การประเมินฤทธิ์ฆ่าแบคทีเรียในหลอดทดลองของยาในกลุ่มอะมิโนกลัยโคซัย ฟลูออโรควิโนโลน และเบต้า แลคแตม ต่อเชื้อแบคทีเรียแกรมลบที่ก่อโรค

นางสาว กิ่งกาญจน์ ภาณุมาศ

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

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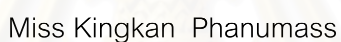
EVALUATION OF *IN VITRO* BACTERICIDAL ACTIVITY OF AMINOGLYCOSIDES, FLUOROQUINOLONE AND BETA LACTAM ON GRAM NEGATIVE PATHOGEN.



Miss Kingkan Phanumass

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

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กิ่งกาญจน์ ภาณุมาศ : การประเมินฤทธิ์ฆ่าแบคทีเรียในหลอดทดลองของยาในกลุ่มอะมิโนกลัยโคซัย ฟลูออโรควิโนโลน และเบต้า แลคแทมต่อเชื้อแบคทีเรียแกรมลบที่ก่อโรค. (EVALUATION OF *IN VITRO* BACTERICIDAL ACTIVITY OF AMINOGLYCOSIDES, FLUOROQUINOLONE AND BETA-LACTAM ON GRAM NEGATIVE PATHOGENS) อาจารย์ที่ปรึกษา : รศ.ศิริภรณ์ ฟุ้งวิทยา, อาจารย์ที่ปรึกษาร่วม: ศ.พญ.นลินี อัศวโภคี, 126 หน้า. ISBN 974-14-3807-9.

ปัจจุบันการรักษาโรคติดเชื้อที่เกิดจากแบคทีเรียแกรมลบพบความล้มเหลวค่อนข้างมาก แม้ว่าจะได้รับการรักษาที่ถูกต้องเหมาะสมแล้วก็ตาม ดังนั้นในการศึกษานี้จึงได้นำยาในกลุ่ม อะมิโนกลัยโคซัย (gentamicin, amikacin, netilmicin), ฟลูออโรควิโนโลน (ofloxacin, ciprofloxacin), และเบต้า แลคแตม (cefotaxime, ceftazidime, amoxicillin/clavulanic acid, piperacillin/tazobactam, ampicillin/sulbactam, cefoperazone/sulbactam, imipenem) ซึ่งใช้ในการรักษาโรคติดเชื้อดังกล่าวมาประเมินฤทธิ์ในการฆ่าเชื้อของยา (bactericidal activity) และศึกษาปัจจัยต่างๆ ที่มีผลต่อการออกฤทธิ์ฆ่าเชื้อ เช่น inoculum effect ต่อแบคทีเรียแกรมลบจำนวน 6 ชนิด (*E. coli, K. pneumoniae, P. mirabilis, Providencia sp. P. aeruginosa* และ *A. buamannii*) ซึ่งสุ่มเลือกจากผู้ป่วย โดยส่วนใหญ่ได้เชื้อที่ไวต่อยายกเว้นเชื้อ *A. buamannii* ที่ได้สายพันธ์ที่มีการดื้อยาหลายชนิด การทดสอบฤทธิ์ในการฆ่าเชื้อโดยใช้วิธี Broth macro dilution ศึกษาค่า MIC, MBC และศึกษาค่า MIC ที่เปลี่ยนไปเมื่อเชื้อเพิ่มจำนวนมากขึ้น (inoculum effect) และใช้วิธี Time kill study เพื่อศึกษาฤทธิ์ในการฆ่าเชื้อเมื่อได้รับยาความเข้มข้นสูง และต่ำกว่าค่า MIC รวมทั้งความเข้มข้นสูงสุด และความเข้มข้นต่ำสุดก่อนการให้ยาครั้งต่อไปในทางคลินิก ผลจากการศึกษาในเชื้อ *E.coli* ซึ่งทำการทดสอบกับยา gentamicin, amikacin, ofloxacin, ciprofloxacin, cefotaxime, amoxicillin/clavulanic acid, piperacillin/tazobactam, ampicillin/sulbactam, cefoperazone/sulbactam, และ imipenem พบว่ายาทุกตัวยังคงมีฤทธิ์เป็น bactericidal โดยมีค่า MBC/MIC อยู่ในช่วง 1-4 และพบว่าพบ inoculum effect กับยา cefotaxime, ampicillin/sulbactam, cefoperazone/sulbactam และการฆ่าเชื้อ *E. coli* ได้โดยไม่มี regrowth ภายใน2ชม.พบในยากลุ่ม aminoglycoside และ fluoroquinolone โดยเริ่มฆ่าเชื้อได้ตั้งแต่ระดับยา MIC ส่วนในยากลุ่มเบต้า แลคแตม พบการฆ่าเชื้อภายใน 6 ชั่วโมงและความเข้มข้นที่ฆ่าเชื้อส่วนมากเริ่มต้นที่ 4MIC สำหรับเชื้อ *K.pneumoniae* ซึ่งทำการทดสอบกับยา gentamicin, amikacin, ciprofloxacin, cefotaxime, piperacillin/tazobactam, และ imipenem พบว่ายาทุกตัวยังคงมีฤทธิ์เป็น bactericidal โดยมีค่า (MBC/MIC อยู่ในช่วง 1-4) และพบไม่พบ inoculum effect กับยาที่ทำการทดสอบและการฆ่าเชื้อ *K. pneumoniae* ได้โดยไม่มี regrowth ภายใน2ชม.พบในยากลุ่ม aminoglycoside และยา imipenem โดยเริ่มฆ่าเชื้อได้ตั้งแต่ระดับยา 4MIC ส่วนในยากลุ่มเบต้า แลคแตม และ ciprofloxacin พบการฆ่าเชื้อภายใน 6 ชั่วโมงและความเข้มข้นที่ฆ่าเชื้อส่วนมากเริ่มต้นที่ 4MIC สำหรับเชื้อ *P. mirabilis* ซึ่งทำการศึกษาต่อยา amikacin, netilmicin, ciprofloxacin, cefotaxime, piperacillin/tazobactam, และ imipenem พบว่ายาทุกตัวยังคงมีฤทธิ์เป็น bactericidal โดยมีค่า (MBC/MIC อยู่ในช่วง 1-4) และพบว่าพบ inoculum effect กับยา cefotaxime piperacillin/tazobactam และการฆ่าเชื้อ *P. mirabilis* ได้โดยไม่มี regrowth ภายใน2ชม.พบในยากลุ่มaminoglycoside โดยเริ่มฆ่าเชื้อได้ตั้งแต่ระดับยา 4MIC ส่วนในยากลุ่มเบต้าแลคแตมและ ciprofloxacin พบการฆ่าเชื้อภายใน 6 ชั่วโมงและความเข้มข้นที่ฆ่าเชื้อส่วนมากเริ่มต้นที่ 4MIC สำหรับเชื้อ *Providencia sp,* ซึ่งทำการทดสอบกับยา amikacin, netilmicin, ciprofloxacin, cefotaxime และ imipenem พบว่ายาทุกตัวยังคงมีฤทธิ์เป็น bactericidal โดยมีค่า (MBC/MIC อยู่ในช่วง 1-4) และพบว่าพบ inoculum effect กับยา imipenem และการฆ่าเชื้อ *Providencia sp,* ได้โดยไม่มี regrowth ภายใน2ชม.พบในยากลุ่มaminoglycoside, ciprofloxacin และ imipenemโดยเริ่มฆ่าเชื้อได้ตั้งแต่ระดับยา 4MIC ส่วนในยา cefotaxime พบการฆ่าเชื้อภายใน 6 ชั่วโมงและความเข้มข้นที่ฆ่าเชื้อส่วนมากเริ่มต้นที่ 8MIC สำหรับเชื้อ *P. aeruginosa* ทำการศึกษากับยา amikacin, netilmicin, ciprofloxacin, ceftazidime, piperacillin/tazobactam และ imipenem พบว่ายาทุกตัวยังคงมีฤทธิ์เป็น bactericidal โดยมีค่า (MBC/MIC อยู่ในช่วง 1-4) ยกเว้น imipenem และพบว่าพบ inoculum effect กับยา piperacillin/tazobactamและ imipenemและการฆ่าเชื้อ *P.aeruginosa* ได้โดยไม่มี regrowth ภายใน2ชม.พบในยากลุ่มaminoglycoside และ ciprofloxacin โดยเริ่มฆ่าเชื้อได้ตั้งแต่ระดับยา 8MIC ส่วนในยากลุ่มเบต้าแลคแตม พบการฆ่าเชื้อภายใน 6 ชั่วโมงและความเข้มข้นที่ฆ่าเชื้อส่วนมากเริ่มต้นที่ 8MIC สำหรับเชื้อ *A. buamannii* ทำการศึกษาต่อยา amikacin, netilmicin, ciprofloxacin, ceftazidime, ampicillin/sulbactam, cefoperazone/sulbactam และ imipenem พบว่าดื้อต่อยาทุกชนิด ไม่พบinoculum effect และการฆ่าเชื้อเกิดขึ้นภายใน 6 ชั่วโมงและไม่มี regrowth นั้นสามารถเกิดได้แต่ระดับยาที่ใช้ในการฆ่าเชื้อส่วนใหญ่พบที่ 8MIC, peak และ peak high dose ซึ่งเป็นระดับยาที่สูงมากจนไม่สามารถนำไปประยุกต์ใช้ในทางคลินิกได้

ภาควิชา ....เภสัชวิทยา	ลายมือชื่อนิสิต..
สาขาวิชา ....เภสัชวิทยา....................	ลายมือชื่ออาจารย์ที่ปรึกษา..............................
ปีการศึกษา 2548	ลายมือชื่ออาจารย์ที่ปรึกษาร่วม...........................

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KEY WORDS: BACTERICIDAL/ GRAM NEGATIVE BACTERIA / AMINOGLYCOSIDE / FLUOROQUINOLONE / BETA-LACTAM

KINGKAN PHANUMASS: EVALUATION OF *IN VITRO* BACTERICIDAL ACTIVITY OF AMINOGLYCOSIDES, FLUOROQUINOLONE AND BETA LACTAM ON GRAM NEGATIVE PATHOGEN. THESIS ADVISOR: ASSOC. PROF. SIRIPORN FUNGWITTHAYA, THESIS COADVISOR: PROF. NALINEE ASWAPOKEE, pp. 126 ISBN 974-14-3807-9.

In present, we should have failure on treatment bacteria infection, however, there should have the appropriate treatment. The purposes of this study were to evaluate the bactericidal activity and to study kinetics of killing of aminoglycoside, fluoroquinolone and beta-lactam antibiotics against common gram negative bacteria. The minor objectives were to determine the influence factors which altered bactericidal activity such as, inoculum effect. In this investigation, the six species of gram negative bacteria which were selected from the clinical isolates (*E.coli, K.pneumoniae, P.mirabilis, Providencia* spp., *P.aeruginosa* and *A.buamannii*) were randomized to the study. From the randomized, the microorganisms which *E.coli, K.pneumoniae, P.mirabilis, Providencia* spp., *P.aeruginosa* were senses to tested antimicrobial agents, but, *A.buamannii* was multi-resistance to most used antibiotics. There had used through the study. Bactericidal activity was tested by broth macro dilution which to determine the MIC, MBC and the alter MIC when increase inoculum (inoculum effect). Time killing study in order to determine the bactericidal activity against selected bacteria which should be varied concentrations (sub-MIC, MIC, above MIC, trough, peak). From the study, it has been found that the antibiotics (gentamicin, amikacin, ofloxacin, ciprofloxacin, cefotaxime, amoxicillin/clavulanic acid, piperacillin/tazobactam, ampicillin/sulbactam, cefoperazone/sulbactam and imipenem) which used to against *E.coli* , should still bactericidal activity (MIC/MBC ranging in 1 to 4) and should have inoculum effect to beta-lactam antibiotics (cefotaxime, ampicillin/sulbactam, and cefoperazone/sulbactam). The 99.9% killing to *E.coli* without regrowth in the first 2 hr, should be found in aminoglycosides and fluoroquinolone which there could start to kill in the concentration at MIC. But beta-lactam should be found the 99.9% killing to *E.coli* without regrowth in the 6th hr. which could start to kill in the concentration at 4MIC. For *K.pneumoniae*, the antibiotics (gentamicin, amikacin, ciprofloxacin, cefotaxime, piperacillin/tazobactam, and imipenem) which used to against *K.pneumoniae*, should still bactericidal activity (MIC/MBC ranging in 1 to 4) and should not have inoculum effect. The 99.9% killing to *K.pneumoniae* without regrowth in the first 2 hr, should be found in aminoglycosides and imipenem which there could start to kill in the concentration at 4MIC. But beta-lactam and ciprofloxacin should be found the 99.9% killing to *K.pneumoniae* without regrowth in the 6th hr. which could start to kill in the concentration at 4MIC. In *P.mirabilis*, the antibiotics (amikacin, netilmicin, ciprofloxacin, cefotaxime, piperacillin/tazobactam, and imipenem) which used to against *P.mirabilis* should still bactericidal activity (MIC/MBC ranging in 1 to 4) and should have inoculum effect to cefotaxime and imipenem. And the 99.9% killing to *P.mirabilis* without regrowth in the first 2 hr, should be found in aminoglycosides which there could start to kill in the concentration at 4MIC. But beta-lactam and ciprofloxacin should be found the 99.9% killing to *P.mirabilis* without regrowth in the 6th hr. which could start to kill in the concentration at 4MIC. For *Providencia* spp., all antibiotics (amikacin, netilmicin, ciprofloxacin, cefotaxime, and imipenem) which used to against *Providencia* spp. should still bactericidal activity (MIC/MBC ranging in 1 to 4) and should have inoculum effect to imipenem. And the 99.9% killing to *Providencia* spp. without regrowth in the first 2 hr, should be found in aminoglycosides ciprofloxacin and imipenem which there could start to kill in the concentration at 4MIC. But cefotaxime should be found the 99.9% killing to *Providencia* spp. without regrowth in the 6th hr. which could start to kill in the concentration at 8MIC. For *P.aeruginosa* all antibiotics (amikacin, netilmicin, ciprofloxacin, ceftazidime, piperacillin/tazobactam and imipenem) which used to against *P.aeruginosa* should still bactericidal activity (MIC/MBC ranging in 1 to 4) except for imipenem and should have inoculum effect to piperacillin/tazobactam and imipenem. And the 99.9% killing to *P.aeruginosa* without regrowth in the first 2 hr, should be found in aminoglycosides ciprofloxacin which there could start to kill in the concentration at 8MIC. But beta-lactam should be found the 99.9% killing to *P.aeruginosa* without regrowth in the 6th hr. which could start to kill in the concentration at 8MIC. For *A.buamannii* should have resist to the antibiotics (amikacin, netilmicin, ciprofloxacin, ceftazidime, ampicillin/sulbactam, cefoperazone/sulbactam and imipenem) which used to against *A.buamannii*, and no inoculum effect. The 8MIC, the peak, and the peak high dose were the concentration which could kill 99.9% of *A.buamannii* but there were high concentration to apply in clinical used.

Department ....Pharmacology............ Student's signature......Kingkan Phanumass
Field of study ....Pharmacology............ Advisor's signature......[illegible]
Academic year ....2005...................... Co-advisor's signature..Nalinee Aswapokee

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LIST OF ABBREVIATIONS

°C	= degree Celsius
A. baumannii	= *Acinetobacter baumannii*
AUC	= Area under the curve
BA24	= Bacteriolytic area of 24 hours
CFU	= colony forming unit
CLSI	= Clinical and Laboratory Standard Institute
E. coli	= *Escherichia coli*
e.g.	= exempli gratia (for example)
et al.	= et alii (and other peoples)
ect.	= et cetera (and other similar things)
Fig.	= Figure
g	= gram
hr	= hour
KR2	= Killing rate of the first 2 hours
K. pneumoniae	= *Klebsiella pneumoniae*
L	= Liter
log	= decimal logarithm
MBC	= Minimum bactericidal concentration
MHA	= Meuller – Hinton agar
MHB	= Meuller – Hinton broth
MIC	= Minimum inhibitory concentration
min	= minute
ml	= milliliter
mm	= millimeter
mol	= mole
NSS	= Normal saline solution
P. aeruginosa	= *Pseudomonas aeruginosa*
PBP	= Penicillin binding protien
P. mirabilis	= *Proteus mirabilis*

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CHAPTER I

INTRODUCTION

Antimicrobial has been characterized by activity of killing in to two groups, the first group is bacteriostatic agents which inhibit microorganism and wait for human defense mechanism to eradicate. The other group is bactericidal agents which directly kill bacteria.

Ultimately, the treatment target should be achievement of good clinical outcome (clinical/bacteriologic cure and no relapse) with the less toxicity. Thus, antimicrobial therapy is to eradicate microbial pathogens at the specific site of infection the critical point is the activity of antibiotic.

For decades, it has been traditional to view an antibiotic with bactericidal activity against a pathogen as a preferable choice over one that exhibits bacteriostatic activity. It now well recognized that antibiotics cannot be categorized in such a simplistic manner, since their type of activity varies against different pathogen and under different conditions. (Quintiliani R., 2004)

The recently reviews, Pankey GA and Sabath LD have to evaluate clinical relevance of bactericidal versus bacteriostatic activity in the treatment of gram positive infection. The authors indicated that invitro bacteriostatic/bactericidal data may provide information on the potential action of antibacterial agents, but this is only one of many factors which are necessary to predict a favorable clinical outcome. However, the bacteriostatic/bactericidal activity is the major concern.

The bactericidal effect is desired because, to put it succinctly, dead bacteria don’t mutate. In the other words, if microbial pathogens causing infection are killed by antimicrobial therapy, rather than inhibited, mutations that might already exist or occur under the selective pressure of the antimicrobial agent are less likely to be promulgated (Stratton CW., 2003).

The tests for bactericidal activity are recommended by some authorities. There were the tests from various cases which were bacterial endocarditis, sepsis in the immunocompromised patient, infections in those unable to mount an immune response,

osteomyelitis, chronically infected implants, and other type of chronic infections (Peterson LR., 1992).

Because of the resistance to currently available antimicrobial agents that are used to treat infections with gram negative bacteria, so there is the problem emergence in clinical treatment which the gram negative bacteria is the most important in nosocomial infection. By the way, the data of bacteriostatic/bactericidal of clinical used antimicrobial against gram negative bacteria were small data, thus, the research question is the antimicrobial, which in clinical used, have bacteriostatic or bactericidal action against common gram negative bacteria.

The major objectives of this investigation were to evaluate antimicrobial activity and study kinetics of killing of aminoglycoside (gentamicin, amikacin, netilmicin), fluorquinolone (ofloxacin, ciprofloxacin) and β- lactam antibiotics (amoxicillin/clavulanic acid, ampiciliin/sulbactam, piperacillin/tazobactam, cefotaxime, ceftazidime, cefoperazone/sulactam, imipenem) against common gram negative bacteria (*E. coli, K. pnuemoniaea, P. mirabilis, Providencia* spp., *P.aeruginosa* and *A. buamannii.*)

The minor objective was to determine the influence factors which altered bacterial activity such as inoculum effect etc.

The benefits of the study, first benefit is the enhancement of knowledge about microbiological activities of antimicrobial agents and second benefit is the origination of general database for decision making in order to select optimized antimicrobial in clinical usage.

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Terms Associated with Bactericidal Testing

MIC. The MIC is the lowest concentration of antimicrobial agent that inhibits growth, as determined visually after standard incubation period (usually 18 to 24 hours)

MBC. The MBC is the lowest concentration of an antibacterial agent that causes at least 3 log_{10} reduction in the number of surviving cells (compared with the initial, preincubation concentration) after incubation (usually 18 to 24 hours).

Tolerance. Tolerance is the phenomenon in which normall bactericidal agents (e.g. β- lactam and vancomycin) appear to selected bacterial strains. The mechanism is often through to impaired bacterial autolytic enzyme activity, although other mechanism likely exists. Many authors also include an MBC/MIC ratio $\geq$ 32 as a part of the definition of tolerance.

Persister. Persisters are small numbers of cells (usually less than 0.1 or 0.01 % of the initial inoculum) that survive the lethal effect of antimicrobial agents at concentrations that exceed the MBC. These “persisting“ cells have the same susceptibility as the original strain.

Killing curve (time kill study). This technique is similar to that used for the MBC test except that only a single concentration is typically studied (usually near the mean achievable blood). Additionally sub cultures to antibiotics free agar are done at multiple times during a 24 hr incubation period. The test permits the actual rate at which the number of viable bacteria decreases from the number of viable bacteria in the original inoculum, and thus the bactericidal rate, is determined.

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CHAPTER II

LITERATURES REVIEW

For decades, it has been traditional to view an antibiotic with bactericidal activity (kill bacteria) against a pathogen as a preferable choice over one that exhibits bacteriostatic activity (inhibit the growth of bacteria). It is now well recognized that antibiotics cannot be categorized in such a simplistic manner, since their type of activity varies against different pathogen and under different conditions. (Quintiliani R., 2004; Pankey GA and Sabath LD., 2004)

However, the detection of resistance is somewhat predictive of poor outcome, although in the normal host this may be less clinically importance due to the interaction of host defenses. The ability of bactericidal activity to influence therapeutic efficacy and clinical outcome has been evaluated in infections that typically are refractory to antimicrobial therapy. These infections include endocarditis, meningitis, osteomyelitis, and infection in the neutropenic host. All are similar in that antimicrobial penetration and host defense mechanisms at the site of infection are limited. However, the relevance of pharmacokinetic and pharmacodynamics has become increasingly recognized. Issues such as drug concentration at the site of infection, bactericidal activity, postantibiotic effect, and duration of therapy needed to achieve these effects are now being considered when antimicrobial agents are selected for the therapy of infections.

High inocula occur in endocarditis, meningitis, septic arthritis, osteomyelitis, abcesses, and other deep-seated infections. In addition, stationary-phase growth, which favors beta-lactamase activity and is detrimental to beta-lactam drug efficacy, also occurs in these infections (Stratton CW, 1991). Despite such concerns, inoculum effect are accepted as relevant by some investigators, at least to the extent that it has been proposed that a major inoculum effect for a compound contraindicates its use in serious infections caused by the pathogen (Livermore DM, 1998).

In an in vitro test the ability of the inoculum to influence the antibacterial activity of a beta-lactam drug is determined primarily by two factors, the intrinsic activity of the drug against the test organism and the susceptibility of the drug to hydrolysis by the beta-lactamase of the organism (Thomson KS and Moland ES, 2001).

Although host factors may allow a bacteriostatic agent to be used successfully in an infected patient, these factors appear to lessen the ability of problem emergence resistance. (Pankey GA and Sabath LD., 2004; Finberg RW. et al., 2004; Rahal and Simberkoff, 1979)

The key to resolve the problem of antibacterial resistance lies in the identifying of the mechanisms that produce it. Among the most important mechanisms adopt by bacterial itself are the ability to against the penetration of antibacterial into its cell wall, the active efflux of antibacterial agents, the inactivation of antibacterial agents, the destruction of antibacterial agents, the alteration of antibacterial target sites, the development of bypass pathways around antibacterial targets, and the constitutive phenotypic variation in bacterial physiology.

Fundamental of many of these mechanisms is the mutation of bacterial DNA. Subsequent exposure of the microorganism to a specific agent may then select the mutant, leading to the emergence of resistance. Some resistance mechanisms, such as bacterial production of beta-lactamase, are inducible or can be derepressed requiring either upreglulation or mutation of genetic material. Thus, if resistance is to be suppressed, the opportunity for bacterial upreglulation or mutation of genetic material must be minimized.

The goal of antimicrobial therapy currently focuses on curing disease while minimizing toxic consequences. To achieve this goal, treatment regimens have been designed to block susceptible cell growth or kill vulnerable cells. Pharmacokinetic and pharmacodynamic considerations based on empiric measurements of clinical outcome have been adopted as the estimation of how far above or how long serum concentrations should remain above the MIC.

Craig WA, 1998, divided the pharmacology of antibiotics into pharmacokinetics (PK) and pharmacodynamics (PD). The former describes the concentration-time profile of drugs in the host, whereas pharmacodynamics describes the antimicrobial effect of an antibiotic at its target pathogen. (Antina Barger, et al, 2003)

The MIC for a pathogen, which is a PD parameter used in most pharmacological indices, can also vary markedly depending on whether it is determined in plasma, in urine or in broth. Usually, it is done in broth and the inoculum in the exponential growth phase. It is well known as physiological *in vitro*, such as nutrient supply and pH, does not correspond with those *in vivo*. In addition, the results of MIC determinations depend on the methods used. Even though essential methodical steps like inoculum, source of the broth, incubation temperature, and incubation time are standardized by the NCCLS or BSAC, variations (for example in the cation concentration in the medium) can result on considerable fluctuations in the MIC values. The media used allow optimal growth, but the generation time of bacteria at the site of infection in biofilms, so these two results yield the effect in the clinical outcomes of antimicrobial agent therapy.

Non microbiological factors affect the response to those therapies. The factors are host defense mechanism, site of infection, underlying disease and an antibacterial agent's critical intrinsic pharmacokinetic and pharmacodynamic properties.

Inadequate penetration of the infection site is one of the principal factors related to failure of antibacterial therapy. The active drug needs to reach the bacteria in appropriate body fluid and tissue concentrations which are necessary to kill or suppress the pathogen's growth. (RW Finberg, 2004)

Because the resistance to currently available antimicrobial agents used to treat infections with gram negative bacteria occurs. For example, the development of resistance is the mutation of *K.pneumoniaea* to produce a multidrug-resistant strain (Podschum R and Ullmann, 1998). Keeping these phenomena in check requires a comprehensive strategy that includes, whenever possible, the selection antimicrobial agents in dosages sufficient to bactericidal. (Stratton CW., 2003) Current approaches to therapy for these infections must be reappraised. Ultimately, the treatment target should be achievement of good clinical outcome (clinical/bacteriologic cure and no relapse) with least toxicity.

Both enter *Enterobacteriaceae* and *Pseudomonas aeruginosa* are not only the gram negative bacteria but also the leading causes of nosocomial bloodstream infections. Antibiotic-resistant strains have emerged among the gram-negative bacilli and are being increasingly recognized. This marked increase in the incidence of infections due to the antibiotic-resistant gram-negative bacilli in recent years is of great concern. It is presumed that infections caused by antibiotic-resistant bacteria result in greater mortality, longer hospitalization, and higher costs than infections caused by antibiotic-susceptible bacteria, although only little data are available to support this intuitive concept. The assumption that infections caused by antibiotic-resistant bacteria are associated with a higher mortality rate which can be decreased. In order to decrease the mortality rate, an appropriate is based on the appropriate antimicrobial therapy. (Kollef HM, 2000)

The recent data from the National Antimicrobial Resistance Surveillance center, Thailand (NARST) reported that the first three bacterial ranking from Top Ten isolates from all region of Thailand are gram negative bacteria (*E.coli* 16%, *P.aeruginosa* 11%, and *K.pneumoniae* 10%). Thus, current gram negative infection is importance.

Table2-1, Top ten isolates from all region of Thailand (2003)

NO	Organism	No. of isolates	%
1	*E.coli*	25,631	16
2	*P.aeruginosa*	18,792	11
3	*K.pneumoniae*	17,014	10
4	*A.baumannii*	10,290	6
5	S.aureus	8,261	5
6	Normal flora	8,106	5
7	Staphylococcus,coaglulase positive	7,168	4
8	Staphylococcus,coaglulase negative	5,920	3
9	*Enterobacter cloaceae*	4,882	3
10	*Proteus mirabilis*	3,985	2
	others	48,251	30

** Data from the National Antimicrobial Resistance Surveillance center, Thailand (NARST)**

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The definitions of "bactericidal" and "bacteriostatic" appear to be straightforward: "bacteriostatic" means that the agent prevents the growth of bacteria and "bactericidal" means that it kills bacteria. In reality, there are not exact categories of antimicrobial agents (one that exclusively kills bacteria and another that only inhibits growth). Rather, those agents that are call "bactericidal "usually fail to kill every organism with in 18-24 hrs. after the tests, and most so-called "bacteriostatic" agents kill some organism with in 18-24 hrs. after the tests often more 90%-99% of the inoculum, but it is not enough (>99.9%) to be called "bactericidal".

Determining Bactericidal activity

There are various *in vitro* microbiological techniques to determine the bactericidal activity of antibacterial agents against different isolates include the minimum bactericidal concentration (MBC), time-kill curve, and serum bactericidal titer. (SBT)

Minimum Bactericidal Concentration (MBC)

Guidelines for performing bactericidal tests were published by NCCLS. Minimum bactericidal concentration (MBC) involves in the continuation of the procedure for conventional broth dilution testing. After incubation and determination of the antimicrobial agent's MIC, an aliquot from each tube or well in the dilution series showing inhibition of visible bacterial growth is subcultured to an enriched agar medium. Following overnight incubation the plates are examined and the CFUs are counted. Knowing the volume of the aliquot sampled and the number of CFUs obtained, the number of viable cells per milliliter for each antimicrobial dilution can be calculated. This number is then compared with the know CFU/mL in the original inoculum. The antimicrobial concentration that results in a 99.9% reduction in CFU/mL compared with the organism concentration in the original inoculum is recorded as the MBC. Critical methodology components for MBC include a standard inoculum of $\geq 5 \times 10^5$ cfu/mL and subculture volume of 0.1 mL to accurately predict whether $\geq$ 99.9% of bacteria were killed. Bactericidal is a $\geq$ 99.9% reduction in viable bacteria density in an 18-24 hrs. Bacteriostatic activity has been defined as a ratio of MBC to MIC of > 4.(Pankey GA and Sabath LD,2004; Peterson LR and Shanholtzer CJ,1992)

Although the clinical significance of MBC results is uncertain, applications of this information include the considering whether a treatment failure could be occurring because the organism's MBC exceeds the serum achievable level of the antimicrobial agent. Alternatively, if an antimicrobial agent's MBC is $\geq$ 32 times higher than the MIC, the organism may be tolerant to that drug.

Tolerance is a phenomenon that most commonly associated with bacterial resistance to beta-lactam antibiotics, additionally, tolerance reflects an organism's ability to be only inhibited by an agent that is usually bactericidal.

TIME-KILL STUDIES

Another approach to examine bactericidal activity involves exposing a bacterial isolation to a concentration of antibiotic in broth medium and measuring the rate of killing over a specified period. By this time-kill analysis samples are taken from the antibiotics broth solution immediately after the inoculum was added and at regular intervals afterward. Each time-sample is plated to agar plates; following incubation, CFU counts are performed as described for MBC testing. The number of viable bacteria from each sample is plotted over time so that the rate of killing can be determined. Generally, a 1000-fold decrease in the number of viable bacteria in the antibiotic-containing broth after a 24 hour period will be compared with the number of bacteria in original inoculum. It is interpreted as bactericidal activity used in the research environment to the study the *in vitro* activity of the antimicrobial agents, the labor intensity, and technical specifications of the procedure precluding its use in most clinical microbiology laboratories for production of data used to manage a patient's infection. (Peterson LR and Shanholtzer CJ., 1992)

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SERUM BACTERICIAL TEST

The serum bactericidal test (SBT) is analogous to the MIC-MBC test except that the used medium is patient's serum that contains the therapeutic antimicrobial agent(s) the patient has been receiving. By using patient serum to detect bacteriostatic and bactericidal activity, the antibacterial impact factors other than the antibiotics (e.g. antibodies and complement) are observed.

The test is performed by using the dilution of a serum sample collected from a patient who is under receiving antimicrobial therapy (usually a "peak" or "trough" sample). The sample is diluted (usually in twofold increments) and a standard inoculum of bacteria is added to each dilution. Samples are incubated for 18-24 hours at a standard temperature: the serum inhibitory (SIT) (or bacteriostatic) titer is read as the greatest dilution of serum that prevents visible growth. The serum bactericidal titer is determined by subculture of dilutions showing no growth on antibiotic free agar. Bactericidal endpoints (i.e., 99.9 % reduction in the initial inoculum) are determined as for the MBC test; the greatest dilution of serum that meets the criteria for bactericidal activity is termed the serum bactericidal titer (SBT) (Dudley MN, 1997, Amsterdam D, 1996)

Antibacterial classes for gram-negative bacterial infections

A. Beta-lactam antibiotics

Beta-lactam antibiotics inactivate a family of enzymes involved in the terminal stages of cell wall synthesis, and in shaping the cell. Members of this family of enzymes are called penicillin binding proteins (PBPs) because they are detected when they bind radiolabeled penicillin. Bacteria usually have four to eight different penicillin binding proteins, and each is involved in a different aspect of cell wall synthesis. For example, *E. coli* has multiple PBPs, and there are essential to the cell for growth and survival. Two most famous are PBP 1a and1b. Transpeptidase is the enzyme that catalyzes cross-linking between linear chains of peptidoglycan. Without proper cross-linking, the cell wall is structurally unstable. Beta-lactam antibiotics are suicide inhibitors of transpeptidase. The beta-lactam ring is a structural analog of D-ala-D-ala, the substrate of transpeptidase. Once the enzyme binds the beta-lactam ring in its active site, the drug

becomes covalently attached to the enzyme, resulting in permanent inactivation. Loss of PBP1 (transpeptidase) function leads to cells that can lyse, due to the high internal osmotic pressure found in bacteria. Lysis is facilitated, in many bacteria, by the activity of autolysins, enzymes that digest the existing cell wall. Other penicillin binding proteins have different functions. PBP2 is responsible for the characteristic rod shape of the *E. coli* cell. Beta-lactams that preferentially inhibit PBP2 lead to the formation of round cells. PBP3 is responsible for septum formation. Inhibition of PBP3 forms huge filamentous cells. Although the various beta-lactam antibiotics interact with the PBPs to different degrees, it is extremely important to realize that any of the events that they mediate are lethal. Beta-lactams are generally bactericidal against growing bacteria with active autolysins. Important clinical situations in which the beta-lactams are not bactericidal include slow growing or dormant bacteria found in endocarditis and chronic osteomyelitis, and against certain bacteria such as enterococci that downregulate autolysin activity.

A.1.The cephalosporins

The cepharosporins are traditionally divided, into four generations. The first generation, they do not have as a broad spectrum of action against gram-negative bacteria, but they have greatest activity against gram positive bacteria. The second generation, they have expanded the activity against gram negative organisms. The increased spectrum of action is due to the increase in affinity for PBP, the increase in penetration through the outer envelope of gram negative bacteria, and the increase in resistance to hydrolysis by gram negative beta lactamase. The third generation, their compounds have substitutions in the basic ring structure, which increases their affinity to PBP, thereby, enhancing activity against gram-negative bacteria. There are five parenterally administrated third generation cepharosporins (Ceftizoxime, cefotaxime, ceftriaxone, ceftazidime, and cefoperazone). Their compounds have an expanded spectrum of activity against gram negative bacilli, and most Enterobacteriaceae are highly susceptible to these drugs. In this class, there are two drugs, ceftazidime and cefoperazone, that have activity against *P.aeruginosa*.

The forth generation, there is the chemical modifying to basic cephem ring in order to enhance the stability of these cephalosporins against beta lactamases.

A.1.1. Cefotaxime

The study is presented by the tracing in healthy adults with normal renal function, a single 500-mg, 1-g, or 2-g dose of cefotaxime given by IV injection over 5 minutes resulted in the serum concentrations of cefotaxime which averaged 37.9 mcg/mL, 102.4 mcg/mL, and 214.1 mcg/mL, respectively, immediately after the injection; the averages of serum concentrations of the drug were at 1 mcg/mL, 1.9 mcg/mL, and 3.3 mcg/mL, respectively, 4 hours after the injection. In a multiple-dose study in healthy adults with normal renal function receiving 1-g doses of cefotaxime every 6 hours by IV infusion over 30 minutes, the steady-state peak serum concentrations of cefotaxime ranged from 40.6–46 mcg/mL and the steady-state trough serum concentrations of the drug ranged from 1.1–1.6 mcg/mL.

Following a single cefotaxime dose of 50 mg/kg given by IV infusion over 10 minutes in average birthweight neonates 1–7 days of age, the average of *microbiologic activity* in serum was 133 mcg/mL immediately after the completion of the infusion. The other average values of serum traced according to the completed infusion time are as the following: 1 hour giving 85 mcg/mL, 4 hours giving 52 mcg/mL, and 6 hours giving 38 mcg/mL.

A.1.2. Ceftazidime

Ceftazidime usually is bactericidal in action. The study is presented via the following IV infusion over 20–30 minutes of a single 0.5- or 1-g dose of ceftazidime in healthy men; the average of peak serum concentrations of the drug at the completion of the infusion was at 42 or 69 mcg/mL. Respectively, the study is presented via the following IV infusion over 20–30 minutes of a single 2-g dose in healthy adults results in the peak serum ceftazidime concentrations at completion of the infusion that present the average at 159–185.5 mcg/mL and the serum concentrations at 0.5, 1, 2, 4, and 6 hours after completion of the infusion that average 87.9, 65.2–70.6, 38.7, 16.7–16.9, and 7.7

mcg/mL. Respectively, the study is presented via the following IV injection over 3–5 minutes of a single 0.5- or 1-g dose of ceftazidime in healthy men, the serum concentrations of the drug at 0.25, 0.5, 1, 2, 4, 6, and 8 hours after giving the dose at the average of 34.1, 24.5, 17.1, 11.2, 5.6, 2.1–2.4, and 0.9–1.3 mcg/mL, respectively, after giving the 0.5-g dose and 59.9–83.3, 45.3–60.9, 32.1–40.9, 22.9–23.2, 9.7, 4.4–5.3, and 1.9–3.2 mcg/mL, respectively, after giving the 1-g dose.

