PHARMACODYNAMIC COMPARISON OF LEVOFLOXACIN GATIFLOXACIN AND MOXIFLOXACIN AGAINST BACTERIAL CAUSING HUMAN SINUSITIS IN AN IN *VITRO* PHARMACODYNAMIC MODEL

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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แบบจำลองเภสัชพลศาสตร์นอกร่างกาย ถูกใช้ในการจำลองระดับยา Levofloxacin, Gatifloxacin และ Moxifloxacin ใน maxillary sinus ในการฆ่าเชื้อแบคทีเรียที่ก่อโรคไซนัสอักเสบในคนซึ่งได้แก่ *M.catarrhalis*, *H.influenzae* และ *S.pneumoniae* จำนวน 6 สายพันธุ์โดยเชื้อจะสัมผัสกับยา Levofloxacin, Gatifloxacin และ Moxifloxacin ในความเข้มข้นเท่ากับความเข้มข้นสูงสุด (C_{max}) จากการบริหารยาครั้งเดียว และการบริหารยาหลายครั้ง ในขนาดรับประทานวันละ 500, 400 และ 400 มิลลิกรัม ตามลำดับ โดยความเข้มข้นของยาจะลดลงตามค่าครึ่งชีวิตของยาแต่ละชนิด. ผลจากการทดลองแสดงให้เห็นว่าในการบริหารยา Moxifloxacin และ Gatifloxacin ครั้งเดียว สามารถฆ่าเชื้อทั้ง 6 สายพันธุ์ได้อย่างรวดเร็ว โดยที่ยา Moxifloxacin และ Gatifloxacin ซึ่งมีค่า AUC_{0-24}/MIC อยู่ในช่วง 155.36-189.50 และ 125.36-259.12 ตามลำดับ และมีค่า Peak/MIC อยู่ในช่วง 12.14-15.10 และ 14.14-28.44 ตามลำดับ สามารถฆ่าเชื้อ *S.pneumoniae* ลงได้ 99.9% ภายในเวลา 2 ชั่วโมง และสามารถกำจัดเชื้อได้หมดภายในเวลา 4-6 ชั่วโมง เร็วกว่ายา Levofloxacin ที่มีค่า AUC_{0-24}/MIC อยู่ในช่วง 67.21-1270.80 และมีค่า Peak/MIC อยู่ในช่วง 6.60-145.20. สำหรับเชื้อ *M.catarrhalis* และ *H.influenzae* ยา Levofloxacin, Gatifloxacin และ Moxifloxacin ซึ่งมีค่า AUC_{0-24}/MIC อยู่ในช่วง 4515.33-9034.61, 2187.66-19862.85 และ 1607.80-50420.00 ตามลำดับ และมีค่า Peak/MIC อยู่ในช่วง 483.33-919.23, 237.86-1980.00 และ 116.20-4433.33 ตามลำดับ มีความสามรถในการฆ่าเชื้อดังกล่าวไม่ต่างกัน. ในการบริหารยาหลายครั้ง ยา Moxifloxacin และ Gatifloxacin ซึ่งมีค่า AUC_{0-24}/MIC อยู่ในช่วง 164.14-209.90 และ 150.66-330.80 ตามลำดับ และมีค่า Peak/MIC อยู่ในช่วง 13.10-15.77 และ 16.24-32.96 ตามลำดับ สามารถฆ่าเชื้อ *S.pneumoniae* ได้เร็วกว่าการบริหารยาครั้งเดียว ส่วนยา Levofloxacin ซึ่งมีค่า AUC_{0-24}/MIC อยู่ในช่วง 70.70-1399.40 และมีค่า Peak/MIC อยู่ในช่วง 6.99-137.80 ให้ผลในการฆ่าเชื้อดังกล่าวไม่ต่างกับการบริหารยาครั้งเดียว. เช่นเดียวกับเชื้อ *M.catarrhalis* และ *H.influenzae* การบริหารยา Moxifloxacin และ Gatifloxacin หลายครั้ง ซึ่งมีค่า AUC_{0-24}/MIC อยู่ในช่วง 1653.80-57680.00 และ 2583.66-22237.14 ตามลำดับ และมีค่า Peak/MIC อยู่ในช่วง 137.20-4553.33 และ 292.33-2440.00 ตามลำดับ สามารถฆ่าเชื้อดังกล่าวได้เร็วกว่าการบริหารยาครั้งเดียว ส่วนยา Levofloxacin ที่มีค่า AUC_{0-24}/MIC อยู่ในช่วง 4340.66-9357.69 และมีค่า Peak/MIC อยู่ในช่วง 463.33-967.94 แสดงการฆ่าเชื้อดังกล่าวไม่แตกต่างกับการให้ยาครั้งเดียว.

สรุปผลการทดลองเมื่อบริหารยา Moxifloxacin และ Gatifloxacin ครั้งเดียวสามารถฆ่าเชื้อ *S.pneumoniae* ได้เร็วกว่ายา Levofloxacin โดยที่ความสามารถในการฆ่าเชื้อ *M.catarrhalis* และ *H.influenzae* ของยาทั้งสามชนิดไม่แตกต่างกัน และเมื่อบริหารยาหลายครั้งยา Moxifloxacin และ Gatifloxacin สามารถฆ่าเชื้อทั้ง 6 สายพันธุ์ได้ดีกว่าการบริหารยาครั้งเดียวเดียวในขณะที่ยา Levofloxacin เมื่อบริหารในครั้งเดียวและบริหารยาหลายครั้งให้ผลในการฆ่าเชื้อทั้ง 6 สายพันธ์ไม่แตกต่างกัน

ภาควิชา เภสัชวิทยา ลายมือชื่อนิสิต .....

สาขาวิชา เภสัชวิทยา ลายมือชื่ออาจารย์ที่ปรึกษา .....

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APICHAT PHETMOREEKUL: PHARMACODYNAMIC COMPARISON OF LEVOFLOXACIN GATIFLOXACIN AND MOXIFLOXACIN AGAINST BACTERIAL CAUSING HUMAN SINUSITIS IN AN *IN VITRO* PHARMACODYNAMIC MODEL. THESIS ADVISOR: ASSOC. PROF. SIRIPORN FUNGWITAYA, THESIS COADVISOR: PROF. NALINEE ASWAPOKEE, 105 pp. ISBN 974-17-5018-8.

