ฤทธิ์ต้านเชื้อของเมอโรพีเนม ไซโพรฟลอกซาซินและโคลิสทิน เมื่อให้ร่วมกันสองและสามชนิด ในหลอดทดลองต่อเชื้อซูโดโมแนส แอรูจิโนซา ที่ดื้อต่อยาเมอโรพีเนม

นางสาวแคทรียา เอกอัครนวกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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IN VITRO ANTIMICROBIAL ACTIVITIES OF DOUBLE AND TRIPLE COMBINATIONS OF MEROPENEM, CIPROFLOXACIN AND COLISTIN AGAINST MEROPENEM-RESISTANT *PSEUDOMONAS AERUGINOSA*

Miss Katareeya Ek-akaranawakul

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แคทรียา เอกอัครนวกุล : ฤทธิ์ต้านเชื้อของเมอโรพีเนม ไซโพรฟลอกซาซินและโคลิสทิน เมื่อให้ร่วมกันสองและสามชนิดในหลอดทดลองต่อเชื้อซูโดโมแนส แอรูจิโนซา ที่ดื้อต่อยาเมอโรพีเนม. (*IN VITRO* ANTIMICROBIAL ACTIVITIES OF DOUBLE AND TRIPLE COMBINATIONS OF MEROPENEM, CIPROFLOXACIN AND COLISTIN AGAINST MEROPENEM-RESISTANT *PSEUDOMONAS AERUGINOSA*) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : อ. ดร.สันทัด จันทร์ประภาพ, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ. ดร.พิณทิพย์ พงษ์เพ็ชร, 213 หน้า.

จากการศึกษาฤทธิ์ต้านเชื้อของยา meropenem ciprofloxacin และ colistin เมื่อให้ร่วมกันสองและสามชนิดต่อเชื้อ *P. aeruginosa* จำนวน 30 ไอโซเลท พบว่าเชื้อ *P. aeruginosa* 26 ไอโซเลท (86.67%) มีการดื้อต่อยาหลายชนิด (multidrug-resistant isolate) และทุกไอโซเลทดื้อต่อยา meropenem (MIC_{90}= 32 µg/mL) เชื้อมีความไวต่อยา ciprofloxacin เพียง 4 ไอโซเลท (13.33%) (MIC_{90}= 128 µg/mL) แต่ยังมีความไวต่อ colistin ทุกไอโซเลท (MIC_{90}= 2 µg/mL) ผลจากการประเมินการใช้ยาดังกล่าวร่วมกันโดยวิธี checkerboard พบว่าเมื่อให้ meropenem ร่วมกับ ciprofloxacin เกิดการเสริมฤทธิ์กัน (synergy) ต่อเชื้อเพียง 9 ไอโซเลท (30%) ที่ความเข้มข้นของ meropenem และ ciprofloxacin ในช่วง 4-16 µg/mL และ 0.25-64 µg/mL ตามลำดับ เมื่อให้ meropenem ร่วมกับ colistin หรือ ciprofloxacin ร่วมกับ colistin เกิดการเสริมฤทธิ์กันต่อเชื้อ 11 ไอโซเลทเท่ากัน โดยการเสริมฤทธิ์กันระหว่าง meropemem และ colistin นั้นเกิดที่ความเข้มข้นของยาทั้งสองในช่วง 1-8 µg/mL และ 0.5-1 µg/mL ตามลำดับ ส่วนการเสริมฤทธิ์กันระหว่าง ciprofloxacin และ colistin นั้นอยู่ในช่วง 0.06-32 µg/mL และ 0.5-1 µg/mL ตามลำดับ ในขณะที่เมื่อให้ยาทั้งสามชนิดนี้ร่วมกัน พบว่าเกิดการเสริมฤทธิ์กันต่อเชื้อ 18 ไอโซเลท (60%) ที่ความเข้มของ meropenem ciprofloxacin และ colistin ในช่วง 1-16 µg/mL, 0.06-64 µg/mL และ 0.06-1 µg/mL ตามลำดับ เมื่อนำเชื้อ *P. aeruginosa* ที่ดื้อต่อยา meropenem ในระดับที่แตกต่างกันจำนวน 5 ไอโซเลท ประเมินผลในการฆ่าเชื้อโดยวิธี time kill พบว่าเมื่อให้ meropenem เดี่ยว (16 และ 25 µg/mL) มีเพียง meropenem เดี่ยว 25 µg/mL ที่สามารถลดจำนวนเชื้อ 2 ไอโซเลทได้ 99.9% ที่เวลา 8 ชั่วโมง แต่เชื้อกลับเจริญขึ้นได้อีกที่เวลา 24 ชั่วโมง ส่วน ciprofloxacin เดี่ยว 4 µg/mL มีเพียง 1 ไอโซเลทที่จำนวนเชื้อลดลงไป 99% ที่เวลา 2 ชั่วโมง แต่เชื้อกลับเจริญขึ้นได้อีกที่ 24 ชั่วโมง ในขณะที่ colistin เดี่ยว 0.5xMIC ลดจำนวนเชื้อได้ 99.9% กับเชื้อทั้ง 5 ไอโซเลทที่เวลา 2 ชั่วโมงจนถึงที่เวลา 8 ชั่วโมงพบลดลงเป็น 4 ไอโซเลท ซึ่งทุกไอโซเลทกลับเจริญขึ้นได้อีกที่ 24 ชั่วโมง เมื่อให้ยา meropenem (16 หรือ 25 µg/mL) ร่วมกับ ciprofloxacin พบว่าส่วนใหญ่ไม่ช่วยเพิ่มฤทธิ์การฆ่าเชื้อ มีเพียง 1 ไอโซเลทเท่านั้นที่จำนวนเชื้อลดลงไปในระดับ 99.9% แต่เชื้อกลับเจริญขึ้นได้อีกที่เวลา 24 ชั่วโมง เมื่อให้ meropenem แต่ละความเข้มข้นร่วมกับ colistin 0.5xMIC สามารถลดจำนวนเชื้อทั้ง 5 ไอโซเลทได้ 99.9% ที่เวลา 2-24 ชั่วโมง ส่วนการให้ ciprofloxacin ร่วมกับ colistin ให้ผลต่อการลดจำนวนเชื้อในลักษณะเดียวกับการให้ colistin เดี่ยว การให้ยาทั้งสามชนิดร่วมกันให้ผลในการลดจำนวนเชื้อคล้ายกับการให้ meropenem ร่วมกับ colistin เมื่อพิจารณาจำนวนเชื้อที่ถูกฆ่าภายใน 24 ชั่วโมงจากค่า BA_{24} พบว่าค่า BA_{24} ของ colistin 0.5xMIC เดี่ยวแตกต่างจาก control อย่างมีนัยสำคัญทางสถิติ ($p<0.05$) การให้ merpenem (16 หรือ 25 µg/mL) ร่วมกับ ciprofloxacin 4 µg/mL มีค่า BA_{24} ไม่แตกต่างจากการให้ meropenem เดี่ยว ($p>0.05$) เช่นเดียวกับการให้ ciprofloxacin ร่วมกับ colistin ไม่แตกต่างจากการให้ colistin เดี่ยว ส่วนการให้ meropenem ร่วมกับ colistin ให้ผลแตกต่างจากการให้ยาเดี่ยวของ meropenem และ colistin ($p<0.05$) แต่ไม่แตกต่างจากการให้ยาร่วมกันสามชนิด ($p>0.05$) สำหรับผลของการเปลี่ยนแปลงสัณฐานวิทยาของเชื้อเมื่อเจริญในยา meropenem ร่วมกับ colistin พบว่าที่ผนังเซลล์ของเชื้อมีการเปลี่ยนแปลงโดยผนังเซลล์มีการถูกทำลายเมื่อเทียบกับเซลล์ที่ไม่ได้รับยา

จากการทดลองแสดงให้เห็นว่าการใช้ meropenem (16 หรือ 25 µg/mL) ร่วมกับ colistin 0.5xMIC มีประสิทธิภาพดีที่สุด จึงอาจเป็นอีกทางเลือกหนึ่งในการรักษาโรคติดเชื้อที่เกิดจาก *P. aeruginosa* ที่ดื้อต่อยา meropenem และอาจช่วยลดขนาดของ colistin ที่จะใช้ในการรักษาเพื่อลดอัตราการเกิดพิษของ colistin ได้

ภาควิชา เภสัชวิทยาและสรีรวิทยา ลายมือชื่อนิสิต .....

สาขาวิชา เภสัชวิทยา ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ปีการศึกษา 2552 ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

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KATAREEYA EK-AKARANAWAKUL : *IN VITRO* ANTIMICROBIAL ACTIVITIES OF DOUBLE AND TRIPLE COMBINATIONS OF MEROPENEM, CIPROFLOXACIN AND COLISTIN AGAINST MEROPENEM-RESISTANT *PSEUDOMONAS AERUGINOSA*. THESIS ADVISOR : SANTAD CHANPRAPAPH, Ph.D., THESIS CO-ADVISOR : ASSOC. PROF. PINTIP PONGPECH, Ph.D., 213 pp.

Determination of *in vitro* antimicrobial activities of double and triple combinations of meropenem, ciprofloxacin and colistin against 30 clinical isolates of *P. aeruginosa* found that twenty-six isolates (86.67%) were multidrug-resistant isolates (MDR) and all isolates were meropenem-resistant (MIC_{90}= 16 µg/mL). Only 4 isolates (13.33%) were susceptible to ciprofloxacin (MIC_{90}=128 µg/mL). Whereas all isolates were susceptible to colistin (MIC_{90}= 2 µg/mL). The results from checkerboard method have shown that the combination of meropenem with ciprofloxacin had synergistic effect against only 9 isolates (30%) at concentrations ranged from 4-16 µg/mL and 0.25-64 µg/mL, respectively. For combination of meropenem and colistin, it had synergistic effect against 11 isolates the same as ciprofloxacin combined with colistin. Synergistic effect of meropenem and colistin was observed at concentrations ranged from 1-8 µg/mL and 0.5-1 µg/mL, respectively, whereas such effect was observed at ciprofloxacin and colistin concentrations ranged from 0.06-32 µg/mL and 0.5-1 µg/mL, respectively. In addition, triple combination of these agents showed synergistic effect against 18 isolates (60%) at meropenem, ciprofloxacin and colistin concentrations ranged from 1-16 µg/mL, 0.06-64 µg/mL and 0.06-1 µg/mL, respectively. In time-kill study, 5 isolates of meropenem-resistant *P. aeruginosa* were tested. When exposed to meropenem 16 and 25 µg/mL alone, 99.9% killing was observed in 2 isolates at 8^{th} hour with meropenem 25 µg/mL and then the regrowth occurred at 24^{th} hour. For ciprofloxacin 4 µg/mL alone, only 1 isolate was killed at level of 99% killing at 2^{nd} hour and the regrowth was observed at 24^{th} hour. Whereas, 0.5xMIC colistin alone, 99.9% killing was observed in all isolates at 2^{nd} hour. However, such effect was observed in only 4 isolates at 8^{th} hour then the regrowth of all isolates occurred at the 24^{th} hour. The combinations of meropenem (16 or 25 µg/mL) and ciprofloxacin seem not to increase antimicrobial activity, only 1 isolate was killed at level of 99.9% killing. On the contrary, the combinations of meropenem (16 or 25 µg/mL) with 0.5xMIC colistin, 99.9% killing was observed in all isolates at 2^{nd}-24^{th} hour. The same results were observed in triple combinations. In addition, ciprofloxacin in combination with colistin showed the same results as colistin alone.

BA_{24} value of 0.5xMIC colistin alone showed significantly different from control ($p<0.05$). BA_{24} values of meropenem (16 or 25 µg/mL) in combination with 4 µg/mL ciprofloxacin were not significantly different from meropenem alone ($p>0.05$) the same as ciprofloxacin in combination with colistin compared to colistin alone. Whereas meropenem (16 or 25 µg/mL) combined with 0.5xMIC colistin were significantly different from meropenem at each concentration alone and colistin alone as well ($p<0.05$). Moreover, there were not significantly different from triple combinations ($p>0.05$). The morphology change of such *P. aeruginosa* exposed to meropenem combined with colistin occurred with alteration of bacterial membrane resulted in cell break and cell debris when compared to control.

The results have shown that antibacterial activity of meropenem (16 or 25 µg/mL) combined with 0.5xMIC colistin were most effective. Therefore, the combination of meropemem with low dose of colistin could be the promising alternative treatment for infections caused by meropenem-resistant *P. aeruginosa*. The lower dose of colistin could also lead to the decreased nephrotoxicity in patients.

Department : Pharmacology and Physiology Student's Signature

Field of Study : Pharmacology Advisor's Signature

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LIST OF ABBREVIATIONS

%	=	percent
β	=	beta
μ	=	micro
°C	=	degree of celsius
μg	=	microgram
ATCC	=	American Type Culture Collection
AUBKC	=	Area under the bacterial killing regrowth curves
BA_{24}	=	Bacteriolytic area of 24 hours
BSI	=	bloodstream infection
CFU	=	colony forming unit
CLSI	=	Clinical and Laboratory Standards Institute
cm	=	centimeter
DHP-1	=	dehydropeptidase-I
E. coli	=	*Escherichia coli*
EDTA	=	ethylenediaminetetraacetic acid
et al.	=	et alii (and other peoples)
FIC	=	Fractional Inhibitory Concentration
g	=	gram
GIM	=	German imipenemase
hr	=	hour
ICU	=	intensive care unit
IMP	=	imipenemase
L	=	liter
log	=	decimal logarithm
MBL	=	metallo-β-lactamase
Mex	=	Multidrug efflux
mg	=	milligram
MDR	=	multidrug-resistant

MHA	=	Mueller-Hinton agar
MHB	=	Mueller-Hinton broth
MIC	=	Minimum Inhibitory Concentration
min	=	minute
mL	=	milliliter
mm	=	millimeter
MPA	=	mercaptopropionic acid
MYSTIC	=	Meropenem Yearly Susceptibility Test Information Collection
NARST	=	National Antimicrobial Resistance Surveillance Center, Thailand
NCCLS	=	The Nation Committee for Clinical Laboratory Standards
NNIS	=	National Nosocomial Infections Surveillance System
No.	=	number of isolates
Opr	=	outer membrane proteins
P. aeruginosa	=	*Pseudomonas aeruginosa*
PBP	=	penicillin-binding protein
RND	=	Resistance-nodulation-division
SIM	=	Seoul imipenemase
SPM	=	São Paulo metallo-β-lactamase
TSA	=	Tryptic soy agar
TRUST	=	Tracking Resistance in the United States Today
UTI	=	Urinary Tract Infection
VAP	=	Ventilator associated pneumonia
VIM	=	Verona imipenemase

CHAPTER I

INTRODUCTION

Background and rationale

Pseudomonas aeruginosa (*P. aeruginosa*) is an opportunistic pathogen that continues to be the major causes of hospital-acquired (nosocomial) infection in seriously ill patients worldwide (NNIS, 1998, 2003, 2004; Hidron *et al*., 2008). It tolerates to a wide variety of physical conditions and can be isolated from nearly any conceivable source within hospitals. The high incidence of *P. aeruginosa* infections was observed in the hospitals in Thailand as well as in other countries all over the world and is an important health problem (Danchaivijitr *et al*., 2005, 2007).

This organism rarely causes infection in healthy persons. Whenever defensives are compromised, it can infect virtually all body systems. In most cases, the process begins with patients who have alteration of normal host defense. This may involve a disruption in the integrity of physical barrier to bacterial invasion, such as the use of urinary catheter, endotracheal tube, intravenous line or injury with burn. In other instances, there is an underlying dysfunction of specific host defense mechanism, such as neutropenia, immunosuppression or immunocompromised (Gales *et al*., 2001). These can cause nosocomial infections such as ventilator associated pneumonia (VAP), urinary tract infection (UTI), bacteremia and surgical site infection. It was the second most common pathogen recovered among intensive care unit (ICU) patients in a study in North America (Streit *et al*., 2004) and the most commonly isolated gram-negative bacteria (18.1%) for nosocomial pneumonia and the second most commonly isolated gram-negative bacteria (16.3%) for nosocomial urinary tract infection in the United State (Gaynes and Edwards, 2005; Tam *et al*., 2007).

Increasing prevalence of multidrug-resistant *P. aeruginosa* has been a problem worldwide (Andrade *et al*., 2003; Goossens, 2003; Tsujii *et al*., 2003; Van Eldere, 2003; NNIS, 2004; Obritsch *et al*., 2004; Paterson *et al*., 2005) and considered as a major cause of morbidity and mortality for hospitalized patients (Carmeli *et al*., 1999; Niederman, 2001; Dimatatac, 2003). According to the 2004 NNIS system report, among ICU patients, the rate of resistance to third-generation/antipseudomonal cephalosporins, imipenem, and quinolone increased by 20%, 15% and 9%, respectively, of *P. aeruginosa* strains isolated in 2003 when compared with the results from 1998 through 2002 (McGowan, 2006).

Carbapenems, mainly imipenem and meropenem, are traditionally one of last lines of agents that are use in the treatment of multidrug-resistant pseudomonal infection. These drugs have considerable β-lactamase stability and overall have the broadest spectrum of activity when compared with other β-lactams. Meropenem differs from imipenem in the fact that it is stable to human renal dehydropeptidase-I (DHP-I) and more potent against gram-negative organism including *P. aeruginosa* (White *et al*., 1996; Blumer,1997; Knapp and English, 2001).

However, the emerging of carbapenem resistance has been documented and trends to increase among *P. aeruginosa* isolates (Goossens, 2003; Van Eldere, 2003; Sasaki *et al*. 2004; Hsueh *et al*., 2005; Hocquet *et al*., 2007; Jones *et al*., 2008). In Thailand, the study at Songklanagarind Hospital showed that 54.71% of *P. aeruginosa* isolates were low level imipenem resistant and 45.28% were high level resistance (Tunyapanit, 2003). Data from National Antimicrobial Resistance Surveillance Center, Thailand (NARST) showed that imipenem susceptibility has been decreased from 85.0% in year 2000 to 82.0% in year 2006. In addition, antimicrobial susceptibility to meropenem was less than 80% in year 2007 (http://narst.dmsc.moph.go.th/). Rising in the prevalence of carbapenem resistance among *P. aeruginosa* has been a clinical problem in worldwide since it can cause life-threatening infection.

Mechanisms of resistance to carbapenems are associated with reduced uptake as a result of qualitative or quantitative alteration of the OprD porin as well as overexpression of multidrug efflux pump system. The transmissible carbapenemase (metallo-β-lactamase: MBL) confers high grade resistance to the carbapenems (Knapp and English, 2001; Livermore, 2001; Hancock and Speert, 2002; Mesaros *et al*., 2007; Farra *et al*, 2008; Giske *et al*., 2008).

While multidrug-resistant *P. aeruginosa*, including resistant to carbapenems have been increased but the novel antimicrobial agents with activity against *P. aeruginosa* will not be available in the near future, making ongoing surveillance of the activities of currently available agent critical. New therapies are needed urgently. The combination therapy with two or more of antimicrobial agents will be alternative treatments, because it can be used to expand the antimicrobial spectrum, to prevent the emergence of resistant mutants, to minimize toxicity, and to obtain synergistic antimicrobial activity (Sader *et al*., 2003; Drago *et al*., 2005; Rahal, 2006; Tan *et al*., 2007). Although, previous data demonstrated the synergistic effects between carbapenems and aminoglycosides or fluoroquinolones (lower toxicity) (Nakamura *et al*., 2000; Ermertcan *et al*., 2001; Oie *et al*., 2003; Solak *et al*., 2003; Kanellakopoulou *et al*., 2008), resistances to each of these agents have also been documented (Nakajima *et al*., 2002; Poole, 2005). This resulted in a limited number of effective antimicrobial agents for clinical use.

Recently, there has been the renewed interest in polymyxin, old class of antimicrobial agent, which had earlier been abandoned because of their serious adverse effects. For example, colistin which has the excellent *in vitro* activity against many species of aerobic gram-negative bacilli was extensively used during the 1960s to the early 1980s because multidrug-resistant *P. aeruginosa* including carbapenem resistant isolates are also susceptible to colistin (Tribuddharat *et al*., 2003; Wongwatcharapaiboon, 2007). In addition, there are several experimentals and clinical studies in the literature regarding safety, efficacy and synergistic effects of colistin and

with other antimicrobial agents against multidrug-resistant gram-negative bacteria (Conway, 1997; Rynn *et al*., 1999; Falagas *et al*., 2005; Lanmand *et al*. 2005; Timurkaynak *et al*., 2006; Cirioni *et al*., 2007; Punkuch *et al*., 2008).

Data from Koomanachai *et al*. (2007) demonstrated the efficacy and safety of colistin (colistimethate sodium) for therapy of infection caused by multidrug-resistant *P. aeruginosa* and *Acinetobacter baumannii* in Siriraj Hospital, Bangkok, Thailand between January 2005 and April 2006. In colistin group, a good clinical and microbiological response were observed. Therefore, colistin appears to be safe and effective for treatment of infections caused by multidrug-resistant *P. aeruginosa* and *A. baumannii* in Thai adult patients.

Hypothesis and aims of this thesis

As previously mentioned, the hypothesis of this study is that the combination of meropenem, ciprofloxacin and colistin could provide synergistic antimicrobial effects against meropenem-resistant *P. aeruginosa*. Therefore, this study will emphasize on the effects of meropenem, ciprofloxacin, colistin and the combinations on the meropenem-resistant *P. aeruginosa* isolated from Thai patients. In order to obtain the informative conclusions on this aspect, the experimental studies are designed to determine:

1. The antimicrobial susceptibility of *P. aeruginosa* from clinical isolates against broad-spectrum antimicrobial agents which are commonly used in the treatment of *P. aeruginosa* infection.

2. The combination effects of double and triple combinations of meropenem, ciprofloxacin and colistin against the clinical isolates of *P. aeruginosa*.

3. Bactericidal activity and morphological changes of meropenem-resistant *P. aeruginosa* after treated with antimicrobial agents alone and in combination.

Anticipated benefit from the study

Obtained the preliminary information of the combination effects between meropenem, ciprofloxacin and colistin useful for the consideration in a treatment of meropenem-resistant *P. aeruginosa*.

CHAPTER II

LITERATURE REVIEWS

Background of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa (*P. aeruginosa*) is a gram-negative, aerobic rod, possessing a strict respiratory metabolism and belong to the bacterial family of *Pseudomonadaceae*. It lives primarily in water, soil, vegetation and animals (including humans). Most isolates produce the water-soluble pigments pyoverdine and pyocyanin, which together confer the bright green color characteristic of the organism (Giske, 2007).

P. aeruginosa rarely infects healthy tissue, but, when defensive are compromised, it can infects virtually all tissue. This bacteria is an important cause of both community-acquired infections and hospital-acquired infections (nosocomial infection). Community-acquired infections include ulcerative keratitis (usually associated with contact lens use or minor trauma), otitis externa of swimmer's ear and typically in immunocompromised hosts such as those with diabetes mellitus, skin and soft tissue infections (including diabetic foot infections). Hospitalized patients may be colonized with *P. aeruginosa* on admission or may acquire the organism during their hospital stay, particularly the patients with compromised host defense mechanism. Hospital-acquired infections caused by *P. aeruginosa* including ventilator associated pneumonia (VAP), urinary tract infections (UTIs), bloodstream infections, wound infections in burn patients. This organism is well-adapted to respiratory tract environment, especially in patients with chronic obstructive bronchopulmonary disease, who are immunocompromised. It also causes chronic colonization of airways of patients suffering from bronchiectasis, chronic obstructive bronchopulmonary disease or cystic fibrosis (CF) (Mesaros *et al*., 2007; Giske, 2007). The main pathologies caused by *P. aeruginosa* are listed in table 2-1.

Table 2-1 Main pathologies caused by *P. aeruginosa*, grouped according to the infection site. (Modified from Mesaros *et al*., 2007).

Infection site	Specific pathologies	Occurrence (at risk population)
Respiratory tract	Acute pneumonia	Frequent (hospital; ICU)
	Chronic lower respiratory tract infections	(Cystic fibrosis)
Blood	Bacteraemia and septicaemia	Frequent
Urinary tract	Acute infections, Chronic infections	Relatively frequent (complication resulting from the presence of foreign bodies)
Ear	Otitis externa ('swimmer's ear), Malignant external otitis, Chronic suppurative otitis media	Frequent
Skin and soft tissue infections	Dermatitis, Wound infections, Burn wound sepsis, Ecthyma gangrenosa, Pyoderma, Folliculitis, Unmanageable forms of acne vulgalis	Relatively frequent (Trauma)
Eye	Keratitis (corneal ulcer), Endophthalmitis, Neonatal ophtalmia	Rare (secondary to trauma)
Central nervous system	Meningitis, Brain abscess	Rare (secondary to neurosurgery or trauma)
Heart	Endocarditis	Rare (drug addicts)
Bone and joint infections	Stenoarticular pyoarthosis, Vertebral osteomyelitis, Symphysis pubis infection, Osteochondritis of the foot, Chronic contiguous osteomyelitis	Rare
Gastrointestinal tract	Necrotising enterocolitis, Peri-rectal infections	Rare

These infections caused by *P. aeruginosa* are not only common, but they should be considered as severe and even life-threatening in specific situations, with the high morbidity and mortality when compared to other bacterial pathogens. Of additional concern is the antibacterial resistance trend that has been noted in large database on nosocomial *P. aeruginosa* isolates (Fridkin and Gaynes, 1999; Gales *et al*., 2001; Tacconelli *et al*., 2002; Andrade *et al*., 2003; Van Eldere, 2003; NNIS, 2003, 2004; Kang, 2005).

Infections by this pathogen are notoriously difficult to treat because it has high intrinsic resistance due to impermeability, β-lactamase production (such as cephalosporinase), and constitutive expression multidrug efflux pumps. Furthermore, it also has a remarkable capacity to develop or acquire new mechanisms of resistance to antibiotic. These mechanisms are often present simultaneously, thereby conferring multiresistant phenotypes (Mesaros *et al.*, 2007; Strateva and Yordanov, 2009). Emerging frequency of multidrug-resistant *P. aeruginosa* strains is concerning as the limited efficacious antimicrobial options. The definition of multidrug-resistant (MDR) *P. aeruginosa* has been established as the isolates that are intermediate resistant or resistant to at least three drugs in the following classes: antipseudomonal β-lactams, aminoglycosides and fluoroquinolones. Reported rates of multidrug-resistant *P. aeruginosa* varied from 0.6% to 32% according to geographic locations and types of surveillance study (Obritsch *et al.*, 2005). Data from a national survey in the USA indicated a persistent decrease in susceptibility to all antipseudomonal agents, with significant declines during the longer period from 1993 to 2002 and also showed that the rate of multidrug-resistant *P. aeruginosa* isolates increase from 4% to 14% (Obritsch *et al.*, 2004). In addition, data from the Tracking Resistance in the United States Today (TRUST) study showed a progressive increase in multidrug-resistant *P. aeruginosa* (resistance to three or more antibiotics) from 7.2% in 2001 to 8.8% and 9.9% in 2002 and 2003, respectively (Karlowsky *et al.*, 2005; McGowan, 2006).

Antibacterial therapy

If any suspicion of pseudomonal infection, therapy should be initiated as soon as clinical samples have been collected, using the best available knowledge to cover the suspected pathogen. Initial therapy for *P. aeruginosa* infection will depend on the patient's risk factors and antimicrobial susceptibility patterns or local epidemiology. Usually, combination therapy has been recommended for *P. aeruginosa* infections. The combination of an anti-pseudomonal β-lactam (antipseudomonal penicillins, extended

spectrum cephalosporins, peniciillin/β-lactamase inhibitor, carbapenems) with an aminoglycoside (gentamicin, amikacin, tobramycin) or a fluoroquinolone (ofloxacin, ciprofloxacin) has been empirical therapy for *P. aeruginosa* infections (Mesaros *et al*., 2007). Fluoroquinolones (preferable ciprofloxacin) was an alternative due to their lower toxicity.

1. Carbapenems

Carbapenems have been introduced since the late 1980s and have excellent activity against most clinically significant gram-positive and gram-negative bacteria. They are often used to treat serious nosocomial infection by gram-negative bacteria which resistant to traditional antimicrobials. There is a class of β-lactam antibiotics that structurally related to penicillins, differing from that group by the substitution of carbon atom for a sulfur atom and by the addition of a double bond to the five-membered ring of the penicillin nucleus. In addition, the presence of the *trans* configuration of hydroxyethyl side chain, in place of the acylamino group shared by penicillins and cephalosporin, confers resistance to most β-lactamase. Also, this class of antibiotics has a broader spectrum of activity than do most other β-lactam antibiotics (Hellinger and Brewer, 1997; Knapp and English, 2001).

The carbapenems which mainly available for the treatment of *P. aeruginosa* infections in many countries are imipenem and meropenem. They are potent agents for the treatment of infections due to MDR *P. aeruginosa*. In general, meropenem is slightly more active *in vitro* against aerobic gram-negative bacilli and slightly less active against aerobic gram-possitive cocci. Like other β-lactam antibiotics, carbapenems binds to penicillin-binding proteins (PBPs), the binding of the β-lactam molecule to PBPs inhibits bacteria from completing transpeptidation (cross-linking) of peptidoglycan strand leading to disrupts bacterial cell wall synthesis (thus preventing the synthesis, and

causes death of susceptible microorganisms). The illustration of mechanism of action of carbapenems is shown in Figure 2-1.

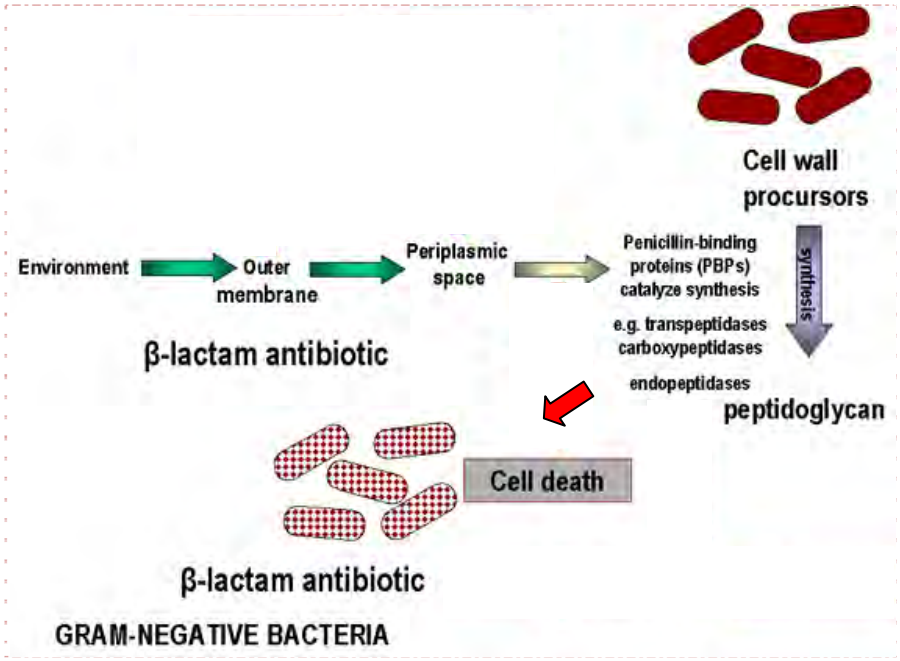

Figure 2-1 Mechanism of action of the carbapenems to binding to PBPs, leading to death of susceptible microorganisms (Picture modified from webpage http://pathmicro.med.sc.edu/fox/antibiotics1.htm, [2009, June, 14]).

1.1 Meropenem

Meropenem is the second carbapenem antibiotic. It is a derivative of thienamycin and its chemical name is (-)-(4R,5S,6S)-3-[[(3S,SS)-5-(dimethylcarbmoyl)3pyrrolidinyl] thio]-6-[(1R)-l-hydroxyethyl]-4-methyl-7-0x0-1-azabicyclo[3,2,0]hept-2-ene-2-carboxylic acid. Its empirical formula is $C_{17}H_{25}N_3O_5S$. Meropenem has pK values of 2.9 and 7.4, and an octanol water partition coefficient of less than 1×10^{-3} over the pH range 3-9. Meropenem trihydrate is available for parenteral clinical use as a sterile white to yellow crystalline powder, blended with anhydrous sodium carbonate to increase its solubility (Blumer, 1997). The structural formula of meropenem is shown in Figure 2-2.

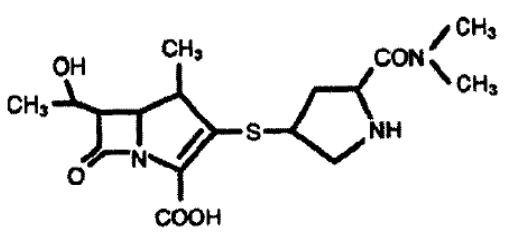

Figure 2-2 Structure of meropenem (Picture modified from Hellinger and Brewer, 1999).

Meropenem is stable to dehydropeptidase-I (DHP-I), renal brush border of human, because of the addition of a methyl group at C-1. Therefore, it does not require the coadministration of DHP-I inhibitor like imipenem. In addition, meropenem presents the pyrroline side chain at C-2 of meropenem which may confers enhanced activity against aerobic gram-negative bacilli including *P. aeruginosa* and its reduce epileptogenic potential in comparison with imipenem (Hellinger and Brewer, 1999; Knapp and English, 2001)

The antibacterial activity of meropenem is excellent *in vitro* for a wide variety of aerobic and anaerobic microorganism. Meropenem readily penetrates cell wall to reach penicillin binding protein (PBP) targets. It has been shown to be active against most isolates of the following microorganisms: 1) gram-positive microorganisms: *Enterococcus faecalis* (excluding vancomycin-resistant isolates), *S. aureus* (excluding methicillin-resistant isolates), *Staphylococcus epidermidis* (excluding methicillin-resistant isolates), *Streptococcus pyogenes, Streptococcus pneumoniae* 2) gram-negative microorganisms: *E. coli*, *Citrobacter freundii, Klebsieilla pneumoniae*, *Enterobacter cloacae, Serratia marcescens, Proteus mirabilis*, *Proteus vulgaris*, *Salmonella* spp., *Morganella morganii*, *Providencia rettgeri*, *Providencia stuartii*, *Haemophilus infuenzae, Neisseria meningitidis*, *Neisseria gonorrhoeae, Moraxella catarrhalis*, *P. aeruginosa*, *Burkholderia cepacia, Acinetobacter calcoaceticus*. Meropenem exhibits a high degree of potency against non-fermenters. It is generally two- to four-fold more active than imipenem

against most *Pseudomonas* spp. and four-fold more potent against *B. cepacia* (Edwards, 1995). Indeed, meropenem was the most potent antimicrobial tested in a study involving 1991 clinical isolates of *P. aeruginosa*; 98.9% of isolates were fully susceptible to meropenem (Chen *et al*., 1995; Blumer, 1997).

Meropenem penetrates well into most body tissue and fluid including the cerebrospinal fluid, and is not highly bound to plasma proteins (approximately 2%). It is excreted primarily by the kidney via glomerular filtration and active tubular secretion. The elimination half-life of meropenem is approximately 1 hour (Blumer, 1997). Approximately 70% of the intravenously administered dose is recovered as unchanged meropenem in the urine over 12 hours, after which little further urinary excretion is detectable. Dosage should be reduced in patients with creatinine clearance less than 51 mL/min.

It is approved for use in both adults and children only 3 months or older including the following infection by susceptible strains: lower respiratory tract infections, urinary tract infections, intra-abdominal and gynecological infections, bacterial septicemia, uncomplicated and complicated skin infections and bacterial meningitis. The dosing range of meropenem in adults is 500 mg to 1 g intravenously every 8 hours, depending on type, the severity of the infection and clinical status of the patients. A dosage of 2 g intravenously every 8 hours is needed for treatment of meningitis. Mean peak plasma concentrations following single intravenous infusions over 30 minutes of meropenem 500 mg and 1 g in healthy volunteers were approximately 25 mg/L and 50 mg/L, respectively (Blumer, 1997; Knapp and English, 2001). Generally, meropenem is well tolerated, the most common adverse clinical effects associated with meropenem were diarrhea (4.3%), nausea (3.6%), vomiting (3.6%), rash (2.3%) and pruritus (1.2%). The most common laboratory abnormalities were mild transient increases in alanine aminotransferase (7.6%) and aspartate aminotransferase (5.6%), thrombocytosis (2.4%) and eosinophilia (1.2%) (Hellinger and Brewer, 1999).

1.2 Carbapenem resistance problems

Carbepenems are currently indicated for treatment of serious infections potentially caused by MDR bacteria including *P. aeruginosa*. However, emerging carbapenem resistant *P. aeruginosa* isolates are of clinical concern worldwide (Paterson *et al*., 2005) since these drugs may represent the last resort in treating life-threatening infection cause by *P. aeruginosa* (Altoparlak, 2005).

Several reports have been documented on carbapenem resistant *P. aeruginosa* and declining carbapenem susceptibility rates of this organism (Livermore, 2002; Goossens, 2003; Van Eldere, 2003; Sasaki *et al*. 2004; Hsueh *et al*., 2005; Hocquet *et al*., 2007). The European MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) study group in intensive care unit (1997-2000) reported that the percentage of meropenem resistance in MDR *P. aeruginosa* was 29.1% and imipenem was 44.9% (Goossens, 2003). Data from the SENTRY Antimicrobial Surveillance Program, 1997-2001, in Latin America medical centres (Table 2-2) showed the decrease in susceptibility of *P. aeruginosa* isolates to various antimicrobial agents including meropenen and imipenem which were 83.0% to 64.4% and 77.1% to 62.0%, respectively (Andrade *et al*., 2003). In addition, in the United States, the resistant rates of meropenem and imipenem in the year 2002 were 4.4% and 7.5%, respectively and increased to 8.6% and 18.3% in the year 2007 (Jones *et al*., 2008).

Table 2-2 Susceptibility to 12 antimicrobial agents of *P. aeruginosa* isolates in Latin America (SENTRY Antimicrobial Surveillance Program, 1997-2001). (Modified from Andrade *et al*., 2003).

Antimicrobial class/agent	% Susceptibility by year (no. tested)					
	1997 (335)	1998 (424)	1999 (371)	2000 (357)	2001 (407)	OR (95 CI %)[a]
β-Lactams						
ceftazidime	66.6	64.4	65.8	59.7	56.3	1.39 (1.02-1.91)
cefepime	66.2	67.9	65.2	59.4	54.8	1.53 (1.12-2.09)
aztreonam	55.5	45.0	46.6	34.7	41.3	1.76 (1.30-2.39)
piperacillin	71.9	66.7	66.6	61.9	60.9	1.64 (1.19-2.27)
piperacillin/tazobactam	79.4	77.1	73.3	66.4	64.9	2.09 (1.48-2.96)
imipenem	77.1	76.7	73.6	70.6	62.2	2.07 (1.47-2.90)
meropenem	83.0	79.7	76.0	71.7	64.4	2.70 (1.88-3.89)
Aminoglycosides						
amikacin	77.7	73.3	69.0	65.0	65.4	1.84 (1.31-2.59)
gentamicin	63.6	61.8	60.1	56.9	49.6	1.77 (1.30-2.41)
Fluoroquinolones						
ciprofloxacin	67.2	60.8	60.6	53.2	49.9	2.06 (1.51-2.81)
gatifloxacin	60.9	57.1	56.3	52.1	46.4	1.80 (1.33-2.44)
levofloxacin	63.6	59.0	59.3	53.5	49.6	1.77 (1.30-2.41)

[a] *P* value was calculated by χ^2 for trend test; all *P* value were <0.001. OR and respective 95% CI refer to comparisons between the years of 1997 and 2001.

In Thailand, the study at Songklanagarind Hospital showed that 54.71% of *P. aeruginosa* isolates were low level imipenem-resistant (MIC 8-32 μg/mL) and 45.28% were high level resistant (MIC > 32 μg/mL) (Tunyapanit, 2003). Percentage of susceptible *P. aeruginosa* at Rajavithi Hostpital (2005-2006) showed that susceptibility rate of ceftazidime, cefepime cefoperazone/sulbactam, ciprofloxacin, gentamicin, amikacin, imipenem and meropenem decreased by 12%, 7%, 20%, 16%, 10%, 15%, 12% and 15%, respectively (Microbiology Laboratory Department of Pathology Rajavithi Hospital, unpublished data). Percentage of imipenem susceptibility of *P. aeruginosa* from National Antimicrobial Resistance Surveillance Center, Thailand (NARST) (http://narst. dmsc.moph.go.th) has decreased from 85.0% in the year 2000 to 82.0% in

the year 2006, and while meropenem susceptibility in the year 2007 was only 78% (Table 2-3).

Table 2-3 Percentage of antimicrobial susceptibility of *P. aeruginosa* during January 2000 to December 2007. (Data from NARST, http://narst. dmsc.moph.go.th).