A.2. The carbapenem

A.2.1. Imipemem

Imipenem usually is bactericidal in action. Like other beta-lactam antibiotics, the antibacterial activity of imipenem results from inhibition of mucopeptide synthesis in the bacterial cell wall. Imipenem has an affinity for and binds to most penicillin-binding proteins (PBPs) of susceptible organisms, including PBPs 1a, 1b, 2, 4, 5, and 6 of *Escherichia coli*; PBPs 1a, 1b, 2, 4, and 5 of *Pseudomonas aeruginosa*; and PBPs 1, 2, 3, and 4 of *S. aureus*. In susceptible gram-negative bacteria, imipenem has the greatest affinity for PBP 2 and the lowest affinity for PBP 3. These properties result in the formation of spheroplasts or ellipsoidal cells without filament formation. Because imipenem also has a high affinity for PBPs 1a and 1b of these organisms, the spheroplasts lyse rapidly. Imipenem is able to penetrate the outer membrane of most gram-negative bacteria and gain access to the PBPs more readily than many other currently available β-lactam antibiotics.

From the following IV infusion over 20–30 minutes of a single 250-mg, 500-mg, or1-g dose of imipenem and cilastatin sodium in healthy adults with normal renal function, the peak serum concentrations of imipenem immediately following completion of the infusion range from 14–24, 21–58, and 41–83 mcg/mL, respectively, the serum concentrations are 4–6 hours after these doses decline to 1.5 mcg/mL or less. In infected adults who receive 500-mg or 1-g doses of imipenem and cilastatin sodium by IV infusion over 30–60 minutes every 6 hours, the peak serum imipenem concentrations are 19.3–38.3 or 16.7–67.3 mcg/mL, respectively, and the average of trough concentrations is at 1 or 3.1 mcg/mL, respectively.

A.3. The beta-lactam/beta-lactamase inhibitor

A.3.1. Ampicillin/sulbactam

Ampicillin sodium and sulbactam sodium usually are bactericidal in action. Concurrent administration of sulbactam does not alter the mechanism of action of ampicillin. However, because sulbactam has a high affinity for and binds to certain beta-lactamases that generally inactivate ampicillin by hydrolyzing the beta-lactam ring, concurrent administration of the drug with ampicillin results in a synergistic bactericidal effect which expands the spectrum of activity of ampicillin against many strains of beta-lactamase-producing bacteria that are resistance to ampicillin alone

Sulbactam generally acts as an irreversible inhibitor and is active against a wide range of bacterial beta-lactamases. The drug is considered a "suicide inhibitor" because the interaction between sulbactam and target beta-lactamases causes both the drug and the enzyme to be incapable of further action. Sulbactam has a much greater affinity for beta-lactamases than does ampicillin or other beta-lactam antibiotics, and the drug quickly forms an enzyme-inhibitor complex with target beta-lactamases; this complex evolves into one or more irreversibly inactivated proteins. Sulbactam inhibition of beta-lactamases is concentration and time dependent. At low sulbactam concentrations, a first-order reaction occurs and at high concentrations a zero-order reaction occurs. Results of *in vitro* studies indicate that ampicillin to sulbactam ratios of 2:1, 1:1, or 1:2 results in optimal beta-lactamase inhibition and antibacterial activity.

Sulbactam inactivates both plasmid- and chromosome-mediated beta-lactamases. *In vitro* studies indicate that sulbactam generally inhibits staphylococcal beta-lactamases and beta-lactamases can be classified as Richmond-Sykes types II, III (TEM type, HSV-1), IV, V (PSE and OXA types), and VI. The drug generally does not inhibit inducible, chromosomally mediated cephalosporinases classified as Richmond-Sykes type I, which may be produced by *Pseudomonas aeruginosa*, *Citrobacter*, *Enterobacter*, and *Serratia*. In addition to its affinity for bacterial beta-lactamases, sulbactam has an affinity for and binds to some bacterial penicillin-binding proteins (PBPs). PBPs are the target enzymes of beta-lactam antibiotics and this binding may be the mechanism of sulbactam's intrinsic antibacterial activity against some organisms. It

also may contribute to the synergistic bactericidal effect that occurs between sulbactam and ampicillin or other beta-lactam anti-infective agents. Sulbactam has a strong affinity for PBP 1a of *Proteus mirabilis* and *Escherichia coli* and PBP 2 of *Acinetobacter*. The drug has a lesser affinity for PBPs of *Staphylococcus aureus*, PBP 1a of *E. coli*, and PBP 2 of *E. coli* or *P. mirabilis*. Unlike clavulanic acid, sulbactam generally does not induce the production of type I chromosomally mediated cephalosporinases in *Pseudomonas* or Enterobacteriaceae, including *Citrobacter*, *Enterobacter*, *Morganella*, and *Serratia marcescens*.

In adults with normal renal function, peak serum concentrations of ampicillin are 40–71 mcg/mL following administration of a 1.5-g dose of ampicillin and sulbactam (1 g of ampicillin and 0.5 g of sulbactam) or 109–150 mcg/mL following a 3-g dose of the drug (2 g of ampicillin and 1 g of sulbactam); peak serum concentrations of sulbactam following these doses are 21–40 or 48–88 mcg/mL, respectively. The elimination half-life of ampicillin averaged 1.4 hours and that of sulbactam averaged 1.6 hours.

A.3.2. Amoxicillin/clavulanic acid

Amoxicillin and clavulanate potassium usually are bactericidal in action. Concurrent administration of clavulanic acid does not alter the mechanism of action of amoxicillin. However, because clavulanic acid has a high affinity for and binds to certain beta-lactamases that generally inactivate amoxicillin by hydrolyzing its beta-lactam ring, concurrent administration of the drug with amoxicillin results in a synergistic bactericidal effect which expands the spectrum of activity of amoxicillin against many strains of beta-lactamase-producing bacteria that are resistance to amoxicillin alone.

Clavulanic acid generally acts as an irreversible, competitive inhibitor of beta-lactamases. The mechanism by which clavulanic acid binds to and inhibits beta-lactamases varies depending on the specific beta-lactamase involved. Because clavulanic acid is structurally similar to penicillins and cephalosporins, it initially acts as a competitive inhibitor and binds to the active site on the beta-lactamase. An inactive acyl intermediate is then formed but it is only transiently inactive since the intermediate can be hydrolyzed, resulting in restoration of beta-lactamase activity and in release of

clavulanic acid degradation products. With many types of beta-lactamases, however, subsequent reactions occur that lead to irreversible inactivation of the beta-lactamase.

Synergism does not generally occur between amoxicillin and clavulanic acid if resistance to aminopenicillins is intrinsic (i.e., results from the presence of a permeability barrier in the outer membrane of the organism or alterations in the properties of the penicillin-binding proteins). Synergism between the drugs also does not generally occur against organisms that are susceptible to amoxicillin alone; however, a slight additive effect has been reported *in vitro* with amoxicillin and clavulanic acid against some non-beta-lactamase-producing strains of *Staphylococcus aureus* and *Haemophilus influenzae* and some strains of *Streptococcus pneumoniae* and group A beta-hemolytic streptococci. This additive effect may result from clavulanic acid's intrinsic antibacterial activity, but this activity generally is inadequate for the drug to be therapeutically used alone.

There were the studies that following up with the oral administration of a single conventional tablet containing 250 mg of amoxicillin and 125 mg of clavulanic acid in healthy, fasting adults, the peak serum concentrations of amoxicillin and of clavulanic acid averaging of 3.7–4.8 mcg/mL and 2.2–3.5 mcg/mL, respectively, and with the oral administration of a single conventional tablet containing 500 mg of amoxicillin and 125 mg of clavulanic acid in healthy, fasting adults, peak serum concentrations of amoxicillin averaging of 6.5–9.7 mcg/mL and peak serum concentrations of clavulanic acid average 2.1–3.9 mcg/mL. The medicine manufacturers state that the serum concentrations of the drugs achieved following oral administration of a single chewable tablet containing 250 mg of amoxicillin and 62.5 mg of clavulanic acid or 2 chewable tablets each containing 125 and 31.25 mg of the drugs, respectively, are similar to those achieved following oral administration of a single equivalent dose of the oral suspension. The manufacturer also states that serum concentrations of amoxicillin achieved following oral administration of conventional preparations or extended-release tablets of amoxicillin and clavulanate potassium are similar to those achieved following oral administration of equivalent doses of amoxicillin alone. Amoxicillin has an elimination half-life of 1–1.3

hours and clavulanic acid has a distribution half-life of 0.28 hours and an elimination half-life of 0.78–1.2 hours

A.3.3. Piperacillin/tazobactam

Piperacillin sodium and tazobactam sodium are a fixed combination of the sodium salts of piperacillin (an extended-spectrum penicillin antibiotic) and tazobactam (a beta-lactamase inhibitor); tazobactam synergistically expands piperacillin's spectrum of activity against many strains of beta-lactamase-producing bacteria.

Extended-spectrum penicillins reportedly vary in their rate of bactericidal action and in the completeness of this effect. This appears to result partly from differences in drug-induced morphologic effects on susceptible bacteria and on subsequent formation of bacterial variants with varying degrees of osmotic stability. *In vitro* studies using susceptible strains of *Pseudomonas aeruginosa* indicate that ticarcillin causes rapid formation of spheroplasts which are unstable and lyse rapidly whereas acylureidopenicillins cause the formation of elongated or filamentous forms of the organism which are more stable and lyse at a slower rate. Preliminary data indicate that acylureidopenicillins usually cause the formation of filamentous forms in susceptible gram-negative bacteria because these derivatives have a high affinity for penicillin-binding protein (PBP) 3 which appears to be responsible for septum formation in these organisms. Although the clinical importance of these differences in morphologic effects is unclear, results of *in vitro* studies with some bacteria (e.g., *Escherichia coli*) indicate that filamentous forms are capable of rapidly resuming growth if the penicillin is removed before the cells lyse. It has been suggested that the observation that acylaminopenicillins may not be as rapidly bactericidal as some other anti-infectives may have negative clinical implications in the use of the drugs in febrile granulocytopenic patients.

Piperacillin sodium and ticarcillin disodium are not appreciably absorbed from the GI tract and must be given parenterally Following IV injection over 2–5 minutes of a 2-g dose of piperacillin or ticarcillin in healthy adults, the average of peak serum concentrations immediately following the injection is at 199–305 mcg/mL for piperacillin and 200–218 mcg/mL for ticarcillin; serum concentrations of the drugs are low or

undetectable 6–8 hours after the injection. Piperacillin reportedly has a $t_{½}β$ of 0.6 hours after a single 1-g dose of the drug, 0.72 hours after a single 2-g dose, and 1.05 hours after a single 6-g dose.

A.3.4.Cefoperazone/sulbactam

Cefoperazone is more susceptible to inactivation by beta-lactamase than are cefoxitin, cefotaxime, cefizoxime and cefuroxime. Cefoperazone is generally resistance to inactivatation by beta-lactamases that act principally as cephalosporinases which are not generally hydrolyzed by enzymes produced by Ps.aeruginosa or S.aureus. However, cefoperazone may be inactivated by certain TEM and SHV-1 type beta-lactamases, Richmond-Sykes type 1 cephalosporinases, and type III and IV penicillinase. Cefoperazone is also generally hydrolyzed by a beta-lactamases produced by B.fagilis.

B. Fluoroquinolone

Fluoroquinolone usually is bactericidal in action. Like other fluoroquinolone anti-infectives, it inhibits DNA synthesis in susceptible organisms via the inhibition of the enzymatic activities of 2 members of the DNA topoisomerase class of enzymes, DNA gyrase and topoisomerase IV. DNA gyrase and topoisomerase IV have distinct essential roles in bacterial DNA replication. DNA gyrase, a type II DNA topoisomerase, was the first identified quinolone target; DNA gyrase is a tetramer composed of 2 GyrA and 2 GyrB subunits. DNA gyrase introduces negative superhelical twists in DNA, an activity important for initiation of DNA replication. DNA gyrase also facilitates DNA replication by removing positive super helical twists. Topoisomerase IV, another type II DNA topoisomerase, is composed of 2 ParC and 2 ParE subunits. DNA gyrase and topoisomerase IV are structurally related; ParC is homologous to GyrA but ParE is homologous to GyrB. Topoisomerase IV acts at the terminal states of DNA replication by allowing for separation of interlinked daughter chromosomes so that the segregation into daughter cells can occur. Fluoroquinolones inhibit these topoisomerase enzymes by stabilizing either the DNA–DNA gyrase complex or the DNA–topoismerase IV complex; these stabilized complexes block the movement of the DNA replication fork and thereby inhibit DNA replication resulting in cell death.

B.1.Ofloxacin

Following oral administration of a single 100-, 200-, 300-, or 400-mg dose of ofloxacin in healthy, fasting adults, the average of peak serum concentrations is at 1–1.3, 1.5–2.7, 2.4–4.6, or 2.9–5.6 mcg/mL, respectively.

B.2.Ciprofloxacin

The oral bioavailability of ciprofloxacin administered as conventional tablets is 50–85% in healthy, fasting adults, and the peak serum concentrations of the drug generally are attained within 0.5–2.3 hours. The peak serum concentrations and area under the serum concentration-time curve (AUC) increase proportionately to the dose over the oral dosage range of 250–1000 mg and are unaffected by gender. Following oral administration of a single 250-, 500-, 750-, or 1000-mg dose of ciprofloxacin as conventional tablets or oral suspension in healthy, fasting adults, the average of peak serum concentrations is at 0.76–1.5, 1.6–2.9, 2.5–4.3, or 3.4–5.4 mcg/mL, respectively; the serum concentrations are 12 hours after giving the dose at the average of 0.1, 0.2, 0.4, or 0.6 mcg/mL, respectively. In adults, oral administration of 500 mg of ciprofloxacin as conventional tablets every 12 hours results in mean peak or trough serum concentrations at steady-state of 2.97 or 0.2 mcg/mL, respectively.

Following IV infusion over 60 minutes of a single 200- or 400-mg dose of ciprofloxacin in healthy adults, the peak serum concentrations average 2.1 and 4.6 mcg/mL, respectively, immediately following the infusion; the serum concentrations 6 hours after the start of infusion (i.e., 5 hours after completion) which the averages are at 0.3 and 0.7 mcg/mL and those 12 hours after the start of infusion average 0.1 and 0.2 mcg/mL, respectively. In adults who receive 400 mg of ciprofloxacin IV every 12 hours, mean peak or trough serum concentrations at steady-state are 4.56 or 0.2 mcg/mL, respectively.

C. Aminoglycoside

Aminoglycosides are usually bactericidal in action. Although the exact mechanism of action has not been fully elucidated, the drugs appear to inhibit protein synthesis in susceptible bacteria by irreversibly binding to 30S ribosomal subunits. The plasma elimination half-lives ($t_{1/2}$s) of aminoglycosides are usually 2–4 hours in adults with normal renal function.

C.1. Gentamicin

Following IM administration of a single dose of gentamicin of 1 mg/kg in adults with normal renal function, the peak plasma gentamicin concentrations of 4–7.6 mcg/mL are attained within 30–90 minutes. When the same dose is administered by IV infusion over 2 hours, the similar peak plasma concentrations of the drug may be attained. In infants, the peak plasma gentamicin concentrations of 3–5 mcg/mL are usually attained within 30–60 minutes following a single IM dose of 2.5 mg/kg.

C.2. Amikacin

Following IM administration of a single dose of amikacin of 7.5 mg/kg in adults with normal renal function, the peak plasma amikacin concentrations of 17–25 mcg/mL are attained within 45 minutes to 2 hours; the average of plasma concentrations of the drug is 2.1 mcg/mL at 10 hours. When the same dose is administered by IV infusion over 1 hour, the average peak plasma concentrations of the drug is at 38 mcg/mL immediately following the infusion, 5.5 mcg/mL at 4 hours, and 1.3 mcg/mL at 8 hours. In one study in neonates, peak serum amikacin concentrations of 17–20 mcg/mL were attained in 30 minutes after a single IM dose of 7.5 mg/kg. In one adult with meningitis, the intrathecal administration of 4 mg of amikacin daily in conjunction with IM administration of 15 mg/kg daily for 2 weeks yielded the result in CSF concentrations of the drug ranging from 7–40 mcg/mL within 12 hours after an intrathecal dose and 1–19 mcg/mL within 24 hours after an intrathecal dose. (ASHF, 2005)

C.3. Netilmicin

Netilmicin is a semisynthetic aminoglycoside that resistant to many of the aminoglycoside modifying enzymes the inactivate gentamicin. The pharmacokinetics and the spectrum of activity of netilmicin are similar to those of gentamicin (Scholar and Pratt, 2000)

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CHAPTER III

MATERIALS AND METHODS

MATERIALS

1. Microorganisms, Chemicals and Reagents

1.1 Microorganisms

The bacterial strains used thoughout this study were *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Providencia* spp., *Pseudomonas aeruginosa* and *Acinetobacter baumannii.* These bacterials were clinical isolates from patients in Siriraj Hospital by randomization during year 2004-2005. Most microorganisms except for *A.baumannii* were high to moderately susceptible to the drugs to be studied (gentamicin, amikacin, netilmicin, ofloxacin, ciprofloxacin, amoxcillin/clavulanic acid, ampicillin/sulbactam, piperacillin/tazobactam,cefotaxime, ceftazidime, cefepime, cefoperazone/sulbactam, imipenem) as tested from disk susceptibility method, which was described by the National Committee for Clinical Laboratory Standard 2004 or NCCLS(2004).A.baumannii was multi-drug resistance to amikacin, netilmicin, ciprofloxacin, ceftazidime, imipenem and intermediate to cefoperazone/sulbactam. The selected microorganisms were sampled from 80 clinical isolates by simple random sampling to collect 1 strain of each specie for further studies in the broth macrodilution test and the time kill test.

1.2 Chemicals

- Standard powders

All antimicrobial agents standard powders were gentamicin, amikacin, netilmicin, ofloxacin, ciprofloxacin, amoxcillin/clavulanic acid, ampicillin/sulbactam, piperacillin/tazobactam,cefotaxime, ceftazidime, cefepime, cefoperazone/sulbactam, imipenem. gentamicin, ampicillin, cefotaxime from Sigma Chemical. Netilmicin, sulbactam, imipenem, cefoperazone, ciprofloxacin were purchased USP. Amikacin, ofloxacin, cefepime, clavulanic acid, amoxicillin, ceftazidime, piperacillin, tazobactam were kindly supported by Fluka Biochemika. Japan, Daiichi Pharmaceutical Co.,LTD., Bristol Myers Squibb. USA., Glaxo-Smithkline Beecham Pharmaceuticals, Wyeth-Ayerst

Reference Std. Working solutions were prepared immediately before the usage, as specified by the manufactures before adding to the working media.

1.3 Reagents

- Mueller-Hinton Broth (MHB) purchased from BBL (Becton, Dickinson, USA). It was used as the test medium for all microorganism.
- Mueller-Hinton Agar (MHA) purchased from Oxoid (Oxoid chemical, England). It was used for susceptibility test.
- Mc.Conkey agar was prepared from Mc.Conkey agar which purchased from from Oxoid (Oxoid chemical, England). It was used in for the time killing test.
- Sterile water, buffer pH 6, buffer pH 7.2 were used as the solvent for the chemical powders. (Table 3-1)

Table 3-1. Solvents and diluents for preparation of stock solutions of antimicrobial agents

antimicrobial	solvent	diluent
gentamicin	Water	
amikacin	Water	
netilmicin	Water	
ofloxacin	1/2 volume of water, then 0.1mol/L NaOH dropwise to dissolve	Water
ciprofloxacin	Water	
cefotaxime	Water	
ceftazidime	Sodium carbonate[c]	Water
amoxicillin	Phosphate buffer pH 6.0, 0.1mol/L	Phosphate buffer pH 6.0, 0.1mol/L
piperacillin	Water	
ampicillin	Phosphate buffer pH 6.0, 0.1mol/L	Phosphate buffer pH 6.0, 0.1mol/L
cefoperazone	Water	
imipenem	Phosphate buffer pH 7.2, 0.1mol/L	Phosphate buffer pH 7.2, 0.1mol/L
clavulanic acid	Phosphate buffer pH 6.0, 0.1mol/L	Phosphate buffer pH 6.0, 0.1mol/L
sulbactam	Water	
tazobactam	Water	

- 0.9% Sterile normal saline (0.9%NSS) was chosen as the diluent of specimens in colony counting procedures of time kill method.

- A $BaSO_4$ 0.5 McFarland standard

To standardize the inoculum density for a susceptibility test, $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard should be used. A $BaSO_4$ 0.5 McFarland standard may be prepared as the following:

A 0.5 ml aliquot of 0.048 mol/L $BaCl_2$ (1.175 % w/v $BaCl_2 . 2H_2O$) was added to 99.5 ml of 0.18 mol/L H_2SO_4 (1% v/v) with constant stirring to maintain a suspension.

The correct density of the turbidity standard should be verified by using a spectrophotometer with a 1-cm light path and matched cuvette to determine the absorbance. The absorbance at 625 nm should be ranged from 0.08 to 0.10 for the 0.5 Mc.Farland standard.

The barium sulfate suspension should be transferred in 4 to 6 ml aliquots into screw-cap tubes of the same size as those used in growing or diluting the bacterial inoculum.

These tubes should be tightly sealed and should be stored in the dark at room temperature.

The barium sulfate turbidity standard should be vigorously agitated on a mechanical vortex mixer before each usage and should be inspected for a uniformly turbid appearance. If large particles appear, the standard should be replaced.

The barium sulfate standards should be replaced or their densities should be monthly verified.

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2. Laboratory Equipment

2.1 Disposable Equipment

- Cotton plugs.
- Aluminum foil.

2.2 Sterile Glass Equipment

- Petri dishes.
- Erlenmeyer flasks 50 mL.
- Glass tubes.
- Pipettes were used in experiment divided into 2 types

  Glass pipettes.

  Micropipette.

2.3 General Equipment

- Chemical spoons.
- General loop.
- Tube rack.

3. Laboratory Instruments

3.1 Temperature Controlling Instruments

- Autoclave was used to sterilize equipment, media, diluent, inoculum, Tygon tube and others throughout this experiment for sterile condition.
- Deep freezer at -80 ^{0}C were used to preserve stock solution of antibiotics before used in all experiment and to maintain antibiotic activity during the research before using in bioassay method.
- Incubator was used to provide the appropriate environmental condition for bacterial growth throughout the procedures such as subculture, bioassay process, inoculum preparation, etc.
- Hot air ovens were used to keep drying and to sterilize all glass equipment before usage.
- Water bath shaker was used to apply appropriate bacterial growth condition of liquid media that simulate human body temperatures in the time killing method.

3.2 General Instruments

- Chemical scale was selected for weighting media and standard powder of antimicrobial agent in preparing procedures of both test media and working standard solutions.
- Spectrophotometer, A-JUST™ turbidity meter of Abbott Laboratories, U.S.A., was applied to adjust turbidity of the inoculum to equivalent with 0.5 McFaland standard solution and 1.0 McFaland standard solution.
- Mechanical vortex mixer was used to mix 0.5 McFaland standard, working standard solutions, which result to homogeneity of suspension before using for further procedures in the experiment.

METHODS

1. Disk diffusion test to determine susceptibility pattern of all microorgranisms.
2. Broth Macrodilution Method to determine minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of gentamicin, amikacin, netilmicin, ofloxacin, ciprofloxacin, amoxicillin/clavulanic acid, ampicillin/sulbactam, piperacillin/tazobactam, cefotaxime, ceftazidime, cefoperazone/sulbactam and imipenem to the selected microorganisms.
3. Time Kill Method to investigate bactericidal activity of gentamicin, amikacin, netilmicin, ofloxacin, ciprofloxacin, amoxicillin/clavulanic acid, ampicillin/sulbactam, piperacillin/tazobactam, cefotaxime, ceftazidime, cefoperazone/sulbactam and imipenem against to the selected microorganisms.

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1. Disk Diffusion Procedures

1.1 Preparation Agar Plate

1.1.1 MHA was prepared from the commercially available dehydrated base according to the manufacture's instruction.

1.1.2 Immediately after autoclaving, allow it to cool at the temperature between 45 to 50 °C

1.1.3 Pour the freshly prepared and cooled medium into glass, flat-bottomed petri dishes on a level, horizontal surface to give a unifrom depth of approximately 4 mm. This process corresponds to 25 to 30 mL for plates with a diameter 100 mm.

1.1.4 The agar medium should be allowed to cool at a room temperature and than it should be kept in refrigerator (2 to 8 °C).

1.1.5 Medium plates should be used within 7 days after preparation and a representative sample of each batch of plates should be examined for sterility by incubating at 30 to 35 °C for 24 hours.

1.2 Inoculum preparation

1.2.1 At least three or five well-isolated colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched within loop, and the growth was transferred into a tube containing 4 to 5 mL of a suitable broth medium

1.2.2 The broth culture was incubated at 35 °C until it achieved or exceeded the turbidity of the 0.5 Mc.Farland standard (usually 2 to 6 hours)

1.2.3 The turbidity of the actively growing broth culture was adjusted with sterile saline or broth to obtain turbidity optically comparable to that of the 0.5 Mc.Farland standard. This attempt resulted in a suspension containing approximately 1 to 2 x 10^8 CFU/mL for E.coli ATCC 25922. To perform this step properly, a photometric device can be used.

1.3 Inoculum 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 times and pressed

firmly on the inside wall of the tube above the fluid level. This process will remove excess inoculum from the swab.

1.3.2 The dried surface of a MHA plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking by two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of agar was swabbed.

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

1.4 Application of Disks to inoculated agar plates

1.4.1 The predetermined battery of antimicrobial disks was dispensed onto the surface of the inoculated agar plate. Each disk must be pressed down to ensure complete contact with agar surface. They must be distributed evenly so that they are no closer than 24 mm from center to center. Because some of the drug diffuses almost instantaneously, a disk should not be relocated once it has come into contact with the agar surface. Instead, place a new disk in another location on the agar.

1.4.2 The plates were inverted and placed in an incubator which the temperature was set at 35°C within 15 minutes after the disks were applied in ambient air. Because of the interpretive standards were developed by using ambient air incubation, CO_2 will significantly alter the size of the inhibitory zones of some agents.

1.5 Reading Plates and Interpreting Results.

1.5.1 After 16 to 18 hours of incubation, each plate was examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. The diameters of the zones of complete inhibition (as judged by the unaided eye) were measure, including the diameter of the disk. Zones were measured to the nearest whole millimeter by using a

ruler, which was held a few inches above a black, nonreflecting background and illuminated with reflected light.

1.5.2 The zone margin should be taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of zone of inhibited growth, was ignored. However, discrete colonies growing within a clear zone of inhibition should be subcultured, re-identified, and retested.

1.5.3 The size of the inhibition zone were interpreted by referring to NCCLS, 2004 and the organisms were reported as either susceptible, intermediate, or resistant to the agents that have been tested (Table 3-2)

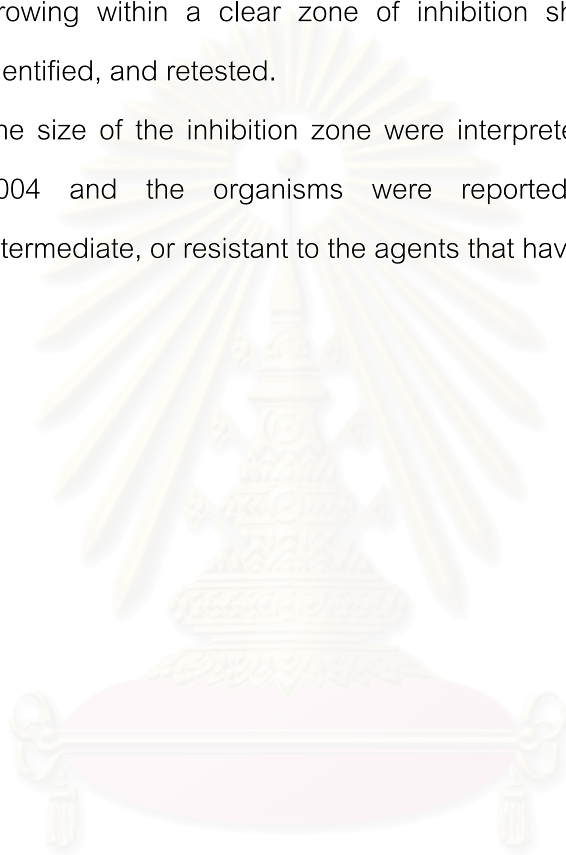

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Table 3-2. Zone diameter interpretive standard breakpoints for Enterobacteriaceae

drug	Disk content (mcg)	Zone diameter			comment
		R[a]	I[b]	S[c]	
amikacin	10	≤ 14	15-16	≥ 17	**
amox/calv.	20/10	≤ 13	14-17	≥ 18	
ampi/sul	10/10	≤ 11	12-14	≥ 15	**
cefepime	30	≤ 14	15-17	≥ 18	**
cefotaxime	30	≤ 14	15-22	≥ 23	**
cefoperazone	75	≤ 15	16-20	≥ 21	**
cefoxitin	30	≤ 14	15-17	≥ 18	
ceftazidime	30	≤ 14	15-17	≥ 18	**
ceftriazone	30	≤ 13	14-20	≥ 21	**
ciprofloxacin	5	≤ 13	16-24	≥ 21	**
colistin	10	≤ 8	9-10	≥ 11	
gentamicin	10	≤ 12	13-14	≥ 15	**
imipenem	10	≤ 13	14-15	≥ 16	**
levofloxacin	5	≤ 13	14-16	≥ 17	**
ofloxacin	5	≤ 12	13-15	≥ 16	
piperacillin/tazobactam	100/10	≤ 17	18-20	≥ 21	*
		≤ 17	-	≥ 18	***
netilmicin	30	≤ 12	13-14	≥ 15	**

* plus *Acinetobacter* spp.

** plus *Acinetobacter* spp. and *Pseudomonas* spp.

*** plus *Pseudomonas* spp.

a = resistance

b = intermediate

c = susceptible

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2. Broth Macrodilution Procedures

2.1 Test Broth

2.1.1 MHB was recommended as the medium of choice for the susceptibility testing of commonly isolated such as Enterobacteriaceae.

2.2 Diluted Antimicrobial Agents Preparation

2.2.2 Standard powder of antimicrobials were dissolved in sterile water for injection to final concentration approximately 1,000 μg/ml. All stock solution was aliquot to 1 ml and stored in the refrigerator at -80 ^{O}C before usage. The quality control strains used for all tests were E.coli ATCC 25922 and P.aeruginosa ATCC 27853. All results were within published ranges. The MICs of the control strains were shown in Table 3-3

Table 3-3. Acceptable Limits for Quality Control Strains used to monitor accuracy of minimal inhibitory concentrations (MICs) (mcg/mL) (NCCLS.2004)

Antimicrobial agents	E.coli ATCC 25922	P.aeruginosa ATCC 27853
Amikacin	0.5 - 4	1 - 4
Amoxicillin/clavulanic acid	2/1 - 8/4	ND
Ampicillin/sulbactam	2 - 8	ND
Cefoperazone(alone)	0.12 - 0.5	2 - 8
Cefotaxime	0.03 - 0.12	8 - 32
Ceftazidime	0.06 - 0.5	1 - 4
Ciprofloxacin	0.004 - 0.016	0.25 - 1
Gentamicin	0.25 - 1	0.5 - 2
Imipenem	0.06 - 0.25	1 - 4
Netilmicin	0.5 - 1	0.5 - 8
Ofloxacin	0.015 - 0.12	1 - 8
Piperacillin/tazobactam	1/4 - 4/4	1/4 - 8/4

ND = not determined.

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2.2.3 Sterile 13- x10-mm test tubes should be used to conduct the test.

2.2.4 A control tube containing broth without antimicrobial agent was used for each organism tested.

2.2.5 The tube can be closed with cotton plugs.

2.2.6 The final twofold dilutions of antimicrobial agents were prepared volumetrically in the broth. Because final volume of 1.0 ml in each tube consisted of the 0.5 ml of broth containing antimicrobial and the 0.5 ml of broth containing a suspension of the organisms to be tested. Thus antimicrobial concentrations used in the initial (stock) solutions should be prepared four-fold in greater than the desired final concentration. The concentrations tested for each antimicrobial typically range from 4 to 5 below the MIC to twice the MIC or higher.(Table 3-4)

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Table 3-4. Scheme for preparing dilutions of Antimicrobial agents to be used in broth dilution susceptibility test (NCCLS, 2004)

Antimicrobial Solution					
Step	Concentration	source	volume	CAMHB volume	Final concentration
1	5120 mcg/mL	stock	1mL	9mL	512 mcg/mL
2	512	step1	1	1	256
3	512	step1	1	3	128
4	512	step1	1	7	64
5	64	step4	1	1	32
6	64	step4	1	3	16
7	64	step4	1	7	8
8	8	step7	1	1	4
9	8	step7	1	3	2
10	8	step7	1	7	1
11	1	step10	1	1	0.5
12	1	step10	1	3	0.25
13	1	step10	1	7	0.12

Note: this table id modified from Ericsson HM, Sherris JC. Antibiotic sensitivity testing. Report of an international collaborative study. Acta Pathol Microbiol Scand. 1971;217 (suppl B): 1-90

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2.3 Broth Dilution Testing

2.3.3 Inoculum preparation

A standardized inoculum for the macrodilution broth method may be prepared by either growing microorganisms or suspending colonies directly to obtain the turbidity of the 0.5 McFarland standard.

2.3.3.1 Growth Method

- At least three to five well-isolated colonies of the same morphological type of all organisms were selected from an agar plate culture. The top of each colony was touch with a loop, and the growth was transferred into a tube containing 4 to 5 ml of a test broth medium.
- The broth culture was incubated at 37 ^{O}C until it achieves or exceeded the turbidity of the 0.5 Mc Faland standard (usually 2 to 6 hours).
- The turbidity of the actively growth broth culture was adjusted with sterile saline or broth to obtain turbidity optically comparable to that of the 0.5 McFaland standard. This result in a suspension containing approximately 1 to 2X10^{8}CFU/ml. A-JUST^{TM} turbidity meter of Abbott Laboratories, U.S.A. is a photometric device used to perform this step property.

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2.3.3.2 Within 15 minutes after the inoculum has been diluted, 0.5 ml of the adjusted inoculum was added to each tube containing the dilution series of antimicrobial agents and the positive control tube containing only broth without antimicrobial agents, each tube was mixed. This process yields the result in a 1:2 dilution of each antimicrobial concentration and 1:2 dilution of the inoculum. Clearly, the inoculum standard established by NCCLS is 5x10^{5} CFU/ML final concentration for broth dilution.

2.3.3.3 The inoculated macrodilution tubes should be incubated at 37 ^{O}C for 16 to 24 hours in an ambient air incubator.

2.3.3.4 Determining MIC End Points

The MIC is the lowest concentration of antimicrobial agents that completely inhibit the growth of the organisms in the tubes as detected by the unaided eye. The amount of growth in the tubes containing the antimicrobial should be compared with the amount of growth in the growth-control tubes (no antimicrobial) used in each set of the tests when determining the end points of the growth.

2.4 Determining MBC End Points

After record the MIC end point, mix again on a vortex mixer and the sample tubes for MBC determination; spread 100 μL samples across the surface of dried TS agar plate with sterile, bent, glass rods.

2.4.1 Incubate plates overnight at 37 ^{o}C for the MBC test.

2.4.1.1 After 1 day (or 2 days), count the number of colonies per plate and average. Determine a colony count that represents 0.1% of the original inoculum (i.e. 99.9%reduction)Count colonies from MBC plates. Any number which is equal to or less than the determined colony count from step 2.3.3.4 is considered as a 99.9%killing or bactericidal result. (Amsterdam D., 1996, Dudley MN, 1997)

2.5 Determining inoclulum effect

For determining inoculum effect, it had been used inoculum higher than standard inoculum (e.g. final concentration of inoculum is $5x10^{7}$ CFU/mL). The inoculum effect can be defined as a significant increased MIC (plus 2 dilutions) when the inoculum size is increased (at least by 0.5 log unit). (Amsterdam D., 1996, Dudley MN, 1997)

3. Time kill method

3.1 Prepare all antimicrobial agent concentration at 1/2MIC, MIC, 4MIC, 8MIC, trough and peak that referred to pharmacokinetic achievable concentration from previously articles (AHSF Drugs information, 2004) to study effect of

concentration on eradication and then prepare concentration at every 0, 2, 4, 6, 8 and 24 hours to effect of the duration of exposure on eradication.

3.2 Dilute the standard inoculum to obtain the final bacterial quantity $5x10^6$ CFU/mL into working media and then control tubes containing broth without antimicrobial agent on water bath shaker at 37 °C

3.3 Collect the samples to detect for colony forming unit at the time 0, 2, 4, 6, 8, and 24 hours after microorgranism exposed to drug in each concentration include the control group.

3.4 Incubate the sample on appropriate solid media for 16 to 18 hours at 37 °C to detect for colony forming unit.

3.5 Calculate the quantity of survival bacteria in each group to obtain the killing curves data.

3.6 Killing curves were constructed by Microsoft Excel 97. The criteria to define the bactericidal property is the decreasing in colony forming unit from the origin point $\geq$ 3 log CFU/mL at 24 hours of exposure. The regrowth is defined as an increase of $\geq$ 2 log CFU/mL after 6 hours. (Amsterdam, 1996, Peterson LR and Shanholtzer CJ.,1992) The qualitative evaluation of antimicrobial effect was calculated as in the published article (Firsov, et al., 1997).

