also found in 80% of isolates at 24th hour. BA_{24} was significantly different from meropenem alone but not different from sulbactam alone. The probable role of sulbactam may be related to its intrinsic activity to bind penicillin-binding protein 2 (PBP2) (Choi et al., 2004). However, meropenem has good affinity for the PBP2 of Gram-negative bacteria, and it has been described that reduced expression of PBP2 is related to reduced susceptibility or resistance to carbapenems (Fernandez-Cuenca et al., 2003). Therefore, it seems possible that other mechanisms might be involved in the synergic effects observed in the present study.

With 0.5 μg/ml colistin alone, 99.9% killing could be observed at the 2nd to 8th hour, but the regrowth was also shown in 80% of isolates at 24th hour. In the same way, Owen et al. (2007) found that colistin showed extremely rapid killing in a concentration-dependent manner; but regrowth was observed as early as the 3rd hour and substantial regrowth at 24th hour even at concentrations up to 32x MIC or 64x MIC for some isolates. These findings may support the fact that the presence of heteroresistant subpopulations in colistin susceptible *A. baumannii* may pose future therapeutic issues (Li, Rayner, et al., 2006) and monotherapy with colistin with extended-interval (e.g. 24 hour) dosage regimens may be problematic for treatment of infections caused by colistin-heteroresistant *A. baumannii* (Owen et al., 2007).

The addition of colistin in the combination of meropenem with colistin and sulbactam with colistin showed the bactericidal activity since the 2nd to 24th hour,

comparing with no bactericidal activity at all in meropenem alone. However, at the 8th hour, the bactericidal activity was shown in sulbactam alone. Even though the BA_{24} of these combinations were not statistically difference from that of colistin alone, the occurrence of bacterial regrowth was lower than that in the colistin alone. The enhancement of meropenem and sulbactam's activity could be explained by the capability of colistin that increases bacterial membrane permeability, allowing enhanced penetration and activity of meropenem and sulbactam. Synergy between colistin and the carbapenems would be expected when carbapenem resistance is due to porin protein defects (Yoon et al., 2004). From the previous study (Panapakdee, 2007); metallo beta-lactamase could not be detected in all these MDR *A. baumannii* isolates indicating that these isolates might use the other resistant mechanism to carbapenems such as class D OXA-type enzymes, the changes in outer membrane protein, alteration of PBPs or multidrug efflux pump. However, from the lower dose of colistin given in this study than in therapeutics, the reduction of the toxicity and prevention of the resistance that could be occurred from colistin were expected. Practically, in clinical used, dosing may be optimized to obtain the appropriate therapies.

The triple combination of meropenem, colistin, and sulbactam was also exhibited bactericidal since 2nd to 24th hour. BA_{24} of the triple combination was significantly different from meropenem and sulbactam alone but not statistically different from colistin alone. Besides, it was not statistically different from all double combinations. Nevertheless, the bacterial regrowth which is significantly clinical concerned was shown in only one isolate at 24th hour when triple combination was given. The ability of colistin to increase the permeability of the cell membrane and allow the penetration of meropenem and sulbactam into cell could be accounted for this bactericidal activity.

The MDR *A. baumannii* morphological changes after 2-hour exposed to 50 μg/ml meropenem, 0.5 μg/ml colistin, and 30 μg/ml sulbactam alone and in combinations were examined by scanning electron microscopy. The results showed that the abnormal form with roughly spherical surface and bacterial cell lysis were observed in the combinations

comparing with the less changes in agent given alone. Especially, the combination of colistin which has the role to increases permeability of the bacterial membrane, broaden the penetration and activity of both meropenem and sulbactam.

In conclusion, there were no statistical difference in BA_{24} between the double combinations and the triple combination and the combination of meropenem and colistin was as effective as the triple combination. However, the regrowth at 24^{th} hour occurred less in the triple combination accompanied with the synergistic effects shown more in the triple combination. Therefore, the triple combination of meropenem, colistin, and sulbactam could be the promising alternative for treatment of infection due to MDR *A. baumannii.* The present *in vitro* results may serve as a guide to future clinical studies which aim at achieving adequate serum/tissue concentrations of combined formulations to act synergistically according to pharmacodynamic of the agents. Further studies, including *in vivo* and clinical trials to evaluate forms of therapy optimization will be necessary to help identify options for patients with *A. baumannii* infections.

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APPENDICES

APPENDIX A

Antimicrobial susceptibility

Table A-1 Susceptibilities of 30 clinical isolates of *A.baumannii* to 5 broad-spectrum antimicrobial agents by disk diffusion method.

Isolate No.	Meropenem		Piperacillin/tazobactam		Ciprofloxacin		Amikacin		Cefepime	
	Zone Diameter (mm)	Interpretation	Zone Diameter(mm)	Interpretation	Zone Diameter (mm)	Interpretation	Zone Diameter (mm)	Interpretation	Zone Diameter (mm)	Interpretation
1	9.13	R	8.99	R	NZ	R	12.95	R	13.79	R
2	9.45	R	8.28	R	NZ	R	13.91	R	13.13	R
3	NZ	R	10.83	R	NZ	R	15.15	I	15.77	I
4	NZ	R	13.28	R	NZ	R	14.58	I	NZ	R
5	7.51	R	9.65	R	NZ	R	15.62	I	13.90	R
6	7.15	R	14.25	R	7.59	R	12.78	R	NZ	R
7	8.37	R	10.60	R	NZ	R	NZ*	R	11.51	R
8	NZ	R	11.55	R	NZ	R	13.54	R	13.55	R
9	7.55	R	11.54	R	6.90	R	11.85	R	NZ	R
10	8.69	R	10.66	R	NZ	R	NZ	R	13.36	R
11	7.93	R	9.70	R	NZ	R	NZ	R	15.81	I
12	7.11	R	8.15	R	NZ	R	13.31	R	8.13	R
13	6.80	R	12.40	R	7.15	R	11.65	R	19.52	S
14	NZ	R	10.04	R	NZ	R	NZ	R	13.62	R
15	8.41	R	11.65	R	NZ	R	NZ	R	7.62	R

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

Table A-1 (continued) Susceptibilities of 30 clinical isolates of *A.baumannii* to 5 broad-spectrum antimicrobial agents by disk diffusion method.

Isolate No.	Meropenem		Piperacillin/tazobactam		Ciprofloxacin		Amikacin		Cefepime	
	Zone Diameter (mm)	Interpretation	Zone Diameter(mm)	Interpretation	Zone Diameter (mm)	Interpretation	Zone Diameter (mm)	Interpretation	Zone Diameter (mm)	Interpretation
16	8.93	R	10.88	R	NZ	R	NZ	R	15.51	I
17	7.07	R	13.73	R	NZ	R	11.98	R	NZ	R
18	8.50	R	13.81	R	NZ	R	18.38	S	13.97	R
19	NZ	R	8,61	R	NZ	R	15.45	I	10.61	R
20	8.23	R	11.99	R	7.92	R	NZ	R	8.59	R
21	10.87	R	14.28	R	NZ	R	NZ	R	11.89	R
22	7.26	R	13.10	R	7.68	R	12.80	R	7.26	R
23	NZ	R	13.44	R	NZ	R	12.41	R	15.68	I
24	NZ	R	7.16	R	NZ	R	NZ	R	NZ	R
25	8.94	R	11.79	R	NZ	R	NZ	R	15.48	I
26	7.75	R	9.61	R	NZ	R	NZ	R	8.44	R
27	9.34	R	10.85	R	NZ	R	NZ	R	15.34	I
28	NZ	R	11.85	R	NZ	R	11.73	R	15.17	I
29	7.19	R	8.34	R	NZ	R	19.06	S	11.90	R
30	7.50	R	8.63	R	7.67	R	13.90	R	7.51	R

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

Table A-2 The minimum inhibitory concentration of meropenem, colistin, and sulbactam of 30 isolates of *A. baumannii* by agar dilution method.

Isolate No.	Meropenem		Colistin		Sulbactam
	MIC (µg/ml)	Interpretation	MIC (µg/ml)	Interpretation	MIC (µg/ml)
1	64	R	0.5	S	32
2	64	R	0.5	S	32
3	256	R	1	S	32
4	128	R	1	S	32
5	128	R	1	S	32
6	128	R	1	S	32
7	128	R	1	S	32
8	128	R	2	S	8
9	128	R	0.5	S	32
10	64	R	1	S	32
11	64	R	1	S	16
12	128	R	0.5	S	32
13	128	R	1	S	32
14	128	R	1	S	32
15	64	R	1	S	32
16	64	R	0.5	S	16
17	128	R	0.5	S	32
18	64	R	1	S	8
19	64	R	1	S	16
20	64	R	1	S	8
21	64	R	1	S	8
22	128	R	1	S	32
23	128	R	1	S	4
24	128	R	1	S	32
25	64	R	1	S	32
26	128	R	0.5	S	32
27	64	R	0.5	S	16
28	128	R	0.5	S	8
29	128	R	0.5	S	16
30	128	R	0.5	S	64

R= resistant, I= intermediate, S= susceptible

Table A-3 The MIC distribution (%) with MIC_{50} and MIC_{90} for meropenem, colistin, and sulbactam against MDR *A. baumannii* 30 isolates by agar dilution method

Meropenem		Colistin		Sulbactam	
MIC range	% (No.)	MIC range	% (No.)	MIC range	% (No.)
0.03	0	0.03	0	0.03	0
0.06	0	0.06	0	0.06	0
0.12	0	0.12	0	0.12	0
0.25	0	0.25	0	0.25	0
0.5	0	0.5	33.67(11)	0.5	0
1	0	$MIC_{50,90}$ 1	60 (18)	1	0
2	0	2	3.33 (1)	2	0
4	0	4	0	4	3.33 (1)
8	0	8	0	8	16.67 (5)
16	0	16	0	16	16.67 (5)
32	0	32	0	$MIC_{50,90}$ 32	60 (18)
64	40 (12)	64	0	64	3.33 (1)
$MIC_{50,90}$ 128	56.67(17)	128	0	128	0
256	3.33 (1)	256	0	256	0

APPENDIX B

Viable cell count, Killing rate, Change in viable cell count, and kinetic parameters of 10 *A. baumannii* isolates

Table B-1 Viable cell counts (log CFU/ml) at various time points in 10 isolates of *A. baumannii.*

Antimicrobial agents	Log viable cell count (log CFU/ml) at time point (hr)					
	0	2	4	6	8	24
A 1						
A=Control	5.989	7.362	8.332	9.061	8.352	21.332
B=MEM 50 µg/ml	5.989	7.041	8.243	8.778	9.088	21.217
C=SUL 30 µg/ml	5.989	6.677	7.954	8.942	8.130	19.423
D=COL 0.5 µg/ml	5.989	3.021	4.301	5.813	4.903	6.169
E=MEM 50 + SUL30	5.989	6.079	5.916	7.031	8.197	13.954
F=MEM 50 + COL 0.5	5.989	1.699	3.301	2.176	5.061	6.398
G=SUL 30 + COL 0.5	5.989	2.352	3.138	3.447	2.477	5.088
H=MEM 50+SUL 30+COL 0.5	5.989	1.699	1.699	1.699	1.699	3.385
A 2						
A=Control	6.070	7.114	8.183	8.183	8.183	8.439
B=MEM 50 µg/ml	6.070	4.778	5.366	6.796	8.088	8.439
C=SUL 30 µg/ml	6.070	5.204	4.106	5.327	6.978	7.778
D=COL 0.5 µg/ml	6.070	2.845	2.628	5.740	6.720	8.284
E=MEM 50 + SUL 30	6.070	4.290	3.720	3.284	2.740	1.699
F=MEM 50 + COL 0.5	6.070	2.512	1.699	1.699	1.699	1.699
G=SUL 30 + COL 0.5	6.070	2.628	2.000	1.699	1.699	1.699
H=MEM 50+SUL 30+COL 0.5	6.070	3.011	2.740	1.699	1.699	1.699
A 8						
A=Control	5.966	7.357	8.261	8.653	8.796	21.954
B=MEM 50 µg/ml	5.966	7.138	8.031	8.130	8.230	7.778
C=SUL 30 µg/ml	5.966	5.249	4.154	3.204	2.602	5.602
D=COL 0.5 µg/ml	5.966	1.699	2.966	2.398	3.477	6.243
E=MEM 50 + SUL 30	5.966	4.760	3.255	2.699	2.544	1.699
F=MEM 50 + COL 0.5	5.966	1.699	1.699	1.699	2.097	3.332
G=SUL 30 + COL 0.5	5.966	2.301	2.813	2.677	2.176	5.337
H=MEM 50+SUL 30+COL 0.5	5.966	1.699	1.699	1.699	1.699	3.041

MEM, meropenem; SUL, sulbactam; COL, colistin

Table B-1 (continued) Log viable cell counts (log CFU/ml) at time point in 10 isolates of *A. baumannii*

Antimicrobial agents	Log viable cell count (log CFU/ml) at time point					
	0	2	4	6	8	24
A 11						
A=Control	6.000	7.061	8.267	8.183	8.342	8.342
B=MEM 50 µg/ml	6.000	4.720	4.966	6.398	7.813	8.183
C=SUL 30 µg/ml	6.000	5.431	3.380	3.903	5.021	7.829
D=COL 0.5 µg/ml	6.000	6.106	5.813	4.484	3.677	6.778
E=MEM 50 + SUL 30	6.000	3.357	2.740	3.491	4.760	7.267
F=MEM 50 + COL 0.5	6.000	3.079	2.097	1.699	1.699	2.720
G=SUL 30 + COL 0.5	6.000	4.875	2.628	1.699	2.000	3.796
H=MER 50+SUL 30+COL 0.5	6.000	3.000	2.097	1.699	2.176	5.051
A 18						
A=Control	6.371	6.860	8.224	8.352	8.813	11.255
B=MEM 50 µg/ml	6.371	5.954	7.301	8.249	8.237	10.211
C=SUL 30 µg/ml	6.371	6.041	4.301	3.415	3.161	5.389
D=COL 0.5 µg/ml	6.371	6.041	5.989	6.190	6.903	10.021
E=MEM 50 + SUL 30	6.371	4.813	2.398	2.544	2.097	6.230
F=MEM 50 + COL 0.5	6.371	2.796	2.000	1.699	1.699	2.740
G=SUL 30 + COL 0.5	6.371	4.431	4.097	2.929	1.699	5.419
H=MEM 50+SUL 30+COL 0.5	6.371	2.860	2.477	1.699	1.699	1.699
A 25						
A=Control	6.000	6.677	8.146	9.088	12.512	23.481
B=MEM 50 µg/ml	6.000	5.929	6.385	7.455	9.357	18.296
C=SUL 30 µg/ml	6.000	5.942	6.255	7.130	8.097	23.435
D=COL 0.5 µg/ml	6.000	4.204	2.942	2.301	2.301	6.813
E=MEM 50 + SUL 30	6.000	5.720	3.394	2.942	2.574	6.061
F=MER 50 + COL 0.5	6.000	3.431	2.477	1.699	1.699	1.699
G=SUL 30 + COL 0.5	6.000	3.431	2.398	1.699	1.699	1.699
H=MEM 50+SUL 30+COL 0.5	6.000	3.491	2.778	2.176	1.699	1.699

MEM, meropenem; SUL, sulbactam; COL, colistin

Table B-1 (continued) Log viable cell counts (log CFU/ml) at time point in 10 isolates of *A. baumannii*

Antimicrobial agents	Log viable cell count (log CFU/ml) at time point					
	0	2	4	6	8	24
A 20						
A=Control	6.061	7.138	8.273	8.407	8.447	22.312
B=MEM 50 µg/ml	6.061	5.301	4.183	4.342	6.312	15.312
C=SUL 30 µg/ml	6.061	6.079	4.903	4.114	3.889	6.978
D=COL 0.5 µg/ml	6.061	5.114	4.296	3.362	4.423	9.114
E=MEM 50 + SUL 30	6.061	4.942	3.273	2.966	3.070	6.061
F=MEM 50 + COL 0.5	6.061	3.284	3.031	2.243	2.000	1.699
G=SUL 30 + COL 0.5	6.061	3.362	4.130	3.011	2.829	5.760
H=MEM 50+SUL 30+COL 0.5	6.061	3.290	3.279	2.653	2.301	1.699
A 24						
A=Control	5.796	7.161	10.000	10.942	11.176	22.712
B=MEM 50 µg/ml	5.796	7.154	9.261	10.106	10.740	22.562
C=SUL 30 µg/ml	5.796	6.699	7.439	8.097	7.813	18.106
D=COL 0.5 µg/ml	5.796	4.051	4.677	4.942	5.653	16.423
E=MEM 50 + SUL 30	5.796	5.677	4.929	6.327	8.011	13.230
F=MEM 50 + COL 0.5	5.796	3.070	2.699	4.327	4.376	6.978
G=SUL 30 + COL 0.5	5.796	3.653	4.079	4.267	5.061	11.357
H=MEM 50+SUL 30+COL 0.5	5.796	3.273	3.217	3.122	3.889	4.954
A 25						
A=Control	5.978	7.267	8.243	8.477	8.699	12.279
B=MEM 50 µg/ml	5.978	4.385	5.230	6.860	8.183	12.267
C=SUL 30 µg/ml	5.978	5.491	3.889	4.574	5.306	9.398
D=COL 0.5 µg/ml	5.978	4.261	2.796	3.829	4.677	9.197
E=MEM 50 + SUL 30	5.978	3.138	2.677	3.740	5.000	6.889
F=MEM 50 + COL 0.5	5.978	1.699	1.699	1.699	1.699	4.628
G=SUL 30 + COL 0.5	5.978	1.699	1.699	2.398	2.875	4.677
H=MEM 50+SUL 30+COL 0.5	5.978	1.699	1.699	1.699	3.154	3.097

MEM, meropenem; SUL, sulbactam; COL, colistin

Table B-1 (continued) Log viable cell counts (log CFU/ml) at time point in 10 isolates of *A. baumannii*

Antimicrobial agents	Log viable cell count (log CFU/ml) at time point					
	0	2	4	6	8	24
A 28						
A=Control	5.574	7.224	8.183	8.431	8.989	15.860
B=MEM 50 µg/ml	5.574	6.860	7.760	7.929	8.079	8.398
C=SUL 30 µg/ml	5.574	5.677	4.161	3.051	2.699	5.290
D=COL 0.5 µg/ml	5.574	1.875	2.966	4.296	2.903	6.273
E=MEM 50 + SUL 30	5.574	4.778	3.273	2.544	2.813	5.114
F=MEM 50 + COL 0.5	5.574	1.699	1.699	1.699	1.699	4.677
G=SUL 30 + COL 0.5	5.574	1.699	1.699	1.699	1.699	1.699
H=MEM 50+SUL 30+ COL 0.5	5.574	1.699	1.699	2.760	1.699	4.197
Average						
A=Control	5.980±0.20	7.122±0.22	8.411±0.56	8.778±0.82	9.231±1.43	16.797±6.24
B=MEM 50 µg/ml	5.980±0.20	5.926±1.09	6.673±1.69	7.504±1.55	8.413±1.15	13.266±5.70
C=SUL 30 µg/ml	5.980±0.20	5.849±0.54	5.054±1.59	5.176±2.14	5.370±2.25	10.923±6.74
D=COL 0.5 µg/ml	5.980±0.20	3.922±1.56	3.937±1.27	4.336±1.38	4.564±1.54	8.532±3.11
E=MEM 50 + SUL 30	5.980±0.20	4.755±0.96	3.558±1.08	3.757±1.60	4.181±2.28	6.820±4.07
F=MEM 50 + COL 0.5	5.980±0.20	2.497±0.73	2.240±0.60	2.064±0.82	2.373±1.26	3.657±1.94
G=SUL 30 + COL 0.5	5.980±0.20	3.043±1.09	2.868±0.97	2.552±0.88	2.421±1.04	4.653±2.87
H=MEM 50+SUL 30+ COL 0.5	5.980±0.20	2.572±0.77	2.338±0.64	2.091±0.55	2.171±0.77	3.052±1.35

MEM, meropenem; SUL, sulbactam; COL, colistin

Table B-2 Log change viable cell counts at various time points and kinetic parameters in 10 isolates of *A. baumannii*

Strain no.	Antimicrobial agents	Change in viable cell count (log CFU/ml)					AUBKC 0-24	BA_{24}
		Δ_2	Δ_4	Δ_6	Δ_8	Δ_{24}		
A 1	A=Control	1.373	2.343	3.072	2.363	15.343	301.328	-
	B=MEM 50 µg/ml	1.052	2.254	2.789	3.099	15.228	305.647	4.681
	C=SUL 30 µg/ml	0.688	1.965	2.953	2.141	13.434	281.694	28.634
	D=COL 0.5 µg/ml	-2.968	-1.688	-0.176	-1.086	0.180	125.737	184.590
	E=MEM 50 + SUL 30	0.090	-0.073	1.042	2.208	7.965	229.453	80.875
	F=MEM 50 + COL 0.5	-4.290	-2.688	-3.813	-0.928	0.409	117.071	193.257
	G=SUL 30 + COL 0.5	-3.637	-2.851	-2.542	-3.512	-0.901	86.863	223.464
	H=MEM50+SUL30+COL0.5	-4.290	-4.290	-4.290	-4.290	-2.604	58.551	251.777
A 2	A=Control	1.044	2.113	2.113	2.113	2.369	194.195	-
	B=MEM 50 µg/ml	-1.292	-0.704	0.726	2.018	2.369	180.259	13.936
	C=SUL 30 µg/ml	-0.866	-1.965	-0.743	0.908	1.708	160.369	33.826
	D=COL 0.5 µg/ml	-3.225	-3.442	-0.330	0.650	2.214	155.255	38.940
	E=MEM 50 + SUL 30	-1.780	-2.350	-2.786	-3.330	-4.371	66.914	127.281
	F=MEM 50 + COL 0.5	-3.558	-4.371	-4.371	-4.371	-4.371	46.772	147.423
	G=SUL 30 + COL 0.5	-3.442	-4.070	-4.371	-4.371	-4.371	47.607	146.588
	H=MEM50+SUL30+COL0.5	-3.059	-3.330	-4.371	-4.371	-4.371	49.853	144.342
A 8	A=Control	1.391	2.295	2.687	2.830	15.988	309.306	-
	B=MEM 50 µg/ml	1.172	2.065	2.164	2.264	1.812	188.865	120.440
	C=SUL 30 µg/ml	-0.717	-1.812	-2.762	-3.364	-0.364	99.415	209.890
	D=COL 0.5 µg/ml	-4.267	-3.000	-3.568	-2.489	0.277	101.331	207.975
	E=MEM 50 + SUL 30	-1.206	-2.711	-3.267	-3.422	-4.267	63.882	245.424
	F=MEM 50 + COL 0.5	-4.267	-4.267	-4.267	-3.869	-2.634	61.692	247.614
	G=SUL 30 + COL 0.5	-3.665	-3.153	-3.289	-3.790	-0.629	83.832	225.474
	H=MEM50+SUL30+COL0.5	-4.267	-4.267	-4.267	-4.267	-2.925	55.782	253.524

MEM, meropenem; SUL, sulbactam; COL, colistin

Table B-2 (continued) Log change viable cell counts at various time points and kinetic parameters in 10 isolates of *A. baumannii*