Antimicrobial agents	Piperacillin/ tazobactam	Ceftazidime	Amikacin	Ciprofloxacin	Imipenem	Meropenem
2000	-	70%	73%	70%	85%	-
2001	-	70%	76%	73%	85%	-
2002	-	73%	78%	76%	86%	-
2003	78%	71%	78%	76%	83%	-
2004	80%	73%	80%	76 %	83%	-
2005	83%	72%	80%	75%	83%	-
2006	84%	71%	79%	73%	71%	
2007	85%	73%	80%	74%	82%	78%

1.3 Carbapenem resistance mechanisms in *P. aeruginosa*

P. aeruginosa is intrinsically resistant to several antibiotics because of the low permeability of its outer membrane, the constitutive expression of various efflux pumps and the production of antibiotic inactivating enzymes (e.g. cephalosporinase). Furthermore, it also has remarkable capacity to develop or acquire new mechanisms of resistance to antibiotics, thus, multidrug-resistance is common in this organism (Mesaros *et al*., 2007).

The most important resistance mechanisms of carbapenems (Figure 2-3) are associated with reduced uptake as a result of qualitative or quantitative alteration of the OprD porin (carbapenem-specific outer membrane porin) as well as overexpression of multidrug efflux pump system. The transmissible carbapenemase (such as metallo-

β-lactamase: MBL) also confer high grade resistance to the carbapenems (Livermore, 2001; Pai, 2001; Quale, 2006; Mesaros *et al*., 2007; Rahal, 2008).

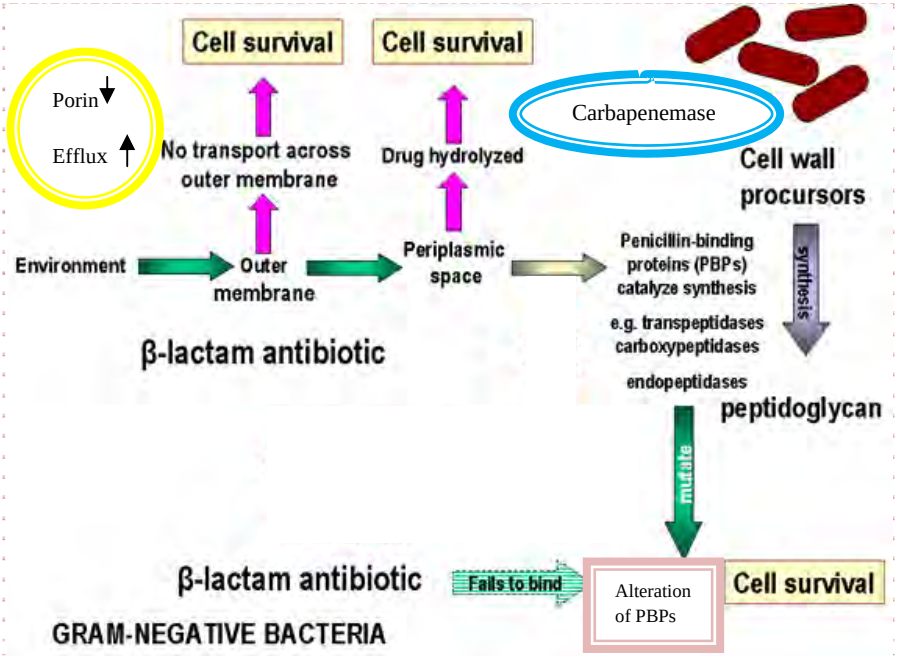

Figure 2-3 Carbapenem resistance mechanisms in *P. aeruginosa* (Picture modified from webpage http://pathmicro.med.sc.edu/fox/antibiotics1.htm, [2009, June, 14]).

The selectivity of the OprD porin for carbapenems is attributable to the chemical resemblance between carbapenems and basic amino acid. Although changes in the carbapenem binding regions of OprD may confer a carbapenem resistant phenotype, the most important mechanism of resistance in clinical isolates is from down regulation of OprD. Typically, imipenem MIC is increased to 8-32 mg/L and meropenem MIC to 2-4 mg/L in down regulation of OprD isolates (Livermore, 2001; Giske, 2007).

Antibacterials may be extruded from within *P. aeruginosa* via multidrug efflux pumps. The major efflux systems involved in *P. aeruginosa* resistance belong to the Hydrophobic/Amphiphilic Efflux1 (HAE1) family, a subclass of the Resistance Nodulation

Division (RND) transporter superfamily (Poole and Srikumar, 2001). These transporters function consist of a pump protein traversing the inner membrane, the periplasmatic membrane fusion protein and the channel forming outer membrane protein (Figure 2-4). Overexpression of a multidrug efflux pump raises the minimum inhibitory concentration (MIC) of any drug susceptible to the pump, and each pump is able to handle multiple antibacterial substrates. Three RND efflux pumps relevant for carbapenem efflux have been described in *P. aeruginosa*, namely MexA-MexB-OprM, MexC-MexD-OprJ and MexX-MexY. MexA-MexB-OprM is regarded the most efficient efflux pump for extrusion of carbapenems. Relatively modestly increased levels of expression of only two-fold the levels of wild-type isolates may be clinically relevant. In contrast, transcription levels of MexX-MexY and MexC-MexD-OprJ probably need to be increased up to ten and seventy-fold, respectively, in order to affect carbapenem susceptibility. Typically, overexpression of MexA-MexB-OprM increases meropenem MIC from the wild-type level of around 0.5 to around 4 µg/mL. In mutants featuring concomitant down regulation of the OprD porin and up regulation of MexA-MexB-OprM, MIC of meropenem increases from wild-type levels up to around 16 µg/mL (Köhler, *et al*. 1999; Giske, 2007).

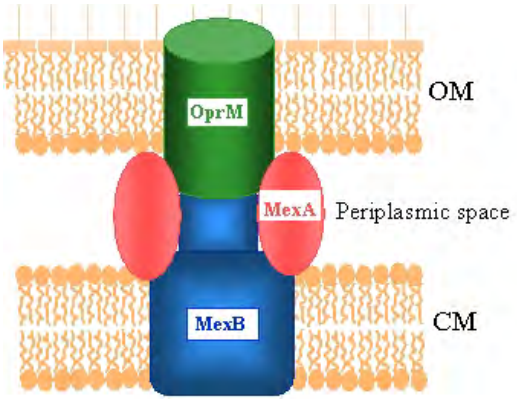

Figure 2-4 MexA-MexB-OprM pump in *P. aeruginosa* (picture from webpage www.phar.cam.ac.uk/ri/venter.html, [2009, June, 14]).

Traditionally, carbapenemase mediated resistance, particularly in *Pseudomonas* and *Acinetobacter* spp., was due to synthesis of carbapenemase. This includes class B metallo-β-lactamase belonging to the IMP, VIM, GIM and SPM groups, class A enzymes belonging to the SME, NMC/IMI and KPC groups and several class D (OXA) enzymes (Table 2-4). Metallo-β-lactamases (MBLs) are prominent carbapenemase enzymes found in *P. aeruginosa*. These enzymes are universally inhibited by ethylenediamine tetraacetic acid (EDTA) or 2-mercaptopropionic acid (MPA). β-lactamase inhibitors such as clavulanic acid, tazobactam and sulbactam are also hydrolysed rather weakly by the MBLs. They hydrolyse virtually all β-lactams except monobactam, aztreonam. Similar to other β-lactamases, MBLs are divided into those normally encoded by chromosomal genes and ones encoded by transferable genes. The transferable *bla* genes are typically found as gene cassettes in class 1 integrons and move between organisms mainly on plasmids but also on transposons (Giske, 2007; Maltezou, 2009).

Metallo-β-lactamases-producing *P. aeruginosa* have been reported in many regions. There are five types of acquired MBLs identified to date, namely IMP, VIM, SPM, GIM and SIM. Four of them have so far been reported in *P. aeruginosa* (IMP, VIM, GIM, SPM), while SIM so far only reported in *Acinetobacter* spp. IMP and VIM types are the prevalent MBLs with a wide geographic distribution (Giske, 2007; Maltezou, 2009).

The IMP group compromises > 22 types now. The first MBL, IMP-1, conferring carbapenem resistance was detected in 1988 in *P. aeruginosa* strain in Japan. It has a very broad resistant pattern, including extended spectrum cephalosporins, carbapenems and the β-lactamases inhibitor clavulanic acid and sulbactam. Recently, IMP-type MBLs have been detected throughout Southeast Asia, The USA, Canada, Brazil and occasionally in England, Italy and Portugal. IMP-type MBLs have been mainly detected in *P. aeruginosa* as well as in *A. baumannii, K. pneumoniae, Klebsialla oxytoca, S. marcescens, C. freundii, E. cloacae, E. coli, Providencia ruttgeri, M. morganii and Alcaligenes* sp. (Queenan and Bush, 2007; Maltezou, 2009)

VIM-types, a total of approximately 12 VIM-type MBLs have been reported, VIM-1 was initially detected in a *P. aeruginosa* strain responsible for nosocomial outbreak in Verona, Italy, in 1997, signalling the emergence of the second group of acquired MBLs. Although the VIM-type MBLs were initially considered as European counterpart of the IMP-type MBL predominant in Southeast Asia, nowadays VIM-type MBLs have established endemicity not only in Europe but also in Korea and other countries, and have also been reported from South and North Americas and recently from Australia, India and Iran.

While GIM-1 and SPM-1 so far have a restricted geographic spread, GIM-1 has been detected in five MDR *P. aeruginosa* isolates from different patients hospitalised during 2002 in a medical centre in Dusseldorf, Germany. GIM-1 was more closely related to IMP-type MBLs, sharing 39%-43% identity with them. Whereas, SPM-1 was initially identified in Brazil in 2001 in a pan-resistant *P. aeruginosa* isolate (susceptible to polymyxin B only) and showed highest identity (35.5%) to IMP-1 (Maltezou, 2009).

Few studies have addressed the putative role of alterations of penicillin-binding proteins in conferring carbapenem resistane in gram-negative bacilli (Sumita and Fukasawa; 1995; Fernandez-Cuenca *et al*. 2003; Ayala *et al*., 2005). Farra *et al*. (2008) suggested that PBPs do not play a role in imipenem resistance in the clinical strains of *P. aeruginosa* in their examination. In addition, Giske *et al*. (2008) investigated the transcription levels of *pbp* 2 and *pbp* 3 in clinical isolates of *P. aeruginosa* with high level resistance to meropenem and/or imipenem. Decrease amount of *pbp* 2 and *pbp* 3 was found, sequencing of *pbp* 2 and *pbp* 3 revealed no amino acid changes potentially leading to conformational changes. Reduced transcription of *pbp* 2 and *pbp* 3 may contribute to the carbapenem resistance. This suggested, although the current data support a potential role of PBP changes in conferring carbapenem resistance in *P. aeruginosa*, further studies of laboratory mutants are warranted to establish the relative contribution of this putative resistant mechanism.

Table 2-4 Selected β-lactamases of Gram-negative bacteria (Modified from Jacoby and Munos-Price, 2005).

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

* Plus signs denote relative sensitivity to inhibition.

1.4 The impact of carbarpenem resistance

The impact of carbarpenem resistance in *P. aeuruginosa* on mortality, morbidity and length of hospital stay was evaluated in few studies. Kang *et al*. (2005) study of 190 patients with bloodstream infection (BSI), whereof 28 patients were infected with imipenem-resistant *P. aeruginosa* (case), revealed that the mortality was 53.6% among resistant case. Furtado *et al*. (2009) reported nosocomial infections caused by imipenem-resistant *P. aeruginosa* with mortality rate 49% compared with 33% for control patients (OR, 1.92; 95% CI, 1.07-3.44; p = 0.02), article in press, corrected proof in Journal of Critical Care.

Increasing carbapenem resistance in *P. aeruginosa* is a serious clinical problem worldwide but the novel antimicrobial agents with activity against *P. aeruginosa* will not be available in the near future, making ongoing surveillance of the activities of currently available agent critical. Thus, new therapies are urgently needed. The combination therapy will provide alternative treatments. Usually, empirical therapy of serious infections caused by *P. aeruginosa* consists of an antipseudomonal β-lactam and an aminoglycoside or a fluoroquinolone because less toxic than aminoglycoside (preferably ciprofloxacin) to provide adequate therapy and improve patient outcomes (Mesaros *et al*., 2007). Ciprofloxacin is the most *in vitro* active anti-*P. aeruginosa* fluoroquinolone and is often used as alternative to the aminoglycosides.

2. Ciprofloxacin

Ciprofloxacin is an antimicrobial agent that member of the fluoroquinolone class which have a broad-spectrum antimicrobial activity. This agent active against both gram-positive and gram-negative bacteria, particularly is the most active fluoroquinolone against *P. aeruginosa* (Neu, 1989). Its chemical name is 1-cyclopropyl-

6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid. Its empirical formula is $C_{17}H_{18}FN_3O_3$ and the structural formula of ciprofloxacin is shown in Figure 2-5.

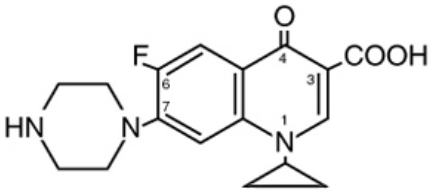

Figure 2-5 Structure of ciprofloxacin (Picture from http://www.drugs.com/pro/ciprofloxacin-injection.html, [2007, June, 14])

Similar to other fluoroquinolones, ciprofloxacin is known to have two enzyme targets, DNA gyrase and topoisomerase IV (both enzymes are type II topoisomerase), in the bacterial cell. Both of these targets are essential for bacterial DNA replication. DNA gyrase is a tetrameric composed of 2 GyrA and 2 GyrB subunits. Topoisomerase IV has a similar structure and is composed of 2 ParC and 2 ParE subunits. DNA gyrase is the only bacterial enzyme that introduces negative superhelical twists into DNA. This negatively twisted DNA is important for initiation of DNA replication. DNA gyrase also facilitates DNA replication by removing positive superhelical twists that accumulate ahead of the replication fork or as a result of the transcription of certain genes. Topoisomerase IV acts in the terminal stages of DNA replication, allowing for the separation of interlinked daughter chromosomes so that segregation into daughter cells can occur. Ciprofloxacin inhibit these enzymes by stabilizing either the DNA–DNA gyrase complex or the DNA–topoisomerase IV complex. The stabilized DNA–DNA gyrase complex blocks movement of the replication fork, causing formerly reversible DNA-enzyme complexes to become irreversible that ultimately result in cell death (Cross, 2001; Hooper, 2001). The illustration of mechanism of action of ciprofloxacin is shown in Figure 2-6.

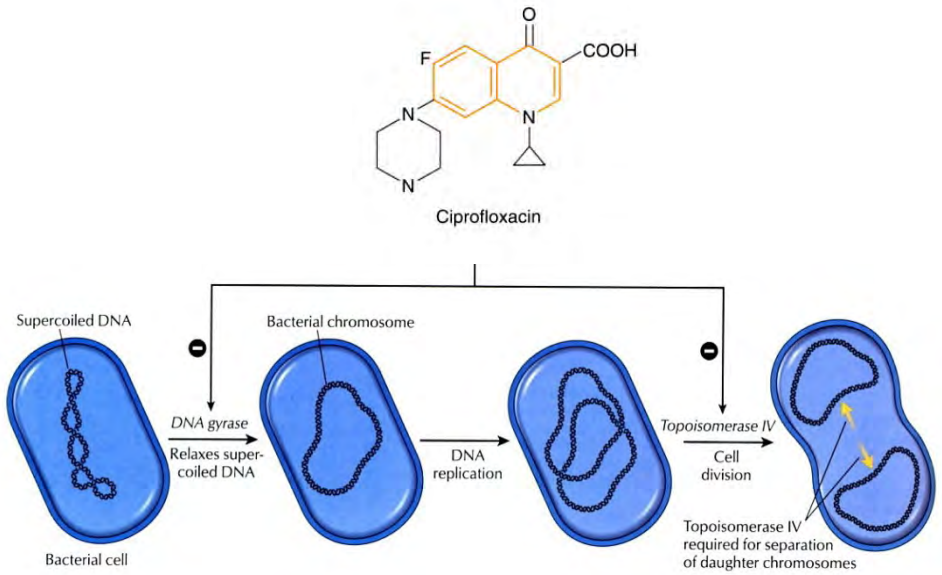

Figure 2-6 Mechanism of action of fluoroquinolone (Picture modified from INTERNATIONAL STUDENT EDITION Netter ' s Illustrated Pharmacology).

Ciprofloxacin attains therapeutic concentrations in most tissues and body fluids. It is effective in the treatment of a wide variety of infections, particularly those caused by gram-negative pathogens. These include complicated urinary tract infections, sexually transmitted diseases (gonorrhoea and chancroid), skin and bone infections, gastrointestinal infections caused by multi-resistant organisms, lower respiratory tract infections (including those in patients with cystic fibrosis), febrile neutropenia (combined with an agent which possesses good activity against gram-positive bacteria), intra-abdominal infections (combined with an anti anaerobic agent) and malignant external otitis (Davis, Markham and Balfour, 1996).

Ciprofloxacin is one of the few broad-spectrum antibacterials available in both intravenous and oral formulations. For most infections the recommended oral dose for adults is 250 mg to 750 mg (immediate release tablets) every 12 hours or 500 mg to 1000 mg (extended release tablets) every 24 hours. The usual intravenous dose is 200 to 400 mg every 8 to12 hours (http://www.medicinenet.com/ciprofloxacin/article.htm).

After intravenous administration, ciprofloxacin is present in saliva, nasal and bronchial secretions, sputum, skin blister fluid, lymph, peritoneal fluid, bile, and prostatic secretions. It has also been detected in the lung, skin, fat, muscle, cartilage, and bone. Following 60-minute intravenous infusions of 200 mg and 400 mg ciprofloxacin to normal volunteers, the mean maximum serum concentrations achieved were 2.1 and 4.6 µg/mL, respectively. The serum elimination half-life is approximately 5 to 6 hours and the total clearance is around 35 L/hr. After intravenous (IV) administration, approximately 50% to 70% of the dose is excreted in the urine as unchanged drug. Following a 200 mg IV dose, concentrations in the urine usually exceed 200 µg/mL at 0 to 2 hours after dosing and are generally greater than 15 µg/mL at 8 to 12 hours after dosing. Following a 400 mg IV dose, urine concentrations generally exceed 400 µg/mL at 0 to 2 hours after dosing and are usually greater than 30 µg/mL at 8 to 12 hours after dosing. The renal clearance is approximately 22 L/hr. The urinary excretion of ciprofloxacin is virtually complete by 24 hours after dosing (http://www. drugs.com/ pro/ciprofloxacin-injection.html.).

The most frequently reported adverse drug reactions from clinical trials of all formulations, all dosages, all drug-therapy durations, and for all indications of ciprofloxacin therapy were nausea (2.5%), diarrhea (1.6%), liver function tests abnormal (1.3%), vomiting (1.0%), and rash (1.0%).

Quinolone resistance mechanisms are involved chromosomal mutations and plasmid-mediated quinolone resistance (plasmid-encoded Qnr protein). However, resistance mechanisms of fluoroquinolone have largely associated with chromosomal mutations in subunits of the target sites or in genes controlling the expression of multidrug efflux pumps. Overexpression of efflux pumps seems to play an important role in *P. aeruginosa* resistant to fluoroquinolones, but generally does not appear sufficient by itself to confer significant resistance. With fluoroquinolones, over expression of efflux pumps typically combines with DNA gyrase (and secondarily, topoisomerase IV)

mutations to confer high level resistance in *P. aeruginosa* (McGowan, 2006). Resistance to these agents are reducing the number of effective available antimicrobial agents.

3. Colistin

The problems of multidrug-resistance gram-negative bacteria causing severe infections are increasing. The significant levels of resistant to carbapenem (meropenem) have already been reported in clinical isolates of *P. aeruginosa*. Unfortunately, there is no novel antibiotic to combat them. Limited therapeutic options have forced to the re-evaluation of polymyxin, an old class of antibiotic which has excellent *in vitro* activity against gram-negative bacteria. It consists of five chemically different compounds, polymyxin A, B, C, D and E (Falagas and Kasiakou, 2006). Only polymyxin B and E have been used in clinical practice. Polymyxin E, also known as colistin, is an old class of antibiotic with significant *in vitro* activity against some multi-resistant gram-negative pathogens, including *P. aeruginosa*, *A. baumannii* and *K. pneumoniae*. When β-lactam, aminoglycoside or fluoroquinolone is ineffective, colistin remain the last resort (Livermore, 2002; Li *et al*., 2005).

Colistin, a cationic polypeptide antibiotic, was first isolated in Japan from *Bacillus polymyxa* subspecies *colistinus* in 1949. It has been available since 1959 for the treatment of infection caused by gram-negative bacteria but was replaced in 1970 by antibiotic agents became available considered less toxic, such as aminoglycoside and other antipseudomonal. Therefore, from the decline in its use in the early 1970s up until the mild 1990s, there have been limited studies on the clinical use of colistin or on its pharmacokinetics and pharmacodynamics (Li *et al*., 2001, 2005).

Colistin is a multicomponent antibiotic consisting of several closely related decapeptides with a general structure compose of a cyclic heptapeptide moiety and a tripeptide side chain acylated at the amino terminus by fatty acid through α amide

linkage (Figure 2-7). The amino acid components in the molecule of colistin are D-leucine, L-threonine and l-α-γ diaminobutiric acid. The two main components, which have been identified by the composition of their amino acids and fatty acids are colistin A (polymyxin E1) and colistin B (polymyxin E2). They have the same amino acids but a different fatty acid (6-methyloctanoic acid and 6-methylheptanoic acid, respectively) (Li *et al*, 2001).

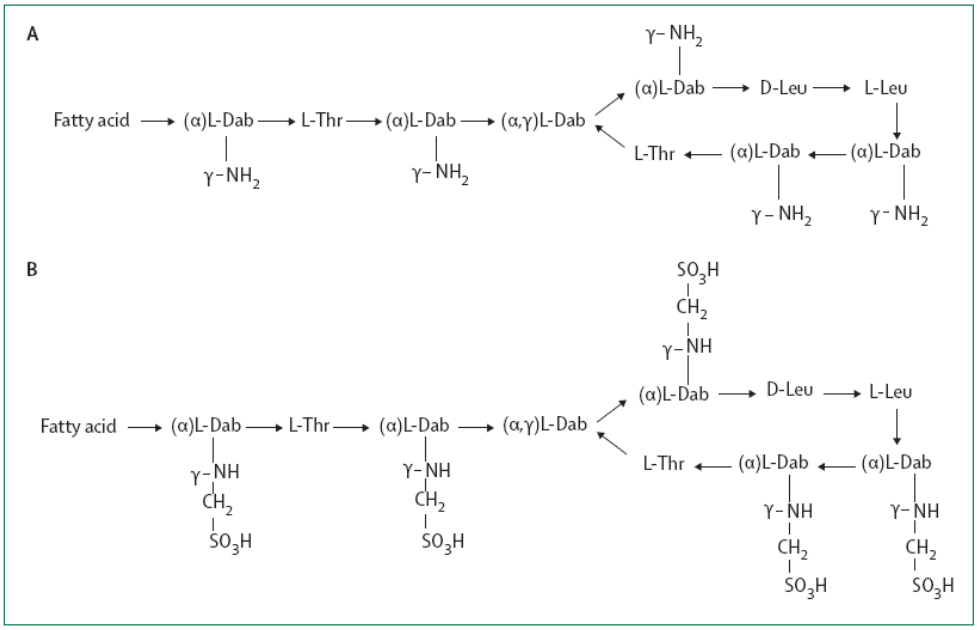

Figure 2-7 Colistin structures (A) structures of colistin A and B; (B) structures of sodium colistin A and B methanesulphonate. Fatty acid: 6-methyloctanoic acid for colistin A and 6-methylheptanoic acid for colistin B; Thr : threonine; Leu : leucine; Dab : α and γ diaminobutyric acid. α and γ indicate the respective –NH2 involved in the peptide linkage. (Picture modified from Li *et al*., 2006)

Colistin exhibits bactericidal activity in concentration dependent manner to gram-negative bacteria by detergent-like mechanism, disturbs the structure and function of the outer and cytoplasmic membranes of bacteria (Li *et al*., 2001). The initial process begins with colistin associated with bacterial membrane through electrostatic interactions

between the cationic polypeptide of colistin and anionic of lipopolysaccharide (LPS) molecules in the outer membrane of the gram-negative bacteria, leading to derangement of the cell membrane. Colistin displaces divalent cations (magnesium and calcium), which normally stabilize the LPS molecules, from the negatively charged LPS (Falagas and Kasiakou, 2005). The result of this process leads to a local disturbance of the outer membrane and increases the permeability of the cell envelope. After that it reaches to cytoplasmic membrane where it exercises bactericidal activity, leakage of cell components, and, subsequently, cell death (Figure 2-8).

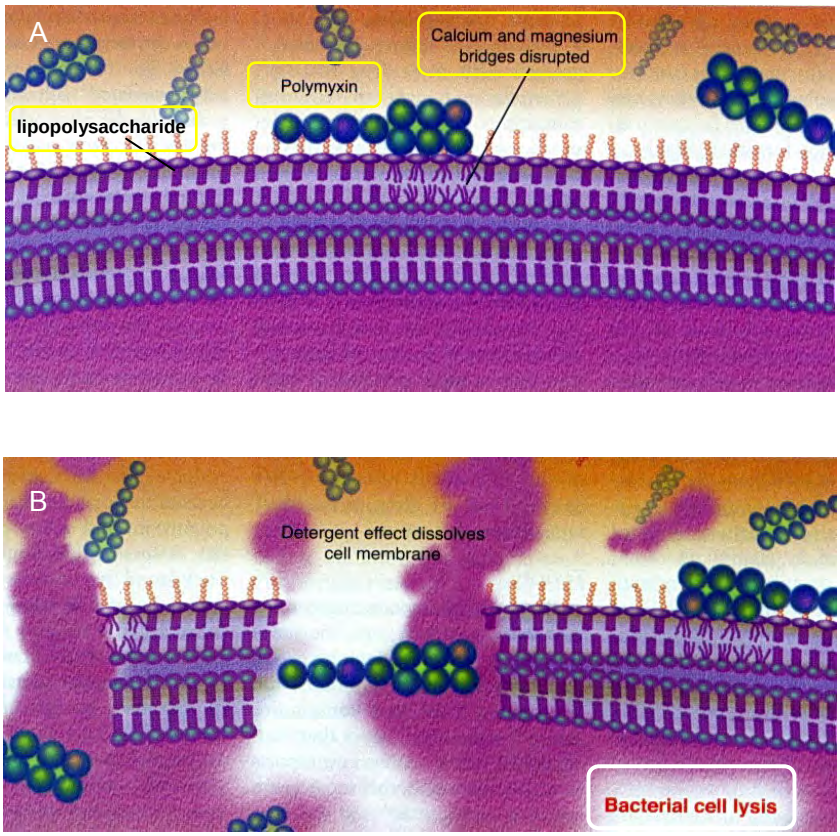

Figure 2-8 The mechanism of action for polymyxins (A) polymyxin binds to negative charge of LPS and displaces divalent cation, leads to a local disturbance of the outer membrane (B) polymyxin has become inserted into the outer membrane, increases the permeability of the cell envelope, leakage of cell components following cell lysis (Picture modified from Arnold *et al*., 2007).

Colistin has excellent bactericidal activity against most gram-negative aerobic bacilli, including *Acinetobacter* species, *P. aeruginosa, Klebsiella* species, *E. coli, Salmonella* species, *Shigella* species, *Citrobacter* species, *Morganella morganii, Yersinia pseudotuberculosis*, and *H. influenzae*. Colistin has also been shown to possess a considerable *in vitro* activity against *Stenotrophomonas maltophilia* strains. It has also been reported to be potentially active against several mycobacterial species, including *Mycobacterium xenopi, Mycobacterium fortuitum, Mycobacterium phlei*, and *Mycobacterium smegmatis*. However, *Burkholderia mallei*, *B. cepacia*, *Proteus* species, *Providencia* species, *Serratia* species, *Edwardsiella* species, and *Brucella* species are all resistant to colistin. In addition, colistin is not active against gram-negative and gram-positive aerobic cocci, gram-positive aerobic bacilli, all anaerobes, fungi, and parasites (Falagas and Kasiakou, 2005).

Currently, two forms of colistin are available commercially: colistin sulphate for oral and topical use, and colistimethate sodium (sodium colistin methanesulphonate, colistin sulfomethate sodium) for parenteral use. Colistimethate sodium is less toxic than colistin sulphate. Both forms are not absorbed by the gastrointestinal tract with oral administration. Colistimethate sodium is not stable *in vitro* or *in vivo,* and is hydrolysed to a series of methanesulphonated derivatives plus colistin (Li *et al*., 2006).

The two most common commercially available parenteral formulations of colistimethate sodium are Colomycin (Dumex-Alpharma A/S, Copenhagen, Denmark) and Coly-Mycin M parenteral (Parkedale Pharmaceuticals, Rochester, USA). The recommended dose for Colomycin for a patient over 60 kg and with normal renal function is 1 to 2 million IU three times daily, equivalent to 240 to 480 mg colistimethate per day. By contrast, Coly-Mycin M parenteral, the recommended doses are 2.5 to 5 mg/kg colistin base activity per day in two to four divided doses; which is equivalent to about 6.67 to 13.3 mg/kg of colistimethate sodium per day. The volume of distribution of colistimethate sodium is very limited, not extensively distributed outside plasma. Its poor

tissue penetration may be a consequence of their relatively high molecular weight and polarity. Elimination of colistimethate sodium occurs mainly by the renal route, approximately 60% of colistimethate sodium is excreted as unchanged drug in the urine during the first 24 hour after dosing (Falagas and Kasiakou 2005; Li *et al*., 2006).

The most common toxicity of colistin involves the renal toxicity (acute tubular necrosis manifested as decreased creatinine clearance, increased serum urea and creatinine level) and neurotoxicity (dizziness, weakness, facial and peripheral paresthesia, vertigo, visual disturbance, confusion, ataxia and neuromuscular blockade, which can lead to respiratory failure or apnea). The incidence of neurotoxicity related to the use polymyxins reported in the old literature was considerably less compared to nephrotoxicity. Both renal and neurological toxicity are considered to be dose dependent and usually reversible after early discontinuation of therapy with the drug, however, there are scare publish reports of irreversible nephrotoxicity after the cessation of colistin treatment (Falagas and Kasiakou, 2005). Because of serious adverse effects colistin systemic utilization has been discouraged. However, recent data between 1995 and 2005, the use of intravenous colistin related nephrotoxicity may be less prominent than previous studies (Falagas and Kasiakou, 2006).

There are limited data on mechanism of resistance to colistin. In literature, studies on *P. aeruginosa* strain have suggested that alterations of the outer membrane of the bacterial cell (reduction in LPS, reduced levels of specific outer membrane proteins, reduction in cell envelope Mg^{2+} and Ca^{2+} contents, and lipid alterations) are related to the development of resistance (Falagas and Kasiakou 2005). Studies on *P. aerugiosa* suggest a role for OprH (or H1) an outer membrane protein which is overexpressed in low Mg^{2+} environments resulting in resistance to polymyxin B and gentamicin was occurred (Li *et al*, 2005). Resistance to colistin is rare because the compound itself can promote its penetration through the cell envelope and cause irreversible disruption of cytoplasmic membrane.

Combination studies of various antimicrobial agents for *P. aeruginosa* isolates

Reasons for the use of combination therapy are to gain synergistic/additive antimicrobial effects, to prevent emergence of resistant bacteria and to expand the spectrum of antibacterial activity beyond that of the individual antibiotic alone and also to permit use of lower dose of one or all of the antimicrobials as well as reduction of toxicity (Sader *et al*., 2003; Song *et al*., 2003; Rahal, 2006; Tan *et al*., 2007).

Several studies reported the synergistic effects between β-lactams and aminoglycosides or fluoroquinolones against gram-positive and gram-negative bacteria including *P. aeruginosa* (Visalli *et al*., 1998; Burgess and Nathisuwan, 2002; Dawis *et al*., 2003; Kanellakopoulou *et al*., 2008). However, the variation in synergy prevalence is most likely due to the variation in the biology of the isolates at each location.

In 2000, Nakamura *et al*. investigated the *in vitro* effects of combinations of meropenem and three aminoglycoside (arbekacin, amikacin, netilmicin) against 50 clinical strains of *P. aeruginosa* by agar dilution checkerboard method. This study showed that the combinations of meropenem and aminoglycosides were effective against most of *P. aeruginosa* tested including meropenem-resistant strains with synergy of 20% to 58% and additive of 40% to 76%. Ermertcan *et al*. (2001) investigated synergistic effects of meropenem and ciprofloxacin against 18 strains of *P. aeruginosa* isolated from patients in intensive care unit (ICU) by time kill methodology. The synergistic effect of meropenem and ciprofloxacin in combination was found to be 22% at 0.5xMIC and 61% at 1xMIC. Two strains (11%) showed synergy at both 0.5 and 1xMIC. Among the 18 *P. aeruginosa* strains, 1 strain (6%) did not show a synergistic effect at either 0.5 or 1xMIC. Oie *et al*. (2003) evaluated the combination effects of antibiotic combinations by agar incorporation inhibitory tests and time kill test on seven geographically and epidemiologically distinct isolates of MDR *P. aeruginosa*. All seven strains were resistant to all agents in this study (piperacillin,

meropenem, ceftazidime, cefoperazone/sulbactam, aztreonam, amikacin and ciprofloxacin). This study showed that the combination of aztreonam and amikacin was the most effective among the two drug combinations. Such combination inhibited 5 of the 7 strains. Among the three drug combination, the combinations of piperacillin, ceftazidime, and amikacin, and that of ceftazidime, aztreonam and amikacin were the most effective. The combinations inhibited all 7 strains. In the killing tests, this three drug combination had bacteriostatic effects on all 7 strains after 2, 4, 6 and 24 hour of drug addition, bactericidal effects on 1 to 2 strains after 4, 6 and 24 hour. Therefore, the author concluded that three drug combination of ceftazidime, aztreonam and amikacin may be effective against *P. aeruginosa* resistant to all commonly used antipseudomonal drugs. Solak *et al*. (2003) investigated the *in vitro* interaction of meropenem and ciprofloxacin against 20 clinical isolates of *P. aeruginosa* by checkerboard method. This study showed that the synergistic interaction between meropenem and ciprofloxacin was 30% (including both meropenem and ciprofloxacin resistant strains) and additive was 70%. In addition, Kanellakopoulou, *et al*. (2008) investigated *in vitro* synergism of β-lactams (imipenenen, meropenem and ceftazidime) with ciprofloxacin and moxifloxacin against 24 genetically distinct multidrug-resistant isolates of *P. aeruginosa* by time kill assay at mean serum levels (conventional dose administration) and their maximum serum levels. The combinations of imipenem with ciprofloxacin or moxifloxacin and the combination of ceftazidime with ciprofloxacin were the most effective (synergistic effect 21%). This study underscore the potential interest of reporting synergism between β-lactams and fluoroquinolones in nosocomial setting when a MDR isolate emerges.

However, resistance to aminoglycoside and fluoroquinolone have been documented and trends to increase worldwide which limited therapeutic option. *P. aeruginosa* is resistant to aminoglycoside via over expression of efflux pumps and aminoglycoside modifying enzyme (Lambert, 2002; Poole, 2005), whereas resistance to fluoroquinolone is via overexpression of efflux pumps and mutation of targeting enzyme

(DNA gyrase and topoisomerase IV) (Hancock and Speert, 2000; Nakajima *et al*., 2002).

Unfortunately, in the past two decades, a marked decline in the discovery and development of novel antibiotics and a remarkable increase in resistance to those currently available have been seen, particulary in MDR *P. aeruginosa*, *A. baumannii, K. pneumoniae.* Polymyxins (such as colistin) are sometimes the only available active agents existing.

There are several experimentals in the literature regarding susceptibility and synergistic activity of colistin with other antimicrobial agent against MDR gram-negative bacteria including *P. aeruginosa* and *A. baumannii*. Tribuddharat *et al*. (2003) evaluated *in vitro* activity of polymyxin B and polymyxin E against 100 clinical isolates of multidrug-resistant *P. aeruginosa* by standard disk diffusion technique. The study showed that all isolates were susceptible to polymyxin B and polymyxin E. Rynn *et al.* (1999) investigated the interactions of colistin with ceftazidime, aztreonam, gentamicin, piperacillin and ciprofloxacin by time kill. This study indicated that colistin plus other antibiotics trend to produce smaller area under bacterial kill curve (AUBKC) than single agents and combinations of colistin and ceftazidime were significantly smaller than those for the single agents. Lanmand *et al*. (2005) used checkerboard testing in 10 MDR *P. aeruginosa* and demonstrated synergy for polymyxin B combined with azithromycin, imipenem and rifampicin in 6, 2 and 1 isolate, respectively. The time kill study exhibited bactericidal activity for the triple combination of polymyxin B plus imipenem and rifampicin against all 10 isolates as well as the bactericidal activity observed from double combination of polymyxin B plus rifampicin or imipenem or azithromycin in 9, 8, 4 isolates, respectively. Timurkaynak *et al*. (2006) found that the combination between colistin and rifampicin produced the synergy effect in 2 out of 5 strains of MDR *P. aeruginosa* by checkerboard method, whereas the combination of colistin with meropenem or azithromycin or doxycycline showed either partial synergy,

or additive or indifferent effects. Cirioni *et al.* (2007) reported the efficacy of combination of colistin and imipenem against two strains of *P. aeruginosa* (one susceptible strain and one multi-resistant strain) both *in vitro* (checkerboard and time kill) and *in vivo* (sepsis mouse model). The combination of colistin with imipenem showed *in vitro* synergistic interaction and significant increase in efficacy was also observed *in vivo*. Punkuch *et al*. (2008) showed that the subinhibitory concentrations of meropenem (0.06 to 128 µg/mL) and ciprofloxacin (0.03 to 32 µg/mL) showed synergy against 34 out of 51 *P. aeruginosa* strains and subinhibitory concentrations of meropenem (0.06 to 8 µg/mL) and colistin (0.12 to 1 µg/mL) showed synergy in 13 isolates in time kill studies at 24 hour. There were 6 meroenem-resistant isolates, the combinations of meropenem with ciprofloxacin and meropenem with colistin showed synergy against 2 and 4 isolates, respectively.

Recently, clinical reports published that colistin either monotherapy or in combination with other antibiotics were effective and safe for treatment of patients infected with multidrug-resistant *P. aeruginosa* and *A. baumannii* (Markou *et al*., 2003; Michalopoulos *et al*., 2005; Pintado *et al*., 2008). One clinical trial of the effectiveness of colistin in 53 cystic fibrosis (CF) patients with exacerbations of chronic pulmonary infection due to MDR *P. aeruginosa* showed that the combinations of colistin with an antipseudomonal agent (azlocillin, piperacillin, aztreonam, ceftazidime, imipenem or ciprofloxacin) was more effective than colistin monotherapy (Conway, 1997). Falagas *et al*. (2005) reported that a pandrug-resistant (PDR) *P. aeruginosa* strain was isolated from bronchial secretion from a patient with pneumonia on the 34th day of the ICU stay. At that time, the medications were combination of colistin, meropenem, ofloxacin and gentamicin. PDR *P. aeruginosa* was not isolated again from subsequent cultures of bronchial secretions and there was an improvement of clinical and microbiological. Tascini *et al*. (2006) reported that the combination between colistin, imipenem and rifampin in the treatment of diabetic foot infection with osteomylitis due to MDR *P. aeruginosa* infection was efficiency and safety.

Data from Koomanachai *et al*. (2007) demonstrated the efficacy and safety of colistin (colistimethate sodium) for therapy of infection caused by MDR *P. aeruginosa* and *A. baumannii* in Siriraj Hospital, Bangkok, Thailand between January 2005 and Apirl 2006. In colistin group which are divided into colisin monotherapy and colistin in combinations with other antibiotics, 80.8% of patients showed good clinical and 94.4% showed good microbiological response. While in non colistin group, received other antibiotics, a good clinical response was only 26.7%. Therefore, colistin appears to be safe and effective for treatment of infections caused by MDR *P. aeruginosa* and *A. baumannii* in Thai adult patients.

CHAPTER III

MATERIALS AND METHODS

Materials

1. Microorganisms

The bacterial isolates used throughout this study were 30 isolates of *P. aeruginosa* clinically isolated from the patients at Siriraj Hospital between 2006 and 2008. All the isolates were kindly provided by Assistant Professor Chanwit Tribuddharat, M.D., Ph.D., Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University. *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as the control strains. All isolates were stored at -20º C in Tryptic soy broth: glycerin (85:15) and subcultured twice before use.