An *in vitro* pharmacokinetic model (IVPM) was used to simulate the peak concentration (C_{max}) of Levofloxacin, Gatifloxacin and Moxifloxacin in human maxillary sinus, when administrate 500, 400 and 400 mg, respectively by oral rout once daily in single dose and multiple doses regimen, against 6 strains of microorganisms causing human maxillary sinusitis such as *M.catarrhalis*, *H.influenzae* and *S.pneumoniae*. The concentration was decreased depend on the elimination half-life in each agents. The results from this experiment exhibit the single dose regimen of Moxifloxacin and Gatifloxacin demonstrated rapid killing of all 6 strains of microorganisms. Moxifloxacin and Gatifloxacin, which have AUC_{0-24}/MIC in the range of 155.39-189.50 and 125.36-259.12 respectively and Peak/MIC in the range of 12.14-15.10 and 14.14-28.44, respectively could decrease 99.9% viable count of *S.pneumoniae* within 2 hours and decrease this microorganism to eradicate within 4-6 hours faster than Levofloxacin, which have AUC_{0-24}/MIC in the range of 67.21-1270.80 and Peak/MIC in the range of 6.60-145.20. Levofloxacin, Gatifloxacin and Moxifloxacin, which have AUC_{0-24}/MIC in the range of 4515.33-9034.61, 2187.66-19862.85 and 1607.80-50420.00, respectively and Peak/MIC in the range of 483.33-919.23, 237.86-1980.00 and 116.20-4433.33, respectively, against *M.catarrhalis* and *H.influenzae* demonstrated the similar killing rate for these microorganisms. The multiple doses regimen of Moxifloxacin and Gatifloxacin, which have AUC_{0-24}/MIC in the range of 164.14-209.90 and 150.66-330.80, respectively and Peak/MIC in the range of 13.10-15.77 and 16.24-32.96 respectively, demonstrated the 99.9% viable count of *S.pneumoniae* decrease which was more than single dose regimen. For Levofloxacin, which have AUC_{0-24}/MIC in the range of 70.70-1399.40 and Peak/MIC in the range of 6.99-137.80, demonstrated killing these microorganisms as the same rate as single dose regimen. Moxifloxacin and Gatifloxacin, which have AUC_{0-24}/MIC in the range of 1653.80-57680.00 and 2583.66-22237.14 respectively and Peak/MIC in the range of 137.20-4553.33 and 292.33-2440.00 respectively, demonstrated killing *M.catarrhalis* and *H.influenzae* faster than single dose regimen. While Levofloxacin, which have AUC_{0-24}/MIC in the range of 4340.66-9357.69 and Peak/MIC in the range 463.33-967.94, demonstrated killing these microorganisms as the same rate as single dose regimen.

The summary, Moxifloxacin and Gatifloxacin single dose regimen demonstrated killing *S.pneumoniae* faster than Levofloxacin. While efficacy of Levofloxacin, Gatifloxacin and Moxifloxacin against *M.catarrhalis* and *H.influenzae* demonstrated not difference. About Moxifloxacin and Gatifloxacin, when administration multiple doses regimen, decreased all 6 strains of microorganisms faster than single dose regimen. In the contrast Levofloxacin multiple doses regimen demonstrated similar killing rate with single dose regimen.

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Field of study Pharmacology ..... Advisor's signature .....

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LIST OF ABBREVIATIONS

ATP	= Adenosine triphosphate
AUC	= Area under the curve
^{O}C	= degree Celsius
CAP	= Community acquires pneumonia
CFU	= Colony forming unit
E.coli	= *Escherichia coli*
e.g.	= exempli gratia (for example)
et al.	= et alii (and other people)
g	= gram
H.influenzae	= *Haemophilus influenzae*
hr	= Hour
IVPM	= *In vitro* pharmacodynamic model
log	= decimal logarithm
MHA	= Meuller-Hinton agar
MHB	=Meuller-Hinton broth
MIC	= Minimum inhibitory concentration
min	= minute
ml	= milliliter
mm	= millimeter
M.catarrhalis	= *Moraxella catarrhalis*
NCCLS	= The National Committee for Clinical Laboratory Standards
NSS	= Normal saline solution
PBP	= Penicillin binding protein
PD_{50}	= The predictive dose that produces half of the maximal effect
Peak	= Peak concentration
PISP	= Penicillin intermediate *streptococcus pneumoniae*
PRSP	= Penicillin resistant *streptococcus pneumoniae*
PSSP	= Penicillin susceptible *streptococcus pneumoniae*
QRDR	= Quinolones resistance-determining region
S.aureus	= *Staphylococcus aureus*

LIST OF ABBREVIATIONS (Cont.)

S.D. = Standard deviation

S.pneumoniae = *Streptococcus pneumoniae*

$T_{1/2}$ = Half-life

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

$T_{eradication}$ = Time required to decrease viable counts below the 10 CFU/ml (1logCFU/ml) limit of detection

μg = microgram

μl = microliter

CHAPTER I

INTRODUCTION

Over the past decade community acquires pneumonia (CAP) is the most important disease for treatment by antimicrobial agents. Because of microorganisms' causes CAP is world wild developing to multi drugs resistance to increase minimal inhibitory concentration (MICs) and decrease efficacy of antimicrobial agents especially to *Streptococcus pneumoniae*. Similar to CAP, sinusitis is causing by three important microorganisms such as *Moraxella catarrhalis*, *Haemophilus influenzae* and *Streptococcus pneumoniae*. Both, of *Moraxella catarrhalis* and *Haemophilus influenzae,* are gram-negative cocci and gram-negative rod respectively while *Streptococcus pneumoniae* are gram-positive cocci. The patient who are infected by *S.pneumoniae* approximately 30-40% while *M.catarrhalis* and *H.influenzae* that can be found in patient approximately 20% by *M.catarrhalis* can found in children about 26% more than adult that can found this pathogen approximately 2%. *H.influenzae* can be found in children about 29% and adult approximately 35% while *S.pneumoniae* can found in children 41% and adult 35% (Dipiro et al., 1999).

The antimicrobial agents such as β-lactam group are chosen as the first line drugs to treat sinusitis although in the current day β-lactam group are of least efficacy to against three microorganisms above because theses microorganisms are develop to resist β-lactam group. The epidemiology study demonstrate *H.influenzae* which isolated from patients in Asia have resist to antimicrobial agents as β-lactam group by produce enzyme β-lactamase 17.2% while *M.catarrhalis* produce this enzyme about 97.6% (Hoban et al., 2002). Similar as *S.pneumoniae* that resists to β-lactam group by change target of penicillin binding protein (PBPs.) approximately 44.5-71.5% (Felmingham et al., 2002). From epidemiology study in Thailand during 1999 to 2000, it is demonstrated that the isolate of *S.pneumoniae* 206 samples can be classified to penicillin susceptible *streptococcus pneumoniae* (PSSP) 33.5%, penicillin intermediate *streptococcus pneumoniae* (PISP) 27.2% and penicillin resistant *streptococcus pneumoniae* (PRSP) 39.3%. The isolates of *H.influenzae* from 305 samples are resist to β-lactam by

producing enzyme β-lactamase about 45.2% while The 39 samples, the isolate of *M.catarrhalis* produce this enzyme 100% (Critchley et al., 2002). β-lactam group maybe not an effective antibiotic against pathogen causing sinusitis in present day.