Isolated no.	Antimicrobial agents	Change in viable cell count (log CFU/ml)					$AUBKC_{0-24}$	BA_{24}
		Δ_2	Δ_4	Δ_6	Δ_8	Δ_{24}		
A 11	A=Control	1.061	2.267	2.183	2.342	2.342	194.843	-
	B=MEM 50 µg/ml	-1.280	-1.034	0.398	1.813	2.183	173.951	20.893
	C=SUL 30 µg/ml	-0.569	-2.620	-2.097	-0.979	1.829	139.254	55.589
	D=COL 0.5 µg/ml	0.106	-0.187	-1.516	-2.323	0.778	126.121	68.723
	E=MEM 50 + SUL 30	-2.643	-3.260	-2.509	-1.240	1.267	126.152	68.692
	F=MEM 50 + COL 0.5	-2.921	-3.903	-4.301	-4.301	-3.280	56.802	138.041
	G=SUL 30 + COL 0.5	-1.125	-3.372	-4.301	-4.000	-2.204	72.772	122.072
	H=MEM50+SUL30+COL0.5	-3.000	-3.903	-4.301	-3.824	-0.949	79.586	115.258
A 18	A=Control	0.489	1.853	1.981	2.442	4.884	222.603	-
	B=MEM 50 µg/ml	-0.417	0.930	1.878	1.866	3.840	205.198	17.405
	C=SUL 30 µg/ml	-0.330	-2.070	-2.956	-3.210	-0.982	105.452	117.151
	D=COL 0.5 µg/ml	-0.330	-0.382	-0.181	0.532	3.650	185.110	37.493
	E=MEM 50 + SUL 30	-1.558	-3.973	-3.827	-4.274	-0.141	94.597	128.006
	F=MEM 50 + COL 0.5	-3.575	-4.371	-4.672	-4.672	-3.631	56.574	166.028
	G=SUL 30 + COL 0.5	-1.940	-2.274	-3.442	-4.672	-0.952	87.930	134.672
	H=MEM50+SUL30+COL0.5	-3.511	-3.894	-4.672	-4.672	-4.672	49.326	173.276
A 19	A=Control	0.677	2.146	3.088	6.512	17.481	354.275	-
	B=MEM 50 µg/ml	-0.071	0.385	1.455	3.357	12.296	276.115	78.159
	C=SUL 30 µg/ml	-0.058	0.255	1.130	2.097	17.435	305.010	49.264
	D=COL 0.5 µg/ml	-1.796	-3.058	-3.699	-3.699	0.813	100.107	254.168
	E=MEM 50 + SUL 30	-0.280	-2.606	-3.058	-3.426	0.061	101.763	252.511
	F=MEM 50 + COL 0.5	-2.569	-3.523	-4.301	-4.301	-4.301	50.097	304.177
	G=SUL 30 + COL 0.5	-2.569	-3.602	-4.301	-4.301	-4.301	49.939	304.336
	H=MEM50+SUL30+COL0.5	-2.509	-3.222	-3.824	-4.301	-4.301	51.774	302.501

MEM, meropenem; SUL, sulbactam; COL, colistin

Table B-2 (continued) Log change viable cell counts at various time points and kinetic parameters in 10 isolates of *A. baumannii*

Isolated no.	Antimicrobial agents	Change in viable cell count (log CFU/ml)					$AUBKC_{0-24}$	BA_{24}
		Δ_2	Δ_4	Δ_6	Δ_8	Δ_{24}		
A 20	A=Control	1.078	2.212	2.346	2.386	16.251	308.215	-
	B=MEM 50 μg/ml	-0.760	-1.877	-1.718	0.251	9.251	213.014	95.201
	C=SUL 30 μg/ml	0.018	-1.158	-1.947	-2.171	0.917	127.079	181.136
	D=COL 0.5 μg/ml	-0.947	-1.765	-2.699	-1.637	3.053	144.324	163.891
	E=MEM 50 + SUL 30	-1.119	-2.788	-3.095	-2.991	0.000	104.539	203.676
	F=MEM 50 + COL 0.5	-2.776	-3.029	-3.818	-4.061	-4.362	54.770	253.445
	G=SUL 30 + COL 0.5	-2.699	-1.930	-3.050	-3.231	-0.301	98.607	209.607
	H=MEM50+SUL30+COL0.5	-2.771	-2.782	-3.407	-3.760	-4.362	58.806	249.409
A 24	A=Control	1.365	4.204	5.146	5.380	16.916	344.282	-
	B=MEM 50 μg/ml	1.358	3.465	4.310	4.944	16.766	335.999	8.283
	C=SUL 30 μg/ml	0.903	1.643	2.301	2.017	12.310	265.427	78.855
	D=COL 0.5 μg/ml	-1.745	-1.119	-0.854	-0.143	10.627	215.400	128.881
	E=MEM 50 + SUL 30	-0.119	-0.866	0.531	2.215	7.435	217.603	126.679
	F=MEM 50 + COL 0.5	-2.726	-3.097	-1.469	-1.420	1.182	121.191	223.091
	G=SUL 30 + COL 0.5	-2.143	-1.717	-1.529	-0.735	5.561	166.197	178.085
	H=MEM50+SUL30+COL0.5	-2.523	-2.578	-2.674	-1.907	-0.842	99.659	244.623
A 25	A=Control	1.289	2.265	2.499	2.721	6.301	230.473	-
	B=MEM 50 μg/ml	-1.593	-0.747	0.883	2.206	6.289	210.716	19.758
	C=SUL 30 μg/ml	-0.486	-2.088	-1.404	-0.671	3.420	156.828	73.645
	D=COL 0.5 μg/ml	-1.716	-3.182	-2.148	-1.301	3.220	143.419	87.054
	E=MEM 50 + SUL 30	-2.839	-3.301	-2.237	-0.978	0.912	125.203	105.270
	F=MEM 50 + COL 0.5	-4.278	-4.278	-4.278	-4.278	-1.349	68.505	161.974
	G=SUL 30 + COL 0.5	-3.978	-4.278	-3.580	-3.103	-1.301	81.463	149.012
	H=MEM50+SUL30+COL0.5	-4.278	-4.278	-4.278	-2.824	-2.881	69.337	161.142

MEM, meropenem; SUL, sulbactam; COL, colistin

Table B-2 (continued) Log change viable cell counts at various time points and kinetic parameters in 10 isolates of *A. baumannii*

Isolated no.	Antimicrobial agents	Change in viable cell count (log CFU/ml)					$AUBKC_{0-24}$	BA_{24}
		Δ_2	Δ_4	Δ_6	Δ_8	Δ_{24}		
A 28	A=Control	1.650	2.609	2.857	3.415	9.415	254.064	-
	B=MEM 50 µg/ml	1.286	2.186	2.355	2.505	2.824	190.569	70.466
	C=SUL 30 µg/ml	0.103	-1.413	-2.523	-2.875	-0.284	97.963	163.072
	D=COL 0.5 µg/ml	-3.699	-2.608	-1.278	-2.671	0.699	100.159	160.876
	E=MEM 50 + SUL 30	-0.796	-2.301	-3.030	-2.761	-0.460	92.992	168.043
	F=MEM 50 + COL 0.5	-3.875	-3.875	-3.875	-3.875	-0.897	68.472	192.563
	G=SUL 30 + COL 0.5	-3.875	-3.875	-3.875	-3.875	-3.875	44.650	216.385
	H=MEM50+SUL30+COL0.5	-3.875	-3.875	-2.814	-3.875	-1.377	66.758	194.277

MEM, meropenem; SUL, sulbactam; COL, colistin

Table B-3 Killing rate of *A.baumannii* 10 isolates by meropenem, colistin, and sulbactam alone and in combinations.

Isolated No.	Antimicrobial Agents	Time (hr) for 3 log killing	Time (hr) for regrowth
A 1	A=Control	-	24
	B=MEM 50 µg/ml	-	24
	C=SUL 30 µg/ml	-	24
	D=COL 0.5 µg/ml	-	-
	E=MEM 50 + SUL 30	-	24
	F=MEM 50 + COL 0.5	2	8
	G=SUL 30 + COL 0.5	2	-
	H=MEM50+SUL30+COL0.5	2	-
A 2	A=Control	-	-
	B=MEM 50 µg/ml	-	-
	C=SUL 30 µg/ml	-	24
	D=COL 0.5 µg/ml	2	24
	E=MEM 50 + SUL 30	8	-
	F=MEM 50 + COL 0.5	2	-
	G=SUL 30 + COL 0.5	2	-
	H=MEM50+SUL30+COL0.5	2	-
A 8	A=Control	-	24
	B=MEM 50 µg/ml	-	-
	C=SUL 30 µg/ml	8	24
	D=COL 0.5 µg/ml	2	24
	E=MEM 50 + SUL 30	6	-
	F=MEM 50 + COL 0.5	2	-
	G=SUL 30 + COL 0.5	2	24
	H=MEM50+SUL30+COL0.5	2	-

MEM, meropenem; SUL, sulbactam; COL, colistin

Table B-3 (continued) Killing rate of *A.baumannii* 10 isolates by meropenem, colistin, and sulbactam alone and in combinations.

Isolated No.	Antimicrobial Agents	Time (hr) for 3 log killing	Time (hr) for regrowth
A 11	A=Control	-	-
	B=MEM 50 µg/ml	-	-
	C=SUL 30 µg/ml	-	24
	D=COL 0.5 µg/ml	-	24
	E=MEM 50 + SUL 30	4	24
	F=MEM 50 + COL 0.5	4	-
	G=SUL 30 + COL 0.5	4	24
	H=MEM50+SUL30+COL0.5	2	24
A 18	A=Control	-	24
	B=MEM 50 µg/ml	-	-
	C=SUL 30 µg/ml	8	-
	D=COL 0.5 µg/ml	-	24
	E=MEM 50 + SUL 30	4	24
	F=MEM 50 + COL 0.5	2	-
	G=SUL 30 + COL 0.5	6	24
	H=MEM50+SUL30+COL0.5	2	-
A 19	A=Control	-	24
	B=MEM 50 µg/ml	-	24
	C=SUL 30 µg/ml	-	24
	D=COL 0.5 µg/ml	4	24
	E=MEM 50 + SUL 30	6	24
	F=MEM 50 + COL 0.5	4	-
	G=SUL 30 + COL 0.5	4	-
	H=MEM50+SUL30+COL0.5	4	-

MEM, meropenem; SUL, sulbactam; COL, colistin

Table B-3 (continued) Killing rate of *A.baumannii* 10 isolates by meropenem, colistin, and sulbactam alone and in combinations.

Isolated No.	Antimicrobial Agents	Time (hr) for 3 log killing	Time (hr) for regrowth
A 20	A=Control	-	24
	B=MEM 50 µg/ml	-	24
	C=SUL 30 µg/ml	-	24
	D=COL 0.5 µg/ml	-	24
	E=MEM 50 + SUL 30	6	24
	F=MEM 50 + COL 0.5	4	-
	G=SUL 30 + COL 0.5	6	24
	H=MEM50+SUL30+COL0.5	6	-
A 24	A=Control	-	24
	B=MEM 50 µg/ml	-	24
	C=SUL 30 µg/ml	-	24
	D=COL 0.5 µg/ml	-	24
	E=MEM 50 + SUL 30	-	24
	F=MEM 50 + COL 0.5	4	24
	G=SUL 30 + COL 0.5	-	24
	H=MEM50+SUL30+COL0.5	-	-
A 25	A=Control	-	24
	B=MEM 50 µg/ml	-	24
	C=SUL 30 µg/ml	-	24
	D=COL 0.5 µg/ml	4	24
	E=MEM 50 + SUL 30	4	24
	F=MEM 50 + COL 0.5	2	24
	G=SUL 30 + COL 0.5	2	24
	H=MEM50+SUL30+COL0.5	2	-

MEM, meropenem; SUL, sulbactam; COL, colistin

Table B-3 (continued) Killing rate of *A.baumannii* 10 isolates by meropenem, colistin, and sulbactam alone and in combinations.

Isolated No.	Antimicrobial Agents	Time (hr) for 3 log killing	Time (hr) for regrowth
A 28	A=Control	-	24
	B=MEM 50 µg/ml	-	-
	C=SUL 30 µg/ml	-	24
	D=COL 0.5 µg/ml	2	-
	E=MEM 50 + SUL 30	6	24
	F=MEM 50 + COL 0.5	2	24
	G=SUL 30 + COL 0.5	2	-
	H=MEM50+SUL30+COL0.5	2	-

MEM, meropenem; SUL, sulbactam; COL, colistin

APPENDIX C

Three dimensional checkerboard results of meropenem, colistin, and sulbactam in *A. baumannii* 30 isolates

Plate 1 – No colistin (0 µg/ml)

Sulbactam x MIC

4/0	4/1/16	4/1/8	4/1/4	4/1/2	4/1	4/2	4/4
2/0	2/1/16	2/1/8	2/1/4	2/1/2	2/1	2/2	2/4
1/0	1/1/16	1/1/8	1/1/4	1/1/2	1/1	1/2	1/4
1/2/0	1/2/1/16	1/2/1/8	1/2/1/4	1/2/1/2	1/2/1	1/2/2	1/2/4
1/4/0	1/4/1/16	1/4/1/8	1/4/1/4	1/4/1/2	1/4/1	1/4/2	1/4/4
1/8/0	1/8/1/16	1/8/1/8	1/8/1/4	1/8/1/2	1/8/1	1/8/2	1/8/4
1/16/0	1/16/1/16	1/16/1/8	1/16/1/4	1/16/1/2	1/16/1	1/16/2	1/16/4
0/0	0/1/16	0/1/8	0/1/4	0/1/2	0/1	0/2	0/4

Meropenem x MIC

Plate 2 – Colistin 1/16 x MIC

Sulbactam x MIC

4/0/1/16	4/1/16/1/16	4/1/8/1/16	4/1/4/1/16	4/1/2/1/16	4/1/1/16	4/2/1/16	4/4/1/16
2/0/1/16	2/1/16/1/16	2/1/8/1/16	2/1/4/1/16	2/1/2/1/16	2/1/1/16	2/2/1/16	2/4/1/16
1/0/1/16	1/1/16/1/16	1/1/8/1/16	1/1/4/1/16	1/1/2/1/16	1/1/1/16	1/2/1/16	1/4/1/16
1/2/0/1/16	1/2/1/16/1/16	1/2/1/8/1/16	1/2/1/4/1/16	1/2/1/2/1/16	1/2/1/1/16	1/2/2/1/16	1/2/4/1/16
1/4/0/1/16	1/4/1/16/1/16	1/4/1/8/1/16	1/4/1/4/1/16	1/4/1/2/1/16	1/4/1/1/16	1/4/2/1/16	1/4/4/1/16
1/8/0/1/16	1/8/1/16/1/16	1/8/1/8/1/16	1/8/1/4/1/16	1/8/1/2/1/16	1/8/1/1/16	1/8/2/1/16	1/8/4/1/16
1/16/0/1/16	1/16/1/16/1/16	1/16/1/8/1/16	1/16/1/4/1/16	1/16/1/2/1/16	1/16/1/1/16	1/16/2/1/16	1/16/4/1/16
0/0/1/16	0/1/16/1/16	0/1/8/1/16	0/1/4/1/16	0/1/2/1/16	0/1/1/16	0/2/1/16	0/4/1/16

Meropenem x MIC

Plate 3 – Colistin 1/8 x MIC

Sulbactam x MIC

4/0/1/8	4/1/16/1/8	4/1/8/1/8	4/1/4/1/8	4/1/2/1/8	4/1/1/8	4/2/1/8	4/4/1/8
2/0/1/8	2/1/16/1/8	2/1/8/1/8	2/1/4/1/8	2/1/2/1/8	2/1/1/8	2/2/1/8	2/4/1/8
1/0/1/8	1/1/16/1/8	1/1/8/1/8	1/1/4/1/8	1/1/2/1/8	1/1/1/8	1/2/1/8	1/4/1/8
1/2/0/1/8	1/2/1/16/1/8	1/2/1/8/1/8	1/2/1/4/1/8	1/2/1/2/1/8	1/2/1/1/8	1/2/2/1/8	1/2/4/1/8
1/4/0/1/8	1/4/1/16/1/8	1/4/1/8/1/8	1/4/1/4/1/8	1/4/1/2/1/8	1/4/1/1/8	1/4/2/1/8	1/4/4/1/8
1/8/0/1/8	1/8/1/16/1/8	1/8/1/8/1/8	1/8/1/4/1/8	1/8/1/2/1/8	1/8/1/1/8	1/8/2/1/8	1/8/4/1/8
1/16/0/1/8	1/16/1/16/1/8	1/16/1/8/1/8	1/16/1/4/1/8	1/16/1/2/1/8	1/16/1/1/8	1/16/2/1/8	1/16/4/1/8
0/0/1/8	0/1/16/1/8	0/1/8/1/8	0/1/4/1/8	0/1/2/1/8	0/1/1/8	0/2/1/8	0/4/1/8

Meropenem x MIC

Figure C-1 The illustrations of three dimensional microdilution checkerboard of meropenem, colistin, and sulbactam using the series of proportional to MICs of the agents being tested (Modified from Eliopoulos and Moellering, 1996, Yoon et al., 2004).

Plate 4 – Colistin 1/4 x MIC

Sulbactam x MIC

4/0/1/4	4/1/16/1/4	4/1/8/1/4	4/1/4/1/4	4/1/2/1/4	4/1/1/4	4/2/1/4	4/4/1/4
2/0/1/4	2/1/16/1/4	2/1/8/1/4	2/1/4/1/4	2/1/2/1/4	2/1/1/4	2/2/1/4	2/4/1/4
1/0/1/4	1/1/16/1/4	1/1/8/1/4	1/1/4/1/4	1/1/2/1/4	1/1/1/4	1/2/1/4	1/4/1/4
1/2/0/1/4	1/2/1/16/1/4	1/2/1/8/1/4	1/2/1/4/1/4	1/2/1/2/1/4	1/2/1/1/4	1/2/2/1/4	1/2/4/1/4
1/4/0/1/4	1/4/1/16/1/4	1/4/1/8/1/4	1/4/1/4/1/4	1/4/1/2/1/4	1/4/1/1/4	1/4/2/1/4	1/4/4/1/4
1/8/0/1/4	1/8/1/16/1/4	1/8/1/8/1/4	1/8/1/4/1/4	1/8/1/2/1/4	1/8/1/1/4	1/8/2/1/4	1/8/4/1/4
1/16/0/1/4	1/16/1/16/1/4	1/16/1/8/1/4	1/16/1/4/1/4	1/16/1/2/1/4	1/16/1/1/4	1/16/2/1/4	1/16/4/1/4
0/0/1/4	0/1/16/1/4	0/1/8/1/4	0/1/4/1/4	0/1/2/1/4	0/1/1/4	0/2/1/4	0/4/1/4

Meropenem x MIC

Plate 5 – Colistin 1/2 x MIC

Sulbactam x MIC

4/0/1/2	4/1/16/1/2	4/1/8/1/2	4/1/4/1/2	4/1/2/1/2	4/1/1/2	4/2/1/2	4/4/1/2
2/0/1/2	2/1/16/1/2	2/1/8/1/2	2/1/4/1/2	2/1/2/1/2	2/1/1/2	2/2/1/2	2/4/1/2
1/0/1/2	1/1/16/1/2	1/1/8/1/2	1/1/4/1/2	1/1/2/1/2	1/1/1/2	1/2/1/2	1/4/1/2
1/2/0/1/2	1/2/1/16/1/2	1/2/1/8/1/2	1/2/1/4/1/2	1/2/1/2/1/2	1/2/1/1/2	1/2/2/1/2	1/2/4/1/2
1/4/0/1/2	1/4/1/16/1/2	1/4/1/8/1/2	1/4/1/4/1/2	1/4/1/2/1/2	1/4/1/1/2	1/4/2/1/2	1/4/4/1/2
1/8/0/1/2	1/8/1/16/1/2	1/8/1/8/1/2	1/8/1/4/1/2	1/8/1/2/1/2	1/8/1/1/2	1/8/2/1/2	1/8/4/1/2
1/16/0/1/2	1/16/1/16/1/2	1/16/1/8/1/2	1/16/1/4/1/2	1/16/1/2/1/2	1/16/1/1/2	1/16/2/1/2	1/16/4/1/2
0/0/1/2	0/1/16/1/2	0/1/8/1/2	0/1/4/1/2	0/1/2/1/2	0/1/1/2	0/2/1/2	0/4/1/2

Meropenem x MIC

Plate 6 – Colistin 1 x MIC

Sulbactam x MIC

4/0/1	4/1/16/1	4/1/8/1	4/1/4/1	4/1/2/1	4/1/1	4/2/1	4/4/1
2/0/1	2/1/16/1	2/1/8/1	2/1/4/1	2/1/2/1	2/1/1	2/2/1	2/4/1
1/0/1	1/1/16/1	1/1/8/1	1/1/4/1	1/1/2/1	1/1/1	1/2/1	1/4/1
1/2/0/1	1/2/1/16/1	1/2/1/8/1	1/2/1/4/1	1/2/1/2/1	1/2/1/1	1/2/2/1	1/2/4/1
1/4/0/1	1/4/1/16/1	1/4/1/8/1	1/4/1/4/1	1/4/1/2/1	1/4/1/1	1/4/2/1	1/4/4/1
1/8/0/1	1/8/1/16/1	1/8/1/8/1	1/8/1/4/1	1/8/1/2/1	1/8/1/1	1/8/2/1	1/8/4/1
1/16/0/1	1/16/1/16/1	1/16/1/8/1	1/16/1/4/1	1/16/1/2/1	1/16/1/1	1/16/2/1	1/16/4/1
0/0/1	0/1/16/1	0/1/8/1	0/1/4/1	0/1/2/1	0/1/1	0/2/1	0/4/1

Meropenem x MIC

Figure C-1 (continued) The illustrations of three dimensional microdilution checkerboard of meropenem, colistin, and sulbactam using the series of proportional to MICs of the agents being tested (Modified from Eliopoulos and Moellering, 1996, Yoon et al., 2004).

Plate 7 – Colistin 2 x MIC

Sulbactam x MIC							
4/0/2	4/1/16/2	4/1/8/2	4/1/4/2	4/1/2/2	4/1/2	4/2/2	4/4/2
2/0/2	2/1/16/2	2/1/8/2	2/1/4/2	2/1/2/2	2/1/2	2/2/2	2/4/2
1/0/2	1/1/16/2	1/1/8/2	1/1/4/2	1/1/2/2	1/1/2	1/2/2	1/4/2
1/2/0/2	1/2/1/16/2	1/2/1/8/2	1/2/1/4/2	1/2/1/2/2	1/2/1/2	1/2/2/2	1/2/4/2
1/4/0/2	1/4/1/16/2	1/4/1/8/2	1/4/1/4/2	1/4/1/2/2	1/4/1/2	1/4/2/2	1/4/4/2
1/8/0/2	1/8/1/16/2	1/8/1/8/2	1/8/1/4/2	1/8/1/2/2	1/8/1/2	1/8/2/2	1/8/4/2
1/16/0/2	1/16/1/16/2	1/16/1/8/2	1/16/1/4/2	1/16/1/2/2	1/16/1/2	1/16/2/2	1/16/4/2
0/0/2	0/1/16/2	0/1/8/2	0/1/4/2	0/1/2/2	0/1/2	0/2/2	0/4/2

Meropenem x MIC

Plate 8 – Colistin 4 x MIC

Sulbactam x MIC							
4/0/4	4/1/16/4	4/1/8/4	4/1/4/4	4/1/2/4	4/1/4	4/2/4	4/4/4
2/0/4	2/1/16/4	2/1/8/4	2/1/4/4	2/1/2/4	2/1/4	2/2/4	2/4/4
1/0/4	1/1/16/4	1/1/8/4	1/1/4/4	1/1/2/4	1/1/4	1/2/4	1/4/4
1/2/0/4	1/2/1/16/4	1/2/1/8/4	1/2/1/4/4	1/2/1/2/4	1/2/1/4	1/2/2/4	1/2/4/4
1/4/0/4	1/4/1/16/4	1/4/1/8/4	1/4/1/4/4	1/4/1/2/4	1/4/1/4	1/4/2/4	1/4/4/4
1/8/0/4	1/8/1/16/4	1/8/1/8/4	1/8/1/4/4	1/8/1/2/4	1/8/1/4	1/8/2/4	1/8/4/4
1/16/0/4	1/16/1/16/4	1/16/1/8/4	1/16/1/4/4	1/16/1/2/4	1/16/1/4	1/16/2/4	1/16/4/4
0/0/4	0/1/16/4	0/1/8/4	0/1/4/4	0/1/2/4	0/1/4	0/2/4	0/4/4

Meropenem x MIC

Figure C-1 (continued) The illustrations of three dimensional microdilution checkerboard of meropenem, colistin, and sulbactam using the series of proportional to MICs of the agents being tested (Modified from Eliopoulos and Moellering, 1996, Yoon et al., 2004).