2. Chemicals

- Three standard laboratory powders were used : colistin sulfate (potency = 19,696 units/mg solid, lot No. 036K1374) was kindly provided from Sigma Aldrich, St. Louis, Mo., meropenem and sodium carbonate (potency = 71.73% on basis, lot No. 07100113746) and ciprofloxacin HCL (potency = 98%, batch cp 347 EOS) were kindly provide from Siam Bhesach Co., Ltd. Bangkok, Thailand used as working standard of colistin, meropenem and ciprofloxacin, respectively. Working standard solutions were prepared immediately prior to use.

- Five antimicrobial disks which were used : amikacin (30 µg), ciprofloxacin (5 µg) were purchased from BBL™ (Beckton Dickinson, USA), cefepime (30 µg), meropenem (10 µg) were purchased from BBL™ (BENEX Limited, USA) and piperacillin/tazobactam (100/10 µg) was purchased from Oxoid (Oxoid, Basingstoke, Hants, England). All of the disks were used to determine antimicrobial susceptibility by Kirby-Bauer disk diffusion method.

3. Media and reagents

- Mueller-Hinton agar (MHA) and Mueller-Hinton broth (MHB) purchased from BBL chemicals (Beckton Dickinson, USA) were used as the test medium for all bacterial isolates and control strains (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853).

- Tryptic soy agar (TSA) purchased from BBL chemicals (Beckton Dickinson, USA) were used as the culture media for *P. aeruginosa* and control strains.

- Sterile water was used as the solvent for the chemical powders to develop the working solution.

- Sterile 0.85% saline solution was used as the diluent of the inoculum in the turbidity adjusting process to quantity the precise numbers of bacteria. This solution was also applied as the diluent of the specimens in colony counting procedures in the time kill method.

4. Laboratory equipment and instruments

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

- Petri dishes were used 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 0.85% saline solution 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 inoculums and specimen, etc.

- Pipettes used in experiment are divided into 2 types;
    1) Glass pipettes were chosen to measure media, inoculums, drugs and solvent as general equipment processes.
    2) Micropipettes was 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, inoculums preparation, etc.

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

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

- Autoclave was used to sterilize equipment, media, diluents, inoculums and 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, inoculums 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 ovens were used to keep drying and sterilize all glass equipment before using.

Methods

1. Kirby-Bauer disk diffusion test was performed to determine the susceptibility of bacterial clinical isolates to antibiotics.
2. Agar dilution method was performed to determine the minimum inhibitory concentration (MIC) of the test agents.
3. Three dimensional checkerboard microdilution method was done to determine the combination effects of meropenem, ciprofloxacin and colistin.
4. Time kill method was done to determine the combined bactericidal activity of meropenem, ciprofloxacin and colistin.
5. Scanning electron microscope was used to observe the morphological cell change of merpenem-resistant *P. aeruginosa* after exposure to each antimicrobial agent alone and in combinations.

1. Kirby-Bauer disk diffusion test to determine the susceptibility of bacterial isolates to antibiotics.

Kirby-Bauer disk susceptible test was performed according to the disk diffusion method by Clinical and Laboratory Standards Institute (CLSI), 2006. All isolates including the control strain were tested to determine susceptibility of the organism against 5 broad-antimicrobial agents.

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, the media was allowed to cool in a water bath at 45 ^{o}C to 50 ^{o}C.

1.1.3 The freshly prepared and cooled medium was poured into plastic, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of media approximately 4 mm. This corresponds to 25 mL of media 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 for the sterility by incubating at 37 ^{o}C for 24 hours.

1.1.5 Unless the plates were used the same day, there were stored in a refrigerator (2 ^{o}C to 8 ^{o}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 to 24 hours *P. aeruginosa* from clinical specimen, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were selected from Tryptic soy agar (TSA) plates and transferred to a tube containing 7 mL of 0.85% saline solution.

1.2.2 The suspension was adjusted to match the turbidity of the 0.5 McFarland standard solution. A suspension containing approximately 1 to 2 x 10^{8} CFU/mL of bacteria was obtained.

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 should be rotated several time and pressed firmly on the inside wall of the tube above the fluid leveling order to remove excess inoculum from the swab for three times.

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 three times, rotating the plate approximately 60° each time to ensure an even distribution of inoculums.

1.3.3 The lid was left agar for 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 inoculated agar plates

1.4.1 The antibiotic disks were applied to the surface of the media with sterile forceps. Each disk was pressed down to ensure complete contact with the agar surface. They were distributed evenly so that they were no closer than 24 mm from center to center of each antibiotic disk.

1.4.2 The plate were inverted and incubated at 37 °C for 18 hours before measuring the zones of inhibition by venier-caliper.

1.5 Reading plates and interpreting results

1.5.1 After 18 hours of incubation, each plate was examined. The diameters of zones of inhibition, including the diameter of the disk were measured with digital sliding venier-caliper.

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

Table 3-1 Zone diameter interpretive standards breakpoints for *P. aeruginosa, E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 to 5 antimicrobial agents (CLSI, 2007).

Drugs	Disk content (µg)	Zone diameter (mm)				
		P. aeruginosa			*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
		R[a]	I[b]	S[c]		
amikacin	30	≤ 14	15-16	≥ 17	19-26	18-26
ciprofloxacin	5	≤ 15	16-20	≥ 21	30-40	25-33
cefepime	30	≤ 14	15-17	≥ 18	31-37	24-30
meropenem	10	≤ 13	14-15	≥ 16	28-34	27-33
piperacillin/tazobactam	100/10	≤ 17	-	≥ 18	24-30	25-333

R[a]= Resistant, I[b]= Intermediate resistant, S[c]= Susceptible

1.6 Screening for the multidrug-resistant isolates

An isolate was considered to be the multidrug-resistant when it was resistant to three or more of the following five broad-spectrum antimicrobial agents : amikacin, ciprofloxacin, cefepime, meropenem and piperacillin/tazobactam (Timurkaynak *et al*., 2006) according to the interpretation of disk diffusion test.

2. Agar dilution method to determine minimum inhibitory concentrations (MICs).

Agar dilution method was performed in duplicate according to CLSI, 2006 in order to determine minimal inhibitory concentrations (MICs) of meropenem, ciprofloxacin and colistin against all tested isolates.

2.1 Preparation of antimicrobial solution

2.1.1 The two-fold dilution of meropenem, ciprofloxacin and colistin solution (0.03 to 256 µg/mL) were prepared by diluting the drug in Mueller-Hinton agar (MHA). The antimicrobial concentrations used in initial solutions were prepared to be ten-fold higher than the desired final concentration (0.3 to 2,560 µg/mL).

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

2.1.3 Immediately after autoclaving, allow it to cool in a 45 ^{o}C to 55 ^{o}C water bath and then pipetted 6 mL of each antimicrobial dilution into bottle contain MHA 54 mL.

2.1.4 The agar and antimicrobial agent solution were mixed thoroughly and then pour into plates by 25 mL/plate.

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

2.2 Preparation of inoculums

2.2.1 The well-isolates colony of each 18 to 24 hours *P. aeruginosa* from clinical specimen, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were selected from Tryptic soy agar (TSA) plates and transferred to a tube containing 7 mL of 0.85% saline solution.

2.2.2 The suspension was adjusted to match the turbidity of the 0.5 McFarland standard solution. This resulted in a bacterial suspension contained approximately 1 to 2 x 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 will then be 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 ^{o}C for 18 hours.

2.5 Test result interpretation

2.5.1 The lowest concentration of antimicrobial agent that completely inhibited the growth of microorganism as detected by the unaided eye represented the MIC.

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

Table 3-2 MICs interpretive standard breakpoints (μg/mL) by agar dilution method (CLSI, 2007).

Drugs	Minimum Inhibitory Concentrations [MICs] (μg/mL)				
	P. aeruginosa			*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
	S[a]	I[b]	R[c]		
meropenem	≤ 4	8	≥ 16	0.008 – 0.06	0.25 - 1
ciprofloxacin	≤ 1	2	≥ 4	0.004-0.015[d]	0.25 - 1
colistin	≤ 2	4	≥ 8	0.25 – 1	0.25 – 2

S[a]= Susceptible, I[b]= Intermediate, R[c]= Resistant

d : Quality control limits for *E. coli* ATCC 25922 with ciprofloxacin, nalidixic acid, minocycline and sulfamethoxazole when test in CAMHB with 2.5 lysed horse blood incubated either in ambient air or 5% CO_2.

3. Three dimensional checkerboard microdilution method to determine combination effects of meropenem, ciprofloxacin and colistin in double and triple combinations.

Three dimensional checkerboard microdilution method was modified from Eliopoulos and Moellering, 1996 and Yoon *et al*., 2004. All isolates were included in the test to determine the combination effect of meropenem, ciprofloxacin and colistin. The concentrations tested for each antimicrobial agents typically range from four dilutions below the MIC to twice the MIC of each isolate, using two-fold dilutions of each antimicrobial agent.

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 to be four-fold higher than the desired final concentration.

3.2 Preparing diluted antimicrobial agents

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

3.2.2 The concentrations of meropenem and colistin used in the initial solutions were prepared to be eight-fold higher than the desired final concentration and ciprofloxacin was four-fold higher 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 may be prepared by suspending colonies directly to obtain the turbidity of the 0.5 McFarland standard. A suspension containing approximately 1 to $2x10^8$ CFU/mL of bacteria was obtained.

3.3.1 The adjusted inoculum suspension was diluted 1:100 in 0.85% saline solution thereafter within 15 minutes after the inoculation, each well contained approximately $5x10^5$ CFU/mL in final volume.

3.3.2 The final volume of 200 µL in each well consisted of 50 µL of MHB, 25 µL of meropenem, 25 µL of colistin, 50 µL of ciprofloxacin and 50 µL of bacterial suspension was obtained.

3.3.3 A series of antimicrobials concentrations were taken to produce the desired range of drug concentration by adding an aliquot of those solution to each well in the appropriate row or column by the three dimensional microdilution checkerboard method, which modified from previous study. Three dimensional checkerboard microdilutions

were principally base on the standard two dimensional checkerboard method. At first plate contained no colistin, increasing the serial two-fold dilution of ciprofloxacin ranging from 1/16 to 4 of the MIC in X axis and meropenem ranging from 1/16 to 4 of the MIC in Y axis in 96-well microtitre plate. The third agent, colistin, was dispended throughout the wells as an overlay in all wells. The subsequent seven plates contained a fix concentration of colistin ranging from 1/16 to 4 of the MIC with increasing the serial two-fold dilution of ciprofloxacin ranging from 1/16 to 4 of the MIC in X axis and meropenem ranging from 1/16 to 4 of the MIC in Y axis.

3.3.4 The rest rows or columns of microtitre plate were used to determine MICs of each antimicrobial agent alone.

3.4 Reading plates and interpreting results

3.4.1 After 18 hours, each panel was examined to determine MIC, the MIC is the lowest concentration of antimicrobial agent that completely inhibits growth of the organism in the panel as detected by the unaided eye. The amount of growth in the microwell 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 were done by reading the first clear well in each row of panel with the antimicrobial agents and calculated the fractional inhibitory concentrations.

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

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

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

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

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

FIC index (∑FIC) = Sum of the FICs of each antibiotic

3.4.5 The smallest FIC value was used to establish the antimicrobial combination interaction for each specific isolate referring to definition of Yoon *et al.*, 2004.

3.4.6 FIC index results for each combination were defined as:

Synergy : $\sum FIC < 1.0$

Additive : $\sum FIC = 1.0$

Antagonist : $\sum FIC > 1$

3.4.7 Results were expressed as FICI range, percentage of isolates with synergy, additive and antagonist.

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

The antibacterial activity of the combinations were performed according to the time kill method (NCCLS, 1999). Five meropenem-resistant *P. aeruginosa* at different antimicrobial susceptibility were tested to determine the bactericidal activity of meropenem, ciprofloxacin and colistin alone and the combinations of double and triple of these agents. The bacterial isolates in time kill method must be correlated with synergistic effect in checkerboard microdilution method which describe previously.

4.1 Determination of bactericidal activity of meropenem, ciprofloxacin and colistin alone and in double and triple combinations.

4.1.1 The concentrations of antimicrobial agents were chosen to reflect clinical relevant levels. The concentration of meropenem was prepared to 16 and 25 µg/mL following Kanellakopoulou *et al*., 2008 These were mean serum level of conventional dose and maximum serum level, respectively. The concentration of ciprofloxacin was 4 µg/mL (maximum serum level). Colistin was prepared to 0.5xMIC of specific isolate (referring to the MICs from checkerboard microdilution). Antimicrobial concentrations used in initial (stock) solutions were prepared to be ten-fold higher than the desired final concentrations.

4.1.2 One mL of each agent was pipetted into 6 mL of Mueller Hinton broth (MHB) for the preparation of the working media before adding the standardized inoculum (final volume of working media = 9 mL). By doing so, there were 12 groups including

1) control (no antimicrobial agents)
2) meropenem 16 µg/mL
3) meropenem 25 µg/mL

4) ciprofloxacin 4 μg/mL

5) colistin 0.5xMIC*

6) meropenem 16 μg/mL plus ciprofloxacin 4 μg/mL

7) meropenem 25 μg/mL plush ciprofloxacin 4 μg/mL

8) meropenem 16 μg/mL plus colistin 0.5xMIC

9) meropenem 25 μg/mL plus colistin 0.5xMIC

10) ciprofloxacin 4 μg/mL plus colistin 0.5xMIC

11) meropenem 16 μg/mL plus ciprofloxacin 4 μg/mL and colistin 0.5xMIC

12) meropenem 25 μg/mL plus ciprofloxacin 4 μg/mL and colistin 0.5xMIC

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 the 9 mL working media which made the final concentration approximately 1 x 10^6 CFU/mL and incubated at 37 ^{o}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 saline solution and 20 μL of each dilution was dropped to the surface of TSA plates in duplicate and then incubated at 37 ^{o}C for 18 hours.

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

* referring to the MICs from checkerboard microdilution method that equal to 1 μg/mL.

4.1.8 Killing curves were constructed by Microsoft Excel 2007 at each time interval. The log change of the viable cell counts compared to the starting inoculums was determined.

4.1.9. The results were analyzed by determining the number of isolate which the yield changes in the log number of CFU/mL of -1, -2 and -3 at 2, 4, 6, 8 and 24 hours compared to the counts at 0 hour. A given concentration of antimicrobial alone or in combination was considered bactericidal if it reduced the original inoculums size by ≥ 3 log CFU/mL (≥ 99.9% killing) at each of the time periods or bacteriostatic if the inoculums size was reduced by 0-< 3 log CFU/mL. The regrowth was defined as an increase of ≥ 2 log CFU/mL after 6 hours (Pankuch, Jacobs and Appelbaum, 1994; Amterdam, 1996; Gunderson *et al*., 2004; Reinert and Al-Lahham, 2005).

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

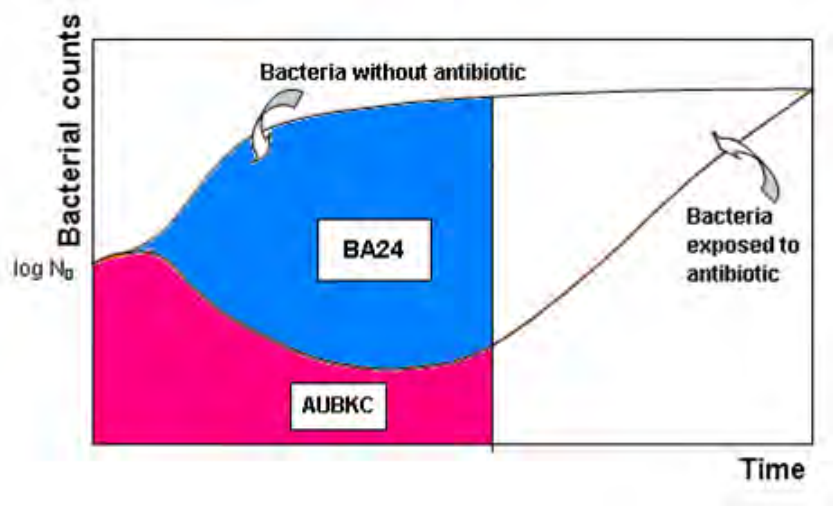

Figure 3-1 Parameters for quantifying bacterial killing, regrowth curve and the antimicrobial effect (Picture modified from Firsov *et al.,* 1997).

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).
- The quantitative evaluation of antimicrobial effect ($AUBKC_{0-24}$, BA_{24}) was calculated as in page 50.

5. Scanning electron microscope was used to observe the morphological cell change of merpenem-resistant *P. aeruginosa* after exposure to each antimicrobial agent alone and in combinations.

The scanning electron microscopy (modified from Kobayashi *et al.,* 2004) was chosen to examine the morphological changes in a representative of meropenem resistant *P. aeruginosa* when exposed to drugs alone and the combination for 2 hours. The *P. aeruginosa* isolate was selected from meropenem-resistant isolates which showed synergistic effect from checkerboard method and bactericidal activity from time kill method. The concentrations of antimicrobial agents and bacterial isolates in this study were correlated to time kill study. Therefore, the morphological changes were observed in meropenem alone, colistin alone, and the combination.

5.1 Meropenem was prepared to 16 µg/mL and colistin concentration was 0.5xMIC. 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 inoculums

(final volume of working media = 9 mL). By doing so, there were 4 groups including

1) control (no antimicrobial agents)
2) meropenem 16 µg/mL
3) colistin 0.5xMIC
4) meropenem 16 µg/mL plus colistin 0.5xMIC

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 which made the final concentration approximately 1 x 10^6 CFU/mL and was then incubated at 37 ^{o}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 0.1 M phosphate buffer pH 7.2 for 2 hours then they were rinsed twice in phosphate buffer for 10 min/each and once in distilled water for 10 minutes.

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

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

5.9 The specimens were observe under a scanning electron microscope (JEOL, model JSM-5410LV) and were photographed.

Statistical analysis

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

CHAPTER IV

RESULTS

Antimicrobial susceptibility test

Antimicrobial susceptibility test was performed by disk diffusion test (Figure 4-1) according to CLSI (2006). The percentage of susceptible isolates, intermediate and resistant to each 5 broad-spectrum antimicrobial agents for *P. aeruginosa* isolates were presented in Table 4-1. It was shown that all isolates were resistant to meropenem whereas 83.33%, 73.33%, 73.33% and 40% of the tested isolates were resistant to ciprofloxacin, amikacin, cefepime and piperacillin/tazobactam, respectively.

Table 4-1 Antimicrobial susceptibility of 5 broad-spectrum antimicrobial agents against 30 clinical isolate of *P. aeruginosa* as tested by disk diffusion method.

Antimicrobial agents	Susceptible	Intermediate	Resistant
	No. of isolates (%)	No. of isolates (%)	No. of isolates (%)
amikacin	7 (23.33)	1 (3.33)	22 (73.33)
ciprofloxacin	4 (13.33)	1 (3.33)	25 (83.33)
cefepime	7 (23.33)	1 (3.33)	22 (73.33)
meropenem	0 (0)	0 (0)	30 (100)
piperacillin/tazobactam	18 (60)	0 (0)	12 (40)

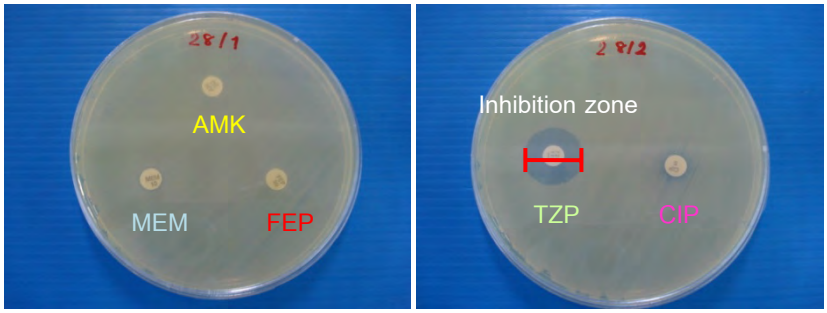

Figure 4-1 Inhibition zone of 5 antimicrobial agents of one *P. aeruginosa* isolate was determined by Kirby-Bauer disk diffusion method (AMK: amikacin, MEM: meropenem, FEP: cefepime, TPZ: piperacillin/tazobactam, CIP: ciprofloxacin)

The screening result for multidrug-resistant (MDR) isolate of *P. aeruginosa* from this part of the study showed that, 26 isolates (86.67%) were MDR *P. aeruginosa* because they were resistant to at least 3 out of 5 of the tested agents, while 4 isolates (13.33%) were non-MDR isolates (Table 4-2). Among the MDR isolates, 3 isolate (11.54%) were resistant to 3 agents, 15 isolates (57.69%) were resistant to 4 agents and 8 isolates (30.77%) were resistant to all 5 tested agents. The distributions of MDR isolates of *P. aeruginosa* according to the number of antimicrobial agents were shown in Figure 4-2.

Table 4-2 Distribution of *P. aeruginosa* according to the number of antimicrobial agents that they were resistant.

No. of antimicrobial	Resistant[a]	% of isolates	Total isolates	%
0 drug	0	0	4 (Non-MDR)	13.33
1 drug	3	10		
2 drugs	1	3.33		
3 drugs	3	10	26 (MDR)	86.67
4 drugs	15	50		
5 drugs	8	26.67		

[a]Intermediate resistant or Resistant

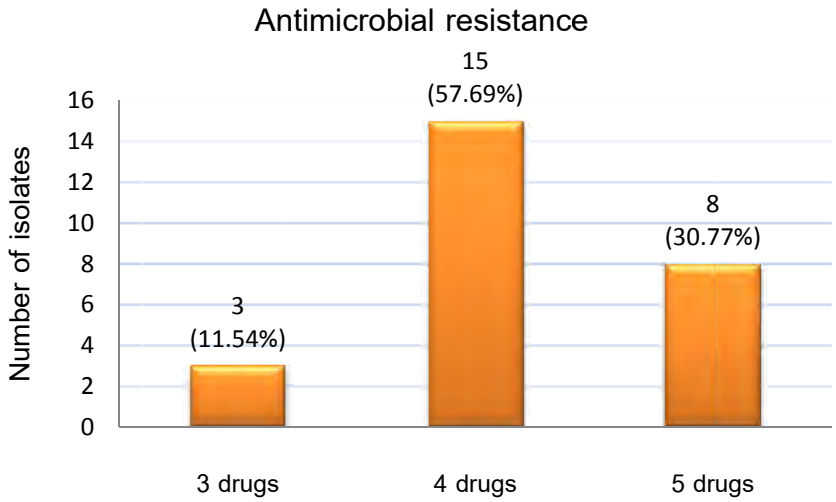

Figure 4-2 Distribution of twenty-six multidrug-resistant isolates of *P. aeruginosa* according to the number of antimicrobials to which they were resistant.

Minimum inhibitory concentrations (MICs) determined by agar dilution method

The minimum inhibitory concentrations (MICs) test was performed by agar dilution (Figure 4-3) according to CLSI (2006). The MICs range, MIC_{50} and MIC_{90} of meropenem, ciprofloxacin and colistin from agar dilution when tested with all 30 isolates of *P. aeruginosa* were shown in Table 4-3. Most of the tested isolates were resistant to both meropenem and ciprofloxacin (73.33% and 86.67%, accordingly) while all of the *P. aeruginosa* isolates were susceptible to colistin. The MICs range of meropenem were 8-32 µg/mL (MIC_{50} 16 µg/mL and MIC_{90} 32 µg/mL) while the MICs range of ciprofloxacin were 0.5-128 µg/mL, MIC_{50} 64 µg/mL and MIC_{90} 128 µg/mL which were the high level resistance (susceptibility breakpoint $\leq$1 µg/mL). Whereas all isolate were very susceptible to colistin with the MICs range of 0.5-2 µg/mL (MIC_{50} 1 µg/mL and MIC_{90} 2 µg/mL).

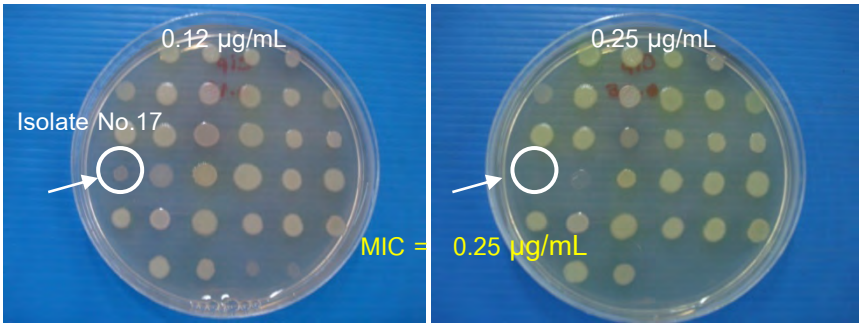

Figure 4-3 Agar dilution method for determination MICs of ciprofloxacin against *P. aeruginosa* 30 isolates.

Table 4-3 Antimicrobial susceptibility, MIC_{50} and MIC_{90} of meropenem, ciprofloxacin and colistin against 30 *P. aeruginosa* isolates.

Susceptibility	Meropenem		Ciprofloxacin		Colistin	
	No. of isolates	%	No. of isolates	%	No. of isolates	%
Susceptible	0	0	4	13.33	30	100
Intermediate	8	26.67	0	0	0	0
Resistant	22	73.33	26	86.67	0	0
Range (µg/mL)	8-32		0.5-128		0.5-2	
MIC_{50} (µg/mL)	16		64		1	
MIC_{90} (µg/mL)	32		128		2	

Susceptibility breakpoint : Meropenem ≤ 4 µg/mL. Ciprofloxacin ≤ 1 µg/mL, Colistin ≤ 2 µg/mL

Combination effects

The combination effects between meropenem, ciprofloxacin and colistin against 30 clinical isolates of *P. aeruginosa* were investigated by the checkerboard microdilution method as shown in Table 4-4 and Table 4-5. The combination effects were evaluated from the Fractional inhibitory concentration (FIC) index.

Table 4-4 Combination effects of meropenem, ciprofloxacin and colistin against 30 clinical isolates of *P. aeruginosa*.

Antimicrobial combinations	FICI range	Synergy (ΣFIC < 1)	Additive (ΣFIC = 1)	Antagonism (ΣFIC > 1)
		No. of isolate (%)	No. of isolate (%)	No. of isolates (%)
MEM + CIP	0.75-1.125	9(30)	15(50)	6(20)
MEM + COL	0.563-1.250	11(36.67)	6(20)	13(43.33)
CIP + COL	0.560-1.060	11(36.67)	7(23.33)	12(40)
MEM + CIP + COL	0.623-1.060	18(60)	2(6.66)	10(33.33)

Abbreviations; MEM, meropenem; CIP, ciprofloxacin; COL, colistin; FICI, fraction inhibitory concentration index.

There were difference in the combination effects for the different antimicrobial combinations against 30 isolates of *P. aeruginosa* (Table 4-4). Among double combinations, meropenem combined with ciprofloxacin showed synergy against only 9 isolates (30%), additive 15 isolates (50%) and antagonism 6 isolates (20%). While meropenem combined with colistin and ciprofloxacin combined with colistin showed the same of synergy rate, 11 isolates (36.67%), additive and antagonism were approximately 20-23.33% and 40-43.33%, respectively. Triple combination of meropenem, ciprofloxacin and colistin showed resulting synergistic on the highest rate, 18 isolates (60%) but additive was 2 isolates (6.66%) and antagonism was 10 isolates (33.33%).

Table 4-5 MICs of meropenem, ciprofloxacin, colistin alone and in combination with FIC index were determined by checkerboard microdilution method.

Isolate No.	MIC[a] (µg/mL)																				
	Alone		Combined			Alone		Combined			Alone		Combined			Alone			Combined			
	MEM	CIP	MEM	CIP	FIC[b] index	MEM	COL	MEM	COL	FIC[b] index	CIP	COL	CIP	COL	FIC[b] index	MEM	CIP	COL	MEM	CIP	COL	FIC[b] index
1	8	64	4	32	1	16	1	16	0.06	1.060	64	1	64	0.06	1.060	16	64	1	8	16	0.12	0.87
2	16	64	8	32	1	32	1	16	0.5	1	64	1	64	0.06	1.060	32	64	1	16	8	0.12	0.745
3	16	64	8	16	0.75	16	2	16	0.12	1.060	128	2	64	1	1	16	128	2	2	64	0.12	0.685
4	16	8	8	2	0.75	16	1	16	0.06	1.060	8	1	8	0.06	1.060	16	8	1	8	2	0.06	0.81
5	32	1	16	0.25	0.75	64	2	8	1	0.625	1	2	0.06	1	0.560	64	1	2	4	0.06	1	0.623
6	8	64	0.5	64	1.063	16	2	8	1	1	64	2	32	1	1	16	64	2	8	32	0.06	1.030
7	16	8	8	2	0.75	32	2	16	1	1	8	2	4	1	1	32	8	2	16	2	0.06	0.78
8	8	64	4	32	1	16	2	1	1	0.563	64	2	32	1	1	16	64	2	4	16	1	1
9	8	64	0.5	64	1.063	16	2	16	0.06	1.030	64	2	64	0.06	1.030	16	64	2	8	32	0.06	1.030
10	16	64	8	32	1	16	2	1	1	0.563	64	2	8	1	0.625	16	64	2	1	8	1	0.688
11	8	64	0.5	64	1.063	16	1	16	0.06	1.060	64	1	64	0.06	1.060	16	64	1	8	16	0.12	0.87
12	8	64	4	16	0.75	16	1	16	0.06	1.060	64	1	64	0.06	1.060	16	64	1	8	16	0.06	0.81
13	32	1	16	0.5	1	64	2	4	1	0.563	1	2	0.12	1	0.620	64	1	2	2	0.12	1	0.651
14	16	64	8	32	1	16	2	2	1	0.625	64	2	4	1	0.563	16	64	2	2	8	1	0.75
15	16	32	8	16	1	16	2	1	1	0.563	64	2	4	1	0.563	16	64	2	2	4	1	0.688

[a] MIC from checkerboard method; Meropenem (MEM) ≤ 4 µg/mL (Susceptible), = 8 µg/mL (Intermediate), ≥ 16 µg/mL (Resistant); Ciprofloxacin (CIP) ≤ 1 µg/mL (Susceptible), = 2 µg/mL (Intermediate), ≥ 4 µg/mL (Resistant); Colistin (COL) ≤ 2 µg/mL (Susceptible), = 4 µg/mL (Intermediate), ≥ 8 µg/mL (Resistant).

[b] FIC index < 1 (Synergy), = 1 (Additive), > 1 (Antagonist), Shade : isolate of *P. aeruginosa* that occurred synergistic effect in each combination.

Table 4-5 (Continued) MICs of meropenem, ciprofloxacin, colistin alone and in combinations with FIC index were determined by checkerboard microdilution method.

Isolate No.	MIC[a] (µg/mL)																				
	Alone		Combined			Alone		Combined			Alone		Combined			Alone			Combined			
	MEM	CIP	MEM	CIP	FIC[b] index	MEM	COL	MEM	COL	FIC[b] index	CIP	COL	CIP	COL	FIC[b] Index	MEM	CIP	COL	MEM	CIP	COL	FIC[b] index
16	16	128	8	64	1	16	2	16	0.06	1.030	128	2	128	0.06	1.030	16	128	2	8	64	0.06	1.030
17	64	0.25	32	0.12	1	64	1	64	0.06	1.060	0.25	1	0.25	0.06	1.060	64	0.25	1	32	0.12	0.06	1.060
18	8	0.5	4	0.25	1	8	2	4	1	1	0.25	2	0.25	0.06	1.030	8	0.25	2	4	0.12	0.06	1.030
19	16	128	4	64	0.75	16	1	1	0.06	1.060	128	1	128	0.06	1.060	16	128	1	4	64	0.06	0.81
20	16	64	1	64	1.063	16	2	16	0.12	1.060	64	2	16	1	0.75	16	64	2	8	32	0.12	1.060
21	16	128	8	64	1	16	2	4	1	0.75	128	2	32	1	0.75	16	128	2	4	32	1	1
22	16	64	8	32	1	16	2	4	1	0.75	64	2	16	1	0.75	16	64	2	8	32	0.06	1.030
23	16	128	8	64	1	16	2	16	0.12	1.060	128	2	128	0.12	1.060	16	128	2	8	64	0.12	1.060
24	16	64	2	64	1.125	32	1	8	0.5	0.75	32	1	8	0.5	0.75	32	32	1	16	8	0.06	0.78
25	16	128	8	64	1	16	2	16	0.5	1.250	128	2	64	1	1	16	128	2	8	64	0.06	1.030
26	32	128	2	128	1.063	16	2	2	1	0.625	256	2	32	1	0.625	16	256	2	2	32	1	0.75
27	16	64	8	32	1	16	2	8	1	1	64	2	64	0.06	1.030	16	64	2	8	32	0.06	1.03
28	16	16	8	4	0.75	16	2	16	0.06	1.030	16	2	8	1	1	16	16	2	8	4	0.06	0.78
29	32	128	16	32	0.75	32	2	16	1	1	128	2	64	1	1	32	128	2	16	8	0.25	0.688
30	32	8	16	2	0.75	32	2	4	1	0.625	8	2	0.5	1	0.563	32	8	2	16	2	0.06	0.78

[a] MIC from checkerboard method; Meropenem (MEM) ≤ 4 µg/mL (Susceptible), = 8 µg/mL (Intermediate), ≥ 16 µg/mL (Resistant); Ciprofloxacin (CIP) ≤ 1 µg/mL (Susceptible), = 2 µg/mL (Intermediate), ≥ 4 µg/mL (Resistant); Colistin (COL) ≤ 2 µg/mL (Susceptible), = 4 µg/mL (Intermediate), ≥ 8 µg/mL (Resistant).

[b] FIC index < 1 (Synergy), = 1 (Additive), > 1 (Antagonist), Shade : isolate of *P. aeruginosa* that occurred synergistic effect in each combination.

As shown in Table 4-5, the synergistic effect from the combination of meropenem and ciprofloxacin was observed only in 9 of 30 isolates (isolate No. 3, 4, 5, 7, 12, 19, 28, 29 and 30) with FIC index 0.75. Synergy were observed with such combination at the range of meropenem and ciprofloxacin concentrations 4-16 μg/mL and 0.25-64 μg/mL, respectively. The MICs of meropenem and ciprofloxacin alone were ranged from 8-32 μg/mL and 1-128 μg/mL, respectively. For the combination of meropenem and colistin, synergistic effect against 11 of 30 isolates (isolate No. 5, 8, 10, 13, 14, 15, 21, 22, 24, 26 and 30) with FIC index 0.563-0.75 was observed at concentrations of meropenem and colistin ranged from 1-8 μg/mL and 0.5-1 μg/mL, respectively. The MICs of meropenem and colistin alone were ranged from 16-64 μg/mL and 1-2 μg/mL, respectively. Synergy of the combination ciprofloxacin with colistin were observed in the same isolates of the combination of meropenem with colistin, except that isolate No. 8 was 20 with FIC index 0.560-0.75. Synergistic effect was observed at concentrations of ciprofloxacin and colistin ranged from 0.06-32 μg/mL and 0.5-1 μg/mL, respectively. The MICs of ciprofloxacin and colistin alone against all these isolate ranged from 1-256 μg/mL and 1-2 μg/mL, respectively. Triple combination exhibited synergistic effect in 18 of 30 isolates (isolates No. 1, 2, 3, 4, 5, 7, 10, 11, 12, 13, 14, 15, 19, 24, 26, 28, 29 and 30) with FIC index ranged from 0.623-0.81 at meropenem concentrations ranged from 1-16 μg/mL while ciprofloxacin and colistin concentrations ranged from 0.06-64 μg/mL and 0.06-1 μg/mL, respectively. The MICs of meropenem, ciprofloxacin and colistin alone ranged from 16-64 μg/mL, 1-256 μg/mL and 1-2 μg/mL, respectively.

Time kill study

Time kill study was performed to evaluate the bactericidal activity of meropenem, ciprofloxacin, colistin and the combinations against meropenem-resistant *P. aeruginosa*. The concentrations of meropenem used were 16 and 25 µg/mL, ciprofloxacin was 4 µg/mL. These concentrations were chosen to reflect clinical relevant levels. The concentration of colistin was 0.5xMIC (referring to the MICs from checkerboard microdilution mehod). The bacterial isolates in time kill method must be correlated with synergistic results in combination effects from checkerboard method which described previously. Five isolates of meropenem-resistant *P. aeruginosa* were tested, including number 10, 15, 21, 26 and 30 as shown in Table 4-6 and Table 4-7.

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

Isolate No.	MICs[a] (µg/mL) for isolates			No. of antimicrobial resistant[b]
	Meropenem	Ciprofloxacin	Colistin	
10	16	64	2	5
15	16	64	2	4
21	16	128	2	5
26	32	128	2	5
30	32	8	2	3

[a]MICs from checkerboard microdilution method; [b]determined by Kirby-Bauer Disk diffusion method

Table 4-7 Minimum inhibitory concentrations (MICs) of meropenem, ciprofloxacin and colistin and combination effects of these agents against 5 isolates of meropenem resistant *P. aeruginosa*.

Isolate No.	MICs[a] (μg/mL) for isolates			FIC index/Combination effects[b]			
	MEM	CIP	COL	MEM+CIP	MEM+COL	CIP+COL	MEM+CIP+COL
10	16	64	2	1/ Add	0.563/ Syn	0.563/ Syn	0.688/ Syn
15	16	64	2	1/ Add	0.563/ Syn	0.563/ Syn	0.688/ Syn
21	16	128	2	1/ Add	0.75/ Syn	0.75/ Syn	1/ Add
26	32	128	2	1.063/ Ant	0.625/ Syn	0.625/ Syn	0.75/ Syn
30	32	8	2	0.75/ Syn	0.625/ Syn	0.563/ Syn	0.78/ Syn

[a]MICs from checkerboard microdilution method ; [b]Syn = Synergy, Add = Additive, Ant = Antagonist

Abbreviations; MER, meropenem; CIP, ciprofloxacin; COL, colistin.

In this study, 5 isolates of meropenem-resistant *P. aeruginosa* were included. The mean (±SD) log decrease of viable cell count and bacteriolytic area for 24 hours (BA_{24}) were determined for the time kill effect of an antimicrobial or of the combinations of meropenem, ciprofloxacin and colistin. Numbers of isolates which were killed at various time intervals were shown in Table 4-8.

Table 4-8 Reduction of viable cell counts of meropenem-resistant *P. aeruginosa* (5 isolates) at various time intervals.

Antimicrobial agents	No. of isolates to be killed at time point															
	2 hr			4 hr			6 hr			8 hr			24 hr			
	-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3	-1	-2	-3	R
MEM (16)	1	-	-	2	-	-	2	-	-	1	-	-	-	-	-	5
MEM (25)	2	-	-	3	1	-	-	1	1	-	-	2	-	-	-	5
CIP (4)	-	(1)	-	(1)	-	-	(1)	-	-	(1)	-	-	-	-	-	5
COL (0.5xMIC)	-	-	5	-	-	5	-	-	5	-	1	4	-	-	-	5
MEM (16) + CIP (4)	1	(1)	-	2	(1)	-	2	-	(1)	1	-	(1)	-	-	-	5
MEM (25) + CIP (4)	2	-	(1)	2	1	(1)	-	1	2(1)	-	-	3(1)	-	-	-	5
MEM (16) + COL (0.5xMIC)	-	-	5	-	-	5	-	-	5	-	-	5	-	-	5	-
MEM (25) + COL (0.5xMIC)	-	-	5	-	-	5	-	-	5	-	-	5	-	-	5	-
CIP (4) + COL (0.5xMIC)	-	-	5	-	-	5	-	-	5	-	-	5	1	-	-	5
MEM (16) + CIP (4) + COL (0.5xMIC)	-	-	5	-	-	5	-	-	5	-	-	5	-	1	4	1
MEM (25) + CIP (4) + COL (0.5xMIC)	-	-	5	-	-	5	-	-	5	-	-	5	-	-	5	-

(1) : isolate No. 30 (MIC of ciprofloxacin = 8 µg/mL), -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

After exposed to meropenem at 16 µg/mL, the bactericidal activity (99.9% killing or -3 log CFU/mL) could not be observed during the time of study. Only 2 out of 5 isolate (isolate No. 10 and 15) were killed at the level of 90% killing (-1 log CFU/mL). One isolate (isolates No. 15) was killed at 2nd to 6th hour while another isolate (isolate No. 10) was killed at 4th to 8th hour. The regrowth was observed at 24th hour. When the concentration of meropenem was increased to 25 µg/mL, 99.9% killing was observed in only 2 isolates, one isolate (isolate No. 10) was killed at 6th to 8th hour and the other one isolate (isolate No. 15) was killed at 8th hour. However, all of the isolates were regrowth in

both concentrations of meropenem at 24th hour. For 4 μg/mL ciprofloxacin, the killing activity was rarely observed during the time of study (< -1 log CFU/mL), only one isolate (isolate No. 30, MIC 8 μg/mL) was killed at the level of 99% killing (-2 log CFU/mL) at 2th hour and declined to 90% killing at 4th to 8th hours, after that the regrowth was also observed at 24th hour. With 0.5xMIC colistin (1 μg/mL), the rapid bacterial killing of all isolates, 99.9% killing could be observed at 2nd hour but at the 8th hour, 99.9% killing was observed only in 4 isolates. Nevertheless, all of them were regrowth at 24th hour.