Therefore the second line drugs are choose by physician for treatment of microorganisms causing sinusitis which resistance to β-lactam group. There are many kinds of the second line drugs to treat sinusitis patients. Fluoroquinolones are one choice that physician can choose to treat sinusitis. Because of efficacy of fluoroquinolones is developed by modification structure to coverage both gram-negative and gram-positive microorganisms. In addition new fluoroquinolones has long half-life thus it is easy to administer the drugs only 1 to 2 times per day by oral rout. Easy administrations are beneficial because it can be improve compliance of patients too. Today fluoroquinolones can be classified in 4 generation as show in table 1-1.

Table 1-1 Quinolones classification (Gootz et al., 1996)

Generation	Gm-negative Bacteria	Gm-positive Bacteria	Mycoplasma Chlamydia Legionella	Anaerobes	Mycobacteria
First					
Nalidixic acid	++	0	0	0	0
Cinoxacin	++	0	0	0	0
Second					
Norfloxacin	++	0	+	0	0
Ofloxacin	+++	++	++	0	++
Ciprofloxacin	++++	++	++	0	++
Third					
Levofloxacin	+++	+++	+++	0	++
Sparfloxacin	+++	+++	+++	0	+++
Gatifloxacin	+++	+++	++++	0	++
Fourth					
Moxifloxacin	+++	++++	++++	++	++++
Gemifloxacin	+++	++++	++++	++	++

New generation fluoroquinolones, which have been approved by FDA in Thailand such as the third generation fluoroquinolones, levofloxacin and gatifloxacin that are improve spectrum to cover gram-positive microorganisms. The fourth generation fluoroquinolones, moxifloxacin has improved efficacy against gram-positive and anaerobe microorganisms action. From structure activity relationship of fluoroquinolones show in figure 1-1. Modifying structure at R1, R5, R7 and X8 position can improve spectrum cover more organisms.

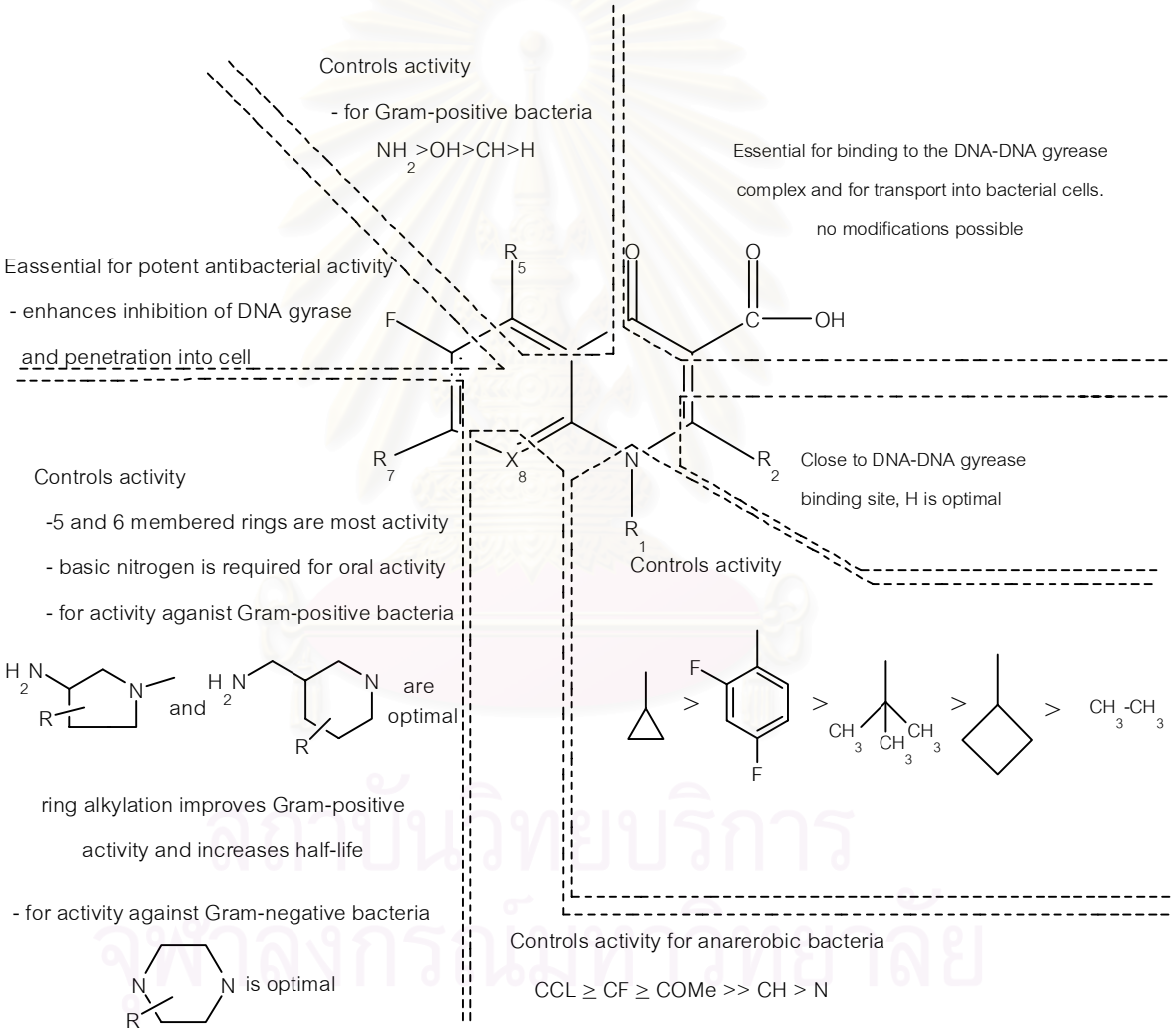


Figure 1-1 Structure activity relationship of fluoroquinolones (modified from Zhanel et at., 2001)