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Isolate No. 1 **Plate 1** – No colistin (0 µg/ml)

Sulbactam								
128	128/4	128/8	128/16	128/32	128/64	128/128	128/256
64	64/4	64/8	64/16	64/32	64/64	64/128	64/256
32	32/4	32/8	32/16	32/32	32/64	32/128	32/256
16	16/4	16/8	16/16	16/32	16/64	16/128	16/256
8	8/4	8/8	8/16	8/32	8/64	8/128	8/256
4	4/4	4/8	4/16	4/32	4/64	4/128	4/256
2	2/4	2/8	2/16	2/32	2/64	2/128	2/256
0	4	8	16	32	64	128	256

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam								
128/0.03	128/4/0.03	128/8/0.03	128/16/0.03	128/32/0.03	128/64/0.03	128/128/0.03	128/256/0.03
64/0.03	64/4/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03	64/256/0.03
32/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03	32/256/0.03
16/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03	16/256/0.03
8/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03	8/256/0.03
4/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03	4/256/0.03
2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03	2/256/0.03
0/0/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03	256/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam								
128/0.06	128/4/0.06	128/8/0.06	128/16/0.06	128/32/0.06	128/64/0.06	128/128/0.06	128/256/0.06
64/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06	64/256/0.06
32/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06
16/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06
8/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06
4/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06
2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06
0/0/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06

Meropenem

Figure C-2 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 1 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 1 (Continued) **Plate 4** – Colistin 0.12 µg/ml

Sulbactam								
128/0.12	128/4/0.12	128/8/0.12	128/16/0.12	128/32/0.12	128/64/0.12	128/128/0.12	128/256/0.12
64/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12	64/256/0.12
32/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12
16/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12
8/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12
4/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12
2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12
0/0/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12

Meropenem

Plate 5 – Colistin 0.25 µg/ml

Sulbactam								
128/0.25	128/4/0.25	128/8/0.25	128/16/0.25	128/32/0.25	128/64/0.25	128/128/0.25	128/256/0.25
64/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25	64/256/0.25
32/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25
16/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25
8/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25
4/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25
2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25
0/0/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25

Meropenem

Plate 6 – Colistin 0.5 µg/ml

Sulbactam							
128/0.5	128/4/0.5	128/8/0.5	128/16/0.5	128/32/0.5	128/64/0.5	128/128/0.5	128/256/0.5
64/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5	64/256/0.5
32/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5
16/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5
8/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5
4/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5
2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5
0/0/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5

Meropenem

Figure C-2 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 1 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 1 (Continued) **Plate 7** – Colistin 1μg/ml

128/1	128/4/1	128/8/1	128/16/1	128/32/1	128/64/1	128/128/1	128/256/1
64/1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1	64/256/1
32/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1
16/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1
8/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1
4/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1
2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1
0/0/1	4/1	8/1	16/1	32/1	64/1	128/1	256/1

Sulbactam

Meropenem

Plate 8 – Colistin 2 μg/ml

128/2	128/4/2	128/8/2	128/16/2	128/32/2	128/64/2	128/128/2	128/256/2
64/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2	64/256/2
32/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2
16/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2
8/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2
4/2	4/4//2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2
2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2
0/0/2	4/2	8/2	16/2	32/2	64/2	128/2	256/2

Sulbactam

Meropenem

Figure C-2 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 1 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 2 **Plate 1** – No colistin (0 µg/ml)

Sulbactam								
128	128/4	128/8	128/16	128/32	128/64	128/128	128/256
64	64/4	64/8	64/16	64/32	64/64	64/128	64/256
32	32/4	32/8	32/16	32/32	32/64	32/128	32/256
16	16/4	16/8	16/16	16/32	16/64	16/128	16/256
8	8/4	8/8	8/16	8/32	8/64	8/128	8/256
4	4/4	4/8	4/16	4/32	4/64	4/128	4/256
2	2/4	2/8	2/16	2/32	2/64	2/128	2/256
0	4	8	16	32	64	128	256

Meropenem

Plate 2 – Colistin 0.06 µg/ml

Sulbactam								
128/0.06	128/4/0.06	128/8/0.06	128/16/0.06	128/32/0.06	128/64/0.06	128/128/0.06	128/256/0.06
64/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06	64/256/0.06
32/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06
16/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06
8/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06
4/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06
2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06
0/0/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06

Meropenem

Plate 3 – Colistin 0.12 µg/ml

Sulbactam								
8/0.12	128/4/0.12	128/8/0.12	128/16/0.12	128/32/0.12	128/64/0.12	128/128/0.12	128/256/0.12
64/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12	64/256/0.12
32/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12
16/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12
8/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12
4/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12
2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12
0/0/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12

Meropenem

Figure C-3 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 2 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 2 (Continued) **Plate 4** – Colistin 0.25 µg/ml

Sulbactam								
8/0.25	128/4/0.25	128/8/0.25	128/16/0.25	128/32/0.25	128/64/0.25	128/128/0.25	128/256/0.25
64/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25	64/256/0.25
32/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25
16/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25
8/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25
4/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25
2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25
0/0/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25

Meropenem

Plate 5 – Colistin 0.5 µg/ml

Sulbactam							
128/0.5	128/4/0.5	128/8/0.5	128/16/0.5	128/32/0.5	128/64/0.5	128/128/0.5	128/256/0.5
64/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5	64/256/0.5
32/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5
16/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5
8/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5
4/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5
2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5
0/0/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5

Meropenem

Plate 6 – Colistin 1 µg/ml

Sulbactam							
128/1	128/4/1	128/8/1	128/16/1	128/32/1	128/64/1	128/128/1	128/256/1
64/1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1	64/256/1
32/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1
16/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1
8/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1
4/1	14/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1
2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1
0/0/1	4/1	8/1	16/1	32/1	64/1	128/1	256/1

Meropenem

Figure C-3 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 2 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 2 (Continued) **Plate 7** – Colistin 2 μg/ml

Sulbactam							
128/2	128/4/2	128/8/2	128/16/2	128/32/2	128/64/2	128/128/2	128/256/2
64/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2	64/256/2
32/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2
16/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2
8/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2
4/2	4/4//2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2
2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2
0/0/2	4/2	8/2	16/2	32/2	64/2	128/2	256/2

Meropenem

Plate 8 – Colistin 4 μg/ml

Sulbactam							
128/4	128/4/4	128/8/4	128/16/4	128/32/4	128/64/4	128/128/4	128/256/4
64/4	64/4/4	64/8/4	64/16/4	64/32/4	64/64/4	64/128//4	64/256/4
32/4	32/4/4	32/8/4	32/16/4	32/32/4	32/64/4	32/128/4	32/256/4
16/4	16/4/4	16/8/4	16/16/4	16/32/4	16/64/4	16/128/4	16/256/4
8/4	8/4/4	8/8/4	8/16/4	8/32/4	8/64/4	8/128/4	8/256/4
4//4	4/4/4	4/8/4	4/16/4	4/32/4	4/64/4	4/128/4	4/256/4
2/4	2/4/4	2/8/4	2/16/4	2/32/4	2/64/4	2/128/4	2/256/4
0/0/4	4/4	8/4	16/4	32/4	64/4	128/4	256/4

Meropenem

Figure C-3 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 2 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 3 **Plate 1** – No colistin (0 µg/ml)

Sulbactam								
128	128/16	128/32	128/64	128/128	128/256	128/512	128/1,024
64	64/16	64/32	64/64	64/128	64/256	64/512	64/1,024
32	32/16	32/32	32/64	32/128	32/256	32/512	32/1,024
16	16/16	16/32	16/64	16/128	16/256	16/512	16/1,024
8	8/16	8/32	8/64	8/128	8/256	8/512	8/1,024
4	4/16	4/32	4/64	4/128	4/256	4/512	4/1,024
2	2/16	2/32	2/64	2/128	2/256	2/512	2/1,024
0	16	32	64	128	256	512	1,024

Meropenem

Plate 2 – Colistin 0.03µg/ml

Sulbactam							
128/0.03	128/16/0.03	128/32/0.03	128/64/0.03	128/128/0.03	128/256/0.03	128/512/0.03	128/1,024/0.03
64/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03	64/256/0.03	64/512/0.03	64/1,024/0.03
32/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03	32/256/0.03	32/512/0.03	32/1,024/0.03
16/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03	16/256/0.03	16/512/0.03	16/1,024/0.03
8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03	8/256/0.03	8/512/0.03	8/1,024/0.03
4/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03	4/256/0.03	4/512/0.03	4/1,024/0.03
2/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03	2/256/0.03	2/512/0.03	2/1,024/0.03
0/0/0.03	16/0.03	32/0.03	64/0.03	128/0.03	256/0.03	512/0.03	1,024/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
128/0.06	128/16/0.06	128/32/0.06	128/64/0.06	128/128/0.06	128/256/0.06	128/512/0.06	128/1,024/0.06
64/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06	64/256/0.06	64/512/0.06	64/1,024/0.06
32/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06	32/512/0.06	32/1,024/0.06
16/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06	16/512/0.06	16/1,024/0.06
8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06	8/512/0.06	8/1,024/0.06
4/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06	4/512/0.06	4/1,024/0.06
2/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06	2/512/0.06	2/1,024/0.06
0/0/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06	512/0.06	1,024/0.06

Meropenem

Figure C-4 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 3 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 3 (Continued) **Plate 4** – Colistin 0.12 µg/ml

128/0.12	128/16/0.12	128/32/0.12	128/64/0.12	128/128/0.12	128/256/0.12	128/512/0.12	128/1,024/0.12
64/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12	64/256/0.12	64/512/0.12	64/1,024/0.12
32/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12	32/512/0.12	32/1,024/0.12
16/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12	16/512/0.12	16/1,024/0.12
8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12	8/512/0.12	8/1,024/0.12
4/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12	4/512/0.12	4/1,024/0.12
2/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12	2/512/0.12	2/1,024/0.12
0/0/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12	512/0.12	1,024/0.12

Sulbactam

Meropenem

Plate 5 – Colistin 0.25 µg/ml

128/0.25	128/16/0.25	128/32/0.25	128/64/0.25	128/128/0.25	128/256/0.25	128/512/0.25	128/1,024/0.25
64/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25	64/256/0.25	64/512/0.25	64/1,024/0.25
32/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25	32/512/0.25	32/1,024/0.25
16/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25	16/512/0.25	16/1,024/0.25
8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25	8/512/0.25	8/1,024/0.25
4/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25	4/512/0.25	4/1,024/0.25
2/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25	2/512/0.25	2/1,024/0.25
0/0/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25	512/0.25	1,024/0.25

Sulbactam

Meropenem

Plate 6 – Colistin 0.5 µg/ml

128/0.5	128/16/0.5	128/32/0.5	128/64/0.5	128/128/0.5	128/256/0.5	128/512/0.5	128/1,024/0.5
64/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5	64/256/0.5	64/512/0.5	64/1,024/0.5
32/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5	32/512/0.5	32/1,024/0.5
16/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5	16/512/0.5	16/1,024/0.5
8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5	8/512/0.5	8/1,024/0.5
4/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5	4/512/0.5	4/1,024/0.5
2/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5	2/512/0.5	2/1,024/0.5
0/0/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5	512/0.5	1,024/0.5

Sulbactam

Meropenem

Figure C-4 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 3 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 3 (Continued) **Plate 7** – Colistin 1 μg/ml

Sulbactam								
128/1	128/16/1	128/32/1	128/64/1	128/128/1	128/256/1	128/512/1	128/1,024/1
64/1	64/16/1	64/32/1	64/64/1	64/128/1	64/256/1	64/512/1	64/1,024/1
32/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1	32/512/1	32/1,024/1
16/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1	16/512/1	16/1,024/1
8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1	8/512/1	8/1,024/1
4/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1	4/512/1	4/1,024/1
2/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1	2/512/1	2/1,024/1
0/0/1	16/1	32/1	64/1	128/1	256/1	512/1	1,024/1

Meropenem

Plate 8 – Colistin 2 μg/ml

Sulbactam								
128/2	128/16/2	128/32/2	128/64/2	128/128/2	128/256/2	128/512/2	128/1,024/2
64/2	64/16/2	64/32/2	64/64/2	64/128/2	64/256/2	64/512/2	64/1,024/2
32/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2	32/512/2	32/1,024/2
16/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2	16/512/2	16/1,024/2
8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2	8/512/2	8/1,024/2
4/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2	4/512/2	4/1,024/2
2/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2	2/512/2	2/1,024/2
0/0/2	16/2	32/2	64/2	128/2	256/2	512/2	1,024/2

Meropenem

Figure C-4 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 3 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 4 **Plate 1** – No Colistin (0 µg/ml)

Sulbactam								
128	128/4	128/8	128/16	128/32	128/64	128/128	128/256
64	64/4	64/8	64/16	64/32	64/64	64/128	64/256
32	32/4	32/8	32/16	32/32	32/64	32/128	32/256
16	16/4	16/8	16/16	16/32	16/64	16/128	16/256
8	8/4	8/8	8/16	8/32	8/64	8/128	8/256
4	4/4	4/8	4/16	4/32	4/64	4/128	4/256
2	2/4	2/8	2/16	2/32	2/64	2/128	2/256
0	4	8	16	32	64	128	256

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam								
128/0.03	128/2/0.03	128/4/0.03	128/8/0.03	128/16/0.03	128/32/0.03	128/64/0.03	128/128/0.03
64/0.03	64/2/0.03	64/4/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03
32/0.03	32/2/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03
16/0.03	16/2/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03
8/0.03	8/2/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03
4/0.03	4/2/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03
2/0.03	2/2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03
0/0/0.03	2/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam								
128/0.06	128/2/0.06	128/4/0.06	128/8/0.06	128/16/0.06	128/32/0.06	128/64/0.06	128/128/0.06
64/0.06	64/2/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06
32/0.06	32/2/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06
16/0.06	16/2/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06
8/0.06	8/2/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06
4/0.06	4/2/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06
2/0.06	2/2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06
0/0/0.06	2/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06

Meropenem

Figure C-5 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 4 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 4 (Continued) **Plate 4** – Colistin 0.12 µg/ml

Sulbactam								
128/0.12	128/2/0.12	128/4/0.12	128/8/0.12	128/16/0.12	128/32/0.12	128/64/0.12	128/128/0.12
64/0.12	64/2/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12
32/0.12	32/2/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12
16/0.12	16/2/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12
8/0.12	8/2/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12
4/0.12	4/2/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12
2/0.12	2/2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12
0/0/0.12	2/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12

Meropenem

Plate 5 – Colistin 0.25 µg/ml

Sulbactam								
128/0.25	128/2/0.25	128/4/0.25	128/8/0.25	128/16/0.25	128/32/0.25	128/64/0.25	128/128/0.25
64/0.25	64/2/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25
32/0.25	32/2/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25
16/0.25	16/2/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25
8/0.25	8/2/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25
4/0.25	4/2/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25
2/0.25	2/2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25
0/0/0.25	2/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25

Meropenem

Plate 6 – Colistin 0.5 µg/ml

Sulbactam								
128/0.5	128/2/0.5	128/4/0.5	128/8/0.5	128/16/0.5	128/32/0.5	128/64/0.5	128/128/0.5
64/0.5	64/2/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5
32/0.5	32/2/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5
16/0.5	16/2/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5
8/0.5	8/2/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5
4/0.5	4/2/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5
2/0.5	2/2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5
0/0/0.5	2/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5

Meropenem

Figure C-5 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 4 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 4 (Continued) **Plate 7** – Colistin 1 µg/ml

Sulbactam								
128/1	128/2/1	128/4/1	128/8/1	128/16/1	128/32/1	128/64/1	128/128/1
64/1	64/2/1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1
32/1	32/2/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1
16/1	16/2/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1
8/1	8/2/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1
4/1	4/2/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1
2/1	2/2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1
0/0/1	2/1	4/1	8/1	16/1	32/1	64/1	128/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam								
128/2	128/2/2	128/4/2	128/8/2	128/16/2	128/32/2	128/64/2	128/128/2
64/2	64/2/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2
32/2	32/2/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2
16/2	16/2/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2
8/2	8/2/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2
4/2	4/2/2	4/4//2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2
2/2	2/2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2
0/0/2	2/2	4/2	8/2	16/2	32/2	64/2	128/2

Meropenem

Figure C-5 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 4 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 5 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
128	128/8	128/16	128/32	128/64	128/128	128/256	128/512
64	64/8	64/16	64/32	64/64	64/128	64/256	64/512
32	32/8	32/16	32/32	32/64	32/128	32/256	32/512
16	16/8	16/16	16/32	16/64	16/128	16/256	16/512
8	8/8	8/16	8/32	8/64	8/128	8/256	8/512
4	4/8	4/16	4/32	4/64	4/128	4/256	4/512
2	2/8	2/16	2/32	2/64	2/128	2/256	2/512
0	8	16	32	64	128	256	512

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
128/0.03	128/4/0.03	128/8/0.03	128/16/0.03	128/32/0.03	128/64/0.03	128/128/0.03	128/256/0.03
64/0.03	64/4/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03	64/256/0.03
32/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03	32/256/0.03
16/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03	16/256/0.03
8/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03	8/256/0.03
4/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03	4/256/0.03
2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03	2/256/0.03
0/0/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03	256/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
128/0.06	128/4/0.06	128/8/0.06	128/16/0.06	128/32/0.06	128/64/0.06	128/128/0.06	128/256/0.06
64/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06	64/256/0.06
32/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06
16/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06
8/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06
4/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06
2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06
0/0/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06

Meropenem

Figure C-6 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 5 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 5 (Continued) **Plate 4** – Colistin 0.12 µg/ml

Sulbactam							
128/0.12	128/4/0.12	128/8/0.12	128/16/0.12	128/32/0.12	128/64/0.12	128/128/0.12	128/256/0.12
64/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12	64/256/0.12
32/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12
16/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12
8/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12
4/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12
2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12
0/0/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12

Meropenem

Plate 5 – Colistin 0.25 µg/ml

Sulbactam							
128/0.25	128/4/0.25	128/8/0.25	128/16/0.25	128/32/0.25	128/64/0.25	128/128/0.25	128/256/0.25
64/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25	64/256/0.25
32/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25
16/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25
8/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25
4/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25
2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25
0/0/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25

Meropenem

Plate 6– Colistin 0.5 µg/ml

Sulbactam							
128/0.5	128/4/0.5	128/8/0.5	128/16/0.5	128/32/0.5	128/64/0.5	128/128/0.5	128/256/0.5
64/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5	64/256/0.5
32/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5
16/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5
8/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5
4/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5
2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5
0/0/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5

Meropenem

Figure C-6 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 5 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 5 (Continued) **Plate 7**– Colistin 1 µg/ml

Sulbactam							
128/1	128/4/1	128/8/1	128/16/1	128/32/1	128/64/1	128/128/1	128/256/1
64/1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1	64/256/1
32/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1
16/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1
8/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1
4/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1
2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1
0/0/1	4/1	8/1	16/1	32/1	64/1	128/1	256/1

Meropenem

Plate 8– Colistin 2 µg/ml

Sulbactam							
128/2	128/4/2	128/8/2	128/16/2	128/32/2	128/64/2	128/128/2	128/256/2
64/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2	64/256/2
32/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2
16/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2
8/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2
4/2	4/4//2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2
2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2
0/0/2	4/2	8/2	16/2	32/2	64/2	128/2	256/2

Meropenem

Figure C-6 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 5 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 6 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
128	128/4	128/8	128/16	128/32	128/64	128/128	128/256
64	64/4	64/8	64/16	64/32	64/64	64/128	64/256
32	32/4	32/8	32/16	32/32	32/64	32/128	32/256
16	16/4	16/8	16/16	16/32	16/64	16/128	16/256
8	8/4	8/8	8/16	8/32	8/64	8/128	8/256
4	4/4	4/8	4/16	4/32	4/64	4/128	4/256
2	2/4	2/8	2/16	2/32	2/64	2/128	2/256
0	4	8	16	32	64	128	256

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
128/0.03	128/4/0.03	128/8/0.03	128/16/0.03	128/32/0.03	128/64/0.03	128/128/0.03	128/256/0.03
64/0.03	64/4/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03	64/256/0.03
32/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03	32/256/0.03
16/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03	16/256/0.03
8/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03	8/256/0.03
4/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03	4/256/0.03
2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03	2/256/0.03
0/0/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03	256/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
128/0.06	128/4/0.06	128/8/0.06	128/16/0.06	128/32/0.06	128/64/0.06	128/128/0.06	128/256/0.06
64/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06	64/256/0.06
32/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06
16/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06
8/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06
4/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06
2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06
0/0/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06

Meropenem

Figure C-7 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 6 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 6 (Continued) **Plate 4** – Colistin 0.12 µg/ml

128/0.12	128/4/0.12	128/8/0.12	128/16/0.12	128/32/0.12	128/64/0.12	128/128/0.12	128/256/0.12
64/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12	64/256/0.12
32/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12
16/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12
8/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12
4/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12
2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12
0/0/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12

Sulbactam

Meropenem

Plate 5 – Colistin 0.25 µg/ml

128/0.25	128/4/0.25	128/8/0.25	128/16/0.25	128/32/0.25	128/64/0.25	128/128/0.25	128/256/0.25
64/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25	64/256/0.25
32/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25
16/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25
8/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25
4/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25
2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25
0/0/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25

Sulbactam

Meropenem

Plate 6 – Colistin 0.5 µg/ml

128/0.5	128/4/0.5	128/8/0.5	128/16/0.5	128/32/0.5	128/64/0.5	128/128/0.5	128/256/0.5
64/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5	64/256/0.5
32/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5
16/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5
8/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5
4/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5
2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5
0/0/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5

Sulbactam

Meropenem

Figure C-7 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 6 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 6 (Continued) **Plate 7** – Colistin 1 µg/ml

128/1	128/4/1	128/8/1	128/16/1	128/32/1	128/64/1	128/128/1	128/256/1
64/1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1	64/256/1
32/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1
16/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1
8/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1
4/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1
2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1
0/0/1	4/1	8/1	16/1	32/1	64/1	128/1	256/1

Sulbactam

Meropenem

Plate 8– Colistin 2 µg/ml

128/2	128/4/2	128/8/2	128/16/2	128/32/2	128/64/2	128/128/2	128/256/2
64/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2	64/256/2
32/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2
16/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2
8/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2
4/2	4/4//2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2
2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2
0/0/2	4/2	8/2	16/2	32/2	64/2	128/2	256/2

Sulbactam

Meropenem

Figure C-7 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 6 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 7 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
128	128/8	128/16	128/32	128/64	128/128	128/256	128/512
64	64/8	64/16	64/32	64/64	64/128	64/256	64/512
32	32/8	32/16	32/32	32/64	32/128	32/256	32/512
16	16/8	16/16	16/32	16/64	16/128	16/256	16/512
8	8/8	8/16	8/32	8/64	8/128	8/256	8/512
4	4/8	4/16	4/32	4/64	4/128	4/256	4/512
2	2/8	2/16	2/32	2/64	2/128	2/256	2/512
0	8	16	32	64	128	256	512