In double combinations, the killing effects of meropenem 16 μg/mL or 25 μg/mL combined with 4 μg/mL ciprofloxacin were demonstrated. Killing effects in most of the isolates did not differ in different concentration of meropenem alone. Only one isolate (Isolate No. 30) was killed at the level of 99% killing during the 2nd to 4th hour and increased to 99.9% killing during the 6th to 8th hour in 16 μg/mL meropenem combined with ciprofloxacin. Whereas, exposure to 25 μg/mL meropenem combined with ciprofloxacin, 99.9% killing of the same isolate was observed during the 2nd to 8th hour. However, bacterial regrowth of all isolates at 24th hour was observed in these combinations. When each meropenem concentrations combined with 0.5xMIC colistin, 99.9% killing of all isolates could be observed during the 2nd to 24th hour without regrowth. For combination of ciprofloxacin and colistin, 99.9% killing of all isolates could be observed during the 2nd to 8th hour. However, at 24th hour, all isolates were also regrowth as in colistin alone.

In triple combinations of each meropenem concentration (16 or 25 μg/mL) combined with ciprofloxacin and colistin, 99.9% killing of all isolates were observed during the 2nd to 24th hour. In addition, the regrowth of one isolate (isolate No. 21) was observed at the 24th hour in the combination of 16 μg/mL meropenem combined with ciprofloxacin and colistin even though 99% killing could be observed.

The mean killing effects of meropenem, ciprofloxacin and colistin alone and the combinations against 5 meropenem-resistant *P. aeruginosa* are shown in Figure 4-4 and Table 4-9.

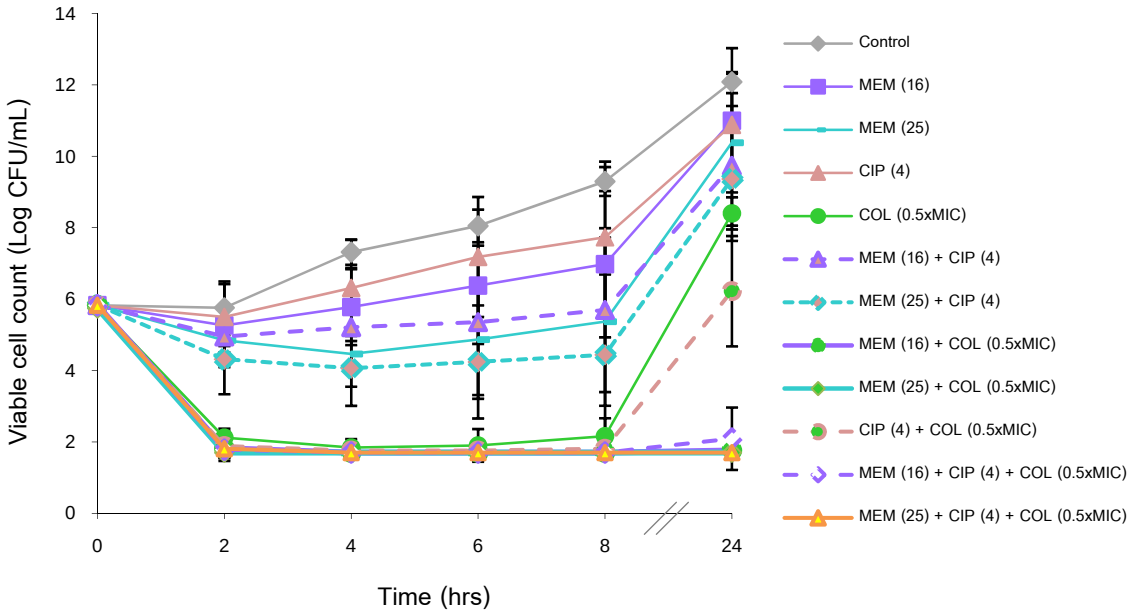

Figure 4-4 Time killing curve showing the antibacterial activity of the combinations against 5 isolates of meropenem-resistant *P. aeruginosa*.

Table 4-9 Mean (±SD) of log change viable cell counts after exposed to meropenem (MEM), ciprofloxacin (CIP), colistin (COL) and the combinations at various time intervals, $AUBKC_{0-24}$ and BA_{24} of 5 isolates of meropenem-resistant *P. aeruginosa*.

Isolate No.	Change in viable cell counts (log CFU/mL) at the following time					$AUBKC_{0-24}$	BA_{24}
	$\Delta 2$	$\Delta 4$	$\Delta 6$	$\Delta 8$	$\Delta 24$		
Average							
CONTROL	-0.07±0.62	1.49±0.30	2.22±0.44	3.47±0.44	6.26±0.97	228.42±12.08	0.00
MEM (16)	-0.57±0.52	-0.05±1.10	0.55±1.67	1.15±2.10	5.17±1.35	190.96±34.15	37.46±30.01[a]
MEM (25)	-0.99±0.09	-1.37±0.42	-0.96±1.60	-0.46±2.39	4.55±1.39	165.21±33.26	63.22±27.23[a,c]
CIP (4)	-0.33±0.95	0.49±1.31	1.35±1.64	1.90±2.09	5.05±1.48	199.15±37.38	29.28±29.27[a]
COL (0.5xMIC)	-3.71±0.29	-3.99±0.27	-3.92±0.49	-3.66±0.55	2.57±0.55	104.25±8.61	124.18±13.46[b]
MEM (16) + CIP (4)	-0.88±0.81	-0.62±1.65	-0.48±2.14	-0.14±2.28	3.91±1.68	165.98±42.50	62.45±33.49[d]
MEM (25) + CIP (4)	-1.51±0.92	-1.77±1.03	-1.59±1.60	-1.39±2.26	3.54±1.74	145.98±39.24	82.49±30.18[e]
MEM (16) + COL (0.5xMIC)	-4.01±0.31	-4.13±0.10	-4.13±0.10	-4.13±0.10	-4.07±0.06	45.63±1.17	182.80±12.60[f,h,i]
MEM (25) + COL (0.5xMIC)	-4.13±0.10	-4.13±0.10	-4.13±0.10	-4.13±0.10	-4.13±0,10	44.90±0.10	183.52±12.08[g]
CIP (4) + COL (0.5xMIC)	-3.94±0.45	-4.07±0.20	-4.07±0.20	-4.02±0.30	0.39±1.61	82.69±14.77	145.74±17.05[j]
MEM (16) + CIP (4) + COL (0.5xMIC)	-4.05±0.22	-4.13±0.10	-4.13±0.10	-4.13±0.10	-3.74±0.90	48.19±7.33	180.23±14.57[k,m]
MEM (25) + CIP (4) + COL (0.5xMIC)	-4.02±0.28	-4.13±0.10	-4.13±0.10	-4.13±0.10	-4.13±0.10	45.12±0.48	183.30±12.12[l]

[a]= p>0.05 compared to control, [b,]= p<0.05 compared to control, [c]=p>0.05 compared to activity of MEM 16 µg/mL, [d]=p>0.05 compared to activity of MEM16 µg/mL, [e]=p>0.05 compared to activity of MEM 25 µg/mL, [f]=p<0.05 compared to activity of MEM 16 µg/mL, [g]= p<0.05 compared to MEM 25 µg/mL, [h]= p>0.05 compared to MEM 25 µg/mL+ COL 0.5xMIC, [i]= p<0.05 compared to COL 0.5xMIC, [j]= p>0.05 compared to COL 0.5xMIC, [k]= p>0.05 compared to MEM 16 µg/mL+COL 0.5xMIC, [l]= p>0.05 compared to MEM 25 µg/mL+COL 0.5xMIC, [m]= p>0.05 compared to MEM 25 µg/mL+CIP 4 µg/mL+COL 0.5xMIC, $AUBKC_{0-24}$= Area under bacterial killing and regrowth curve for 24 hours, BA_{24}= Bacteriolytic area for 24 hour.

Concerning of activities of meropenem, ciprofloxacin, colistin alone and the combinations against all 5 isolates, it was found that meropenem alone at concentration 16 µg/mL showed bacterial killing effect less than 90% killing against all 5 meropenem resistant isolates. At merpenem concentration 25 µg/mL, 90% killing was observed at the 4th hour. BA_{24} of 16 and 25 µg/mL meropenem were 37.46±30.01 and 63.22±27.23 log CFU/mL.h, respectively considered not significantly different between these two concentrations ($p>0.05$). In addition, these BA_{24} values were not significantly different from control ($p>0.05$). Ciprofloxacin 4 µg/mL demonstrated low killing effect (BA_{24} = 29.28±29.27 log CFU/mL.h) which was consistent with meropenem and considered not to be significantly different from control group as well ($p>0.05$). For colistin at 0.5xMIC (1µg/mL), bactericidal activity (-3 log CFU/mL, 99.9% killing) was observed at 2nd to 8th hour and the bacterial regrowth of the pathogens were observed after that. BA_{24} of colistin was 124.18±13.46 log CFU/mL.h. This result was significantly different when compared to control ($p=0.002$).

In double combinations, the combination of meropenem at each concentration (16 or 25 µg/mL) with 4 µg/mL ciprofloxacin demonstrated 90% killing during 2nd to 8th hour only in the combination with higher concentration of meropenem (25 µg/mL) with ciprofloxacin. BA_{24} of 16 or 25 µg/mL meropenem combined with ciprofloxacin were 62.45±33.49 and 82.49±30.18 log CFU/mL.h, respectively. These were not significantly different ($p>0.05$) when compared to meropenem at each concentration alone. When meropenem at each concentration was combined with 0.5xMIC colistin, bactericidal activity was observed at 2nd hour without any bacterial regrowth during the time of study. BA_{24} of 16 or 25 µg/mL meropenem combined with colistin were 182.80±12.60 and 183.52±12.08 log CFU/mL.h, respectively. BA_{24} value of meropenem 16 µg/mL in combination with colistin was significantly different from 16 µg/mL meropenem alone ($p=0.008$) and colistin alone as well ($p=0.007$). The result same as meropenem 25 µg/mL in combination with colistin was significantly different from 25 µg/mL meropenem alone ($p=0.011$) and colistin alone ($p=0.006$). However, it was not significantly different when

compared to each other. Bactericidal activity of ciprofloxacin combined with colistin was found at 2nd to 8th hour and regrowth of the pathogens was observed at the 24th hour (the same as in colistin alone). Even though BA_{24} value from ciprofloxacin in combination with colistin was more than BA_{24} of colistin alone (145.74±17.05 and 124.18±13.46 log CFU/mL.h), there is no statistical significance between them ($p>0.05$).

In triple combinations, meropenem at each concentration (16 or 25 µg/mL) combined with ciprofloxacin and colistin, bactericidal activity was observed at 2nd until to 24th hour without any bacterial regrowth. BA_{24} of these combinations were 180.23±14.57 and 183.30±12.12 log CFU/mL.h, respectively. There was no significant difference between these BA_{24} values ($p>0.05$). In addition, these BA_{24} values were not significantly different when compared to each of double combinations of meropenem (16 or 25 µg/mL) and colistin as well ($p>0.05$).

Morphological cell structure change of meropenem-resistant *P. aeruginosa* after exposure to each antimicrobial agent alone and in combination.

The morphological cell structure changes of the meropenem-resistant *P. aeruginosa* isolate No.15 (MIC of meropenem and colistin were 16 and 2 µg/mL, respectively) after exposed to meropenem 16 µg/mL, colistin 0.5xMIC alone and the combination of the two drugs for 2 hours were shown in Figure 4-5. The normal morphological structure of bacterial cell was also observed in the control broth culture (no drug) (Figure 4-5A). Meropenem alone (16 µg/mL) exhibited minor morphological alterations (Figure 4-5B). On the contrary, after exposed to colistin alone (0.5xMIC), projections appeared all over on the cell surface (Figure 4-5C). Furthermore, the combination of both agents appeared numerous projections on cell surface including the destructed cell and cell debris, resulted in part of the cytoplasmic material was released (Figure 4-5D).

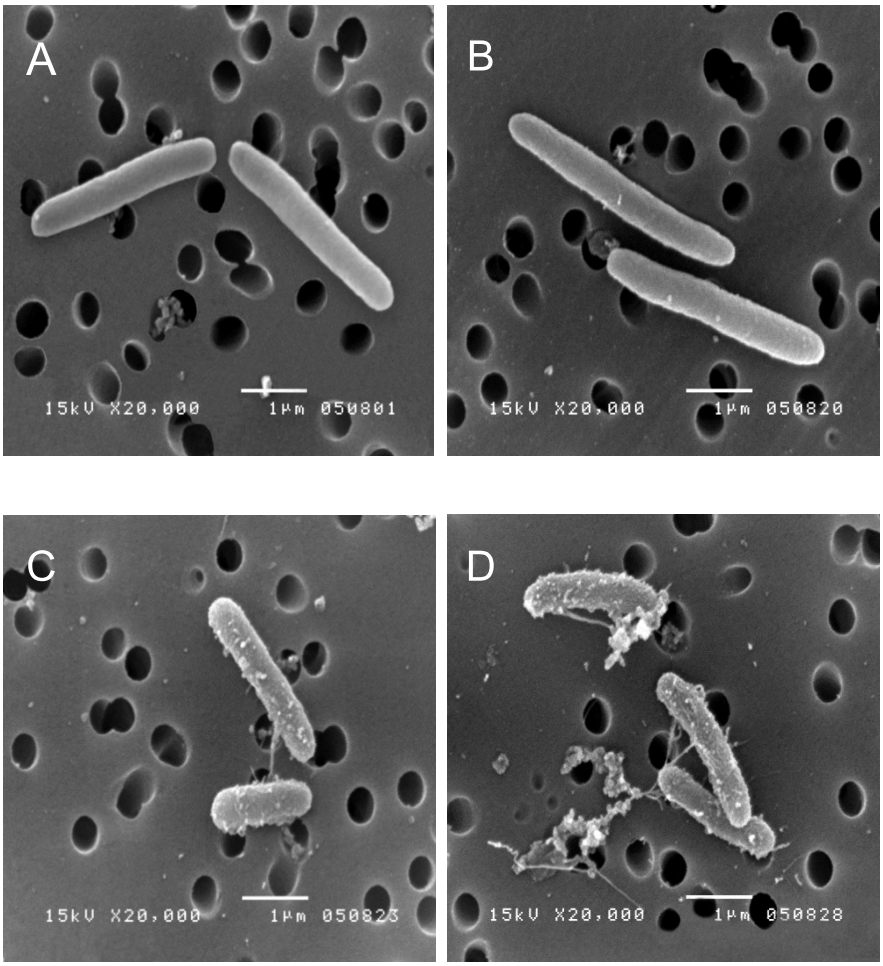

Figure 4-5 Scanning electron micrographs of *P. aeruginosa* isolate No. 15 after exposed to (A) no antibiotic, (B) meropenem 16 µg/mL, (C) colistin 0.5xMIC and (D) combination between meropenem 16 µg/mL and colistin 0.5xMIC for 2 hours.

CHAPTER V

DISCUSSIONS AND CONCLUSION

P. aeruginosa, a clinically relevant pathogen, is a frequent cause of nosocomial infections. It is well documented as a multidrug-resistant pathogen which is difficult to treat since this organism has high intrinsic resistance and impressive capacity to develop or acquisition of genetic resistance determinants. Therefore, *P. aeruginosa* infections cause high morbidity and mortality (Carmeli *et al*., 1999; Niederman, 2001; Dimatatac, 2003).

Carbapenems (mainly imipenem, meropenem) are traditionally one of last lines of agents that are utilized for treating multidrug-resistant pseudomonal infections. Resistance to these agents can cause life-threatening infections. However, carbapenems resistant *P. aeruginosa* is also emerging and increasing (Goossens, 2003; Van Eldere, 2003; Sasaki *et al*., 2004; Hsueh *et al*., 2005; Hocquet *et al*., 2007; Jones *et al*., 2008). This is of great concern for clinicians in the treatment due to limited therapeutic options. The mechanism involved the carbapenem resistance is attributed to loss of OprD porin associated with an increase in drug efflux from overexpression of multidrug efflux pump system including carbapenem-hydrolysing-β-lactamase which confer high grade resistance to the carbapenems. On the other hand, alteration of penicillin-binding proteins is not clear (Farra *et al*., 2008; Gike *et al*., 2008). Increasing in the incidence of carbapenems resistant *P. aeruginosa*, particularly meropenem, mays lead to the decrease of the number of effective antimicrobial agents for the treatment of the organism because meropenem are potent agent against gram-negative organisms including *P. aeruginosa* (White *et al*., 1996; Blumer, 1997; Knapp and English, 2001).

In this study we found that all 30 clinical isolates of *P. aeruginosa* were highly resistant to almost of 5 broad-spectrum antimicrobial agents that commonly used in the treatment of *P. aeruginosa* infections. In addition, 86.67% were multidrug-resistant isolates and most of isolates are resistant to 4 in 5 broad-spectrum antimicrobial agents. Furthermore, all isolates were resistant to meropenem. This may be caused by interplay of various mechanisms of resistance such as antibiotic-inactivating enzyme (β-lactamases, aminoglycoside-modifying enzymes), target modifications (mutation in topoisomerase) and permeability alterations (multidrug efflux pump) associated OprD loss (the uptake pathway for carbapenems). However, low level of meropenem resistance with the MICs range 8-32 μg/mL, MIC_{50} and MIC_{90} were 16 and 32 μg/mL, respectively were observed. Köhler *et al*. (1999), reported overexpression of multidrug efflux pump (MexA-MexB-OprM) increases meropenem MIC from the wild-type level of around 0.5 to around 4 μg/mL. *P. aeruginosa* in mutant featuring concomitant down regulation of the OprD porin and up regulation of MexA-MexB-OprM, MIC of meropenem increases from wild-type levels up to around 16 μg/mL. Therefore, low level meropenem resistance of these isolates may caused by loss of OprD associated overexpression of multidrug efflux pump.

Previous studies reported the synergistic effects between carbapenems with aminogycosides and/or fluoroquinolones against *P. aeruginosa* (Nakamura *et al*., 2000; Ermertcan *et al*.,2001, Solak *et al*., 2003). However, resistances to these agents have been documented which limited therapeutic options. The results from this study have shown that 26 in 30 isolates (86.67%) were resistant to ciprofloxacin with high level (MICs range 0.25-128 μg/mL, MIC_{50} and MIC_{90} were 64 and 128 μg/mL, respectively). For fluoroquinolones, overexpression of efflux pumps typically together with target sites mutation may attribute high level to resistance (McGowan, 2006). Unfortunately, nowadays there is no new antimicrobial agent with activity against *P. aeruginosa* and still not be available in the near future. Therefore, new therapies are urgently needed. Recently, nontraditional antibiotic such as colistin has been brought back in the treatment of the infections caused by this organism as a stopgap measure until novel

antimicrobial agent can be developed. Colistin shows the most marked antimicrobial effects against multidrug-resistant gram-negative bacilli. This study showed that all isolates were very susceptible to colistin with low MICs (range from 0.5-2 μg/mL, MIC_{50} and MIC_{90} were 1 and 2 μg/mL, respectively). The result is consistent with the previous study results from Tribuddharat *et al*. (2003) which evaluated the *in vitro* activity of polymyxin B and colistin against 100 clinical isolates of multidrug-resistant *P. aeruginosa* by standard disk diffusion technique and showed that all isolates were susceptible to polymyxin B and colistin.

Colistin is an old antimicrobial belongs to the polymyxin family. Despite of the effectiveness against most gram-negative bacteria including *A. baumannii* and *P. aeruginosa*, the use of colistin as parenteral therapy is limited owing to poor pharmacokinetics, neurotoxicity and nephrotoxicity (Kasiakou and Michalopoulos, 2005; Li *et al*., 2005; Falagas *et al*., 2005, 2006). However, the recent studies demonstrated synergistic effects of colistin with other antimicrobial agents against multidrug-resistant gram-negative bacteria. In addition, several literatures reported the safety and efficacy of either colistin monotherapy or in the combination with other antimicrobial agents (Conway *et al*., 1997; Levin, *et al*., 1999; Linden *et al*., 2003; Michalopoulos *et al*., 2004; Falagas *et al*., 2005; Kasiakou *et al*., 2005; Koomanachai *et al*., 2007; Pintado *et al*., 2008). Tascini *et al.* (2004) showed that colistin in combination with rifampicin had bactericidal activity and was clinically effective against multidrug-resistant pseudomonas infections. In addition, the authors concluded that this combination could also be effective in preventing the development of resistance.

With respect to synergy, it is suggested that colistin probably causes rapid permeabilization of the outer cell membrane, which allows enhanced penetration of the other antibiotics in the combination into cell (Gunderson *et al*., 2003; Tascini *et al*., 2004 Yoon *et. al*., 2004; Timurkaynak *et al*., 2006). The present study has shown *in vitro* antimicrobial activities of meropenem, ciprofloxacin, colistin and the combinations

against clinical isolates of meropenem-resistant *P. aeruginosa* which is also the first study performed in our country.

Combination effects were investigated by checkerboard microdilution and bactericidal activity was determined by time kill method. Although, combination of meropenem and ciprofloxacin showed synergy in 9 out of 30 isolates (30%) with FIC index 0.75 (Table 4-5). However, concerning with an application of drug combination therapy, the therapeutic serum concentration level should be taken into account as well. Only 5 out of 9 isolates had synergistic effects at the concentrations of ciprofloxacin within therapeutic serum level (ciprofloxacin 400 mg intravenous infusion every 8 to 12 hour, mean peak serum level was approximately 4 µg/mL). Whereas for the rest of the isolates, the synergy was observed when ciprofloxacin concentrations were higher than the therapeutic serum level. Such isolates were resistant to ciprofloxacin at high level. The present findings should be interpreted with caution for the management with meropenem combined with ciprofloxacin in *P. aeruginosa* infections.

The combinations of meropenem with colistin and ciprofloxacin with colistin showed the same synergy rate (36.67%, 11 of 30 isolates) with FIC index 0.563-0.75 and 0.560-0.75, respectively. This may be because of the same resistant mechanisms of both meropenem and ciprofloxacin at bacterial cell membrane (overexpression of multidrug efflux pump). The actions of colistin are probably the disturbance of membrane and the increase in the permeability of the cell envelope, which allows the enhanced penetration of the antibiotics into cell. However, synergistic effects in the combination of ciprofloxacin with colistin were observed at ciprofloxacin concentrations within therapeutic serum level against 5 out of 11 isolates. For the other 6 isolates, synergy were observed only when the ciprofloxacin concentrations were out of range of therapeutic serum level. These isolates were highly resistant to ciprofloxacin (MICs range 32-256 µg/mL). Although, colistin may enhance ciprofloxacin into the bacterial cell but the drug may not be able to bind to the target site due to the target alteration. However, this hypothesis needs to be elucidated in further studies. In the combination of

meropenem and colistin, all synergistic effects were occurred at the concentrations of meropenem within therapeutic serum level (meropenem 500 mg intravenous infusion every 8 hour, mean peak plasma level was approximately 25 μg/mL). In triple combination, the result showed the highest synergistic effects against 18 isolates (60%) with FIC index of 0.623-0.81. However, 11 of 18 isolates were synergy at the concentrations of ciprofloxacin out of therapeutic serum level. Taken into account for all combinations, the combination of meropenem and colistin may be the best combination for the alternative treatment of meropenem-resistant *P. aeruginosa* infection.

Antagonism (FIC index > 1) was also observed in various combinations in this study. However, there was no change in MIC values when tested with drug alone and drug in combinations.

It has been known that the bactericidal effect of β-lactam antibiotics is closely related to the time which the serum concentration of the antibiotic remains above the minimal inhibitory concentration against the target pathogen. Several studies have demonstrated that continuous infusion (CI) of β-lactam antibiotics is an effective dosing strategy. The study of Thalhammer *et al*. (1999) reported that meropenem 2 g intravenous loading dose followed by a 3 g continuous infusion over 24 hours showed the concentration at steady state was 11.9±5.0 μg/mL. However, the recommended dose of meropenem were 500 mg to 1 g intravenous infusions every 8 hour or up to 2 g intravenous infusions every 8 hour depending on severity of infections. Conte Jr. *et al*. (2005) reported that meropenem 500 mg intravenously infusion every 8 hour showed maximum concentration serum level was 25.8±5.8 μg/mL. Kanellakopoulou *et al*. (2008) investigated *in vitro* combinations effects of β-lactams with fluoroquinolones against multidrug-resistant *P. aeruginosa* by time kill assay which used meropenem concentrations were 16 and 25 μg/mL. These concentrations reflected to the mean serum level following administration of a conventional dose (Thalhammer *et al*., 1999) and maximum serum level (Conte Jr. *et al*, 2005). Therefore, in this study, we used these two concentrations of meropenem in time kill study.

Apart from gaining synergistic/additive effect, dose reduction of each agent in combination and reduction in the occurrence of bacterial resistance should also be expected in clinical setting. The time kill study was performed with colistin 0.25xMIC (0.5 µg/mL). There seems to be no enhancement in the activity of drugs in the combinations. Therefore, colistin was adjusted to 0.5xMIC (1 µg/mL) in further studies for determining bactericidal activity of drug alone and in combinations against 5 meropenem-resistant *P. aeruginosa*. BA_{24} (Bacteriolytic area for 24 hours) was used to evaluate the quantitative of total antibacterial effect during 24 hours in time kill study. The present study has shown that BA_{24} value from the colistin 0.5xMIC alone was significantly different from that of the control ($p<0.05$). This may be due to the fact that this organism was highly susceptible to colistin. Although colistin alone showed the bactericidal activity at 2^{nd} to 8^{th} hour but the regrowth was observed at 24^{th} hour. Since, colistin exhibits bactericidal activity in concentration dependent manner. Li *et al*. (2001) investigated time kill kinetic of colistin sulphate and colistin methansulphonate with two clinical isolates of *P. aeruginosa* and *P. aeruginosa* ATCC 27853. It was shown that colistin sulphate required concentration of 64xMIC for complete eradication of the microorganism within 5 minutes and colistin methanesulphonate required concentration of 16xMIC to achieve complete killing with in 24 hour. The combination of meropenem (16 or 25 µg/mL) with 4 µg/mL ciprofloxacin seems not to render much more antimicrobial activity comparing with meropenem alone as well as ciprofloxacin in combination with colistin comparing colistin alone. The BA_{24} values of the combination of meropenem (16 or 25 µg/mL) with 4 µg/mL ciprofloxacin were not significantly different from that of meropenem at each concentration alone ($p>0.05$). The result same as the combination of ciprofloxacin with ciprofloxacin showed BA_{24} value was not significantly different from colistin alone ($p>0.05$). It may caused by most of this organism was high level resistant to ciprofloxacin. Whereas when meropenem (16 or 25 µg/mL) in combination with colistin (0.5xMIC), the bactericidal activity was observed at 2^{nd} to 24^{th} hour without regrowth in all test isolates. The BA_{24} values were significantly different from these of the meropenem alone and colistin alone as well ($p<0.05$), even though they were not significantly different when compared to each other. In addition, meropenem

(16 or 25 μg/mL) in combination with 0.5xMIC colistin were comparable with triple combinations. The BA_{24} values of meropenem (16 or 25 μg/mL) in combination with 0.5xMIC colistin were not significantly different from triple combinations of meropenem, ciprofloxacin and colistin ($p>0.05$). The result from this study suggested the use combination of meropenem with colistin as alternative promising treatment for meropenem-resistant *P. aeruginosa* infections. Furthermore, such combination may reduce dose of colistin leading to the decrease in the nephrotoxicity and neurotoxicity.

For determining morphological change of meropenem-resistant *P. aeruginosa* in broth culture containing 16 μg/mL meropenem alone, 0.5xMIC colistin and the combination. After 2 hours of exposure to 16 μg/mL meropenem alone, the morphological change of such pathogen did not really occur and was quite similar to control. Whereas after the exposure to colistin alone, abnormal cells with projections on bacterial cell surface were observed. This result was consistent with time kill study in the fact that meropenem alone showed antibacterial activity less than colistin alone (rapid bactericidal activity). In addition, after the exposure to meropenem combined with colistin, abnormal cells with numerous projections including destructed cells and cell debris were observed. Morphological change in colistin alone and in the combination of meropenem with colistin are quite comparable, this may be due to the fact that colistin has an excellent bactericidal activity by itself. Therefore, morphological changes in combination of meropenem and colistin were not significantly different from colistin alone in this study. Since colistin has very potent antibacterial activity, it may lead to the rapid permeabilization of outer membrane resulting in the penetration of meropenem into bacterial cell in a very short time. Consequently, significant morphological changes probably occurred before 2 hour. This hypothesis needs to be elucidated in further studies. Interestingly, combination could enhance bactericidal activity without bacterial regrowth in time kill assay whereas bacterial regrowth was observed at 24^{th} hour in colistin alone owing to the existing of some bacterial cells.

In conclusion, this study indicated that the combinations of meropenem (16 or 25 μg/mL) and 0.5xMIC colistin were the most effective against meropenem resistant *P. aeruginosa* without any regrowth within 24 hours of the study. Therefore, the combination of meropenem with colistin offers the promising alternative treatment for infection due to meropenem-resistant *P. aeruginosa*. However, further *in vitro* and *in vivo* assessment are needed before the application in clinical setting can be established.

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APPENDICES

Appendix A

Antimicrobial susceptibility

Table A-1 Susceptibility of 30 *P. aeruginosa* isolates to 5 broad-spectrum antimicrobial agents by disk diffusion method.

Isolate No.	Inhibition Zone (mm)										No. of antimicrobial resistant[a]
	Amikacin (AMK-30)		Ciprofloxacin (CIP-5)		Cefepime (FEP-30)		Meropenem (MEM-10)		Piperacillin/tazobactam (TZP-100/10)		
1	13.20	R	NZ	R	NZ	R	9.45	R	24.40	S	4
2	11.13	R	NZ	R	NZ	R	7.93	R	20.91	S	4
3	13.55	R	NZ	R	NZ	R	8.9	R	22.55	S	4
4	26.10	S	13.70	R	29.50	S	11.65	R	28.50	S	2
5	21.05	S	28.65	S	22.90	S	NZ	R	20.70	S	1
6	12.05	R	NZ	R	NZ	R	8.13	R	20.32	S	4
7	19.40	S	9.40	R	16.50	I	9.40	R	16.35	R	4
8	12.05	R	NZ	R	NZ	R	8.15	R	23.30	S	4
9	12.35	R	NZ	R	NZ	R	10.45	R	21.30	S	4
10	11.60	R	NZ	R	NZ	R	8.75	R	16.60	R	5
11	13.55	R	NZ	R	NZ	R	9.35	R	21.35	S	4
12	19.15	S	10.95	R	18.70	S	12.30	R	14.70	R	3
13	19.40	S	24.55	S	23.65	S	NZ	R	25.45	S	1
14	14.0	R	NZ	R	NZ	R	9.30	R	20.40	S	4
15	11.45	R	NZ	R	NZ	R	10.20	R	21.60	S	4
16	13.88	R	NZ	R	NZ	R	8.60	R	11.10	R	5
17	32.20	S	31.40	S	22.7	S	NZ	R	31.40	S	1

[a]Intermediate resistant or Resistant; S=Susceptible, I=Intermediate, R=Resistant, NZ=no zone

Table A-1 (Continued) Susceptibility of 30 *P. aeruginosa* isolates to 5 broad-spectrum antimicrobial agents by disk diffusion method.

Isolate No.	Inhibition Zone (mm)										No. of antimicrobial resistant[a]
	Amikacin (AMK-30)		Ciprofloxacin (CIP-5)		Cefepime (FEP-30)		Meropenem (MEM-10)		Piperacillin/tazobactam (PTZ-100/10)		
18	NZ	R	27.83	S	NZ	R	9.47	R	15.63	R	4
19	15.97	I	NZ	R	6.96	R	7.85	R	16.89	R	5
20	12.97	R	NZ	R	NZ	R	9.08	R	19.70	S	4
21	12.68	R	NZ	R	6.39	R	7.26	R	12.75	R	5
22	12.81	R	NZ	R	NZ	R	8.77	R	22.43	S	4
23	9.97	R	NZ	R	NZ	R	9.44	R	16.51	R	5
24	10.43	R	NZ	R	19.96	S	6.80	R	23.18	S	3
25	11.37	R	NZ	R	NZ	R	8.91	R	18.30	S	4
26	13.15	R	NZ	R	NZ	R	7.91	R	13.43	R	5
27	12.76	R	NZ	R	NZ	R	9.50	R	22.78	S	4
28	NZ	R	7.79	R	NZ	R	6.41	R	16.97	R	5
29	13.02	R	NZ	R	NZ	R	7.35	R	12.97	R	5
30	19.95	S	16.28	I	18.00	S	7.26	R	17.00	R	3
E. coli ATCC 25922	19.00	-	32.11	-	33.06	-	31.10	-	24.78	-	-
P. aeruginosa ATCC 27853	23.44	-	27.77	-	29.46	-	29.29	-	30.13	-	-

[a]Intermediate resistant or Resistant; S=Susceptible, I=Intermediate, R=Resistant, NZ=no zone

Table A-2 The minimum inhibitory concentrations (MICs) of meropenem, ciprofloxacin and colistin against 30 *P. aeruginosa* isolates.

Isolate No.	MIC (μg / mL)					
	Meropenem		Ciprofloxacin		Colistin	
1	8	I	64	R	1	S
2	32	R	64	R	1	S
3	8	I	64	R	2	S
4	8	I	8	R	1	S
5	32	R	1	S	1	S
6	8	I	64	R	1	S
7	16	R	8	R	1	S
8	8	I	64	R	1	S
9	8	I	64	R	1	S
10	16	R	64	R	1	S
11	8	I	64	R	1	S
12	8	I	64	R	1	S
13	32	R	1	S	1	S
14	16	R	64	R	1	S
15	16	R	64	R	1	S
16	16	R	128	R	1	S
17	32	R	0.5	S	1	S
18	16	R	0.5	S	1	S
19	16	R	128	R	1	S
20	16	R	64	R	2	S
21	32	R	128	R	1	S
22	16	R	64	R	1	S
23	16	R	128	R	2	S
24	32	R	32	R	0.5	S
25	16	R	128	R	1	S
26	32	R	128	R	2	S
27	16	R	64	R	1	S
28	16	R	16	R	2	S
29	32	R	128	R	1	S
30	32	R	4	R	1	S
E. coli ATCC 25922	< 0.03	-	ND	-	0.5	-
P. aeruginosa ATCC 27853	0.25	-	0.25	-	1	-

R= Resistant, I= Intermediate, S= Susceptible, ND = not determine

Table A-3 MICs distribution and MIC_{50}, MIC_{90} of meropenem, ciprofloxacin and colistin from agar dilution method.

	Meropenem			Ciprofloxacin			Colistin	
	MIC range	No. (%)		MIC range	No. (%)		MIC range	No. (%)
	0.03	0 (0)		0.03	0 (0)		0.03	0 (0)
	0.06	0 (0)		0.06	0 (0)		0.06	0 (0)
	0.12	0 (0)		0.12	0 (0)		0.12	0 (0)
	0.25	0 (0)		0.25	0 (0)		0.25	0 (0)
	0.5	0 (0)		0.5	2 (6.67)		0.5	2(6.67)
	1	0 (0)		1	2 (6.67)	MIC_{50}	1	24 (80)
	2	0 (0)		2	0	MIC_{90}	2	4 (13.33)
	4	0 (0)		4	1 (3.33)		4	0 (0)
	8	8 (26.67)		8	2 (6.67)		8	0 (0)
MIC_{50}	16	13 (43.33)		16	1 (3.33)		16	0 (0)
MIC_{90}	32	9 (30)		32	1 (3.33)		32	0 (0)
	64	0 (0)	MIC_{50}	64	14 (46.67)		64	0 (0)
	128	0 (0)	MIC_{90}	128	7 (23.33)		128	0 (0)
	256	0 (0)		256	0		256	0 (0)

Appendix B

Viable cell count, Killing rate, Changes in viable cell count and kinetic parameters of 5 *P. aeruginosa* isolates

Table B-1 Viable cell count (log CFU/mL) of 5 *P. aeruginosa* isolates at various time intervals.

Isolate No.	Viable cell count (log CFU/mL) at the following time					
	0 hr	2 hr	4 hr	6 hr	8 hr	24 hr
*Ps.*10						
CONTROL	6.00	6.01	7.54	7.99	8.98	11.22
MEM (16)	6.00	5.17	4.68	4.57	4.60	9.68
MEM (25)	6.00	5.03	3.98	2.94	2.95	9.54
CIP (4)	6.00	5.93	6.57	7.68	8.06	10.07
COL (0.5xMIC)	6.00	1.88	1.70	1.70	1.70	7.72
MEM (16) + CIP (4)	6.00	5.15	4.65	4.30	4.78	8.48
MEM (25) + CIP (4)	6.00	5.04	3.81	2.93	2.98	7.93
MEM (16) + COL (0.5xMIC)	6.00	1.70	1.70	1.70	1.70	2.00
MEM (25) + COL (0.5xMIC)	6.00	1.70	1.70	1.70	1.70	1.70
CIP (4) + COL (0.5xMIC)	6.00	1.70	1.70	1.70	1.70	4.63
MEM (16) + CIP (4) + COL (0.5xMIC)	6.00	1.70	1.70	1.70	1.70	1.70
MEM (25) + CIP (4) + COL (0.5xMIC)	6.00	1.70	1.70	1.70	1.70	1.70
Ps. 15						
CONTROL	5.76	5.83	6.88	7.99	9.51	12.20
MEM (16)	5.76	4.76	4.74	4.72	4.92	9.72
MEM (25)	5.76	4.72	4.26	3.70	2.76	9.70
CIP (4)	5.76	5.72	6.48	7.94	9.02	11.81
COL (0.5xMIC)	5.76	1.88	1.70	1.70	2.48	8.70
MEM (16) + CIP (4)	5.76	4.74	4.70	4.65	4.93	8.95
MEM (25) + CIP (4)	5.76	4.70	4.18	3.70	2.72	8.88
MEM (16) + COL (0.5xMIC)	5.76	1.70	1.70	1.70	1.70	1.70
MEM (25) + COL (0.5xMIC)	5.76	1.70	1.70	1.70	1.70	1.70
CIP (4) + COL (0.5xMIC)	5.76	1.70	2.00	2.00	2.24	8.38
MEM (16) + CIP (4) + COL (0.5xMIC)	5.76	1.70	1.70	1.70	1.70	1.70
MEM (25) + CIP (4) + COL (0.5xMIC)	5.76	1.70	1.70	1.70	1.70	1.70

MEM, meropenem; CIP, ciprofloxacin; COL, colistin

Table B-1 (Continued) Viable cell count (log CFU/mL) of 5 *P. aeruginosa* isolates at various time intervals.

Isolate No.	Viable cell count (log CFU/mL) at the following time					
	0 hr	2 hr	4 hr	6 hr	8 hr	24 hr
Ps. 21						
CONTROL	5.80	5.68	7.12	8.04	8.85	12.48
MEM (16)	5.80	5.57	6.63	7.83	8.65	11.65
MEM (25)	5.80	4.68	4.78	6.11	7.15	11.40
CIP (4)	5.80	6.02	6.92	7.92	8.44	11.10
COL (0.5xMIC)	5.80	2.35	2.24	2.72	2.85	8.81
MEM (16) + CIP (4)	5.80	5.22	6.70	7.37	7.93	10.80
MEM (25) + CIP (4)	5.80	4.24	4.11	5.65	6.76	10.51
MEM (16) + COL (0.5xMIC)	5.80	2.30	1.70	1.70	1.70	1.70
MEM (25) + COL (0.5xMIC)	5.80	1.70	1.70	1.70	1.70	1.70
CIP (4) + COL (0.5xMIC)	5.80	2.63	1.70	1.70	1.70	7.24
MEM (16) + CIP (4) + COL (0.5xMIC)	5.80	2.10	1.70	1.70	1.70	3.65
MEM (25) + CIP (4) + COL (0.5xMIC)	5.80	2.24	1.70	1.70	1.70	1.70
Ps. 26						
CONTROL	5.85	6.54	7.78	8.76	9.85	13.41
MEM (16)	5.85	6.02	7.01	7.89	8.72	12.76
MEM (25)	5.85	4.97	4.86	6.60	7.74	12.28
CIP (4)	5.85	6.10	7.57	8.18	9.12	12.60
COL (0.5xMIC)	5.85	2.40	1.88	1.70	2.10	8.18
MEM (16) + CIP (4)	5.85	5.98	7.02	7.74	8.11	12.15
MEM (25) + CIP (4)	5.85	4.95	5.57	6.18	7.04	11.76
MEM (16) + COL (0.5xMIC)	5.85	1.70	1.70	1.70	1.70	1.70
MEM (25) + COL (0.5xMIC)	5.85	1.70	1.70	1.70	1.70	1.70
CIP (4) + COL (0.5xMIC)	5.85	1.70	1.70	1.70	1.70	5.68
MEM (16) + CIP (4) + COL (0.5xMIC)	5.85	1.70	1.70	1.70	1.70	1.70
MEM (25) + CIP (4) + COL (0.5xMIC)	5.85	1.70	1.70	1.70	1.70	1.70

MEM, meropenem; CIP, ciprofloxacin; COL, colistin

Table B-1 (Continued) Viable cell count (log CFU/mL) of 5 *P. aeruginosa* isolates at various time intervals.