The Quantitative Evaluation of Antimicrobial Effect (Firsov, et al., 1997)

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

$T_{90\%}$ = The time to reduce the initial inoculum 10 fold (90% kill of the inoculum).

$T_{99\%}$ = The time to reduce the initial inoculum 100 fold (99% kill of the inoculum).

$T_{99.9\%}$ = The time to reduce the initial inoculum 1000 fold (99.9% kill of the inoculum).

T_E = The time shift between the normal growth and the re-growth curves

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

N_{min} = The minimum number of bacteria resulting from exposure to antibiotic.

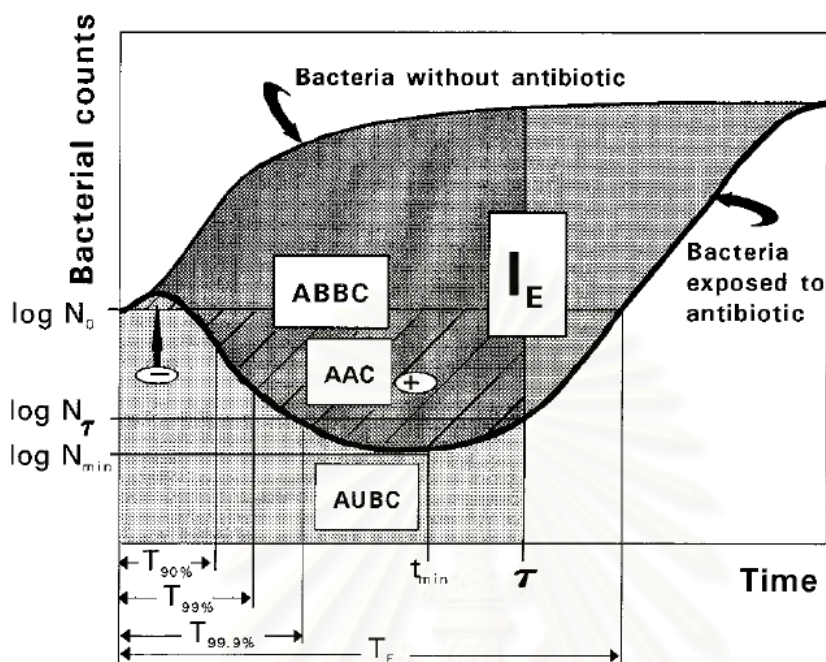

Figure 3-1. Parameters for quantitative bacterial killing and regrowth curves and the antimicrobial effect.

The special parameter T is the time at the end of administration period that usually mimicked the dosing interval. This data referred to the registered monograph of each agent, which was approved by the Food and Drugs Administration of Thailand. The N_T was determined by extrapolation of killing curves as shown in the Figure.

T = The time at the end of the administration period that usually mimicked the dosing interval.

N_T = The number of viable counts at the end of administration period that usually mimicked the dosing interval.

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

Δ log CFU 2 hours = The difference between the number of viable counts at time zero versus the number of viable counts after exposed to antimicrobial for 2 hours.

Δ log CFU 4 hours = The difference between the number of viable counts at time zero versus the number of viable counts after exposed to antimicrobial for 4 hours.

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

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

Δ log CFU 6 to 24 hours = The difference between the number of viable counts at the 6th hours versus the number of viable counts after exposed to antimicrobial for 24 hours.

3.8 The following parameters were calculated by various methodologies as followed:

AUC 24 hours = Area under the control curve or the bacterial killing and regrowth curves that calculated by the trapezoidal rule which is generally accepted as a standard method to determine the AUC for the pharmacokinetic model.

Bateriolytic area for 24 hours = The area among control growth curve, and the bacterial killing and regrowth curves (AUC24 of the control growth curve subtracted by AUC24 of the bacterial killing and regrowth curve).

CHAPTER IV

RESULT

Susceptibility Testing

Susceptibility testing, the MICs and MBCs of all antimicrobial agents against selected strains were presented in Table 4-1.

Gentamicin had the low MIC for *E.coli, P.mirabilis* (MIC= 1mcg/mL) and *K.pneumoniae* (MIC=0.5mcg/mL) and the bactericidal activity of gentamicin is confirmed by the observation of MBC higher than MIC for twofold in a selected strains.

Amikacin had the lower MIC for *Providencia* spp. (MIC=1mcg/mL). Amikacin against *K.pneumoniae* and *P.mirabilis* had the same MIC (MIC=2mcg/mL). Amikacin against *E.coli* and *P.aeruginosa* had same MIC (MIC = 4mcg/mL). Amikacin presented equal MBC to MIC for *K.pneumoniae*. But the MBC was higher than MIC for twofold in *Providencia* spp. The MBC/MIC ratio was higher than four which could be found in *P.aeruginasa*, *E.coli,* and *P.mirabilis*.

Netilmicin had lower MIC for *Providencia* spp. (MIC = 0.015mcg/mL) but could not determine both MIC and MBC for *A.baumannii*. (MIC, MBC > 8192mcg/mL)

For fluoroquinolone, **ofloxacin** MBC of ofloxacin was equal to MIC value (MIC, MBC = 0.06mcg/mL)

Ciprofloxacin had the same MIC for *E.coli* and *Providencia* spp. (MIC = 0.015mcg/mL). For the other organism, the MIC had increased in series of dilution, started from *P.mirabilis*, *K.pneumoiae* to *P.aeruginosa*. All strains presented at the level higher than MIC for twofold, except for in *A.baumannii* which was highest in MIC and its MBC was higher than MIC for one fold.

Cefotaxime had the same MIC (MIC=0.06mcg/mL) for *E.coli* and *K.pneumoniae*. The lowest MIC found in *P.mirabilis* (0.015mcg/mL) and the highest value could be found in *Providencia* spp. (0.12mcg/mL). Cefotaxime showed the MBC higher than MIC for twofold in *E.coli, K.pneumoniae,* and *P.mirabilis*, but *Providencia* spp presented 33 fold.

Ceftazidime had low MIC for *P.aeruginosa* (MIC= 2mcg/mL) but *A.baumannii* had high MIC (MIC=128mcg/mL), however, had equal value of MBC and MIC.

Amoxicillin/clavulanic acid, the MBC was equal to MIC value (MIC, MBC = 4/2 mcg/mL)

Ampicillin/sulbactam had low MIC for *E.coli* (MIC=8/4mcg/mL) but high MIC for *A.baumannii* (MIC=64/32mcg/mL). The MBC was equal MIC in *E.coli* and higher than MIC for twofold in *A.baumannii*.

Piperacillin/tazobactam was low in MIC in *E.coli, K.pneumoniae, P.mirabilis*, and *P.aeruginosa* (MIC = 1/2, 2/2, 0.25/2, 4/2 mcg/mL) and the MBC was equal to MIC for all microorganism.

Cefoperazone/sulbactam had low MIC for *E.coli* (MIC=1mcg/mL) and high MIC for *A.baumannii*. (MIC= 32mcg/mL) The MBC was equal to MIC for both.

Imipenem had low MIC for *E.coli, K.pneumoniae, P.mirabilis, Providencia* spp. and *P.aeruginosa*. The MBC was equal to MIC for *E.coli*, and *K.pneumoniae*. The MBC was higher than MIC for twofold in *Providencia* spp and P. mirabilis. Higher in MBC than in MIC 16 fold could be found in *P.aeruginosa*. The MIC of *A.buamannii* was high (MIC = 32mcg/mL) and the MBC was equal to MIC.

The bactericidal activity of gentamicin, amikacin, ofloxacin, ciprofloxacin, cefotaxime, amoxicillin/clavulanic acid, piperacillin/tazobactam, ampicillin/sulbactam, cefoperazone/sulbactam, and imipenem are confirmed by observation of MBC is $\leq$ 4 time than the MIC against selected strains.

Imipenem had only bacteriostatic activity against *P.aeruginosa* that presented by the MBC was 16 times to MIC value.

Inoculum effect

In this study, increasing the inoculum size from10^5 to 10^7 CFU/mL had no significant effect on activity of gentamicin against single isolate of *E.coli*, *K.pneumoniae,* and *P.mirabilis* in this study. (Table 4- 2)

Amikacin against *E.coli*, *P.mirabilis,* and *Providencia* spp should not have inoculum effect.

Netilmicin against *Providencia* spp, *P.aeruginosa* and *A.buamannii*, should not have inoculum effect.

Ofloxacin against *E.coli* should not have inoculum effect.

Ciprofloxacin against *E.coli*, *K.pneumoniae, P.mirabilis, Providencia* spp, *P.aeruginosa* and *A.buamannii* should not have inoculum effect.

Cefotaxime against *E.coli*, and *P.mirabilis* had inoculum effect that confirmed by the MIC was increased for 8.3 fold and 33 fold, respectively. But cefotaxime should not have inoculum effect against *K.pneumoniae, Providencia* spp, *P.aeruginosa* and *A.buamannii*.

Ceftazidime against *P.aeruginosa* and *A.buamannii*, should not have inoculum effect.

Amoxicllin/clavulanic acid against *E.coli* should not have inoculum effect.

Piperacillin/tazobactam against *P.mirabilis*, and *P.aeruginosa* had inoculum effect that confirmed by the MIC was increased for eight fold.

Ampicillin/sulbactam against *E.coli*, had inoculum effect that confirmed by the MIC was increased for eight fold.

cefoperazon/sulbactam against *E.coli*, had inoculum effect that confirmed by the MIC was increased for eight fold.

Imipenem against *Providencia* spp. and *P.aeruginosa*, had inoculum effect that confirmed by the MIC was increased for 8.3 fold and eight fold.

Time-kill study

From the time kill studies, the results from the studies are as the following :

Gantamicin

In the time kill assay (as shown in Figure 4-1to 4-3 and Table 4-3 to 4-5), gentamicin showed concentration-dependent killing at concentration just above MIC (4MIC, 8MIC) and the peak concentration at standard and high dose (7mcg/mL, 20mcg/mL) against *E.coli*, *K.pneumoniae,* and *P.mirabilis.* The 99.9% killing in < 2 hours(T99.9% = 0.91 -1.08 hr) without bacterial regrowth at 24 hours.

Amikacin

In the time kill assay (as shown in Figure 4-4 to 4-7 and Table 4-6 to 4-9), amikacin showed concentration-dependent killing at concentration just above MIC (4MIC, 8MIC) and the peak concentration at standard and high dose (38 mcg/mL, 60mcg/mL) against *E.coli*, *K.pneumoniae, Providencia* spp and *P.aeruginosa.* The 99.9% killing in < 2 hours (T99.9% = 1.2 - 2 hr) without bacterial regrowth at 24 hours.

Netilmicin

In the time kill assay (as shown in Figure 4-8 to 4-9 and Table 4-10 to 4-11), netilmicin showed concentration-dependent killing at concentration just above MIC (4MIC, 8MIC) and the peak concentration at standard and high dose (7mcg/mL, 20mcg/mL) against *Providencia* spp and *P.aeruginosa.* The 99.9% killing in 2 to 4 hours (T99.9% = 1.41-3.16 hr) without bacterial regrowth at 24 hours.

Ofloxacin

As presented in Figure 4-10 and Table 4-12, ofloxacin against *E.coli* at the first 2 hours, most of the concentrations could reach 99.9% killing except for the ½ MIC (0.015mcg/mL) and MIC (0.03mcg/mL). Then at the sixth hour, the MIC could reach 99.9% killing and the killing ability still existed during 24 hour period after taking the medicine.

Ciprofloxacin

As illustrate in Figure 4-11 to 4-16 and Table 4-13 to 4-18, ciprofloxacin showed concentration-dependent killing at concentration. The concentration just above MIC (4MIC, 8MIC) and peak oral (2.1mcg/mL), peak IV (2.4mcg/mL), peak IV high dose (4.8mcg/mL) of ciprofloxacin against *K.pneumoniae, P.mirabilis*, and *Providencia* spp. The 99.9% killing in < 2 hour (T99.9% = 1.5 - 2 hr) without bacterial regrowth at 24 hours. Anyway, ciprofloxacin against *P.aeruginosa* should have 99.9% killing at the first 2 hour at the concentration of the 4MIC, 8MIC and all peaks. But there were regrowth except for the peak IV and the peak IV high dose.

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The peak IV and the peak IV high dose of ciprofloxacin against E.coli could kill 99.9% within the first 2 hour and without regrowth during 24 hours. Moreover, the 4MIC and 8MIC could kill 99.9% within the first 2 hour and without regrowth during 24 hours.

Cefotaxime

As demonstrated in Figure 4-17 to 4-20 and Table 4-19 to 4-22, cefotaxime showed time-dependent killing at concentration. The killing started at the MIC, 4MIC, 8MIC, trough and the peak concentration at standard and high dose against *E.coli*, *K.pneumoniae,*and *P.mirabilis*. The 99.9% killing in 6 hours (T99.9% = 1.3 - 6 hr) without bacterial regrowth at 24 hours. Nevertheless, the 8MIC of cefotaxime against *Providencia* spp. could reach 99.9% killing in the 6th hour and the killing ability still existed during 24 hour period after taking the medicine.

Ceftazidime

As demonstrated in Figure 4-21 to 4-22 and Table 4-16 to 4-17, ceftazidime presented time-dependent killing at concentration. The killing started at more over MIC, (4MIC, 8MIC) and the peak concentration at standard and high dose against *P.aeruginosa*, although, the peak high dose of ceftazidime against *A.baumannii*. The 99.9% killing in 6 hours (T99.9% = 1.84 – 5.3 hr), however, there should have bacterial regrowth at 24 hours.

Amoxicillin/clavulanic acid

In Figure 4-23 and Table 4-32, amoxicillin/clavulanic acid against *E.coli* at the first 2 hour, all concentrations could not reach 99.9% killing. Then at the sixth hour, the 4MIC, 8MIC and peak high dose (8.1/3mcg/mL) could reach 99.9% killing. At 24th hour, the 4MIC, 8MIC, and peak high dose still could reach 99.9% killing.

Ampicillin/sulbactam

In Figure 4-24 and Table 4-26, ampicilin/sulbactam against *E.coli* at the first 2 hour, most of the concentrations could not reach 99.9% killing except for the 4MIC(32/16 mcg/mL). Then at the sixth hour, the 4MIC and peak high dose (130/68mcg/mL) could reach 99.9% killing. At 24th hour, the 8MIC and peak high dose still reached 99.9% killing but the other concentration could not reach 99.9% killing and the 4MIC had regrowth.

As presented in Figure 4-25 and Table 4-27, ampicillin/sulbactam against *A.buamannii* at the first 2 hour, most of the concentrations could not reach 99.9% killing but the 8MIC (512/256mcg/mL). Then at the sixth hour, the 4MIC and 8MIC could reach 99.9% killing. At 24th hour, the 4MIC, 8MIC, and peak high dose still reached 99.9% killing but the 4MIC had regrowth.

Piperacillin/tazobactam

In Figure 4-26 and Table 4-28, piperacillin/tazobactam against *E.coli* at the first 2 hour, all concentration could not reach 99.9% killing. Then at the sixth hour, the peak (120mcg/mL) could reach 99.9% killing. At 24th hour, almost of concentrations could not reach 99.9% killing except for the peak which had still could reach.

As presented in Figure 4-27 and Table 4-29, piperacillin/tazobactam against *K.pneumoniae* at the first 2 hour, all concentrations could not reach 99.9% killing. Then at the fourth hour, the 4MIC (8mcg/mL), 8MIC(16mcg/mL), trough(35mcg/mL), trough high dose(64mcg/mL), peak(120mcg/mL) and peak high dose(237mcg/mL) could reach 99.9% killing and still kill during 24 hour period after taking the medicine.

In Figure 4-28 and Table 4-30, piperacillin/tazobactam against *P.mirabilis* at the first 2 hour, all concentrations could not reach 99.9% killing. Then at the fourth hour, trough (35mcg/mL), trough high dose (64mcg/mL), peak (120mcg/mL) and peak high dose (237mcg/mL) reached 99.9% killing and at the sixth hours, all concentration reached 99.9% killing exception in the ½ MIC and still killed during 24 hours.

In Figure 4-29 and Table 4-31, piperacillin/tazobactam against *P.aeruginosa* at the first 2 hour, all concentrations could not reach 99.9% killing. Then at the fourth hour, 8MIC (32mcg/mL) could reach 99.9% killing and still could kill during 24 hour period after taking the medicine.

Cefoperazone/sulbactam

As shown in Figure 4-30 and Table 4-32, cefoperazone/sulbactam against *E.coli* at the first 2 hour, the peak (153mcg/mL) and peak high dose (256mcg/mL) could reach 99.9% killing. Then at the sixth hour, the 4MIC (4mcg/mL), 8MIC (8mcg/mL) could reach 99.9% killing and the other concentrations still could kill. At the 24th hour, the 4MIC, 8MIC, peak, and peak high dose still could reach 99.9%killing but the others had regrowth.

In Figure 4-31 and Table 4-33, cefoperazone/sulbactam against *A.baumannii* at the first 2 hour, the 4MIC (128mcg/mL), 8MIC (256mcg/mL), the peak (153mcg/mL) and peak high dose (252mcg/mL) could reach 99.9% killing. At the sixth hour and the 24th hour, it did not change from the first 2 hour.

Imipenem

As illustrate in Figure 4-32 to 4-37 and Table 4-34 to 4-39, the imipenem presented the time-dependent killing. The concentration just above MIC (4MIC, 8MIC) and peak (28.8mcg/mL), peak high dose (42mcg/mL) of ciprofloxacin against *K.pneumoniae*,*P.mirabilis*, and *Providencia* spp. The 99.9% killing presented in 6 hour (T99.9% = 1.16 - 4 hr) without bacterial regrowth at 24 hours. Anyway, the concentration of the MIC, 4MIC, 8MIC and all peaks of imipenem against *E.coli* should have 99.9% killing at the first 6 hour and should be no regrowth. The peak and the peak high dose of imipenem against *P.aeruginosa* could kill 99.9% within the 6th hour and without regrowth during 24 hours (T99.9% = 2.5 hr). Moreover, the 4MIC and 8MIC of imipenem against *A.baumannii* could kill 99.9% within the 6th hour and without regrowth during 24 hours. (T99.9% = 1.66 hr)

Table 4-1 The MIC, MBC (mcg/mL) of selected antibiotics against selected organism and bactericidal activity[a].

	E.coli			*K.pneumoniaea*			*P.mirabilis*			*Providencia spp.*			*A.buamannii*			*P.aeruginosa*		
antimicrobial	MIC μg/mL	MBC μg/mL	MBC/ MIC	MIC μg/mL	MBC μg/mL	MBC/ MIC	MIC μg/mL	MBC μg/mL	MBC/ MIC	MIC μg/mL	MBC μg/mL	MBC/ MIC	MIC μg/mL	MBC μg/mL	MBC/ MIC	MIC μg/mL	MBC μg/mL	MBC/ MIC
gentamicin	1	2	2	0.5	2	4	1	2	2									
amikacin	4	16	4	2	2	1	2	8	4	1	2	2	8192	>8192	1	4	8	2
netilmicin										0.5	1	2	>8192	>8192	1	4	8	2
ofloxacin	0.06	0.06	1															
ciprofloxacin	0.015	0.03	2	0.12	0.25	2	0.03	0.06	2	0.015	0.03	2	128	128	1	0.25	0.5	2
cefotaxime	0.06	0.12	2	0.06	0.12	2	0.015	0.03	2	0.25	0.25	1						
ceftazidime													128	128	1	2	2	1
amox/clav.	4/2	4/2	1															
pip/taz	1/2	1/2	1	2/2	2/2	1	0.25/2	0.25/2	1									
ampi/sul	8/4	8/4	1										64/32	128/64	2			
cfp/sul	1	1	1										32	32	1			
imipenem	2	2	1	1	1	1	4	8	2	0.12	0.25	2	32	32	1	1	16	16

bactericidal activity[a] define as a ratio of MBC/MIC $\leq$ 4

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Table 4-2. The inoculum effect[c] of the selected antibiotics against selected organism.

Antimicrobial	E.coli			K.pneumoniaea			P.mirabilis			Providencia spp.			A.buamannii			P.aeruginosa		
	MIC[a] μg/mL	MIC[b] μg/mL	Fold of MIC	MIC[a] μg/mL	MIC[b] μg/mL	Fold of MIC	MIC[a] μg/mL	MIC[b] μg/mL	Fold of MIC	MIC[a] μg/mL	MIC[b] μg/mL	Fold of MIC	MIC[a] μg/mL	MIC[b] μg/mL	Fold of MIC	MIC[a] μg/mL	MIC[b] μg/mL	Fold of MIC
gentamicin	1	2	2	0.5	0.5	1	1	2	2									
amikacin	4	16	4	2	4	2	2	8	4	8	8	1	8192	8192	1	4	8	2
netilmicin										0.5	1	2	>8192	>8192	1	4	16	4
ofloxacin	0.06	0.06	1															
ciprofloxacin	0.015	0.03	2	0.12	0.3	2	0.03	0.03	1	0.015	0.015	1	128	128	1	0.25	1	4
cefotaxime	0.06	0.5	8.3	0.06	0.1	1	0.015	0.5	33.33	0.25	0.25	1			1			
ceftazidime													128	128		2	8	4
amox/clav.	4/2	8/4	2															
pip/taz	1/2	2/2	2	2/2	2/2	1	0.25/2	4/2	16							4/2	32/2	8
ampi/sul	8/4	64/32	8										64/32	128/64	2			
cfp/sul	1	8	8										32	64	2			
imipenem	2	2	1	1	1	1	4	4	1	0.12	1	8.3	32	64	2	1	8	8

MIC[a] means the MIC of antibiotics against selected organism at the inoculum size at 10^5 CFU/mL

MIC[b] means the MIC of antibiotics against selected organism at the inoculum size at 10^7 CFU/mL

Inoculum effect[c] defines as the fold of MIC > 4

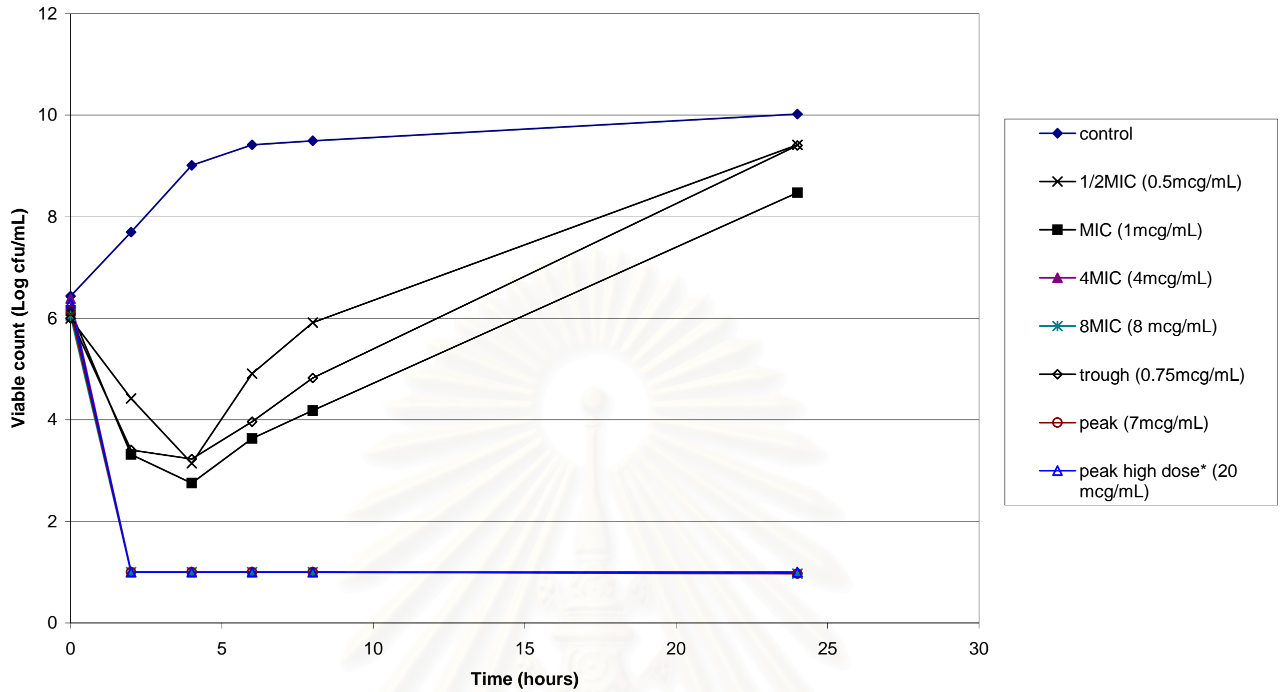

Figure. 4-1 The time-kill curve of gentamicin against *E.coli*.

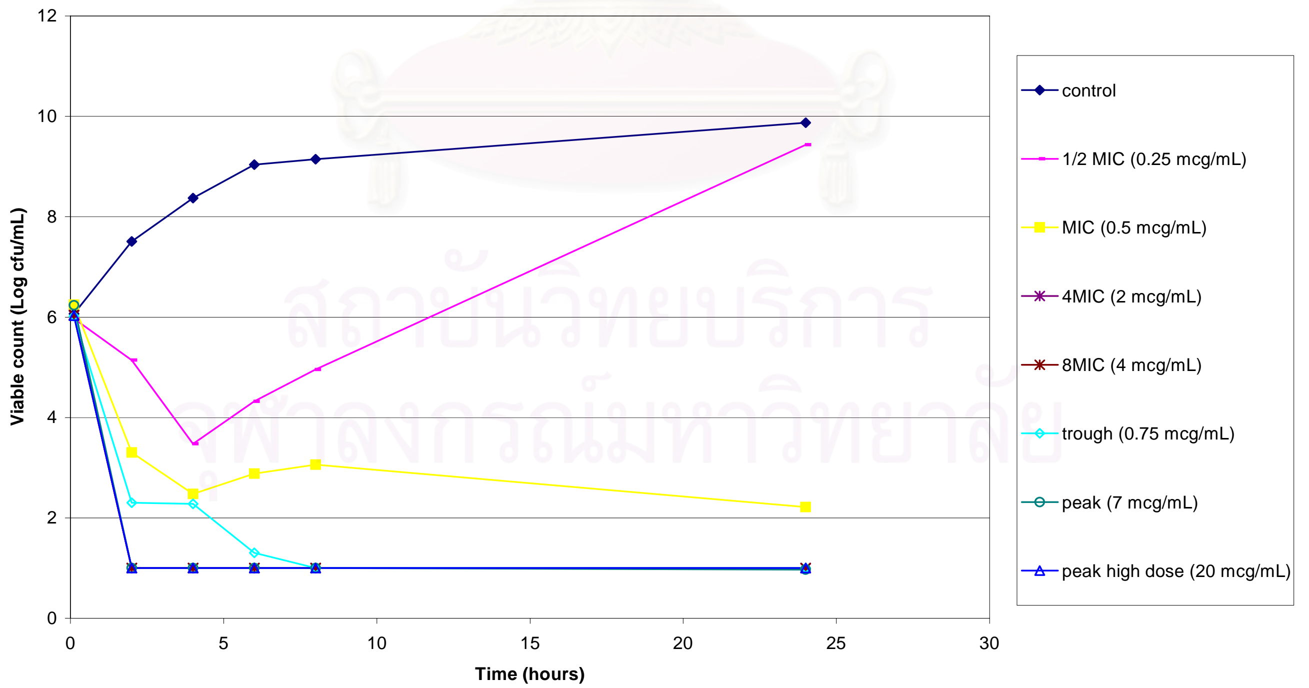

Figure. 4-2 The time-kill curve of gentamicin against *K.pneumoniaea*.

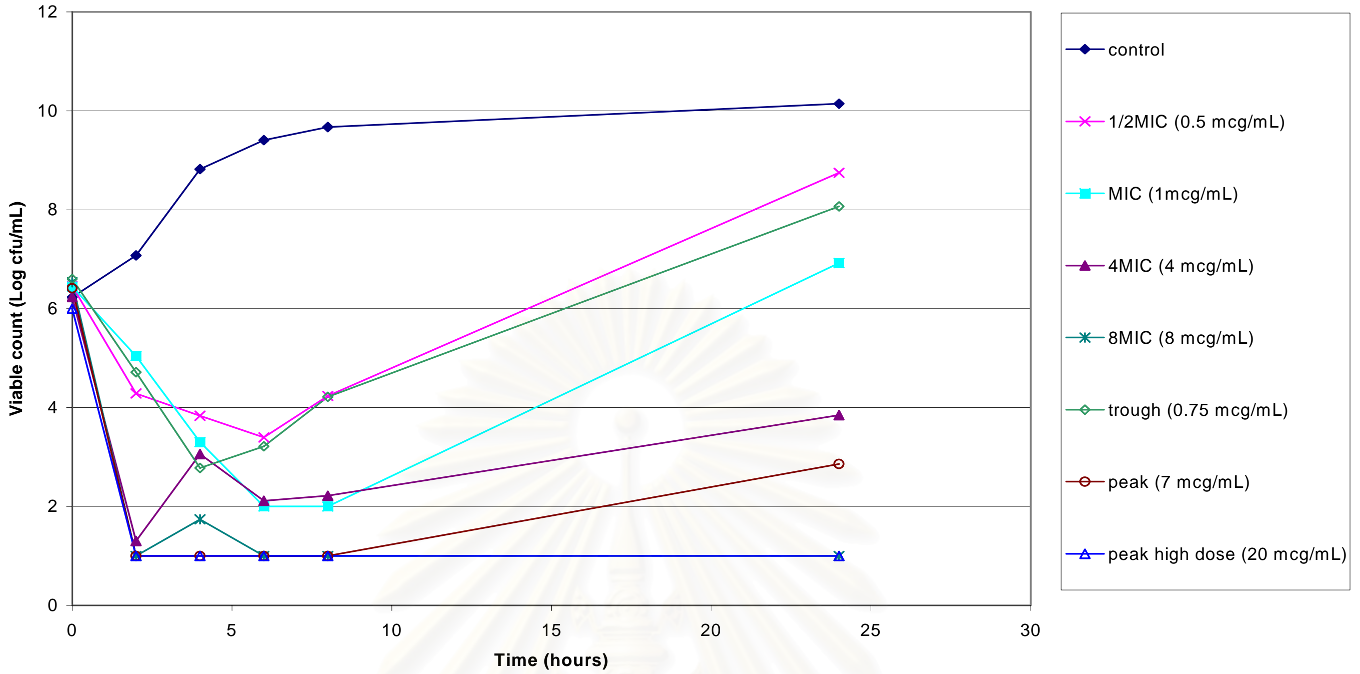

Figure. 4-3 The time-kill curve of gentamicin against *P.mirabilis*.

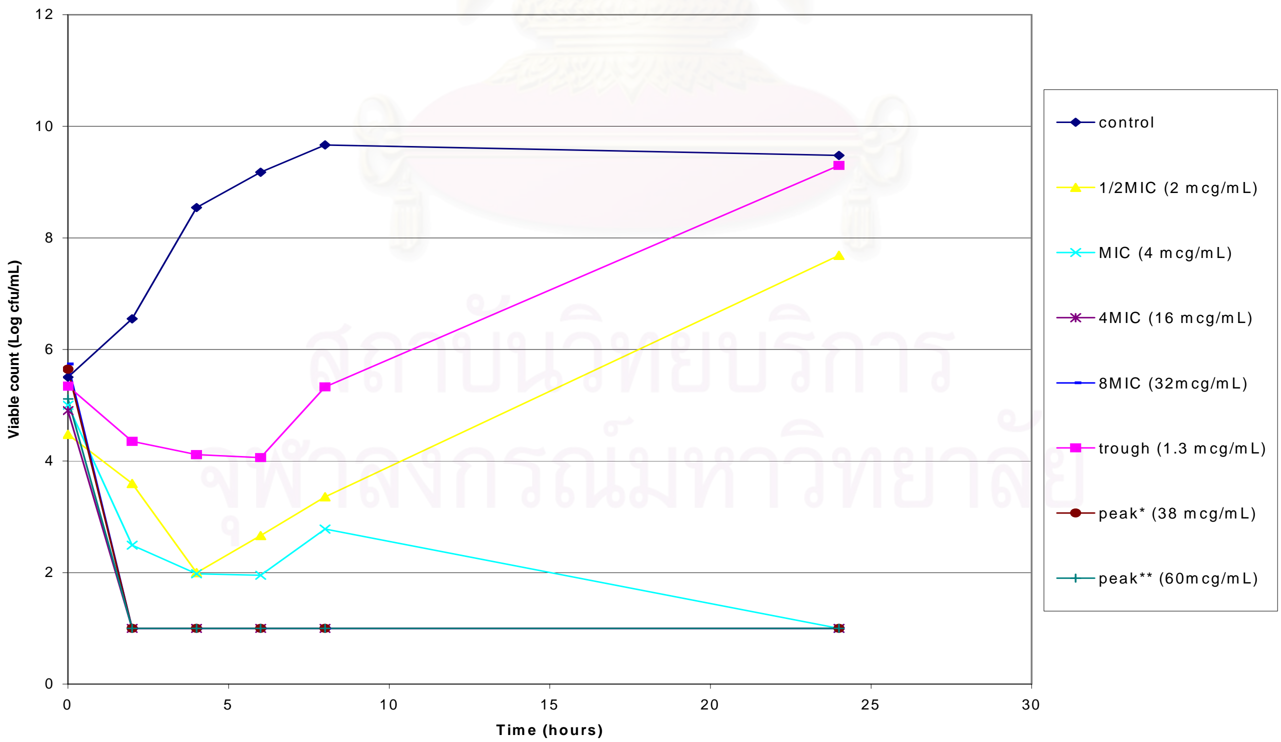

Figure.4-4 The time-kill curve of amikacin against *E.coli*.