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
128/0.03	128/8/0.03	128/16/0.03	128/32/0.03	128/64/0.03	128/128/0.03	128/256/0.03	128/512/0.03
64/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03	64/256/0.03	64/512/0.03
32/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03	32/256/0.03	32/512/0.03
16/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03	16/256/0.03	16/512/0.03
8/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03	8/256/0.03	8/512/0.03
4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03	4/256/0.03	4/512/0.03
2/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03	2/256/0.03	2/512/0.03
0/0/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03	256/0.03	512/0.03

Meropenem

Plate 3– Colistin 0.06 µg/ml

Sulbactam							
128/0.06	128/8/0.06	128/16/0.06	128/32/0.06	128/64/0.06	128/128/0.06	128/256/0.06	128/512/0.06
64/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06	64/256/0.06	64/512/0.06
32/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06	32/512/0.06
16/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06	16/512/0.06
8/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06	8/512/0.06
4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06	4/512/0.06
2/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06	2/512/0.06
0/0/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06	512/0.06

Meropenem

Figure C-8 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 7 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 7 (Continued) **Plate 4** – Colistin 0.12 µg/ml

128/0.12	128/8/0.12	128/16/0.12	128/32/0.12	128/64/0.12	128/128/0.12	128/256/0.12	128/512/0.12
64/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12	64/256/0.12	64/512/0.12
32/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12	32/512/0.12
16/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12	16/512/0.12
8/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12	8/512/0.12
4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12	4/512/0.12
2/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12	2/512/0.12
0/0/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12	512/0.12

Sulbactam

Meropenem

Plate 5 – Colistin 0.25 µg/ml

128/0.25	128/8/0.25	128/16/0.25	128/32/0.25	128/64/0.25	128/128/0.25	128/256/0.25	128/512/0.25
64/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25	64/256/0.25	64/512/0.25
32/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25	32/512/0.25
16/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25	16/512/0.25
8/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25	8/512/0.25
4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25	4/512/0.25
2/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25	2/512/0.25
0/0/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25	512/0.25

Sulbactam

Meropenem

Plate 6 – Colistin 0.5 µg/ml

128/0.5	128/8/0.5	128/16/0.5	128/32/0.5	128/64/0.5	128/128/0.5	128/256/0.5	128/512/0.5
64/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5	64/256/0.5	64/512/0.5
32/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5	32/512/0.5
16/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5	16/512/0.5
8/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5	8/512/0.5
4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5	4/512/0.5
2/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5	2/512/0.5
0/0/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5	512/0.5

Sulbactam

Meropenem

Figure C-8 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 7 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 7 (Continued) **Plate 7**– Colistin 1 µg/ml

Sulbactam								
128/1	128/8/1	128/16/1	128/32/1	128/64/1	128/128/1	128/256/1	128/512/1
64/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1	64/256/1	64/512/1
32/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1	32/512/1
16/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1	16/512/1
8/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1	8/512/1
4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1	4/512/1
2/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1	2/512/1
0/0/1	8/1	16/1	32/1	64/1	128/1	256/1	512/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam								
128/2	128/8/2	128/16/2	128/32/2	128/64/2	128/128/2	128/256/2	128/512/2
64/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2	64/256/2	64/512/2
32/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2	32/512/2
16/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2	16/512/2
8/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2	8/512/2
4/2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2	4/512/2
2/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2	2/512/2
0/0/2	8/2	16/2	32/2	64/2	128/2	256/2	512/2

Meropenem

Figure C-8 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 7 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 8 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
32	32/4	32/8	32/16	32/32	32/64	32/128	32 /256
16	16/4	16/8	16/16	16/32	16/64	16/128	16/256
8	8/4	8/8	8/16	8/32	8/64	8/128	8/256
4	4/4	4/8	4/16	4/32	4/64	4/128	4/256
2	2/4	2/8	2/16	2/32	2/64	2/128	2/256
1	1/4	1/8	1/16	1/32	1/64	1/128	1/256
0.5	0.5/4	0.5/8	0.5/16	0.5/32	0.5/64	0.5/128	0.5/256
0	4	8	16	32	64	128	256

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
32/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03	32 /256/0.03
16/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03	16/256/0.03
8/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03	8/256/0.03
4/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03	4/256/0.03
2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03	2/256/0.03
1/0.03	1/4/0.03	1/8/0.03	1/16/0.03	1/32/0.03	1/64/0.03	1/128/0.03	1/256/0.03
0.5/0.03	0.5/4/0.03	0.5/8/0.03	0.5/16/0.03	0.5/32/0.03	0.5/64/0.03	0.5/128/0.03	0.5/256/0.03
0/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03	256/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
32/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32 /256/0.06
16/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06
8/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06
4/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06
2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06
1/0.06	1/4/0.06	1/8/0.06	1/16/0.06	1/32/0.06	1/64/0.06	1/128/0.06	1/256/0.06
0.5/0.06	0.5/4/0.06	0.5/8/0.06	0.5/16/0.06	0.5/32/0.06	0.5/64/0.06	0.5/128/0.06	0.5/256/0.06
0/0/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06

Meropenem

Figure C-9 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 8 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 8 (Continued) **Plate 4** – Colistin 0.12 µg/ml

Sulbactam								
32/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32 /256/0.12
16/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12
8/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12
4/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12
2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12
1/0.12	1/4/0.12	1/8/0.12	1/16/0.12	1/32/0.12	1/64/0.12	1/128/0.12	1/256/0.12
0.5/0.12	0.5/4/0.12	0.5/8/0.12	0.5/16/0.12	0.5/32/0.12	0.5/64/0.12	0.5/128/0.12	0.5/256/0.12
0/0/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12

Meropenem

Plate 5 – Colistin 0.25 µg/ml

Sulbactam								
32/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32 /256/0.25
16/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25
8/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25
4/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25
2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25
1/0.25	1/4/0.25	1/8/0.25	1/16/0.25	1/32/0.25	1/64/0.25	1/128/0.25	1/256/0.25
0.5/0.25	0.5/4/0.25	0.5/8/0.25	0.5/16/0.25	0.5/32/0.25	0.5/64/0.25	0.5/128/0.25	0.5/256/0.25
0/0/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25

Meropenem

Plate 6 – Colistin 0.5 µg/ml

Sulbactam								
32/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32 /256/0.5
16/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5
8/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5
4/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5
2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5
1/0.5	1/4/0.5	1/8/0.5	1/16/0.5	1/32/0.5	1/64/0.5	1/128/0.5	1/256/0.5
0.5/0.5	0.5/4/0.5	0.5/8/0.5	0.5/16/0.5	0.5/32/0.5	0.5/64/0.5	0.5/128/0.5	0.5/256/0.5
0/0/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5

Meropenem

Figure C-9 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 8 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 8 (Continued) **Plate 7** – Colistin 1 µg/ml

Sulbactam							
32/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32 /256/1
16/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1
8/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1
4/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1
2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1
1/1	1/4/1	1/8/1	1/16/1	1/32/1	1/64/1	1/128/1	1/256/1
0.5/1	0.5/4/1	0.5/8/1	0.5/16/1	0.5/32/1	0.5/64/1	0.5/128/1	0.5/256/1
0/0/1	4/1	8/1	16/1	32/1	64/1	128/1	256/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam							
32/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32 /256/2
16/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2
8/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2
4/2	4/4/2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2
2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2
1/2	1/4/2	1/8/2	1/16/2	1/32/2	1/64/2	1/128/2	1/256/2
0.5/2	0.5/4/2	0.5/8/2	0.5/16/2	0.5/32/2	0.5/64/2	0.5/128/2	0.5/256/2
0/0/2	4/2	8/2	16/2	32/2	64/2	128/2	256/2

Meropenem

Figure C-9 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 8 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 9 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
128	128/4	128/8	128/16	128/32	128/64	128/128	128/256
64	64/4	64/8	64/16	64/32	64/64	64/128	64/256
32	32/4	32/8	32/16	32/32	32/64	32/128	32/256
16	16/4	16/8	16/16	16/32	16/64	16/128	16/256
8	8/4	8/8	8/16	8/32	8/64	8/128	8/256
4	4/4	4/8	4/16	4/32	4/64	4/128	4/256
2	2/4	2/8	2/16	2/32	2/64	2/128	2/256
0	4	8	16	32	64	128	256

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
128/0.03	128/4/0.03	128/8/0.03	128/16/0.03	128/32/0.03	128/64/0.03	128/128/0.03	128/256/0.03
64/0.03	64/4/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03	64/256/0.03
32/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03	32/256/0.03
16/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03	16/256/0.03
8/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03	8/256/0.03
4/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03	4/256/0.03
2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03	2/256/0.03
0/0/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03	256/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
128/0.06	128/4/0.06	128/8/0.06	128/16/0.06	128/32/0.06	128/64/0.06	128/128/0.06	128/256/0.06
64/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06	64/256/0.06
32/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06
16/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06
8/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06
4/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06
2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06
0/0/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06

Meropenem

Figure C-10 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 9 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 9 (Continued) **Plate 4** – Colistin 0.12 µg/ml

Sulbactam							
128/0.12	128/4/0.12	128/8/0.12	128/16/0.12	128/32/0.12	128/64/0.12	128/128/0.12	128/256/0.12
64/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12	64/256/0.12
32/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12
16/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12
8/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12
4/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12
2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12
0/0/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12

Meropenem

Plate 5– Colistin 0.25 µg/ml

Sulbactam							
128/0.25	128/4/0.25	128/8/0.25	128/16/0.25	128/32/0.25	128/64/0.25	128/128/0.25	128/256/0.25
64/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25	64/256/0.25
32/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25
16/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25
8/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25
4/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25
2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25
0/0/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25

Meropenem

Plate 6– Colistin 0.5 µg/ml

Sulbactam							
128/0.5	128/4/0.5	128/8/0.5	128/16/0.5	128/32/0.5	128/64/0.5	128/128/0.5	128/256/0.5
64/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5	64/256/0.5
32/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5
16/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5
8/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5
4/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5
2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5
0/0/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5

Meropenem

Figure C-10 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 9 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 9 (Continued) **Plate 7** – Colistin 1 µg/ml

Sulbactam							
128/1	128/4/1	128/8/1	128/16/1	128/32/1	128/64/1	128/128/1	128/256/1
64/1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1	64/256/1
32/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1
16/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1
8/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1
4/1	14/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1
2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1
0/0/1	4/1	8/1	16/1	32/1	64/1	128/1	256/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam							
128/2	128/4/2	128/8/2	128/16/2	128/32/2	128/64/2	128/128/2	128/256/2
64/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2	64/256/2
32/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2
16/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2
8/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2
4/2	4/4//2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2
2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2
0/0/2	4/2	8/2	16/2	32/2	64/2	128/2	256/2

Meropenem

Figure C-10 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 9 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 10 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
128	128/4	128/8	128/16	128/32	128/64	128/128	128/256
64	64/4	64/8	64/16	64/32	64/64	64/128	64/256
32	32/4	32/8	32/16	32/32	32/64	32/128	32/256
16	16/4	16/8	16/16	16/32	16/64	16/128	16/256
8	8/4	8/8	8/16	8/32	8/64	8/128	8/256
4	4/4	4/8	4/16	4/32	4/64	4/128	4/256
2	2/4	2/8	2/16	2/32	2/64	2/128	2/256
0	4	8	16	32	64	128	256

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
128/0.03	128/4/0.03	128/8/0.03	128/16/0.03	128/32/0.03	128/64/0.03	128/128/0.03	128/256/0.03
64/0.03	64/4/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03	64/256/0.03
32/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03	32/256/0.03
16/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03	16/256/0.03
8/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03	8/256/0.03
4/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03	4/256/0.03
2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03	2/256/0.03
0/0/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03	256/0.03

Meropenem

Plate 3– Colistin 0.06 µg/ml

Sulbactam							
128/0.06	128/4/0.06	128/8/0.06	128/16/0.06	128/32/0.06	128/64/0.06	128/128/0.06	128/256/0.06
64/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06	64/256/0.06
32/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06
16/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06
8/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06
4/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06
2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06
0/0/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06

Meropenem

Figure C-11 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 10 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 10 (Continued) **Plate 4** – Colistin 0.12 µg/ml

Sulbactam								
128/0.12	128/4/0.12	128/8/0.12	128/16/0.12	128/32/0.12	128/64/0.12	128/128/0.12	128/256/0.12
64/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12	64/256/0.12
32/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12
16/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12
8/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12
4/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12
2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12
0/0/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12

Meropenem

Plate 5 – Colistin 0.25µg/ml

Sulbactam							
128/0.25	128/4/0.25	128/8/0.25	128/16/0.25	128/32/0.25	128/64/0.25	128/128/0.25	128/256/0.25
64/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25	64/256/0.25
32/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25
16/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25
8/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25
4/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25
2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25
0/0/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25

Meropenem

Plate 6 – Colistin 0.5 µg/ml

Sulbactam							
128/0.5	128/4/0.5	128/8/0.5	128/16/0.5	128/32/0.5	128/64/0.5	128/128/0.5	128/256/0.5
64/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5	64/256/0.5
32/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5
16/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5
8/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5
4/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5
2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5
0/0/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5

Meropenem

Figure C-11 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 10 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 10 (Continued) **Plate 7** – Colistin 1 µg/ml

Sulbactam							
128/1	128/4/1	128/8/1	128/16/1	128/32/1	128/64/1	128/128/1	128/256/1
64/1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1	64/256/1
32/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1
16/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1
8/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1
4/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1
2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1
0/0/1	4/1	8/1	16/1	32/1	64/1	128/1	256/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam							
128/2	128/4/2	128/8/2	128/16/2	128/32/2	128/64/2	128/128/2	128/256/2
64/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2	64/256/2
32/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2
16/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2
8/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2
4/2	4/4//2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2
2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2
0/0/2	4/2	8/2	16/2	32/2	64/2	128/2	256/2

Meropenem

Figure C-11 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 10 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 11 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
64	64/2	64/4	64/8	64/16	64/32	64/64	64/128
32	32/2	32/4	32/8	32/16	32/32	32/64	32/128
16	16/2	16/4	16/8	16/16	16/32	16/64	16/128
8	8/2	8/4	8/8	8/16	8/32	8/64	8/128
4	4/2	4/4	4/8	4/16	4/32	4/64	4/128
2	2/2	2/4	2/8	2/16	2/32	2/64	2/128
1	1/2	1/4	1/8	1/16	1/32	1/64	1/128
0	2	4	8	16	32	64	128

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
64 /0.03	64/2/0.03	64/4/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03
32/0.03	32/2/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03
16/0.03	16/2/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03
8/0.03	8/2/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03
4/0.03	4/2/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03
2/0.03	2/2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03
1/0.03	1/2/0.03	1/4/0.03	1/8/0.03	1/16/0.03	1/32/0.03	1/64/0.03	1/128/0.03
0/0/0.03	2/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
64/0.06	64/2/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06
32/0.06	32/2/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06
16/0.06	16/2/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06
8/0.06	8/2/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06
4/0.06	4/2/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06
2/0.06	2/2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06
1/0.06	1/2/0.06	1/4/0.06	1/8/0.06	1/16/0.06	1/32/0.06	1/64/0.06	1/128/0.06
0/0/0.06	2/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06

Meropenem

Figure C-12 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 11 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 11 (Continued) **Plate 4** – Colistin 0.12μg/ml

Sulbactam								
	64/0.12	64/2/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12
	32/0.12	32/2/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12
	16/0.12	16/2/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12
	8/0.12	8/2/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12
	4/0.12	4/2/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12
	2/0.12	2/2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12
	1/0.12	1/2/0.12	1/4/0.12	1/8/0.12	1/16/0.12	1/32/0.12	1/64/0.12	1/128/0.12
	0/0/0.12	2/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12

Meropenem

Plate 5 – Colistin 0.25 μg/ml

Sulbactam								
	64/0.25	64/2/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25
	32/0.25	32/2/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25
	16/0.25	16/2/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25
	8/0.25	8/2/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25
	4/0.25	4/2/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25
	2/0.25	2/2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25
	1/0.25	1/2/0,25	1/4/0.25	1/8/0.25	1/16/0.25	1/32/0.25	1/64/0.25	1/128/0.25
	0/0/0.25	2/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25

Meropenem

Plate 6 – Colistin 0.5 μg/ml

Sulbactam								
	64/0.5	64/2/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5
	32/0.5	32/2/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5
	16/0.5	16/2/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5
	8/0.5	8/2/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5
	4/0.5	4/2/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5
	2/0.5	2/2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5
	1/0.5	1/0.5/2	1/4/0.5	1/8/0.5	1/16/0.5	1/32/0.5	1/64/0.5	1/128/0.5
	0/0/0.5	2/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5

Meropenem

Figure C-12 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 11 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 11 (Continued) **Plate 7** – Colistin 1 μg/ml

Sulbactam							
64 /1	64/2/1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1
32/1	32/2/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1
16/1	16/2/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1
8/1	8/2/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1
4/1	4/2/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1
2/1	2/2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1
1/1	1/2/1	1/4/1	1/8/1	1/16/1	1/32/1	1/64/1	1/128/1
0/0/1	2/1	4/1	8/1	16/1	32/1	64/1	128/1

Meropenem

Plate 8 – Colistin 2 μg/ml

Sulbactam							
64/2	64/2/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2
32/2	32/2/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2
16/2	16/2/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2
8/2	8/2/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2
4/2	4/2/2	4/4/2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2
2/2	2/2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2
1/2	1/2/2	1/4/2	1/8/2	1/16/2	1/32/2	1/64/2	1/128/2
0/0/2	2/2	4/2	8/2	16/2	32/2	64/2	128/2

Meropenem

Figure C-12 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 11 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 12 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
128	128/4	128/8	128/16	128/32	128/64	128/128	128/256
64	64/4	64/8	64/16	64/32	64/64	64/128	64/256
32	32/4	32/8	32/16	32/32	32/64	32/128	32/256
16	16/4	16/8	16/16	16/32	16/64	16/128	16/256
8	8/4	8/8	8/16	8/32	8/64	8/128	8/256
4	4/4	4/8	4/16	4/32	4/64	4/128	4/256
2	2/4	2/8	2/16	2/32	2/64	2/128	2/256
0	4	8	16	32	64	128	256

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam								
128/0.03	128/4/0.03	128/8/0.03	128/16/0.03	128/32/0.03	128/64/0.03	128/128/0.03	128/256/0.03
64/0.03	64/4/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03	64/256/0.03
32/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03	32/256/0.03
16/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03	16/256/0.03
8/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03	8/256/0.03
4/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03	4/256/0.03
2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03	2/256/0.03
0/0/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03	256/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam								
128/0.06	128/4/0.06	128/8/0.06	128/16/0.06	128/32/0.06	128/64/0.06	128/128/0.06	128/256/0.06
64/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06	64/256/0.06
32/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06
16/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06
8/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06
4/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06
2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06
0/0/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06

Meropenem

Figure C-13 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 12 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 12 (Continued) **Plate 4** – Colistin 0.12 µg/ml

128/0.12	128/4/0.12	128/8/0.12	128/16/0.12	128/32/0.12	128/64/0.12	128/128/0.12	128/256/0.12
64/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12	64/256/0.12
32/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12
16/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12
8/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12
4/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12
2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12
0/0/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12

Sulbactam

Meropenem

Plate 5 – Colistin 0.25 µg/ml

128/0.25	128/4/0.25	128/8/0.25	128/16/0.25	128/32/0.25	128/64/0.25	128/128/0.25	128/256/0.25
64/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25	64/256/0.25
32/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25
16/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25
8/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25
4/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25
2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25
0/0/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25

Sulbactam

Meropenem

Plate 6 – Colistin 0.5 µg/ml

128/0.5	128/4/0.5	128/8/0.5	128/16/0.5	128/32/0.5	128/64/0.5	128/128/0.5	128/256/0.5
64/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5	64/256/0.5
32/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5
16/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5
8/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5
4/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5
2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5
0/0/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5

Sulbactam

Meropenem

Figure C-13 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 12 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 7 – Colistin 1 µg/ml

Sulbactam							
128/1	128/4/1	128/8/1	128/16/1	128/32/1	128/64/1	128/128/1	128/256/1
64/1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1	64/256/1
32/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1
16/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1
8/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1
4/1	14/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1
2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1
0/0/1	4/1	8/1	16/1	32/1	64/1	128/1	256/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam							
128/2	128/4/2	128/8/2	128/16/2	128/32/2	128/64/2	128/128/2	128/256/2
64/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2	64/256/2
32/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2
16/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2
8/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2
4/2	4/4//2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2
2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2
0/0/2	4/2	8/2	16/2	32/2	64/2	128/2	256/2

Meropenem

Figure C-13 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 12 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolates No. 13 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
256	256/4	256/8	256/16	256/32	256/64	256/128	256/256
128	128/4	128/8	128/16	128/32	128/64	128/128	128/256
64	64/4	64/8	64/16	64/32	64/64	64/128	64/256
32	32/4	32/8	32/16	32/32	32/64	32/128	32/256
16	16/4	16/8	16/16	16/32	16/64	16/128	16/256
8	8/4	8/8	8/16	8/32	8/64	8/128	8/256
4	4/4	4/8	4/16	4/32	4/64	4/128	4/256
0	4	8	16	32	64	128	256

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
256/0.03	256/4/0.03	256/8/0.03	256/16/0.03	256/32/0.03	256/64/0.03	256/128/0.03	256/256/0.03
128/0.03	128/4/0.03	128/8/0.03	128/16/0.03	128/32/0.03	128/64/0.03	128/128/0.03	128/256/0.03
64/0.03	64/4/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03	64/256/0.03
32/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03	32/256/0.03
16/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03	16/256/0.03
8/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03	8/256/0.03
4/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03	4/256/0.03
0/0/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03	256/0.03

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
256/0.06	256/4/0.06	256/8/0.06	256/16/0.06	256/32/0.06	256/64/0.06	256/128/0.06	256/256/0.06
128/0.06	128/4/0.06	128/8/0.06	128/16/0.06	128/32/0.06	128/64/0.06	128/128/0.06	128/256/0.06
64/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06	64/256/0.06
32/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06
16/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06
8/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06
4/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06
0/0/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06

Meropenem

Figure C-14 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 13 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 4 – Colistin 0.12 µg/ml

Sulbactam							
256/0.12	256/4/0.12	256/8/0.12	256/16/0.12	256/32/0.12	256/64/0.12	256/128/0.12	256/256/0.12
128/0.12	128/4/0.12	128/8/0.12	128/16/0.12	128/32/0.12	128/64/0.12	128/128/0.12	128/256/0.12
64/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12	64/256/0.12
32/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12
16/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12
8/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12
4/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12
0/0/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12

Meropenem

Plate 5 – Colistin 0.25 µg/ml

Sulbactam							
256/0.25	256/4/0.25	256/8/0.25	256/16/0.25	256/32/0.25	256/64/0.25	256/128/0.25	256/256/0.25
128/0.25	128/4/0.25	128/8/0.25	128/16/0.25	128/32/0.25	128/64/0.25	128/128/0.25	128/256/0.25
64/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25	64/256/0.25
32/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25
16/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25
8/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25
4/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25
0/0/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25

Meropenem

Plate 6 – Colistin 0.5 µg/ml

Sulbactam							
256/0.5	256/4/0.5	256/8/0.5	256/16/0.5	256/32/0.5	256/64/0.5	256/128/0.5	256/256/0.5
128/0.5	128/4/0.5	128/8/0.5	128/16/0.5	128/32/0.5	128/64/0.5	128/128/0.5	128/256/0.5
64/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5	64/256/0.5
32/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5
16/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5
8/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5
4/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5
0/0/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5