Isolate No.	Viable cell count (log CFU/mL) at the following time					
	0 hr	2 hr	4 hr	6 hr	8 hr	24 hr
*Ps.*30						
CONTROL	5.74	4.72	7.26	7.48	9.30	11.12
MEM (16)	5.74	4.78	5.83	6.88	8.01	11.18
MEM (25)	5.74	4.81	4.44	4.98	6.24	8.99
CIP (4)	5.74	3.74	4.03	4.19	4.02	8.83
COL (0.5xMIC)	5.74	2.10	1.70	1.70	1.70	8.60
MEM (16) + CIP (4)	5.74	3.65	2.98	2.70	2.72	8.30
MEM (25) + CIP (4)	5.74	2.65	2.63	2.74	2.70	7.74
MEM (16) + COL (0.5xMIC)	5.74	1.70	1.70	1.70	1.70	1.70
MEM (25) + COL (0.5xMIC)	5.74	1.70	1.70	1.70	1.70	1.70
CIP (4) + COL (0.5xMIC)	5.74	1.70	1.70	1.70	1.70	5.20
MEM (16) + CIP (4) + COL (0.5xMIC)	5.74	1.70	1.70	1.70	1.70	1.70
MEM (25) + CIP (4) + COL (0.5xMIC)	5.74	1.70	1.70	1.70	1.70	1.70
Average[a]						
CONTROL	5.83±0.10	5.76±0.67	7.32±0.35	8.05±0.46	9.30±0.40	12.09±0.95
MEM (16)	5.83±0.10	5.26±0.54	5.78±1.06	6.38±1.63	6.98±2.05	11.00±1.32
MEM (25)	5.83±0.10	4.84±0.15	4.67±0.37	4.87±1.55	5.37±2.36	10.38±1.39
CIP (4)	5.83±0.10	5.50±0.99	6.31±1.35	7.18±1.68	7.73±2.12	10.88±1.48
COL (0.5xMIC)	5.83±0.10	2.12±0.25	1.84±0.24	1.90±0.46	2.16±0.50	8.40±0.45
MEM (16) + CIP (4)	5.83±0.10	4.95±0.85	5.21±1.66	5.35±2.15	5.69±2.30	9.74±1.68
MEM (25) + CIP (4)	5.83±0.10	4.32±0.98	4.06±1.05	4.24±1.58	4.44±2.25	9.36±1.73
MEM (16) + COL (0.5xMIC)	5.83±0.10	1.82±0.27	1.70±0.00	1.70±0.00	1.70±0.00	1.76±0.13
MEM (25) + COL (0.5xMIC)	5.83±0.10	1.70±0.00	1.70±0.00	1.70±0.00	1.70±0.00	1.70±0.00
CIP (4) + COL (0.5xMIC)	5.83±0.10	1.88±0.42	1.76±0.13	1.76±0.13	1.81±0.24	6.22±1.55
MEM (16) + CIP (4) + COL (0.5xMIC)	5.83±0.10	1.78±0.18	1.7±0.00	1.7±0.00	1.7±0.00	2.09±0.87
MEM (25) + CIP (4) + COL 0.(5xMIC)	5.83±0.10	1.81±0.24	1.7±0.00	1.7±0.00	1.7±0.00	1.7±0.00

[a]Mean±SD; MEM, meropenem; CIP, ciprofloxacin; COL, colistin

Table B-2 Killing rate by meropenem, ciprofloxacin and colistin alone and the combinations against 5 *P. aeruginosa* isolates.

Isolate No.	Time (hr) for 3 log killing	Time (hr) for regrowth
*Ps.*10		
MEM (16)	-	24
MEM (25)	6	24
CIP (4)	-	UD
COL (0.5xMIC)	2	24
MEM (16) + CIP (4)	-	24
MEM (25) + CIP (4)	6	24
MEM (16) + COL (0.5xMIC)	2	-
MEM (25) + COL (0.5xMIC)	2	-
CIP (4) + COL (0.5xMIC)	2	24
MEM (16) + CIP (4) + COL (0.5xMIC)	2	-
MEM (25) + CIP (4) + COL (0.5xMIC)	2	-
Ps. 15		
MEM (16)	-	24
MEM (25)	8	24
CIP (4)	-	UD
COL (0.5xMIC)	2	24
MEM (16) + CIP (4)	-	24
MEM (25) + CIP (4)	8	24
MEM (16) + COL (0.5xMIC)	2	-
MEM (25) + COL (0.5xMIC)	2	-
CIP (4) + COL (0.5xMIC)	2	24
MEM (16) + CIP (4) + COL (0.5xMIC)	2	-
MEM (25) + CIP (4) + COL (0.5xMIC)	2	-

UD = Undetectable; MEM, meropenem; CIP, ciprofloxacin; COL, colistin

Table B-2 (Continued) Killing rate by meropenem, ciprofloxacin and colistin alone and the combinations against 5 *P. aeruginosa* isolates.

Isolate No.	Time (hr) for 3 log killing	Time (hr) for regrowth
*Ps.*21		
MEM (16)	-	24
MEM (25)	-	24
CIP (4)	-	UD
COL (0.5xMIC)	2	24
MEM (16) + CIP (4)	-	24
MEM (25) + CIP (4)	-	24
MEM (16) + COL (0.5xMIC)	2	-
MEM (25) + COL (0.5xMIC)	2	-
CIP (4) + COL (0.5xMIC)	2	24
MEM (16) + CIP (4) + COL (0.5xMIC)	2	24
MEM (25) + CIP (4) + COL (0.5xMIC)	2	-
Ps. 26		
MEM (16)	-	24
MEM (25)	-	24
CIP (4)	-	UD
COL (0.5xMIC)	2	24
MEM (16) + CIP (4)	-	24
MEM (25) + CIP (4)	-	24
MEM (16) + COL (0.5xMIC)	2	-
MEM (25) + COL (0.5xMIC)	2	-
CIP (4) + COL (0.5xMIC)	2	24
MEM (16) + CIP (4) + COL (0.5xMIC)	2	-
MEM (25) + CIP (4) + COL (0.5xMIC)	2	-

UD = Undetectable; MEM, meropenem; CIP, ciprofloxacin; COL, colistin

Table B-2 (Continued) Killing rate by meropenem, ciprofloxacin and colistin alone and the combinations against 5 *P. aeruginosa* isolates.

Isolate No.	Time (hr) for 3 log killing	Time (hr) for regrowth
*Ps.*30		
MEM (16)	-	24
MEM (25)	-	24
CIP (4)	-	24
COL (0.5xMIC)	2	24
MEM (16) + CIP (4)	6	24
MEM (25) + CIP (4)	2	24
MEM (16) + COL (0.5xMIC)	2	-
MEM (25) + COL (0.5xMIC)	2	-
CIP (4) + COL (0.5xMIC)	2	24
MEM (16) + CIP (4) + (COL 0.5xMIC)	2	-
MEM (25) + CIP (4) + COL (0.5xMIC)	2	-

MEM, meropenem; CIP, ciprofloxacin; COL, colistin

Table B-3 Changes in viable cell count (log CFU/mL) at various time intervals and kinetic parameters ($AUBKC_{0-24}$, BA_{24}) of *P. aeruginosa* (5 isolates).

Isolate No.	Change in viable cell counts (log CFU/mL) at the following time					$AUBKC_{0-24}$	BA_{24}
	Δ_2	Δ_4	Δ_6	Δ_8	Δ_{24}		
Ps. 10							
CONTROL	0.01	1.54	1.99	2.98	5.22	219.68	-
MEM (16)	-0.83	-1.32	-1.43	-1.40	3.68	153.67	66.01
MEM (25)	-0.97	-2.02	-3.06	-3.05	3.54	132.84	86.84
CIP (4)	-0.07	0.57	1.68	2.06	4.07	199.47	20.21
COL (0.5xMIC)	-4.12	-4.30	-4.30	-4.30	1.72	93.60	126.08
MEM (16) + CIP (4)	-0.85	-1.35	-1.70	-1.22	2.48	145.04	74.64
MEM (25) + CIP (4)	-0.96	-2.19	-3.07	-3.02	1.93	119.80	99.88
MEM (16) + COL (0.5xMIC)	-4.30	-4.30	-4.30	-4.30	-4.00	47.48	172.19
MEM (25) + COL (0.5xMIC)	-4.30	-4.30	-4.30	-4.30	-4.30	45.08	174.60
CIP (4) + COL (0.5xMIC)	-4.30	-4.30	-4.30	-4.30	-1.37	68.51	151.17
MEM (16) + CIP (4) + COL (0.5xMIC)	-4.30	-4.30	-4.30	-4.30	-4.30	45.08	174.60
MEM (25) + CIP (4) + COL (0.5xMIC)	-4.30	-4.30	-4.30	-4.30	-4.30	45.08	174.60

MEM, meropenem; CIP, ciprofloxacin; COL, colistin

Table B-3 (Continued) Changes in viable cell count (log CFU/mL) at various time intervals and kinetic parameters ($AUBKC_{0-24}$, BA_{24}) of *P. aeruginosa* (5 isolates).

Isolate No.	Change in viable cell counts (log CFU/mL) at the following time					$AUBKC_{0-24}$	BA_{24}
	Δ_2	Δ_4	Δ_6	Δ_8	Δ_{24}		
Ps. 15							
CONTROL	0.07	1.12	2.23	3.75	6.44	230.33	-
MEM (16)	-1.00	-1.02	-1.04	-0.84	3.96	156.21	74.12
MEM (25)	-1.04	-1.50	-2.06	-3.00	3.94	133.54	96.79
CIP (4)	-0.04	0.72	2.18	3.26	6.05	221.73	8.60
COL (0.5xMIC)	-3.88	-4.06	-4.06	-3.28	2.94	108.19	122.14
MEM (16) + CIP (4)	-1.02	-1.06	-1.11	-0.83	3.19	149.94	80.39
MEM (25) + CIP (4)	-1.06	-1.58	-2.06	-3.04	3.12	126.40	103.93
MEM (16) + COL (0.5xMIC)	-4.06	-4.06	-4.06	-4.06	-4.06	44.84	185.50
MEM (25) + COL (0.5xMIC)	-4.06	-4.06	-4.06	-4.06	-4.06	44.84	185.50
CIP (4) + COL (0.5xMIC)	-4.06	-3.76	-3.76	-3.52	2.62	104.35	125.98
MEM (16) + CIP (4) + COL (0.5xMIC)	-4.06	-4.06	-4.06	-4.06	-4.06	44.84	185.50
MEM (25) + CIP (4) + COL (0.5xMIC)	-4.06	-4.06	-4.06	-4.06	-4.06	44.84	185.50

MEM, meropenem; CIP, ciprofloxacin; COL, colistin

Table B-3 (Continued) Changes in viable cell count (log CFU/mL) at various time intervals and kinetic parameters ($AUBKC_{0-24}$, BA_{24}) of *P. aeruginosa* (5 isolates).

Isolate No.	Change in viable cell counts (log CFU/mL) at the following time					$AUBKC_{0-24}$	BA_{24}
	Δ_2	Δ_4	Δ_6	Δ_8	Δ_{24}		
PS. 21							
CONTROL	-0.12	1.33	2.25	3.05	6.68	226.90	-
MEM (16)	-0.22	0.83	2.03	2.86	5.86	214.41	12.49
MEM (25)	-1.12	-1.02	0.31	1.35	5.60	190.76	36.14
CIP (4)	0.23	1.12	2.12	2.64	5.30	205.58	21.32
COL (0.5xMIC)	-3.44	-3.55	-3.08	-2.95	3.02	116.54	110.36
MEM (16) + CIP (4)	-0.58	0.90	1.58	2.13	5.00	202.10	24.80
MEM (25) + CIP (4)	-1.56	-1.68	-0.14	0.96	4.72	178.74	48.16
MEM (16) + COL (0.5xMIC)	-3.49	-4.10	-4.10	-4.10	-4.10	46.08	180.82
MEM (25) + COL (0.5xMIC)	-4.10	-4.10	-4.10	-4.10	-4.10	44.87	182.03
CIP (4) + COL (0.5xMIC)	-3.17	-4.10	-4.10	-4.10	1.44	91.03	135.87
MEM (16) + CIP (4) + COL (0.5xMIC)	-3.70	-4.10	-4.10	-4.10	-2.14	61.30	165.60
MEM (25) + CIP (4) + COL (0.5xMIC)	-3.55	-4.10	-4.10	-4.10	-4.10	45.96	180.94

MEM, meropenem; CIP, ciprofloxacin; COL, colistin

Table B-3 (Continued) Changes in viable cell count (log CFU/mL) at various time intervals and kinetic parameters ($AUBKC_{0-24}$, BA_{24}) of *P. aeruginosa* (5 isolates).

Isolate No.	Change in viable cell counts (log CFU/mL) at the following time					$AUBKC_{0-24}$	BA_{24}
	Δ_2	Δ_4	Δ_6	Δ_8	Δ_{24}		
PS. 26							
CONTROL	0.70	1.93	2.91	4.00	7.56	247.87	-
MEM (16)	0.18	1.17	2.04	2.88	6.91	228.25	19.62
MEM (25)	-0.88	-0.98	0.76	1.90	6.43	206.60	41.27
CIP (4)	0.25	1.73	2.34	3.28	6.76	232.47	15.40
COL (0.5xMIC)	-3.45	-3.97	-4.15	-3.75	2.33	102.07	145.80
MEM (16) + CIP (4)	0.13	1.18	1.90	2.26	6.31	217.50	30.36
MEM (25) + CIP (4)	-0.89	-0.27	0.34	1.20	5.91	196.72	51.15
MEM (16) + COL (0.5xMIC)	-4.15	-4.15	-4.15	-4.15	-4.15	44.92	202.95
MEM (25) + COL (0.5xMIC)	-4.15	-4.15	-4.15	-4.15	-4.15	44.92	202.95
CIP (4) + COL (0.5xMIC)	-4.15	-4.15	-4.15	-4.15	-0.17	76.74	171.12
MEM (16) + CIP (4) + COL (0.5xMIC)	-4.15	-4.15	-4.15	-4.15	-4.15	44.92	202.95
MEM (25) + CIP (4) + COL (0.5xMIC)	-4.15	-4.15	-4.15	-4.15	-4.15	44.92	202.95

MEM, meropenem; CIP, ciprofloxacin; COL, colistin

Table B-3 (Continued) Changes in viable cell count (log CFU/mL) at various time interval and kinetic parameters ($AUBKC_{0-24}$, BA_{24}) of *P. aeruginosa* (5 isolates).

Isolate No.	Change in viable cell counts (log CFU/mL) at the following time					$AUBKC_{0-24}$	BA_{24}
	Δ_2	Δ_4	Δ_6	Δ_8	Δ_{24}		
PS. 30							
CONTROL	-1.02	1.52	1.74	3.56	5.38	217.34	-
MEM (16)	-0.96	0.09	1.13	2.27	5.44	202.27	15.08
MEM (25)	-0.93	-1.30	-0.76	0.50	3.25	162.30	55.04
CIP (4)	-2.00	-1.71	-1.55	-1.72	3.09	136.49	80.85
COL (0.5xMIC)	-3.64	-4.04	-4.04	-4.04	2.86	100.84	116.51
MEM (16) + CIP (4)	-2.09	-2.76	-3.04	-3.02	2.56	115.29	102.05
MEM (25) + CIP (4)	-3.09	-3.11	-3.00	-3.04	2.00	108.00	109.35
MEM (16) + COL (0.5xMIC)	-4.04	-4.04	-4.04	-4.04	-4.04	44.82	172.53
MEM (25) + COL (0.5xMIC)	-4.04	-4.04	-4.04	-4.04	-4.04	44.82	172.53
CIP (4) + COL (0.5xMIC)	-4.04	-4.04	-4.04	-4.04	-0.54	72.80	144.54
MEM (16) + CIP (4) + COL (0.5xMIC)	-4.04	-4.04	-4.04	-4.04	-4.04	44.82	172.53
MEM (25) + CIP (4) + COL (0.5xMIC)	-4.04	-4.04	-4.04	-4.04	-4.04	44.82	172.53

MEM, meropenem; CIP, ciprofloxacin; COL, colistin

Table B-3 (Continued) Changes in viable cell count (log CFU/mL) at various time interval and kinetic parameters ($AUBKC_{0-24}$, BA_{24}) of *P. aeruginosa* (5 isolates).

Isolate No.	Change in viable cell counts (log CFU/mL) at the following time					$AUBKC_{0-24}$	BA_{24}
	Δ_2	Δ_4	Δ_6	Δ_8	Δ_{24}		
Average[a]							
CONTROL	-0.07±0.62	1.49±0.30	2.22±0.44	3.47±0.44	6.26±0.97	228.42±12.08	0.00
MEM (16)	-0.57±0.52	-0.05±1.10	0.55±1.67	1.15±2.10	5.17±1.35	190.96±34.15	37.46±30.01
MEM (25)	-0.99±0.09	-1.37±0.42	-0.96±1.60	-0.46±2.39	4.55±1.39	165.21±33.26	63.22±27.23
CIP (4)	-0.33±0.95	0.49±1.31	1.35±1.64	1.90±2.09	5.05±1.48	199.15±37.38	29.28±29.27
COL (0.5xMIC)	-3.71±0.29	-3.99±0.27	-3.92±0.49	-3.66±0.55	2.57±0.55	104.25±8.61	124.18±13.46
MEM (16) + CIP (4)	-0.88±0.81	-0.62±1.65	-0.48±2.14	-0.14±2.28	3.91±1.68	165.98±42.50	62.45±33.49
MEM (25) + CIP (4)	-1.51±0.92	-1.77±1.03	-1.59±1.60	-1.39±2.26	3.54±1.74	145.98±39.24	82.49±30.18
MEM (16) + COL (0.5xMIC)	-4.01±0.31	-4.13±0.10	-4.13±0.10	-4.13±0.10	-4.07±0.06	45.63±1.17	182.80±12.60
MEM (25) + COL (0.5xMIC)	-4.13±0.10	-4.13±0.10	-4.13±0.10	-4.13±0.10	-4.13±0,10	44.90±0.10	183.52±12.08
CIP (4) + COL (0.5xMIC)	-3.94±0.45	-4.07±0.20	-4.07±0.20	-4.02±0.30	0.39±1.61	82.69±14.77	145.74±17.05
MEM (16) + CIP (4) + (COL 0.5xMIC)	-4.05±0.22	-4.13±0.10	-4.13±0.10	-4.13±0.10	-3.74±0.90	48.19±7.33	180.23±14.57
MEM (25) + CIP (4) + (COL 0.5xMIC)	-4.02±0.28	-4.13±0.10	-4.13±0.10	-4.13±0.10	-4.13±0.10	45.12±0.48	183.30±12.12

Mean±SD; MEM, meropenem; CIP, ciprofloxacin; COL, colistin

Appendix C

Distribution of antimicrobial resistance of *P. aeruginosa*

and illustration of agar dilution method for determination MICs of antimicrobials

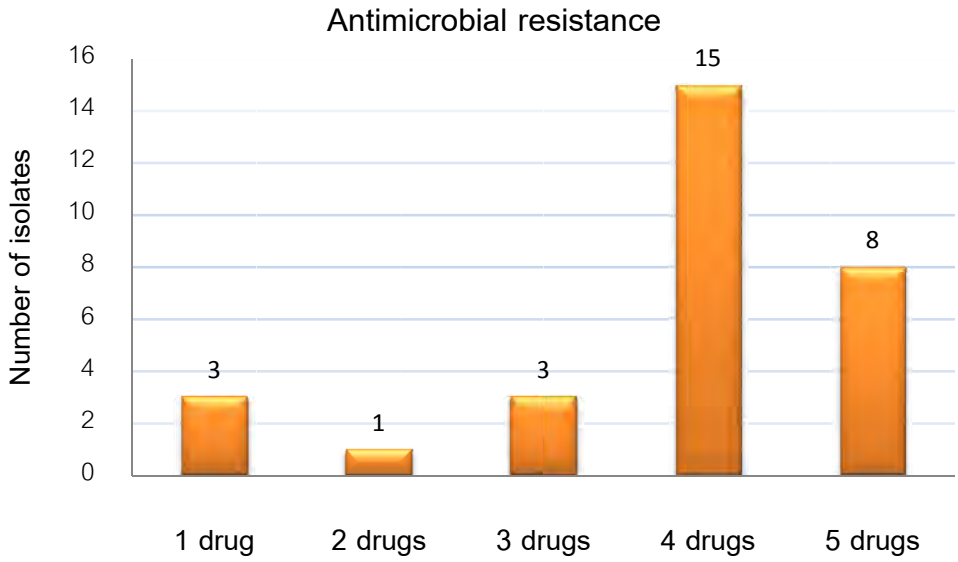

Figure C-1 Distribution of antimicrobial resistance of *P. aeruginosa* according to the number of antimicrobial to which they showed resistance.

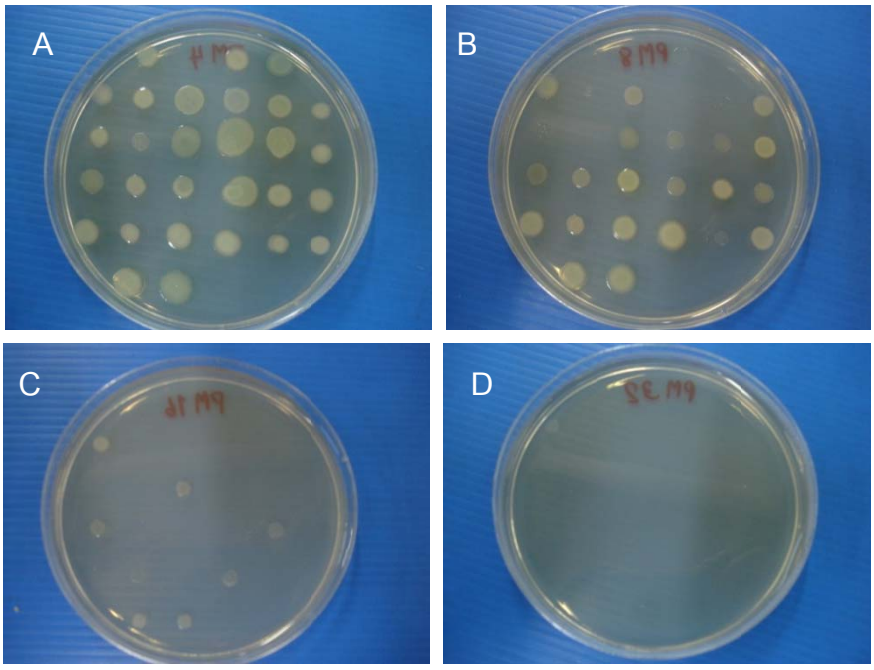

Figure C-2 An Illustration of agar dilution method for determination MICs of meropenem against 30 *P. aeruginosa* isolates, (A) meropenem at 4 µg/mL, (B) meropenem at 8 µg/mL, (C) meropenem at 16 µg/mL, (D) meropenem at 32 µg/mL.

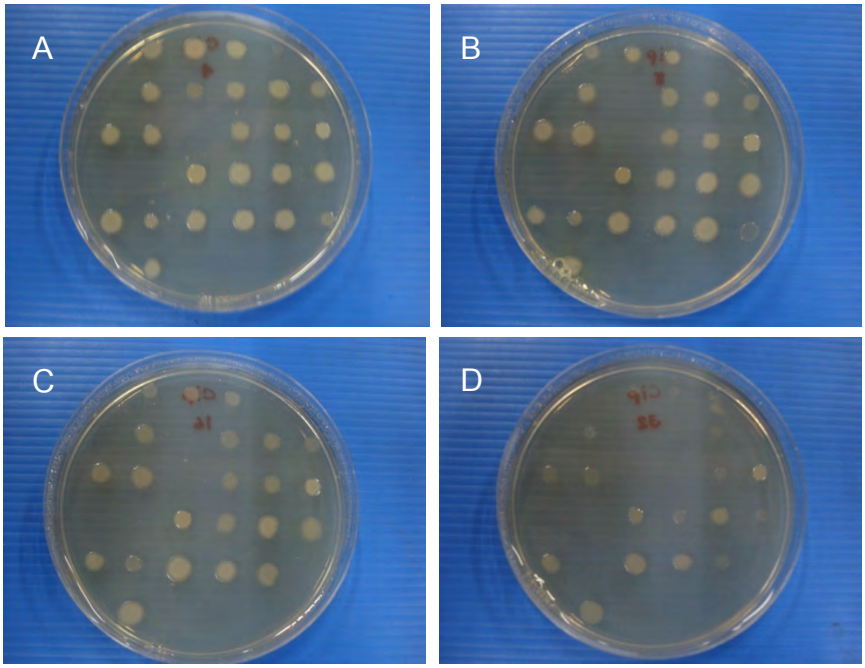

Figure C-3 An Illustration of agar dilution method for determination MICs of ciprofloxacin against 30 *P. aeruginosa* isolates, (A) ciprofloxacin at 4 µg/mL, (B) ciprofloxacin at 8 µg/mL, (C) ciprofloxacin at 16 µg/mL, (D) ciprofloxacin at 32 µg/mL.

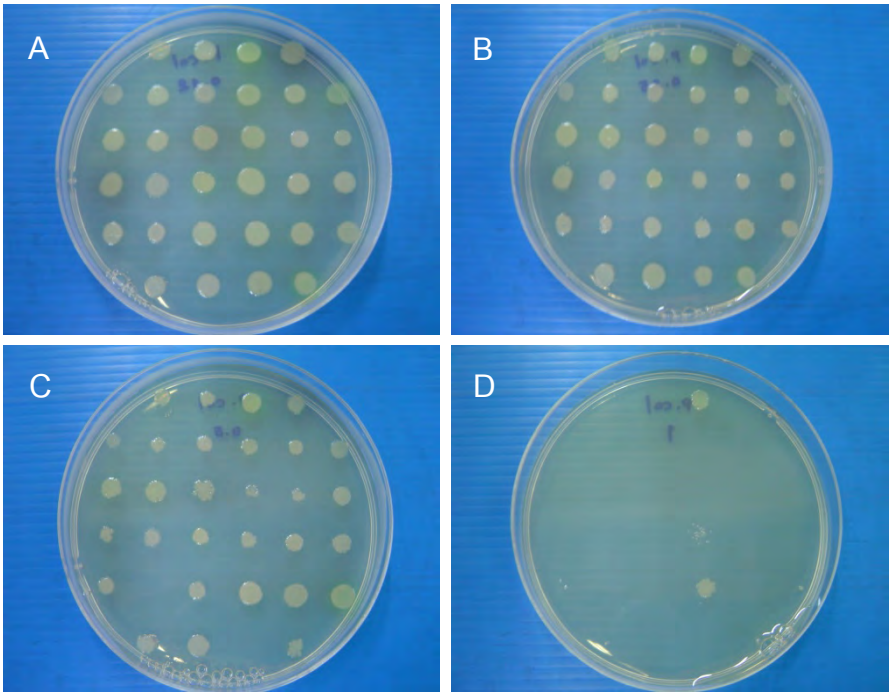

Figure C-4 An Illustration of agar dilution method for determination MICs of colistin against 30 *P. aeruginosa* isolates, (A) colistin at 0.12 µg/mL, (B) colistin at 0.25 µg/mL, (C) colistin at 0.5 µg/mL, (D) colistin at 1 µg/mL.

Appendix D

Illustration of checkerboard technique and three dimensional checkerboard results of meropenem, ciprofloxacin and colistin in *P. aeruginosa* isolates.

Plate 1 – No colistin (0 µg/ml)

Meropenem x MIC

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

Ciprofloxacin x MIC

Plate 2 – Colistin 1/16 x MIC

Meropenem 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	1/2/ 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
1/2/ 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/ 1/8 /	1/16/ 1/4 /	1/16/ 1/2 /	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

Ciprofloxacin x MIC

Plate 3 – Colistin 1/8 x MIC

Meropenem 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	1/2/ 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
1/2/ 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

Ciprofloxacin x MIC

Figure D-1 An illustration of checkerboard technique, three dimensional checkerboard microdilution of meropenem, ciprofloxacin and colistin were performed using the series of proportional to MICs of the drugs being tested (Modified from Eliopoulos and Moellering, 1996, Yoon *et al.*, 2004).

Plate 4 – Colistin 1/4 x MIC

Meropenem 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	1/2/ 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
1/2/ 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

Ciprofloxacin x MIC

Plate 5 – Colistin 1/2 x MIC

Meropenem 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	1/2/ 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
1/2/ 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

Ciprofloxacin x MIC

Plate 6 – Colistin 1 x MIC

Meropenem 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	1/2/ 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
1/2/ 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

Ciprofloxacin x MIC

Figure D-1 (Continued) An illustration of checkerboard technique, three dimensional checkerboard microdilution of meropenem, ciprofloxacin and colistin were performed using the series of proportional to MICs of the drugs being tested (Modified from Eliopoulos and Moellering, 1996, Yoon *et al*., 2004).

Plate 7 – Colistin 2 x MIC

Meropenem 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	1/2/ 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
1/2/ 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

Ciprofloxacin x MIC

Plate 8 – Colistin 4 x MIC

Meropenem 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	1/2/ 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
1/2/ 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

Ciprofloxacin x MIC

Figure D-1 (Continued) An illustration of checkerboard technique, three dimensional checkerboard microdilution of meropenem, ciprofloxacin and colistin were performed using the series of proportional to MICs of the drugs being tested (Modified from Eliopoulos and Moellering, 1996, Yoon *et al.*, 2004).

Isolate No. 1 **Plate 1-** No Colistin (0 µg/mL)

Meropenem

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/ 0/ 0	4	8	16	32	64	128	256

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

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

Ciprofloxacin

Plate 3 – Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

32	32/ 4/0.12	32/ 8/ 0.12	32/ 16/0.12	32/ 32/0.12	32/ 64/ 0.12	32/ 1280.12	32/ 256/0.12	256
16	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	128
8	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	64
4	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	32
2	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	16
1	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	8
0.5	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	4
0/ 0/ 0.12	4	8	16	32	64	128	256	C

Ciprofloxacin

Figure D-2 Three dimentional microdilution checkerboard result of *P. aeruginosa* isolate No.1 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate NO. 1 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem								
32	32/ 4/0.25	32/ 8/ 0.25	32/ 16/0.25	32/ 32/0.25	32/ 64/ 0.25	32/ 1280.25	32/ 256/0.25	4
16	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	2
8	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	1
4	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	0.5
2	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	0.25
1	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	0.12
0.5	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	0.06
0/ 0/ 0.25	4	8	16	32	64	128	256	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem							
32	32/ 4/0.5	32/ 8/ 0.5	32/ 16/0.5	32/ 32/0.5	32/ 64/ 0.5	32/ 1280.5	32/ 256/0.5
16	16/ 4/0.5	16/ 8/0.5	16/ 16/0.25	16/ 32/0.25	16/ 64/0.25	16/ 128/0.25	16/ 256/0.25
8	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	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	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	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/ 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	8	16	32	64	128	256

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

Meropenem							
32	32/ 4/1	32/ 8/ 1	32/ 16/1	32/ 32/1	32/ 64/ 1	32/ 1281	32/ 256/1
16	16/ 4/1	16/ 8/1	16/ 16/1	16/ 32/1	16/ 64/1	16/ 128/1	16/ 256/1
8	8/ 4/1	8/ 8/1	8/ 16/1	8/ 32/1	8/ 64/1	8/ 128/1	8/256/1
4	4/ 4/1	4/ 8/1	4/ 16/1	4/ 32/1	4/ 64/1	4/ 128/1	4/ 256/1
2	2/ 4/1	2/ 8/1	2/ 16/1	2/ 32/1	2/ 64/1	2/ 128/1	2/ 256/1
1	1/ 4/1	1/ 8/1	1/ 16/1	1/ 32/1	1/ 64/1	1/ 128/1	1/ 256/1
0.5	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	8	16	32	64	128	256

Ciprofloxacin

Figure D-2 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.1 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 1 (Continued) **Plate 7** - Colistin 2 µg/mL

32	32/ 4/2	32/ 8/ 2	32/ 16/2	32/ 32/2	32/ 64/ 2	32/ 1282	32/ 256/2
16	16/ 4/2	16/ 8/2	16/ 16/2	16/ 32/2	16/ 64/2	16/ 128/2	16/ 256/2
8	8/ 4/2	8/ 8/2	8/ 16/2	8/ 32/2	8/ 64/2	8/ 128/2	8/256/2
4	4/ 4/2	4/ 8/2	4/ 16/2	4/ 32/2	4/ 64/2	4/ 128/2	4/ 256/2
2	2/ 4/2	2/ 8/2	2/ 16/2	2/ 32/2	2/ 64/2	2/ 128/2	2/ 256/2
1	1/ 4/2	1/ 8/2	1/ 16/2	1/ 32/2	1/ 64/2	1/ 128/2	1/ 256/2
0.5	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	8	16	32	64	128	256

Meropenem

Ciprofloxacin

8. **Plate 8** – Colistin 4 µg/mL

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

Meropenem

Ciprofloxacin

Figure D-2 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.1 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1

FIC index for meropenem + colistin = 1.060

FIC index for ciprofloxacin + colistin = 1.060

FIC index for meropenem + ciprofloxacin + colistin = 0.87

Isolate No. 2 **Plate 1-** No Colistin (0 µg/mL)

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

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem	128	128/ 4/0.06	128/ 8/ 0.06	128/ 16/0.06	128/ 32/0.06	128/ 64/ 0.06	128/ 1280.06	128/ 256/0.06	128
	64	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	64
	32	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	32
	16	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	16
	8	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	8
	4	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	4
	2	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	2
	0/ 0/ 0.06	4	8	16	32	64	128	256	C

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem	128	128/ 4/0.12	128/ 8/ 0.12	128/ 16/0.12	128/ 32/0.12	128/ 64/ 0.12	128/ 1280.12	128/ 256/0.12	256
	64	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	128
	32	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	64
	16	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	32
	8	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	16
	4	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	8
	2	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	4
	0/ 0/ 0.12	4	8	16	32	64	128	256	C

Ciprofloxacin

Figure D-3 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.2 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 2 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem								
128	128/ 4/0.25	128/ 8/ 0.25	128/ 16/0.25	128/ 32/0.25	128/ 64/ 0.25	128/ 1280.25	128/ 256/0.25	4
64	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	2
32	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	1
16	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	0.5
8	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	0.25
4	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	0.12
2	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	0.06
0/ 0/ 0.25	4	8	16	32	64	128	256	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem							
128	128/ 4/0.5	128/ 8/ 0.5	128/ 16/0.5	128/ 32/0.5	128/ 64/ 0.5	128/ 1280.5	128/ 256/0.5
64	64/ 4/0.5	64/ 8/0.5	64/ 16/0.25	64/ 32/0.25	64/ 64/0.25	64/ 128/0.25	64/ 256/0.25
32	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	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	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	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	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	8	16	32	64	128	256

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

Meropenem							
128	128/ 4/1	128/ 8/ 1	128/ 16/1	128/ 32/1	128/ 64/ 1	128/ 1281	128/ 256/1
64	64/ 4/1	64/ 8/1	64/ 16/1	64/ 32/1	64/ 64/1	64/ 128/1	64/ 256/1
32	32/ 4/1	32/ 8/1	32/ 16/1	32/ 32/1	32/ 64/1	32/ 128/1	32/256/1
16	16/ 4/1	16/ 8/1	16/ 16/1	16/ 32/1	16/ 64/1	16/ 128/1	16/ 256/1
8	8/ 4/1	8/ 8/1	8/ 16/1	8/ 32/1	8/ 64/1	8/ 128/1	8/ 256/1
4	4/ 4/1	4/ 8/1	4/ 16/1	4/ 32/1	4/ 64/1	4/ 128/1	4/ 256/1
2	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	8	16	32	64	128	256

Ciprofloxacin

Figure D-3 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.2 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 2 (Continued) **Plate 7** - Colistin 2 µg/mL

Meropenem								
128	128/ 4/2	128/ 8/ 2	128/ 16/2	128/ 32/2	128/ 64/ 2	128/ 1282	128/ 256/2
64	64/ 4/2	64/ 8/2	64/ 16/2	64/ 32/2	64/ 64/2	64/ 128/2	64/ 256/2
32	32/ 4/2	32/ 8/2	32/ 16/2	32/ 32/2	32/ 64/2	32/ 128/2	32/256/2
16	16/ 4/2	16/ 8/2	16/ 16/2	16/ 32/2	16/ 64/2	16/ 128/2	16/ 256/2
8	8/ 4/2	8/ 8/2	8/ 16/2	8/ 32/2	8/ 64/2	8/ 128/2	8/ 256/2
4	4/ 4/2	4/ 8/2	4/ 16/2	4/ 32/2	4/ 64/2	4/ 128/2	4/ 256/2
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	8	16	32	64	128	256

Ciprofloxacin

Plate 8 – Colistin 4 µg/mL

Meropenem							
128	128/ 4/4	128/ 8/ 4	128/ 16/4	128/ 32/4	128/ 64/ 4	128/ 1284	128/ 256/4
64	64/ 4/4	64/ 8/4	64/ 16/4	64/ 32/4	64/ 64/4	64/ 128/4	64/ 256/4
32	32/ 4/4	32/ 8/4	32/ 16/4	32/ 32/4	32/ 64/4	32/ 128/4	32/256/4
16	16/ 4/4	16/ 8/4	16/ 16/4	16/ 32/4	16/ 64/4	16/ 128/4	16/ 256/4
8	8/ 4/4	8/ 8/2	8/ 16/4	8/ 32/4	8/ 64/4	8/ 128/4	8/ 256/4
4	4/ 4/4	4/ 8/4	4/ 16/4	4/ 32/4	4/ 64/4	4/ 128/4	4/ 256/4
2	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	8	16	32	64	128	256

Ciprofloxacin

Figure D-3 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 2 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1

FIC index for meropenem + colistin = 1

FIC index for ciprofloxacin + colistin = 1.060

FIC index for meropenem + ciprofloxacin **+** colistin = 0.745

Isolate No. 3 **Plate 1-** No Colistin (0 µg/mL)

Meropenem

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/ 0/ 0	4	8	16	32	64	128	256

Ciprofloxacin

Plate 2 - Colistin 0.12 µg/mL

Meropenem alone

Meropenem

32	32/ 4/0.12	32/ 8/ 0.12	32/ 16/0.12	32/ 32/0.12	32/ 64/ 0.12	32/ 1280.12	32/ 256/0.12	32
16	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	16
8	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	8
4	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	4
2	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	2
1	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	1
0.5	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	0.5
0/ 0/ 0.12	4	8	16	32	64	128	256	C

Ciprofloxacin

Plate 3 - Colistin 0.25 µg/mL

Ciprofloxacin alone

Meropenem

32	32/ 4/0.25	32/ 8/ 0.25	32/ 16/0.25	32/ 32/0.25	32/ 64/ 0.25	32/ 1280.25	32/ 256/0.25	256
16	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	128
8	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	64
4	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	32
2	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	16
1	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	8
0.5	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	4
0/ 0/ 0.25	4	8	16	32	64	128	256	C

Ciprofloxacin

Figure D-4 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.3 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate 3 (Continued) **Plate 4** – Colistin 0.5 µg/mL

Colistin alone

32	32/ 4/0.5	32/ 8/ 0.5	32/ 16/0.5	32/ 32/0.5	32/ 64/	32/ 128	32/ 256/	8
16	16/ 4/0.5	16/ 8/0.5	16/ 16/0.25	16/ 32/0.25	16/ 64/	16/ 128/	16/ 256/	4
8	8/ 4/0.5	8/ 8/0.5	8/ 16/0.5	8/ 32/0.5	8/ 64/0.5	8/ 128/	8/256/	2
4	4/ 4/0.5	4/ 8/0.5	4/ 16/0.5	4/ 32/0.5	4/ 64/0.5	4/ 128/	4/ 256/	1
2	2/ 4/0.5	2/ 8/0.5	2/ 16/0.5	2/ 32/0.5	2/ 64/0.5	2/ 128/	2/ 256/	0.5
1	1/ 4/0.5	1/ 8/0.5	1/ 16/0.5	1/ 32/0.5	1/ 64/0.5	1/ 128/	1/ 256/	0.25
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/ 256/	0.12
0/ 0/ 0.5	4	8	16	32	64	128	256	C