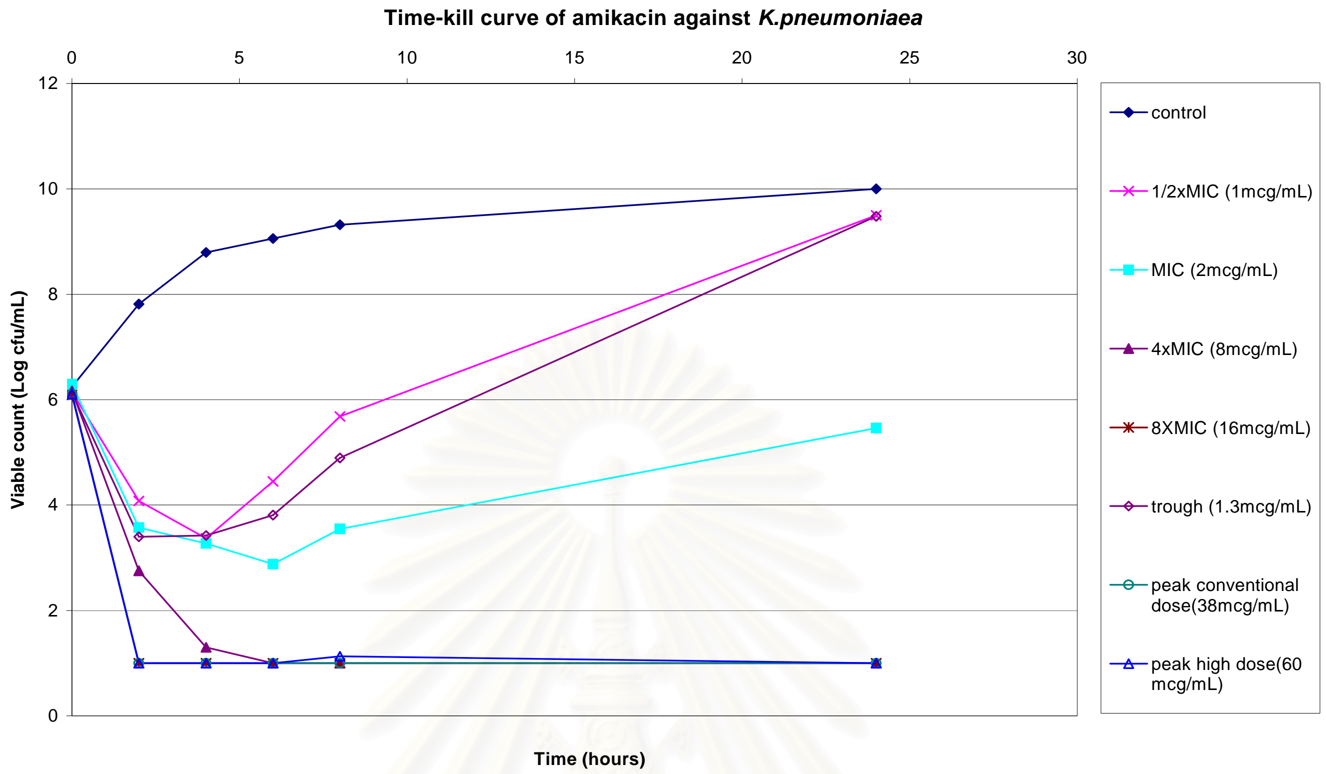

Figure. 4-5 The time-kill curve of amikacin against *K.pneumoniaea.*

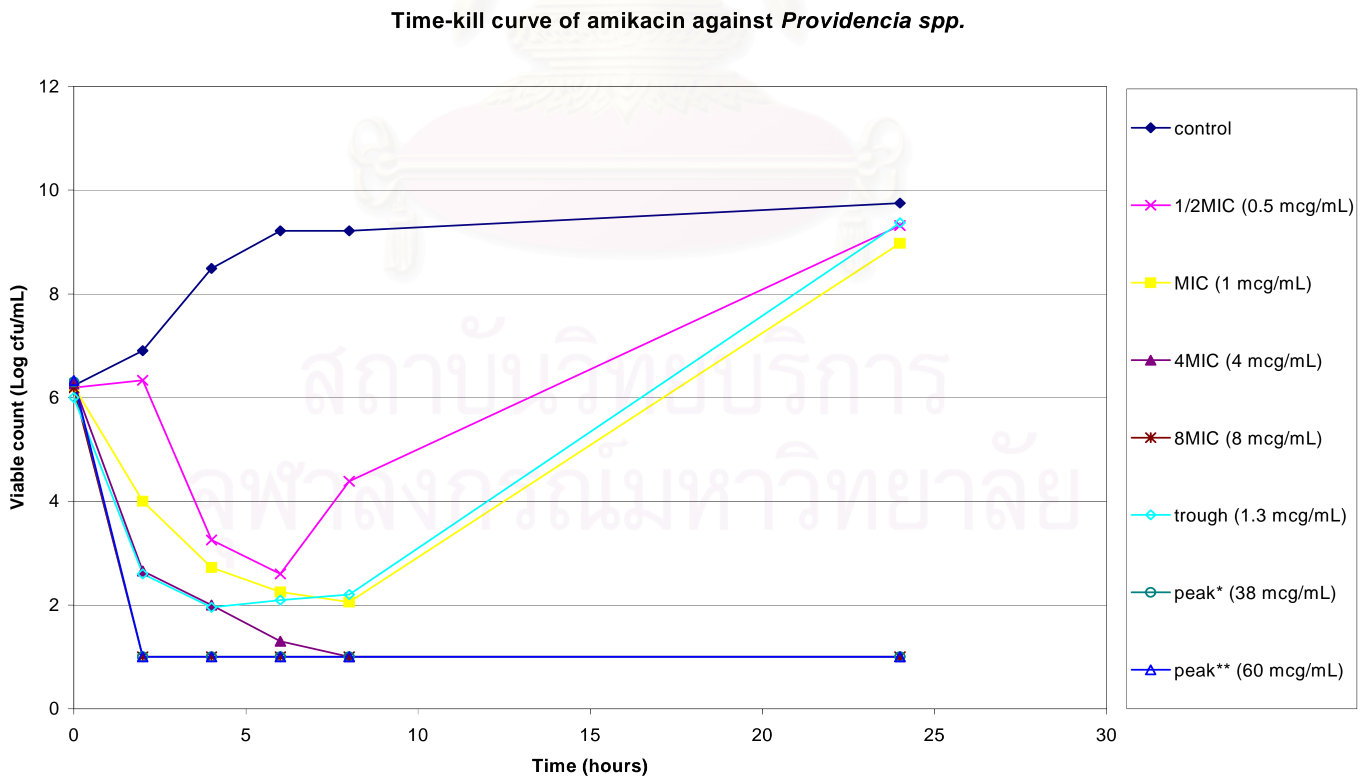

Figure. 4-6 The time-kill curve of amikacin against *Providencia spp.*

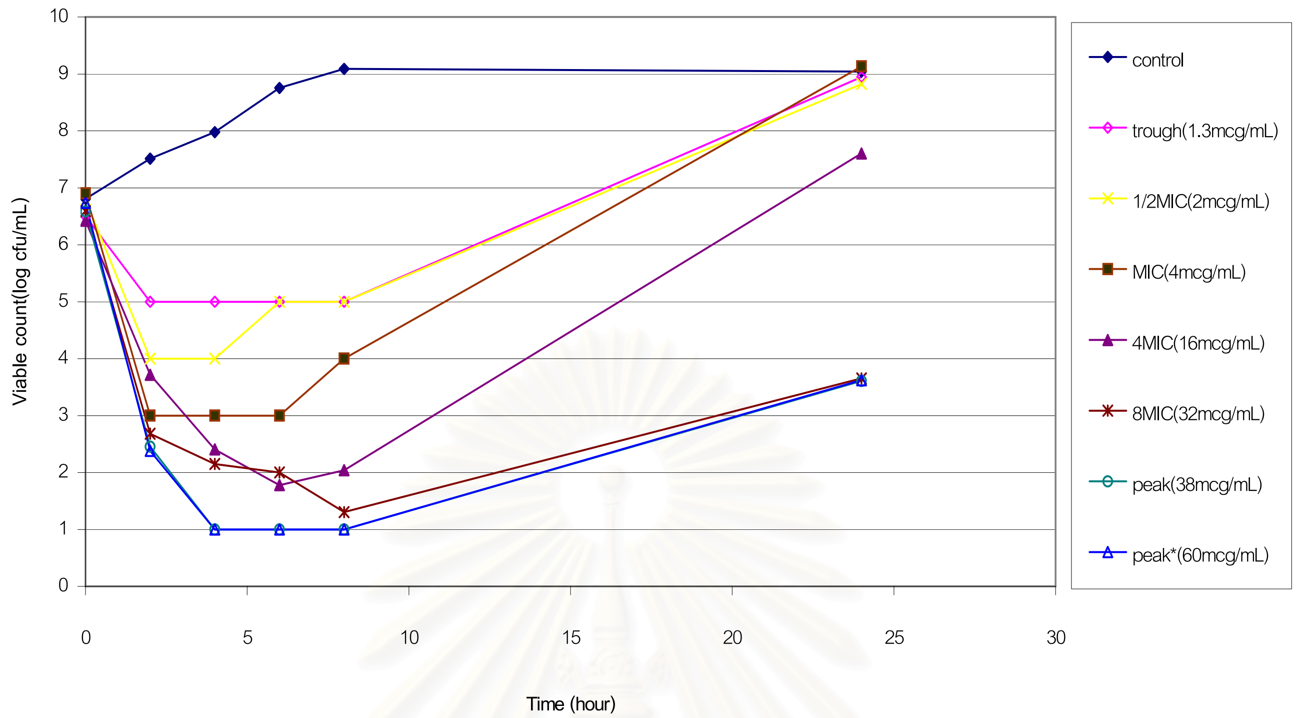

Figure. 4-7 The time-kill curve of amikacin against *P.aeruginosa*

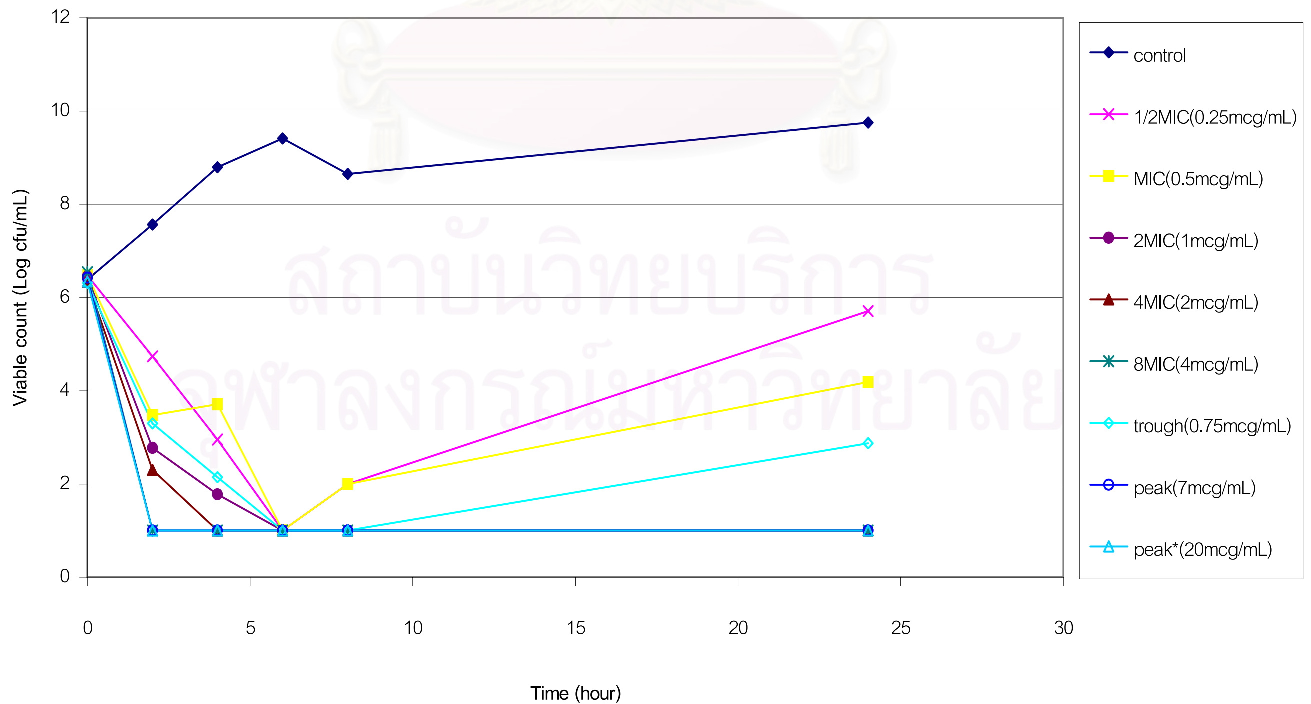

Figure. 4-8 The time-kill curve of netilmicin against *Providencia spp.*

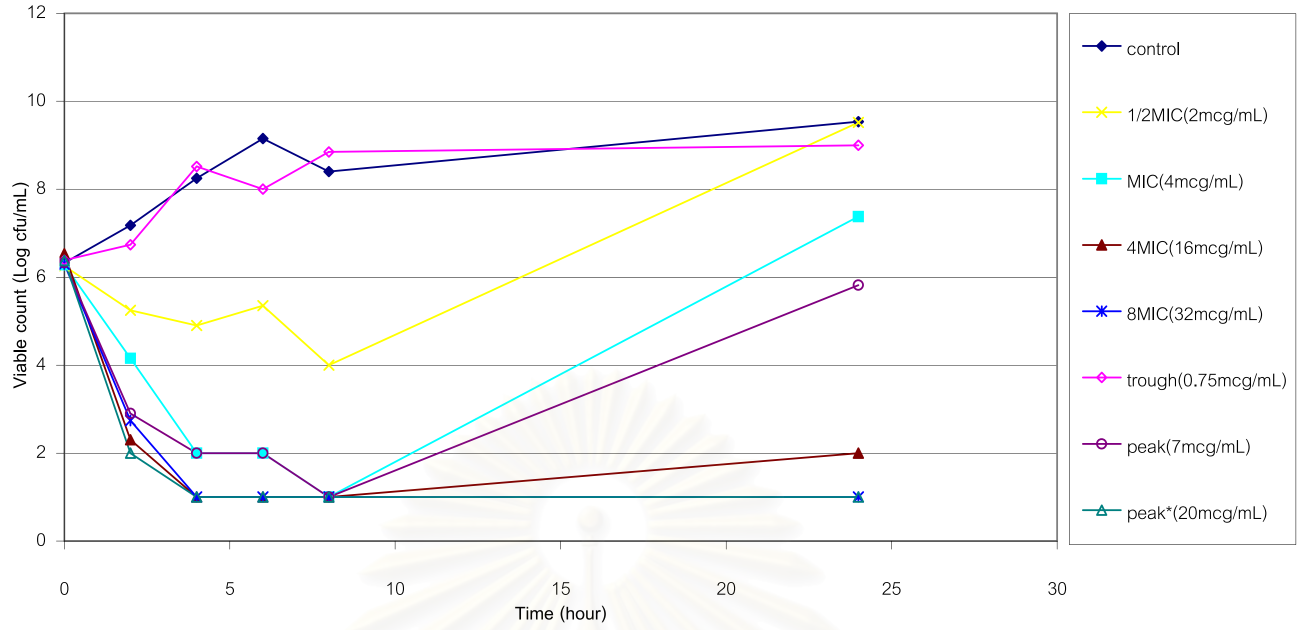

Figure. 4-9 The time-kill curve of netilmicin against *P.aeruginosa.*

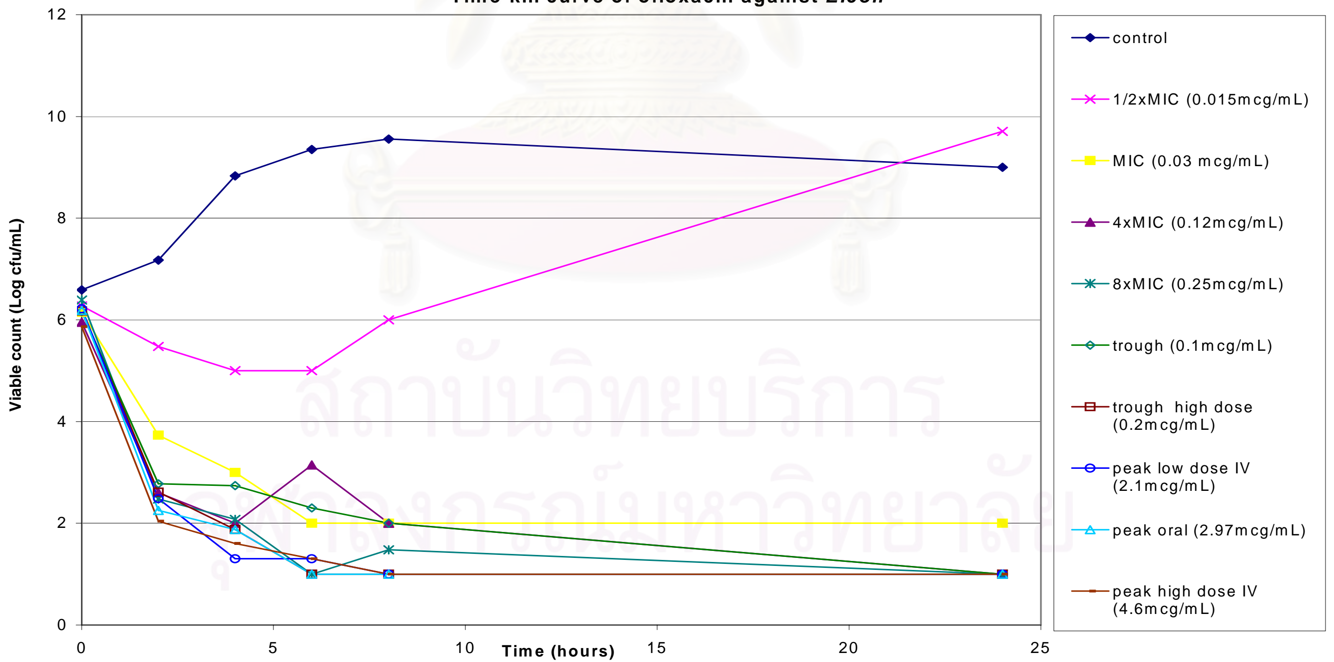

Figure. 4-10 The time-kill curve of ofloxacin against *E.coli.*

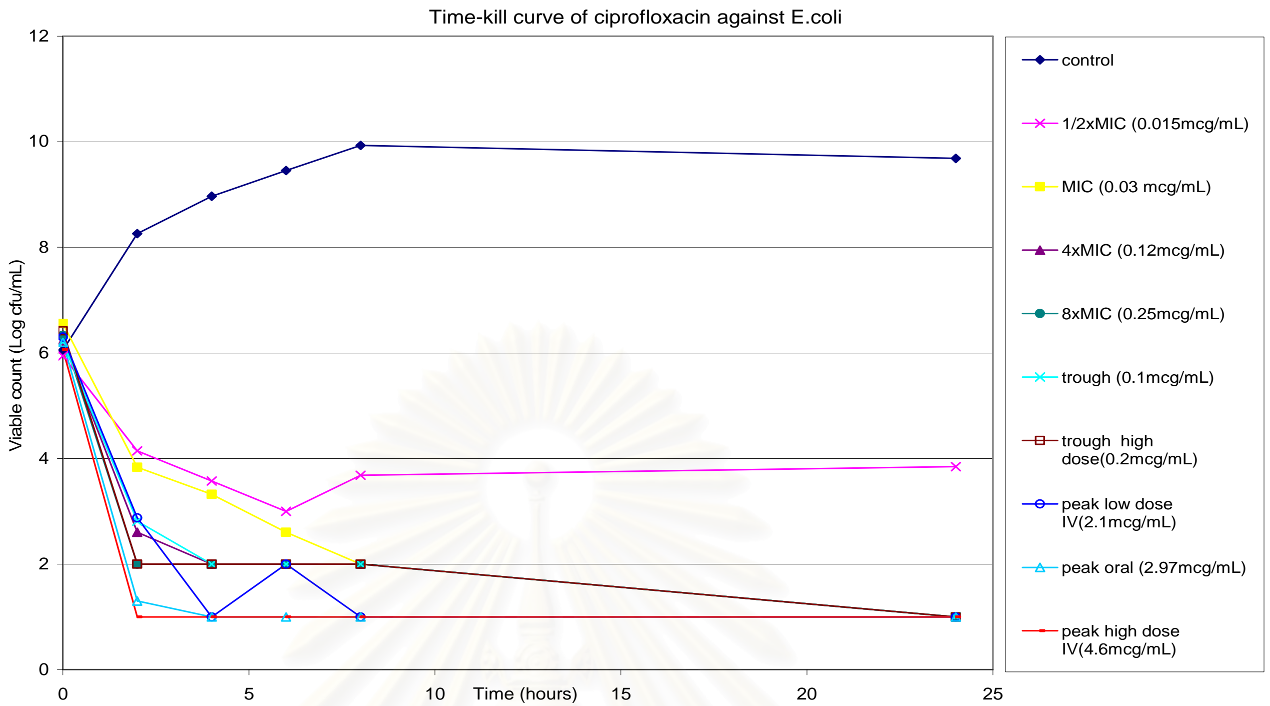

Figure. 4-11 The time-kill curve of ciprofloxacin against *E.coli.*

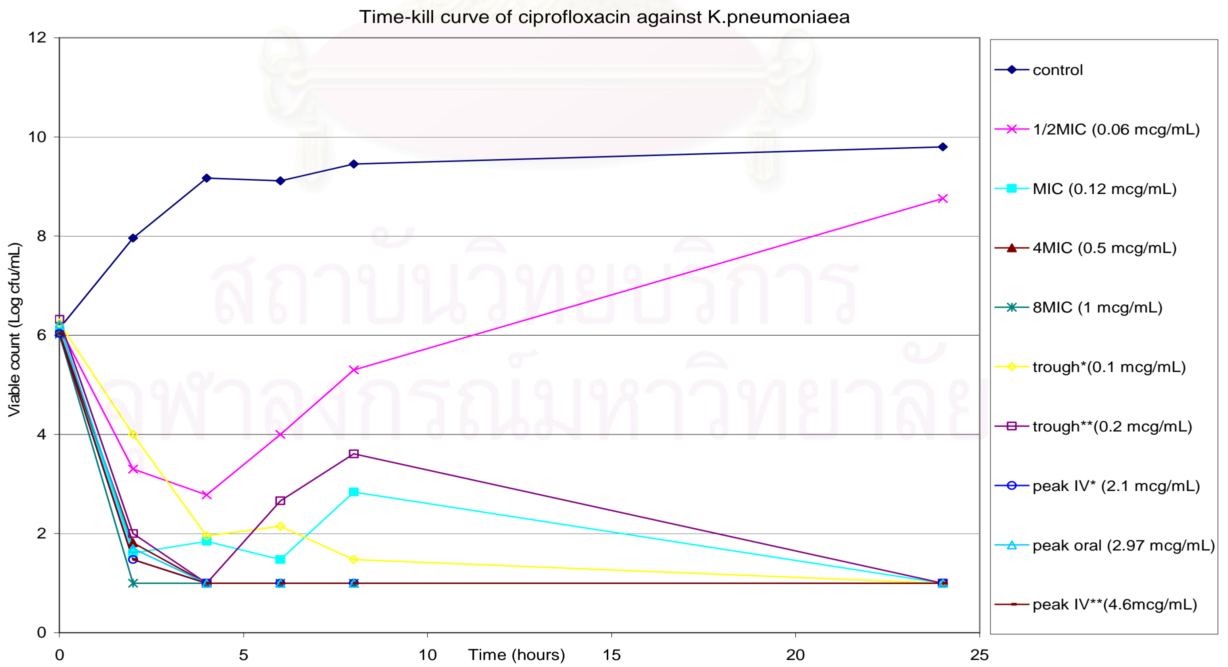

Figure. 4-12 The time-kill curve of ciprofloxacin against *K.pneumoniaea*

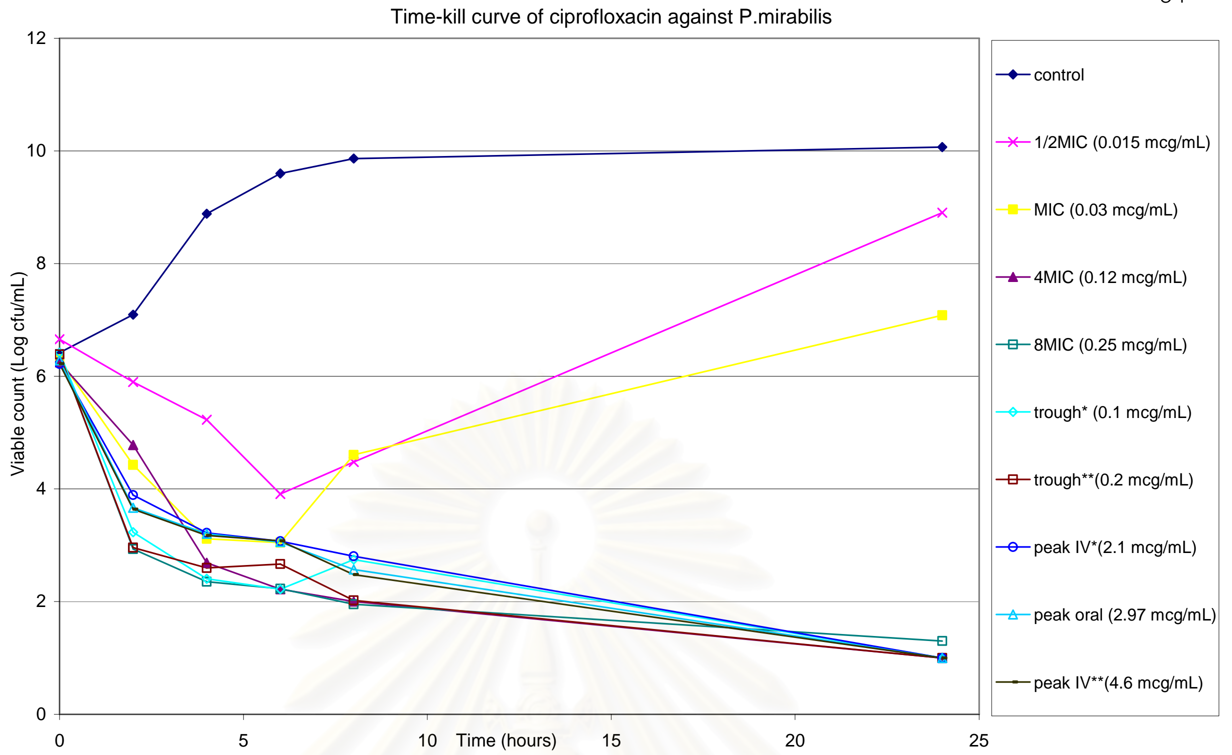

Figure. 4-13 The time-kill curve of ciprofloxacin against *P.mirabilis*

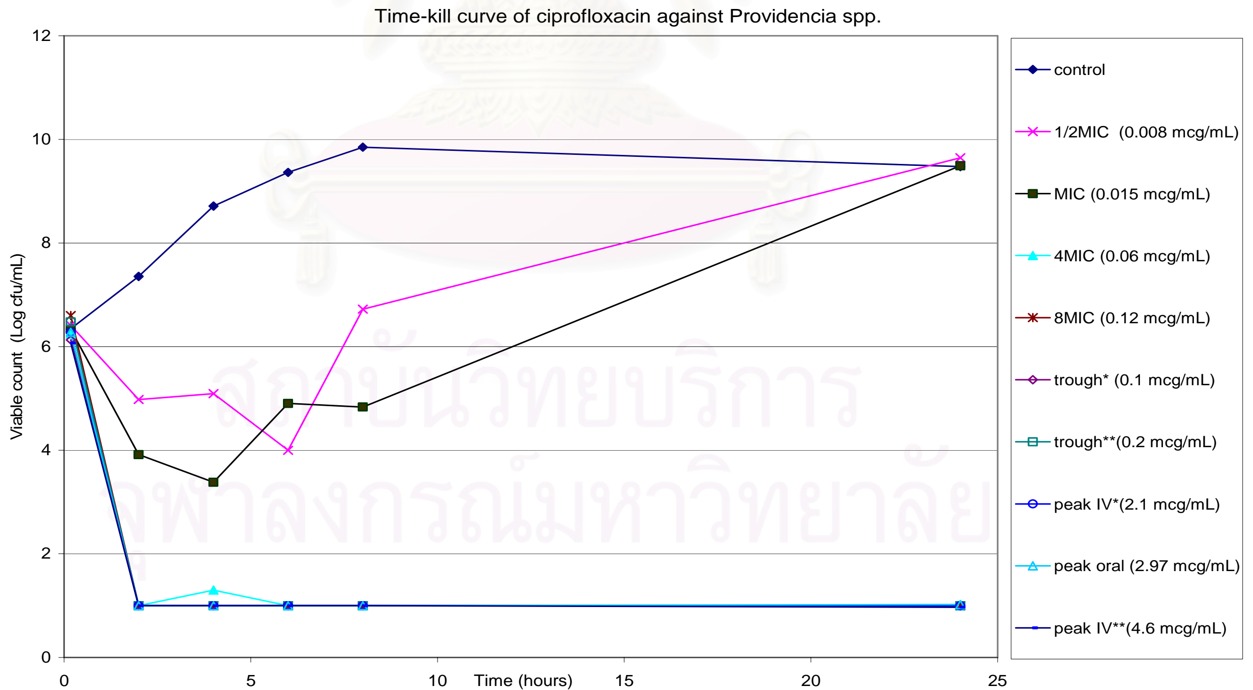

Figure. 4-14 The time-kill curve of ciprofloxacin against *Providencia spp.*

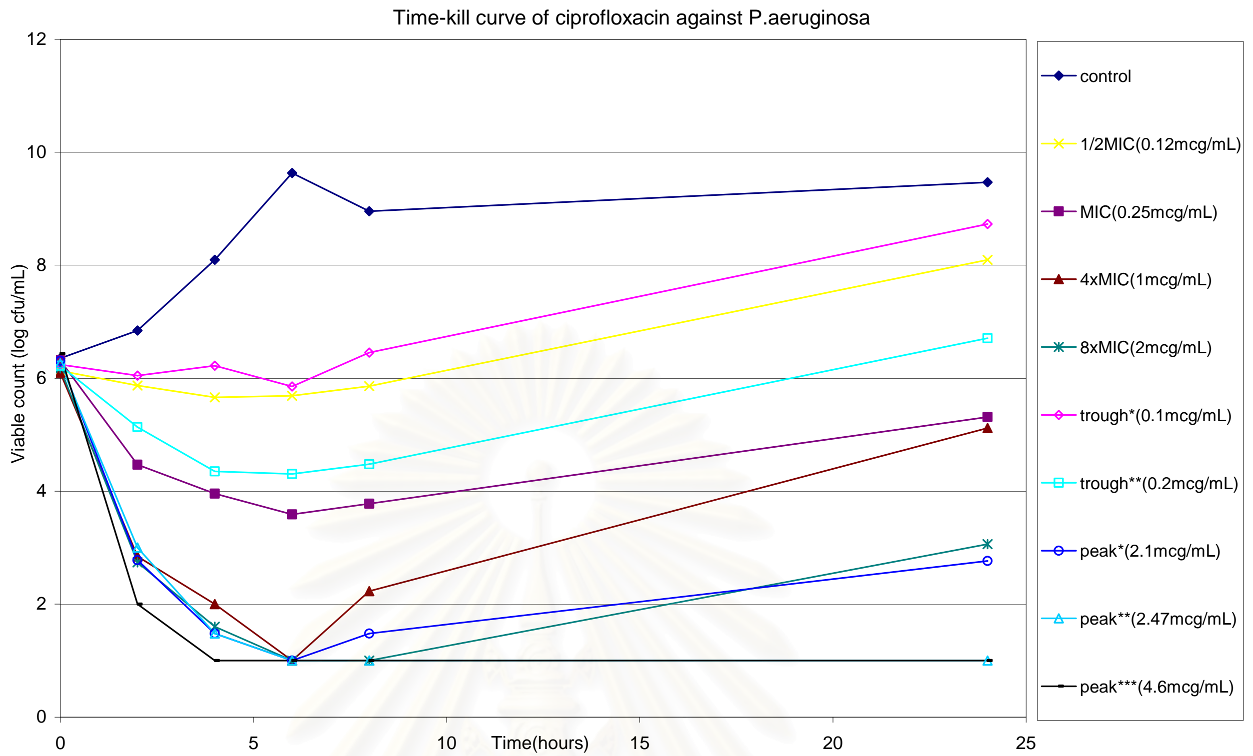

Figure. 4-15 The time-kill curve of ciprofloxacin against *P.aeruginosa*.

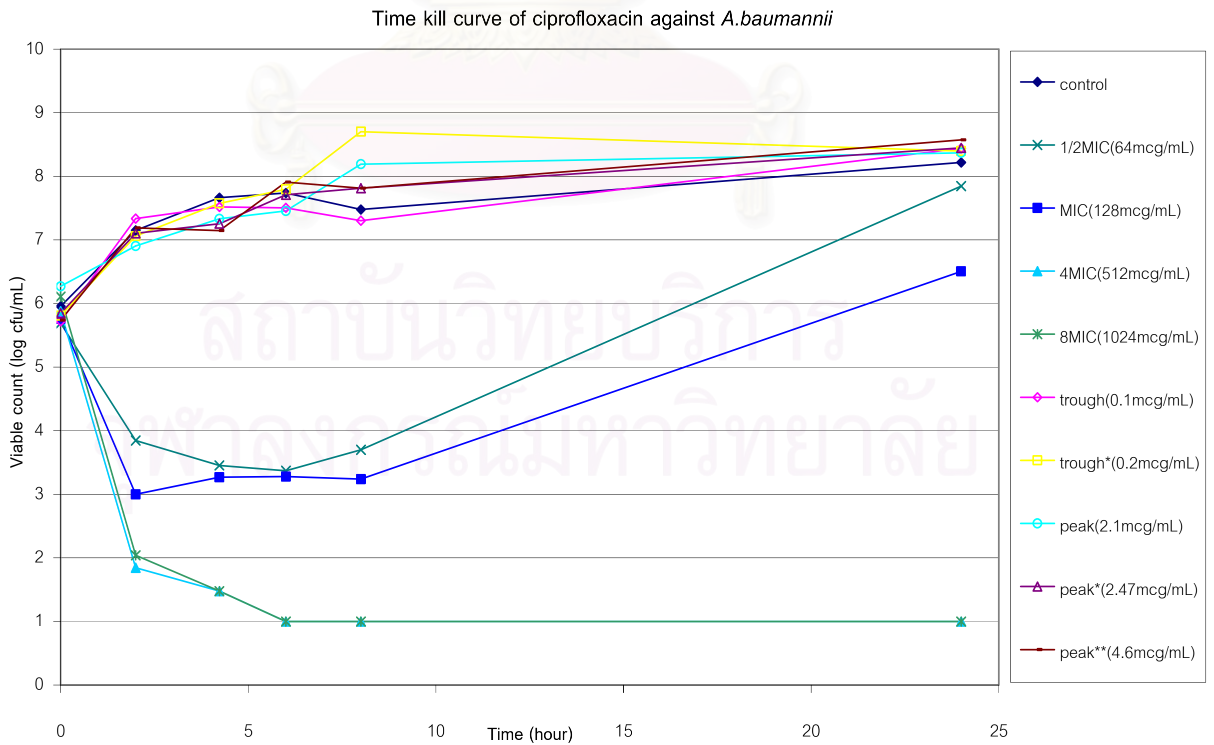

Figure. 4-16 The time-kill curve of ciprofloxacin against *A.buamannii*.