Meropenem

Figure C-14 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 13 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 7 – Colistin 1 µg/ml

Sulbactam								
256/1	256/4/1	256/8/1	256/16/1	256/32/1	256/64/1	256/128/1	256/256/1
128/1	128/4/1	128/8/1	128/16/1	128/32/1	128/64/1	128/128/1	128/256/1
64/1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1	64/256/1
32/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1
16/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1
8/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1
4/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1
0/0/1	4/1	8/1	16/1	32/1	64/1	128/1	256/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam								
256/2	256/4/2	256/8/2	256/16/2	256/32/2	256/64/2	256/128/2	256/256/2
128/2	128/4/2	128/8/2	128/16/2	128/32/2	128/64/2	128/128/2	128/256/2
64/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2	64/256/2
32/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2
16/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2
8/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2
4/2	4/4//2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2
2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2
0/0/2	4/2	8/2	16/2	32/2	64/2	128/2	256/2

Meropenem

Figure C-14 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 13 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolates No. 14 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
128	128/4	128/8	128/16	128/32	128/64	128/128	128/256
64	64/4	64/8	64/16	64/32	64/64	64/128	64/256
32	32/4	32/8	32/16	32/32	32/64	32/128	32/256
16	16/4	16/8	16/16	16/32	16/64	16/128	16/256
8	8/4	8/8	8/16	8/32	8/64	8/128	8/256
4	4/4	4/8	4/16	4/32	4/64	4/128	4/256
2	2/4	2/8	2/16	2/32	2/64	2/128	2/256
0	4	8	16	32	64	128	256

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
128/0.03	128/4/0.03	128/8/0.03	128/16/0.03	128/32/0.03	128/64/0.03	128/128/0.03	128/256/0.03
64/0.03	64/4/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03	64/256/0.03
32/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03	32/256/0.03
16/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03	16/256/0.03
8/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03	8/256/0.03
4/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03	4/256/0.03
2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03	2/256/0.03
0/0/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03	256/0.03

Meropenem

Plate 4 – Colistin 0.06 µg/ml

Sulbactam							
128/0.06	128/4/0.06	128/8/0.06	128/16/0.06	128/32/0.06	128/64/0.06	128/128/0.06	128/256/0.06
64/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06	64/256/0.06
32/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06
16/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06
8/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06
4/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06
2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06
0/0/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06

Meropenem

Figure C-15 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 14 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 4 – Colistin 0.12 µg/ml

Sulbactam								
128/0.12	128/4/0.12	128/8/0.12	128/16/0.12	128/32/0.12	128/64/0.12	128/128/0.12	128/256/0.12
64/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12	64/256/0.12
32/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12
16/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12
8/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12
4/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12
2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12
0/0/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12

Meropenem

Plate 5 – Colistin 0.25 µg/ml

Sulbactam								
128/0.25	128/4/0.25	128/8/0.25	128/16/0.25	128/32/0.25	128/64/0.25	128/128/0.25	128/256/0.25
64/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25	64/256/0.25
32/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25
16/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25
8/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25
4/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25
2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25
0/0/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25

Meropenem

Plate 6 – Colistin 0.5 µg/ml

Sulbactam								
128/0.5	128/4/0.5	128/8/0.5	128/16/0.5	128/32/0.5	128/64/0.5	128/128/0.5	128/256/0.5
64/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5	64/256/0.5
32/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5
16/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5
8/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5
4/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5
2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5
0/0/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5

Meropenem

Figure C-15 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 14 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 7 – Colistin 1 µg/ml

Sulbactam							
128/1	128/4/1	128/8/1	128/16/1	128/32/1	128/64/1	128/128/1	128/256/1
64/1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1	64/256/1
32/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1
16/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1
8/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1
4/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1
2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1
0/0/1	4/1	8/1	16/1	32/1	64/1	128/1	256/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam							
128/2	128/4/2	128/8/2	128/16/2	128/32/2	128/64/2	128/128/2	128/256/2
64/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2	64/256/2
32/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2
16/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2
8/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2
4/2	4/4//2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2
2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2
0/0/2	4/2	8/2	16/2	32/2	64/2	128/2	256/2

Meropenem

Figure C-15 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 14 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolates No. 15 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
128	128/4	128/8	128/16	128/32	128/64	128/128	128/256
64	64/4	64/8	64/16	64/32	64/64	64/128	64/256
32	32/4	32/8	32/16	32/32	32/64	32/128	32/256
16	16/4	16/8	16/16	16/32	16/64	16/128	16/256
8	8/4	8/8	8/16	8/32	8/64	8/128	8/256
4	4/4	4/8	4/16	4/32	4/64	4/128	4/256
2	2/4	2/8	2/16	2/32	2/64	2/128	2/256
0	4	8	16	32	64	128	256

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam								
128/0.03	128/2/0.03	128/4/0.03	128/8/0.03	128/16/0.03	128/32/0.03	128/64/0.03	128/128/0.03
64/0.03	64/2/0.03	64/4/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03
32/0.03	32/2/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03
16/0.03	16/2/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03
8/0.03	8/2/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03
4/0.03	4/2/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03
2/0.03	2/2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03
0/0/0.03	2/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam								
128/0.06	128/2/0.06	128/4/0.06	128/8/0.06	128/16/0.06	128/32/0.06	128/64/0.06	128/128/0.06
64/0.06	64/2/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06
32/0.06	32/2/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06
16/0.06	16/2/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06
8/0.06	8/2/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06
4/0.06	4/2/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06
2/0.06	2/2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06
0/0/0.06	2/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06

Meropenem

Figure C-16 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 15 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 4 – Colistin 0.12 µg/ml

Sulbactam								
128/0.12	128/2/0.12	128/4/0.12	128/8/0.12	128/16/0.12	128/32/0.12	128/64/0.12	128/128/0.12
64/0.12	64/2/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12
32/0.12	32/2/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12
16/0.12	16/2/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12
8/0.12	8/2/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12
4/0.12	4/2/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12
2/0.12	2/2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12
0/0/0.12	2/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12

Meropenem

Plate 5 – Colistin 0.25 µg/ml

Sulbactam								
128/0.25	128/2/0.25	128/4/0.25	128/8/0.25	128/16/0.25	128/32/0.25	128/64/0.25	128/128/0.25
64/0.25	64/2/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25
32/0.25	32/2/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25
16/0.25	16/2/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25
8/0.25	8/2/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25
4/0.25	4/2/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25
2/0.25	2/2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25
0/0/0.25	2/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25

Meropenem

Plate 6 – Colistin 0.5 µg/ml

Sulbactam								
128/0.5	128/2/0.5	128/4/0.5	128/8/0.5	128/16/0.5	128/32/0.5	128/64/0.5	128/128/0.5
64/0.5	64/2/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5
32/0.5	32/2/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5
16/0.5	16/2/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5
8/0.5	8/2/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5
4/0.5	4/2/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5
2/0.5	2/2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5
0/0/0.5	2/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5

Meropenem

Figure C-16 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 15 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 7 – Colistin 1 µg/ml

Sulbactam								
128/1	128/2/1	128/4/1	128/8/1	128/16/1	128/32/1	128/64/1	128/128/1
64/1	64/2/1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1
32/1	32/2/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1
16/1	16/2/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1
8/1	8/2/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1
4/1	4/2/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1
2/1	2/2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1
0/0/1	2/1	4/1	8/1	16/1	32/1	64/1	128/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam								
128/2	128/2/2	128/4/2	128/8/2	128/16/2	128/32/2	128/64/2	128/128/2
64/2	64/2/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2
32/2	32/2/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2
16/2	16/2/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2
8/2	8/2/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2
4/2	4/2/2	4/4//2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2
2/2	2/2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2
0/0/2	2/2	4/2	8/2	16/2	32/2	64/2	128/2

Meropenem

Figure C-16 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 15 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolates No. 16 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
64	64/4	64/8	64/16	64/32	64/64	64/128	64/256
32	32/4	32/8	32/16	32/32	32/64	32/128	32/256
16	16/4	16/8	16/16	16/32	16/64	16/128	16/256
8	8/4	8/8	8/16	8/32	8/64	8/128	8/256
4	4/4	4/8	4/16	4/32	4/64	4/128	4/256
2	2/4	2/8	2/16	2/32	2/64	2/128	2/256
1	1/4	1/8	1/16	1/32	1/64	1/128	1/256
0	4	8	16	32	64	128	256

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
64 /0.03	64/4/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03	64/256/0.03
32/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03	32/256/0.03
16/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03	16/256/0.03
8/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03	8/256/0.03
4/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03	4/256/0.03
2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03	2/256/0.03
1/0.03	1/4/0.03	1/8/0.03	1/16/0.03	1/32/0.03	1/64/0.03	1/128/0.03	1/256/0.03
0/0/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03	256/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
64/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06	64/256/0.06
32/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06
16/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06
8/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06
4/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06
2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06
1/0.06	1/4/0.06	1/8/0.06	1/16/0.06	1/32/0.06	1/64/0.06	1/128/0.06	1/256/0.06
0/0/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06

Meropenem

Figure C-17 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 16 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 4 – Colistin 0.12 µg/ml

Sulbactam								
64/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12	64/256/0.12
32/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12
16/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12
8/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12
4/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12
2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12
1/0.12	1/4/0.12	1/8/0.12	1/16/0.12	1/32/0.12	1/64/0.12	1/128/0.12	1/256/0.12
0/0/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12

Meropenem

Plate 5 – Colistin 0.25 µg/ml

Sulbactam								
64/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25	64/256/0.25
32/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25
16/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25
8/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25
4/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25
2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25
1/0.25	1/4/0.25	1/8/0.25	1/16/0.25	1/32/0.25	1/64/0.25	1/128/0.25	1/256/0.25
0/0/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25

Meropenem

Plate 6 – Colistin 0.5 µg/ml

Sulbactam								
64/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5	64/256/0.5
32/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5
16/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5
8/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5
4/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5
2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5
1/0.5	1/4/0.5	1/8/0.5	1/16/0.5	1/32/0.5	1/64/0.5	1/128/0.5	1/256/0.5
0/0/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5

Meropenem

Figure C-17 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 16 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 7 – Colistin 1 µg/ml

Sulbactam							
64 /1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1	64/256/1
32/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1
16/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1
8/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1
4/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1
2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1
1/1	1/4/1	1/8/1	1/16/1	1/32/1	1/64/1	1/128/1	1/256/1
0/0/1	4/1	8/1	16/1	32/1	64/1	128/1	256/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam							
64/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2	64/256/2
32/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2
16/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2
8/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2
4/2	4/4/2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2
2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2
1/2	1/4/2	1/8/2	1/16/2	1/32/2	1/64/2	1/128/2	1/256/2
0/0/2	4/2	8/2	16/2	32/2	64/2	128/2	256/2

Meropenem

Figure C-17 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 16 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolates No. 17 **plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
128	128/4	128/8	128/16	128/32	128/64	128/128	128/256
64	64/4	64/8	64/16	64/32	64/64	64/128	64/256
32	32/4	32/8	32/16	32/32	32/64	32/128	32/256
16	16/4	16/8	16/16	16/32	16/64	16/128	16/256
8	8/4	8/8	8/16	8/32	8/64	8/128	8/256
4	4/4	4/8	4/16	4/32	4/64	4/128	4/256
2	2/4	2/8	2/16	2/32	2/64	2/128	2/256
0	4	8	16	32	64	128	256

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
128/0.03	128/4/0.03	128/8/0.03	128/16/0.03	128/32/0.03	128/64/0.03	128/128/0.03	128/256/0.03
64/0.03	64/4/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03	64/256/0.03
32/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03	32/256/0.03
16/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03	16/256/0.03
8/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03	8/256/0.03
4/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03	4/256/0.03
2/0.03	2/40.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03	2/256/0.03
0/0/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03	256/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
128/0.06	128/4/0.06	128/8/0.06	128/16/0.06	128/32/0.06	128/64/0.06	128/128/0.06	128/256/0.06
64/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06	64/256/0.06
32/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06
16/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06
8/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06
4/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06
2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06
0/0/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06

Meropenem

Figure C-18 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 17 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 4 – Colistin 0.12 µg/ml

Sulbactam								
128/0.12	128/4/0.12	128/8/0.12	128/16/0.12	128/32/0.12	128/64/0.12	128/128/0.12	128/256/0.12
64/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12	64/256/0.12
32/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12
16/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12
8/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12
4/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12
2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12
0/0/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12

Meropenem

Plate 5 – Colistin 0.25 µg/ml

Sulbactam								
128/0.25	128/4/0.25	128/8/0.25	128/16/0.25	128/32/0.25	128/64/0.25	128/128/0.25	128/256/0.25
64/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25	64/256/0.25
32/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25
16/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25
8/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25
4/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25
2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25
0/0/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25

Meropenem

Plate 6 – Colistin 0.5 µg/ml

Sulbactam								
128/0.5	128/4/0.5	128/8/0.5	128/16/0.5	128/32/0.5	128/64/0.5	128/128/0.5	128/256/0.5
64/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5	64/256/0.5
32/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5
16/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5
8/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5
4/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5
2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5
0/0/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5

Meropenem

Figure C-18 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 17 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 7 – Colistin 1 µg/ml

Sulbactam								
128/1	128/4/1	128/8/1	128/16/1	128/32/1	128/64/1	128/128/1	128/256/1
64 /1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1	64/256/1
32/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1
16/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1
8/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1
4/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1
2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1
0/0/1	4/1	8/1	16/1	32/1	64/1	128/1	256/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam								
128/2	128/4/2	128/8/2	128/16/2	128/32/2	128/64/2	128/128/2	128/256/2
64/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2	64/256/2
32/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2
16/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2
8/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2
4/2	4/4/2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2
2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2
0/0/2	4/2	8/2	16/2	32/2	64/2	128/2	256/2

Meropenem

Figure C-18 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 17 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolates No. 18 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
32	32/2	32/4	32/8	32/16	32/32	32/64	32/128
16	16/2	16/4	16/8	16/16	16/32	16/64	16/128
8	8/2	8/4	8/8	8/16	8/32	8/64	8/128
4	4/2	4/4	4/8	4/16	4/32	4/64	4/128
2	2/2	2/4	2/8	2/16	2/32	2/64	2/128
1	1/2	1/4	1/8	1/16	1/32	1/64	1/128
0.5	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32	0.5/64	0.5/128
0	2	4	8	16	32	64	128

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
32/0.03	32/2/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03
16/0.03	16/2/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03
8/0.03	8/2/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03
4/0.03	4/2/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03
2/0.03	2/2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03
1/0.03	1/2/0.03	1/4/0.03	1/8/0.03	1/16/0.03	1/32/0.03	1/64/0.03	1/128/0.03
0.5/0.03	0.5/2/0.03	0.5/4/0.03	0.5/8/0.03	0.5/16/0.03	0.5/32/0.03	0.5/64/0.03	0.5/128/0.03
0/0/0.03	2/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
32/0.06	32/2/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06
16/0.06	16/2/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06
8/0.06	8/2/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06
4/0.06	4/2/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06
2/0.06	2/2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06
1/0.06	1/2/0.06	1/4/0.06	1/8/0.06	1/16/0.06	1/32/0.06	1/64/0.06	1/128/0.06
0.5/0.06	0.5/2/0.06	0.5/4/0.06	0.5/8/0.06	0.5/16/0.06	0.5/32/0.06	0.5/64/0.06	0.5/128/0.06
0/0/0.06	2/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06

Meropenem

Figure C-19 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 18 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 4 – Colistin 0.12 µg/ml

Sulbactam							
32/0.12	32/2/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12
16/0.12	16/2/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12
8/0.12	8/2/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12
4/0.12	4/2/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12
2/0.12	2/2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12
1/0.12	1/2/0.12	1/4/0.12	1/8/0.12	1/16/0.12	1/32/0.12	1/64/0.12	1/128/0.12
0.5/0.12	0.5/2/0.12	0.5/4/0.12	0.5/8/0.12	0.5/16/0.12	0.5/32/0.12	0.5/64/0.12	0.5/128/0.12
0/0/0.12	2/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12

Meropenem

Plate 5 – Colistin 0.25 µg/ml

Sulbactam							
32/0.25	32/2/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25
16/0.25	16/2/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25
8/0.25	8/2/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25
4/0.25	4/2/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25
2/0.25	2/2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25
1/0.25	1/2/0.25	1/4/0.25	1/8/0.25	1/16/0.25	1/32/0.25	1/64/0.25	1/128/0.25
0.5/0.25	0.5/2/0.25	0.5/4/0.25	0.5/8/0.25	0.5/16/0.25	0.5/32/0.25	0.5/64/0.25	0.5/128/0.25
0/0/0.25	2/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25

Meropenem

Plate 6 – Colistin 0.5 µg/ml

Sulbactam							
32/0.5	32/2/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5
16/0.5	16/2/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5
8/0.5	8/2/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5
4/0.5	4/2/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5
2/0.5	2/2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5
1/0.5	1/2/0.5	1/4/0.5	1/8/0.5	1/16/0.5	1/32/0.5	1/64/0.5	1/128/0.5
0.5/0.5	0.5/2/0.5	0.5/4/0.5	0.5/8/0.5	0.5/16/0.5	0.5/32/0.5	0.5/64/0.5	0.5/128/0.5
0/0/0.5	2/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5

Meropenem

Figure C-19 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 18 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 7 – Colistin 1 µg/ml

Sulbactam							
32/1	32/2/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1
16/1	16/2/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1
8/1	8/2/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1
4/1	4/2/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1
2/1	2/2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1
1/1	1/2/1	1/4/1	1/8/1	1/16/1	1/32/1	1/64/1	1/128/1
0.5/1	0.5/2/1	0.5/4/1	0.5/8/1	0.5/16/1	0.5/32/1	0.5/64/1	0.5/128/1
0/0/1	2/1	4/1	8/1	16/1	32/1	64/1	128/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam							
32/2	32/2/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2
16/2	16/2/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2
8/2	8/2/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2
4/2	4/2/2	4/4/2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2
2/2	2/2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2
1/2	1/2/2	1/4/2	1/8/2	1/16/2	1/32/2	1/64/2	1/128/2
0.5/2	0.5/2/2	0.5/4/2	0.5/8/2	0.5/16/2	0.5/32/2	0.5/64/2	0.5/128/2
0/0/2	2/2	4/2	8/2	16/2	32/2	64/2	128/2

Meropenem

Figure C-19 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 18 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolates No. 19 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
64	64/2	64/4	64/8	64/16	64/32	64/64	64/128
32	32/2	32/4	32/8	32/16	32/32	32/64	32/128
16	16/2	16/4	16/8	16/16	16/32	16/64	16/128
8	8/2	8/4	8/8	8/16	8/32	8/64	8/128
4	4/2	4/4	4/8	4/16	4/32	4/64	4/128
2	2/2	2/4	2/8	2/16	2/32	2/64	2/128
1	1/2	1/4	1/8	1/16	1/32	1/64	1/128
0	2	4	8	16	32	64	128

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
64 /0.03	64/2/0.03	64/4/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03
32/0.03	32/2/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03
16/0.03	16/2/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03
8/0.03	8/2/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03
4/0.03	4/2/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03
2/0.03	2/2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03
1/0.03	1/2/0.03	1/4/0.03	1/8/0.03	1/16/0.03	1/32/0.03	1/64/0.03	1/128/0.03
0/0/0.03	2/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
64/0.06	64/2/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06
32/0.06	32/2/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06
16/0.06	16/2/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06
8/0.06	8/2/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06
4/0.06	4/2/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06
2/0.06	2/2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06
1/0.06	1/2/0.06	1/4/0.06	1/8/0.06	1/16/0.06	1/32/0.06	1/64/0.06	1/128/0.06
0/0/0.06	2/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06

Meropenem

Figure C-20 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 19 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 4 – Colistin 0.12 µg/ml

Sulbactam								
	64/0.12	64/2/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12
	32/0.12	32/2/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12
	16/0.12	16/2/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12
	8/0.12	8/2/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12
	4/0.12	4/2/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12
	2/0.12	2/2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12
	1/0.12	1/2/0.12	1/4/0.12	1/8/0.12	1/16/0.12	1/32/0.12	1/64/0.12	1/128/0.12
	0/0/0.12	2/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12

Meropenem

Plate 5 – Colistin 0.25 µg/ml

Sulbactam								
	64/0.25	64/2/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25
	32/0.25	32/2/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25
	16/0.25	16/2/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25
	8/0.25	8/2/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25
	4/0.25	4/2/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25
	2/0.25	2/2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25
	1/0.25	1/2/0,25	1/4/0.25	1/8/0.25	1/16/0.25	1/32/0.25	1/64/0.25	1/128/0.25
	0/0/0.25	2/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25

Meropenem

Plate 6 – Colistin 0.5 µg/ml

Sulbactam								
	64/0.5	64/2/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5
	32/0.5	32/2/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5
	16/0.5	16/2/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5
	8/0.5	8/2/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5
	4/0.5	4/2/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5
	2/0.5	2/2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5
	1/0.5	1/0.5/2	1/4/0.5	1/8/0.5	1/16/0.5	1/32/0.5	1/64/0.5	1/128/0.5
	0/0/0.5	2/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5

Meropenem

Figure C-20 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 19 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 7 – Colistin 1 µg/ml

Sulbactam							
64 /1	64/2/1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1
32/1	32/2/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1
16/1	16/2/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1
8/1	8/2/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1
4/1	4/2/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1
2/1	2/2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1
1/1	1/2/1	1/4/1	1/8/1	1/16/1	1/32/1	1/64/1	1/128/1
0/0/1	2/1	4/1	8/1	16/1	32/1	64/1	128/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam							
64/2	64/2/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2
32/2	32/2/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2
16/2	16/2/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2
8/2	8/2/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2
4/2	4/2/2	4/4/2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2
2/2	2/2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2
1/2	1/2/2	1/4/2	1/8/2	1/16/2	1/32/2	1/64/2	1/128/2
0/0/2	2/2	4/2	8/2	16/2	32/2	64/2	128/2

Meropenem

Figure C-20 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 19 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolates No. 20 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
32	32/2	32/4	32/8	32/16	32/32	32/64	32/128
16	16/2	16/4	16/8	16/16	16/32	16/64	16/128
8	8/2	8/4	8/8	8/16	8/32	8/64	8/128
4	4/2	4/4	4/8	4/16	4/32	4/64	4/128
2	2/2	2/4	2/8	2/16	2/32	2/64	2/128
1	1/2	1/4	1/8	1/16	1/32	1/64	1/128
0.5	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32	0.5/64	0.5/128
0	2	4	8	16	32	64	128

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
32/0.03	32/2/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03
16/0.03	16/2/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03
8/0.03	8/2/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03
4/0.03	4/2/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03
2/0.03	2/2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03
1/0.03	1/2/0.03	1/4/0.03	1/8/0.03	1/16/0.03	1/32/0.03	1/64/0.03	1/128/0.03
0.5/0.03	0.5/2/0.03	0.5/4/0.03	0.5/8/0.03	0.5/16/0.03	0.5/32/0.03	0.5/64/0.03	0.5/128/0.03
0/0/0.03	2/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
32/0.06	32/2/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06
16/0.06	16/2/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06
8/0.06	8/2/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06
4/0.06	4/2/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06
2/0.06	2/2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06
1/0.06	1/2/0.06	1/4/0.06	1/8/0.06	1/16/0.06	1/32/0.06	1/64/0.06	1/128/0.06
0.5/0.06	0.5/2/0.06	0.5/4/0.06	0.5/8/0.06	0.5/16/0.06	0.5/32/0.06	0.5/64/0.06	0.5/128/0.06
0/0/0.06	2/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06

Meropenem

Figure C-21 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 20 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 4 – Colistin 0.12 µg/ml