Meropenem

Ciprofloxacin

Plate 5 – Colistin 1 µg/mL

32	32/ 4/1	32/ 8/ 1	32/ 16/1	32/ 32/1	32/ 64/ 1	32/ 1281	32/ 256/1
16	16/ 4/1	16/ 8/1	16/ 16/1	16/ 32/1	16/ 64/1	16/ 128/1	16/ 256/1
8	8/ 4/1	8/ 8/1	8/ 16/1	8/ 32/1	8/ 64/1	8/ 128/1	8/256/1
4	4/ 4/1	4/ 8/1	4/ 16/1	4/ 32/1	4/ 64/1	4/ 128/1	4/ 256/1
2	2/ 4/1	2/ 8/1	2/ 16/1	2/ 32/1	2/ 64/1	2/ 128/1	2/ 256/1
1	1/ 4/1	1/ 8/1	1/ 16/1	1/ 32/1	1/ 64/1	1/ 128/1	1/ 256/1
0.5	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	8	16	32	64	128	256

Meropenem

Ciprofloxacin

Plate 6 – Colistin 2 µg/mL

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

Meroenem

Ciprofloxacin

Figure D-4 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.3 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 3 (Continued) **Plate 7** – Colistin 4 µg/mL

32	32/ 4/4	32/ 8/ 4	32/ 16/4	32/ 32/4	32/ 64/ 4	32/ 1284	32/ 256/4
16	16/ 4/4	16/ 8/4	16/ 16/4	16/ 32/4	16/ 64/4	16/ 128/4	16/ 256/4
8	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/ 8/4	4/ 16/4	4/ 32/4	4/ 64/4	4/ 128/4	4/ 256/4
2	2/ 4/4	2/ 8/2	2/ 16/4	2/ 32/4	2/ 64/4	2/ 128/4	2/ 256/4
1	1/ 4/4	1/ 8/4	1/ 16/4	1/ 32/4	1/ 64/4	1/ 128/4	1/ 256/4
0.5	0.5/ 4/4	0.5/ 8/4	0.5/ 16/4	0.5/ 32/4	0.5/ 64/4	0.5/ 128/4	0.5/ 256/4
0/ 0/ 4	4	8	16	32	64	128	256

Meropenem

Ciprofloxacin

Plate 8 - Colistin 8 µg/mL

32	32/ 4/8	32/ 8/ 8	32/ 16/8	32/ 32/8	32/ 64/ 8	32/ 1288	32/ 256/8
16	16/ 4/8	16/ 8/8	16/ 16/8	16/ 32/8	16/ 64/8	16/ 128/8	16/ 256/8
8	8/ 4/8	8/ 8/8	8/ 16/8	8/ 32/8	8/ 64/8	8/ 128/8	8/256/8
4	4/ 4/8	4/ 8/8	4/ 16/8	4/ 32/8	4/ 64/8	4/ 128/8	4/ 256/8
2	2/ 4/8	2/ 8/8	2/ 16/8	2/ 32/8	2/ 64/8	2/ 128/8	2/ 256/8
1	1/ 4/8	1/ 8/8	1/ 16/8	1/ 32/8	1/ 64/8	1/ 128/8	1/ 256/8
0.5	0.5/ 4/8	0.5/ 8/8	0.5/ 16/8	0.5/ 32/8	0.5/ 64/8	0.5/ 128/8	0.5/ 256/8
0/ 0/ 8	4	8	16	32	64	128	256

Meropenem

Ciprofloxacin

Figur D-4 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 3 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 0.75

FIC index for meropenem + colistin = 1.060

FIC index for ciprofloxacin + colistin = 1

FIC index for meropenem + ciprofloxacin **+** colistin = 0.685

Isolate No. 4 **Plate 1**- No Colistin (0 µg/mL)

Meropenem								
32	32/ 0.5	32/ 1	32/ 2	32/ 4	32/ 8	32/ 16	32/ 32
16	16/ 0.5	16/ 1	16/ 2	16/ 4	16/ 8	16/ 16	16/ 32
8	8/ 0.5	8/ 1	8/ 2	8/ 4	8/ 8	8/ 16	8/ 32
4	4/ 0.5	4/ 1	4/ 2	4/ 4	4/ 8	4/ 16	4/ 32
2	2/ 0.5	2/ 1	2/ 2	2/ 4	2/ 8	2/ 16	2/ 32
1	1/ 0.5	1/ 1	1/ 2	1/ 4	1/ 8	1/ 16	1/ 32
0.5	0.5/ 0.5	0.5/ 1	0.5/ 2	0.5/ 4	0.5/ 8	0.5/ 16	0.5/ 32
0/ 0/ 0	0.5	1	2	4	8	16	32

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem								
32	32/ 0.5/0.06	32/ 1/ 0.06	32/ 2/0.06	32/ 4/0.06	32/ 8/ 0.06	32/ 16/0.06	32/ 32/0.06	32
16	16/ 0.5/0.06	16/ 1/0.06	16/ 2/0.06	16/ 4/0.06	16/ 8/0.06	16/ 16/0.06	16/ 32/0.06	16
8	8/ 0.5/0.06	8/ 1/0.06	8/ 2/0.06	8/ 4/0.06	8/ 8/0.06	8/ 16/0.06	8/ 32/0.06	8
4	4/ 0.5/0.06	4/ 1/0.06	4/ 2/0.06	4/ 4/0.06	4/ 8/0.06	4/ 16/0.06	4/ 32/0.06	4
2	2/ 0.5/0.06	2/ 1/0.06	2/ 2/0.06	2/ 4/0.06	2/ 8/0.06	2/ 16/0.06	2/ 32/.06	2
1	1/ 0.5/0.06	1/ 1/0.06	1/ 2/0.06	1/ 4/0.06	1/ 8/0.06	1/ 16/0.06	1/ 32/0.06	1
0.5	0.5/ 0.5/0.06	0.5/ 1/0.06	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
0/ 0/ 0.06	0.5	1	2	4	8	16	32	C

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem								
32	32/ 0.5/0.12	32/ 1/ 0.12	32/ 2/0.12	32/ 4/0.12	32/ 8/ 0.12	32/ 16/0.12	32/ 32/0.12	32
16	16/ 0.5/0.12	16/ 1/0.12	16/ 2/0.12	16/ 4/0.12	16/ 8/0.12	16/ 16/0.12	16/ 32/0.12	16
8	8/ 0.5/0.12	8/ 1/0.12	8/ 2/0.12	8/ 4/0.12	8/ 8/0.12	8/ 16/0.12	8/ 32/0.12	8
4	4/ 0.5/0.12	4/ 1/0.12	4/ 2/0.12	4/ 4/0.12	4/ 8/0.12	4/ 16/0.12	4/ 32/0.12	4
2	2/ 0.5/0.12	2/ 1/0.12	2/ 2/0.12	2/ 4/0.12	2/ 8/0.12	2/ 16/0.12	2/ 32/0.12	2
1	1/ 0.5/0.12	1/ 1/0.12	1/ 2/0.12	1/ 4/0.12	1/ 8/0.12	1/ 16/0.12	1/ 32/0.12	1
0.5	0.5/ 0.5/0.12	0.5/ 1/0.12	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
0/ 0/ 0.12	0.5	1	2	4	8	16	32	C

Ciprofloxacin

Figure D-5 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 4 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 4 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem								
32	32/ 0.5/0.25	32/ 1/ 0.25	32/ 2/0.25	32/ 4/0.25	32/ 8/ 0.25	32/ 16/0.25	32/ 32/0.25	4
16	16/ 0.5/0.25	16/ 1/0.25	16/ 2/0.25	16/ 4/0.25	16/ 8/0.25	16/ 16/0.25	16/ 32/0.25	2
8	8/ 0.5/0.25	8/ 1/0.25	8/ 2/0.25	8/ 4/0.25	8/ 8/0.25	8/ 16/0.25	8/ 32/0.25	1
4	4/ 0.5/0.25	4/ 1/0.25	4/ 2/0.25	4/ 4/0.25	4/ 8/0.25	4/ 16/0.25	4/ 32/0.25	0.5
2	2/ 0.5/0.25	2/ 1/0.25	2/ 2/0.25	2/ 4/0.25	2/ 8/0.25	2/ 16/0.25	2/ 32/0.25	0.25
1	1/ 0.5/0.25	1/ 1/0.25	1/ 2/0.25	1/ 4/0.25	1/ 8/0.25	1/ 16/0.25	1/ 32/0.25	0.12
0.5	0.5/ 0.5/0.25	0.5/ 1/0.25	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	0.06
0/ 0/ 0.25	0.5	1	2	4	8	16	32	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem							
32	32/ 0.5/0.5	32/ 1/ 0.5	32/ 2/0.5	32/ 4/0.5	32/ 8/ 0.5	32/ 16/0.5	32/ 32/0.5
16	16/ 0.5/0.5	16/ 1/0.5	16/ 2/0.25	16/ 4/0.25	16/ 8/0.25	16/ 16/0.25	16/ 32/0.25
8	8/ 0.5/0.5	8/ 1/0.5	8/ 2/0.5	8/ 4/0.5	8/ 8/0.5	8/ 16/0.5	8/ 32/0.5
4	4/ 0.5/0.5	4/ 1/0.5	4/ 2/0.5	4/ 4/0.5	4/ 8/0.5	4/ 16/0.5	4/ 32/0.5
2	2/ 0.5/0.5	2/ 1/0.5	2/ 2/0.5	2/ 4/0.5	2/ 8/0.5	2/ 16/0. 5	2/ 32/0.5
1	1/ 0.5/0.5	1/ 1/0.5	1/ 2/0.5	1/ 4/0.5	1/ 8/0.5	1/ 16/0.5	1/ 32/0.5
0.5	0.5/ 0.5/0.5	0.5/ 1/0.5	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/ 0/ 0.5	0.5	1	2	4	8	16	32

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

Meropenem							
32	32/ 0.5/1	32/ 1/ 1	32/ 2/1	32/ 4/1	32/ 8/ 1	32/ 16/1	32/ 32/1
16	16/ 0.5/1	16/ 8/1	16/ 2/1	16/ 4/1	16/ 8/1	16/ 16/1	16/ 32/1
8	8/ 0.5/1	8/ 1/1	8/ 2/1	8/ 4/1	8/ 8/1	8/ 16/1	8/ 32/1
4	4/ 0.5/1	4/ 1/1	4/ 2/1	4/ 4/1	4/ 8/1	4/ 16/1	4/ 32/1
2	2/ 0.5/1	2/ 1/1	2/ 2/1	2/ 4/1	2/ 8/1	2/ 16/1	2/ 32/1
1	1/ 0.5/1	1/ 1/1	1/ 2/1	1/ 4/1	1/ 8/1	1/ 16/1	1/ 32/1
0.5	0.5/ 0.5/1	0.5/ 1/1	0.5/ 2/1	0.5/ 4/1	0.5/ 8/1	0.5/ 16/1	0.5/ 32/1
0/ 0/ 1	0.5	1	2	4	8	16	32

Ciprofloxacin

Figure D-5 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 4 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 4 (Continued) **Plate 7** - Colistin 2 µg/mL

Meropenem							
32	32/ 0.5/2	32/ 1/ 2	32/ 2/2	32/ 4/2	32/ 8/ 2	32/ 16/2	32/ 32/2
16	16/ 0.5/2	16/ 1/2	16/ 2/2	16/ 4/2	16/ 8/2	16/ 16/2	16/ 32/2
8	8/ 0.5/2	8/ 1/2	8/ 2/2	8/ 4/2	8/ 8/2	8/ 16/2	8/ 32/2
4	4/ 0.5/2	4/ 1/2	4/ 2/2	4/ 4/2	4/ 8/2	4/ 16/2	4/ 32/2
2	2/ 0.5/2	2/ 1/2	2/ 2/2	2/ 4/2	2/ 8/2	2/ 16/2	2/ 32/2
1	1/ 0.5/2	1/ 1/2	1/ 2/2	1/ 4/2	1/ 8/2	1/ 16/2	1/ 32/2
0.5	0.5/ 0.5/2	0.5/ 1/2	0.5/ 2/2	0.5/ 4/2	0.5/ 8/2	0.5/ 16/2	0.5/ 32/2
0/ 0/ 2	0.5	1	2	4	8	16	32

Ciprofloxacin

Plate 8 – Colistin 4 µg/mL

Meropenem							
32	32/ 0.5/4	32/ 18/ 4	32/ 2/4	32/ 4/4	32/ 8/ 4	32/ 16/4	32/ 32/4
16	16/ 0.5/4	16/ 1/4	16/ 2/4	16/ 4/4	16/ 8/4	16/ 16/4	16/ 32/4
8	8/ 0.5/4	8/ 1/4	8/ 2/4	8/ 4/4	8/ 8/4	8/ 16/4	8/ 32/4
4	4/ 0.5/4	4/ 1/4	4/ 2/4	4/ 4/4	4/ 8/4	4/ 16/4	4/ 32/4
2	2/ 0.5/4	2/ 1/2	2/ 2/4	2/ 4/4	2/ 8/4	2/ 16/4	2/ 32/4
1	1/ 0.5/4	1/ 1/4	1/ 2/4	1/ 4/4	1/ 8/4	1/ 16/4	1/ 32/4
0.5	0.5/ 0.5/4	0.5/ 1/4	0.5/ 2/4	0.5/ 4/4	0.5/ 8/4	0.5/ 16/4	0.5/ 32/4
0/ 0/ 4	0.5	1	2	4	8	16	32

Ciprofloxacin

Figure D-5 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 4 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed. .

FIC index for meropenem + ciprofloxacin = 0.75

FIC index for meropenem + colistin = 1.060

FIC index for ciprofloxacin + colistin = 1.060

FIC index for meropenem + ciprofloxacin + colistin = 0.81

Isolate No. 5 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

128	128/ 0.06	128/ 0.12	128/ 0.25	128/ 0.5	128/ 1	128/ 2	128/ 4
64	64/ 0.06	64/ 0.12	64/ 0.25	64/ 0.5	64/ 1	64/ 2	64/ 4
32	32/ 0.06	32/ 0.12	32/ 0.25	32/ 0.5	32/ 1	32/ 2	32/ 4
16	16/ 0.06	16/ 0.12	16/ 0.25	16/ 0.5	16/ 1	16/ 2	16/ 4
8	8/ 0.06	8/ 0.12	8/ 0.25	8/ 0.5	8/ 1	8/ 2	8/ 4
4	4/ 0.06	4/ 0.12	4/ 0.25	4/ 0.5	4/ 1	4/ 2	4/ 4
2	2/ 0.06	2/ 0.12	2/ 0.25	2/ 0.5	2/ 1	2/ 2	2/ 4
0/ 0/ 0	0.06	0.12	0.25	0.5	1	2	4

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

128	128/ 0.06/0.06	128/ 0.12/	128/ 0.25/0.06	128/ 0.5/0.06	128/ 1/ 0.06	128/ 2/0.06	128/ 4/0.06	128
64	64/ 0.06/0.06	64/ 0.12/0.06	64/ 0.25/0.06	64/ 0.5/0.06	64/ 1/0.06	64/ 2/0.06	64/ 4/0.06	64
32	32/ 0.06/0.06	32/ 0.12/0.06	32/ 0.25/0.06	32/ 0.5/0.06	32/ 1/0.06	32/ 2/0.06	32/ 4/0.06	32
16	16/ 0.06/0.06	16/ 0.12/0.06	16/ 0.25/0.06	16/ 0.5/0.06	16/ 1/0.06	16/ 2/0.06	16/ 4/0.06	16
8	8/ 0.06/0.06	8/ 0.12/0.06	8/ 0.25/0.06	8/ 0.5/0.06	8/ 1/0.06	8/ 2/0.06	8/ 4/0.06	8
4	4/ 0.06/0.06	4/ 0.12/0.06	4/ 0.25/0.06	4/ 0.5/0.06	4/ 1/0.06	4/ 2/0.06	4/ 4/0.06	4
2	2/ 0.06/0.06	2/ 0.12/0.06	2/ 0.25/0.06	2/ 0.5/0.06	2/ 1/0.06	2/ 2/0.06	2/ 4/0.06	2
0/ 0/ 0.06	0.06	0.12	0.25	0.5	1	2	4	C

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

128	128/ 0.06/0.12	128/ 0.12/0.12	128/ 0.25/0.12	128/ 0.5/0.12	128/ 1/ 0.12	128/ 2/0.12	128/ 4/0.12	4
64	64/ 0.06/0.12	64/ 0.12/0.12	64/ 0.25/0.12	64/ 0.5/0.12	64/ 1/0.12	64/ 2/0.12	64/ 4/0.12	2
32	32/ 0.06/0.12	32/ 0.12/0.12	32/ 0.25/0.12	32/ 0.5/0.12	32/ 1/0.12	32/ 2/0.12	32/ 4/0.12	1
16	16/ 0.06/0.12	16/ 0.12/0.12	16/ 0.25/0.12	16/ 0.5/0.12	16/ 1/0.12	16/ 2/0.12	16/ 4/0.12	0.5
8	8/ 0.06/0.12	8/ 0.12/0.12	8/ 0.25/0.12	8/ 0.5/0.12	8/ 1/0.12	8/ 2/0.12	8/ 4/0.12	0.25
4	4/ 0.06/0.12	4/ 0.12/0.12	4/ 0.25/0.12	4/ 0.5/0.12	4/ 1/0.12	4/ 2/0.12	4/ 4/0.12	0.12
2	2/ 0.06/0.12	2/ 0.12/0.12	2/ 0.25/0.12	2/ 0.5/0.12	2/ 1/0.12	2/ 2/0.12	2/ 4/0.12	0.06
0/ 0/ 0.12	0.06	0.12	0.25	0.5	1	2	4	C

Ciprofloxacin

Figure D-6 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.5 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 5 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem								
128	128/ 0.06/0.25	128/ 0.12/ 0.25	128/ 0.25/0.25	128/ 0.5/0.25	128/ 1/ 0.25	128/ 2/0.25	128/ 4/0.25	4
64	64/ 4/0.25	64/ 0.12/0.25	64/ 0.25/0.25	64/ 0.5/0.25	64/ 1/0.25	64/ 2/0.25	64/ 4/0.25	2
32	32/ 0.06/0.25	32/ 0.12/0.25	32/ 0.25/0.25	32/ 0.5/0.25	32/ 1/0.25	32/ 2/0.25	32/ 4/0.25	1
16	16/ 0.06/0.25	16/ 0.12/0.25	16/ 0.25/0.25	16/ 0.5/0.25	16/ 1/0.25	16/ 2/0.25	16/ 4/0.25	0.5
8	8/ 0.06/0.25	8/ 0.12/0.25	8/ 0.25/0.25	8/ 0.5/0.25	8/ 1/0.25	8/ 2/0.25	8/ 4/0.25	0.25
4	4/ 0.06/0.25	4/ 0.12/0.25	4/ 0.25/0.25	4/ 0.5/0.25	4/ 1/0.25	4/ 2/0.25	4/ 4/0.25	0.12
2	2/ 0.06/0.25	2/ 0.12/0.25	2/ 0.25/0.25	2/ 0.5/0.25	2/ 1/0.25	2/ 2/0.25	2/ 4/0.25	0.06
0/ 0/ 0.25	0.06	0.12	0.25	0.5	1	2	4	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem							
128	128/ 0.06/0.5	128/ 0.12/ 0.5	128/ 0.25/0.5	128/ 0.5/0.5	128/ 1/ 0.5	128/ 2/0.5	128/ 4/0.5
64	64/ 0.06/0.5	64/ 0.12/0.5	64/ 0.25/0.5	64/ 0.5/0.5	64/ 1/0.5	64/ 2/0.5	64/ 4/0.5
32	32/ 0.06/0.5	32/ 0.12/0.5	32/ 0.25/0.5	32/ 0.5/0.5	32/ 1/0.5	32/ 2/0.5	32/ 4/0.5
16	16/ 0.06/0.5	16/ 0.12/0.5	16/ 0.25/0.5	16/ 0.5/0.5	16/ 1/0.5	16/ 2/0.5	16/ 4/0.5
8	8/ 0.06/0.5	8/ 0.12/0.5	8/ 0.25/0.5	8/ 0.5/0.5	8/ 1/0.5	8/ 2/0. 5	8/ 4/0.5
4	4/ 0.06/0.5	4/ 0.12/0.5	4/ 0.25/0.5	4/ 0.5/0.5	4/ 1/0.5	4/ 2/0.5	4/ 4/0.5
2	2/ 0.06/0.5	2/ 0.12/0.5	2/ 0.25/0.5	2/ 0.5/0.5	2/ 1/0.5	2/ 2/0.5	2/ 4/0.5
0/ 0/ 0.5	0.06	0.12	0.25	0.5	1	2	4

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

Meropenem							
128	128/ 0.06/1	128/ 0.12/ 1	128/ 0.25/1	128/ 0.5/1	128/ 1/ 1	128/ 2/1	128/ 4/1
64	64/ 0.06/1	64/ 0.12/1	64/ 0.25/1	64/ 0.5/1	64/ 1/1	64/ 2/1	64/ 4/1
32	32/ 0.06/1	32/ 0.12/1	32/ 0.25/1	32/ 0.5/1	32/ 1/1	32/ 2/1	32/ 4/1
16	16/ 0.06/1	16/ 0.12/1	16/ 0.25/1	16/ 0.5/1	16/ 1/1	16/ 2/1	16/ 4/1
8	8/ 0.06/1	8/ 0.12/1	8/ 0.25/1	8/ 0.5/1	8/ 1/1	8/ 2/1	8/ 4/1
4	4/ 0.06/1	4/ 0.12/1	4/ 0.25/1	4/ 0.5/1	4/ 1/1	4/ 2/1	4/ 4/1
2	2/ 0.06/1	2/ 0.12/1	2/ 0.25/1	2/ 0.5/1	2/ 1/1	2/ 2/1	2/ 4/1
0/ 0/ 1	0.06	0.12	0.25	0.5	1	2	4

Ciprofloxacin

Figure D-6 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.5 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 5 (Continued) **Plate 7** - Colistin 2 µg/mL

Meropenem							
128	128/ 0.06/2	128/ 0.12/ 2	128/ 0.25/2	128/ 0.5/2	128/ 1/ 2	128/ 2/2	128/ 4/2
64	64/ 0.06/2	64/ 0.12/2	64/ 0.25/2	64/ 0.5/2	64/ 1/2	64/ 2/2	64/ 4/2
32	32/ 0.06/2	32/ 0.12/2	32/ 0.25/2	32/ 0.5/2	32/ 1/2	32/ 2/2	32/ 4/2
16	16/ 0.06/2	16/ 0.12/2	16/ 0.25/2	16/ 0.5/2	16/ 1/2	16/ 2/2	16/ 4/2
8	8/ 0.06/2	8/ 0.12/2	8/ 0.25/2	8/ 0.5/2	8/ 1/2	8/ 2/2	8/ 4/2
4	4/ 0.06/2	4/ 0.12/2	4/ 0.25/2	4/ 0.5/2	4/ 1/2	4/ 2/2	4/ 4/2
2	2/ 0.06/2	2/ 0.12/2	2/ 0.25/2	2/ 0.5/2	2/ 1/2	2/ 2/2	2/ 4/2
0/ 0/ 2	0.06	0.12	0.25	0.5	1	2	4

Ciprofloxacin

Plate 8 - Colistin 4 µg/mL

Meropenem							
128	128/ 0.06/4	128/ 0.12/ 4	128/ 0.25/4	128/ 0.5/4	128/ 1/ 4	128/ 2/4	128/ 4/4
64	64/ 0.06/4	64/ 0.12/4	64/ 0.25/4	64/ 0.5/4	64/ 1/4	64/ 2/4	64/ 4/4
32	32/ 0.06/4	32/ 0.12/4	32/ 0.25/4	32/ 0.5/4	32/ 1/4	32/ 2/4	32/ 4/4
16	16/ 0.06/4	16/ 0.12/4	16/ 0.25/4	16/ 0.5/4	16/ 1/4	16/ 2/4	16/ 4/4
8	8/ 0.06/4	8/ 0.12/4	8/ 0.25/4	8/ 0.5/4	8/ 1/4	8/ 2/4	8/ 4/4
4	4/ 0.06/4	4/ 0.12/4	4/ 0.25/4	4/ 0.5/4	4/ 1/4	4/ 2/4	4/ 4/4
2	2/ 0.06/4	2/ 0.12/4	2/ 0.25/4	2/ 0.5/4	2/ 1/4	2/ 2/4	2/ 4/4
0/ 0/ 4	0.06	0.12	0.25	0.5	1	2	4

Ciprofloxacin

Figure D-6 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 5 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed. .

FIC index for meropenem + ciprofloxacin = 0.75

FIC index for meropenem + colistin = 0.625

FIC index for ciprofloxacin + colistin = 0.56

FIC index for meropenem + ciprofloxacin + colistin = 0.623

Isolate No. 6 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

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/ 0/ 0	4	8	16	32	64	128	256

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

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

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

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

Ciprofloxacin

Figure D-7 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.6 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 6 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem								
32	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	4
16	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	2
8	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	1
4	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	0.5
2	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	0.25
1	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	0.12
0.5	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	0.06
0/ 0/ 0.25	4	8	16	32	64	128	256	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem							
32	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	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	8/ 4/0.5	8/ 8/0.5	8/ 16/0.5	8/ 32/0.5	8/ 64/0.5	8/ 128/. 5	8/ 256/0.5
4	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	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	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/ 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	8	16	32	64	128	256

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

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

Ciprofloxacin

Figure D-7 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.6 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 6 (Continued) **Plate 7** - Colistin 2 µg/mL

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

Ciprofloxacin

Plate 8 – Colistin 4 µg/mL

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

Ciprofloxacin

Figure D-7 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 6 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1.063

FIC index for meropenem + colistin = 1

FIC index for ciprofloxacin + colistin = 1

FIC index for meropenem + ciprofloxacin + colistin = 1.030

Isolate No. 7 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

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

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

64	64/ 0.5/0.06	64/ 1/ 0.06	64/ 2/0.06	64/ 4/0.06	64/ 8/ 0.06	64/ 16/0.06	64/ 32/0.06	64
32	32/ 0.5/0.06	32/ 1/0.06	32/ 2/0.06	32/ 4/0.06	32/ 8/0.06	32/ 16/0.06	32/ 32/0.06	32
16	16/ 0.5/0.06	16/ 1/0.06	16/ 2/0.06	16/ 4/0.06	16/ 8/0.06	16/ 16/0.06	16/ 32/0.06	16
8	6/ 0.5/0.06	8/ 1/0.06	8/ 2/0.06	8/ 4/0.06	8/ 8/0.06	8/ 16/0.06	8/ 32/0.06	8
4	4/ 0.5/0.06	4/ 1/0.06	4/ 2/0.06	4/ 4/0.06	4/ 8/0.06	4/ 16/0.06	4/ 32/0.06	4
2	2/ 0.5/0.06	2/ 1/0.06	2/ 2/0.06	2/ 4/0.06	2/ 8/0.06	2/ 16/0.06	2/ 32/0.06	2
1	1/ 0.5/0.06	1/ 1/0.06	1/ 2/0.06	1/ 4/0.06	1/ 8/0.06	1/ 16/0.06	1/ 32/0.06	1
0/ 0/ 0.06	0.5	1	2	4	8	16	32	C

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

64	64/ 0.5/0.12	64/ 1/ 0.12	64/ 2/0.12	64/ 4/0.12	64/ 8/ 0.12	64/ 16/0.12	64/ 32/0.12	32
32	32/ 0.5/0.12	32/ 1/0.12	32/ 2/0.12	32/ 4/0.12	32/ 8/0.12	32/ 16/0.12	32/ 32/0.12	16
16	16/ 0.5/0.12	16/ 1/0.12	16/ 2/0.12	16/ 4/0.12	16/ 8/0.12	16/ 16/0.12	16/ 32/0.12	8
8	6/ 0.5/0.12	8/ 1/0.12	8/ 2/0.12	8/ 4/0.12	8/ 8/0.12	8/ 16/0.12	8/ 32/0.12	4
4	4/ 0.5/0.12	4/ 1/0.12	4/ 2/0.12	4/ 4/0.12	4/ 8/0.12	4/ 16/0.12	4/ 32/0.12	2
2	2/ 0.5/0.12	2/ 1/0.12	2/ 2/0.12	2/ 4/0.12	2/ 8/0.12	2/ 16/0.12	2/ 32/0.12	1
1	1/ 0.5/0.12	1/ 1/0.12	1/ 2/0.12	1/ 4/0.12	1/ 8/0.12	1/ 16/0.12	1/ 32/0.12	0.5
0/ 0/ 0.12	0.5	1	2	4	8	16	32	C

Ciprofloxacin

Figure D-8 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.7 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 7 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem								
64	64/ 0.5/0.25	64/ 1/ 0.25	64/ 2/0.25	64/ 4/0.25	64/ 8/ 0.25	64/ 16/0.25	64/ 32/0.25	4
32	32/ 0.5/0.25	32/ 1/0.25	32/ 2/0.25	32/ 4/0.25	32/ 8/0.25	32/ 16/0.25	32/ 32/0.25	2
16	16/ 0.5/0.25	16/ 1/0.25	16/ 2/0.25	16/ 4/0.25	16/ 8/0.25	16/ 16/0.25	16/ 32/0.25	1
8	6/ 0.5/0.25	8/ 1/0.25	8/ 2/0.25	8/ 4/0.25	8/ 8/0.25	8/ 16/0.25	8/ 32/0.25	0.5
4	4/ 0.5/0.25	4/ 1/0.25	4/ 2/0.25	4/ 4/	4/ 8/0.25	4/ 16/0.25	4/ 32/0.25	0.25
2	2/ 0.5/0.25	2/ 1/0.25	2/ 2/0.25	2/ 4/0.25	2/ 8/0.25	2/ 16/0.25	2/ 32/0.25	0.12
1	1/ 0.5/0.25	1/ 1/0.25	1/ 2/0.25	1/ 4/0.25	1/ 8/0.25	1/ 16/0.25	1/ 32/0.25	0.06
0/ 0/ 0.25	0.5	1	2	4	8	16	32	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem							
64	64/ 0.5/0.5	64/ 1/ 0.5	64/ 2/0.5	64/ 4/0.5	64/ 8/ 0.5	64/ 16/0.5	64/ 32/0.5
32	32/ 0.5/0.5	32/ 1/0.5	32/ 2/0.5	32/ 4/0.5	32/ 8/0.5	32/ 16/0.5	32/ 32/0.5
16	16/ 0.5/0.5	16/ 1/0.5	16/ 2/0.5	16/ 4/0.5	16/ 8/0.5	16/ 16/0.5	16/ 32/0.5
8	6/ 0.5/0.5	8/ 1/0.5	8/ 2/0.5	8/ 4/0.5	8/ 8/0.5	8/ 16/0.5	8/ 32/0.5
4	4/ 0.5/0.5	4/ 1/0.5	4/ 2/0.5	4/ 4/0.5	4/ 8/0.5	4/ 16/0.5	4/ 32/0.5
2	2/ 0.5/0.5	2/ 1/0.5	2/ 2/0.5	2/ 4/0.5	2/ 8/0.5	2/ 16/0.5	2/ 32/0.5
1	1/ 0.5/0.5	1/ 1/0.5	1/ 2/0.5	1/ 4/0.5	1/ 8/0.5	1/ 16/0.5	1/ 32/0.5
0/ 0/ 0.5	0.5	1	2	4	8	16	32

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

Meropenem							
64	64/ 0.5/1	64/ 1/ 1	64/ 2/1	64/ 4/1	64/ 8/ 1	64/ 16/1	64/ 32/1
32	32/ 0.5/1	32/ 1/1	32/ 2/1	32/ 4/1	32/ 8/1	32/ 16/1	32/ 32/1
16	16/ 0.5/1	16/ 1/1	816/ 2/1	16/ 4/1	16/ 8/1	16/ 16/1	16/ 32/1
8	6/ 0.5/1	8/ 1/1	8/ 2/1	8/ 4/1	8/ 8/1	8/ 16/1	8/ 32/1
4	4/ 0.5/1	4/ 1/1	4/ 2/1	4/ 4/1	4/ 8/1	4/ 16/1	4/ 32/1
2	2/ 0.5/1	2/ 1/1	2/ 2/1	2/ 4/1	2/ 8/1	2/ 16/1	2/ 32/1
1	1/ 0.5/1	1/ 1/1	1/ 2/1	1/ 4/1	1/ 8/1	1/ 16/1	1/ 32/1
0/ 0/ 1	0.5	1	2	4	8	16	32

Ciprofloxacin

Figure D-8 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.7 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 7 (Continued) **Plate 7** - Colistin 2 µg/mL

Meropenem							
64	64/ 0.5/2	64/ 1/ 2	64/ 2/2	64/ 4/2	64/ 8/ 2	64/ 16/2	64/ 32/2
32	32/ 0.5/2	32/ 1/2	32/ 2/2	32/ 4/2	32/ 8/2	32/ 16/2	32/ 32/2
16	16/ 0.5/2	16/ 1/2	816/ 2/2	16/ 4/2	16/ 8/2	16/ 16/2	16/ 32/2
8	6/ 0.5/2	8/ 1/2	8/ 2/2	8/ 4/2	8/ 8/2	8/ 16/2	8/ 32/2
4	4/ 0.5/2	4/ 1/2	4/ 2/2	4/ 4/2	4/ 8/2	4/ 16/2	4/ 32/2
2	2/ 0.5/2	2/ 1/2	2/ 2/2	2/ 4/2	2/ 8/2	2/ 16/2	2/ 32/2
1	1/ 0.5/2	1/ 1/2	1/ 2/2	1/ 4/2	1/ 8/2	1/ 16/2	1/ 32/2
0/ 0/ 2	0.5	1	2	4	8	16	32

Ciprofloxacin

Plate 8 – Colistin 4 µg/mL

Meropenem							
64	64/ 0.5/4	64/ 1/ 4	64/ 2/4	64/ 4/4	64/ 8/ 4	64/ 16/4	64/ 32/4
32	32/ 0.5/4	32/ 1/4	32/ 2/4	32/ 4/4	32/ 8/4	32/ 16/4	32/ 32/4
16	16/ 0.5/4	16/ 1/4	816/ 2/4	16/ 4/4	16/ 8/4	16/ 16/4	16/ 32/4
8	6/ 0.5/4	8/ 1/4	8/ 2/4	8/ 4/4	8/ 8/4	8/ 16/4	8/ 32/4
4	4/ 0.5/4	4/ 1/4	4/ 2/4	4/ 4/4	4/ 8/4	4/ 16/4	4/ 32/4
2	2/ 0.5/4	2/ 1/4	2/ 2/4	2/ 4/4	2/ 8/4	2/ 16/4	2/ 32/4
1	1/ 0.5/4	1/ 1/4	1/ 2/4	1/ 4/4	1/ 8/4	1/ 16/4	1/ 32/4
0/ 0/ 4	0.5	1	2	4	8	16	32

Ciprofloxacin

Figure D-8 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 7 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 0.75

FIC index for meropenem + colistin = 1

FIC index for ciprofloxacin + colistin = 1

FIC index for meropenem + ciprofloxacin + colistin = 0.78

Isolate No. 8 **Plate 1-** No Colistin (0 µg/mL)

Meropenem

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/ 0/ 0	4	8	16	32	64	128	256

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

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

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

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

Ciprofloxacin

Figure D-9 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.8 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 8 (Continued) **Plate 4** – Colistin 0.25 µg/mL

Colistin alone

Meropenem								
32	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	4
16	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	2
8	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	1
4	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	0.5
2	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	0.25
1	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	0.12
0.5	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	0.06
0/ 0/ 0.25	4	8	16	32	64	128	256	C

Ciprofloxacin

Plate 5 – Colistin 0.5 µg/mL

Meropenem							
32	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	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	8/ 4/0.5	8/ 8/0.5	8/ 16/0.5	8/ 32/0.5	8/ 64/0.5	8/ 128/. 5	8/ 256/0.5
4	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	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	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/ 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	8	16	32	64	128	256

Ciprofloxacin

Plate 6 – Colistin 1 µg/mL

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

Ciprofloxacin

Figure D-9 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.8 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 8 (Continued) **Plate 7** - Colistin 2 µg/mL

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

Ciprofloxacin

Plate 8 – Colistin 4 µg/mL

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

Ciprofloxacin

Figure D-9 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.8 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1

FIC index for meropenem + colistin = 0.563

FIC index for ciprofloxacin + colistin = 1

FIC index for meropenem + ciprofloxacin + colistin = 1

Isolate No. 9 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

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/ 0/ 0	4	8	16	32	64	128	256

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

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

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

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

Ciprofloxacin

Figure D-10 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.9 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No.9 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem

32	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	4
16	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	2
8	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	1
4	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	0.5
2	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	0.25
1	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	0.12
0.5	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	0.06
0/ 0/ 0.25	4	8	16	32	64	128	256	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem

32	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	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	8/ 4/0.5	8/ 8/0.5	8/ 16/0.5	8/ 32/0.5	8/ 64/0.5	8/ 128/. 5	8/ 256/0.5
4	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	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	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/ 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	8	16	32	64	128	256

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

Meropenem

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

Ciprofloxacin

Figure D-10 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.9 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 9 (Continued) **Plate 7** - Colistin 2 µg/mL

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

Meropenem

Ciprofloxacin

Plate 8 – Colistin 4 µg/mL

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

Meropenem

Ciprofloxacin

Figure D-10 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.9 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1.063

FIC index for meropenem + colistin = 1.030

FIC index for ciprofloxacin + colistin = 1.030

FIC index for meropenem + ciprofloxacin + colistin = 1.030

Isolate No. 10 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

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/ 0/ 0	4	8	16	32	64	128	256

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

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

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

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

Ciprofloxacin

Figure D-11 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.10 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 10 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem								
64	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	4
32	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	2
16	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	1
8	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	0.5
4	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	0.25
2	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	0.12
1	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	0.06
0/ 0/ 0.25	4	8	16	32	64	128	256	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem							
64	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	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	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	8/ 4/0.5	8/ 8/0.5	8/ 16/0.5	8/ 32/0.5	8/ 64/0.5	8/ 128/. 5	8/ 256/0.5
4	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	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	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	8	16	32	64	128	256

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

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

Ciprofloxacin

Figure A-11 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.10 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 10 (Continued) **Plate 7** - Colistin 2 µg/mL

Meropenem

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

Ciprofloxacin

Plate 8 – Colistin 4 µg/mL

Meropenem

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

Ciprofloxacin

Figure A-11 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.10 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1

FIC index for meropenem + colistin = 0.563

FIC index for ciprofloxacin + colistin = 0.625

FIC index for meropenem + ciprofloxacin + colistin = 0.688

Isolate No. 11 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

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/ 0/ 0	4	8	16	32	64	128	256

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

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

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

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

Ciprofloxacin

Figure D-12 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.11 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 11 (Continued) **Plate 4** - Colistin 0.25 µg/mL Colistin alone

Meropenem								
32	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	4
16	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	2
8	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	1
4	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	0.5
2	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	0.25
1	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	0.12
0.5	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	0.06
0/ 0/ 0.25	4	8	16	32	64	128	256	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem							
32	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	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	8/ 4/0.5	8/ 8/0.5	8/ 16/0.5	8/ 32/0.5	8/ 64/0.5	8/ 128/. 5	8/ 256/0.5
4	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	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	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/ 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	8	16	32	64	128	256

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

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

Ciprofloxacin

Figure D-12 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.11 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 11 (Continued) **Plate 7** - Colistin 2 µg/mL

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

Ciprofloxacin

Plate 8 – Colistin 4 µg/mL

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

Ciprofloxacin

Figure D-12 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.11 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1.063

FIC index for meropenem + colistin = 1.060

FIC index for ciprofloxacin + colistin = 1.060

FIC index for meropenem + ciprofloxacin + colistin = 0.87

Isolate No. 12 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

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/ 0/ 0	4	8	16	32	64	128	256

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

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

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

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

Ciprofloxacin

Figure D-13 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.12 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 12 (Continued) **Plate 4** - Colistin 0.25 µg/mL Colistin alone

Meropenem

32	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	4
16	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	2
8	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	1
4	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	0.5
2	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	0.25
1	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	0.12
0.5	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	0.06
0/ 0/ 0.25	4	8	16	32	64	128	256	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem

32	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	16/ 4/0.5	16/ 8/0.5	16/ 16/0.5	16/ 32/0.5	16/ 64/0.5	16/ 128/	16/ 256/0.5
8	8/ 4/0.5	8/ 8/0.5	8/ 16/0.5	8/ 32/0.5	8/ 64/0.5	8/ 128/. 5	8/ 256/0.5
4	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	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	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/ 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	8	16	32	64	128	256

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

Meropenem

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

Ciprofloxacin

Figure D-13 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.12 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 12 (Continued) **Plate 7** - Colistin 2 µg/mL

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

Meropenem

Ciprofloxacin

Plate 8 – Colistin 4 µg/mL

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

Meropenem

Ciprofloxacin

Figure D-13 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.12 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 0.75