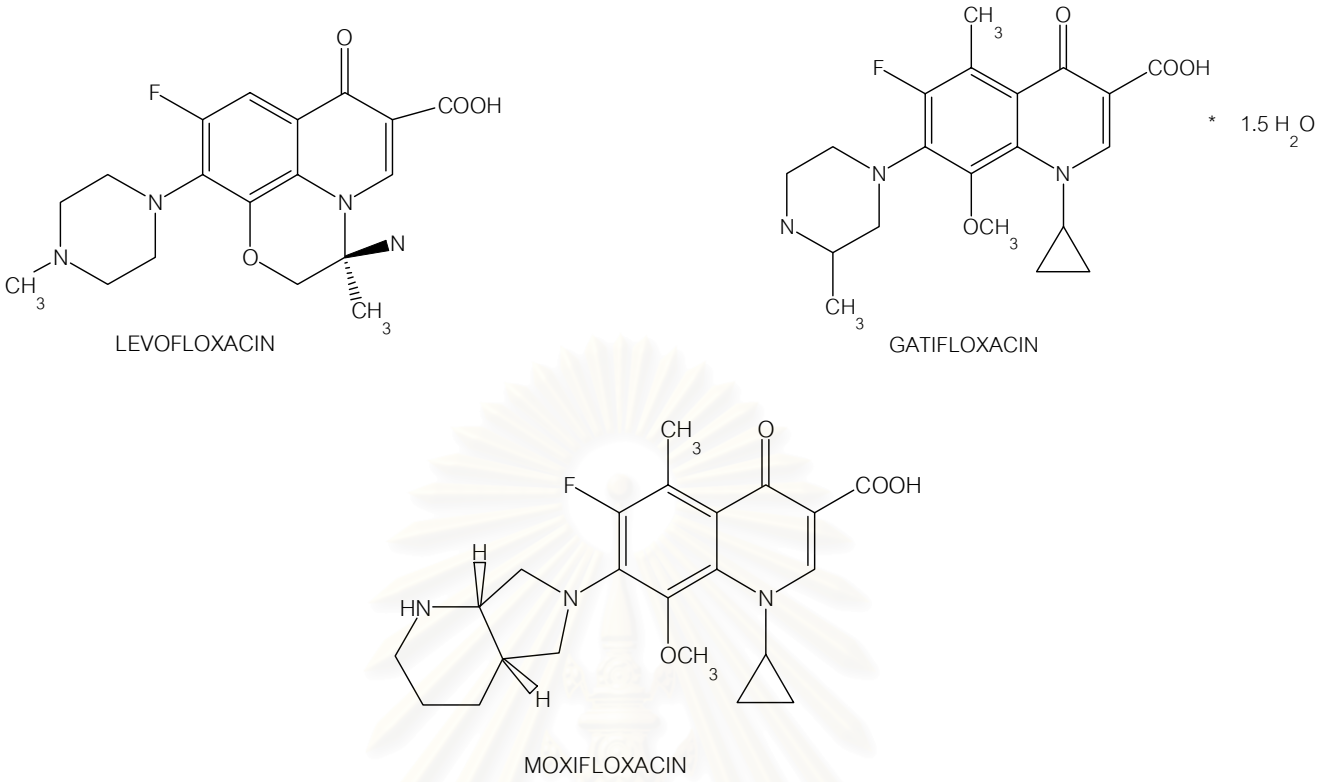


Figure 1-2 Structure of LEVOFLOXACIN, GATIFLOXACIN and MOXIFLOXACIN

Levofloxacin, the levo-isomer of the D, L-racemate ofloxacin has modifying structure by substitute oxygen group at X8 position and compatible with R1 position to be heterocyclic ring show in figure 1-2. From modifying structure of levofloxacin which make its spectrum cover gram-positive microorganisms more than ofloxacin and efficacy of levofloxacin are more than ofloxacin 8-128 times. Levofloxacin can be administered by oral rout and their bioavailability approximately 99% extensive penetration into tissues and body fluids and an elimination half-life about 5.5-8.1 hours. Therefore can administration levofloxacin can be administered 500 mg once daily (North et al., 1998).

Gatifloxacin and moxifloxacin have been modifying structure by substitute methoxy group at X8 position show in figure 1-2 and improve their spectrum are coverage anaerobic microorganisms and cyclopropyl group at R1 are enhance gram-negative activity and provide some gram-positive activity (Fish et al., 2001). Moxifloxacin have modifying structure differ from gatifloxacin by moxifloxacin replace methyl

piperazine group at R7 position by azablicyclo is associated with substantially improved gram-positive activity. In study of *S.aureus*, the presence of methoxy group at the C-8 position was associated with a decreased propensity for development of resistance (Zhao et al., 1998). Both of gatifloxacin and moxifloxacin can be administered by oral rout and their bioavailability is about 96% and 89% respectively (Balfour et al., 1998). Half-life of gatifloxacin is approximately 6.5-7.6 hours and half-life of moxifloxacin approximately 10-16 hours. In clinical use these drugs can be administered once daily.

Mechanisms of action of fluoroquinolones are binding to a specific target site on bacterial DNA. When molecule of fluoroquinolones penetrate into the cell membrane of microorganism, the antimicrobial action of fluoroquinolones is mediated through the inhibition of two type II DNA topoisomerase enzyme such as DNA gyrase and topoisomerase IV (Zhanel et al., 2002). DNA gyrase, a tetrameric enzyme consisting of two GyrA and two GyrB subunits, which are the products of the *gyrA* and *gyrB* genes, respectively. Topoisomerase IV also has two pairs of subunits, ParC and ParE subunits are encoded by the *parC* and *parE* genes, respectively. The DNA gyrase is responsible for introducing and removing DNA supercoils and for unlinking (decatenating) interlocked DNA circles. This action proceeds ahead of the activity moving replication fork. Topoisomerase IV have major action by removal of DNA supercoils and separation of newly built daughter DNA after replication complete (Bearden et al., 2001). Levofloxacin have primary target is *par*C on topoisomerase IV while gatifloxacin and moxifloxacin have primary target is *gyr*A on DNA gyrase (Fukuda et al., 1999). These enzymes are needed in replication of microorganisms.

Fluoroquinolones typically have excellent bioavailability, large volumes of distribution, and extensive tissue penetration. Bioavailability of oral levofloxacin, gatifloxacin and moxifloxacin is in excess of 85%. As indicated by their large volumes of distribution that most fluoroquinolones penetrate rapidly and efficiently through the body, achieving tissue and fluid concentration that are generally higher than those in plasma. The maxillary sinus mucosa concentration of levofloxacin, gatifloxacin and moxifloxacin were 1.15 (North et al., 1998), 1.81 and 2.00 times) respectively higher than serum plasma concentration (Rodvold et al., 2001). In addition fluoroquinolones have accumulative effect by when administration this agent for multiple doses the molecule of

agents can be accumulated to steady state into site of action. Because of course of treatment sinusitis for levofloxacin is 7 days therefore when administration levofloxacin 500 mg/day single dose in the first day its concentration in maxillary sinus approximately 6.9 μg/ml for multiple doses the steady state concentration in day 7 approximately 7.0 μg/ml. While course of treatment for sinusitis of gatifloxacin and moxifloxacin is 10 and 5 days respectively. When administration gatifloxacin single dose in the first dose its concentration in maxillary sinus approximately 6.7 μg/ml about multiple doses the steady state concentration in maxillary sinus when administration for 10 days its concentration approximately 8.1 μg/ml. On the other hand moxifloxacin when administration single dose its concentration in maxillary sinus approximately 6.0 μg/ml for multiple doses the steady state concentration in maxillary sinus when administration for 5 days approximately 6.4 μg/ml (Rodvold et al., 2001).