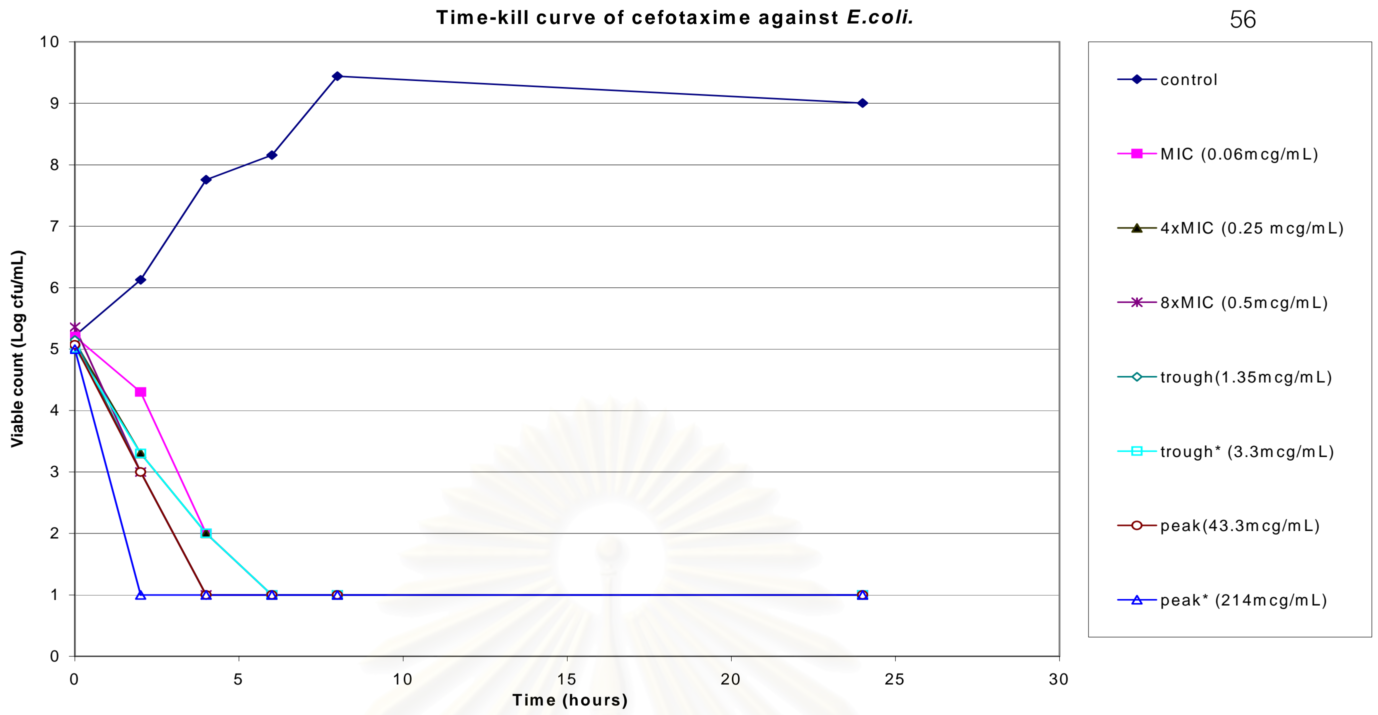

Figure. 4-17 The time-kill curve of cefotaxime against *E.coli.*

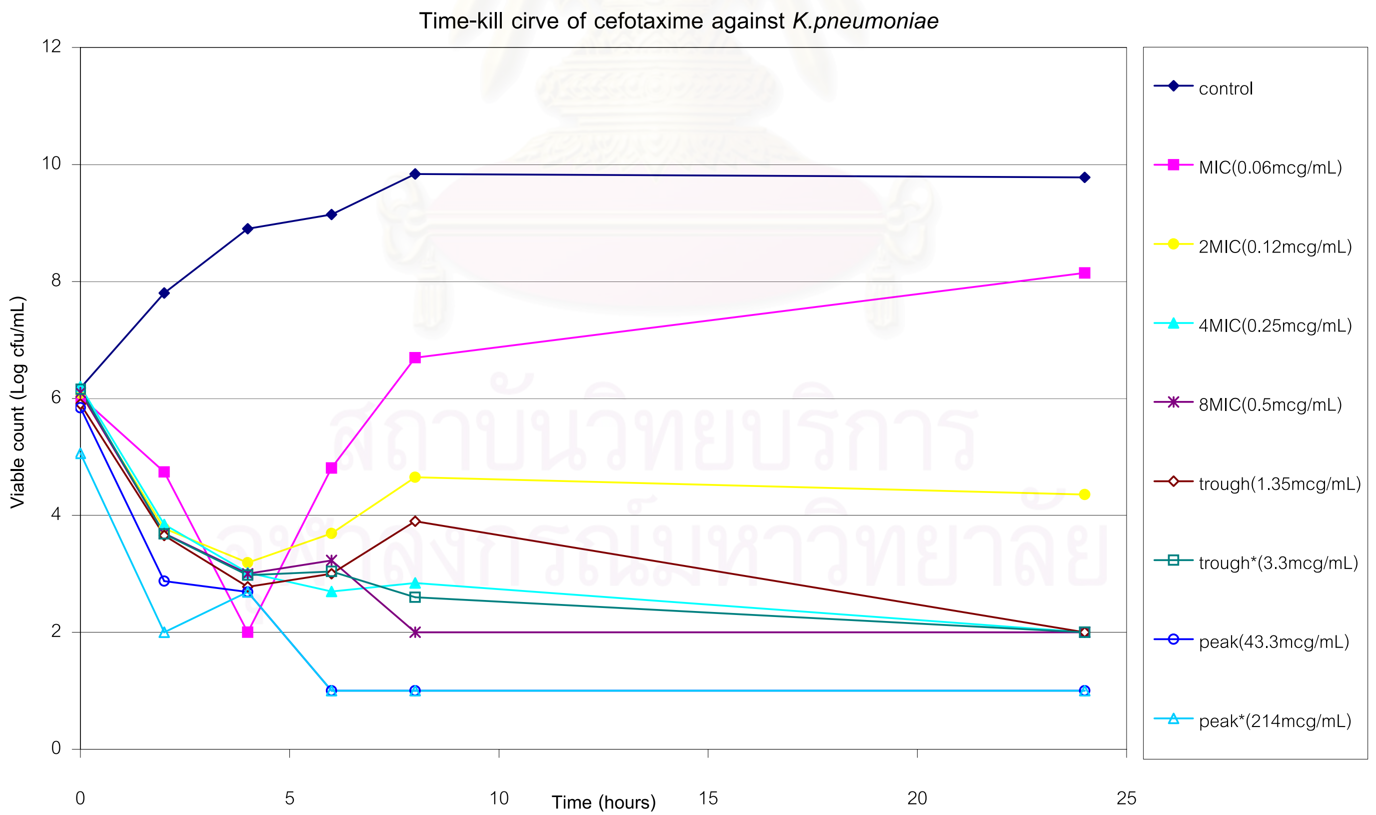

Figure. 4-18 The time-kill curve of cefotaxime against *K.pneumoniaea*

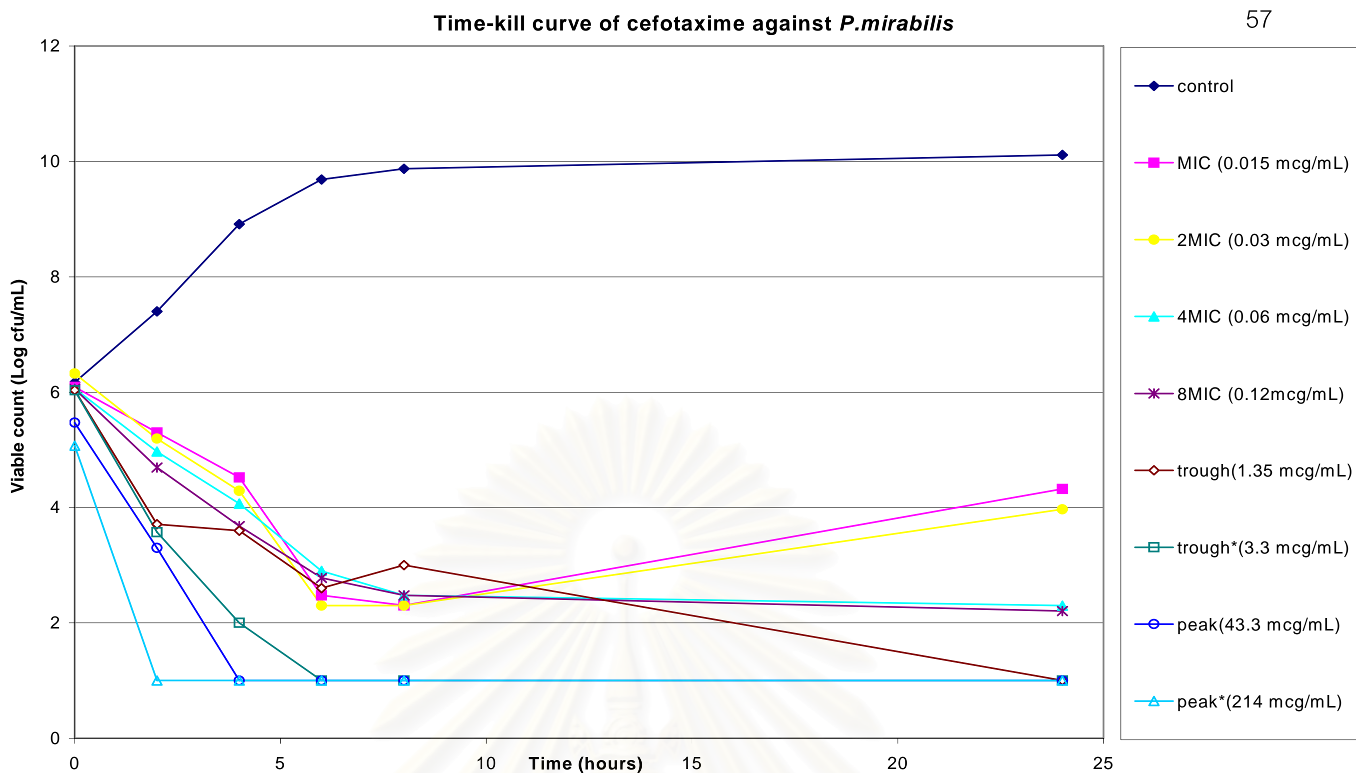

Figure. 4-19 The time-kill curve of cefotaxime against *P.mirabilis.*

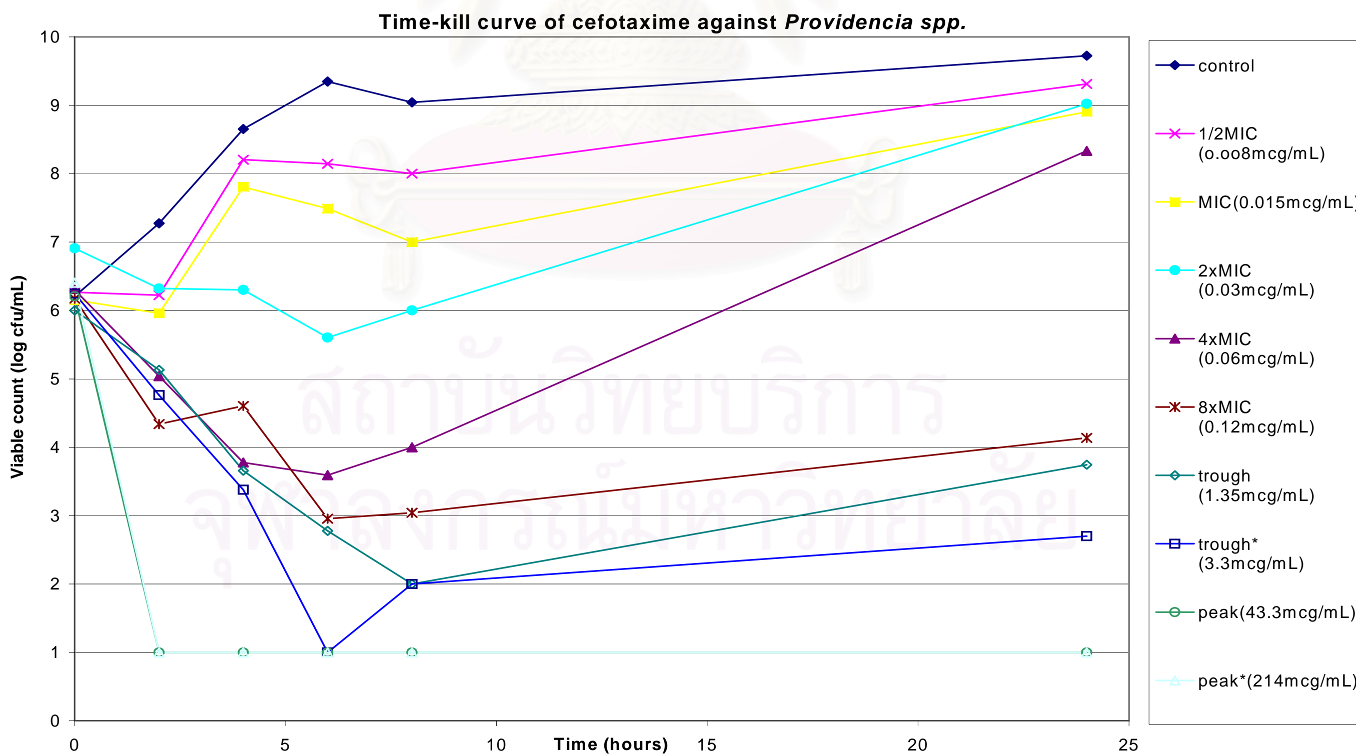

Figure. 4-20 The time-kill curve of cefotaxime against *Providencia spp.*

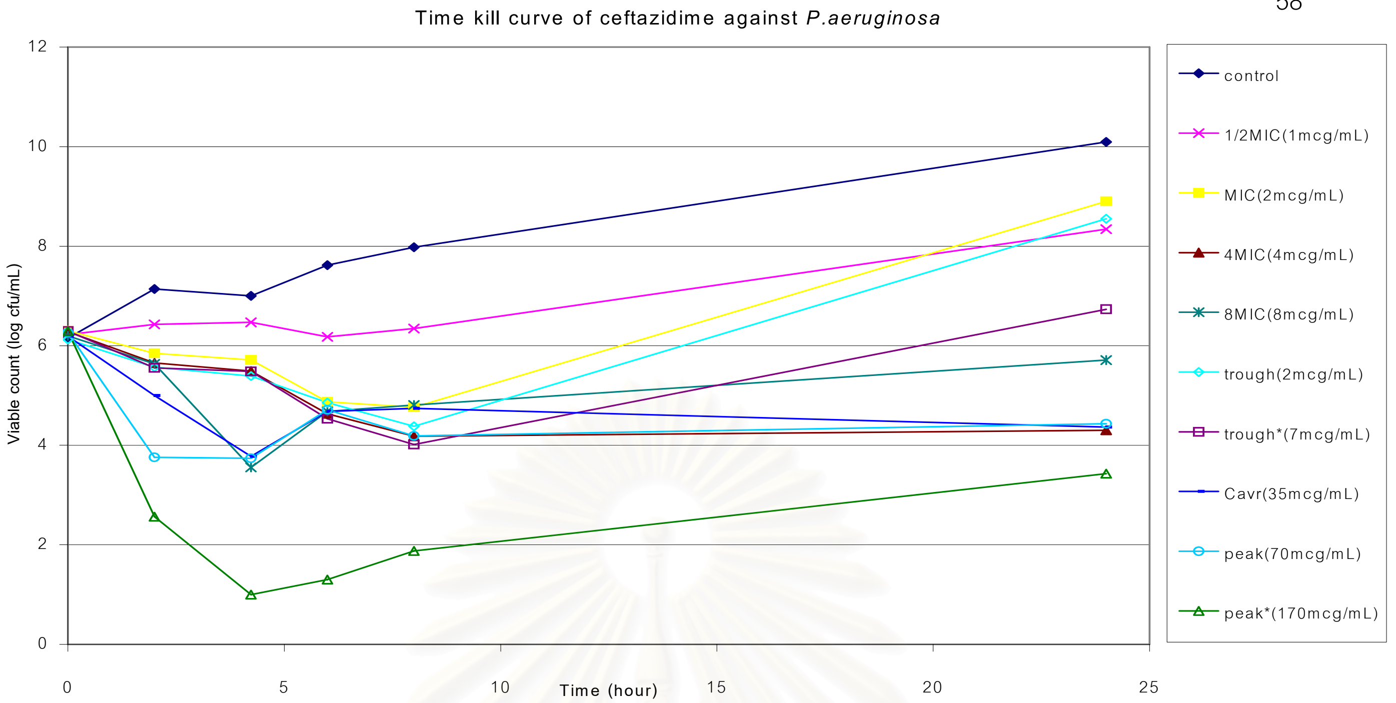

Figure. 4-21 The time-kill curve of ceftazidime against *P.aeruginosa.*

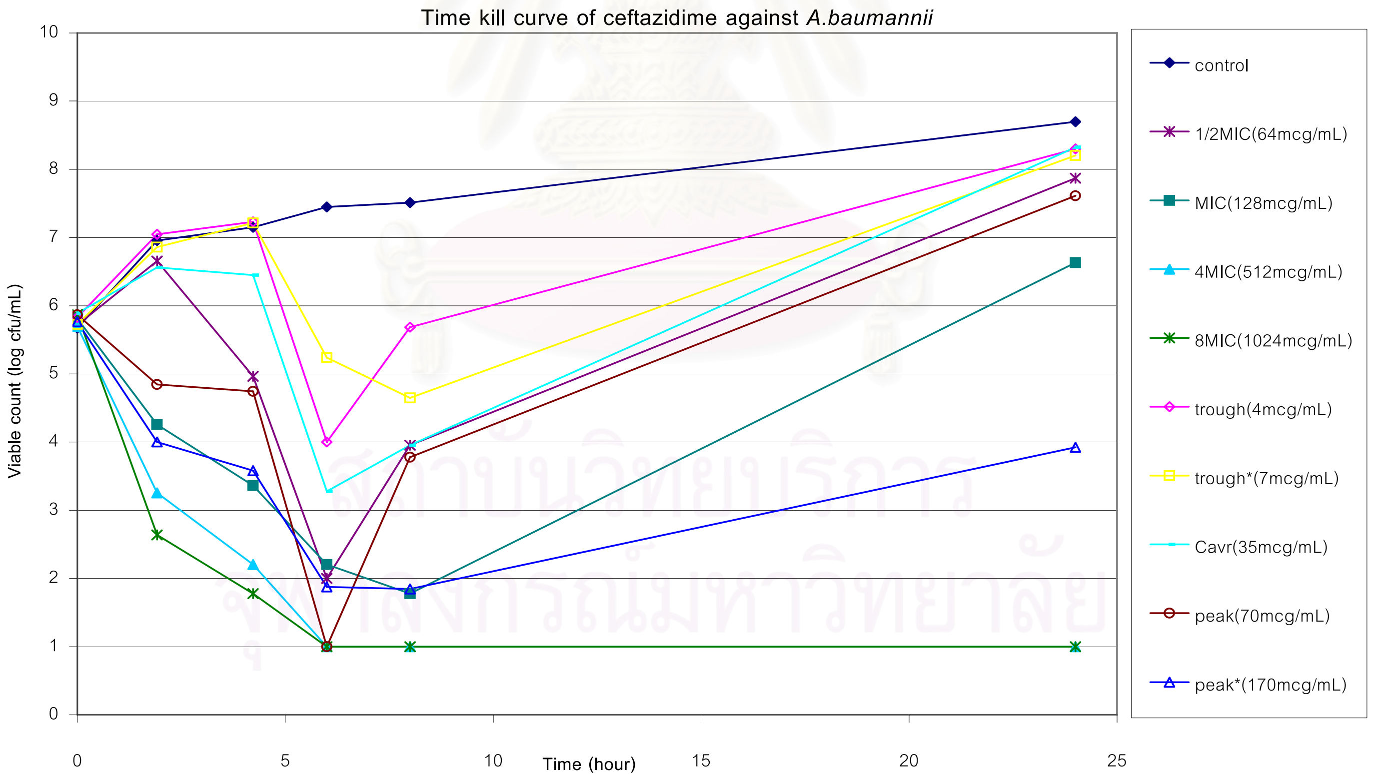

Figure. 4-22 The time-kill curve of ceftazidime against *A.buamannii.*

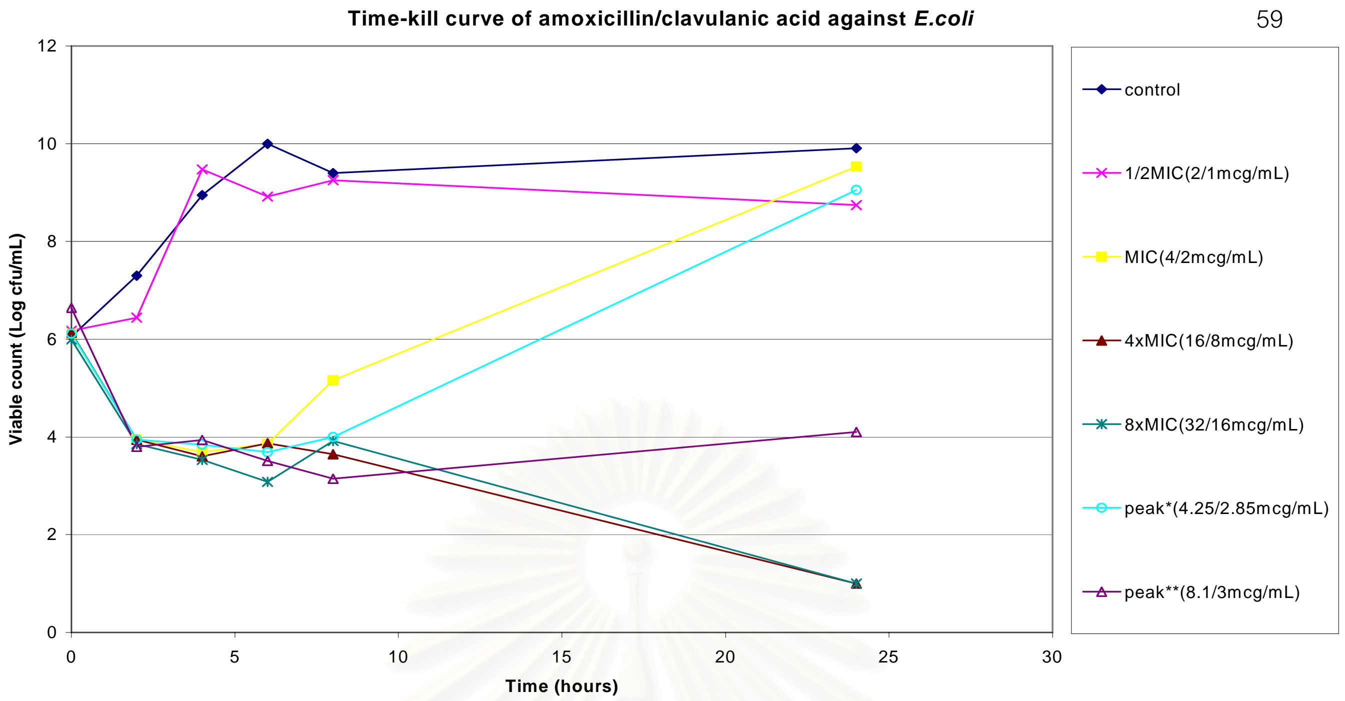

Figure. 4-23 The time-kill curve of amoxicillin/clavulanic acid against *E.coli.*

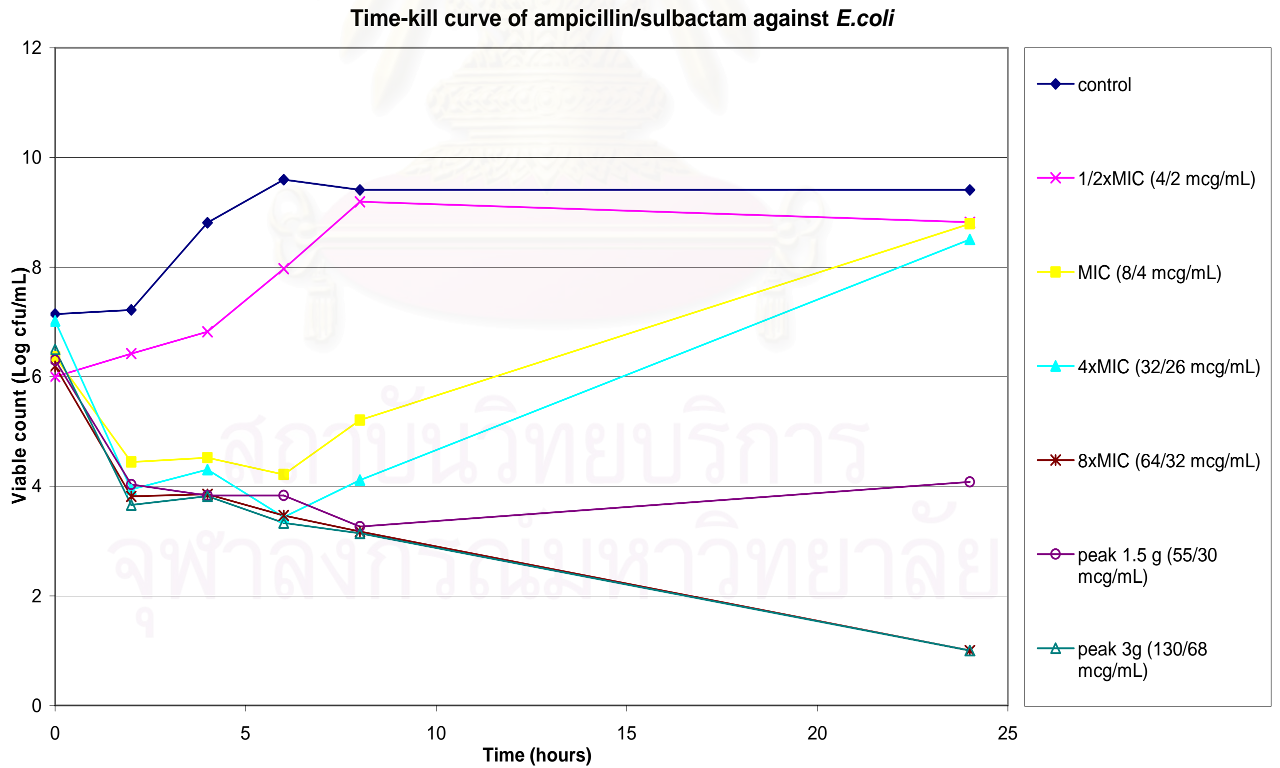

Figure. 4-24 The time-kill curve of ampicillin/sulbactam acid against *E.coli.*

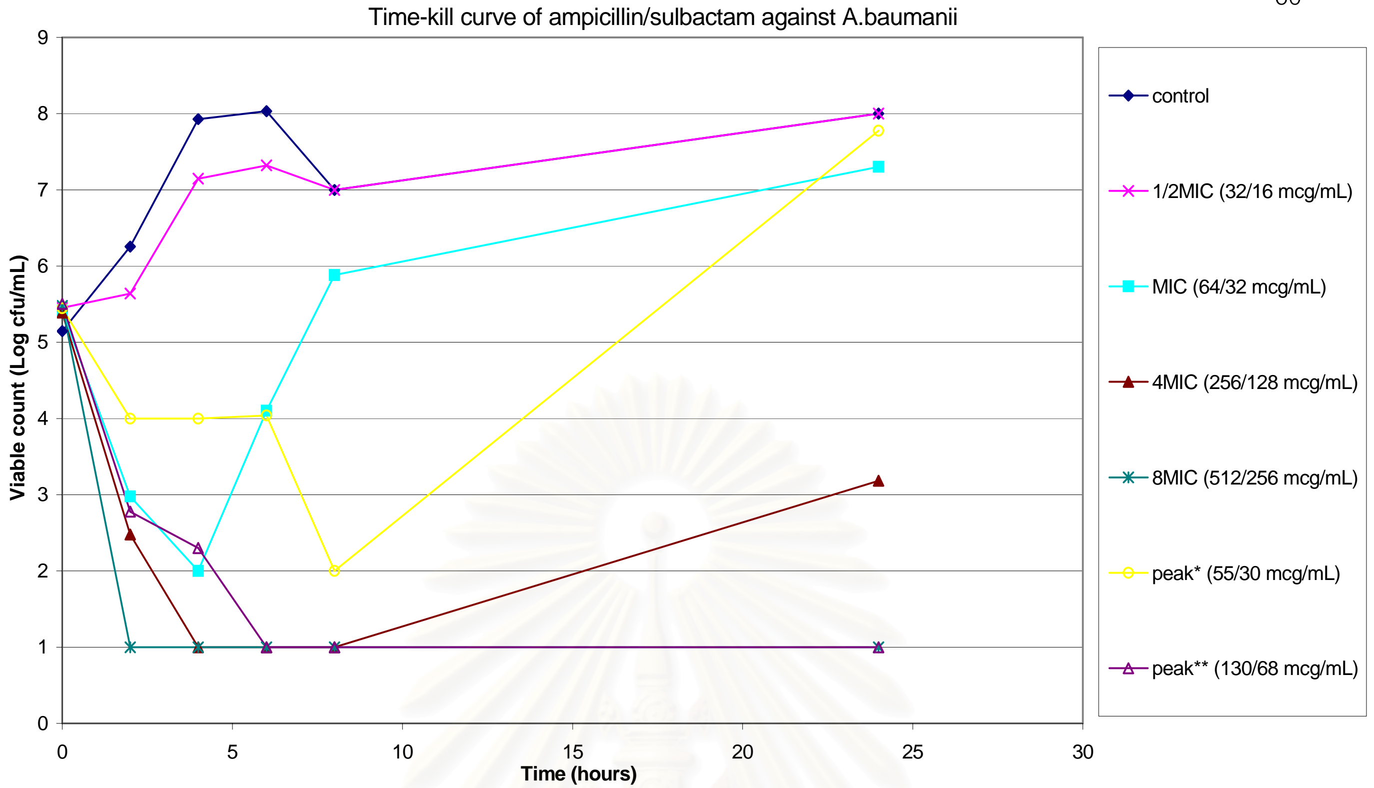

Figure. 4-25 The time-kill curve of ampicillin/sulbactam against *A.buamannii.*

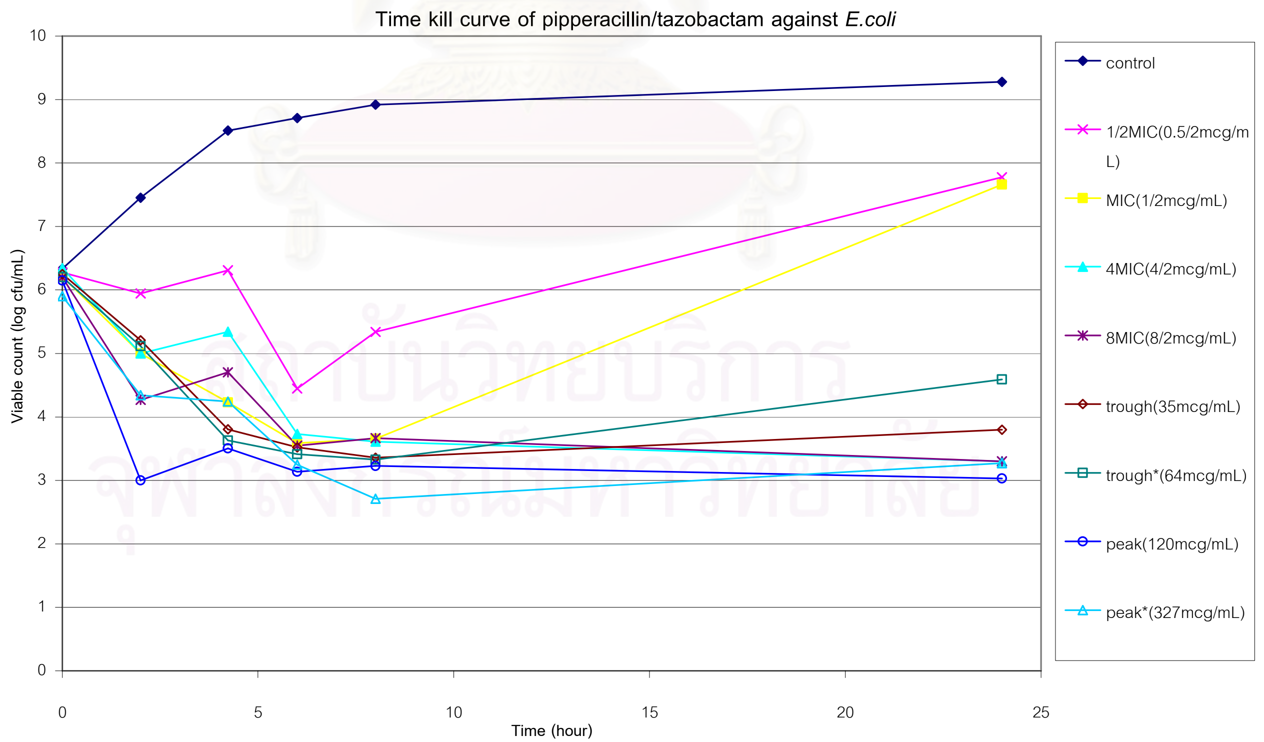

Figure.4-26 The time-kill curve of piperacillin/tazobactam against *E.coli.*

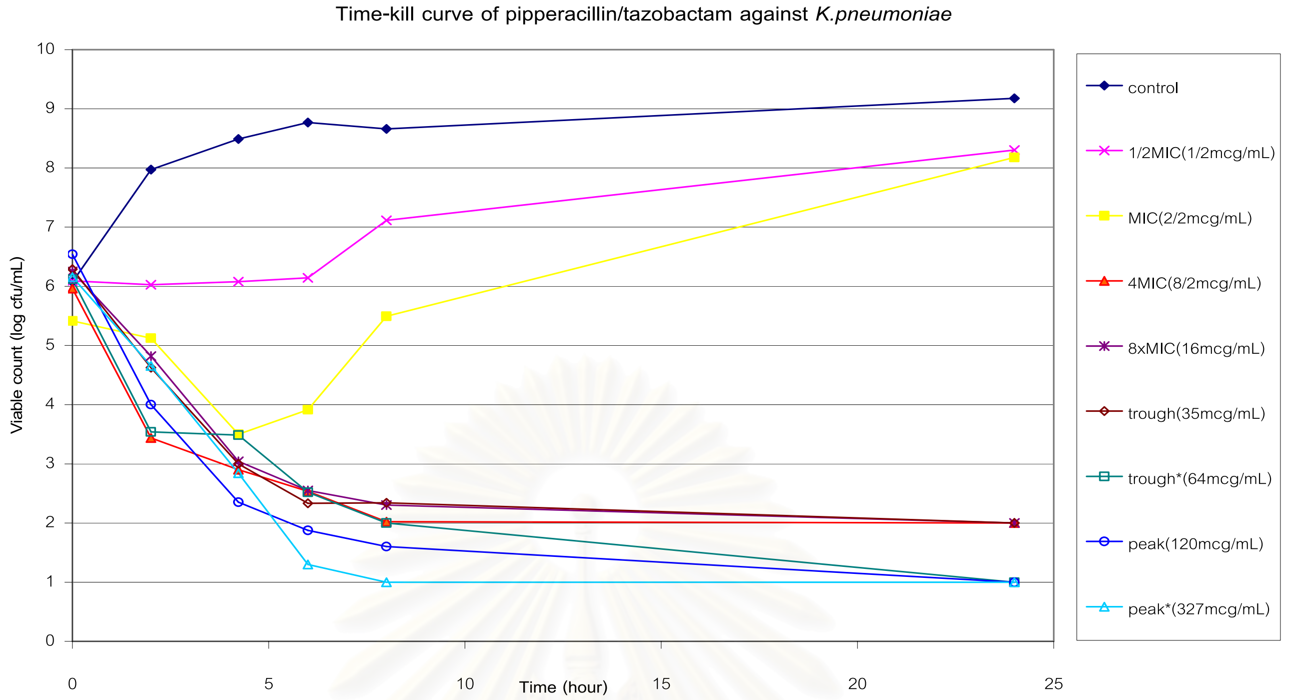

Figure. 4-27 The time-kill curve of piperacillin/tazobactam against *K.pneumoniaea*

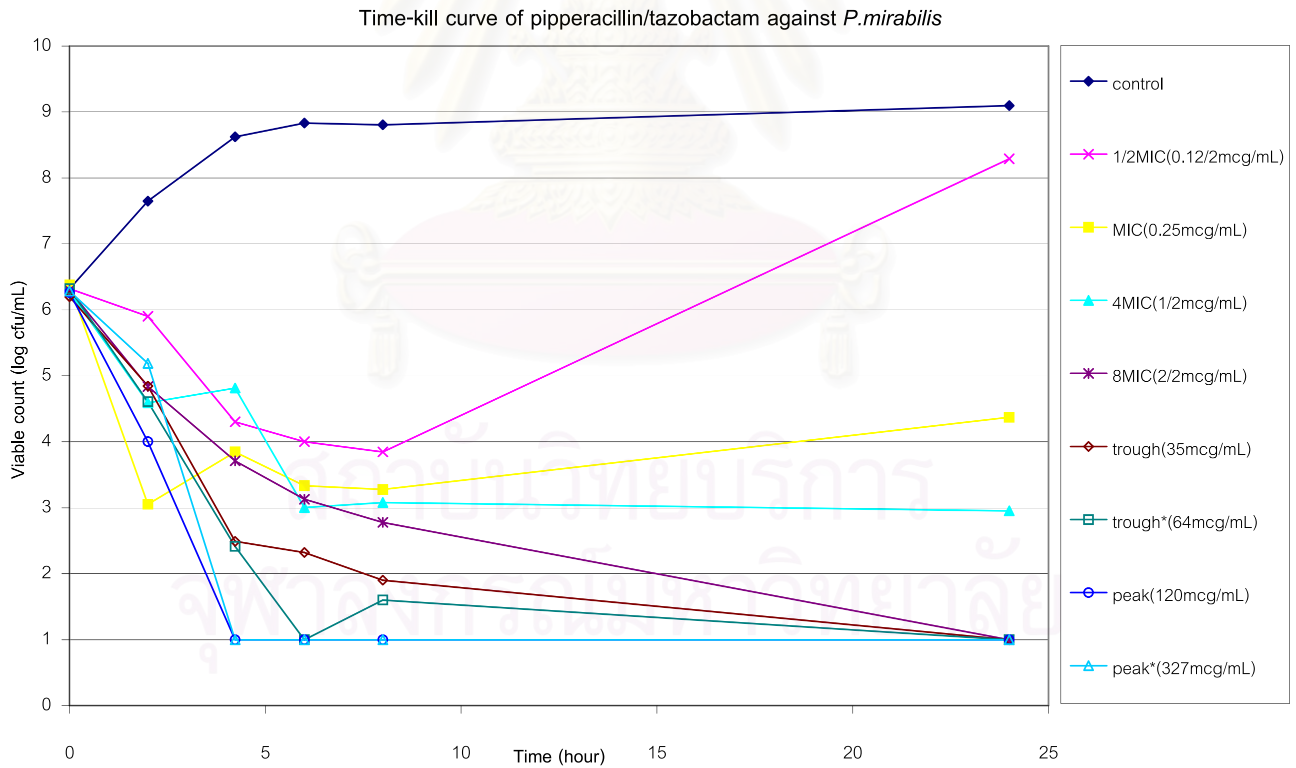

Figure. 4-28 The time-kill curve of piperacillin/tazobactam against *P.mirabilis*

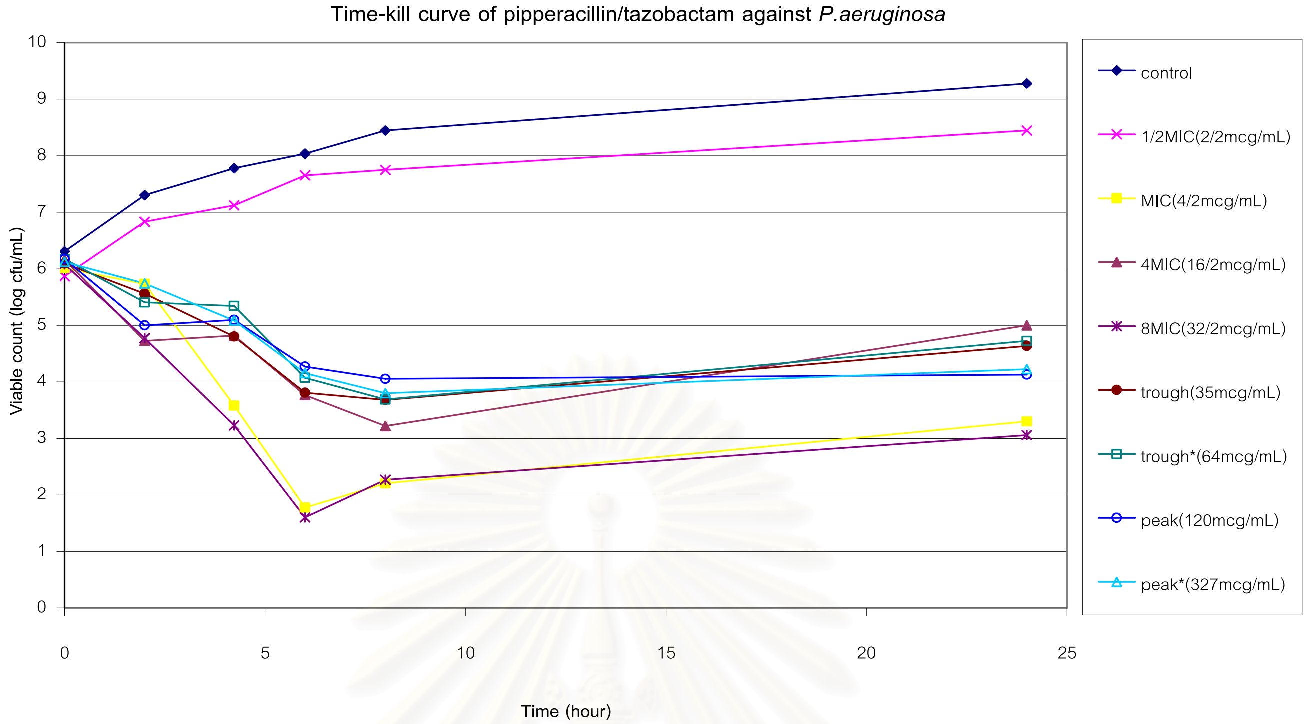

Figure. 4-29 The time-kill curve of piperacillin/tazobactam against *P.aeruginosa*.