FIC index for meropenem + colistin = 1.060

FIC index for ciprofloxacin + colistin = 1.060

FIC index for meropenem + ciprofloxacin + colistin = 0.81

Isolate No. 13 **Plate 1**- No Colistin (0 µg/mL)

Meropenem	128	128/ 0.06	128/ 0.12	128/ 0.25	128/ 0.5	128/ 1	128/ 2	128/ 4
	64	64/ 0.06	64/ 0.12	64/ 0.25	64/ 0.5	64/ 1	64/ 2	64/ 4
	32	32/ 0.06	32/ 0.12	32/ 0.25	32/ 0.5	32/ 1	32/ 2	32/ 4
	16	16/ 0.06	16/ 0.12	16/ 0.25	16/ 0.5	16/ 1	16/ 2	16/ 4
	8	8/ 0.06	8/ 0.12	8/ 0.25	8/ 0.5	8/ 1	8/ 2	8/ 4
	4	4/ 0.06	4/ 0.12	4/ 0.25	4/ 0.5	4/ 1	4/ 2	4/ 4
	2	2/ 0.06	2/ 0.12	2/ 0.25	2/ 0.5	2/ 1	2/ 2	2/ 4
	0/ 0/ 0	0.06	0.12	0.25	0.5	1	2	4

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem	128	128/ 0.06/0.06	128/ 0.12/	128/ 0.25/0.06	128/ 0.5/0.06	128/ 1/ 0.06	128/ 2/0.06	128/ 4/0.06	128
	64	64/ 0.06/0.06	64/ 0.12/0.06	64/ 0.25/0.06	64/ 0.5/0.06	64/ 1/0.06	64/ 2/0.06	64/ 4/0.06	64
	32	32/ 0.06/0.06	32/ 0.12/0.06	32/ 0.25/0.06	32/ 0.5/0.06	32/ 1/0.06	32/ 2/0.06	32/ 4/0.06	32
	16	16/ 0.06/0.06	16/ 0.12/0.06	16/ 0.25/0.06	16/ 0.5/0.06	16/ 1/0.06	16/ 2/0.06	16/ 4/0.06	16
	8	8/ 0.06/0.06	8/ 0.12/0.06	8/ 0.25/0.06	8/ 0.5/0.06	8/ 1/0.06	8/ 2/0.06	8/ 4/0.06	8
	4	4/ 0.06/0.06	4/ 0.12/0.06	4/ 0.25/0.06	4/ 0.5/0.06	4/ 1/0.06	4/ 2/0.06	4/ 4/0.06	4
	2	2/ 0.06/0.06	2/ 0.12/0.06	2/ 0.25/0.06	2/ 0.5/0.06	2/ 1/0.06	2/ 2/0.06	2/ 4/0.06	2
	0/ 0/ 0.06	0.06	0.12	0.25	0.5	1	2	4	C

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem	128	128/ 0.06/0.12	128/ 0.12/	128/ 0.25/0.12	128/ 0.5/0.12	128/ 1/ 0.12	128/ 2/0.12	128/ 4/0.12	4
	64	64/ 0.06/0.12	64/ 0.12/0.12	64/ 0.25/0.12	64/ 0.5/0.12	64/ 1/0.12	64/ 2/0.12	64/ 4/0.12	2
	32	32/ 0.06/0.12	32/ 0.12/0.12	32/ 0.25/0.12	32/ 0.5/0.12	32/ 1/0.12	32/ 2/0.12	32/ 4/0.12	1
	16	16/ 0.06/0.12	16/ 0.12/0.12	16/ 0.25/0.12	16/ 0.5/0.12	16/ 1/0.12	16/ 2/0.12	16/ 4/0.12	0.5
	8	8/ 0.06/0.12	8/ 0.12/0.12	8/ 0.25/0.12	8/ 0.5/0.12	8/ 1/0.12	8/ 2/0.12	8/ 4/0.12	0.25
	4	4/ 0.06/0.12	4/ 0.12/0.12	4/ 0.25/0.12	4/ 0.5/0.12	4/ 1/0.12	4/ 2/0.12	4/ 4/0.12	0.12
	2	2/ 0.06/0.12	2/ 0.12/0.12	2/ 0.25/0.12	2/ 0.5/0.12	2/ 1/0.12	2/ 2/0.12	2/ 4/0.12	0.06
	0/ 0/ 0.12	0.06	0.12	0.25	0.5	1	2	4	C

Ciprofloxacin

Figure D-14 Three dimentional microdilution checkerboard result of *P. aeruginosa* isolate No.13 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 13 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem								
128	128/ 0.06/0.25	128/ 0.12/	128/ 0.25/0.25	128/ 0.5/0.25	128/ 1/ 0.25	128/ 2/0.25	128/ 4/0.25	4
64	64/ 4/0.25	64/ 0.12/0.25	64/ 0.25/0.25	64/ 0.5/0.25	64/ 1/0.25	64/ 2/0.25	64/ 4/0.25	2
32	32/ 0.06/0.25	32/ 0.12/0.25	32/ 0.25/0.25	32/ 0.5/0.25	32/ 1/0.25	32/ 2/0.25	32/ 4/0.25	1
16	16/ 0.06/0.25	16/ 0.12/0.25	16/ 0.25/0.25	16/ 0.5/0.25	16/ 1/0.25	16/ 2/0.25	16/ 4/0.25	0.5
8	8/ 0.06/0.25	8/ 0.12/0.25	8/ 0.25/0.25	8/ 0.5/0.25	8/ 1/0.25	8/ 2/0.25	8/ 4/0.25	0.25
4	4/ 0.06/0.25	4/ 0.12/0.25	4/ 0.25/0.25	4/ 0.5/0.25	4/ 1/0.25	4/ 2/0.25	4/ 4/0.25	0.12
2	2/ 0.06/0.25	2/ 0.12/0.25	2/ 0.25/0.25	2/ 0.5/0.25	2/ 1/0.25	2/ 2/0.25	2/ 4/0.25	0.06
0/ 0/ 0.25	0.06	0.12	0.25	0.5	1	2	4	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem							
128	128/ 0.06/0.5	128/ 0.12/ 0.5	128/ 0.25/0.5	128/ 0.5/0.5	128/ 1/ 0.5	128/ 2/0.5	128/ 4/0.5
64	64/ 0.06/0.5	64/ 0.12/0.5	64/ 0.25/0.5	64/ 0.5/0.5	64/ 1/0.5	64/ 2/0.5	64/ 4/0.5
32	32/ 0.06/0.5	32/ 0.12/0.5	32/ 0.25/0.5	32/ 0.5/0.5	32/ 1/0.5	32/ 2/0.5	32/ 4/0.5
16	16/ 0.06/0.5	16/ 0.12/0.5	16/ 0.25/0.5	16/ 0.5/0.5	16/ 1/0.5	16/ 2/0.5	16/ 4/0.5
8	8/ 0.06/0.5	8/ 0.12/0.5	8/ 0.25/0.5	8/ 0.5/0.5	8/ 1/0.5	8/ 2/0. 5	8/ 4/0.5
4	4/ 0.06/0.5	4/ 0.12/0.5	4/ 0.25/0.5	4/ 0.5/0.5	4/ 1/0.5	4/ 2/0.5	4/ 4/0.5
2	2/ 0.06/0.5	2/ 0.12/0.5	2/ 0.25/0.5	2/ 0.5/0.5	2/ 1/0.5	2/ 2/0.5	2/ 4/0.5
0/ 0/ 0.5	0.06	0.12	0.25	0.5	1	2	4

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

Meropenem							
128	128/ 0.06/1	128/ 0.12/ 1	128/ 0.25/1	128/ 0.5/1	128/ 1/ 1	128/ 2/1	128/ 4/1
64	64/ 0.06/1	64/ 0.12/1	64/ 0.25/1	64/ 0.5/1	64/ 1/1	64/ 2/1	64/ 4/1
32	32/ 0.06/1	32/ 0.12/1	32/ 0.25/1	32/ 0.5/1	32/ 1/1	32/ 2/1	32/ 4/1
16	16/ 0.06/1	16/ 0.12/1	16/ 0.25/1	16/ 0.5/1	16/ 1/1	16/ 2/1	16/ 4/1
8	8/ 0.06/1	8/ 0.12/1	8/ 0.25/1	8/ 0.5/1	8/ 1/1	8/ 2/1	8/ 4/1
4	4/ 0.06/1	4/ 0.12/1	4/ 0.25/1	4/ 0.5/1	4/ 1/1	4/ 2/1	4/ 4/1
2	2/ 0.06/1	2/ 0.12/1	2/ 0.25/1	2/ 0.5/1	2/ 1/1	2/ 2/1	2/ 4/1
0/ 0/ 1	0.06	0.12	0.25	0.5	1	2	4

Ciprofloxacin

Figure D-14 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.13 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 13 (Continued) **Plate 7** - Colistin 2 µg/mL

128	128/ 0.06/2	128/ 0.12/ 2	128/ 0.25/2	128/ 0.5/2	128/ 1/ 2	128/ 2/2	128/ 4/2
64	64/ 0.06/2	64/ 0.12/2	64/ 0.25/2	64/ 0.5/2	64/ 1/2	64/ 2/2	64/ 4/2
32	32/ 0.06/2	32/ 0.12/2	32/ 0.25/2	32/ 0.5/2	32/ 1/2	32/ 2/2	32/ 4/2
16	16/ 0.06/2	16/ 0.12/2	16/ 0.25/2	16/ 0.5/2	16/ 1/2	16/ 2/2	16/ 4/2
8	8/ 0.06/2	8/ 0.12/2	8/ 0.25/2	8/ 0.5/2	8/ 1/2	8/ 2/2	8/ 4/2
4	4/ 0.06/2	4/ 0.12/2	4/ 0.25/2	4/ 0.5/2	4/ 1/2	4/ 2/2	4/ 4/2
2	2/ 0.06/2	2/ 0.12/2	2/ 0.25/2	2/ 0.5/2	2/ 1/2	2/ 2/2	2/ 4/2
0/ 0/ 2	0.06	0.12	0.25	0.5	1	2	4

Meropenem

Ciprofloxacin

Plate 8 - Colistin 4 µg/mL

128	128/ 0.06/4	128/ 0.12/ 4	128/ 0.25/4	128/ 0.5/4	128/ 1/ 4	128/ 2/4	128/ 4/4
64	64/ 0.06/4	64/ 0.12/4	64/ 0.25/4	64/ 0.5/4	64/ 1/4	64/ 2/4	64/ 4/4
32	32/ 0.06/4	32/ 0.12/4	32/ 0.25/4	32/ 0.5/4	32/ 1/4	32/ 2/4	32/ 4/4
16	16/ 0.06/4	16/ 0.12/4	16/ 0.25/4	16/ 0.5/4	16/ 1/4	16/ 2/4	16/ 4/4
8	8/ 0.06/4	8/ 0.12/4	8/ 0.25/4	8/ 0.5/4	8/ 1/4	8/ 2/4	8/ 4/4
4	4/ 0.06/4	4/ 0.12/4	4/ 0.25/4	4/ 0.5/4	4/ 1/4	4/ 2/4	4/ 4/4
2	2/ 0.06/4	2/ 0.12/4	2/ 0.25/4	2/ 0.5/4	2/ 1/4	2/ 2/4	2/ 4/4
0/ 0/ 4	0.06	0.12	0.25	0.5	1	2	4

Meropenem

Ciprofloxacin

Figure D-14 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.13 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1

FIC index for meropenem + colistin = 0.563

FIC index for ciprofloxacin + colistin = 0.620

FIC index for meropenem + ciprofloxacin + colistin = 0.651

Isolate No. 14 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

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/ 0/ 0	4	8	16	32	64	128	256

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

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

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

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

Ciprofloxacin

Figure D-15 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.14 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 14 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem

64	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	4
32	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	2
16	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	1
8	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	0.5
4	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	0.25
2	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	0.12
1	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	0.06
0/ 0/ 0.25	4	8	16	32	64	128	256	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem

64	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	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	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	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	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	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	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	8	16	32	64	128	256

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

Meropenem

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

Ciprofloxacin

Figure D-15 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.14 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 14 (Continued) **Plate 7** - Colistin 2 µg/mL

Meropenem

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

Ciprofloxacin

Plate 8 – Colistin 4 µg/mL

Meropenem

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

Ciprofloxacin

Figure D-15 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.14 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1

FIC index for meropenem + colistin = 0.625

FIC index for ciprofloxacin + colistin = 0.563

FIC index for meropenem + ciprofloxacin + colistin = 0.75

Isolate No. 15 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

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/ 0/ 0	4	8	16	32	64	128	256

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

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

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

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

Ciprofloxacin

Figure D-16 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.15 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 15 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem

64	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	4
32	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	2
16	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	1
8	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	0.5
4	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	0.25
2	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	0.12
1	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	0.06
0/ 0/ 0.25	4	8	16	32	64	128	256	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem

64	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	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	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	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	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	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	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	8	16	32	64	128	256

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

Meropenem

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

Ciprofloxacin

Figure D-16 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.15 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 15 (Continued) **Plate 7** - Colistin 2 µg/mL

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

Meropenem

Ciprofloxacin

Plate 8 – Colistin 4 µg/mL

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

Meropenem

Ciprofloxacin

Figure D-16 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.15 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

.FIC index for meropenem + ciprofloxacin = 1

FIC index for meropenem + colistin = 0.563

FIC index for ciprofloxacin + colistin = 0.563

FIC index for meropenem + ciprofloxacin + colistin = 0.688

Isolate No. 16 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

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

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

64	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	64
32	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	32
16	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	16
8	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	8
4	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	4
2	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	2
1	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	1
0/ 0/ 0.06	8	16	32	64	128	256	512	C

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

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

Ciprofloxacin

Figure D-17 Three dimentional microdilution checkerboard resultsof *P. aeruginosa* isolate No.16 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 16 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem								
64	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	4
32	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	2
16	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	1
8	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	0.5
4	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	0.25
2	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	0.12
1	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	0.06
0/ 0/ 0.25	8	16	32	64	128	256	512	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem							
64	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	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	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	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	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	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	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/ 0/ 0.5	8	16	32	64	128	256	512

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

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

Ciprofloxacin

Figure D-17 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.16 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 16 (Continued) **Plate 7** - Colistin 2 µg/mL

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

Ciprofloxacin

Plate 8 – Colistin 4 µg/mL

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

Ciprofloxacin

Figure D-17 (C0ntinued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.16 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1

FIC index for meropenem + colistin = 1.030

FIC index for ciprofloxacin + colistin = 1.030

FIC index for meropenem + ciprofloxacin + colistin = 1.030

Isolate No. 17 **Plate 1**- No Colistin (0 µg/mL)

128	128/ 0.03	128/ 0.06	128/ 0.12	128/ 0.25	128/ 0.5	128/ 1	128/ 2
64	64/ 0.03	64/ 0.06	64/ 0.12	64/ 0.25	64/ 0.5	64/ 1	64/ 2
32	32/ 0.03	32/ 0.06	32/ 0.12	32/ 0.25	32/ 0.5	32/ 1	32/ 2
16	16/ 0.03	16/ 0.06	16/ 0.12	16/ 0.25	16/ 0.5	16/ 1	16/ 2
8	8/ 0.03	8/ 0.06	8/ 0.12	8/ 0.25	8/ 0.5	8/ 1	8/ 2
4	4/ 0.03	4/ 0.06	4/ 0.12	4/ 0.25	4/ 0.5	4/ 1	4/ 2
2	2/ 0.03	2/ 0.06	2/ 0.12	2/ 0.25	2/ 0.5	2/ 1	2/ 2
0/ 0/ 0	0.03	0.06	0.12	0.25	0.5	1	2

Meropenem

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

128	128/ 0.03/0.06	128/ 0.06/0.06	128/ 0.12/0.06	128/ 0.25/0.06	128/ 0.5/ 0.06	128/ 1/0.06	128/ 2/0.06	128
64	64/ 0.25/0.06	64/ 0.06/0.06	64/ 0.12/0.06	64/ 0.25/0.06	64/ 0.5/0.06	64/ 1/0.06	64/ 2/0.06	64
32	32/ 0.03/0.06	32/ 0.06/0.06	32/ 0.12/0.06	32/ 0.25/0.06	32/ 0.5/0.06	32/ 1/0.06	32/ 2/0.06	32
16	16/ 0.03/0.06	16/ 0.06/0.06	16/ 0.12/0.06	16/ 0.25/0.06	16/ 0.5/0.06	16/ 1/0.06	16/ 2/0.06	16
8	8/ 0.03/0.06	8/ 0.06/0.06	8/ 0.12/0.06	8/ 0.25/0.06	8/ 0.5/0.06	8/ 1/0.06	8/ 2/0.06	8
4	4/ 0.03/0.06	4/ 0.06/0.06	4/ 0.12/0.06	4/ 0.25/0.06	4/ 0.5/0.06	4/ 1/0.06	4/ 2/0.06	4
2	2/ 0.03/0.06	2/ 0.06/0.06	2/ 0.12/0.06	2/ 0.25/0.06	2/ 0.5/0.06	2/ 1/0.06	2/ 2/0.06	2
0/ 0/ 0.06	0.03	0.06	0.12	0.25	0.5	1	2	C

Meropenem

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

128	128/ 0.03/0.12	128/ 0.06/0.12	128/ 0.12/0.12	128/ 0.25/0.12	128/ 0.5/ 0.12	128/ 1/0.12	128/ 2/0.12	2
64	64/ 0.25/0.12	64/ 0.06/0.12	64/ 0.12/0.12	64/ 0.25/0.12	64/ 0.5/0.12	64/ 1/0.12	64/ 2/0.12	1
32	32/ 0.03/0.12	32/ 0.06/0.12	32/ 0.12/0.12	32/ 0.25/0.12	32/ 0.5/0.12	32/ 1/0.12	32/ 2/0.12	0.5
16	16/ 0.03/0.12	16/ 0.06/0.12	16/ 0.12/0.12	16/ 0.25/0.12	16/ 0.5/0.12	16/ 1/0.12	16/ 2/0.12	0.25
8	8/ 0.03/0.12	8/ 0.06/0.12	8/ 0.12/0.12	8/ 0.25/0.12	8/ 0.5/0.12	8/ 1/0.12	8/ 2/0.12	0.12
4	4/ 0.03/0.12	4/ 0.06/0.12	4/ 0.12/0.12	4/ 0.25/0.12	4/ 0.5/0.12	4/ 1/0.12	4/ 2/0.12	0.06
2	2/ 0.03/0.12	2/ 0.06/0.12	2/ 0.12/0.12	2/ 0.25/0.12	2/ 0.5/0.12	2/ 1/0.12	2/ 2/0.12	0.03
0/ 0/ 0.12	0.03	0.06	0.12	0.25	0.5	1	2	C

Meropenem

Ciprofloxacin

Figure D-18 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.17 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 17 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem								
128	128/	128/ 0.06/	128/	128/	128/ 0.5/ 0.25	128/ 1/0.25	128/ 2/0.25	4
64	64/ 0.25/0.25	64/ 0.06/0.25	64/ 0.12/0.25	64/ 0.25/0.25	64/ 0.5/0.25	64/ 1/0.25	64/ 2/0.25	2
32	32/ 0.03/0.25	32/ 0.06/0.25	32/ 0.12/0.25	32/ 0.25/0.25	32/ 0.5/0.25	32/ 1/0.25	32/ 2/0.25	1
16	16/ 0.03/0.25	16/ 0.06/0.25	16/ 0.12/0.25	16/ 0.25/0.25	16/ 0.5/0.25	16/ 1/0.25	16/ 2/0.25	0.5
8	8/ 0.03/0.25	8/ 0.06/0.25	8/ 0.12/0.25	8/ 0.25/0.25	8/ 0.5/0.25	8/ 1/0.25	8/ 2/0.25	0.25
4	4/ 0.03/0.25	4/ 0.06/0.25	4/ 0.12/0.25	4/ 0.25/0.25	4/ 0.5/0.25	4/ 1/0.25	4/ 2/0.25	0.12
2	2/ 0.03/0.25	2/ 0.06/0.25	2/ 0.12/0.25	2/ 0.25/0.25	2/ 0.5/0.25	2/ 1/0.25	2/ 2/0.25	0.06
0/ 0/ 0.25	0.03	0.06	0.12	0.25	0.5	1	2	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem							
128	128/ 0.03/0.5	128/ 0.06/ 0.5	128/ 0.12/0.5	128/ 0.25/0.5	128/ 0.5/ 0.5	128/ 1/0.5	128/ 2/0.5
64	64/ 0.25/0.5	64/ 0.06/0.5	64/ 0.12/0.5	64/ 0.25/0.5	64/ 0.5/0.5	64/ 1/0.5	64/ 2/0.5
32	32/ 0.03/0.5	32/ 0.06/0.5	32/ 0.12/0.5	32/ 0.25/0.5	32/ 0.5/0.5	32/ 1/0.5	32/ 2/0.5
16	16/ 0.03/0.5	16/ 0.06/0.5	16/ 0.12/0.5	16/ 0.25/0.5	16/ 0.5/0.5	16/ 1/0.5	16/ 2/0.5
8	8/ 0.03/0.5	8/ 0.06/0.5	8/ 0.12/0.5	8/ 0.25/0.5	8/ 0.5/0.5	8/ 1/0.5	8/ 2/0.5
4	4/ 0.03/0.5	4/ 0.06/0.5	4/ 0.12/0.5	4/ 0.25/0.5	4/ 0.5/0.5	4/ 1/0.5	4/ 2/0.5
2	2/ 0.03/0.5	2/ 0.06/0.5	2/ 0.12/0.5	2/ 0.25/0.5	2/ 0.5/0.5	2/ 1/0.5	2/ 2/0.5
0/ 0/ 0.5	0.03	0.06	0.12	0.25	0.5	1	2

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

Meropenem							
128	128/ 0.03/1	128/ 0.06/ 1	128/ 0.12/1	128/ 0.25/1	128/ 0.5/ 1	128/ 1/1	128/ 2/1
64	64/ 0.25/1	64/ 0.06/1	64/ 0.12/1	64/ 0.25/1	64/ 0.5/1	64/ 1/1	64/ 2/1
32	32/ 0.03/1	32/ 0.06/1	32/ 0.12/1	32/ 0.25/1	32/ 0.5/1	32/ 1/1	32/ 2/1
16	16/ 0.03/1	16/ 0.06/1	16/ 0.12/1	16/ 0.25/1	16/ 0.5/1	16/ 1/1	16/ 2/1
8	8/ 0.03/1	8/ 0.06/1	8/ 0.12/1	8/ 0.25/1	8/ 0.5/1	8/ 1/1	8/ 2/1
4	4/ 0.03/1	4/ 0.06/1	4/ 0.12/1	4/ 0.25/1	4/ 0.5/1	4/ 1/1	4/ 2/1
2	2/ 0.03/1	2/ 0.06/1	2/ 0.12/1	2/ 0.25/1	2/ 0.5/1	2/ 1/1	2/ 2/1
0/ 0/ 1	0.03	0.06	0.12	0.25	0.5	1	2

Ciprofloxacin

Figure D-18 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.17 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 17 (Continued) **Plate 7** - Colistin 2 µg/mL

128	128/ 0.03/2	128/ 0.06/ 2	128/ 0.12/2	128/ 0.25/2	128/ 0.5/ 2	128/ 1/2	128/ 2/2
64	64/ 0.25/2	64/ 0.06/2	64/ 0.12/2	64/ 0.25/2	64/ 0.5/2	64/ 1/2	64/ 2/2
32	32/ 0.03/2	32/ 0.06/2	32/ 0.12/2	32/ 0.25/2	32/ 0.5/2	32/ 1/2	32/ 2/2
16	16/ 0.03/2	16/ 0.06/2	16/ 0.12/2	16/ 0.25/2	16/ 0.5/2	16/ 1/2	16/ 2/2
8	8/ 0.03/2	8/ 0.06/2	8/ 0.12/2	8/ 0.25/2	8/ 0.5/2	8/ 1/2	8/ 2/2
4	4/ 0.03/2	4/ 0.06/2	4/ 0.12/2	4/ 0.25/2	4/ 0.5/2	4/ 1/2	4/ 2/2
2	2/ 0.03/2	2/ 0.06/2	2/ 0.12/2	2/ 0.25/2	2/ 0.5/2	2/ 1/2	2/ 2/2
0/ 0/ 2	0.03	0.06	0.12	0.25	0.5	1	2

Meropenem

Ciprofloxacin

Plate 8 - Colistin 4 µg/mL

128	128/ 0.03/4	128/ 0.06/ 4	128/ 0.12/4	128/ 0.25/4	128/ 0.5/ 4	128/ 1/4	128/ 2/4
64	64/ 0.25/4	64/ 0.06/4	64/ 0.12/4	64/ 0.25/4	64/ 0.5/4	64/ 1/4	64/ 2/4
32	32/ 0.03/4	32/ 0.06/4	32/ 0.12/4	32/ 0.25/4	32/ 0.5/4	32/ 1/4	32/ 2/4
16	16/ 0.03/4	16/ 0.06/4	16/ 0.12/4	16/ 0.25/4	16/ 0.5/4	16/ 1/4	16/ 2/4
8	8/ 0.03/4	8/ 0.06/4	8/ 0.12/4	8/ 0.25/4	8/ 0.5/4	8/ 1/4	8/ 2/4
4	4/ 0.03/4	4/ 0.06/4	4/ 0.12/4	4/ 0.25/4	4/ 0.5/4	4/ 1/4	4/ 2/4
2	2/ 0.03/4	2/ 0.06/4	2/ 0.12/4	2/ 0.25/4	2/ 0.5/4	2/ 1/4	2/ 2/4
0/ 0/ 4	0.03	0.06	0.12	0.25	0.5	1	2

Meropenem

Ciprofloxacin

Figure D-18 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 17 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1

FIC index for meropenem + colistin = 1.060

FIC index for ciprofloxacin + colistin = 1.060

FIC index for meropenem + ciprofloxacin + colistin = 1.060

Isolate No. 18 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

64	64/ 0.03	64/ 0.06	64/ 0.12	64/ 0.25	64/ 0.5	64/ 1	64/ 2
32	32/ 0.03	32/ 0.06	32/ 0.12	32/ 0.25	32/ 0.5	32/ 1	32/ 2
16	16/ 0.03	16/ 0.06	16/ 0.12	16/ 0.25	16/ 0.5	16/ 1	16/ 2
8	8/ 0.03	8/ 0.06	8/ 0.12	8/ 0.25	8/ 0.5	8/ 1	8/ 2
4	4/ 0.03	4/ 0.06	4/ 0.12	4/ 0.25	4/ 0.5	4/ 1	4/ 2
2	2/ 0.03	2/ 0.06	2/ 0.12	2/ 0.25	2/ 0.5	2/ 1	2/ 2
1	1/ 0.03	1/ 0.06	1/ 0.12	1/ 0.25	1/ 0.5	1/ 1	1/ 2
0/ 0/ 0	0.03	0.06	0.12	0.25	0.5	1	2

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

64	64/ 0.25/0.06	64/ 0.06/0.06	64/ 0.12/0.06	64/ 0.25/0.06	64/ 0.5/0.06	64/ 1/0.06	64/ 2/0.06	64
32	32/ 0.03/0.06	32/ 0.06/0.06	32/ 0.12/0.06	32/ 0.25/0.06	32/ 0.5/0.06	32/ 1/0.06	32/ 2/0.06	32
16	16/ 0.03/0.06	16/ 0.06/0.06	16/ 0.12/0.06	16/ 0.25/0.06	16/ 0.5/0.06	16/ 1/0.06	16/ 2/0.06	16
8	8/ 0.03/0.06	8/ 0.06/0.06	8/ 0.12/0.06	8/ 0.25/0.06	8/ 0.5/0.06	8/ 1/0.06	8/ 2/0.06	8
4	4/ 0.03/0.06	4/ 0.06/0.06	4/ 0.12/0.06	4/ 0.25/0.06	4/ 0.5/0.06	4/ 1/0.06	4/ 2/0.06	4
2	2/ 0.03/0.06	2/ 0.06/0.06	2/ 0.12/0.06	2/ 0.25/0.06	2/ 0.5/0.06	2/ 1/0.06	2/ 2/0.06	2
1	1/ 0.03/0.06	1/ 0.06/0.06	1/ 0.12/0.06	1/ 0.25/0.06	1/ 0.5/0.06	1/ 1/0.06	2/ 2/0.06	1
0/ 0/ 0.06	0.03	0.06	0.12	0.25	0.5	1	2	C

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

64	64/ 0.25/0.12	64/ 0.06/0.12	64/ 0.12/0.12	64/ 0.25/0.12	64/ 0.5/0.12	64/ 1/0.12	64/ 2/0.12	2
32	32/ 0.03/0.12	32/ 0.06/0.12	32/ 0.12/0.12	32/ 0.25/0.12	32/ 0.5/0.12	32/ 1/0.12	32/ 2/0.12	1
16	16/ 0.03/0.12	16/ 0.06/0.12	16/ 0.12/0.12	16/ 0.25/0.12	16/ 0.5/0.12	16/ 1/0.12	16/ 2/0.12	0.5
8	8/ 0.03/0.12	8/ 0.06/0.12	8/ 0.12/0.12	8/ 0.25/0.12	8/ 0.5/0.12	8/ 1/0.12	8/ 2/0.12	0.25
4	4/ 0.03/0.12	4/ 0.06/0.12	4/ 0.12/0.12	4/ 0.25/0.12	4/ 0.5/0.12	4/ 1/0.12	4/ 2/0.12	0.12
2	2/ 0.03/0.12	2/ 0.06/0.12	2/ 0.12/0.12	2/ 0.25/0.12	2/ 0.5/0.12	2/ 1/0.12	2/ 2/0.12	0.06
1	1/ 0.03/0.12	1/ 0.06/0.12	1/ 0.12/0.12	1/ 0.25/0.12	1/ 0.5/0.12	1/ 1/0.12	2/ 2/0.12	0.03
0/ 0/ 0.12	0.03	0.06	0.12	0.25	0.5	1	2	C

Ciprofloxacin

Figure D-19 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.18 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 18 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem								
64	64/ 0.25/0.25	64/ 0.06/0.25	64/ 0.12/0.25	64/ 0.25/0.25	64/ 0.5/0.25	64/ 1/0.25	64/ 2/0.25	4
32	32/ 0.03/0.25	32/ 0.06/0.25	32/ 0.12/0.25	32/ 0.25/0.25	32/ 0.5/0.25	32/ 1/0.25	32/ 2/0.25	2
16	16/ 0.03/0.25	16/ 0.06/0.25	16/ 0.12/0.25	16/ 0.25/0.25	16/ 0.5/0.25	16/ 1/0.25	16/ 2/0.25	1
8	8/ 0.03/0.25	8/ 0.06/0.25	8/ 0.12/0.25	8/ 0.25/0.25	8/ 0.5/0.25	8/ 1/0.25	8/ 2/0.25	0.5
4	4/ 0.03/0.25	4/ 0.06/0.25	4/ 0.12/0.25	4/ 0.25/0.25	4/ 0.5/0.25	4/ 1/0.25	4/ 2/0.25	0.25
64	64/ 0.25/0.25	64/ 0.06/0.25	64/ 0.12/0.25	64/ 0.25/0.25	64/ 0.5/0.25	64/ 1/0.25	64/ 2/0.25	0.12
1	1/ 0.03/0.25	1/ 0.06/0.25	1/ 0.12/0.25	1/ 0.25/0.25	1/ 0.5/0.25	1/ 1/0.25	1/ 2/0.25	0.06
0/ 0/ 25	0.03	0.06	0.12	0.25	0.5	1	2	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem							
64	64/ 0.25/0.5	64/ 0.06/0.5	64/ 0.12/0.5	64/ 0.25/0.5	64/ 0.5/0.5	64/ 1/0.5	128/ 2/
32	32/ 0.03/0.5	32/ 0.06/0.5	32/ 0.12/0.5	32/ 0.25/0.5	32/ 0.5/0.5	32/ 1/0.5	64/ 2/0.5
16	16/ 0.03/0.5	16/ 0.06/0.5	16/ 0.12/0.5	16/ 0.25/0.5	16/ 0.5/0.5	16/ 1/0.5	32/ 2/0.5
8	8/ 0.03/0.5	8/ 0.06/0.5	8/ 0.12/0.5	8/ 0.25/0.5	8/ 0.5/0.5	8/ 1/0.5	16/ 2/0.5
4	4/ 0.03/0.5	4/ 0.06/0.5	4/ 0.12/0.5	4/ 0.25/0.5	4/ 0.5/0.5	4/ 1/0.5	8/ 2/0.5
2	2/ 0.03/0.5	2/ 0.06/0.5	2/ 0.12/0.5	2/ 0.25/0.5	2/ 0.5/0.5	2/ 1/0.5	4/ 2/0.5
1	1/ 0.03/0.5	1/ 0.06/0.5	1/ 0.12/0.5	1/ 0.25/0.5	1/ 0.5/0.5	1/ 1/0.5	1/ 2/0.5
0/ 0/ 0.5	0.03	0.06	0.12	0.25	0.5	1	2

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

Meropenem							
64	64/ 0.25/1	64/ 0.06/1	64/ 0.12/1	64/ 0.25/1	64/ 0.5/1	64/ 1/1	64/ 2/1
32	32/ 0.03/1	32/ 0.06/1	32/ 0.12/1	32/ 0.25/1	32/ 0.5/1	32/ 1/1	32/ 2/1
16	16/ 0.03/1	16/ 0.06/1	16/ 0.12/1	16/ 0.25/1	16/ 0.5/1	16/ 1/1	16/ 2/1
8	8/ 0.03/1	8/ 0.06/1	8/ 0.12/1	8/ 0.25/1	8/ 0.5/	8/ 1/1	8/ 2/1
4	4/ 0.03/1	4/ 0.06/1	4/ 0.12/1	4/ 0.25/1	4/ 0.5/1	4/ 1/1	4/ 2/1
2	2/ 0.03/1	2/ 0.06/1	2/ 0.12/1	2/ 0.25/1	2/ 0.5/1	2/ 1/1	2/ 2/1
1	1/ 0.03/1	1/ 0.06/1	1/ 0.12/1	1/ 0.25/1	1/ 0.5/1	1/ 1/1	2/ 2/1
0/ 0/ 1	0.03	0.06	0.12	0.25	0.5	1	2

Ciprofloxacin

Figure D-19 (Continuedd) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.18 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 18 (Continued) **Plate 7** - Colistin 2 µg/mL

Meropenem							
64	64/ 0.25/2	64/ 0.06/2	64/ 0.12/2	64/ 0.25/	64/ 0.5/2	64/ 1/2	64/ 2/2
32	32/ 0.03/2	32/ 0.06/2	32/ 0.12/2	32/ 0.25/	32/ 0.5/2	32/ 1/2	32/ 2/2
16	16/ 0.03/2	16/ 0.06/2	16/ 0.12/2	16/ 0.25/	16/ 0.5/2	16/ 1/2	16/ 2/2
8	8/ 0.03/2	8/ 0.06/2	8/ 0.12/2	8/ 0.25/	8/ 0.5/2	8/ 1/2	8/ 2/2
4	4/ 0.03/2	4/ 0.06/2	4/ 0.12/2	4/ 0.25/	4/ 0.5/2	4/ 1/2	4/ 2/2
2	2/ 0.03/2	2/ 0.06/2	2/ 0.12/2	2/ 0.25/	2/ 0.5/2	2/ 1/2	2/ 2/2
1	1/ 0.03/2	1/ 0.06/2	1/ 0.12/2	1/ 0.25/2	1/ 0.5/2	1/ 1/2	1/ 2/2
0/ 0/ 2	0.03	0.06	0.12	0.25	0.5	1	2

Ciprofloxacin

Plate 8 - Colistin 4 µg/mL

Meropenem							
64	64/ 0.25/4	64/ 0.06/4	64/ 0.12/4	64/ 0.25/4	64/ 0.5/4	64/ 1/4	64/ 2/4
32	32/ 0.03/4	32/ 0.06/4	32/ 0.12/4	32/ 0.25/4	32/ 0.5/4	32/ 1/4	32/ 2/4
16	16/ 0.03/4	16/ 0.06/4	16/ 0.12/4	16/ 0.25/4	16/ 0.5/4	16/ 1/4	16/ 2/4
8	8/ 0.03/4	8/ 0.06/4	8/ 0.12/4	8/ 0.25/4	8/ 0.5/4	8/ 1/4	8/ 2/4
4	4/ 0.03/4	4/ 0.06/4	4/ 0.12/4	4/ 0.25/4	4/ 0.5/4	4/ 1/4	4/ 2/4
2	2/ 0.03/4	2/ 0.06/4	2/ 0.12/4	2/ 0.25/4	2/ 0.5/4	2/ 1/4	2/ 2/4
1	1/ 0.03/4	1/ 0.06/4	1/ 0.12/4	1/ 0.25/4	1/ 0.5/4	1/ 1/4	2/ 2/4
0/ 0/ 4	0.03	0.06	0.12	0.25	0.5	1	2

Ciprofloxacin

Figure D-19 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 18 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1

FIC index for meropenem + colistin = 1

FIC index for ciprofloxacin + colistin = 1.030

FIC index for meropenem + ciprofloxacin + colistin = 1.030

Isolate No. 19 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

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

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

64	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	64
32	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	32
16	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	16
8	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	8
4	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	4
2	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	2
1	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	1
0/ 0/ 0.06	8	16	32	64	128	256	512	C

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

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

Ciprofloxacin

Figure D-20 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.19 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 19 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem

64	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	4
32	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	2
16	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	1
8	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	0.5
4	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	0.25
2	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	0.12
1	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	0.06
0/ 0/ 0.25	8	16	32	64	128	256	512	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem

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

Ciprofloxacin

Plate 6 - Colistin 1 µg/mlL

Meropenem

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

Ciprofloxacin

Figure D-20 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.19 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 19 (Continued) **Plate 7** - Colistin 2 µg/mL

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

Ciprofloxacin

Plate 8 – Colistin 4 µg/mL

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

Ciprofloxacin

Figure D-20 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.19 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 0.75

FIC index for meropenem + colistin = 1.060

FIC index for ciprofloxacin + colistin = 1.060

FIC index for meropenem + ciprofloxacin + colistin = 0.81

Isolate No. 20 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

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/ 0/ 0	4	8	16	32	64	128	256

Ciprofloxacin

Plate 2 - Colistin 0.12 µg/mL

Meropenem alone

Meropenem

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

Ciprofloxacin

Plate 3 - Colistin 0.25 µg/mL

Ciprofloxacin alone

Meropenem

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

Ciprofloxacin

Figure D-21 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 20 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 20 (Continued) **Plate 4** - Colistin 0.5 µg/mL

Colistin alone

Meropenem								
64	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	8
32	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	4
16	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	2
8	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	1
4	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	0.5
2	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	0.25
1	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	0.12
0/ 0/ 0.5	4	8	16	32	64	128	256	C

Ciprofloxacin

Plate 5 - Colistin 1 µg/mL

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

Ciprofloxacin

Plate 6 - Colistin 2 µg/mL

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

Ciprofloxacin

Figure D-21 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.20 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 20 (Continued) **Plate 7** - Colistin 4 µg/mL

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

Ciprofloxacin

Plate 8 – Colistin 8 µg/mL

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

Ciprofloxacin

Figure D-21 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.20 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1.063

FIC index for meropenem + colistin = 1.060

FIC index for ciprofloxacin + colistin = 0.75

FIC index for meropenem + ciprofloxacin + colistin = 1.060

Isolate No. 21 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

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/ 0/ 0	8	16	32	64	128	256	512

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

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

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

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

Ciprofloxacin

Figure D-22 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.21 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 21 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem

128	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	4
64	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	2
32	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	1
16	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	0.5
8	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	0.25
4	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	0.12
2	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	0.06
0/ 0/ 0.25	8	16	32	64	128	256	512	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem

128	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	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	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	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	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	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	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	16	32	64	128	256	512

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

Meropenem

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

Ciprofloxacin

Figure D-22 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.21 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 21 (Continued) **Plate 7** - Colistin 2 µg/mL

Meropenem							
128	128/ 8/2	128/ 16/2	128/ 32/2	128/ 64/2	128/ 128/2	128/256/2	128/512/2
64	64/ 8/2	64/ 16/2	64/ 32/2	64/ 64/2	64/ 128/2	64/256/2	64/512/2
32	32/ 8/2	32/ 16/2	32/ 32/2	32/ 64/2	32/ 128/2	32/256/2	32/512/2
16	16/ 8/2	16/ 16/2	16/ 32/2	16/ 64/2	16/ 128/2	16/ 256/2	16/ 512/2
8	8/ 8/2	8/ 16/2	8/ 32/2	8/ 64/2	8/ 128/2	8/ 256/2	8/ 512/2
4	4/ 8/2	4/ 16/2	4/ 32/2	4/ 64/2	4/ 128/2	4/ 256/2	4/ 512/2
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	16	32	64	128	256	512