As fluoroquinolones have concentration dependent killing, the AUC_{0-24}/MIC ratio and/or Peak/MIC ratio as the pharmacokinetic and pharmacodynamic parameters predictive of microbiologic and clinical outcome (Schentag et al.2003). Three important break points for AUC_{0-24}/MIC can be defined. At AUC_{0-24}/MIC values <30-50 or Peak/MIC ratio in the range of 5:1, the actions of fluoroquinolones are essentially bacteriostatic (Forrest et al., 1993). Any observed bacterial killing is primarily measuring the effects of host factors such as neutrophils and macrophages. In the middle of the AUC_{0-24}/MIC range (>100 but <250), the organisms are killed at a slower rate and usually by day 7 of treatment. At an AUC_{0-24}/MIC >250 or Peak/MIC of 25:1, however, the fluoroquinolones demonstrated rapid concentration-dependent killing, and bacterial eradication occurs within 24 hours (Schentag et al.2003).

Previous study demonstrates that AUC_{0-24}/MIC of fluoroquinolones approximately 125 are achieved to treat gram-negative microorganisms (Forrest et al., 1993). For gram-positive microorganism the AUC_{0-24}/MIC approximately 30 are enough to achieve bactericidal effect of fluoroquinolones (Lacy et al., 1999). In addition the AUC_{0-24}/MIC in the range of 30-50 describe bacteriostatic action. Values of AUC_{0-24}/MIC > 100 and in creasing to 250 are bactericidal and > 250 the AUC_{0-24}/MIC progressively approach maximal killing rate (Schentag et al., 2003).

In this study *in vitro* pharmacodynamic model (IVPM) was used to determine antimicrobial activity and pharmacodynamics of levofloxacin, gatifloxacin and moxifloxacin by focusing on inhibitory growth and killing bacterial pathogen that cause human sinusitis. In these models, bacteria are exposed to fluctuating concentrations of drug adjusted to peak concentration in maxillary sinus and reduced as a function of time (t) in an exponential manner with the elimination half-life ($T_{1/2}$) of the test antimicrobial agents.

The previous study were narrow in their focus and were not designed to determine antimicrobial activity of levofloxacin, gatifloxacin and moxifloxacin against pathogen causing maxillary sinusitis impact of the concentration-time curve with minimum inhibitory concentration (AUC_{0-24}/MIC) on their pharmacodynamic.

In the present study one-compartment IVPM was used to expose clinical isolates of *M.catarrhalis*, *H.influenzae* and *S.pneumoniae*, which are pathogen of maxillary sinusitis, to levofloxacin, gatifloxacin and moxifloxacin to evaluate the impacts of their AUC_{0-24}/MIC ratio from the model.

There are many experiments with in IVPM have indicated that the AUC_{0-24}/MIC of each antimicrobial agent was variation. The reasons for these discrepant results are not clear but may be related to methodological variations in the models used, different strains used, or different choices of antimicrobial effect measures (Macgowan, AP et al., 2003). In this study can be compare bacterial activity of levofloxacin, gatifloxacin and moxifloxacin agents against *M.catarrhalis*, *H.influenzae* and *S.pneumoniae*. Because of the same IVPM and the same strain are using in all experiments.

CHAPTEER II

LITERATURES REVIEW

1. Bacterial DNA Synthesis

The DNA synthesis has two important enzymes involve nucleic acid synthesis, topoisomerase II (DNA gyrase) and topoisomerase IV. As a class, topoisomerase are essential in controlling the topological state of DNA by catalyzing supercoiling, relaxing, knotting, and catenation reactions that are vital for DNA replication, transcription, recombination and repair (Saiki et al., 1999). The DNA gyrase is the bacterial enzymes that introduce negative supercoils into DNA. The protein binds to DNA as a tetramer in which two A-subunits and two B-subunits. The A-subunits encoded by the *gyrA* gene, carry out two activities, introducing single-strand breaks on the bacterial chromosome, and resealing the chromosome strands after supercoiling. After the chromosomal strands are resealed, The two B-subunits, encoded by the *gyrB* gene, are adenosine triphosphate (ATP) hydrolysis dependent and introduce negative supercoils into the DNA strand (Hooper, 1995). Inhibitor of gyrase such as chemical or temperature also lowers supercoiling. Some study about *E.coli* gyrase demonstrated that this organism also responses to changes in DNA twist elicited by intercalating agents and by temperature change within the normal growth range (Goldstein et al., 1984). Thus, gyrase probably certain levels of supercoiling are important for cell growth.

Topoisomerase IV acts in a manner similar to that of DNA gyrase. It has two subunits, ParC and ParE subunits, which encoded by *parC* and *parE* genes respectively (Levine et al., 1998). The major actions of Topoisomerase IV are removal of DNA supercoils and separation of newly built daughter DNA after replication is complete (Hooper, 1998). These actions occur primarily behind the advancing replication fork.

The pair of type II topoisomerase thus works both before and behind the replication fork to provide a properly supercoiled environment for DNA synthesis and to release newly replicated DNA (figure 2-1).

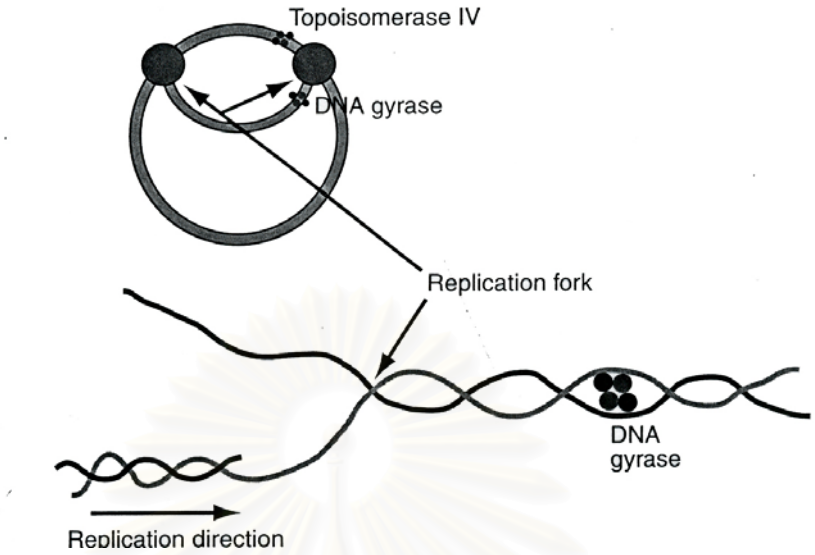


Figure 2-1 Topoisomerase and DNA replication. (Modified from Bearden et al., 2001).