Sulbactam							
32/0.12	32/2/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12
16/0.12	16/2/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12
8/0.12	8/2/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12
4/0.12	4/2/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12
2/0.12	2/2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12
1/0.12	1/2/0.12	1/4/0.12	1/8/0.12	1/16/0.12	1/32/0.12	1/64/0.12	1/128/0.12
0.5/0.12	0.5/2/0.12	0.5/4/0.12	0.5/8/0.12	0.5/16/0.12	0.5/32/0.12	0.5/64/0.12	0.5/128/0.12
0/0/0.12	2/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12

Meropenem

Plate 5 – Colistin 0.25 µg/ml

Sulbactam							
32/0.25	32/2/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25
16/0.25	16/2/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25
8/0.25	8/2/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25
4/0.25	4/2/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25
2/0.25	2/2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25
1/0.25	1/2/0.25	1/4/0.25	1/8/0.25	1/16/0.25	1/32/0.25	1/64/0.25	1/128/0.25
0.5/0.25	0.5/2/0.25	0.5/4/0.25	0.5/8/0.25	0.5/16/0.25	0.5/32/0.25	0.5/64/0.25	0.5/128/0.25
0/0/0.25	2/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25

Meropenem

Plate 2 – Colistin 0.5 µg/ml

Sulbactam							
32/0.5	32/2/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5
16/0.5	16/2/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5
8/0.5	8/2/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5
4/0.5	4/2/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5
2/0.5	2/2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5
1/0.5	1/2/0.5	1/4/0.5	1/8/0.5	1/16/0.5	1/32/0.5	1/64/0.5	1/128/0.5
0.5/0.5	0.5/2/0.5	0.5/4/0.5	0.5/8/0.5	0.5/16/0.5	0.5/32/0.5	0.5/64/0.5	0.5/128/0.5
0/0/0.5	2/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5

Meropenem

Figure C-21 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 20 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 7 – Colistin 1 µg/ml

Sulbactam							
32/1	32/2/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1
16/1	16/2/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1
8/1	8/2/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1
4/1	4/2/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1
2/1	2/2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1
1/1	1/2/1	1/4/1	1/8/1	1/16/1	1/32/1	1/64/1	1/128/1
0.5/1	0.5/2/1	0.5/4/1	0.5/8/1	0.5/16/1	0.5/32/1	0.5/64/1	0.5/128/1
0/0/1	2/1	4/1	8/1	16/1	32/1	64/1	128/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam							
32/2	32/2/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2
16/2	16/2/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2
8/2	8/2/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2
4/2	4/2/2	4/4/2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2
2/2	2/2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2
1/2	1/2/2	1/4/2	1/8/2	1/16/2	1/32/2	1/64/2	1/128/2
0.5/2	0.5/2/2	0.5/4/2	0.5/8/2	0.5/16/2	0.5/32/2	0.5/64/2	0.5/128/2
0/0/2	2/2	4/2	8/2	16/2	32/2	64/2	128/2

Meropenem

Figure C-21 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 20 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolates No. 21 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
32	32/2	32/4	32/8	32/16	32/32	32/64	32/128
16	16/2	16/4	16/8	16/16	16/32	16/64	16/128
8	8/2	8/4	8/8	8/16	8/32	8/64	8/128
4	4/2	4/4	4/8	4/16	4/32	4/64	4/128
2	2/2	2/4	2/8	2/16	2/32	2/64	2/128
1	1/2	1/4	1/8	1/16	1/32	1/64	1/128
0.5	0.5/2	0.5/4	0.5/8	0.5/16	0.5/32	0.5/64	0.5/128
0	2	4	8	16	32	64	128

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
32/0.03	32/2/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03
16/0.03	16/2/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03
8/0.03	8/2/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03
4/0.03	4/2/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03
2/0.03	2/2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03
1/0.03	1/2/0.03	1/4/0.03	1/8/0.03	1/16/0.03	1/32/0.03	1/64/0.03	1/128/0.03
0.5/0.03	0.5/2/0.03	0.5/4/0.03	0.5/8/0.03	0.5/16/0.03	0.5/32/0.03	0.5/64/0.03	0.5/128/0.03
0/0/0.03	2/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
32/0.06	32/2/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06
16/0.06	16/2/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06
8/0.06	8/2/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06
4/0.06	4/2/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06
2/0.06	2/2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06
1/0.06	1/2/0.06	1/4/0.06	1/8/0.06	1/16/0.06	1/32/0.06	1/64/0.06	1/128/0.06
0.5/0.06	0.5/2/0.06	0.5/4/0.06	0.5/8/0.06	0.5/16/0.06	0.5/32/0.06	0.5/64/0.06	0.5/128/0.06
0/0/0.06	2/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06

Meropenem

Figure C-22 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 21 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 4 – Colistin 0.12 µg/ml

Sulbactam							
32/0.12	32/2/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12
16/0.12	16/2/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12
8/0.12	8/2/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12
4/0.12	4/2/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12
2/0.12	2/2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12
1/0.12	1/2/0.12	1/4/0.12	1/8/0.12	1/16/0.12	1/32/0.12	1/64/0.12	1/128/0.12
0.5/0.12	0.5/2/0.12	0.5/4/0.12	0.5/8/0.12	0.5/16/0.12	0.5/32/0.12	0.5/64/0.12	0.5/128/0.12
0/0/0.12	2/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12

Meropenem

Plate 5 – Colistin 0.25 µg/ml

Sulbactam							
32/0.25	32/2/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25
16/0.25	16/2/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25
8/0.25	8/2/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25
4/0.25	4/2/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25
2/0.25	2/2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25
1/0.25	1/2/0.25	1/4/0.25	1/8/0.25	1/16/0.25	1/32/0.25	1/64/0.25	1/128/0.25
0.5/0.25	0.5/2/0.25	0.5/4/0.25	0.5/8/0.25	0.5/16/0.25	0.5/32/0.25	0.5/64/0.25	0.5/128/0.25
0/0/0.25	2/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25

Meropenem

Plate 6 – Colistin 0.5 µg/ml

Sulbactam							
32/0.5	32/2/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5
16/0.5	16/2/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5
8/0.5	8/2/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5
4/0.5	4/2/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5
2/0.5	2/2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5
1/0.5	1/2/0.5	1/4/0.5	1/8/0.5	1/16/0.5	1/32/0.5	1/64/0.5	1/128/0.5
0.5/0.5	0.5/2/0.5	0.5/4/0.5	0.5/8/0.5	0.5/16/0.5	0.5/32/0.5	0.5/64/0.5	0.5/128/0.5
0/0/0.5	2/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5

Meropenem

Figure C-22 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 21 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 7 – Colistin 1 µg/ml

32/1	32/2/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1
16/1	16/2/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1
8/1	8/2/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1
4/1	4/2/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1
2/1	2/2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1
1/1	1/2/1	1/4/1	1/8/1	1/16/1	1/32/1	1/64/1	1/128/1
0.5/1	0.5/2/1	0.5/4/1	0.5/8/1	0.5/16/1	0.5/32/1	0.5/64/1	0.5/128/1
0/0/1	2/1	4/1	8/1	16/1	32/1	64/1	128/1

Sulbactam

Meropenem

Plate 8 – Colistin 2 µg/ml

32/2	32/2/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2
16/2	16/2/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2
8/2	8/2/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2
4/2	4/2/2	4/4/2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2
2/2	2/2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2
1/2	1/2/2	1/4/2	1/8/2	1/16/2	1/32/2	1/64/2	1/128/2
0.5/2	0.5/2/2	0.5/4/2	0.5/8/2	0.5/16/2	0.5/32/2	0.5/64/2	0.5/128/2
0/0/2	2/2	4/2	8/2	16/2	32/2	64/2	128/2

Sulbactam

Meropenem

Figure C-22 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 21 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolates No. 22 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
128	128/8	128/16	128/32	128/64	128/128	128/256	128/512
64	64/8	64/16	64/32	64/64	64/128	64/256	64/512
32	32/8	32/16	32/32	32/64	32/128	32/256	32/512
16	16/8	16/16	16/32	16/64	16/128	16/256	16/512
8	8/8	8/16	8/32	8/64	8/128	8/256	8/512
4	4/8	4/16	4/32	4/64	4/128	4/256	4/512
2	2/8	2/16	2/32	2/64	2/128	2/256	2/512
0	8	16	32	64	128	256	512

Meropenem

Plate 2 – Colistin 0.06 µg/ml

Sulbactam							
128/0.06	128/8/0.06	128/16/0.06	128/32/0.06	128/64/0.06	128/128/0.06	128/256/0.06	128/512/0.06
64/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06	64/256/0.06	64/512/0.06
32/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06	32/512/0.06
16/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06	16/512/0.06
8/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06	8/512/0.06
4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06	4/512/0.06
2/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06	2/512/0.06
0/0/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06	512/0.06

Meropenem

Plate 3 – Colistin 0.12 µg/ml

Sulbactam							
128/0.12	128/8/0.12	128/16/0.12	128/32/0.12	128/64/0.12	128/128/0.12	128/256/0.12	128/512/0.12
64/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12	64/256/0.12	64/512/0.12
32/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12	32/512/0.12
16/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12	16/512/0.12
8/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12	8/512/0.12
4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12	4/512/0.12
2/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12	2/512/0.12
0/0/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12	512/0.12

Meropenem

Figure C-23 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 22 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 4 – Colistin 0.25 µg/ml

128/0.25	128/8/0.25	128/16/0.25	128/32/0.25	128/64/0.25	128/128/0.25	128/256/0.25	128/512/0.25
64/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25	64/256/0.25	64/512/0.25
32/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25	32/512/0.25
16/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25	16/512/0.25
8/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25	8/512/0.25
4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25	4/512/0.25
2/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25	2/512/0.25
0/0/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25	512/0.25

Sulbactam

Meropenem

Plate 5 – Colistin 0.5 µg/ml

128/0.5	128/8/0.5	128/16/0.5	128/32/0.5	128/64/0.5	128/128/0.5	128/256/0.5	128/512/0.5
64/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5	64/256/0.5	64/512/0.5
32/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5	32/512/0.5
16/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5	16/512/0.5
8/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5	8/512/0.5
4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5	4/512/0.5
2/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5	2/512/0.5
0/0/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5	512/0.5

Sulbactam

Meropenem

Plate 6 – Colistin 1 µg/ml

128/1	128/8/1	128/16/1	128/32/1	128/64/1	128/128/1	128/256/1	128/512/1
64/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1	64/256/1	64/512/1
32/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1	32/512/1
16/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1	16/512/1
8/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1	8/512/1
4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1	4/512/1
2/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1	2/512/1
0/0/1	8/1	16/1	32/1	64/1	128/1	256/1	512/1

Sulbactam

Meropenem

Figure C-23 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 22 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 7 – Colistin 2 µg/ml

Sulbactam								
128/2	128/8/2	128/16/2	128/32/2	128/64/2	128/128/2	128/256/2	128/512/2
64/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2	64/256/2	64/512/2
32/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2	32/512/2
16/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2	16/512/2
8/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2	8/512/2
4/2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2	4/512/2
2/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2	2/512/2
0/0/2	8/2	16/2	32/2	64/2	128/2	256/2	512/2

Meropenem

Plate 8 – Colistin 4 µg/ml

Sulbactam								
128/4	128/8/4	128/16/4	128/32/4	128/64/4	128/128/4	128/256/4	128/512/4
64/4	64/8/4	64/16/4	64/32/4	64/64/4	64/128/4	64/256/4	64/512/4
32/4	32/8/4	32/16/4	32/32/4	32/64/4	32/128/4	32/256/4	32/512/4
16/4	16/8/4	16/16/4	16/32/4	16/64/4	16/128/4	16/256/4	16/512/4
8/4	8/8/4	8/16/4	8/32/4	8/64/4	8/128/4	8/256/4	8/512/4
4/4	4/8/4	4/16/4	4/32/4	4/64/4	4/128/4	4/256/4	4/512/4
2/4	2/8/4	2/16/4	2/32/4	2/64/4	2/128/4	2/256/4	2/512/4
0/0/4	8/4	16/4	32/4	64/4	128/4	256/4	512/4

Meropenem

Figure C-23 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 22 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolates No. 23 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
32	32 /8	32 /16	32 /32	32 /64	32 /128	32 /256	32 /512
16	16/8	16/16	16/32	16/64	16/128	16/256	16/512
8	8/8	8/16	8/32	8/64	8/128	8/256	8/512
4	4/8	4/16	4/32	4/64	4/128	4/256	4/512
2	2/8	2/16	2/32	2/64	2/128	2/256	2/512
1	1/8	1/16	1/32	1/64	1/128	1/256	1/512
0.5	0.5/8	0.5/16	0.5/32	0.5/64	0.5/128	0.5/256	0.5/512
0	8	16	32	64	128	256	512

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
32/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03	32/256/0.03	32/512/0.03
16/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03	16/256/0.03	16/512/0.03
8/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03	8/256/0.03	8/512/0.03
4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03	4/256/0.03	4/512/0.03
2/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03	2/256/0.03	2/512/0.03
1/0.03	1/8/0.03	1/16/0.03	1/32/0.03	1/64/0.03	1/128/0.03	1/256/0.03	1/512/0.03
0.5/0.03	0.5/8/0.03	0.5/16/0.03	0.5/32/0.03	0.5/64/0.03	0.5/128/0.03	0.5/256/0.03	0.5/512/0.03
0/0/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03	256/0.03	512/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
32/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06	32/512/0.06
16/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06	16/512/0.06
8/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06	8/512/0.06
4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06	4/512/0.06
2/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06	2/512/0.06
1/0.06	1/8/0.06	1/16/0.06	1/32/0.06	1/64/0.06	1/128/0.06	1/256/0.06	1/512/0.06
0.5/0.06	0.5/8/0.06	0.5/82/0.06	0.5/32/0.06	0.5/64/0.06	0.5/128/0.06	0.5/256/0.06	0.5/512/0.06
0/0/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06	512/0.06

Meropenem

Figure C-24 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 23 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 4 – Colistin 0.12 µg/ml

32/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12	32/512/0.12
16/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12	16/512/0.12
8/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12	8/512/0.12
4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12	4/512/0.12
2/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12	2/512/0.12
1/0.12	1/8/0.12	1/16/0.12	1/32/0.12	1/64/0.12	1/128/0.12	1/256/0.12	1/512/0.12
0.5/0.12	0.5/8/0.12	0.5/82/0.12	0.5/32/0.12	0.5/64/0.12	0.5/128/0.12	0.5/256/0.12	0.5/512/0.12
0/0/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12	512/0.12

Sulbactam

Meropenem

Plate 5 – Colistin 0.25 µg/ml

32/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25	32/512/0.25
16/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25	16/512/0.25
8/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25	8/512/0.25
4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25	4/512/0.25
2/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25	2/512/0.25
1/0.25	1/8/0.25	1/16/0.25	1/32/0.25	1/64/0.25	1/128/0.25	1/256/0.25	1/512/0.25
0.5/0.25	0.5/8/0.25	0.5/16/0.25	0.5/32/0.25	0.5/64/0.25	0.5/128/0.25	0.5/256/0.25	0.5/512/0.25
0/0/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25	512/0.25

Sulbactam

Meropenem

Plate 6 – Colistin 0.5 µg/ml

32/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5	32/512/0.5
16/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5	16/512/0.5
8/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5	8/512/0.5
4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5	4/512/0.5
2/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5	2/512/0.5
1/0.5	1/8/0.5	1/16/0.5	1/32/0.5	1/64/0.5	1/128/0.5	1/256/0.5	1/512/0.5
0.5/0.5	0.5/8/0.5	0.5/16/0.5	0.5/32/0.5	0.5/64/0.5	0.5/128/0.5	0.5/256/0.5	0.5/512/0.5
0/0/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5	512/0.5

Sulbactam

Meropenem

Figure C-24 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 23 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 7 – Colistin 1 µg/ml

Sulbactam								
32/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1	32/512/1
16/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1	16/512/1
8/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1	8/512/1
4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1	4/512/1
2/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1	2/512/1
1/1	1/8/1	1/16/1	1/32/1	1/64/1	1/128/1	1/256/1	1/512/1
0.5/1	0.5/8/1	0.5/16/1	0.5/32/1	0.5/64/1	0.5/128/1	0.5/256/1	0.5/512/1
0/0/1	8/1	16/1	32/1	64/1	128/1	256/1	512/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam								
32/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2	32/512/2
16/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2	16/512/2
8/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2	8/512/2
4/2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2	4/512/2
2/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2	2/512/2
1/2	1/8/2	1/16/2	1/32/2	1/64/2	1/128/2	1/256/2	1/512/2
0.5/2	0.5/8/2	0.5/16/2	0.5/32/2	0.5/64/2	0.5/128/2	0.5/256/2	0.5/512/2
0/0/2	8/2	16/2	32/2	64/2	128/2	256/2	512/2

Meropenem

Figure C-24 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 23 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolates No. 24 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
128	128/8	128/16	128/32	128/64	128/128	128/256	128/512
64	64/8	64/16	64/32	64/64	64/128	64/256	64/512
32	32/8	32/16	32/32	32/64	32/128	32/256	32/512
16	16/8	16/16	16/32	16/64	16/128	16/256	16/512
8	8/8	8/16	8/32	8/64	8/128	8/256	8/512
4	4/8	4/16	4/32	4/64	4/128	4/256	4/512
2	2/8	2/16	2/32	2/64	2/128	2/256	2/512
0	8	16	32	64	128	256	512

Meropenem

Plate 2 – Colistin 0.06 µg/ml

Sulbactam							
128/0.06	128/8/0.06	128/16/0.06	128/32/0.06	128/64/0.06	128/128/0.06	128/256/0.06	128/512/0.06
64/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06	64/256/0.06	64/512/0.06
32/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06	32/512/0.06
16/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06	16/512/0.06
8/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06	8/512/0.06
4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06	4/512/0.06
2/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06	2/512/0.06
0/0/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06	512/0.06

Meropenem

Plate 3 – Colistin 0.12 µg/ml

Sulbactam							
128/0.12	128/8/0.12	128/16/0.12	128/32/0.12	128/64/0.12	128/128/0.12	128/256/0.12	128/512/0.12
64/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12	64/256/0.12	64/512/0.12
32/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12	32/512/0.12
16/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12	16/512/0.12
8/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12	8/512/0.12
4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12	4/512/0.12
2/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12	2/512/0.12
0/0/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12	512/0.12

Meropenem

Figure C-25 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 24 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 4 – Colistin 0.25 µg/ml

Sulbactam								
128/0.25	128/8/0.25	128/16/0.25	128/32/0.25	128/64/0.25	128/128/0.25	128/256/0.25	128/512/0.25
64/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25	64/256/0.25	64/512/0.25
32/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25	32/512/0.25
16/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25	16/512/0.25
8/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25	8/512/0.25
4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25	4/512/0.25
2/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25	2/512/0.25
0/0/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25	512/0.25

Meropenem

Plate 5 – Colistin 0.5 µg/ml

Sulbactam							
128/0.5	128/8/0.5	128/16/0.5	128/32/0.5	128/64/0.5	128/128/0.5	128/256/0.5	128/512/0.5
64/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5	64/256/0.5	64/512/0.5
32/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5	32/512/0.5
16/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5	16/512/0.5
8/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5	8/512/0.5
4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5	4/512/0.5
2/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5	2/512/0.5
0/0/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5	512/0.5

Meropenem

Plate 6 – Colistin 1 µg/ml

Sulbactam							
128/1	128/8/1	128/16/1	128/32/1	128/64/1	128/128/1	128/256/1	128/512/1
64/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1	64/256/1	64/512/1
32/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1	32/512/1
16/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1	16/512/1
8/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1	8/512/1
4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1	4/512/1
2/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1	2/512/1
0/0/1	8/1	16/1	32/1	64/1	128/1	256/1	512/1

Meropenem

Figure C-25 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 24 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 7 – Colistin 2 µg/ml

Sulbactam								
128/2	128/8/2	128/16/2	128/32/2	128/64/2	128/128/2	128/256/2	128/512/2
64/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2	64/256/2	64/512/2
32/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2	32/512/2
16/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2	16/512/2
8/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2	8/512/2
4/2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2	4/512/2
2/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2	2/512/2
0/0/2	8/2	16/2	32/2	64/2	128/2	256/2	512/2

Meropenem

Plate 8 – Colistin 4 µg/ml

Sulbactam								
128/4	128/8/4	128/16/4	128/32/4	128/64/4	128/128/4	128/256/4	128/512/4
64/4	64/8/4	64/16/4	64/32/4	64/64/4	64/128/4	64/256/4	64/512/4
32/4	32/8/4	32/16/4	32/32/4	32/64/4	32/128/4	32/256/4	32/512/4
16/4	16/8/4	16/16/4	16/32/4	16/64/4	16/128/4	16/256/4	16/512/4
8/4	8/8/4	8/16/4	8/32/4	8/64/4	8/128/4	8/256/4	8/512/4
4/4	4/8/4	4/16/4	4/32/4	4/64/4	4/128/4	4/256/4	4/512/4
2/4	2/8/4	2/16/4	2/32/4	2/64/4	2/128/4	2/256/4	2/512/4
0/0/4	8/4	16/4	32/4	64/4	128/4	256/4	512/4

Meropenem

Figure C-25 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 24 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 25 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
128	128/4	128/8	128/16	128/32	128/64	128/128	128/256
64	64/4	64/8	64/16	64/32	64/64	64/128	64/256
32	32/4	32/8	32/16	32/32	32/64	32/128	32/256
16	16/4	16/8	16/16	16/32	16/64	16/128	16/256
8	8/4	8/8	8/16	8/32	8/64	8/128	8/256
4	4/4	4/8	4/16	4/32	4/64	4/128	4/256
2	2/4	2/8	2/16	2/32	2/64	2/128	2/256
0	4	8	16	32	64	128	256

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
128/0.03	128/4/0.03	128/8/0.03	128/16/0.03	128/32/0.03	128/64/0.03	128/128/0.03	128/256/0.03
64/0.03	64/4/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03	64/256/0.03
32/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03	32/256/0.03
16/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03	16/256/0.03
8/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03	8/256/0.03
4/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03	4/256/0.03
2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03	2/256/0.03
0/0/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03	256/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
128/0.06	128/4/0.06	128/8/0.06	128/16/0.06	128/32/0.06	128/64/0.06	128/128/0.06	128/256/0.06
64/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06	64/256/0.06
32/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06
16/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06
8/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06
4/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06
2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06
0/0/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06

Meropenem

Figure C-26 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 25 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 4 – Colistin 0.12 µg/ml

Sulbactam								
8/0.12	128/4/0.12	128/8/0.12	128/16/0.12	128/32/0.12	128/64/0.12	128/128/0.12	128/256/0.12
64/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12	64/256/0.12
32/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12
16/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12
8/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12
4/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12
2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12
0/0/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12

Meropenem

Plate 5 – Colistin 0.25 µg/ml

Sulbactam								
8/0.25	128/4/0.25	128/8/0.25	128/16/0.25	128/32/0.25	128/64/0.25	128/128/0.25	128/256/0.25
64/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25	64/256/0.25
32/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25
16/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25
8/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25
4/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25
2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25
0/0/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25

Meropenem

Plate 6 – Colistin 0.5 µg/ml

Sulbactam								
128/0.5	128/4/0.5	128/8/0.5	128/16/0.5	128/32/0.5	128/64/0.5	128/128/0.5	128/256/0.5
64/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5	64/256/0.5
32/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5
16/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5
8/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5
4/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5
2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5
0/0/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5

Meropenem

Figure C-26 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 25 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 7 – Colistin 1 µg/ml