Ciprofloxacin

Plate 8 – Colistin 4 µg/mL

Meropenem							
128	128/ 8/4	128/ 16/4	128/ 32/4	128/ 64/4	128/ 128/4	128/256/4	128/512/4
64	64/ 8/4	64/ 16/4	64/ 32/4	64/ 64/4	64/ 128/4	64/256/4	64/512/4
32	32/ 8/4	32/ 16/4	32/ 32/4	32/ 64/4	32/ 128/4	32/256/4	32/512/4
16	16/ 8/4	16/ 16/4	16/ 32/4	16/ 64/4	16/ 128/4	16/ 256/4	16/ 512/4
8	8/ 8/4	8/ 16/4	8/ 32/4	8/ 64/4	8/ 128/4	8/ 256/4	8/ 512/4
4	4/ 8/4	4/ 16/4	4/ 32/4	4/ 64/4	4/ 128/4	4/ 256/4	4/ 512/4
2	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	16	32	64	128	256	512

Ciprofloxacin

Figure D-22 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 21 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1

FIC index for meropenem + colistin = 0.75

FIC index for ciprofloxacin + colistin = 0.75

FIC index for meropenem + ciprofloxacin + colistin = 1

Isolate No. 22 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

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/ 0/ 0	4	8	16	32	64	128	256

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

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

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

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

Ciprofloxacin

Figure D-23 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 22 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 22 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem								
64	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	4
32	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	2
16	16/ 4/0.25	16/ 8/0.25	16/ 16/0.25	16/ 320.25	16/ 64/0.25	16/ 128/0.25	16/ 256/0.25	1
8	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	0.5
4	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	0.25
2	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	0.12
1	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	0.06
0/ 0/ 0.25	4	8	16	32	64	128	256	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem							
64	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	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	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	8/ 4/0.5	8/ 8/0.5	8/ 16/0.5	8/ 32/0.5	8/ 64/0.5	8/ 128/. 5	8/ 256/0.5
4	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	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	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	8	16	32	64	128	256

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

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

Ciprofloxacin

Figure D-23 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 22 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 22 (Continued) **Plate 7** - Colistin 2 µg/mL

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

Ciprofloxacin

Plate 8 – Colistin 4 µg/mL

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

Ciprofloxacin

Figure D-23 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 22 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1

FIC index for meropenem + colistin = 0.75

FIC index for ciprofloxacin + colistin = 0.75

FIC index for meropenem + ciprofloxacin + colistin = 1.030

Isolate No. 23 **Plate 1**- No Colistin (0 µg/mL)

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

Meropenem

Ciprofloxacin

Plate 2 - Colistin 0.12 µg/mL Meropenem alone

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

Meropenem

Ciprofloxacin

Plate 3 - Colistin 0.25 µg/mL Ciprofloxacin alone

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

Meropenem

Ciprofloxacin

Figure D-24 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 23 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 23 (Continued) **Plate 4** - Colistin 0.5 µg/mL

Colistin alone

Meropenem								
64	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	8
32	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	4
16	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	2
8	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	1
4	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	0.5
2	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	0.25
1	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	0.12
0/ 0/ 0.5	8	16	32	64	128	256	512	C

Ciprofloxacin

Plate 5 - Colistin 1 µg/mL

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

Ciprofloxacin

Plate 6 - Colistin 2 µg/mL

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

Ciprofloxacin

Figure D-24 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 23 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 23 (Continued) **Plate 7** - Colistin 4 µg/mL

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

Ciprofloxacin

Plate 8 – Colistin 8 µg/mL

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

Ciprofloxacin

Figure D-24 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.23 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1

FIC index for meropenem + colistin = 1.060

FIC index for ciprofloxacin + colistin = 1.060

FIC index for meropenem + ciprofloxacin + colistin = 1.060

Isolate No. 24 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

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

Ciprofloxacin

Plate 2 - Colistin 0.03 µg/mL

Meropenem alone

Meropenem

128	128/ 4/0.03	128/ 8/ 0.03	128/ 16/0.03	128/ 32/0.03	128/ 64/ 0.03	128/ 1280.03	128/ 256/0.03	128
64	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	64
32	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	32
16	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	16
8	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	8
4	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	4
2	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	2
0/ 0/ 0.03	4	8	16	32	64	128	256	C

Ciprofloxacin

Plate 3 - Colistin 0.06 µg/mL

Ciprofloxacin alone

Meropenem

128	128/ 4/0.06	128/ 8/ 0.06	128/ 16/0.06	128/ 32/0.06	128/ 64/ 0.06	128/ 1280.06	128/ 256/0.06	256
64	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	128
32	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	64
16	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	32
8	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	16
4	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	8
2	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	4
0/ 0/ 0.06	4	8	16	32	64	128	256	C

Ciprofloxacin

Figure D-25 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 24 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 24 (Continued) **Plate 4** - Colistin 0.12 µg/mL

Colistin alone

Meropenem								
128	128/ 4/0.12	128/ 8/ 0.12	128/ 16/0.12	128/ 32/0.12	128/ 64/ 0.12	128/ 1280.12	128/ 256/0.12	2
64	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	1
32	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	0.5
16	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	0.25
8	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	0.12
4	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	0.06
2	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	0.03
0/ 0/ 0.12	4	8	16	32	64	128	256	C

Ciprofloxacin

Plate 5 - Colistin 0.25 µg/mL

Meropenem							
128	128/ 4/0.25	128/ 8/ 0.25	128/ 16/0.25	128/ 32/0.25	128/ 64/ 0.25	128/ 1280.25	128/ 256/0.25
64	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	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	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	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	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	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	8	16	32	64	128	256

Ciprofloxacin

Plate 6 - Colistin 0.5 µg/mL

Meropenem							
128	128/ 4/0.5	128/ 8/ 0.5	128/ 16/0.5	128/ 32/0.5	128/ 64/ 0.5	128/ 1280.5	128/ 256/0.5
64	64/ 4/0.5	64/ 8/0.5	64/ 16/0.25	64/ 32/0.25	64/ 64/0.25	64/ 128/0.25	64/ 256/0.25
32	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	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	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	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	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	8	16	32	64	128	256

Ciprofloxacin

Figure D-25 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 24 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 24 (Continued) **Plate 7** - Colistin 1 µg/mL

128	128/ 4/1	128/ 8/ 1	128/ 16/1	128/ 32/1	128/ 64/ 1	128/ 1281	128/ 256/1
64	64/ 4/1	64/ 8/1	64/ 16/1	64/ 32/1	64/ 64/1	64/ 128/1	64/ 256/1
32	32/ 4/1	32/ 8/1	32/ 16/1	32/ 32/1	32/ 64/1	32/ 128/1	32/256/1
16	16/ 4/1	16/ 8/1	16/ 16/1	16/ 32/1	16/ 64/1	16/ 128/1	16/ 256/1
8	8/ 4/1	8/ 8/1	8/ 16/1	8/ 32/1	8/ 64/1	8/ 128/1	8/ 256/1
4	4/ 4/1	4/ 8/1	4/ 16/1	4/ 32/1	4/ 64/1	4/ 128/1	4/ 256/1
2	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	8	16	32	64	128	256

Meropenem

Ciprofloxacin

Plate 8 – Colistin 2 µg/mL

128	128/ 4/2	128/ 8/ 2	128/ 16/2	128/ 32/2	128/ 64/ 2	128/ 1282	128/ 256/2
64	64/ 4/2	64/ 8/2	64/ 16/2	64/ 32/2	64/ 64/2	64/ 128/2	64/ 256/2
32	32/ 4/2	32/ 8/2	32/ 16/2	32/ 32/2	32/ 64/2	32/ 128/2	32/256/2
16	16/ 4/2	16/ 8/2	16/ 16/2	16/ 32/2	16/ 64/2	16/ 128/2	16/ 256/2
8	8/ 4/2	8/ 8/2	8/ 16/2	8/ 32/2	8/ 64/2	8/ 128/2	8/ 256/2
4	4/ 4/2	4/ 8/2	4/ 16/2	4/ 32/2	4/ 64/2	4/ 128/2	4/ 256/2
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	8	16	32	64	128	256

Meropenem

Ciprofloxacin

Figure D-25 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 24 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1.125

FIC index for meropenem + colistin = 0.75

FIC index for ciprofloxacin + colistin = 0.75

FIC index for meropenem + ciprofloxacin + colistin = 0.78

Isolate No. 25 **Plate 1**- No Colistin (0 µg/mL)

Meropenem	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
	1	1/ 8	1/ 16	1/ 32	1/ 64	1/ 128	1/ 256	1/ 512
	0/ 0/ 0	8	16	32	64	128	256	512

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL Meropenem alone

Meropenem	64	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	64
	32	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	32
	16	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	16
	8	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	8
	4	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	4
	2	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	2
	1	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	1
	0/ 0/ 0.06	8	16	32	64	128	256	512	C

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL Ciprofloxacin alone

Meropenem	64	64/ 8/0.12	64/ 16/0.12	64/ 32/0.12	64/ 64/0.12	64/ 128/0.12	64/256/0.12	64/256/0.12	512
	32	32/ 8/0.12	32/ 16/0.12	32/ 32/0.12	32/ 640.12	32/ 128/0.12	32/256/0.12	32/256/0.12	256
	16	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/ 256/0.12	128
	8	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/ 256/0.12	64
	4	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/ 256/0.12	32
	2	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/ 256/0.12	16
	1	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/ 256/0.12	8
	0/ 0/ 0.12	8	16	32	64	128	256	512	C

Ciprofloxacin

Figure D-26 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 25 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 25 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem

64	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	4
32	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	2
16	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	1
8	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	0.5
4	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	0.25
2	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	0.12
1	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	0.06
0/ 0/ 0.25	8	16	32	64	128	256	512	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem

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

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

Meropenem

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

Ciprofloxacin

Figure D-26 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 25 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 25 (Continued) **Plate 7** - Colistin 2 µg/mL

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

Ciprofloxacin

Plate 8 – Colistin 4 µg/mL

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

Ciprofloxacin

Figure D-26 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate no. 25 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1

FIC index for meropenem + colistin = 1.250

FIC index for ciprofloxacin + colistin = 1

FIC index for meropenem + ciprofloxacin + colistin = 1.030

Isolate No. 26 **Plate 1**- No Colistin (0 µg/mL)

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/ 0/ 0	8	16	32	64	128	256	512

Meropenem

Ciprofloxacin

Plate 2 - Colistin 0.12 µg/mL

Meropenem alone

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

Meropenem

Ciprofloxacin

Plate 3 - Colistin 0.25 µg/mL

Ciprofloxacin alone

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

Meropenem

Ciprofloxacin

Figure D-27 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 26 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 26 (Continued) **Plate 4** - Colistin 0.5 µg/mL

Colistin alone

Meropenem								
128	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	8
64	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	4
32	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	2
16	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	1
8	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	0.5
4	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	0.25
2	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	0.12
0/ 0/ 0.5	8	16	32	64	128	256	512	C

Ciprofloxacin

Plate 5 - Colistin 1 µg/mL

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

Ciprofloxacin

Plate 6 - Colistin 2 µg/mL

Meropenem							
128	128/ 8/2	128/ 16/2	128/ 32/2	128/ 64/2	128/ 128/2	128/256/2	128/512/2
64	64/ 8/2	64/ 16/2	64/ 32/2	64/ 64/2	64/ 128/2	64/256/2	64/512/2
32	32/ 8/2	32/ 16/2	32/ 32/2	32/ 64/2	32/ 128/2	32/256/2	32/512/2
16	16/ 8/2	16/ 16/2	16/ 32/2	16/ 64/2	16/ 128/2	16/ 256/2	16/ 512/2
8	8/ 8/2	8/ 16/2	8/ 32/2	8/ 64/2	8/ 128/2	8/ 256/2	8/ 512/2
4	4/ 8/2	4/ 16/2	4/ 32/2	4/ 64/2	4/ 128/2	4/ 256/2	4/ 512/2
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	16	32	64	128	256	512

Ciprofloxacin

Figure D-27 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 26 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 26 (Continued) **Plate 7** - Colistin 4 µg/mL

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

Meropenem

Ciprofloxacin

Plate 8 – Colistin 8 µg/mL

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

Meropenem

Ciprofloxacin

Figure D-27 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 26 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1.063

FIC index for meropenem + colistin = 0.625

FIC index for ciprofloxacin + colistin = 0.625

FIC index for meropenem + ciprofloxacin + colistin = 0.75

Isolate No. 27 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

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/ 0/ 0	4	8	16	32	64	128	256

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

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

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

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

Ciprofloxacin

Figure D-28 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 26 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 27 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem								
64	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	4
32	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	2
16	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	1
8	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	0.5
4	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	0.25
2	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	0.12
1	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	0.06
0/ 0/ 0.25	4	8	16	32	64	128	256	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem							
64	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	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	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	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	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	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	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	8	16	32	64	128	256

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

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

Ciprofloxacin

Figure D-28 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 27 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 27 (Continued) **Plate 7** - Colistin 2 μg/mL

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

Ciprofloxacin

Plate 8 – Colistin 4 μg/mL

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

Ciprofloxacin

Figure D-28 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 27 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 1

FIC index for meropenem + colistin = 1

FIC index for ciprofloxacin + colistin = 1.030

FIC index for meropenem + ciprofloxacin + colistin = 1.030

Isolate No. 28 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

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

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

64	64/ 1/0.06	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
32	32/ 1/0.06	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
16	16/ 1/0.06	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
8	6/ 1/0.06	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
4	4/ 1/0.06	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
2	2/ 1/0.06	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
1	1/ 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
0/ 0/ 0.06	1	2	4	8	16	32	64	C

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

64	64/ 1/0.12	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
32	32/ 1/0.12	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
16	16/ 1/0.12	816/ 2/0.12	16/ 4/0.12	16/ 8/0.12	16/ 16/0.12	16/ 32/0.12	16/ 64/0.12	16
8	6/ 1/0.12	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
4	4/ 1/0.12	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
2	2/ 1/0.12	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
1	1/ 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
0/ 0/ 0.12	1	2	4	8	16	32	64	C

Ciprofloxacin

Figure D-29 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 28 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate NO. 28 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem								
64	64/ 1/0.25	64/ 2/0.25	64/ 4/0.25	64/ 8/ 0.25	64/ 16/0.25	64/ 32/0.25	64/ 64/0.25	4
32	32/ 1/0.25	32/ 2/0.25	32/ 4/0.25	32/ 8/0.25	32/ 16/0.25	32/ 32/0.25	32/ 64/0.25	2
16	16/ 1/0.25	816/ 2/0.25	16/ 4/0.25	16/ 8/0.25	16/ 16/0.25	16/ 32/0.25	16/ 64/0.25	1
8	6/ 1/0.25	8/ 2/0.25	8/ 4/0.25	8/ 8/0.25	8/ 16/0.25	8/ 32/0.25	8/ 64/0.25	0.5
4	4/ 1/0.25	4/ 2/0.25	4/ 4/0.25	4/ 8/0.25	4/ 16/0.25	4/ 32/0.25	4/ 64/0.25	0.25
2	2/ 1/0.25	2/ 2/0.25	2/ 4/0.25	2/ 8/0.25	2/ 16/0.25	2/ 32/0.25	2/ 64/0.25	0.12
1	1/ 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	0.06
0/ 0/ 0.25	1	2	4	8	16	32	64	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem							
64	64/ 1/ 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
32	32/ 1/0.5	32/ 2/0.5	32/ 4/0.5	32/ 8/0.5	32/ 16/0.5	32/ 32/0.5	32/ 64/0.5
16	16/ 1/0.5	816/ 2/0.5	16/ 4/0.5	16/ 8/0.5	16/ 16/0.5	16/ 32/0.5	16/ 64/0.5
8	8/ 1/0.5	8/ 2/0.5	8/ 4/0.5	8/ 8/0.5	8/ 16/0.5	8/ 32/0.5	8/ 64/0.5
4	4/ 1/0.5	4/ 2/0.5	4/ 4/0.5	4/ 8/0.5	4/ 16/0.5	4/ 32/0.5	4/ 64/0.5
2	2/ 1/0.5	2/ 2/0.5	2/ 4/0.5	2/ 8/0.5	2/ 16/0.5	2/ 32/0.5	2/ 64/0.5
1	1/ 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
0/ 0/ 0.5	1	2	4	8	16	32	64

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

Meropenem							
64	64/ 1/ 1	64/ 2/1	64/ 4/1	64/ 8/ 1	64/ 16/1	64/ 32/1	64/ 64/1
32	32/ 1/1	32/ 2/1	32/ 4/1	32/ 8/1	32/ 16/1	32/ 32/1	32/ 64/1
16	16/ 1/1	816/ 2/1	16/ 4/1	16/ 8/1	16/ 16/1	16/ 32/1	16/ 64/1
8	8/ 1/1	8/ 2/1	8/ 4/1	8/ 8/1	8/ 16/1	8/ 32/1	8/ 64/1
4	4/ 1/1	4/ 2/1	4/ 4/1	4/ 8/1	4/ 16/1	4/ 32/1	4/ 64/1
2	2/ 1/1	2/ 2/1	2/ 4/1	2/ 8/1	2/ 16/1	2/ 32/1	2/ 64/1
1	1/ 1/1	1/ 2/1	1/ 4/1	1/ 8/1	1/ 16/1	1/ 32/1	1/ 64/1
0/ 0/ 1	1	2	4	8	16	32	64

Ciprofloxacin

Figure D-29 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 28 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 28 (Continued) **Plate 7** - Colistin 2 µg/mL

64	64/ 1/ 2	64/ 2/2	64/ 4/2	64/ 8/ 2	64/ 16/2	64/ 32/2	64/ 64/2
32	32/ 1/2	32/ 2/2	32/ 4/2	32/ 8/2	32/ 16/2	32/ 32/2	32/ 64/2
16	16/ 1/2	16/ 2/2	16/ 4/2	16/ 8/2	16/ 16/2	16/ 32/2	16/ 64/2
8	8/ 1/2	8/ 2/2	8/ 4/2	8/ 8/2	8/ 16/2	8/ 32/2	8/ 64/2
4	4/ 1/2	4/ 2/2	4/ 4/2	4/ 8/2	4/ 16/2	4/ 32/2	4/ 64/2
2	2/ 1/2	2/ 2/2	2/ 4/2	2/ 8/2	2/ 16/2	2/ 32/2	2/ 64/2
1	1/ 1/2	1/ 2/2	1/ 4/2	1/ 8/2	1/ 16/2	1/ 32/2	1/ 64/2
0/ 0/ 02	1	2	4	8	16	32	64

Ciprofloxacin

Plate 8 – Colistin 4 µg/mL

64	64/ 1/ 4	64/ 2/4	64/ 4/4	64/ 8/ 4	64/ 16/4	64/ 32/4	64/ 64/4
32	32/ 1/4	32/ 2/4	32/ 4/4	32/ 8/4	32/ 16/4	32/ 32/4	32/ 64/4
16	16/ 1/4	816/ 2/4	16/ 4/4	16/ 8/4	16/ 16/4	16/ 32/4	16/ 64/4
8	8/ 1/4	8/ 2/4	8/ 4/4	8/ 8/4	8/ 16/4	8/ 32/4	8/ 64/4
4	4/ 1/4	4/ 2/4	4/ 4/4	4/ 8/4	4/ 16/4	4/ 32/4	4/ 64/4
2	2/ 1/4	2/ 2/4	2/ 4/4	2/ 8/4	2/ 16/4	2/ 32/4	2/ 64/4
1	1/ 1/4	1/ 2/4	1/ 4/4	1/ 8/4	1/ 16/4	1/ 32/4	1/ 64/4
0/ 0/ 4	1	2	4	8	16	32	64

Ciprofloxacin

Figure D-29 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 28 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 0.75

FIC index for meropenem + colistin = 1.030

FIC index for ciprofloxacin + colistin = 1

FIC index for meropenem + ciprofloxacin + colistin = 0.78

Isolate No. 29 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

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/ 0/ 0	8	16	32	64	128	256	512

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

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

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

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

Ciprofloxacin

Figure D-30 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 29 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 29 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem								
128	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	4
64	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	2
32	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	1
16	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	0.5
8	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	0.25
4	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	0.12
2	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	0.06
0/ 0/ 0.25	8	16	32	64	128	256	512	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem							
128	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	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	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	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	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	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	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	16	32	64	128	256	512

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

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

Ciprofloxacin

Figure D-30 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 29 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 29 (Continued) **Plate 7** - Colistin 2 µg/mL

Meropenem							
128	128/ 8/2	128/ 16/2	128/ 32/2	128/ 64/2	128/ 128/2	128/256/2	128/512/2
64	64/ 8/2	64/ 16/2	64/ 32/2	64/ 64/2	64/ 128/2	64/256/2	64/512/2
32	32/ 8/2	32/ 16/2	32/ 32/2	32/ 64/2	32/ 128/2	32/256/2	32/512/2
16	16/ 8/2	16/ 16/2	16/ 32/2	16/ 64/2	16/ 128/2	16/ 256/2	16/ 512/2
8	8/ 8/2	8/ 16/2	8/ 32/2	8/ 64/2	8/ 128/2	8/ 256/2	8/ 512/2
4	4/ 8/2	4/ 16/2	4/ 32/2	4/ 64/2	4/ 128/2	4/ 256/2	4/ 512/2
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	16	32	64	128	256	512

Ciprofloxacin

Plate 8 – Colistin 4 µg/mL

Meropenem							
128	128/ 8/4	128/ 16/4	128/ 32/4	128/ 64/4	128/ 128/4	128/256/4	128/512/4
64	64/ 8/4	64/ 16/4	64/ 32/4	64/ 64/4	64/ 128/4	64/256/4	64/512/4
32	32/ 8/4	32/ 16/4	32/ 32/4	32/ 64/4	32/ 128/	32/256/4	32/512/4
16	16/ 8/4	16/ 16/4	16/ 32/4	16/ 64/4	16/ 128/4	16/ 256/4	16/ 512/4
8	8/ 8/4	8/ 16/4	8/ 32/4	8/ 64/4	8/ 128/4	8/ 256/4	8/ 512/4
4	4/ 8/4	4/ 16/4	4/ 32/4	4/ 64/4	4/ 128/4	4/ 256/4	4/ 512/4
2	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	16	32	64	128	256	512

Ciprofloxacin

Figure D-30 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No.29 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 0.75

FIC index for meropenem + colistin = 1

FIC index for ciprofloxacin + colistin = 1

FIC index for meropenem + ciprofloxacin + colistin = 0.688

Isolate No. 30 **Plate 1**- No Colistin (0 µg/mL)

Meropenem

128	128/ 0.25	128/ 0.5	128/ 1	128/ 2	128/ 4	128/ 8	128/ 16
64	64/ 0.25	64/ 0.5	64/ 1	64/ 2	64/ 4	64/ 8	64/ 16
32	32/ 0.25	32/ 0.5	32/ 1	32/ 2	32/ 4	32/ 8	32/ 16
16	16/ 0.25	16/ 0.5	16/ 1	16/ 2	16/ 4	16/ 8	16/ 16
8	8/ 0.25	8/ 0.5	8/ 1	8/ 2	8/ 4	8/ 8	8/ 16
4	4/ 0.25	4/ 0.5	4/ 1	4/ 2	4/ 4	4/ 8	4/ 16
2	2/ 0.25	2/ 0.5	2/ 1	2/ 2	2/ 4	2/ 8	2/ 16
C	0.25	0.5	1	2	4	8	16

Ciprofloxacin

Plate 2 - Colistin 0.06 µg/mL

Meropenem alone

Meropenem

128	128/ 0.25/0.06	128/ 0.5/ 0.06	128/ 1/0.06	128/ 2/0.06	128/ 4/ 0.06	128/ 8/0.06	128/ 16/0.06	128
64	64/ 0.25/0.06	64/ 0.5/0.06	64/ 1/0.06	64/ 2/0.06	64/ 4/0.06	64/ 8/0.06	64/ 16/0.06	64
32	32/ 0.25/0.06	32/ 0.5/0.06	32/ 1/0.06	32/ 2/0.06	32/ 4/0.06	32/ 8/0.06	32/ 16/0.06	32
16	16/ 0.25/0.06	16/ 0.5/0.06	16/ 1/0.06	16/ 2/0.06	16/ 4/0.06	16/ 8/0.06	16/ 16/0.06	16
8	8/ 0.25/0.06	8/ 0.5/0.06	8/ 1/0.06	8/ 2/0.06	8/ 4/0.06	8/ 8/0.06	8/ 16/0.06	8
4	4/ 0.25/0.06	4/ 0.5/0.06	4/ 1/0.06	4/ 2/0.06	4/ 4/0.06	4/ 8/0.06	4/ 16/0.06	4
2	2/ 0.25/0.06	2/ 0.5/0.06	2/ 1/0.06	2/ 2/0.06	2/ 4/0.06	2/ 2/80.06	2/ 16/0.06	2
C	0.25	0.5	1	2	4	8	16	C

Ciprofloxacin

Plate 3 - Colistin 0.12 µg/mL

Ciprofloxacin alone

Meropenem

128	128/	128/ 0.5/ 0.12	128/ 1/0.12	128/ 2/0.12	128/ 4/ 0.12	128/ 8/0.12	128/ 16/0.12	16
64	64/ 0.25/0.12	64/ 0.5/0.12	64/ 1/0.12	64/ 2/0.12	64/ 4/0.12	64/ 8/0.12	64/ 16/0.12	8
32	32/ 0.25/0.12	32/ 0.5/0.12	32/ 1/0.12	32/ 2/0.12	32/ 4/0.12	32/ 8/0.12	32/ 16/0.12	4
16	16/ 0.25/0.12	16/ 0.5/0.12	16/ 1/0.12	16/ 2/0.12	16/ 4/0.12	16/ 8/0.12	16/ 16/0.12	2
8	8/ 0.25/0.12	8/ 0.5/0.12	8/ 1/0.12	8/ 2/0.12	8/ 4/0.12	8/ 8/0.12	8/ 16/0.12	1
4	4/ 0.25/0.12	4/ 0.5/0.12	4/ 1/0.12	4/ 2/0.12	4/ 4/0.12	4/ 8/0.12	4/ 16/0.12	0.5
2	2/ 0.25/0.12	2/ 0.5/0.12	2/ 1/0.12	2/ 2/0.12	2/ 4/0.12	2/ 2/0.12	2/ 16/0.12	0.25
C	0.25	0.5	1	2	4	8	16	C

Ciprofloxacin

Figure D-31 Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 30 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 30 (Continued) **Plate 4** - Colistin 0.25 µg/mL

Colistin alone

Meropenem

128	128/ 0.25/0.25	128/ 0.5/ 0.25	128/ 1/0.25	128/ 2/0.25	128/ 4/ 0.25	128/ 8/0.25	128/ 16/0.25	4
64	64/ 0.25/0.25	64/ 0.5/0.25	64/ 1/0.25	64/ 2/0.25	64/ 4/0.25	64/ 8/0.25	64/ 16/0.25	2
32	32/ 0.25/0.25	32/ 0.5/0.25	32/ 1/0.25	32/ 2/0.25	32/ 4/0.25	32/ 8/0.25	32/ 16/0.25	1
16	16/ 0.25/0.25	16/ 0.5/0.25	16/ 1/0.25	16/ 2/0.25	16/ 4/0.25	16/ 8/0.25	16/ 16/0.25	0.5
8	8/ 0.25/0.25	8/ 0.5/0.25	8/ 1/0.25	8/ 2/0.25	8/ 4/0.25	8/ 8/0.25	8/ 16/0.25	0.25
4	4/ 0.25/0.25	4/ 0.5/0.25	4/ 1/0.25	4/ 2/0.25	4/ 4/0.25	4/ 8/0.25	4/ 16/0.25	0.12
2	2/ 0.25/0.25	2/ 0.5/0.25	2/ 1/0.25	2/ 2/0.25	2/ 4/0.25	2/ 2/0.25	2/ 16/0.25	0.06
0/ 0/ 0.25	0.25	0.5	1	2	4	8	16	C

Ciprofloxacin

Plate 5 - Colistin 0.5 µg/mL

Meropenem

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

Ciprofloxacin

Plate 6 - Colistin 1 µg/mL

Meropenem

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

Ciprofloxacin

Figure D-31 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 30 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

Isolate No. 30 (Continued) **Plate 7** - Colistin 2 µg/mL

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

Meropenem

Ciprofloxacin

Plate 8 - Colistin 4 µg/mL

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

Meropenem

Ciprofloxacin

Figure D-31 (Continued) Three dimentional microdilution checkerboard results of *P. aeruginosa* isolate No. 30 at any series of proportional concentrations of meropenem, ciprofloxacin and colistin; gray zone: visible microorganism growth, white zone: no microorganism growth were observed.

FIC index for meropenem + ciprofloxacin = 0.75

FIC index for meropenem + colistin = 0.625

FIC index for ciprofloxacin + colistin = 0.563

FIC index for meropenem + ciprofloxacin + colistin = 0.78

Appendix E

Time kill curve of 5 meropenem-resistant isolates

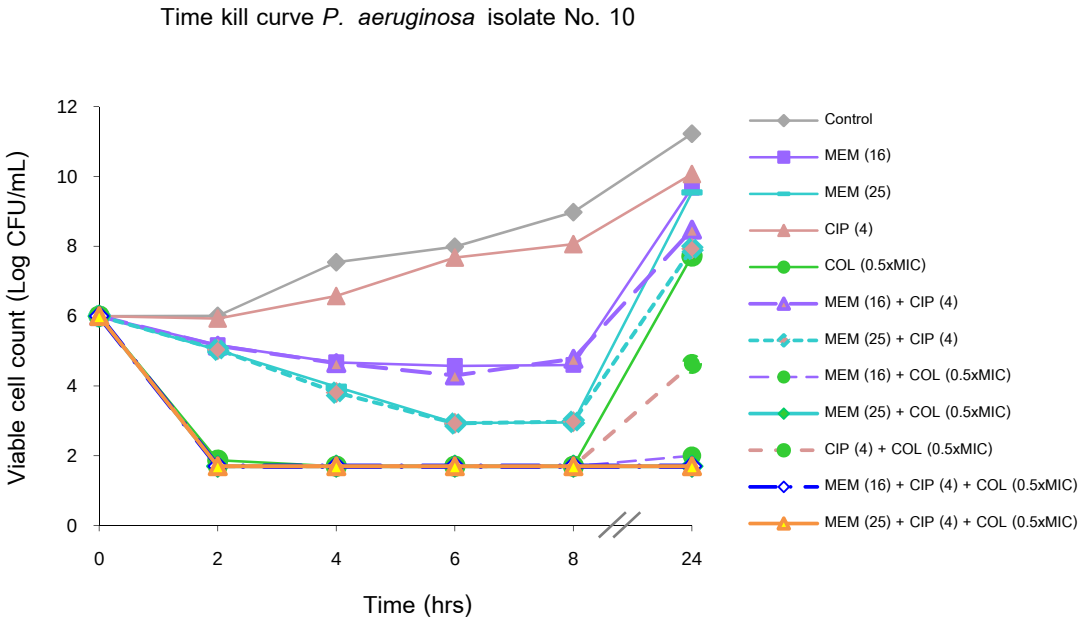

Figure E-1 Time-killing curve of meropenem 16 µg/mL, meropenem 25 µg/mL, ciprofloxacin 4 µg/mL and colistin 0.5xMIC alone and the combinations against *P. aeruginosa* isolate No.10

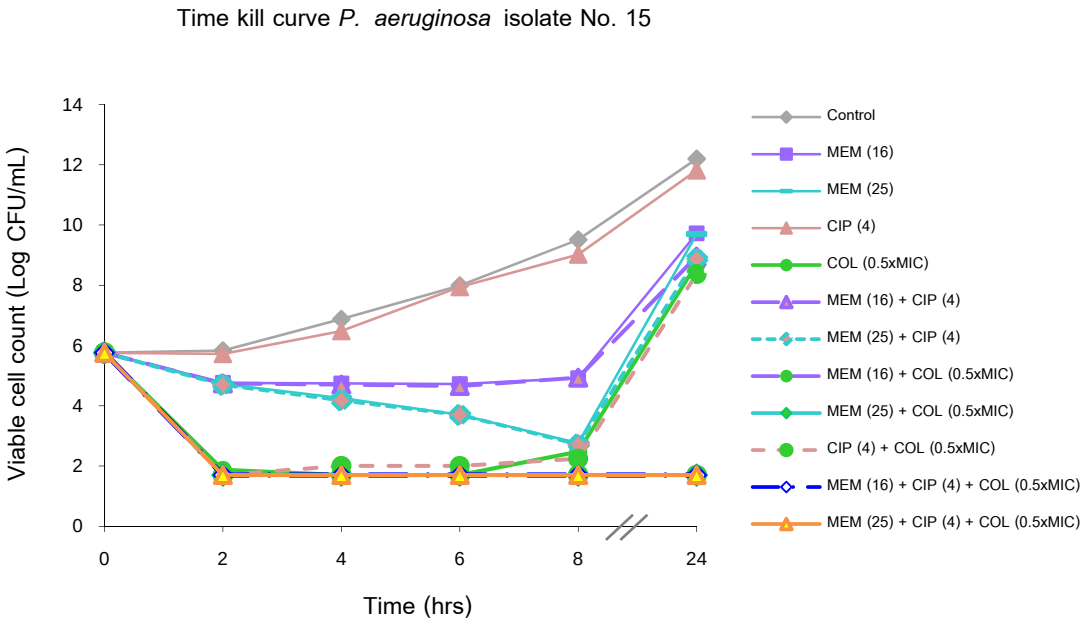

Figure E-2 Time-killing curve of meropenem 16 µg/mL, meropenem 25 µg/mL, ciprofloxacin 4 µg/mL and colistin 0.5xMIC alone and the combinations against *P. aeruginosa* isolate No.15

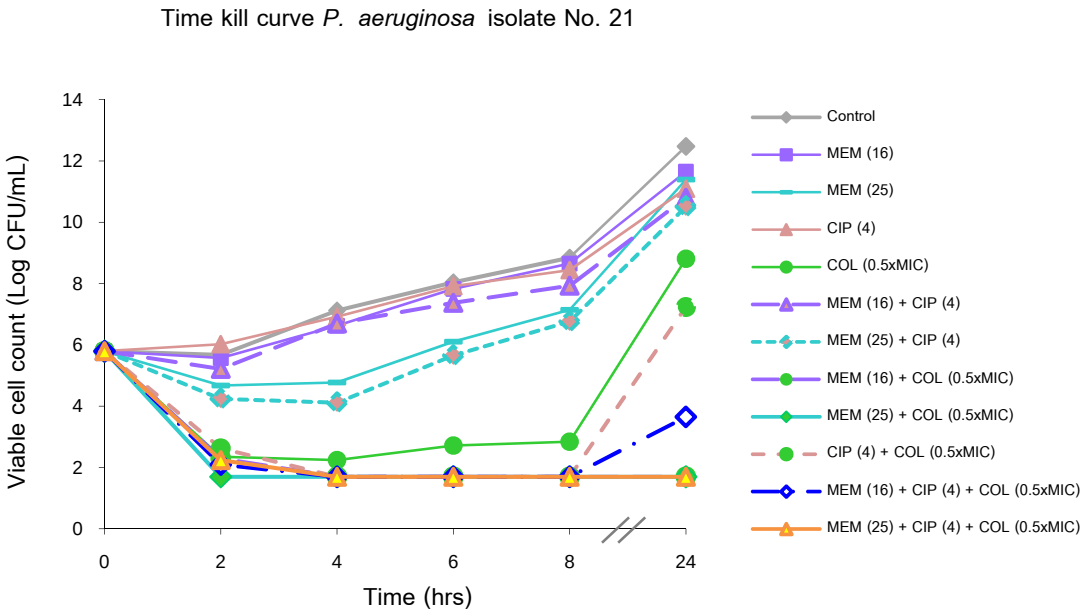

Figure E-3 Time-killing curve of meropenem 16 µg/mL, meropenem 25 µg/mL, ciprofloxacin 4 µg/mL and colistin 0.5xMIC alone and the combinations against *P. aeruginosa* isolate No.21

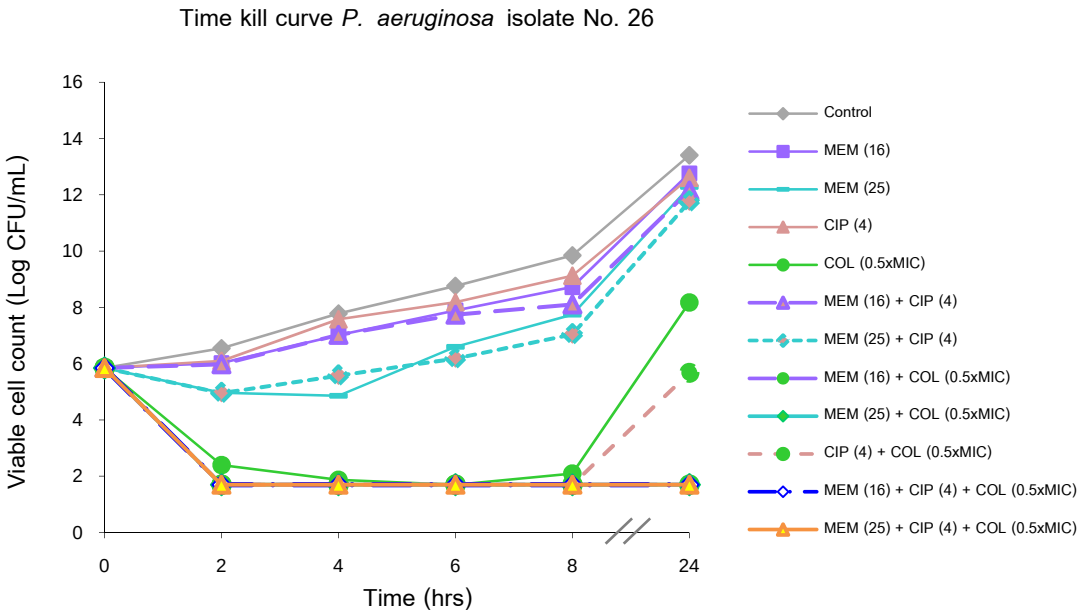

Figure E-4 Time-killing curve of meropenem 16 µg/mL, meropenem 25 µg/mL, ciprofloxacin 4 µg/mL and colistin 0.5xMIC alone and the combinations against *P. aeruginosa* isolate No.26

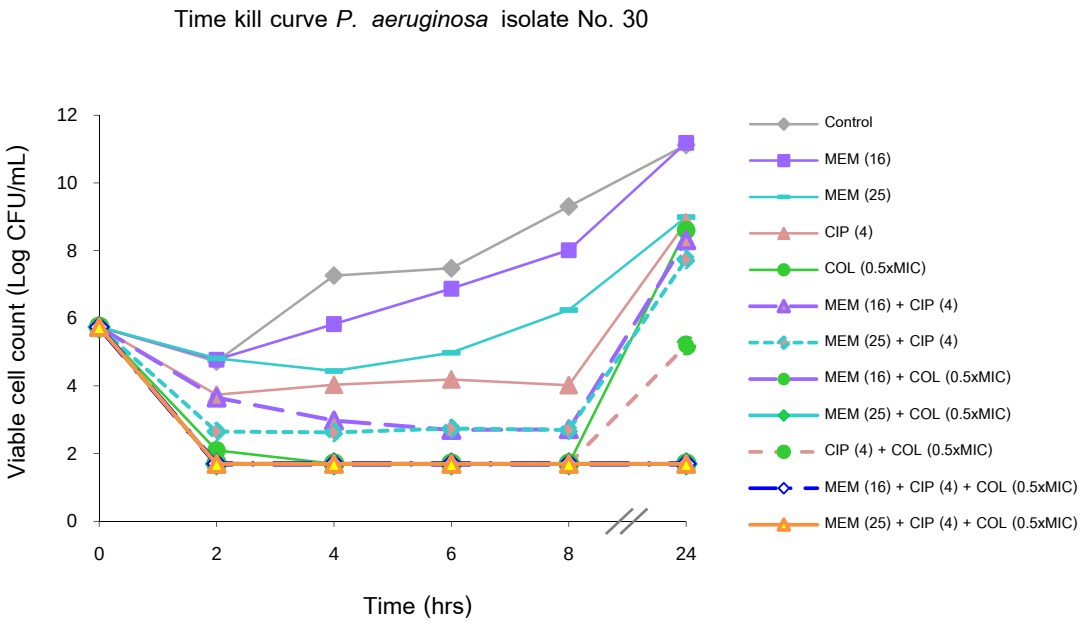

Figure E-5 Time-killing curve of meropenem 16 µg/mL, meropenem 25 µg/mL, ciprofloxacin 4 µg/mL and colistin 0.5xMIC alone and the combinations against *P. aeruginosa* isolate No.30

BIOGRAPHY

Miss Katareeya Ek-akaranawakul was born in September 06, 1978 in Suphanburi, Thailand. She graduated with a Bachelor of Pharmacy in 2002 from the Faculty of Pharmaceutical Science, Silpakorn University, Thailand.