2. Mechanisms of Fluoroquinolones Antimicrobials.

The mechanism of fluoroquinolones has been proposed bind to a specific target site on bacterial DNA. The fluoroquinolones have two closely related intracellular targets, DNA gyrase and topoisomerase IV, these enzymes involved in bacterial DNA replication and maintaining the superhelical density of DNA. Fluoroquinolones bind to the DNA/DNA-gyrase complex (Hooper, 1995) and inhibit to the A-subunits of the enzyme, preventing the bacterial chromosome from rejoining (Yoshida et al., 1993). For topoisomerase IV when fluoroquinolones binding with this enzyme it preventing partitioning replicated chromosomal DNA during cell division and in DNA relaxation and decatenation reaction (Peng et al., 1993). Complex between gyrase and DNA are trapped by the quinolones in a reversible reaction that blocks DNA synthesis and cell growth (figure 2-2, pathway b). Cell death then arises in two ways. One lethal pathway involves removal of gyrase-drugs complexes from DNA and liberation of lethal double-strand DNA breaks (figure 2-2, pathway c). The second mode to gyrase subunit dissociation while the enzyme is complexed to DNA (figure 2-2, pathway d). This event is expected to release DNA ends, albeit with the gyrase subunits attached. Moreover the investigator suggest that the second mode occurs when cell are treated with high concentrations of fluoroquinolones such as ciprofloxacin and that the lethal event is insensitive to inhibition of RNA or protein synthesis. Lethal effects arising from this chloramphenical-insensitive mode of quinolones action become more prominent as the quinolones concentration increases (Chen et al., 1996.).

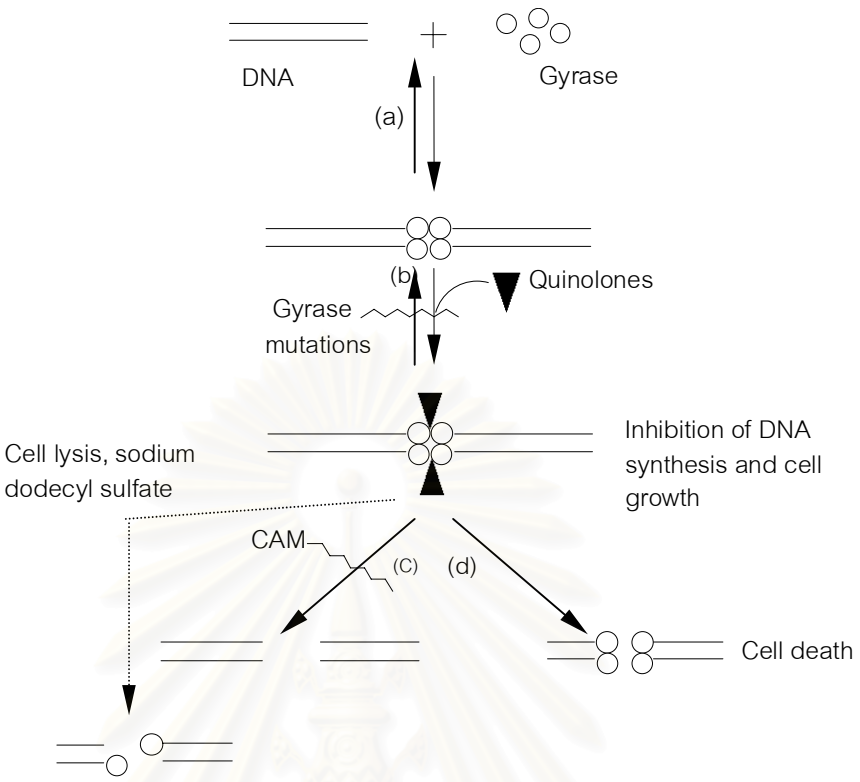


Figure 2-2 Intracellular action of quinolones. (a) DNA gyrase and DNA interact to from a cleaved complex. (b) Quinolones trap the cleaved complex. Gyrase mutaion prevent trapping by the quinolones block DNA synthesis and cell growth. (c) A putative trapped complexs block releases lethal double-strand DNA breaks from the complexes. Chloramphenicol (CAM) or rifampin treatment of cell blocks this reaction. (d) Fluoroquinolones at high concentration stimulate gyrase subunit dissociation, which releases lethal double-strand breaks. The dotted line indicates releases of staggered double-strand DNA breaks when cell lysates are treated with ionic detergents such as sodium dodecyl sulfate (Modified from Chen et al., 1996.).

Many studies have demonstrated that the primary target of fluoroquinolones such as levofloxacin in certain gram-negative species for example *E.coli* is DNA gyrase, with topoisomerase IV serving as the secondary target. Gram-positive microorganisms have topoisomerase IV for primary target of fluoroquinolones have been demonstrated in previous studies (Hooper, 1995). Recent reports have indicated that the molecular structure of the respective fluoroquinolones agent plays a role in differential target selection between DNA gyrase and topoisomerase IV in certain bacteria. Fukuda and Hiramatsu investigated primary targets of fluoroquinolones in *S.pneumoniae* suggest that

in wild-type *S.pneumoniae* the primary target of levofloxacin, trovafloxacin, norfloxacin, and ciprofloxacin is topoisomerase IV whereas the primary target of gatifloxacin and sparfloxacin is DNA gyrase (Fukuda and Hiramatsu, 1999). On the other hand moxifloxacin and which have 8-methoxyfluoroquinolones similar to gatifloxacin demonstrated primary target the GryA subunit of DNA gyrase in *S.pneumoniae* (Pestova et al., 2000). In the current day new fluoroquinolones, which modified structure by substitutes methoxy group at C-8 position was enhancement activity to cover both gram-positive and gram-negative more than old generation fluoroquinolones. Fukuda et al demonstrated that gatifloxacin increases the level of target inhibition, especially against DNA gyrase, so that it is nearly the same topoisomerase IV inhibition in bacteria cell, leading to potent antibacterial activity and low level of resistance selectivity in *S.pneumoniae* (Fukuda et al., 2001). Moreover the study of Stein et al demonstrated that moxifloxacin and gatifloxacin have bactericidal activities which both aerobic and anaerobic microorganisms (Stein et al., 2003).