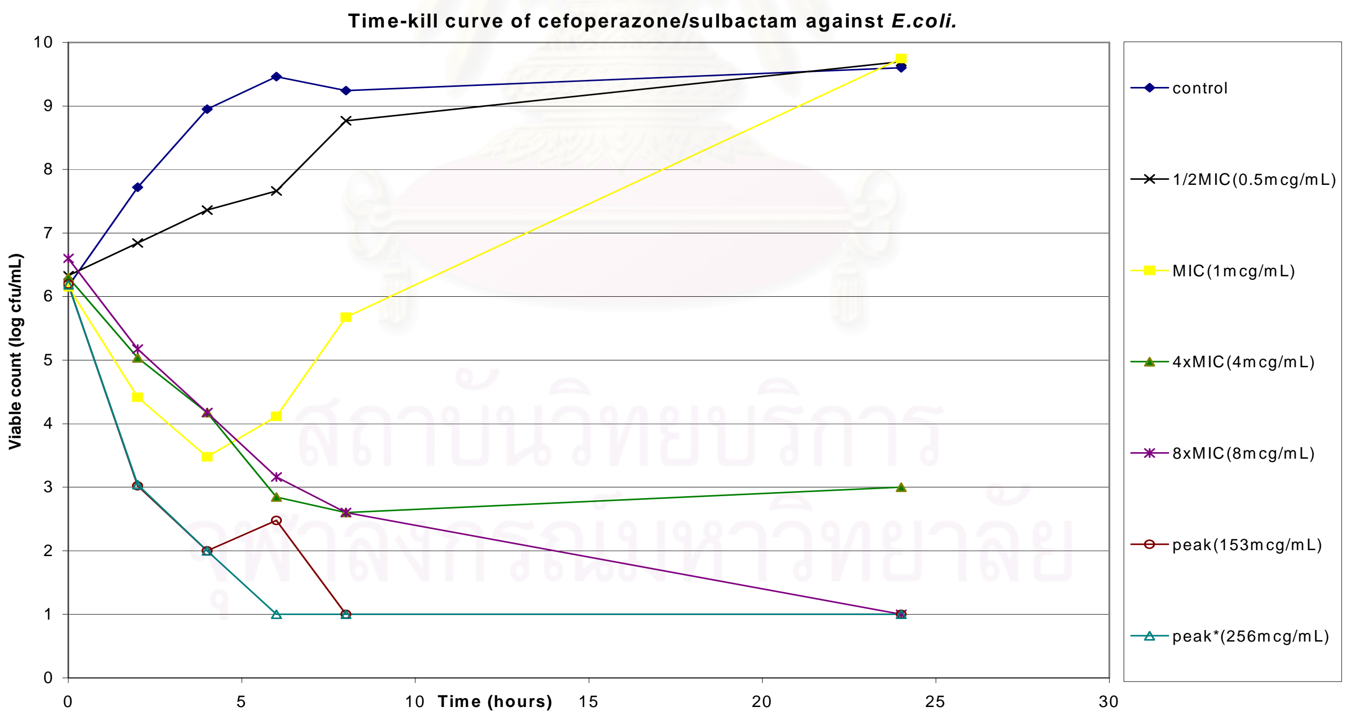

Figure. 4-30 The time-kill curve of cefoperazone/sulbactam against *E.coli*.

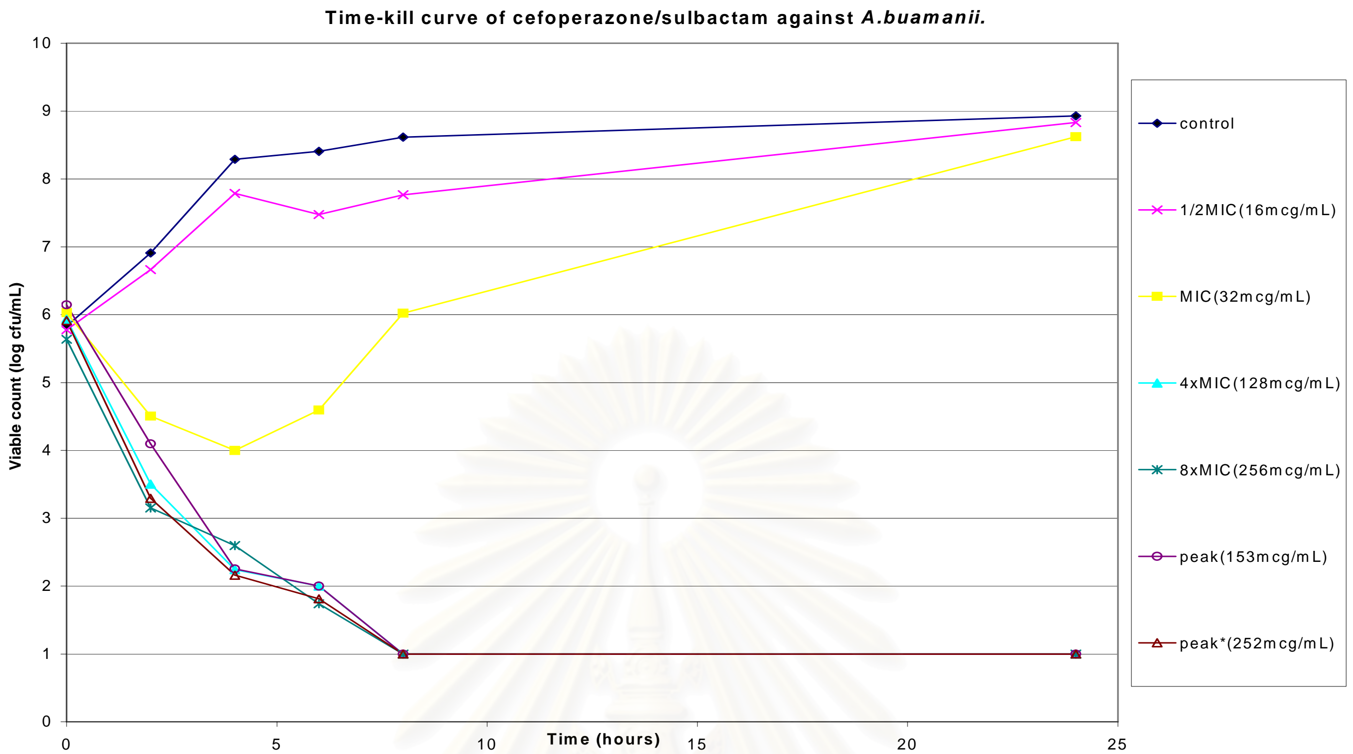

Figure. 4-31 Time-kill curve of cefoperazone/sulbactam against *A.baumannii.*

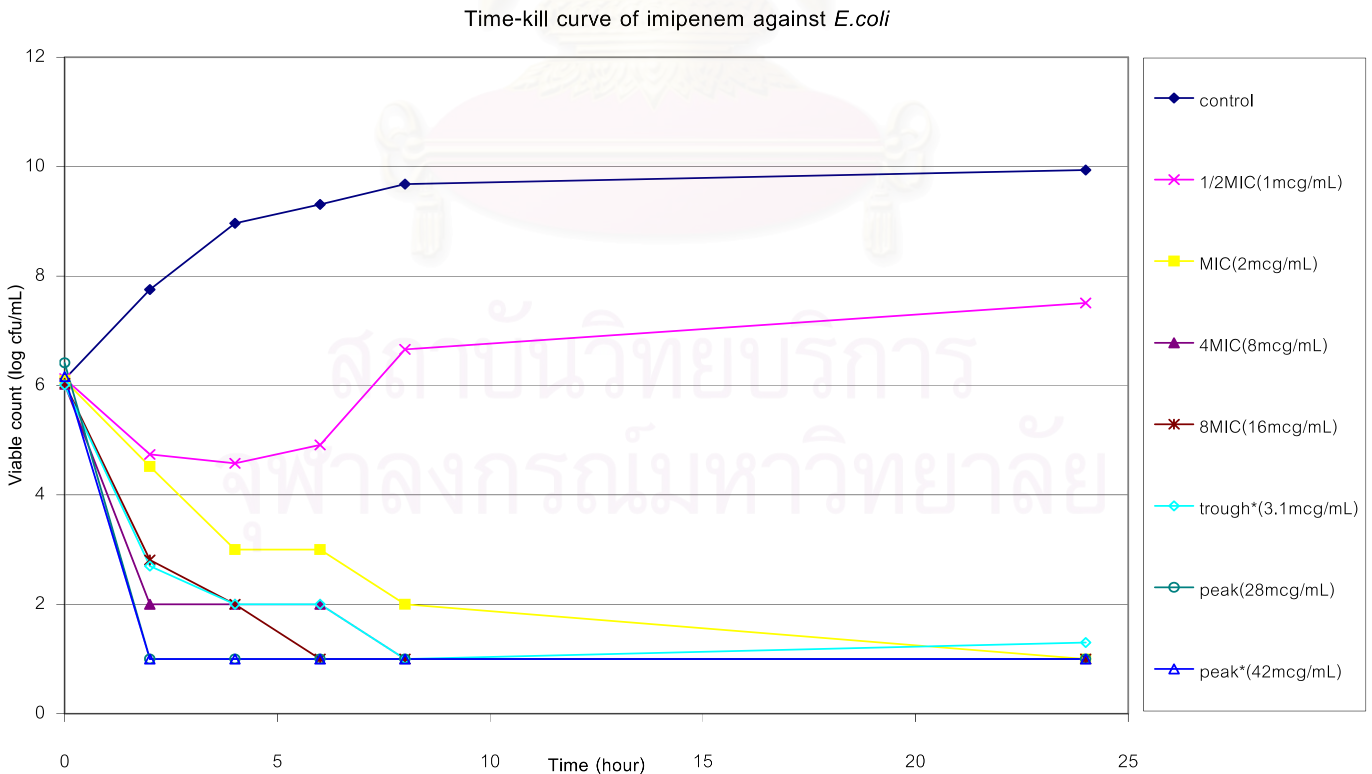

Figure. 4-32 Time-kill curve of imipenem against *E.coli.*

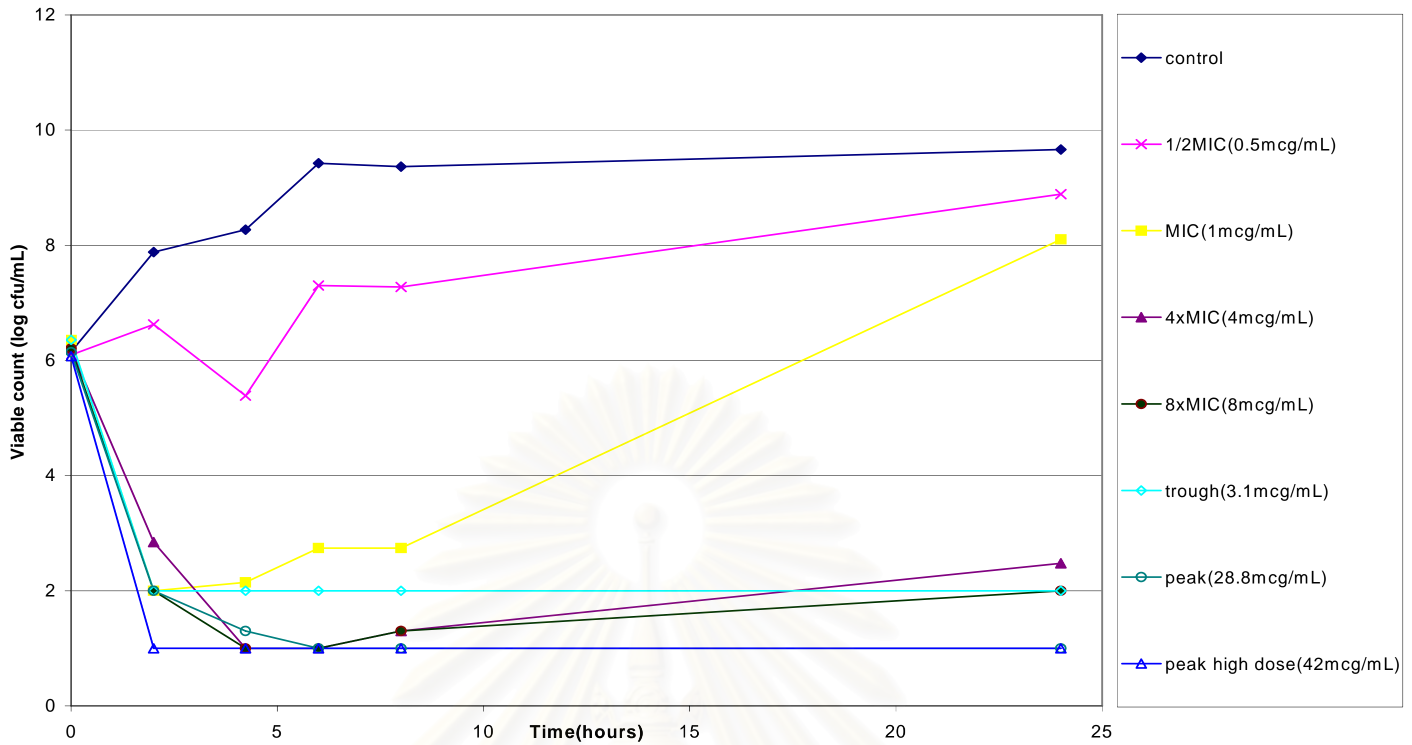

Figure. 4-33 Time-kill curve of imipenem against *K.pnuemoniae.*

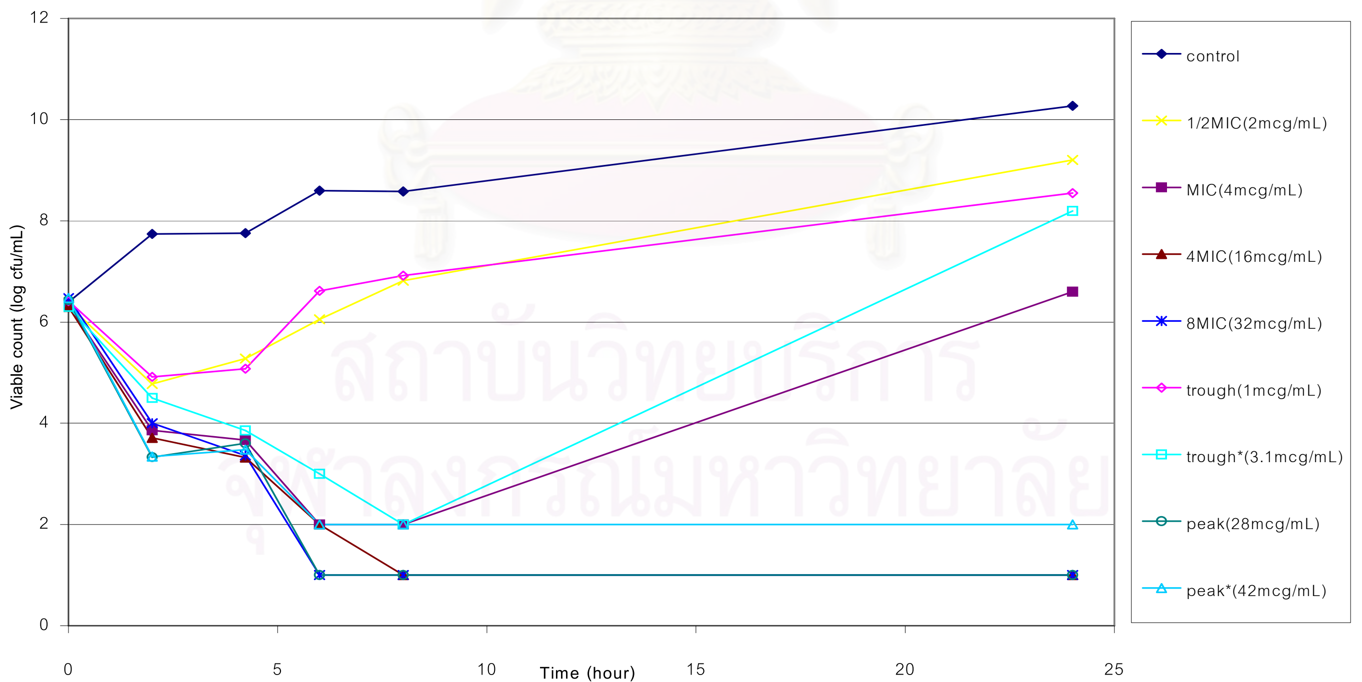

Figure. 4-34 The time-kill curve of imipenem against *P.mirabilis.*

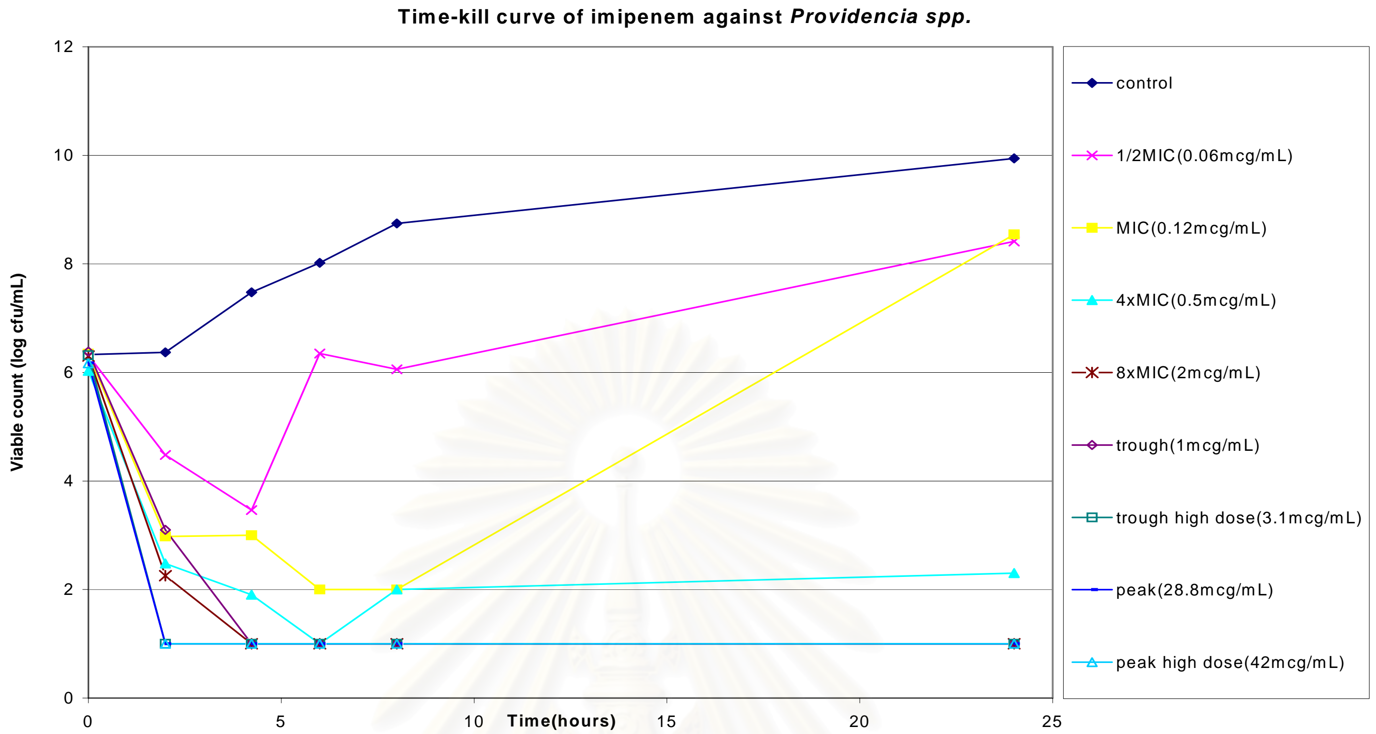

Figure. 4-35 The time-kill curve of imipenem against *Providencia* spp.

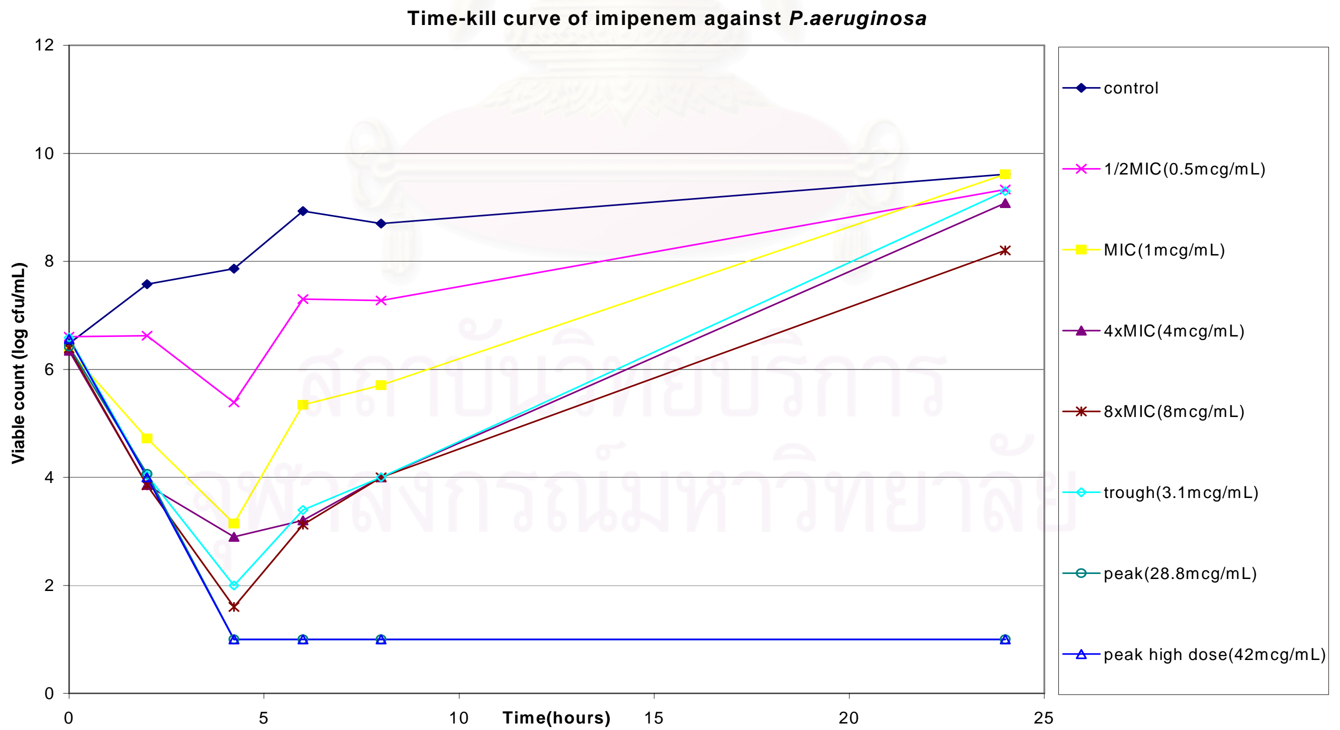

Figure. 4-36 Time-kill curve of imipenem against *P.aeruginosa*.

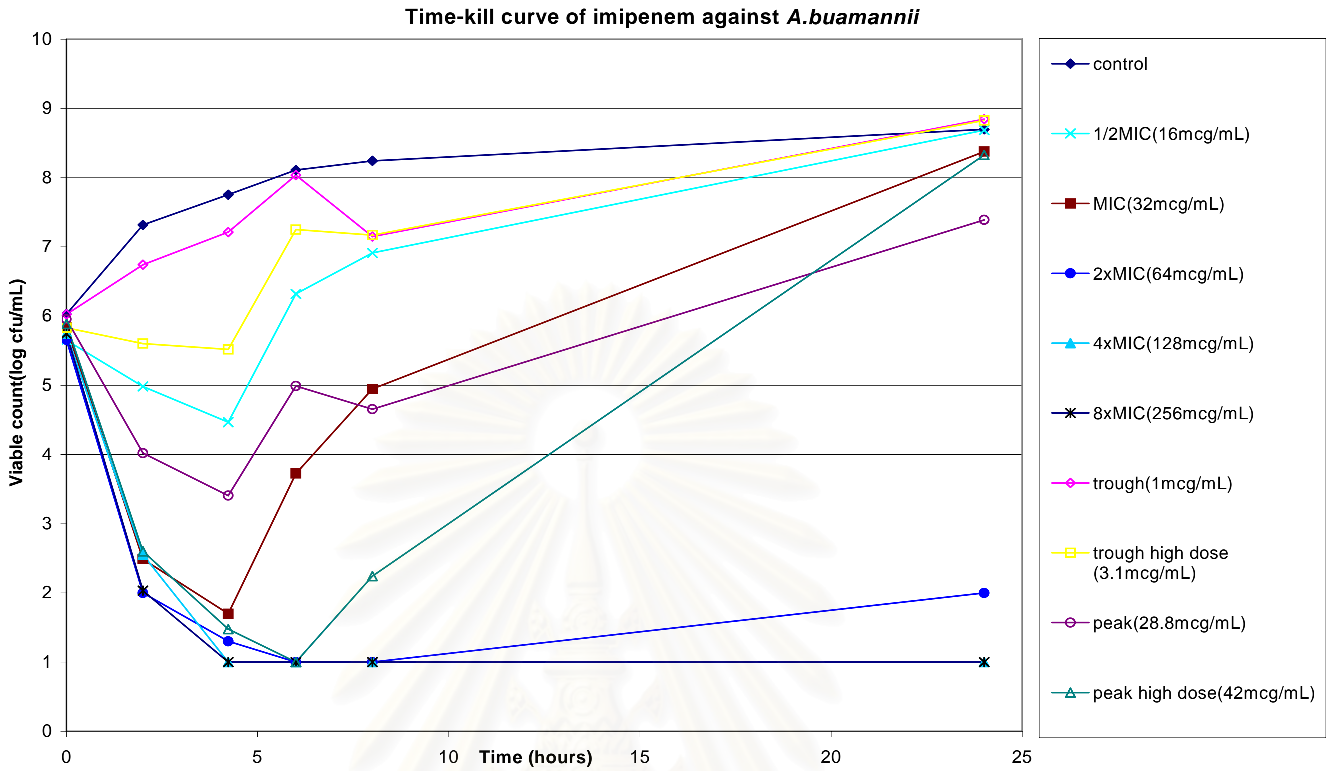

Figure. 4-37 The time-kill curve of imipenem against *A.buamannii.*

Table 4-3 The killing kinetics and regrowth parameters of gentamicin against *E.coli.*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Peak*	Peak**
T90%	-	1.25	0.66	0.33	0.41	0.83	0.33	0.33
T99%	-	2.75	1.41	0.75	0.75	1.5	0.75	0.75
T99.9%	-	-	-	1.08	1.08	-	1.08	0.91
T*E*	-	7.8	15.16	-	-	12	-	-
Tmin	0	4	4	2	2	4	2	2
log Nmin	6.43	3.15	2.75	1	1	3.23	1	1
Δ log Nmin	0	2.85	3.39	5.38	5.15	2.77	5.053	5.296
T	24	24	24	24	24	24	24	24
log N*T*	10.02	9.42	8.47	1	1	9.401	1	1
Δ log N2	-1.268	1.57	2.85	5.38	5.15	2.6	5.05	5.296
Δ log N4	-2.58	2.85	3.4	5.38	5.15	2.77	5.05	5.296
Δ log N6	-2.98	1.08	2.52	5.38	5.15	2.04	5.05	5.296
Δ log N24	-3.58	-3.421	-3.40	5.38	5.15	-3.401	5.053	5.296
Δ log N6-N24	-0.60	-4.5	-5.44	0	0	-5.441	0	0
Killing rate 2	-	0.79	1.43	2.69	2.57	1.3	2.525	2.645
AUC 24	224.32	159.53	131.0	29.385	28.915	145.83	28.813	29.29
Bacteriolytic area	-	64.80	99.33	194.95	195.42	78.50	195.529	195.05

Table 4-4 The killing kinetics and regrowth parameters of gentamicin against *K.pneumoniae.*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Peak*	Peak**
T90%	-	2.25	0.75	0.66	0.66	0.66	0.66	0.66
T99%	-	3.41	1	0.83	0.83	1	0.83	0.83
T99.9%	-	-	2.5	1.25	1.25	1.6	1.25	1.25
T*E*	-	11.25	-	-	-	-	-	-
Tmin	0	4	24	2	2	8	2	2
log Nmin	6.06	3.477	2.21	1	1	1	1	1
Δ log Nmin	0	2.423	4.04	5.03	5.04	5.01	5.23	5.02
T	24	24	24	24	24	24	24	24
log N*T*	9.875	9.4345	2.21	1	1	1	1	1
Δ log N2	-1.447	0.76	2.95	5.03	5.04	3.71	5.23	5.02
Δ log N4	-2.311	2.496	2.530	5.03	5.04	3.745	5.23	5.02
Δ log N6	-2.98	1.578	3.37	5.03	5.04	4.71	5.23	5.02
Δ log N24	-3.81	-3.461	4.03	5.03	5.04	5.01	5.23	5.02
Δ log N6-N24	-0.834	-5.112	0.68	0	0	0.301	0	0
Killing rate 2	-	0.38	1.475	2.515	2.525	1.855	2.615	2.513
AUC 24	217.21	151.91	68.82	29.035	29.045	34.78	28.99	29.02
Bacteriolytic area	-	65.30	148.39	188.18	188.77	182.43	188.22	188.19
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Table4.5 The killing kinetics and regrowth parameters of gentamicin against *P.mirabilis*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Peak*	Peak**
T90%	-	1	1.58	0.66	0.25	1.08	0.25	0.25
T99%	-	1.91	2.75	0.83	0.58	2.16	0.58	0.58
T99.9%	-	6	3.83	1.25	1	3.08	1	1
T*E*	-	15.9	22.83	-	-	18.03	-	-
Tmin	0	6	6	2	2	4	2	2
log Nmin	6.227	3.394	2	1.301	1	2.77	1	1
Δ log Nmin	0	3.056	4.4	4.935	5.53	3.82	5.412	5
T	24	24	24	24	24	24	24	24
log N*T*	10.146	8.747	6.924	3.845	1	8.07	2.86	1
Δ log N2	-0.85	2.179	1.4	4.93	5.53	1.89	5.412	5
Δ log N4	-2.6	2.625	3.146	3.17	5.53	3.82	5.412	5
Δ log N6	-3.18	3.069	4.447	4.112	5.53	3.39	5.412	5
Δ log N24	-3.926	-2.288	-0.477	2.39	5.53	-1.468	3.55	5
Δ log N6-N24	-0.74	-5.353	-4.924	-1.731	0	-4.85	-1.86	0
Killing rate 2	-	1.08	0.7	2.46	2.76	0.946	2.70	2.5
AUC 24	225.057	137.581	100.52	69.89	31.01	130.53	44.29	29
Bacteriolytic area	-	87.476	124.53	155.16	194.04	94.51	180.76	196.05

Table 4-6 The killing kinetics and regrowth parameters of amikacin against *E.coli*.

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Peak*	Peak**
T90%	-	2.3	0.83	0.83	0.5	2.3	0.5	0.41
T99%	-	3.6	1.25	1.25	0.83	-	0.83	0.66
T99.9%	-	-	1.75	1.75	1.33	-	1.33	1.2
T*E*	-	12	-	-	-	8.5	-	-
Tmin	0	4	24	2	2	6	2	2
log Nmin	5.5	2	1	1	1	4.06	1	1
Δ log Nmin	0	2.477	4	3.9	4.11	1.282	4.64	4.74
T	24	24	2.4	24	24	24	24	24
log N*T*	9.477	7.68	1	1	1	1.3	1	1
Δ log N2	-1.045	0.874	2.51	3.9	4.11	0.99	4.6	4.74
Δ log N4	-3.039	2.47	3.023	3.9	4.11	1.232	4.6	4.74
Δ log N6	-3.676	1.81	3.05	3.9	4.11	1.282	4.6	4.74
Δ log N24	-3.978	-3.20	4	3.9	4.11	-3.959	4.6	4.74
Δ log N6-N24	-0.301	-5.02	0.95	0	0	-5.241	0	0
Killing rate 2	-	0.437	1.255	1.95	2.055	0.495	2.3	2.37
AUC 24	216.85	112.70	50.85	27.90	28.11	152.75	28.646	28.74
Bacteriolytic area	-	104.14	165.99	188.94	288.73	64.09	188.20	188.11

Table 4-7 The killing kinetics and regrowth parameters of amikacin against *K.pneumoniae.*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Peak*	Peak**
T90%	-	1.25	0.66	0.33	0.41	0.83	0.33	0.33
T99%	-	2.75	1.41	0.75	0.75	1.5	0.75	0.75
T99.9%	-	-	-	1.08	1.08	-	1.08	0.91
T*E*	-	7.8	15.16	-	-	12	-	-
Tmin	0	4	4	2	2	4	2	2
log Nmin	6.43	3.15	2.75	1	1	3.23	1	1
Δ log Nmin	0	2.85	3.39	5.38	5.15	2.77	5.053	5.296
T	24	24	24	24	24	24	24	24
log N*T*	10.02	9.42	8.47	1	1	9.401	1	1
Δ log N2	-1.268	1.57	2.85	5.38	5.15	2.6	5.05	5.296
Δ log N4	-2.58	2.85	3.4	5.38	5.15	2.77	5.05	5.296
Δ log N6	-2.98	1.08	2.52	5.38	5.15	2.04	5.05	5.296
Δ log N24	-3.58	-3.421	-3.40	5.38	5.15	-3.401	5.053	5.296
Δ log N6-N24	-0.60	-4.5	-5.44	0	0	-5.441	0	0
Killing rate 2	-	0.79	1.43	2.69	2.57	1.3	2.525	2.645
AUC 24	224.32	159.53	131.0	29.385	28.915	145.83	28.813	29.29
Bacteriolytic area	-	64.80	99.33	194.95	195.42	78.50	195.529	195.05

Table 4-8 The killing kinetics and regrowth parameters of amikacin against *Providencia* spp.

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Peak*	Peak**
T90%	-	1.33	1	0.83	0.66	1.08	0.66	0.66
T99%	-	2.16	1.83	1.33	0.83	1.66	0.83	0.83
T99.9%	-	-	5.6	2	133	-	1.33	1.33
T*E*	-	10.5	-	-	-	13.8	-	-
Tmin	0	4	6	6	2	2	2	2
log Nmin	6.24	3.37	2.88	1	1	3.397	1	1
Δ log Nmin	0	2.77	3.41	5.16	5.08	2.733	5.11	5.11
T	24	24	24	24	24	24	24	24
log N*T*	10	9.5	5.46	1	1	9.48	1	1
Δ log N2	-0.74	2.07	2.71	3.41	5.08	2.74	5.11	5.11
Δ log N4	-2.55	2.77	3.03	4.86	5.08	2.71	5.11	5.11
Δ log N6	-2.82	1.77	3.41	5.16	5.08	2.33	5.11	5.11
Δ log N24	-3.76	-3.355	0.830	5.96	5.08	-3.344	5.11	5.11
Δ log N6-N24	-0.94	-5.05	2.53	0	0	-5.67	0	0
Killing rate 2	-	1.04	1.358	1.70	2.54	1.37	2.55	2.55
AUC 24	221.47	157.08	101.36	33.27	29.08	147.28	28.64	30.26
Bacteriolytic area	-	64.39	120.11	188.20	192.39	74.19	192.83	191.21

Table4-9 The killing kinetics and regrowth parameters of amikacin against *P.aeruginosa*.

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Peak*	Peak*
T90%	-	0.75	0.5	0.5	0.5	1.5	0.5	0.5
T99%	-	1.58	1.08	1.41	1.2	-	1.2	1.2
T99.9%	-	-	2	2	1.58	-	1.58	1.58
T*E*	-	15	19.3	21.5	-	14.1	-	-
Tmin	0	2	2	6	8	2	4	4
log Nmin	6.80	4	3	1.77	1.3	5	1	1
Δ log Nmin	0	2.79	3.9	4.65	5.28	1.5	5.58	5.73
T	24	24	24	24	24	24	24	24
log N*T*	9.041	8.82	9.13	7.6	3.65	8.94	3.6	3.62
Δ log N2	-0.71	2.79	3.9	2.71	3.9	1.5	5.58	4.35
Δ log N4	-1.1	2.79	3.9	4.02	4.44	1.5	5.58	5.73
Δ log N6	-1.96	1.79	3.9	4.65	4.58	1.5	5.58	5.73
Δ log N24	-2.24	-2.03	-2.23	-1.18	2.93	-2.44	2.98	3.11
Δ log N6-N24	-0.29	-3.82	-6.13	-5.83	-1.65	-3.94	-2.6	-2.62
Killing rate 2	-	1.395	1.95	1.355	1.95	0.75	2.79	2.17
AUC 24	209.42	148.37	133.95	101.42	61.16	153.05	53.37	53.46
Bacteriolytic area	-	61.04	75.46	107.99	148.25	56.36	156.05	155.95

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Table 4-10 The killing kinetics and regrowth parameters of netilmicin against *Providencia spp.*

Parameter/conc.	control	1/2MIC	MIC	2MIC	4MIC	8MIC	Trough*	Peak*	Peak**
T90%	-	1.5	0.83	0.5	0.41	0.45	0.75	0.4	0.4
T99%	-	3.6	2.5	1.66	1.5	1.16	2	1.16	1.16
T99.9%	-	5.83	5.66	4.75	3.16	1.83	5.33	1.83	1.83
T*E*	-	-	-	-	-	-	-	-	-
Tmin	0	6	6	6	4	2	6	2	2
log Nmin	6.38	1	1	1	1	1	1	1	1
Δ log Nmin	0	5.47	5.48	5.44	5.33	6.54	5.3	5.42	5.3
T	12	12	12	12	12	12	12	12	12
log N*T*	7.87	3	2.5	1	1	1	1.62	1	1
Δ log N2	-1.18	1.73	3.0	3.67	4.04	5.54	3.2	5.42	5.3
Δ log N4	-2.41	3.52	2.78	4.67	5.34	5.54	4.18	5.42	5.3
Δ log N6	-3.03	5.87	5.48	5.44	5.34	5.54	5.32	5.42	5.3
Δ log N24	-3.3	0.768	2.29	5.44	5.34	5.54	3.44	5.42	5.3
Δ log N6-N24	-0.03	-4.70	-3.18	0	0	0	-1.875	0	0
Killing rate 2	-	0.86	1.5	1.84	2.02	2.77	1.6	2.71	2.67
AUC 24	213.85	87.526	74.36	34.56	31.94	29.54	51.21	29.42	29.34
Bacteriolytic area	-	126.32	139.49	179.29	181.91	184.30	162.63	184.42	184.50

Table 4-11 The killing kinetics and regrowth parameters of netilmicin against *P.aeruginosa*.