Sulbactam							
128/1	128/4/1	128/8/1	128/16/1	128/32/1	128/64/1	128/128/1	128/256/1
64/1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1	64/256/1
32/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1
16/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1
8/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1
4/1	14/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1
2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1
0/0/1	4/1	8/1	16/1	32/1	64/1	128/1	256/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam							
128/2	128/4/2	128/8/2	128/16/2	128/32/2	128/64/2	128/128/2	128/256/2
64/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2	64/256/2
32/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2
16/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2
8/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2
4/2	4/4//2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2
2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2
0/0/2	4/2	8/2	16/2	32/2	64/2	128/2	256/2

Meropenem

Figure C-26 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 25 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolates No. 26 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
128	128/4	128/8	128/16	128/32	128/64	128/128	128/256
64	64/4	64/8	64/16	64/32	64/64	64/128	64/256
32	32/4	32/8	32/16	32/32	32/64	32/128	32/256
16	16/4	16/8	16/16	16/32	16/64	16/128	16/256
8	8/4	8/8	8/16	8/32	8/64	8/128	8/256
4	4/4	4/8	4/16	4/32	4/64	4/128	4/256
2	2/4	2/8	2/16	2/32	2/64	2/128	2/256
0	4	8	16	32	64	128	256

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
128/0.03	128/4/0.03	128/8/0.03	128/16/0.03	128/32/0.03	128/64/0.03	128/128/0.03	128/256/0.03
64/0.03	64/4/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03	64/256/0.03
32/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03	32/256/0.03
16/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03	16/256/0.03
8/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03	8/256/0.03
4/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03	4/256/0.03
2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03	2/256/0.03
0/0/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03	256/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
128/0.06	128/4/0.06	128/8/0.06	128/16/0.06	128/32/0.06	128/64/0.06	128/128/0.06	128/256/0.06
64/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06	64/256/0.06
32/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06
16/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06
8/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06
4/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06
2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06
0/0/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06

Meropenem

Figure C-27 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 26 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 4 – Colistin 0.12 µg/ml

Sulbactam								
128/0.12	128/4/0.12	128/8/0.12	128/16/0.12	128/32/0.12	128/64/0.12	128/128/0.12	128/256/0.12
64/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12	64/256/0.12
32/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12
16/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12
8/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12
4/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12
2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12
0/0/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12

Meropenem

Plate 5 – Colistin 0.25 µg/ml

Sulbactam								
128/0.25	128/4/0.25	128/8/0.25	128/16/0.25	128/32/0.25	128/64/0.25	128/128/0.25	128/256/0.25
64/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25	64/256/0.25
32/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25
16/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25
8/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25
4/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25
2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25
0/0/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25

Meropenem

Plate 6 – Colistin 0.5 µg/ml

Sulbactam								
128/0.5	128/4/0.5	128/8/0.5	128/16/0.5	128/32/0.5	128/64/0.5	128/128/0.5	128/256/0.5
64/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5	64/256/0.5
32/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5
16/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5
8/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5
4/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5
2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5
0/0/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5

Meropenem

Figure C-27 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 26 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 7 – Colistin 1 µg/ml

Sulbactam								
128/1	128/4/1	128/8/1	128/16/1	128/32/1	128/64/1	128/128/1	128/256/1
64/1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1	64/256/1
32/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1
16/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1
8/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1
4/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1
2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1
0/0/1	4/1	8/1	16/1	32/1	64/1	128/1	256/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam								
128/2	128/4/2	128/8/2	128/16/2	128/32/2	128/64/2	128/128/2	128/256/2
64/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2	64/256/2
32/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2
16/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2
8/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2
4/2	4/4//2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2
2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2
0/0/2	4/2	8/2	16/2	32/2	64/2	128/2	256/2

Meropenem

Figure C-27 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 26 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolates No. 27 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
64	64/2	64/4	64/8	64/16	64/32	64/64	64/128
32	32/2	32/4	32/8	32/16	32/32	32/64	32/128
16	16/2	16/4	16/8	16/16	16/32	16/64	16/128
8	8/2	8/4	8/8	8/16	8/32	8/64	8/128
4	4/2	4/4	4/8	4/16	4/32	4/64	4/128
2	2/2	2/4	2/8	2/16	2/32	2/64	2/128
1	1/2	1/4	1/8	1/16	1/32	1/64	1/128
0	2	4	8	16	32	64	128

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
64 /0.03	64/2/0.03	64/4/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03
32/0.03	32/2/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03
16/0.03	16/2/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03
8/0.03	8/2/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03
4/0.03	4/2/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03
2/0.03	2/2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03
1/0.03	1/2/0.03	1/4/0.03	1/8/0.03	1/16/0.03	1/32/0.03	1/64/0.03	1/128/0.03
0/0/0.03	2/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
64/0.06	64/2/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06
32/0.06	32/2/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06
16/0.06	16/2/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06
8/0.06	8/2/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06
4/0.06	4/2/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06
2/0.06	2/2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06
1/0.06	1/2/0.06	1/4/0.06	1/8/0.06	1/16/0.06	1/32/0.06	1/64/0.06	1/128/0.06
0/0/0.06	2/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06

Meropenem

Figure C-28 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 27 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 4 – Colistin 0.12 µg/ml

Sulbactam								
	64/0.12	64/2/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12
	32/0.12	32/2/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12
	16/0.12	16/2/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12
	8/0.12	8/2/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12
	4/0.12	4/2/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12
	2/0.12	2/2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12
	1/0.12	1/2/0.12	1/4/0.12	1/8/0.12	1/16/0.12	1/32/0.12	1/64/0.12	1/128/0.12
	0/0/0.12	2/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12

Meropenem

Plate 5 – Colistin 0.25 µg/ml

Sulbactam								
	64/0.25	64/2/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25
	32/0.25	32/2/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25
	16/0.25	16/2/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25
	8/0.25	8/2/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25
	4/0.25	4/2/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25
	2/0.25	2/2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25
	1/0.25	1/2/0,25	1/4/0.25	1/8/0.25	1/16/0.25	1/32/0.25	1/64/0.25	1/128/0.25
	0/0/0.25	2/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25

Meropenem

Plate 6 – Colistin 0.5 µg/ml

Sulbactam								
	64/0.5	64/2/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5
	32/0.5	32/2/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5
	16/0.5	16/2/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5
	8/0.5	8/2/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5
	4/0.5	4/2/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5
	2/0.5	2/2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5
	1/0.5	1/2/0.5	1/4/0.5	1/8/0.5	1/16/0.5	1/32/0.5	1/64/0.5	1/128/0.5
	0/0/0.5	2/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5

Meropenem

Figure C-28 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 27 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 7 – Colistin 1 µg/ml

Sulbactam							
64 /1	64/2/1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1
32/1	32/2/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1
16/1	16/2/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1
8/1	8/2/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1
4/1	4/2/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1
2/1	2/2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1
1/1	1/2/1	1/4/1	1/8/1	1/16/1	1/32/1	1/64/1	1/128/1
0/0/1	2/1	4/1	8/1	16/1	32/1	64/1	128/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam							
64/2	64/2/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2
32/2	32/2/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2
16/2	16/2/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2
8/2	8/2/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2
4/2	4/2/2	4/4/2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2
2/2	2/2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2
1/2	1/2/2	1/4/2	1/8/2	1/16/2	1/32/2	1/64/2	1/128/2
0/0/2	2/2	4/2	8/2	16/2	32/2	64/2	128/2

Meropenem

Figure C-28 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 27 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 28 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
32	32 /8	32 /16	32 /32	32 /64	32 /128	32 /256	32 /512
16	16/8	16/16	16/32	16/64	16/128	16/256	16/512
8	8/8	8/16	8/32	8/64	8/128	8/256	8/512
4	4/8	4/16	4/32	4/64	4/128	4/256	4/512
2	2/8	2/16	2/32	2/64	2/128	2/256	2/512
1	1/8	1/16	1/32	1/64	1/128	1/256	1/512
0.5	0.5/8	0.5/16	0.5/32	0.5/64	0.5/128	0.5/256	0.5/512
0	8	16	32	64	128	256	512
	Meropenem						

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
32/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03	32/256/0.03	32/512/0.03
16/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03	16/256/0.03	16/512/0.03
8/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03	8/256/0.03	8/512/0.03
4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03	4/256/0.03	4/512/0.03
2/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03	2/256/0.03	2/512/0.03
1/0.03	1/8/0.03	1/16/0.03	1/32/0.03	1/64/0.03	1/128/0.03	1/256/0.03	1/512/0.03
0.5/0.03	0.5/8/0.03	0.5/16/0.03	0.5/32/0.03	0.5/64/0.03	0.5/128/0.03	0.5/256/0.03	0.5/512/0.03
0/0/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03	256/0.03	512/0.03
	Meropenem						

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
32/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06	32/512/0.06
16/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06	16/512/0.06
8/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06	8/512/0.06
4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06	4/512/0.06
2/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06	2/512/0.06
1/0.06	1/8/0.06	1/16/0.06	1/32/0.06	1/64/0.06	1/128/0.06	1/256/0.06	1/512/0.06
0.5/0.06	0.5/8/0.06	0.5/82/0.06	0.5/32/0.06	0.5/64/0.06	0.5/128/0.06	0.5/256/0.06	0.5/512/0.06
0/0/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06	512/0.06
	Meropenem						

Figure C-29 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 28 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 4 – Colistin 0.12 µg/ml

Sulbactam							
32/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12	32/512/0.12
16/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12	16/512/0.12
8/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12	8/512/0.12
4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12	4/512/0.12
2/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12	2/512/0.12
1/0.12	1/8/0.12	1/16/0.12	1/32/0.12	1/64/0.12	1/128/0.12	1/256/0.12	1/512/0.12
0.5/0.12	0.5/8/0.12	0.5/82/0.12	0.5/32/0.12	0.5/64/0.12	0.5/128/0.12	0.5/256/0.12	0.5/512/0.12
0/0/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12	512/0.12

Meropenem

Plate 5 – Colistin 0.25 µg/ml

Sulbactam							
32/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25	32/512/0.25
16/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25	16/512/0.25
8/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25	8/512/0.25
4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25	4/512/0.25
2/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25	2/512/0.25
1/0.25	1/8/0.25	1/16/0.25	1/32/0.25	1/64/0.25	1/128/0.25	1/256/0.25	1/512/0.25
0.5/0.25	0.5/8/0.25	0.5/16/0.25	0.5/32/0.25	0.5/64/0.25	0.5/128/0.25	0.5/256/0.25	0.5/512/0.25
0/0/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25	512/0.25

Meropenem

Plate 6 – Colistin 0.5 µg/ml

Sulbactam							
32/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5	32/512/0.5
16/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5	16/512/0.5
8/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5	8/512/0.5
4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5	4/512/0.5
2/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5	2/512/0.5
1/0.5	1/8/0.5	1/16/0.5	1/32/0.5	1/64/0.5	1/128/0.5	1/256/0.5	1/512/0.5
0.5/0.5	0.5/8/0.5	0.5/16/0.5	0.5/32/0.5	0.5/64/0.5	0.5/128/0.5	0.5/256/0.5	0.5/512/0.5
0/0/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5	512/0.5

Meropenem

Figure C-29 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 28 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 7 – Colistin 1 µg/ml

Sulbactam								
32/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1	32/512/1
16/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1	16/512/1
8/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1	8/512/1
4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1	4/512/1
2/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1	2/512/1
1/1	1/8/1	1/16/1	1/32/1	1/64/1	1/128/1	1/256/1	1/512/1
0.5/1	0.5/8/1	0.5/16/1	0.5/32/1	0.5/64/1	0.5/128/1	0.5/256/1	0.5/512/1
0/0/1	8/1	16/1	32/1	64/1	128/1	256/1	512/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam								
32/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2	32/512/2
16/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2	16/512/2
8/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2	8/512/2
4/2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2	4/512/2
2/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2	2/512/2
1/2	1/8/2	1/16/2	1/32/2	1/64/2	1/128/2	1/256/2	1/512/2
0.5/2	0.5/8/2	0.5/16/2	0.5/32/2	0.5/64/2	0.5/128/2	0.5/256/2	0.5/512/2
0/0/2	8/2	16/2	32/2	64/2	128/2	256/2	512/2

Meropenem

Figure C-29 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 28 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 29 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
64	64/4	64/8	64/16	64/32	64/64	64/128	64/256
32	32/4	32/8	32/16	32/32	32/64	32/128	32/256
16	16/4	16/8	16/16	16/32	16/64	16/128	16/256
8	8/4	8/8	8/16	8/32	8/64	8/128	8/256
4	4/4	4/8	4/16	4/32	4/64	4/128	4/256
2	2/4	2/8	2/16	2/32	2/64	2/128	2/256
1	1/4	1/8	1/16	1/32	1/64	1/128	1/256
0	4	8	16	32	64	128	256

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
64 /0.03	64/4/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03	64/256/0.03
32/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03	32/256/0.03
16/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03	16/256/0.03
8/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03	8/256/0.03
4/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03	4/256/0.03
2/0.03	2/4/0.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03	2/256/0.03
1/0.03	1/4/0.03	1/8/0.03	1/16/0.03	1/32/0.03	1/64/0.03	1/128/0.03	1/256/0.03
0/0/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03	256/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
64/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06	64/256/0.06
32/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06
16/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06
8/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06
4/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06
2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06
1/0.06	1/4/0.06	1/8/0.06	1/16/0.06	1/32/0.06	1/64/0.06	1/128/0.06	1/256/0.06
0/0/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06

Meropenem

Figure C-30 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 29 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 4 – Colistin 0.12 µg/ml

64/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12	64/256/0.12
32/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12
16/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12
8/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12
4/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12
2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12
1/0.12	1/4/0.12	1/8/0.12	1/16/0.12	1/32/0.12	1/64/0.12	1/128/0.12	1/256/0.12
0/0/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12

Sulbactam

Meropenem

Plate 5 – Colistin 0.25 µg/ml

64/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25	64/256/0.25
32/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25
16/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25
8/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25
4/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25
2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25
1/0.25	1/4/0.25	1/8/0.25	1/16/0.25	1/32/0.25	1/64/0.25	1/128/0.25	1/256/0.25
0/0/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25

Sulbactam

Meropenem

Plate 6 – Colistin 0.5 µg/ml

64/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5	64/256/0.5
32/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5
16/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5
8/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5
4/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5
2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5
1/0.5	1/4/0.5	1/8/0.5	1/16/0.5	1/32/0.5	1/64/0.5	1/128/0.5	1/256/0.5
0/0/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5

Sulbactam

Meropenem

Figure C-30 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 29 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 7 – Colistin 1 µg/ml

Sulbactam							
64 /1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1	64/256/1
32/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1
16/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1
8/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1
4/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1
2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1
1/1	1/4/1	1/8/1	1/16/1	1/32/1	1/64/1	1/128/1	1/256/1
0/0/1	4/1	8/1	16/1	32/1	64/1	128/1	256/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam							
64/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2	64/256/2
32/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2
16/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2
8/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2
4/2	4/4/2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2
2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2
1/2	1/4/2	1/8/2	1/16/2	1/32/2	1/64/2	1/128/2	1/256/2
0/0/2	4/2	8/2	16/2	32/2	64/2	128/2	256/2

Meropenem

Figure C-30 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 29 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Isolate No. 30 **Plate 1 – No Colistin (0 µg/ml)**

Sulbactam								
128	128/4	128/8	128/16	128/32	128/64	128/128	128/256
64	64/4	64/8	64/16	64/32	64/64	64/128	64/256
32	32/4	32/8	32/16	32/32	32/64	32/128	32/256
16	16/4	16/8	16/16	16/32	16/64	16/128	16/256
8	8/4	8/8	8/16	8/32	8/64	8/128	8/256
4	4/4	4/8	4/16	4/32	4/64	4/128	4/256
2	2/4	2/8	2/16	2/32	2/64	2/128	2/256
0	4	8	16	32	64	128	256

Meropenem

Plate 2 – Colistin 0.03 µg/ml

Sulbactam							
128/0.03	128/4/0.03	128/8/0.03	128/16/0.03	128/32/0.03	128/64/0.03	128/128/0.03	128/256/0.03
64/0.03	64/4/0.03	64/8/0.03	64/16/0.03	64/32/0.03	64/64/0.03	64/128/0.03	64/256/0.03
32/0.03	32/4/0.03	32/8/0.03	32/16/0.03	32/32/0.03	32/64/0.03	32/128/0.03	32/256/0.03
16/0.03	16/4/0.03	16/8/0.03	16/16/0.03	16/32/0.03	16/64/0.03	16/128/0.03	16/256/0.03
8/0.03	8/4/0.03	8/8/0.03	8/16/0.03	8/32/0.03	8/64/0.03	8/128/0.03	8/256/0.03
4/0.03	4/4/0.03	4/8/0.03	4/16/0.03	4/32/0.03	4/64/0.03	4/128/0.03	4/256/0.03
2/0.03	2/40.03	2/8/0.03	2/16/0.03	2/32/0.03	2/64/0.03	2/128/0.03	2/256/0.03
0/0/0.03	4/0.03	8/0.03	16/0.03	32/0.03	64/0.03	128/0.03	256/0.03

Meropenem

Plate 3 – Colistin 0.06 µg/ml

Sulbactam							
128/0.06	128/4/0.06	128/8/0.06	128/16/0.06	128/32/0.06	128/64/0.06	128/128/0.06	128/256/0.06
64/0.06	64/4/0.06	64/8/0.06	64/16/0.06	64/32/0.06	64/64/0.06	64/128/0.06	64/256/0.06
32/0.06	32/4/0.06	32/8/0.06	32/16/0.06	32/32/0.06	32/64/0.06	32/128/0.06	32/256/0.06
16/0.06	16/4/0.06	16/8/0.06	16/16/0.06	16/32/0.06	16/64/0.06	16/128/0.06	16/256/0.06
8/0.06	8/4/0.06	8/8/0.06	8/16/0.06	8/32/0.06	8/64/0.06	8/128/0.06	8/256/0.06
4/0.06	4/4/0.06	4/8/0.06	4/16/0.06	4/32/0.06	4/64/0.06	4/128/0.06	4/256/0.06
2/0.06	2/4/0.06	2/8/0.06	2/16/0.06	2/32/0.06	2/64/0.06	2/128/0.06	2/256/0.06
0/0/0.06	4/0.06	8/0.06	16/0.06	32/0.06	64/0.06	128/0.06	256/0.06

Meropenem

Figure C-31 Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 30 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 4 – Colistin 0.12 µg/ml

Sulbactam								
128/0.12	128/4/0.12	128/8/0.12	128/16/0.12	128/32/0.12	128/64/0.12	128/128/0.12	128/256/0.12
64/0.12	64/4/0.12	64/8/0.12	64/16/0.12	64/32/0.12	64/64/0.12	64/128/0.12	64/256/0.12
32/0.12	32/4/0.12	32/8/0.12	32/16/0.12	32/32/0.12	32/64/0.12	32/128/0.12	32/256/0.12
16/0.12	16/4/0.12	16/8/0.12	16/16/0.12	16/32/0.12	16/64/0.12	16/128/0.12	16/256/0.12
8/0.12	8/4/0.12	8/8/0.12	8/16/0.12	8/32/0.12	8/64/0.12	8/128/0.12	8/256/0.12
4/0.12	4/4/0.12	4/8/0.12	4/16/0.12	4/32/0.12	4/64/0.12	4/128/0.12	4/256/0.12
2/0.12	2/4/0.12	2/8/0.12	2/16/0.12	2/32/0.12	2/64/0.12	2/128/0.12	2/256/0.12
0/0/0.12	4/0.12	8/0.12	16/0.12	32/0.12	64/0.12	128/0.12	256/0.12

Meropenem

Plate 5 – Colistin 0.25 µg/ml

Sulbactam								
128/0.25	128/4/0.25	128/8/0.25	128/16/0.25	128/32/0.25	128/64/0.25	128/128/0.25	128/256/0.25
64/0.25	64/4/0.25	64/8/0.25	64/16/0.25	64/32/0.25	64/64/0.25	64/128/0.25	64/256/0.25
32/0.25	32/4/0.25	32/8/0.25	32/16/0.25	32/32/0.25	32/64/0.25	32/128/0.25	32/256/0.25
16/0.25	16/4/0.25	16/8/0.25	16/16/0.25	16/32/0.25	16/64/0.25	16/128/0.25	16/256/0.25
8/0.25	8/4/0.25	8/8/0.25	8/16/0.25	8/32/0.25	8/64/0.25	8/128/0.25	8/256/0.25
4/0.25	4/4/0.25	4/8/0.25	4/16/0.25	4/32/0.25	4/64/0.25	4/128/0.25	4/256/0.25
2/0.25	2/4/0.25	2/8/0.25	2/16/0.25	2/32/0.25	2/64/0.25	2/128/0.25	2/256/0.25
0/0/0.25	4/0.25	8/0.25	16/0.25	32/0.25	64/0.25	128/0.25	256/0.25

Meropenem

Plate 6 – Colistin 0.5 µg/ml

Sulbactam								
128/0.5	128/4/0.5	128/8/0.5	128/16/0.5	128/32/0.5	128/64/0.5	128/128/0.5	128/256/0.5
64/0.5	64/4/0.5	64/8/0.5	64/16/0.5	64/32/0.5	64/64/0.5	64/128/0.5	64/256/0.5
32/0.5	32/4/0.5	32/8/0.5	32/16/0.5	32/32/0.5	32/64/0.5	32/128/0.5	32/256/0.5
16/0.5	16/4/0.5	16/8/0.5	16/16/0.5	16/32/0.5	16/64/0.5	16/128/0.5	16/256/0.5
8/0.5	8/4/0.5	8/8/0.5	8/16/0.5	8/32/0.5	8/64/0.5	8/128/0.5	8/256/0.5
4/0.5	4/4/0.5	4/8/0.5	4/16/0.5	4/32/0.5	4/64/0.5	4/128/0.5	4/256/0.5
2/0.5	2/4/0.5	2/8/0.5	2/16/0.5	2/32/0.5	2/64/0.5	2/128/0.5	2/256/0.5
0/0/0.5	4/0.5	8/0.5	16/0.5	32/0.5	64/0.5	128/0.5	256/0.5

Meropenem

Figure C-31 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 30 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

Plate 7 – Colistin 1 µg/ml

Sulbactam								
128/1	128/4/1	128/8/1	128/16/1	128/32/1	128/64/1	128/128/1	128/256/1
64 /1	64/4/1	64/8/1	64/16/1	64/32/1	64/64/1	64/128/1	64/256/1
32/1	32/4/1	32/8/1	32/16/1	32/32/1	32/64/1	32/128/1	32/256/1
16/1	16/4/1	16/8/1	16/16/1	16/32/1	16/64/1	16/128/1	16/256/1
8/1	8/4/1	8/8/1	8/16/1	8/32/1	8/64/1	8/128/1	8/256/1
4/1	4/4/1	4/8/1	4/16/1	4/32/1	4/64/1	4/128/1	4/256/1
2/1	2/4/1	2/8/1	2/16/1	2/32/1	2/64/1	2/128/1	2/256/1
0/0/1	4/1	8/1	16/1	32/1	64/1	128/1	256/1

Meropenem

Plate 8 – Colistin 2 µg/ml

Sulbactam								
128/2	128/4/2	128/8/2	128/16/2	128/32/2	128/64/2	128/128/2	128/256/2
64/2	64/4/2	64/8/2	64/16/2	64/32/2	64/64/2	64/128/2	64/256/2
32/2	32/4/2	32/8/2	32/16/2	32/32/2	32/64/2	32/128/2	32/256/2
16/2	16/4/2	16/8/2	16/16/2	16/32/2	16/64/2	16/128/2	16/256/2
8/2	8/4/2	8/8/2	8/16/2	8/32/2	8/64/2	8/128/2	8/256/2
4/2	4/4/2	4/8/2	4/16/2	4/32/2	4/64/2	4/128/2	4/256/2
2/2	2/4/2	2/8/2	2/16/2	2/32/2	2/64/2	2/128/2	2/256/2
0/0/2	4/2	8/2	16/2	32/2	64/2	128/2	256/2

Meropenem

Figure C-31 (continued) Three dimensional microdilution checkerboard result of *A. baumannii* isolate No. 30 at any series of proportional concentrations of meropenem, colistin, and sulbactam; shadow zone: visible microorganism growth, white zone: no microorganism growth was observed.