3. Mechanisms of Resistance

Bacteria can develop resistance to fluoroquinolones through chromosomal mutation in the target enzymes of fluoroquinolones action, DNA gyrase and topoisomerase IV.

The study of Yoshida and colleague demonstrated that the primary mechanism of resistance to fluoroquinolones in gram-negative microorganisms such as *E.coli* result from an alteration in a defined region of the GyrA protein (Tyr-122 in *E.coli*). This region, extending between amino acids 67 to 106, is called the quinolones resistance-determining region (QRDR) (Yoshida et al., 1990). A similar region is likely to exist in the ParC protein in topoisomerase IV (Belland et al., 1994). Mutation in the highly conserved residues Ser-83 and Asp-87 are seen with notable frequency. These residues are close to the catalytic Tyr-122, which is involved in the transient DNA breakage and reunion (Reece et al., 1991). Some study demonstrated that when *gyrA* of *E.coli* were mutated of two codons, Ser-83 and Asp-87 give the greatest reduction in susceptibility as show in table 2-1. Mutation of Ser-83 to a hydrophobic amino acid generally confers more resistance than dose mutation at position 87. When both sites are mutated, levels of resistance can be two- to threefold higher than one position is mutated as show in *N.gonorrhoeae* in table 2-1. For topoisomerase IV *E.coli* demonstrated inhibit fluoroquinolones only after DNA gyrase has become refractory to owing to the presence of at least one mutation within the *gyrA* QRDR (Kumagai et al., 1996). The pervious data have been show that gyrase is the primary target in *E.coli*, with *parC* mediated resistance being detectable only in *gyrA* mutants and at high fluoroquinolones concentrations (Chen et al., 1996). Accordingly, *gyrA* and *parC* double mutants are less susceptible to fluoroquinolones than *gyrA* single mutants in *E.coli*, *H.influenzae*, and *N.gonorrhoes* as show in table 2-1.

As similar above, topoisomerase IV mutants do not by themselves confer resistance. Data of *S.aureus*, which primary target is topoisomerase IV, apparently because *S.aureus* gyrase is much less susceptible to inhibition than is *E.coli* gyrase. Therefore *parC* mutations confer low-level resistance to ciprofloxacin, with an addition *gyrA* mutation increasing resistance data show in table 2-1. The situation is similar in *S.pneumoniae*, gram-positive microorganism, when ciprofloxacin is examined (table2-1). *S.pneumoniae* can be resisting fluoroquinolones at QRDR of both *gyrA* and *parC* genes. In surveillance

study of fluoroquinolones resistance in *S.pneumoniae,* the most frequent mutations that contributed significantly to resistance in *gyrA* and *par C* were Ser-81 Phe or Tyr and Ser-79 Try, respectively. (Jone et al., 2000). Affirming that these two loci in the respective genes are important regions in the development of resistance.

In general, mutation of Ser-83 of GyrA protein is associated with moderate-level resistance, addition of one or two *parC* mutations correlates with increased resistance, three mutation (two *gyrA* and one *parC*) are associated with high-level resistance, and four mutations (two *gyrA* and two *parC*) are associated with very high-level resistance (Vila et al., 1996).

Lu et al. demonstrated that C-8 methoxy fluoroquinolones, new generation fluoroquinolones such as gatifloxacin and moxifloxacin are more effective than fluoroquinolones that without C-8 methoxy fluoroquinolones against microorganism that have *gyrA parC* double mutants (Lu et al., 1999). Moreover, C-8 methoxy fluoroquinolones enhance ability to block mutant growth and kill mutant cell is expected to restrict the selection of resistant mutant.

Table 2-1 Effect of laboratory-generated *gyrA* and *parC* mutations on fluoroquinolones resistance (Modified from Drlica et al., 1997).

Organism	Mutation[a]		Relative resistance[b]
	gyrA	*parC*	
Escherichia coli	Q106H		4
	A67S		4
	G81C		8
	A84P		8
	D87N		16
	S83W		32
	S83L		32
	S83L		10[c]
	S83L	S80L	60[c]
	S83L	E84K	100[c]
	S83L+D87G	S80I	>4,000
Haemophilus influenzae	D88N		8
	D88Y	S84I	20
	S84L	S84I	40
	S84Y+D88N	E88K	320
Neisseria gonorrhoea	S91F		125
	S91F + D95N		250
	S91F + D95N	S88P	2,000
	S91F + D95N	S88P + E91K	8,000
Staphylococcus aureus		S80F	4
		S80Y[e]	4[d]
		E84K[e]	4[d]
		E84K[f, g]	8[d]
		S80Y[f, g]	8[d]
		E84K[f, g]	8[d]
	E88K	S80Y[f, h]	128[d]
	S84L	S80Y[g, i]	256[d]
	S84L	E84L[g, i]	256[d]
		S80F	4
		E84K	4
		S80F + E84K[k]	4
	S84L	S80F + E84K[k]	200
	S84L		1
Streptococcus pneumoniae		K137N	2
		D83Y	4
		S79Y	4-16
		A84T	8
	S83Y	A83T	16
	S83Y	S79Y	32-64
	S83F	S79F + K137N	32
	E87K	S79Y	64

[a] Abbreviations represent the wild-type amino acid (in single-letter code), position number, and mutant amino acid., [b] Numbers indicate the ciprofloxacin MIC for mutant cells relative to the MIC observed for wild-type cells., [c] MIC obtained with norfloxacin rather than ciprofloxacin., [d] MIC obtained with sparfloxacin rather than ciprofloxacin., [e] First-step mutant., [f] Second-step mutant., [g] Mutant exhibits reduced accumulation of norfloxacin., [h] Mutant exhibits intermediate level of norfloxacin accumulation., [i] Third-step mutant., [j] Expressed from a plasmid..