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Peak*	Peak**
T90%	-	2	1.08	0.6	0.5	-2.6	0.58	0.5
T99%	-	8	2	1.16	1.08	-3.75	1.08	1.08
T99.9%	-	3	1.91	1.66	1.41	-	1.58	1.41
T*E*	-	14	19	-	-	-	-	-
Tmin	0	8	8	4	4	0	4	4
log Nmin	6.33	4	1	1	1	6.38	1	1
Δ log Nmin	0	2.25	5.28	5.53	5.29	0	5.37	5.29
T	8	8	8	8	8	8	8	8
log N*T*	8.397	4	1	1	1	8.84	1	1
Δ log N2	-0.85	1.01	2.11	4.23	3.59	-0.35	4.37	3.59
Δ log N4	-1.92	1.35	4.28	5.53	5.29	-2.13	5.37	5.29
Δ log N6	-2.83	0.9	4.28	5.53	5.29	-1.62	5.37	5.29
Δ log N24	-3.3	-3.26	-1.093	4.53	5.29	-2.616	5.37	5.29
Δ log N6-N24	-1.5	-4.16	-5.374	-1	0	-1	0	0
Killing rate 2	-	0.5	1.05	2.11	1.79	-	2.185	1.79
AUC 24	207.32	142.4	90.58	40.134	32.77	204.49	31.37	32.77
Bacteriolytic area	-	57.91	116.73	167.18	174.54	2.825	175.94	174.54

Table 4-12 The killing kinetics and regrowth parameters of ofloxacin against *E.coli*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**	Peak***	Pesk****
T90%	-	0.75	0.66	0.66	0.66	0.66	0.66	0.58	0.5	0.5	0.5
T99%	-	1	1	1	1	1	1	0.91	0.83	0.83	0.83
T99.9%	-	1.25	1.2	1.16	1.16	1.16	1.16	1.08	1	1	1
T*E*	-	8	-	-	-	-	-	-	-	-	-
Tmin	0	4	6	24	6	24	6	6	8	4	2
log Nmin	6.58	5	2	1	1	1	1	1	1	1	1
Δ log Nmin	0	1.3	4.1	5.1	5.1	4.9	5.4	5.1	4.8	4.8	4.9
T	12	12	12	12	12	12	12	12	12	12	12
log N*T*	9.4	6.8	2	2	1.4	1.8	1	1	1	1	1
Δ log N2	-0.58	0.8	2.41	3.39	3.56	3.35	3.91	3.93	3.82	3.78	4.97
Δ log N4	-2.24	1.27	4.14	3.42	4.3	3.95	4.32	4.31	4.26	4.89	4.97
Δ log N6	-2.77	1.27	4.14	3.86	5.17	2.81	5.39	5.18	4.56	4.89	4.97
Δ log N24	-2.42	-3.43	4.14	5.16	5.17	4.95	5.35	5.21	4.86	4.89	4.97
Δ log N6-N24	0.35	-4.70	0	2.14	0	2.14	0	0	0.301	0	0
Killing rate 2	-	0.4	0.91	1.69	1.78	1.67	1.95	1.86	1.91	1.89	2.48
AUC 24	215.30	168.87	57.61	47.80	34.15	47.45	38.79	33.39	32.75	31.12	28.97
Bacteriolytic area	-	46.43	157.69	167.50	181.16	167.85	176.50	181.91	182.55	182.18	186.33

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Table 4-13 The killing kinetics and regrowth parameters of ciprofloxacin against *E.coli*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**	Peak***
T90%	-	2	1.3	1	0.8	1	1	1	0.66	0.66
T99%	-	2.8	3.3	3	1.6	2.16	3	1.6	1	1
T99.9%	-	-	6.6	20	14.1	14.2	16.6	14.1	1.5	1.5
T*E*	-	-	-	-	-	-	-	-	-	-
Tmin	0	6	24	24	24	24	24	4	4	2
log Nmin	6.054	3	1	1	1	1	1	1	1	1
Δ log Nmin	0	2.94	4.55	5.23	5.33	5.2	5.42	5.301	4.9	5.06
T	8	8	8	8	8	8	8	8	8	8
log N*T*	9.9	3.68	2	2	2	2	2	1	1	1
Δ log N2	-2.20	1.79	2.72	3.63	4.33	3.4	4.42	3.55	4.9	5.06
Δ log N4	-2.91	2.37	3.23	4.2	4.33	4.2	4.42	4.30	5.2	5.06
Δ log N6	-3.4	2.94	3.95	4.2	4.33	4.2	4.42	3.30	5.2	5.06
Δ log N24	-3.63	2.09	5.55	5.2	5.33	5.2	5.42	4.30	5.2	5.06
Δ log N6-N24	-0.23	2.1	1.06	1	1	1	1	1	0	0
Killing rate 2	-	0.89	1.36	1.81	2.16	1.7	2.21	1.77	2.6	2.53
AUC 24	226.27	91.27	52.07	45.82	45.43	44.42	44.37	35.05	29.80	29.06
Bacteriolytic area	-	135.0	174.20	180.85	181.94	180.45	181.85	191.22	196.47	197.21

Table 4-14 The killing kinetics and regrowth parameters of ciprofloxacin against *K.pnuemoniae.*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**	Peak***
T90%	-	1	0.75	0.66	0.66	0.83	0.75	0.5	0.5	0.5
T99%	-	1.5	1	1.16	1.16	2	1	1	1	1
T99.9%	-	2.83	1.75	1.66	1.66	3	1.75	1.5	1.5	1.5
T*E*	-	11.3	-	-	-	-	-	-	-	-
Tmin	0	4	24	4	2	24	4	4	4	4
log Nmin	6.13	2.77	1	1	1	1	1	1	1	1
Δ log Nmin	0	3.4	5.11	4.65	3.71	5.24	5.32	3.63	3.51	3.03
T	8	8	8	8	8	8	8	8	8	8
log N*T*	9.45	5.30	2.83	1	1	1.42	3.60	1	1	1
Δ log N2	-1.82	2.87	4.5	4.24	5.01	2.2	4.32	5.03	5.21	5.03
Δ log N4	-3.04	3.4	4.27	5.05	5.01	4.29	5.32	5.03	5.21	5.03
Δ log N6	-2.98	2.17	4.63	5.05	5.01	4.1	3.67	5.03	5.21	5.03
Δ log N24	-3.66	-2.58	5.11	5.05	5.01	5.24	5.32	5.03	5.21	5.03
Δ log N6-N24	-0.68	-4.75	0.477	0	0	1.14	1.65	0	0	0
Killing rate 2	-	1.435	2.25	2.525	2.50	1.1	2.16	2.515	2.60	2.51
AUC 24	222.10	104.10	49.51	30.67	29.01	43.74	58.10	29.99	30.61	29.88
Bacteriolytic area	-	78.00	172.55	141.42	193.68	178.36	164.10	192.11	191.45	192.11

Table 4-15 The killing kinetics and regrowth parameters of ciprofloxacin against *P.mirabilis*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**	Peak***
T90%	-	3.1	1.14	1.14	0.78	1	1	0.71	0.71	0.71
T99%	-	5.14	2.15	1.72	1.57	1.72	1.72	1.14	1.14	1.14
T99.9%	-	-	4	3.5	2	3.5	2	1.86	1.86	1.86
T*E*	-	15.5	14.5	-	-	-	-	-	-	-
Tmin	0	6	6	24	24	24	24	24	24	24
log Nmin	6.41	3.91	3.04	1	1.301	1	1	1	1	2
Δ log Nmin	0	3.74	5.25	4.74	4.09	4.81	4.38	4.22	4.28	3.28
T	8	8	8	8	8	8	8	8	8	8
log N*T*	9.86	4.47	4.60	2	1.95	2.74	2.02	2.866	2.56	2.47
Δ log N2	-0.67	0.765	1.88	1.47	3.46	3.18	3.43	2.59	2.62	2.59
Δ log N4	-2.47	1.42	3.19	3.55	4.04	4.01	3.73	3	3.08	3.05
Δ log N6	-3.09	2.74	3.25	4.04	4.16	4.2	3.72	3.15	3.23	3.16
Δ log N24	-3.66	-2.24	-2.25	5.24	5.08	5.42	5.38	5.22	5.28	5.22
Δ log N6-N24	-0.48	-4.49	-4.03	1.21	0.92	1.21	1.66	2.07	2.05	2.07
Killing rate 2	-	0.38	0.94	0.73	1.73	1.59	1.71	1.44	1.34	1.29
AUC 24	226.92	148.24	125.55	51.61	49.41	54.85	49.01	59.84	57.24	56.29
Bacteriolytic area	-	78.68	101.37	175.30	177.5	172.06	177.91	107.08	169.68	170.62

Table 4-16 The killing kinetics and regrowth parameters of ciprofloxacin against *Providencia spp.*

Parameter/conc.	control	1/2MIC	MIC	2MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**
T90%	-	1.86	1.21	0.78	0.78	0.66	0.66	0.57	0.57	0.57
T99%	-	6.02	2.08	1.5	1.5	1.5	1.33	1.33	1.33	1.33
T99.9%	-	-	-	1.29	1.29	1.29	1.29	1.21	1.21	1.21
T*E*	-	7	13.3	-	-	-	-	-	-	-
Tmin	0	6	4	2	2	2	2	2	2	2
log Nmin	6.34	4	3.38	1	1	1	1	1	1	1
Δ log Nmin	0	2.41	2.95	5.24	5.13	5.00	5.47	5.28	5.28	5.07
T	8	8	8	8	8	8	8	8	8	8
log N*T*	9.84	6.72	4.83	1	1	1	1	5.28	5.28	1
Δ log N2	-1.01	1.43	2.42	5.24	5.13	5.60	5.47	5.28	5.28	5.07
Δ log N4	-2.37	1.32	2.95	4.99	5.13	5.60	5.47	5.28	5.28	5.07
Δ log N6	-3.02	2.41	1.43	5.24	5.13	5.60	5.47	5.28	5.28	5.07
Δ log N24	-3.13	-3.23	-3.15	5.24	5.13	5.6	5.47	5.28	5.28	5.07
Δ log N6-N24	-0.11	-5.64	-4.57	0	0	0	0	0	0	0
Killing rate 2	-	0.717	1.21	2.62	2.56	2.8	2.73	2.64	2.64	2.53
AUC 24	221.65	172.25	150.15	29.89	29.13	29.6	29.47	29.28	29.49	28.83
Bacteriolytic area	-	49.42	71.49	191.80	192.52	192.05	192.17	192.37	192.21	192.82

Table 4-17 The killing kinetics and regrowth parameters of ciprofloxacin against *P.aeruginosa.*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**	Peak***
T90%	-	-	1.16	1	0.75	-	2.16	0.75	0.75	0.66
T99%	-	-	2.8	1.5	1.33	-	-	1.33	1.33	1
T99.9%	-	-	-	2	2	-	-	1.83	2	1.5
T*E*	-	9.5	-	-	-	4	21.3	-	-	-
Tmin	0	4	6	6	6	2	6	6	6	4
log Nmin	6.35	5.658	3.58	1	1	6.04	4.305	1	1	1
Δ log Nmin	0	0.472	2.72	5.09	5.17	0.2	1.91	5.03	5.26	5.43
T	8	8	8	8	8	8	8	8	8	8
log N*T*	8.95	5.86	3.77	2.23	1	6.45	4.47	1.47	1	1
Δ log N2	-0.49	0.27	1.85	3.25	3.43	0.2	1.09	3.53	3.26	4.43
Δ log N4	-1.74	0.48	2.36	4.09	4.57	0.03	1.88	4.57	4.79	5.43
Δ log N6	-3.28	0.45	2.73	5.09	5.09	0.39	1.92	5.17	5.26	5.43
Δ log N24	-3.11	-1.96	1.00	0.98	3.10	-2.48	-0.48	3.54	5.16	5.43
Δ log N6-N24	0.164	-2.40	-1.71	-4.11	-2.06	-2.87	-2.403	-1.76	0	0
Killing rate 2	-	0.135	0.925	1.62	1.71	0.1	0.925	1.76	1.63	2.215
AUC 24	211.84	158.07	106.83	78.8	50.37	170.39	106.83	52.2	34.21	31.43
Bacteriolytic area	-	53.76	105.0	133.0	161.47	41.44	105.0	159.62	177.62	180.41

Table 4-18 The killing kinetics and regrowth parameters of ciprofloxacin against *Abuamannii*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**	Peak***
T90%	-	1.41	0.85	0.7	0.56	-	-	-	-	1.25
T99%	5.8	3.4	1.7	1.146	1.275	5.8	5.8	5.8	5.8	5.8
T99.9%	-	-	-	1.84	1.7	-	-	-	-	-
T*E*	-	17.7	20.82	-	-	-	-	-	-	-
Tmin	0	4	2	6	6	0	0	0	0	0
log Nmin	5.95	3.45	3	1	1	5.72	5.8	5.8	5.8	5.7
Δ log Nmin	0	2.14	2.75	4.87	5.10	0	0	0	0	0
T	8	8	8	8	8	8	8	8	8	8
log N*T*	7.74	3.69	3.23	1	1	7.30	8.70	8.19	7.81	7.81
Δ log N2	-1.2	1.85	2.75	4.03	5.10	-1.61	-1.26	-0.64	-1.26	-1.45
Δ log N4	-1.71	2.24	2.49	4.4	4.06	-1.79	-1.77	-1.07	-1.41	-1.41
Δ log N6	-1.78	2.32	2.48	4.87	4.63	-1.78	-1.99	-1.16	-1.87	-2.17
Δ log N24	-2.26	-2.15	-0.75	4.87	5.10	-2.71	-2.59	-2.08	-2.6	-2.84
Δ log N6-N24	-0.48	0.42	-3.23	0	0	-0.93	-0.6	-0.92	-0.74	-0.67
Killing rate 2	-	0.92	1.375	2.05	2.55	-	-	-	-	-
AUC 24	184.01	123.08	106.03	31.52	32.14	183.66	196.18	190.33	187.88	189.13
Bacteriolytic area	-	61.0	78.05	152.13	164.03	0.427	-12.09	-6.24	-3.79	-5.04

Table4-19 The killing kinetics and regrowth parameters of cefotaxime against *E.coli*.

Parameter/conc.	control	MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**
T90%	-	1.2	1.08	1.16	1.16	1	0.91	0.66
T99%	-	2.8	2.33	2.66	1.83	1.83	1.91	1
T99.9%	-	4	3.66	4	2.83	2.83	2.83	1.5
T*E*	-	-	-	-	-	-	-	-
Tmin	0	6	6	6	4	4	4	1
log Nmin	5.216	1	1	1	1	1	1	1
Δ log Nmin	0	4.2	4.12	4.01	4.35	4.06	4.14	4
T	12	12	12	12	12	12	12	12
log N*T*	9.33	1	1	1	1	1	1	1
Δ log N2	-0.91	0.9	1.82	1.71	2.357	2.06	2.15	4
Δ log N4	-1.62	1.95	2.46	2.35	3.357	3.06	3.15	4
Δ log N6	-2.95	4.2	4.12	4.01	4.357	4.06	4.15	4
Δ log N24	-3.05	4.2	4.12	4.01	4.357	4.06	4.15	4
Δ log N6-N24	-0.10	0	0	0	0	0	0	0
Killing rate 2	-	0.45	0.91	0.57	1.11	1.02	1.04	1.33
AUC 24	206.30	36.806	34.72	34.617	32.06	32.06	32.15	28
Bacteriolytic area	-	169.50	171.58	171.69	173.95	174.24	174.16	178.3

Table4-20 The killing kinetics and regrowth parameters of cefotaxime against *K.pnuemoniae.*

Parameter/conc.	control	MIC	2MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**
T90%	-	1.5	1	0.83	0.75	0.91	0.75	0.75	0.75
T99%	-	2.5	1.83	1.83	1.58	1.83	1.5	1.3	1.16
T99.9%	-	3.3	-	3.5	3.16	3.83	4	2.5	2
T*E*	-	7.3	-	-	-	-	-	-	-
Tmin	0	4	4	24	8	24	24	6	6
log Nmin	-	2	3.19	2	4.1	2	2	1	1
Δ log Nmin	-	4.028	2.89	4.20	12	3.9	4.156	4.84	4.06
T	12	12	12	12	12	12	12	12	12
log N*T*	9.75	7	4.56	2.68	2	3.43	2.5	1	1
Δ log N2	-1.624	1.288	2.306	2.35	2.41	2.25	2.473	2.96	3.06
Δ log N4	-2.136	2.528	2.52	2.82	2.725	2.72	2.78	3.03	2.75
Δ log N6	-2.97	1.219	2.39	3.50	2.87	2.903	3.11	4.84	4.06
Δ log N24	-2.11	1.72	4.20	4.1	3.903	4.84	4.84	4.84	4.06
Δ log N6-N24	-3.334	-0.567	0.698	1.23	1	0	0	0	0
Killing rate 2	-	0.644	1.153	1.178	1.205	1.175	1.23	1.23	1.48
AUC 24	224.63	154.53	104.13	66.93	59.94	75.86	64.95	35.97	33.44
Bacteriolytic area	-	70.1	120.50	157.69	164.69	148.76	159.67	188.66	191.19

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Table 4-21 The killing kinetics and regrowth parameters of cefotaxime against *Providencia spp.*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**
T90%	-	-	-	1.5	1.16	2.3	1	0.5	0.41
T99%	-	-	-	3.3	4.83	3.8	3	1	0.83
T99.9%	-	-	-	-	5.8	5.8	4.5	1.33	1.16
T*E*	-	2.33	2.3	16.5	-	-	-	-	-
Tmin	0	2	2	6	6	8	6	2	2
log Nmin	6.206	6.22	5.95	3.59	2.95	2	1	1	1
Δ log Nmin	0	0.04	0.2	2.71	3.22	4	5.2	5.2	5.4
T	12	12	12	12	12	12	12	12	12
log N*T*	9.166	8.33	7.41	5.08	3.25	2.42	2.16	1	1
Δ log N2	-1.07	0.04	0.2	1.26	1.84	0.88	1.48	5.2	5.4
Δ log N4	-1.63	-0.82	-0.59	1.88	1.75	1.58	2.07	5.2	5.4
Δ log N6	-3.147	-1.88	-1.33	2.71	3.22	3.23	5.24	5.2	5.4
Δ log N24	-3.50	0	-2.744	-2.03	2.036	2.255	3.54	5.2	5.4
Δ log N6-N24	-0.46	-1.167	-1.415	-4.74	-1.182	-0.69	-1.698	0	0
Killing rate 2	-	0.02	0.1	0.63	0.92	0.44	0.74	2.6	2.6
AUC 24	215.92	197.90	182.88	133.78	90.43	77.09	64.12	29.20	29.40
Bacteriolytic area	-	18.01	33.03	82.13	125.48	138.83	151.79	186.20	186.40

Table 4-22 The killing kinetics and regrowth parameters of cefotaxime against *P.mirabilis*

Parameter/conc.	control	MIC	2MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**
T90%	-	2.83	2	1.25	1	1	0.91	1	0.66
T99%	-	4.75	4.33	4.33	3.5	1.83	1	2	1.08
T99.9%	-	5.75	5.3	6.1	5.8	5.66	2.83	2.83	1.58
T*E*	-	-	-	-	-	-	-	-	-
Tmin	0	8	6	24	24	24	6	4	2
log Nmin	6.16	2.301	2.3	2.3	2.2	1	1	1	1
Δ log Nmin	0	3.7	4	3.7	3.8	5	5	5	5
T	12	12	12	12	12	12	12	12	12
log N*T*	9.9	2.85	2.7	2.4	2.4	2.6	1	1	1
Δ log N2	-1.23	0.78	1.13	1.09	1.36	2.26	2.46	2.16	4.07
Δ log N4	-2.03	1.05	1.57	1.45	1.85	2.43	3.23	3.31	4.07
Δ log N6	-3.51	3.61	4.02	3.15	3.28	3.43	5.03	4.46	4.07
Δ log N24	-3.6	1.76	2.353	3.75	3.85	5.03	5.03	4.46	4.07
Δ log N6-N24	-0.1	-1.84	-1.66	0.6	0.57	1.602	0	0	0
Killing rate 2	-	0.39	0.565	0.545	0.68	1.18	1.23	1.08	2.035
AUC 24	227.91	85.96	82.33	70.624	68.27	60.85	36.17	33.07	28.07
Bacteriolytic area	-	141.94	145.58	157.28	159.63	167.05	191.74	194.84	199.84

Table 4-23 The killing kinetics and regrowth parameters of ceftazidime against *P.aeruginosa*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**
T90%	-	-	4.88	4.67	2.26	4.5	4.5	0.5	0.49
T99%	-	-	-	6.9	3.25	7.2	6.51	1.55	1.06
T99.9%	-	-	-	-	-	-	-	-	2.26
T*E*	-	5.3	13.4	-	-	14.87	20.9	-	-
Tmin	0	6	6	8	4	8	8	4	4
log Nmin	6.15	6.17	4.87	4.18	3.55	4.38	4.01	3.73	1
Δ log Nmin	0	0.05	1.42	2.1	2.64	1.74	2.28	2.53	5.29
T	8	8	8	8	8	8	8	8	8
log N*T*	7.97	6.43	4.76	4.18	4.8	4.38	4.01	4.19	1.87
Δ log N2	-0.98	-0.2	0.45	0.63	0.56	0.56	0.74	2.51	3.73
Δ log N4	-0.85	-0.25	0.58	0.79	2.64	0.73	0.81	2.53	5.29
Δ log N6	-1.46	0.05	1.42	1.65	1.51	1.27	1.76	1.55	4.99
Δ log N24	-3.94	-2.12	-2.60	-0.43	0.48	-2.42	-0.437	1.82	2.85
Δ log N6-N24	-2.48	-2.16	-4.03	0.329	-1.02	-3.69	-2.20	0.278	-2.13
Killing rate 2	-	-	0.225	0.315	0.28	0.28	0.37	1.255	1.86
AUC 24	202.24	168.21	153.25	109.87	122.88	145.55	127.41	103.86	60.34
Bacteriolytic area	-	34.03	48.98	92.36	79.36	56.68	74.83	98.38	141.9

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Table 4-24 The killing kinetics and regrowth parameters of ceftazidime against *A.buamannii*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**
T90%	-	4.25	1.41	0.7	0.56	-	7.36	2	2.12
T99%	-	5.1	3.25	3.11	1.13	-	-	4.5	4.53
T99.9%	-	5.3	4.96	4.81	1.84	-	-	5.1	5.1
T*E*	-	2.9	20.25	-	-	4.81	5.3	16.15	-
Tmin	0	6	8	6	6	6	8	6	8
log Nmin	5.67	2	1.77	1	1	4	4.64	1	1.84
Δ log Nmin	0	3.71	4.04	4.69	4.87	1.82	1.08	4.86	3.92
T	8	8	8	8	8	8	8	8	8
log N*T*	7.51	3.95	1.77	1	1	5.68	4.64	3.77	1.84
Δ log N2	-1.28	-0.94	1.56	2.44	3.24	-1.22	-1.13	1.02	1.76
Δ log N4	-1.47	0.75	2.45	3.49	4.1	-1.41	-1.49	1.11	2.23
Δ log N6	-1.77	3.71	3.61	4.69	4.87	1.82	0.48	4.86	3.93
Δ log N24	-2.6	-2.15	-0.82	4.69	4.87	-2.47	-2.47	-1.74	1.83
Δ log N6-N24	-0.9	-5.86	-4.42	0	0	-4.301	-2.96	-6.61	-2.04
Killing rate 2	-	-	0.78	1.22	1.62	-	-	0.51	0.88
AUC 24	185.97	131.49	94.52	35.61	33.7	159.95	151.82	121.95	72367
Bacteriolytic area	-	54.4	91.44	150.36	152.27	26.01	34.14	64.02	113.29

Table 4-25 The killing kinetics and regrowth parameters of amoxicillin/clavulanate against *E.coli*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Peak*	Peak**
T90%	-	-	1.16	0.83	1	1	0.6
T99%	-	-	2	1.83	1	1.83	1.16
T99.9%	-	-	-	11.1	12.1	-	5.6
T*E*	-	-	16.8	-	-	14.2	-
Tmin	0	0	4	24	24	6	8
log Nmin	6.05	6.17	3.69	1	1	3.69	3.14
Δ log Nmin	0	0	2.41	5.13	4.99	2.42	3.49
T	8	8	8	8	8	8	8
log N*T*	9.39	9.25	5.15	3.64	3.91	4	3.14
Δ log N2	-1.25	-0.27	2.16	2.18	2.11	2.17	2.84
Δ log N4	-2.85	-3.3	2.41	2.52	2.46	2.27	2.70
Δ log N6	-3.95	-2.74	2.343	2.26	2.92	2.43	3.13
Δ log N24	-3.85	-2.56	-3.42	5.13	4.99	-2.94	2.53
Δ log N6-N24	0.1	0.174	-5.67	2.87	2.07	-5.36	-0.59
Killing rate 2	-	-	1.08	1.092	1.055	1.085	1.42
AUC 24	222.40	209.09	151.76	69.77	70.19	137.50	90.27
Bacteriolytic area	-	13.3	70.63	152.62	152.20	84.90	132.12

Table 4-26 The killing kinetics and regrowth parameters of ampicillin/sulbactam against *E.coli*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Peak*	Peak**
T90%	-	-	1	0.75	1	1	0.83
T99%	-	-	5	1.41	2	1.5	1.41
T99.9%	-	-	-	2	9.83	7.5	5.5
T*E*	-	-	11.1	18.3	-	-	-
Tmin	0	0	6	6	8	8	24
log Nmin	7.143	6	4.212	3.43	3.17	3.26	1
Δ log Nmin	0	0	1.78	3.58	3.02	3.04	5.49
T	8	8	8	8	8	8	8
log N*T*	9.40	9.19	5.20	4.11	3.17	3.26	3.13
Δ log N2	-0.07	-0.42	1.92	3.08	2.38	2.27	2.84
Δ log N4	-1.66	-0.819	1.85	2.71	2.34	2.4	2.68
Δ log N6	-2.45	-1.968	1.78	3.58	2.73	2.46	3.15
Δ log N24	-2.25	-2.217	-4.42	-1.48	5.19	2.22	5.49
Δ log N6-N24	0.196	-0.024	-6.57	-5.06	2.46	-0.24	2.33
Killing rate 2	-	-	0.96	1.54	1.19	1.13	1.42
AUC 24	218.29	196.86	165.88	135.43	65.0	91.6	64.37
Bacteriolytic area	-	21.43	52.40	82.86	153.26	126.62	153.92

Table 4-27 The killing kinetics and regrowth parameters of ampicillin/sulbactam against *A,baumannii.*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Peak*	Peak**
T90%	-	-	1	0.75	0.5	1.5	0.83
T99%	-	-	1.83	1.33	1	7	1.5
T99.9%	-	-	3.1	2	1.41	7.8	3.5
T*E*	-	-	7.8	-	-	19.1	-
Tmin	0	0	4	4	2	8	6
log Nmin	5.14	5.45	2	1	1	2	1
Δ log Nmin	0	0	3.41	4.38	4.41	3.44	4.50
T	8	8	8	8	8	8	8
log N*T*	7	7	5.88	1	1	2	1
Δ log N2	-1.11	-0.18	2.41	2.91	4.47	1.44	2.53
Δ log N4	-2.78	-1.69	3.41	4.38	4.47	1.44	3.20
Δ log N6	-2.89	-1.87	1.31	4.38	4.47	0.72	4.50
Δ log N24	-3.4	-2.54	-1.88	2.206	4.47	-2.33	4.50
Δ log N6-N24	-0.6	-0.67	-3.19	-2.78	0	-3.73	0
Killing rate 2	-	-	1.22	1.455	2.235	0.72	1.26
AUC 24	176.58	172.66	134.92	48.80	28.47	109.52	34.66
Bacteriolytic area	-	3.91	41.6	127.77	148.10	66.82	141.91

Table 4-28 The killing kinetics and regrowth parameters of piperacillin/tazobactam against *E.coli*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**
T90%	-	5.42	1.9	2.2	1.9	1.9	0.88	0.738	0.88
T99%	-	-	4.4	5.5	5.13	4.6	3.52	1.32	3.52
T99.9%	-	-	-	24	-	-	-	1.9	6.6
T*E*	-	14.6	19	-	-	-	-	-	-
Tmin	0	6	6	24	24	8	8	24	8
log Nmin	6.33	4.44	3.58	3.3	3.3	3.35	3.32	3.02	2.71
Δ log Nmin	0	1.83	2.63	3.04	2.89	2.9	2.88	3.12	3.189
T	8	8	8	8	8	8	8	8	8
log N*T*	8.91	5.33	3.65	3.6	3.66	3.35	3.32	3.22	2.71
Δ log N2	-1.12	0.33	1.21	1.34	1.92	1.05	1.09	2.14	1.56
Δ log N4	-2.18	-0.04	1.98	1	1.49	2.45	2.58	2.64	1.66
Δ log N6	-2.37	1.83	2.63	2.61	2.65	2.73	2.79	3.01	2.66
Δ log N24	-3.2	-1.49	-1.45	3.04	2.89	2.45	1.609	3.12	2.63
Δ log N6-N24	-0.9	-3.33	-4.07	0.431	0.24	-0.27	-1.179	0.1	-0.02
Killing rate 2	-	0.165	0.605	0.67	0.96	0.525	0.545	1.07	0.78
AUC 24	210.18	149.96	126.05	93.37	90.64	91.89	97.19	78.73	80.13
Bacteriolytic area	-	60.21	84.13	116.80	119.53	118.28	112.98	131.45	130.04

Table 4-29 The killing kinetics and regrowth parameters of piperacillin/tazobactam against *K.pneumoniae*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**
T90%	-	-	3.08	0.8	1.76	1.46	0.8	0.88	1.46
T99%	-	-	4.4	1.76	3.08	2.78	1.76	2	2.78
T99.9%	-	-	-	3.52	4.2	4.2	4.2	2.78	3.96
T*E*	-	4.10	7.7	-	-	-	-	-	-
Tmin	0	2	4	24	24	24	24	24	24
log Nmin	6.07	6.02	3.49	2	2	2	1	1	1
Δ log Nmin	0	0.065	1.92	3.961	4.24	4.29	5.11	5.54	5.15
T	8	8	8	8	8	8	8	8	8
log N*T*	8.65	7.11	5.49	2.02	2.3	2.34	2	1.60	1
Δ log N2	-1.9	0.065	0.29	2.53	1.43	1.67	2.58	2.54	1.5
Δ log N4	-2.41	0.02	1.92	3.06	3.2	3.29	2.63	4.19	3.31
Δ log N6	-2.69	-0.05	1.51	3.43	3.69	3.96	3.6	4.67	4.85
Δ log N24	-3.3	-2.20	-2.76	3.981	4.249	4.29	5.11	5.54	5.15
Δ log N6-N24	-0.6	-2.15	-4.26	0.536	0.55	0.33	1.51	0.875	0.301
Killing rate 2	-	0.032	0.145	1.26	0.715	1.185	1.29	1.27	0.75
AUC 24	207.89	173.02	145.34	57.91	53.78	63.28	51.20	45.41	40.75
Bacteriolytic area	-	34.84	62.51	149.95	144.08	144.57	156.66	162.45	132.26

Table 4-30 The killing kinetics and regrowth parameters of piperacillin/tazobactam against *P.mirabilis*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**
T90%	-	3.08	0.73	1.17	1.17	1.46	1.17	0.88	0.88
T99%	-	4.5	1.32	4.9	3.2	2.64	2.4	1.9	1.76
T99.9%	-	-	2.05	5.8	6.16	3.52	3.37	2.64	2.64
T*E*	-	16.1	-	-	-	-	-	-	-
Tmin	0	8	8	24	24	24	6	4	4
log Nmin	6.31	3.84	3.27	2.95	1	1	1	1	1
Δ log Nmin	0	2.48	3.1	3.32	5.29	5.20	5.32	5.26	5.28
T	8	8	8	8	8	8	4	8	8
log N*T*	8.806	3.48	3.27	3.07	2.77	1.90	1.6	1	1
Δ log N2	-1.33	0.42	1.32	1.68	1.46	1.36	1.72	2.26	1.1
Δ log N4	-2.31	2.02	2.53	146	2.58	3.71	3.91	5.26	5.28
Δ log N6	-2.52	2.321	3.04	3.27	3.17	3.88	5.32	5.26	5.28
Δ log N24	-3.33	-1.967	2.00	3.31	5.29	5.20	5.32	5.26	5.28
Δ log N6-N24	-0.8	-4.28	-1.03	0.043	2.12	1.32	0	0	0
Killing rate 2	-	0.21	0.66	0.84	0.73	0.68	0.86	1.13	0.55
AUC 24	208.55	135.64	91.31	82.43	62.66	50.64	44.77	35.26	37.66
Bacteriolytic area	-	72.90	117.24	126.11	145.89	157.90	163.77	173.29	97.98

Table 4-31 The killing kinetics and regrowth parameters of piperacillin/tazobactam against *P.aeruginosa*.

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**
T90%	-	-	2.9	1.76	1.76	3.3	3.96	1.46	3.96
T99%	-	-	3.8	5.4	3.08	5.4	6.3	6.3	6.3
T99.9%	-	-	4.9	-	4.5	-	-	-	-
T*E*	-	-	-	-	-	-	-	-	-
Tmin	0	0	6	8	6	8	8	8	8
log Nmin	6.307	5.869	1.77	3.21	1.602	3.68	3.68	4.05	3.79
Δ log Nmin	0	0	4.23	2.94	4.47	2.41	2.48	2.12	2.32
T	8	8	8	8	8	8	8	8	8
log N*T*	8.447	7.748	2.2	3.21	2.26	3.68	3.68	4.05	3.79
Δ log N2	-0.99	-0.961	0.28	1.44	1.31	0.54	0.77	1.17	0.38
Δ log N4	-1.463	-1.25	2.44	1.35	2.85	1.29	0.83	1.08	1.04
Δ log N6	-1.723	-1.781	4.24	2.4	4.48	2.29	2.1	1.91	1.97
Δ log N24	-2.3	-2.57	2.71	1.16	3.02	1.45	1.45	2.04	1.89
Δ log N6-N24	-0.6	-0.79	-1.52	1.23	-1.99	-0.83	-0.65	0.14	-0.07
Killing rate 2	-	-	0.14	0.72	0.695	0.27	0.385	0.585	0.19
AUC 24	202.79	186.39	74.43	101.73	70.18	104.65	106.79	104.4	104.07
Bacteriolytic area	-	16.39	128.35	101.05	132.61	98.14	96.0	98.38	82.32

Table 4-32 The killing kinetics and regrowth parameters of cefoperazone/sulbactam against *E.coli*.