APPENDIX D

Time kill curves of 10 isolates of *A. baumannii*

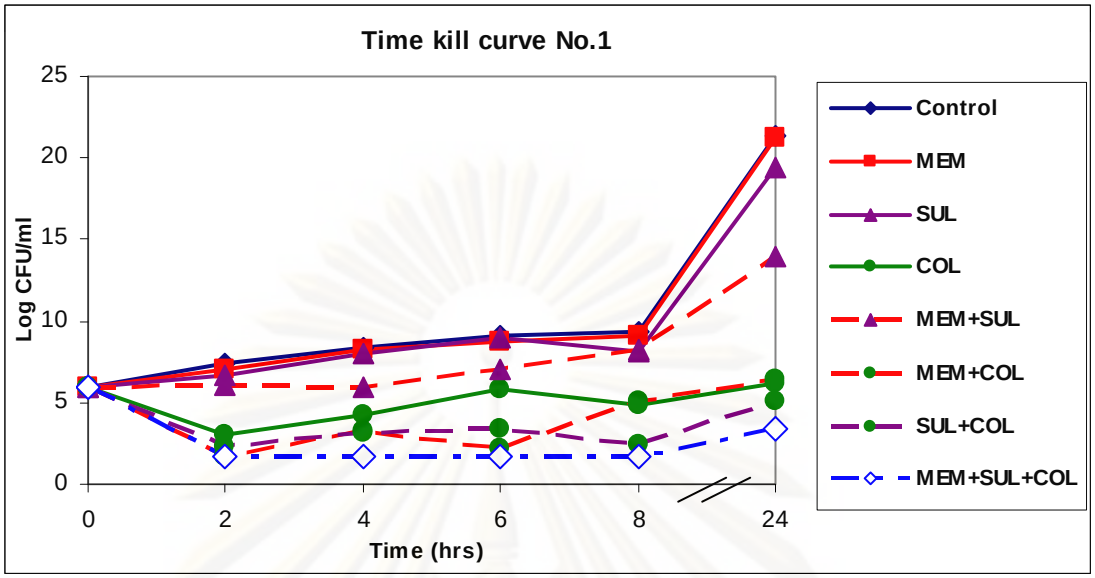

Figure D-1 Time-kill curve of meropenem 50 µg/ml, sulbactam 30 µg/ml, and colistin 0.5 µg/ml alone and in combinations against *A. baumannii* isolates no. 1.

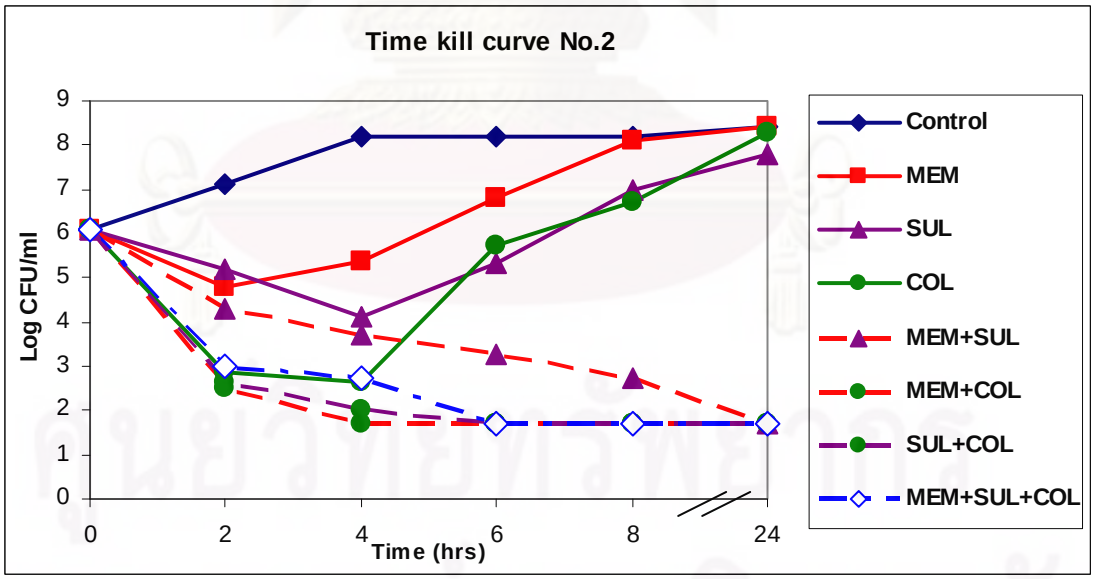

Figure D-2 Time-kill curve of meropenem 50 µg/ml, sulbactam 30 µg/ml, and colistin 0.5 µg/ml alone and in combinations against *A. baumannii* isolates no. 2.

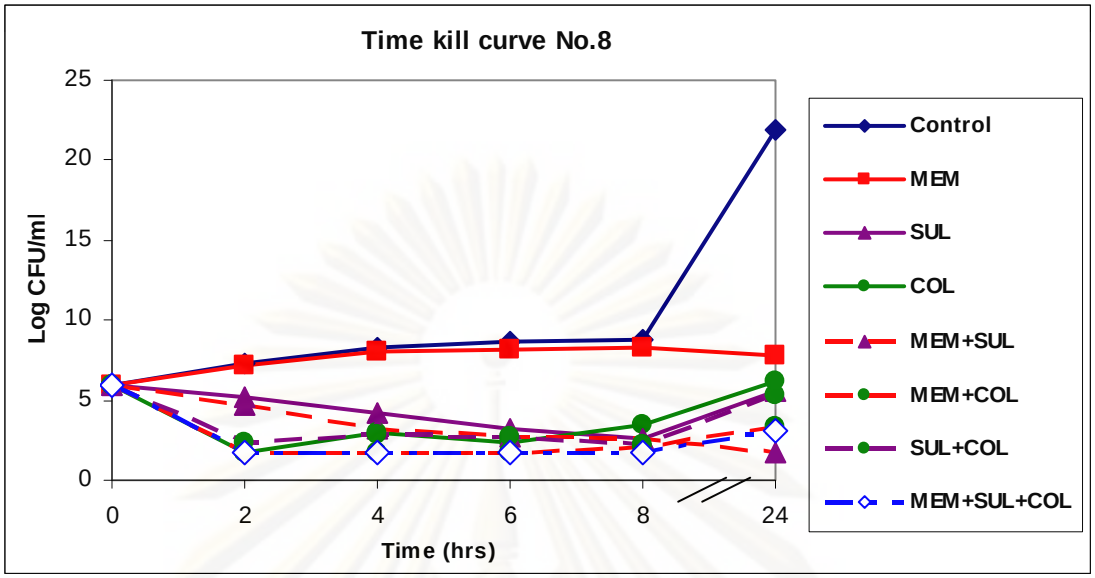

Figure D-3 Time-kill curve of meropenem 50 µg/ml, sulbactam 30 µg/ml, and colistin 0.5 µg/ml alone and in combinations against *A. baumannii* isolates no. 8.

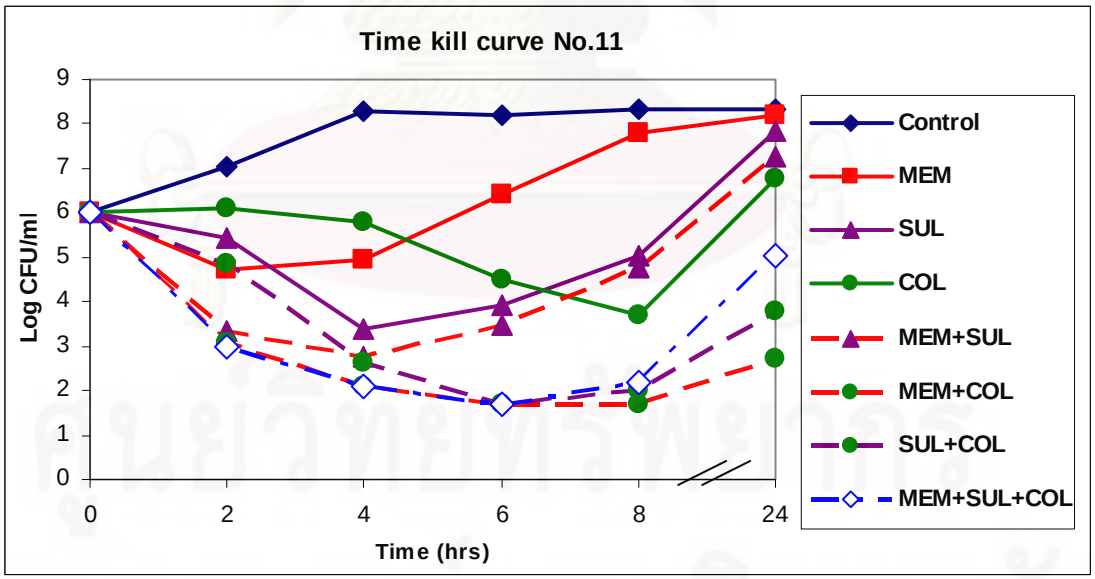

Figure D-4 Time-kill curve of meropenem 50 µg/ml, sulbactam 30 µg/ml, and colistin 0.5 µg/ml alone and in combinations against *A. baumannii* isolates no. 11.

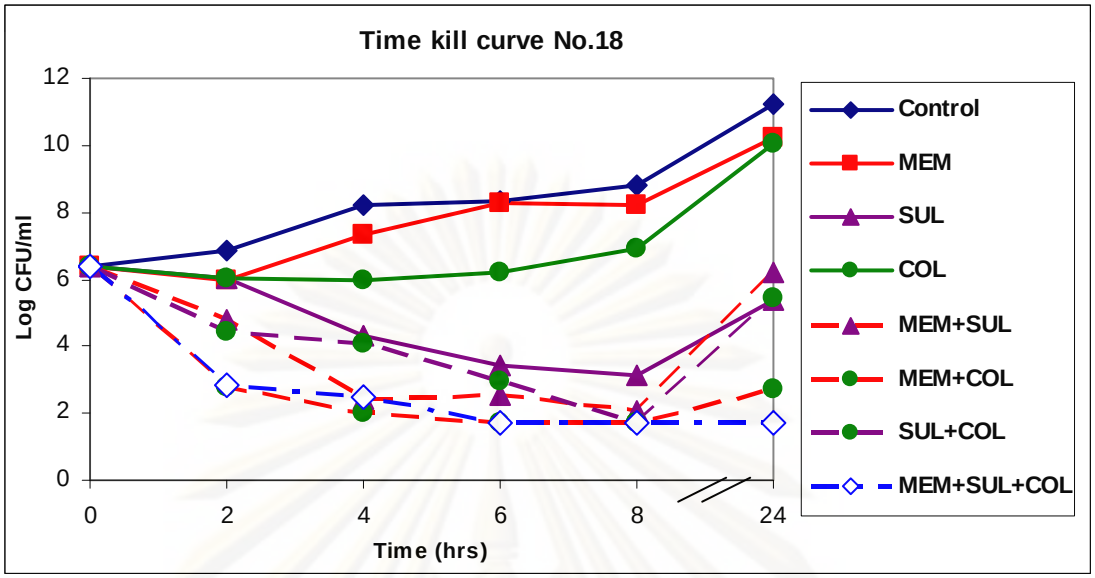

Figure D-5 Time-kill curve of meropenem 50 µg/ml, sulbactam 30 µg/ml, and colistin 0.5 µg/ml alone and in combinations against *A. baumannii* isolates no. 18.

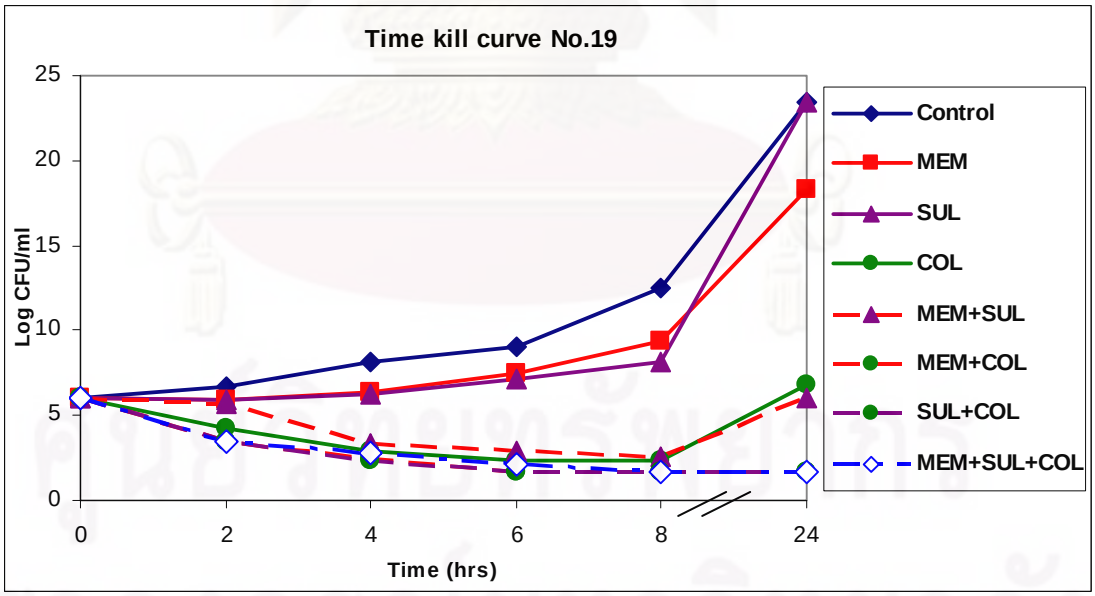

Figure D-6 Time-kill curve of meropenem 50 µg/ml, sulbactam 30 µg/ml, and colistin 0.5 µg/ml alone and in combinations against *A. baumannii* isolates no. 19.

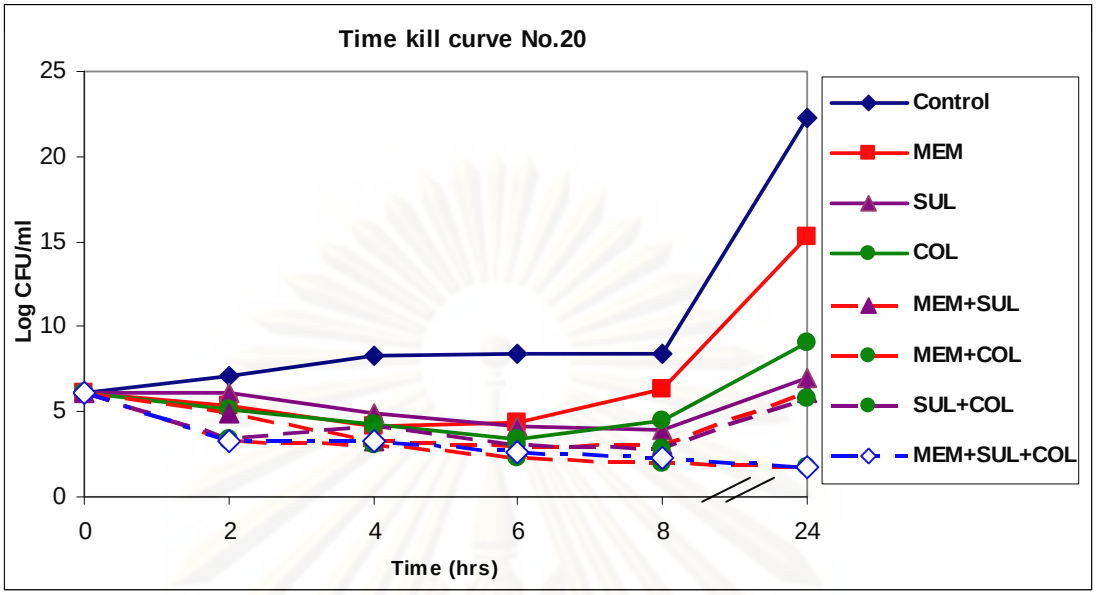

Figure D-7 Time-kill curve of meropenem 50 µg/ml, sulbactam 30 µg/ml, and colistin 0.5 µg/ml alone and in combinations against *A. baumannii* isolates no. 20.

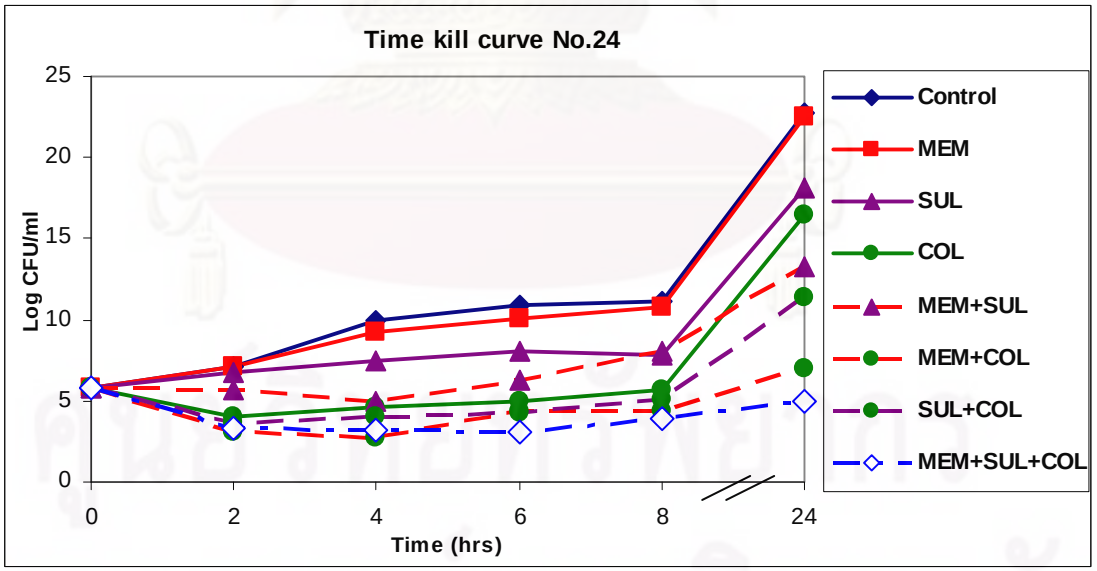

Figure D-8 Time-kill curve of meropenem 50 µg/ml, sulbactam 30 µg/ml, and colistin 0.5 µg/ml alone and in combinations against *A. baumannii* isolates no. 24.

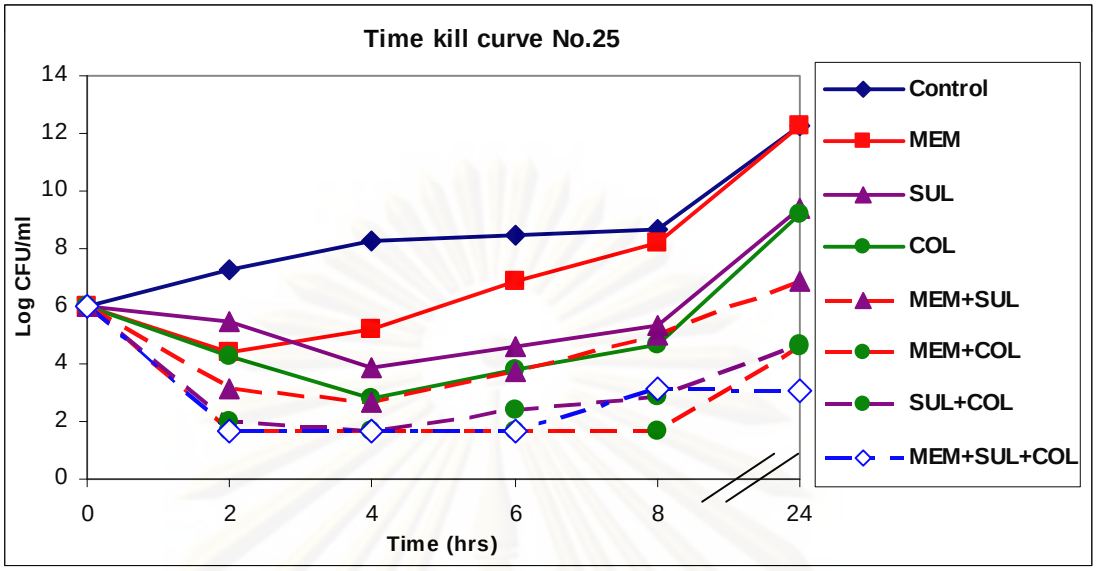

Figure D-9 Time-kill curve of meropenem 50 µg/ml, sulbactam 30 µg/ml, and colistin 0.5 µg/ml alone and in combinations against *A. baumannii* isolates no. 25.

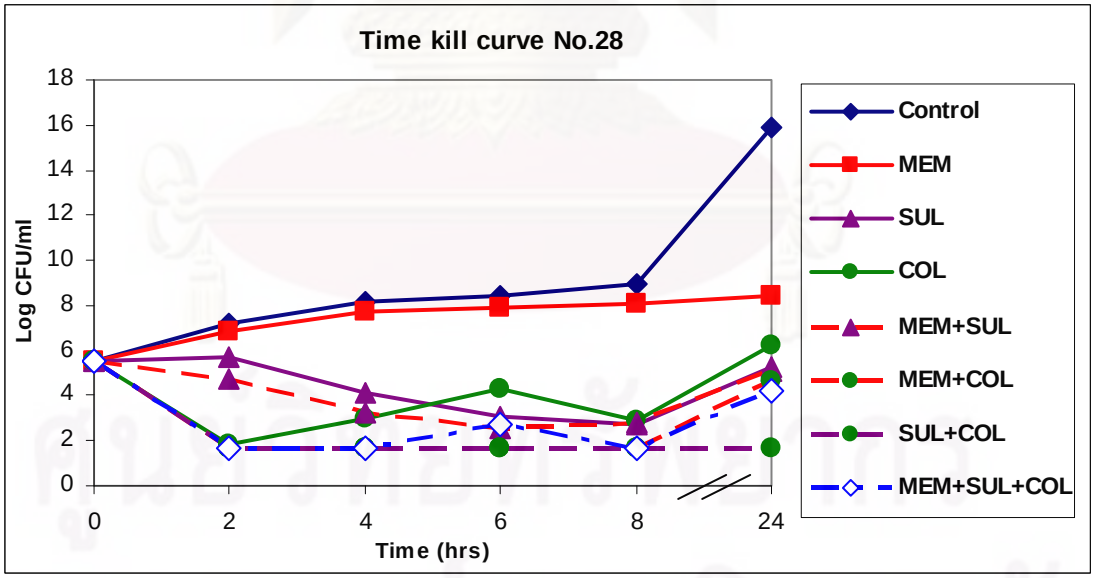

Figure D-10 Time-kill curve of meropenem 50 µg/ml, sulbactam 30 µg/ml, and colistin 0.5 µg/ml alone and in combinations against *A. baumannii* isolates no. 28.

BIOGRAPHY

My name is Suparak Amornnopparattanakul. I was born in August 13, 1979 in Uttaradit, Thailand. I graduated the Bachelor of Pharmacy from the Faculty of Pharmaceutical Sciences, Mahidol University and have enrolled for the Master of Science in Pharmacy Program in Pharmacology in Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University since June, 2007.