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Peak*	Peak**
T90%	-	-	1.24	1.7	1.39	0.62	0.62
T99%	-	-	2.48	3.56	3.25	1.24	1.24
T99.9%	-	-	-	5.11	5.27	1.86	1.86
T*E*	-	-	9.92	-	-	-	-
Tmin	0	0	4	8	24	8	6
log Nmin	7.14	6.33	3.47	2.60	1	1	1
Δ log Nmin	0	0	2.68	3.7	5.6	5.19	5.18
T	8	8	8	8	8	8	8
log N*T*	9.406	8.76	5.67	2.6	2.6	14	1
Δ log N2	-0.07	-0.51	1.74	1.27	1.43	3.17	3.14
Δ log N4	-1.66	-1.03	2.68	2.13	2.43	4.19	4.18
Δ log N6	-2.453	-1.33	2.04	3.46	3.44	3.72	5.18
Δ log N24	-2.253	-3.36	-3.59	3.301	5.602	5.19	5.18
Δ log N6-N24	0.196	-2.03	-5.63	-0.155	2.161	1.477	0
Killing rate 2	-	-	0.87	0.635	0.715	1.58	1.57
AUC 24	218.29	206.57	159.225	77.83	63.041	38.19	35.27
Bacteriolytic area	-	11.85	59.20	140.59	155.6	180.23	183.16

Table 4-33 The killing kinetics and regrowth parameters of cefoperazone/sulbactam against *A.baumannii.*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Peak*	Peak**
T90%	-	-	1.39	0.93	0.62	1.24	1
T99%	-	-	4.3	1.705	1.55	2.17	2
T99.9%	-	-	-	2.945	2.71	3.41	3.2
T*E*	-	-	8	-	-	-	-
Tmin	0	0	4	8	8	8	8
log Nmin	5.83	5.77	4	1	1	1	1
Δ log Nmin	0	0	2.02	4.93	5.14	4.91	4.63
T	8	8	8	8	8	8	8
log N*T*	8.61	7.76	6.02	1	1	1	1
Δ log N2	-1.08	-0.86	1.52	2.43	2.05	2.62	2.48
Δ log N4	-2.46	-2.01	2.02	3.69	3.89	3.75	3.04
Δ log N6	-2.57	-1.7	1.42	3.93	4.14	4.1	3.89
Δ log N24	-3.08	-3.05	-2.59	4.93	5.14	4.413	4.638
Δ log N6-N24	-0.52	-1.355	-4.02	0	0	0.812	0.74
Killing rate 2	-	-	0.76	1.215	1.027	1.31	1.24
AUC 24	202.04	190.20	155.41	38.43	39.85	37.45	37.61
Bacteriolytic area	-	11.83	46.6	163.61	162.19	164.59	164.42

Table 4-34 The killing kinetics and regrowth parameters of imipenem against *E.coli*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Peak*	Peak**
T90%	-	1.5	1.5	0.75	0.75	0.75	0.5	0.5
T99%	-	-	2.75	1.16	1.33	1.33	1	1
T99.9%	-	-	4	2	2	2	1.33	1.33
T*E*	-	7.5	-	-	-	-	-	-
Tmin	0	4	24	8	6	8	2	2
log Nmin	6.11	4.57	1	1	1	1	1	1
Δ log Nmin	0	1.56	5.08	5.02	5.03	5	5.41	5.16
T	8	8	8	8	8	8	8	8
log N*T*	9.68	6.65	2	1	1	1	1	1
Δ log N2	-1.64	1.38	1.57	4.02	2.14	3.31	5.41	5.16
Δ log N4	-2.85	1.56	3.08	4.02	4.03	4	5.41	5.16
Δ log N6	-3.18	1.21	3.08	4.02	5.03	4	5.41	5.16
Δ log N24	-3.82	-1.37	5.08	5.02	5.03	4.07	5.41	5.16
Δ log N6-N24	-0.63	-2.59	2	1	0	0.699	0	0
Killing rate 2	-	0.69	0.78	2.01	1.07	1.65	2.70	2.58
AUC 24	224.81	154.56	53.12	35.02	34.66	38.82	29.41	29.16
Bacteriolytic area	-	70.24	171.68	189.79	190.15	186.0	195.40	195.65

Table 4-35 The killing kinetics and regrowth parameters of imipenem against *K.pnuemoniae*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Peak*	Peak**
T90%	-	-	0.66	0.83	0.66	0.66	0.66	0.5
T99%	-	-	1	1.33	1	1	1	0.83
T99.9%	-	-	1.5	1.83	1.5	1.5	1.5	1.16
T*E*	-	3	18	-	-	-	-	-
Tmin	0	4	2	4	4	2	6	2
log Nmin	6.15	5.38	2	1	1	2	1	1
Δ log Nmin	0	0.71	4.35	5.19	5.2	4.35	5.14	5.07
T	8	8	8	8	8	8	8	8
log N*T*	9.36	7.27	2.74	1.30	1.30	2	1	1
Δ log N2	-1.73	-0.53	4.35	3.345	4.20	4.35	4.14	5.07
Δ log N4	-2.11	0.71	4.21	5.19	5.20	4.35	4.84	5.07
Δ log N6	-3.27	-1.21	3.61	5.19	5.20	4.35	5.14	5.07
Δ log N24	-3.51	-2.78	-1.74	1.67	4.20	4.35	5.14	5.07
Δ log N6-N24	-	-1.58	-5.35	3.77	-1	0	0	0
Killing rate 2	-	-	2.175	-	2.10	2.175	2.07	2.53
AUC 24	218.89	181.30	109.59	47.40	41.91	52.35	31.74	29.07
Bacteriolytic area	-	37.59	109.30	171.49	176.98	166.54	187.15	189.82

Table 4-36 The killing kinetics and regrowth parameters of imipenem against *Providencia spp.*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**
T90%	-	1.16	0.75	0.66	0.5	0.66	0.5	0.41	0.41
T99%	-	2.5	1.33	1.5	1	1	0.83	0.83	0.83
T99.9%	-	-	2	1.83	1.5	1.58	1.16	1.16	1.16
T*E*	-	6	18	-	-	-	-	-	-
Tmin	0	4	6	6	4	4	2	2	2
log Nmin	6.33	3.46	2	1	1	1	1	1	1
Δ log Nmin	0	2.88	4.34	5.02	5.29	5.38	5.31	5.17	5.16
T	8	8	8	8	8	8	8	8	8
log N*T*	3.74	6.05	2	2	1	1	1	1	1
Δ log N2	-0.04	1.66	3.37	3.54	4.04	3.29	5.31	5.17	5.16
Δ log N4	-1.14	2.88	3.34	4.12	5.29	5.38	5.31	5.17	5.16
Δ log N6	-1.69	-0.03	4.34	5.02	5.29	5.38	5.31	5.17	5.16
Δ log N24	-3.61	-2.09	-2.20	3.72	5.29	5.38	5.31	5.17	5.16
Δ log N6-N24	-1.92	-2.06	-6.54	-1.30	0	0	0	0	0
Killing rate 2	-	0.83	1.685	1.77	2.02	1.64	2.655	2.58	2.58
AUC 24	208.35	156.42	108.65	53.19	31.80	33.57	29.31	29.17	29.16
Bacteriolytic area	-	51.62	99.70	155.15	176.54	178.77	179.03	179.17	127.55

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Table 4.37 The killing kinetics and regrowth parameters of imipenem against *P.mirabilis*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**
T90%	-	1.33	1	0.75	0.66	1.41	1.16	0.75	0.66
T99%	-	-	1.5	1.41	1.33	-	2.5	1.33	1.33
T99.9%	-	-	4.6	4.5	2	-	5.6	3.8	2
T*E*	-	6.5	23.75	-	-	6	20.1	-	-
Tmin	0	2	6	8	6	2	8	6	6
log Nmin	6.39	4.72	2	1	1	4.91	2	1	2
Δ log Nmin	0	1.56	4.33	5.3	5.47	1.5	4.3	5.43	4.47
T	8	8	8	8	8	8	8	8	8
log N*T*	8.57	6.81	2	1	1	6.91	2	1	2
Δ log N2	-1.35	1.56	2.47	2.59	2.47	1.5	1.81	3.1	3.13
Δ log N4	-1.35	1.06	2.67	2.98	3.11	1.34	2.45	2.83	3
Δ log N6	-2.2	0.28	4.33	4.30	5.47	-0.2	3.3	5.43	4.47
Δ log N24	-3.88	-2.87	-0.27	5.30	5.47	-2.13	-1.89	5.43	4.47
Δ log N6-N24	-1.68	-3.15	-4.60	1	0	-1.93	-5.19	0	0
Killing rate 2	-	0.78	1.23	1.29	1.238	0.75	0.90	1.55	1.56
AUC 24	213.96	173.56	96.20	41.37	40.20	170.29	112.57	39.30	58.12
Bacteriolytic area	-	40.40	117.76	172.59	173.76	43.67	101.38	174.66	155.84

Table 4-38 The killing kinetics and regrowth parameters of imipenem against *P.aeruginosa*

Parameter/conc.	control	1/2MIC	MIC	4MIC	8MIC	Trough*	Peak*	Peak**
T90%	-	4	1.33	1	0.91	1	0.91	0.91
T99%	-	-	2.83	1.83	1.66	1.83	1.58	1.58
T99.9%	-	-	4.16	3	2.66	2.66	2.5	2.5
T*E*	-	5.3	11.6	16.2	16.8	15.8	-	-
Tmin	0	4	4	4	4	4	4	4
log Nmin	6.477	5.38	3.14	2.89	1.6	2	1	1
Δ log Nmin	0	1.22	3.21	3.45	4.79	4.58	5.41	5.56
T	8	8	8	8	8	8	8	8
log N*T*	8.69	7.27	5.7	4	4	4	1	1
Δ log N2	-1.1	-0.02	1.63	2.48	2.54	2.55	2.35	2.56
Δ log N4	-1.38	1.22	3.21	3.45	4.79	4.58	5.41	5.56
Δ log N6	-2.45	-0.7	1.01	3.14	3.27	3.19	5.41	5.56
Δ log N24	-3.13	-2.72	-3.26	-2.733	-1.81	-2.71	5.41	5.56
Δ log N6-N24	-0.68	-2.03	-4.27	-5.87	-5.07	-5.90	0	0
Killing rate 2	-	-	0.81	1.24	1.27	1.275	1.175	1.28
AUC 24	210.41	185.37	161.03	134.90	125.18	135.86	35.55	35.56
Bacteriolytic area	-	25.03	49.37	75.50	85.23	74.54	174.86	174.85

Table 4-39 The killing kinetics and regrowth parameters of imipenem against *A.buamannii.*

Parameter/conc.	control	1/2MIC	MIC	2MIC	4MIC	8MIC	Trough*	Trough**	Peak*	Peak**
T90%	-	3.6	0.75	0.66	0.66	0.66	-	-	1.16	0.75
T99%	-	-	1.33	1.16	1.16	1.16	-	-	2.33	1.33
T99.9%	-	-	2	1.66	1.66	1.66	-	-	-	2
T*E*	-	5.5	12.6	-	-	-	-	4.3	14.8	15
Tmin	0	4	4	6	4	4	0	4	4	6
log Nmin	6.02	4.46	1.69	1	1	1	6.02	5.51	3.406	1
Δ log Nmin	0	1.18	4.14	4.65	4.74	4.74	0	1.05	2.55	4.88
T	8	8	8	8	8	8	8	8	8	8
log N*T*	8.24	6.91	4.94	1	1	1	7.14	7.17	4.65	2.24
Δ log N2	-1.29	0.67	3.35	3.65	3.197	3.71	-0.72	0.23	1.93	3.28
Δ log N4	-1.73	1.18	4.14	4.35	4.74	4.74	-1.19	1.05	2.55	4.88
Δ log N6	-2.09	-0.67	2.11	4.65	4.74	4.74	-2.01	-1.41	0.97	4.88
Δ log N24	-2.67	-3.03	-2.54	3.65	4.74	4.74	-2.819	-2.99	-1.43	-2.44
Δ log N6-N24	-0.58	-2.36	-4.65	1	0	0	-0.80	-1.57	-2.45	-7.32
Killing rate 2	-	0.335	1.675	1.825	1.598	1.858	-	0.115	0.965	1.64
AUC 24	196.17	168.90	133.19	39.26	31.85	30.81	185.12	177.72	131.78	102.86
Bacteriolytic area	-	27.27	62.98	156.91	164.32	165.36	11.05	18.45	64.98	93.31

CHAPTER V

DISCUSSION AND CONCLUSION

DISCUSSION

From the study of gentamicin and amikacin that are in the aminoglycoside group, there presented their bactericidal activity against *E.coli*. Nevertheless, they presented no inoculum effect. To kill the bacteria at 99.9%, the concentration of the medicine needs to reach the levels of MIC, 4MIC, 8MIC, trough, peak, and peak high dose of amikacin. Additionally, the study presented that in order to kill the bacteria at 99.9% level via using gentamicin, the medicine concentration levels started from 4MIC, 8MIC, trough, peak, and peak high dose of gentamicin. (Table 5-1)

The current clinical usage usually gives a patient aminoglycoside with once daily model that usage provides the peak to MIC ratio higher than 8 possibly killing all bacteria and preventing the treatment failure. Hence, to apply these results to the current clinical usage, the precaution must be taken place in order to use the medicines against the bacteria consisted high MIC although there is the susceptibility in both medicines. The treatment failure concerning from the resistance effect should still be observant. (Kim MK and Nicolau DP, 2002)

Considering, the medicine under the Fluoroquinolone group against *E.coli*, the study indicated that the medicine still could keep its bactericidal activity and generated no inoculum effect. When its concentration is over MIC, it will be able to kill the bacteria at 99.9%.

This result is unified to the previous knowledge that Fluoroquinolone exhibit concentration-dependent killing, thus, it can be concluded that regimens of large doses (resulting in high AUCs and AUICs) given at infrequent intervals (thus relying on the PAE) might be most efficacious in terms of bacterial killing, eradication time, and reducing the selection of resistant bacteria. (Lode H et al., 1998)

Regarding the medicines under the beta lactam group against *E.coli*, the study presented that the medicines still have their bactericidal activity ability. However, the

usage of the cefotaxime, ampicillin/sulbactam and cefoperazone/sulbactam against *E.coli* caused inoculum effect. This result is consistent with a previous study in that piperacillin/tazobactam is the less subject to an inoculum effect than the cephalosporins and aztreonam (Thomson and Moland, 2001). There are at least two hypotheses that attempt to explain the mechanism of the inoculum effect: first, drug destruction by beta-lactamases, and second, filamentous transformation with continued growth. The former mechanism is more probable one for beta-lactamase-unstable- penicillins even when protected by beta-lactamase inhibitors. In this study, it appeared that the inoculum effect for those agents (Goldstein et al., 1991)

Additionally, in order to kill the bacteria at the level of 99.9% which giving the no regrowth effect and regrowth is similarly, the drug levels needed to reach 4MIC and at the level of medicine in a patient blood at peak, and peak high dose. Those results are except for imipenem.

This result is unified to the previous knowledge that beta lactam exhibit time-dependent killing, thus, maximum killing is usually achieved at 3 to 4 times the MIC and the T>MIC is an important predictors for efficacy. It should be possible to compare outcomes with the MICs for the causative pathogens. In the setting of profound neutropenia, penicillin and cephalosporin levels need to exceed the MIC for 90%-100% of the dosing interval for efficacy against gram negative bacilli. Bacteriostatic drug levels may be all that is required in the nonneutropenic host, in which case levels need exceed the MIC for only approximately 20%, 25%-30%, and 25%-40% of the dosing interval for carbapenems, penicillins, and cephalosporins, respectively (Turnidge, 1998).

According to my opinion coming from the study and working experience, howbeit, the bacteria susceptibility and low resistance presented, the bacteria present the inoculum effect in beta lactam antibiotic. Thus, *E.coli* infection which the site of infection were higher inoculum, should be need higher dose more than normal. Thereby, the close tracing on the treatment should be processed.

From the study of amikacin and gentamicin against *K.pneumoniae* presented bactericidal effect. Nevertheless, they presented no inoculum effect. To kill the bacteria at 99.9%, the concentration of the medicine needs to reach the levels of MIC, 4MIC,

8MIC, trough, peak, and peak high dose of amikacin. However, the study showed the regrowth effect after giving the medicine for 6 hrs at the medicine concentration levels at ½ MIC, MIC and trough. Additionally, the study presented that in order to kill the bacteria at 99.9% level via using gentamicin, the medicine concentration levels started from MIC, 4MIC, 8MIC, trough, peak, and peak high dose of gentamicin. However, the study displayed the regrowth effect after giving the medicine for 6 hrs at the medicine concentrations at ½ MIC and MIC. (Table 5-2)

The current clinical usage usually gives a patient aminoglycoside with once daily model that usage provides the peak to MIC ratio higher than 8 possibly killing all bacteria and preventing the treatment failure. Hence, to apply these results to the current clinical usage, the precaution must be taken place in order to use the medicines against the bacteria consisted high MIC although there is the susceptibility in both medicines. The treatment failure concerning from the resistance effect should still be observant.

Considering, the medicine under the Fluoroquinolone group against *K.pneumoniae*, the study indicated that the medicine still could keep its bactericidal activity and generated no inoculum effect. When its concentration is over MIC, it will be able to kill the bacteria at 99.9%.

This result is unified to the previous knowledge that Fluoroquinolone exhibit concentration-dependent killing, thus, it can be concluded that regimens of large doses (resulting in high AUCs and AUICs) given at infrequent intervals (thus relying on the PAE) might be most efficacious in terms of bacterial killing, eradication time, and reducing the selection of resistant bacteria. (Lode H et al., 1998)

Regarding the medicines under the Beta lactam group against *K. pneumoniae*, the study presented that the medicines still have their bactericidal activity ability. However, the usage of the imipenem against *K.pneumoniae* caused no inoculum effect. Additionally, in order to kill the bacteria at the level of 99.9% and giving no regrowth effect, the medicines needed to reach 4MIC and at the level of medicine in a patient blood at trough, peak, and peak high dose. (Table 5-2)

This result is unified to the previous knowledge that in the setting of profound neutropenia, penicillin and cephalosporin levels need to exceed the MIC for 90%-100%

of the dosing interval for efficacy against gram negative bacilli. Bacteriostatic drug levels may be all that is required in the nonneutropenic host, in which case levels need exceed the MIC for only approximately 20%, 25%-30%, and 25%-40% of the dosing interval for carbapenems, penicillins, and cephalosporins, respectively (Turnidge, 1998).

According to my opinion coming from the study and working experience, howbeit, the bacteria susceptibility and low resistance are presented, the bacteria has the ability to generate the ESBL enzyme that can inactivate the medicine. Thus, the close tracing on the treatment should be processed. With use of a simple mathematical formula, T> MIC percentages can be readily calculated on the basic of pharmacokinetics in healthy volunteers and can be used for comparison with current dosage schedules and target T>MIC persentages. Thereby, adjustment of dosage schedules should be considered if these percentages are not achieved, in an aim to give lower doses more frequently, as this is more effective than simply increasing doses (Turnidge, 1998).

From the study of *P.mirabilis*, all medicines, which are amikacin and gentamicin that are in the aminoglycoside group presented their bactericidal activity. Nevertheless, they presented no inoculum effect. To kill the bacteria at 99.9%, the concentration of the medicine needs to reach the levels of 4MIC, 8MIC, trough, peak, and peak high dose of amikacin. However, the study showed the regrowth effect after giving the medicine for 6 hrs at the medicine concentration levels at 4MIC and trough. Additionally, the study presented that in order to kill the bacteria at 99.9% level via using gentamicin, the medicine concentration levels started from MIC, 4MIC, 8MIC, trough, peak, and peak high dose of gentamicin. However, the study displayed the regrowth effect after giving the medicine for 6 hrs at the medicine concentrations at 4MIC (Table 5.3).

The current clinical usage usually gives a patient aminoglycoside with once daily model that usage provides the peak to MIC ratio higher than 8 possibly killing all bacteria and preventing the treatment failure. Hence, to apply these results to the current clinical usage, the precaution must be taken place in order to use the medicines against the bacteria consisted high MIC although there is the susceptibility in both

medicines. The treatment failure concerning from the resistance effect should still be observant.

Considering, the medicine under the Fluoroquinolone group against P. mirabilis, the study indicated that the medicine still could keep its bactericidal activity and generated no inoculum effect. When its concentration is over MIC, it will be able to kill the bacteria at 99.9%.

This result is unified to the previous knowledge that Fluoroquinolone exhibit concentration-dependent killing, thus, it can be concluded that regimens of large doses (resulting in high AUCs and AUICs) given at infrequent intervals (thus relying on the PAE) might be most efficacious in terms of bacterial killing, eradication time, and reducing the selection of resistant bacteria. (Lode H et al., 1998)

Regarding the medicines under the Beta lactam group against *P. mirabilis*, the study presented that the medicines still have their bactericidal activity ability. However, the usage of the ceftotaxime and piperacilin/tazobactam against *P.mirabilis* caused no inoculum effect (Eng RH et al., 1985; Thomson KS and Moland ES, 2001). Additionally, in order to kill the bacteria at the level of 99.9% and presented no regrowth effect, the medicines needed to reach 4MIC and at the level of medicine in a patient blood at trough, peak, and peak high dose.

This result is unified to the previous knowledge that beta lactam exhibit time-dependent killing, thus, maximum killing is usually achieved at 3 to 4 times the MIC and the T>MIC is an important predictors for efficacy. It should be possible to compare outcomes with the MICs for the causative pathogens. In the setting of profound neutropenia, penicillin and cephalosporin levels need to exceed the MIC for 90%-100% of the dosing interval for efficacy against gram negative bacilli. Bacteriostatic drug levels may be all that is required in the nonneutropenic host, in which case levels need exceed the MIC for only approximately 20%, 25%-30%, and 25%-40% of the dosing interval for carbapenems, penicillins, and cephalosporins, respectively (Turnidge, 1998).

According to my opinion coming from the study and working experience, notwithstanding, the bacteria susceptibility and low resistance are presented, the bacteria has the ability to generate the ESBL enzyme that can inactivate the medicine. Thus, the close tracing on the treatment should be processed.

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From the study of *Providencia spp*., all medicines, which are amikacin and netilmicin that are in the aminoglycoside group, presented their bactericidal effect. In contrast, they presented no inoculum effect. To kill the bacteria at 99.9%, the concentration of the medicine needs to reach the levels of ½ MIC, MIC, 4MIC, 8MIC, trough, peak, and peak high dose of amikacin. However, the study showed the regrowth effect after giving the medicine for 6 hrs at the medicine concentration levels at ½ MIC, MIC and trough. Additionally, the study presented that in order to kill the bacteria at 99.9% level via using netilmicin, the medicine concentration levels started from ½ MIC, MIC, 4MIC, 8MIC, trough, peak, and peak high dose of netilmicin. However, the study displayed the regrowth effect after giving the medicine for 6 hrs at the medicine concentrations at ½ MIC and MIC (Table 5-4).

The current clinical usage usually gives a patient aminoglycoside with once daily model that usage provides the peak to MIC higher than 8 possibly killing all bacteria and preventing the treatment failure. Consequently, to apply these results to the current clinical usage, the precaution must be taken place in order to use the medicines against a bacteria consisted high MIC although there is the susceptibility in both medicines. The treatment failure concerning from the resistance effect should still be aware.

Considering, the medicine under the Fluoroquinolone group against Providencia spp., the study indicated that the medicine still could keep its bactericidal activity and generated no inoculum effect. When its concentration is over 4MIC, it will be able to kill the bacteria at 99.9%.

This result is unified to the previous knowledge that Fluoroquinolone exhibit concentration-dependent killing, thus, it can be concluded that regimens of large doses (resulting in high AUCs and AUICs) given at infrequent intervals (thus relying on the PAE) might be most efficacious in terms of bacterial killing, eradication time, and reducing the selection of resistant bacteria. (Lode H et al., 1998)

Regarding the medicines under the Beta lactam group against *Providencia* spp., the study presented that the medicines still have their bactericidal activity ability. However, the usage of the imipenem against *Providencia* spp. caused the inoculum effect that the effect did not show via using the other medicines in this group. So, in

order to treat a serious infected patient who needs the rapid treatment, the level of medicine is needed to reach the optimum quantity that may be higher than normal level usage especially at the area that is infected presented the high inoculum site (Thomson KS and Moland ES, 2001). Additionally, in order to kill the bacteria at the level of 99.9% and receiving no regrowth effect, the medicines needed to reach eight times of MIC and at the level of medicine in a patient blood at trough, peak, and peak high dose.

This result is unified to the previous knowledge that beta lactam exhibit time-dependent killing, thus, maximum killing is usually achieved at 3 to 4 times the MIC and the T>MIC is an important predictors for efficacy. It should be possible to compare outcomes with the MICs for the causative pathogens. In the setting of profound neutropenia, penicillin and cephalosporin levels need to exceed the MIC for 90%-100% of the dosing interval for efficacy against gram negative bacilli. Bacteriostatic drug levels may be all that is required in the nonneutropenic host, in which case levels need exceed the MIC for only approximately 20%, 25%-30%, and 25%-40% of the dosing interval for carbapenems, penicillins, and cephalosporins, respectively (Turnidge, 1998).

According to my opinion coming from the study and working experiences, the level of medicine in the patient blood should be more than 4MIC and the T>MIC should be more than 100% if the patient got the *Providencia* spp., and presented the status of immunocompromise host, and needed to use Beta lactam.

From the study of *P.aeruginosa*, all medicines, which are amikacin and netilmicin that are in the aminoglyvoside group, presented their bactericidal effect. But they presented no inoculum effect. To kill the bacteria at 99.9%, there was regrowth effect at every level of concentration for the usage of amikacin against *P.aeruginosa.* Additionally, the study displayed that in order to kill the bacteria at 99.9% level via using netilmicin, the medicine concentration levels started from MIC, 4MIC, 8MIC, peak, and peak high dose of netilmicin. However, the study displayed the regrowth effect after giving the medicine for 6 hrs at the medicine concentrations at MIC (Table 5-5).

The current clinical usage usually gives a patient who infected with *P.aeruginosa* amikacin. The clinical usage presented no good effect on this kind of treatment.

Besides, the prone to get the treatment failure is high in clinical usage albeit the bacteria presented the susceptibility to the medicine in the laboratory.

Adaptive resistance is the decreased drug uptake that occurs in bacteria that survive an initial, suboptimal aminoglycoside dose. Thus, drug regimens that allow for longer drug-free intervals should help protect the bactericidal activity of aminoglycosides by decreasing adaptive resistance. High dose aminoglycoside therapy, if properly designed (i.e. with peak to MIC ratios of ~10), helps suppress the survival of high-MIC mutants within a population of generally susceptible organisms. The lack of emergence of resistant organisms during therapy is a major advantaqge to high dose aminoglycoside regimens (Lacy MK et al., 1998)

However, using netilimicin can treat this kind of infection and indicates good clinical outcome with the usage at 4MIC level. Consequently, in order to treat a patient who infected with *P.aeruginosa*, the suggestion is the usage of netilimicin as the first line therapy.

In consideration of the medicine under the Fluoroquinolone group against *P.aeruginosa*, the study manifested that the medicine still could keep its bactericidal activity and generated no inoculum effect. When its concentration is the peak, it will be able to kill the bacteria at 99.9%.

This result is unified to the previous knowledge that Fluoroquinolone exhibit concentration-dependent killing, thus, it can be concluded that regimens of large doses (resulting in high AUCs and AUICs) given at infrequent intervals (thus relying on the PAE) might be most efficacious in terms of bacterial killing, eradication time, and reducing the selection of resistant bacteria. (Lode H et al., 1998)

With respect to the medicines under the Beta lactam group against *P.aeruginosa*, the study exhibited that the medicines still have their bactericidal activity ability but the using the imipenem against *P.aeruginosa*. Concerning the inoculum effect, there was the inoculum effect after the usage of piperacilin/tazobactam and imipenem. From the study of ceftazidime, there is the bacteriacidal activity ability and presented no inoculum effect, through there is not any concentration that could kill the bacteria at the level of 99.9%.

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The use of piperacilin/tazobactam could kill the bacteria at the level of 99.9% if the level reached 8MIC. Concerning the clinical usage, there is the inoculum effect from the use of the medicine at the level of 8MIC, which may cause the treatment failure.This case may be found from the usage of imipenem as well as imipenem has the bacteriostatic and present inoculum effect.

This result brought the conception that is harmonious to the previous studies that are as the following: the study of Kovasc and Song (Kovasc K et al., 1998 and Song W et al., 2002). My conception obtaining from the study is that to treat a patient who infected with *P.aeruginosa*, the combination therapy should be used for example the use of ceftazidime and amikacin, and the use of imipenem and amikacin.

From the study of *A.buamannii* which was the multi-drug resistant isolates. Amikacin and netilmicin which there are highly resistant for *A baumannii*. Ciprofloxacin, ceftazidime, ampicillin/sulbactam, cefoperazone/sulbactam, and imipenem present bactericidal activity ability but the clinical using cloud not be utilize because of the concentration which present these ability cloud be higher than the therapeutic levels and their might be toxic for the patient (Table 5-6).

According to my opinion coming from the study and working experiences, treatment for the multi-drug resistant *A.baumannii* infection, the combination therapy should be used for example the use of piperacillin/tazobactam (or cefepime) combined with moxifloxacin, polymixin B combined with other antimicrobial, cefoperazone/sulbactam, ampicillin/sulbactam,

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CONCLUSION

From the investigation on the effect of drugs concentrations and duration of exposure on eradication, we can conclude that aminoglycoside, fluoroquinolone, and beta lactam against *E.coli* have bactericidal activity and have persistent kinetic of killing. Beta lactam, cefotaxime, exhibits the inoculum effect.

We can conclude that aminoglycoside, fluoroquinolone, and beta lactam against *K. pneumoniae* have bactericidal activity and have persistent kinetic of killing. *K. pneumoniae* exhibits no inoculum effect.

We can conclude that the aminoglycoside, fluoroquinolone, and beta lactam against *P. mirabilis* have bactericidal activity and have persistent kinetic of killing. Beta lactam, cefotaxime and piperacillin/tazobactam exhibits the inoculum effect .

We can conclude that the aminoglycoside, fluoroquinolone, and beta lactam against *Providencia* spp. have bactericidal activity and have persistent kinetic of killing. Beta lactam, imipenem, exhibits the inoculum effect.

We can conclude that the aminoglycoside, fluoroquinolone, and beta lactam against *P.aeruginosa* have bactericidal activity and have persistent kinetic of killing. Beta lactam, piperacillin/tazobactam exhibits the inoculum effect.

We can conclude that the aminoglycoside, fluoroquinolone, and beta lactam against the multi-drug resistant *A.baumannii* could not have bactericidal activity against and could not have persistent kinetic of killing. In clinical setting, A.baumannii infection should not be use all groups of antimicrobials as the monotherapy.

Further studies will illustrate whether adjusted in-vitro susceptibility tests would contribute enhanced directions for the clinician's option of the most apropos antibiotic therapy.

Table 5-1 Antimicrobial activity of antimicrobial agants against *E.coli*.

Antimicrobial	MBC/MIC	inoculum effect (flod of MIC)	Concentration at T99.9% with regrowth	Concentration at T99.9% without regrowth
gentamicin	2	2	4MIC, 8MIC, Peak, Peak*	4MIC, 8MIC, Peak, Peak*
amikacin	4	4	MIC,4MIC, 8MIC, Peak, Peak*	MIC,4MIC, 8MIC, Peak, Peak*
netilmicin				
ofloxacin	1	1	1/2MIC,MIC,4MIC, 8MIC, Trough,Trough*,all Peak	MIC,4MIC, 8MIC,Trough, Trough*,all Peak
ciprofloxacin	2	2	MIC,4MIC, 8MIC,Trough, Trough*,all Peak	MIC,4MIC, 8MIC,Trough, Trough*,all Peak
cefotaxime	2	8.3	4MIC, 8MIC, Peak, Peak*	4MIC, 8MIC, Peak, Peak*
ceftazidime				
amox/clav.	1	2	4MIC, 8MIC, Peak, Peak*	4MIC, 8MIC, Peak, Peak*
pip/taz	1	2	Peak, Peak*	Peak, Peak*
ampi/sul	1	8	4MIC, 8MIC, Peak, Peak*	8MIC, Peak, Peak*
cfp/sul	1	8	4MIC, 8MIC, Peak, Peak*	4MIC, 8MIC, Peak, Peak*
imipenem	1	1	MIC,4MIC, 8MIC, Peak, Peak*	MIC,4MIC, 8MIC, Peak, Peak*

Table 5-1 Antimicrobial activity of antimicrobial agents against *K.pneumoniae*.

Antimicrobial	MBC/MIC	inoculum effect (flod of MIC)	Concentration at T99.9% with regrowth	Concentration at T99.9% without regrowth
gentamicin	4	1	MIC,4MIC, 8MIC, Trough, Peak, Peak*	4MIC, 8MIC, Trough, Peak, Peak*
amikacin	1	2	1/2MIC, MIC,4MIC, 8MIC, Trough,Peak, Peak*	4MIC, 8MIC, Peak, Peak*
netilmicin				
ofloxacin				
ciprofloxacin	2	2	MIC,4MIC, 8MIC,Trough, Trough*,all Peak	MIC,4MIC, 8MIC,Trough, Trough*,all Peak
cefotaxime	2	2	MIC, 4MIC, 8MIC,Trough, Trough*,Peak, Peak*	4MIC, 8MIC,Trough, Trough*,Peak, Peak*
ceftazidime				
amox/clav.				
pip/taz	1	1	4MIC, 8MIC,Trough, Trough*,Peak, Peak*	4MIC, 8MIC,Trough, Trough*,Peak, Peak*
ampi/sul				
cfp/sul				
imipenem	1	1	MIC,4MIC, 8MIC,Trough, Peak, Peak*	4MIC, 8MIC,Trough, Peak, Peak*

Table 5-3 Antimicrobial activity of antimicrobial agents against *P.mirabilis*.

Antimicrobial	MBC/MIC	inoculum effect (flod of MIC)	Concentration at T99.9% with regrowth	Concentration at T99.9% without regrowth
gentamicin	2	2	1/2MIC, MIC,4MIC, 8MIC, Trough, Peak, Peak*	4MIC, 8MIC, Peak, Peak*
amikacin	4	4	MIC,4MIC, 8MIC, Peak, Peak*	4MIC, 8MIC, Peak, Peak*
netilmicin				
ofloxacin				
ciprofloxacin	2	1	MIC,4MIC, 8MIC,Trough, Trough*,all Peak	4MIC, 8MIC,Trough, Trough*,all Peak
cefotaxime	2	33.33	MIC, 4MIC, 8MIC,Trough, Trough*,Peak, Peak*	4MIC, 8MIC, Peak, Peak*
ceftazidime				
amox/clav.				
pip/taz	1	16	4MIC, 8MIC,Trough, Trough*,Peak, Peak*	MIC, 4MIC, 8MIC,Trough, Trough*,Peak, Peak*
ampi/sul				
cfp/sul				
imipenem	2	1	MIC,4MIC, 8MIC,Trough, Peak, Peak*	4MIC, 8MIC, Peak, Peak*

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Table 5-3 Antimicrobial activity of antimicrobial agents against *Providencia* spp.

Antimicrobial	MBC/MIC	inoculum effect (flod of MIC)	Concentration at T99.9% with regrowth	Concentration at T99.9% without regrowth
gentamicin				
amikacin	2	1	1/2MIC,MIC,4MIC, 8MIC, Trough,Peak, Peak*	4MIC, 8MIC, Peak, Peak*
netilmicin	2	2	1/2MIC,MIC,4MIC, 8MIC, Trough,Peak, Peak*	2MIC,4MIC, 8MIC, Trough,Peak, Peak*
ofloxacin				
ciprofloxacin	2	1	MIC,4MIC, 8MIC,Trough, Trough*,all Peak	4MIC, 8MIC,Trough, Trough*,all Peak
cefotaxime	1	1	8MIC, Trough, Trough*Peak, Peak*	8MIC, Trough, Trough*Peak, Peak*
ceftazidime				
amox/clav.				
pip/taz				
ampi/sul				
cfp/sul				
imipenem	2	8.3	MIC,4MIC, 8MIC,Trough, Peak, Peak*	4MIC, 8MIC,Trough, Trough*,all Peak

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Table 5-3 Antimicrobial activity of antimicrobial agents against *P.aeruginosa*

Antimicrobial	MBC/MIC	inoculum effect (flod of MIC)	Concentration at T99.9% with regrowth	Concentration at T99.9% without regrowth
gentamicin				
amikacin	2	2	MIC,4MIC, 8MIC, Peak, Peak*	8MIC
netilmicin	2	4	1/2MIC,MIC,4MIC, 8MIC, Trough,Peak, Peak*	4MIC, 8MIC, Peak, Peak*
ofloxacin				
ciprofloxacin	2	4	4MIC, 8MIC, all Peak	all Peak
cefotaxime			Peak	
ceftazidime	1	4		
amox/clav.				
pip/taz	1	8	4MIC, 8MIC	8MIC
ampi/sul				
cfp/sul				
imipenem	16	8	MIC,4MIC, 8MIC,Trough, Peak, Peak*	Peak, Peak*

Table 5-3 Antimicrobial activity of antimicrobial agents against *A.buamannii.*

Antimicrobial	MBC/MIC	inoculum effect (flod of MIC)	Concentration at T99.9% with regrowth	Concentration at T99.9% without regrowth
gentamicin				
amikacin	ND	ND	-	-
netilmicin	ND	ND	-	-
ofloxacin				
ciprofloxacin	1	1	4MIC, 8MIC	4MIC, 8MIC
cefotaxime			1/2MIC, MIC, 4MIC, 8MIC,Peak, Peak*	-
ceftazidime	1	1		
amox/clav.				
pip/taz				8MIC
ampi/sul	2	2	MIC, 4MIC, 8MIC,Peak,Peak*	8MIC, Peak*
cfp/sul	1	2	4MIC, 8MIC,Peak,Peak*	4MIC, 8MIC,Peak,Peak*
imipenem	1	2	MIC,4MIC, 8MIC, Peak, Peak*	4MIC, 8MIC

Reference

AHFS Drug Information. Aminoglycoside, penicillin, cephalosporin, carbapenem and fluoroquinolone (n. p.), 2005

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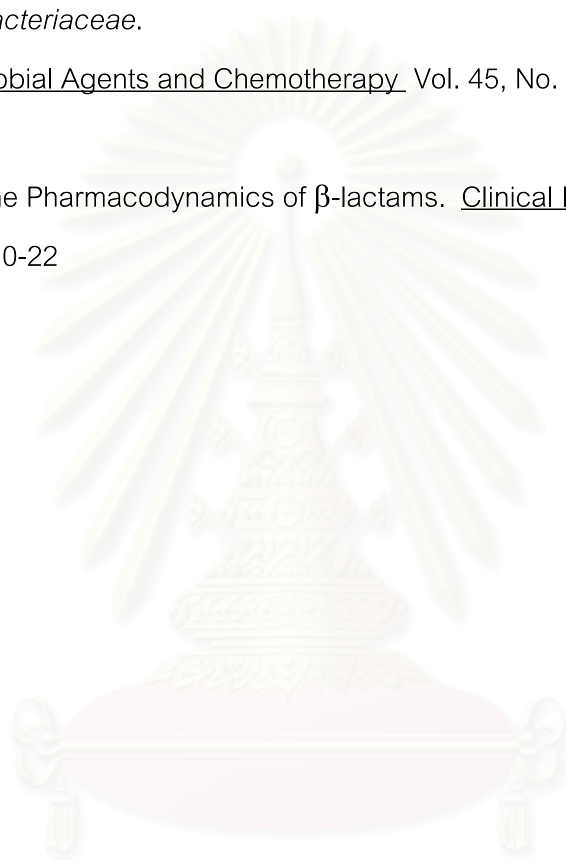
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