4. Prevalence of Sinusitis

The paranasal sinuses are aerated cavities in the bones of the face that develop as outpouches of the nasal cavity and communicate with this cavity throughout life. The maxillary and ethmoidal sinuses are present at birth; the frontal and sphenoidal sinuses develop after ages 2 and 7 years, respectively (Durand et al., 1998). The sinuses participate in warming and humidification of inspired air, and add resonance to the voice. The sinuses are lined with ciliated pseudostratified epithelium containing mucus-producing goblet cells, similar to the lining of the nasal cavity but with less density (Gwaltney, 1998). Obstruction of the ostia and/or delay in mucociliary transport leads to accumulation of secretions and subsequently to the development of sinusitis. Unlike the nasal passages, the paranasal sinuses are normally sterile. The mechanisms maintaining this sterility include the mucociliary clearance system, the immune system, and possible nitric oxide production within the sinus cavity (Palm et al., 2000.) The classification of sinusitis can be based on a number of factors, including the patient's immune status, the causative pathogen (viral, bacterial, fungal), and the duration of the sinusitis (acute, subacute, chronic, and recurrent). Acute sinusitis is defined as sinusitis lasting 4 weeks or less, while sinusitis is considered subacute when symptoms persist for 4 to 12 weeks and chronic when symptoms last longer than 12 weeks. During the first 7 to 10 days of illness, it may be difficult to determine whether the symptoms are caused by a viral or bacterial pathogen and to decide whether antimicrobial agents are indicated. Appropriate classification of the cause is important so that the correct treatment can be defined and instituted and treatment outcomes can be evaluated.

Bacterial infection sinusitis is the most problem of treatment in the current day. The 3 major bacterial pathogens in acute community-acquired sinusitis are *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. Moreover their pathogen have developed to multi drugs resistance. The recent study about epidemiology of sinusitis in primary care during 1999-2000. This study was taken over 10-month periods during 1999-2000 respiratory infection in 16,213 patients who have acute bacterial rhinosinusitis. This study demonstrated four pathogens accounted for 79.7% of all identifiable isolates: *Streptococcus pneumoniae* (11.3%), *Haemophilus influenzae* (21.7%), *Moraxella catarrhalis* (28.9%), and *Staphylococcus aureus* (17.9%).

When test for resistant to penicillin was found for *S.pneumoniae* (16% fully resistant, 20% intermediate resistance, and resists to erythromycin, azithromycin, and clarithromycin in the range of 32% to 35%. H.influenzae was found high rate of resistance to clarithromycin (36%). M.catarrhalis had 15% rate of resistance to erythromycin and 95% rate of resistance to penicillin. Moreover this study show low levels of resistance of *S.pneumoniae* to new fluoroquinolones such as levofloxacin and gatifloxacin by MIC of levofloxacin was 2 μg/ml while MIC of gatifloxacin was 0.5 μg/ml (Sokol , 2001).

5. Pharmacokinetic/Pharmacodynamic parameter of Fluoroquinolones

Assessment of pharmacodynamic activity from standard in vitro minimum inhibitory concentration (MICs) alone is insufficient to predict in vivo potency. Although the minimum inhibitory concentration (MIC) is useful predictor of the activity of an antimicrobial against a microorganism, it dose not necessarily indicate relative in vivo potency. For example, two antimicrobial agents with an MIC of 1.0 μg/ml should be evaluated quit differently if one has a peak concentration of 2 μg/ml and the other a peak of 20 μg/ml. Thus, pharmacokinetic parameter must be considered for proper assessment of a drug and dosage.

Some studies demonstrate the relationship between drug concentration and pharmacologic effect. For pharmacodynamic activity can be decried as concentration dependent or time dependent. Fluoroquinolones have concentration-dependent killing. The pharmacodynamic values that best correlate with efficacy are area under the curve in 24 hours (AUC_{0-24})/ MIC and the peak: MIC. Forrest et al was observe form 74 patients who have acutely ill and treated intravenous ciprofloxacin at dosages ranging between 200 mg every 12 hours and 400 mg every 8 hours. The result shows that an AUC_{0-24}/ MIC at 125 (log_{10}=2.1), the percent probabilities of clinical cures were 69%. At an AUC_{0-24}/ MIC at 250 (log_{10}=2.4), the probabilities were 80% (figure 2-3) In addition, when daily cultures were obtained to derive time to bacterial eradication. AUC_{0-24}/ MIC of 125-250 show effective bacterial was killing in 7 days. When AUC_{0-24}/ MIC values were 250, bacterial was rapid killing within 2 days. Although AUC_{0-24}/ MIC value were increase, bacterial killing rate of eradication did not increased (figure 2-4) (Forrest et al., 1993). Preston et al was finding the relationship between plasma levels of levofloxacin and successful clinical and microbiological outcome of patients with clinical signs and symptoms of bacterial infections of the respiratory tract, skin, or urinary tract. The result of this study demonstrated that the clinical outcome was predicted by the ratio of peak plasma concentration to MIC (Peak/MIC) and site of infection ($P<.001$). Microbiological eradication was predicted by the Peak/MIC ratio ($P<.001$). Both clinical and microbiological outcomes were most likely to be favorable if the Peak/MIC ratio was at least 12.2 (Preston et al., 1998). Understanding this parameter can facilitate selection of effective antimicrobial agents with optimal regimen to hasten response, prevent

treatment failures, and minimize the development of resistance. The most common methodologies are direct comparison of different dosing schedules in vitro in animals and humans. The fluoroquinolones have concentration-dependent killing, the Peak/MIC or AUC_{0-24}/MIC would be most predictive of outcome.

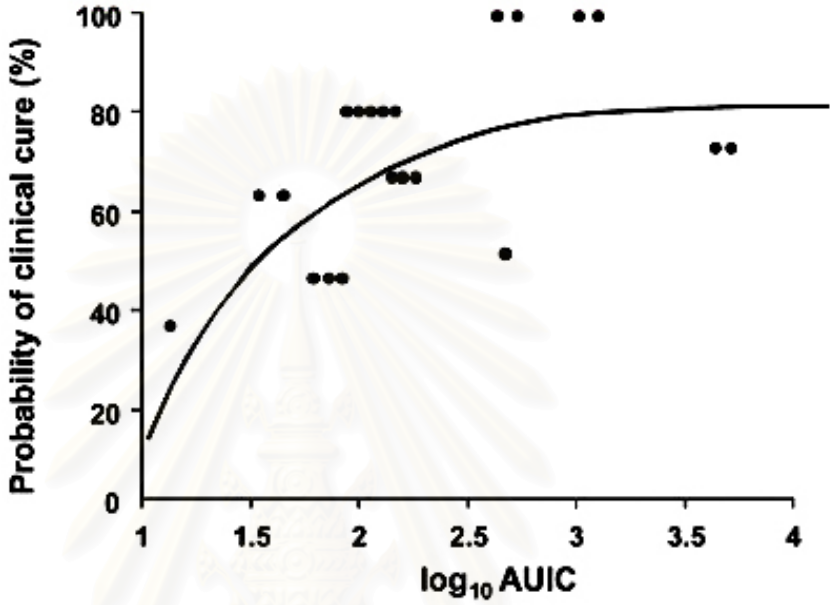


Figure 2-3 Percentage probability of a clinical cure versus AUC_{0-24} fit to a modified Hill equation. The curve is the fitted relation ship; each point represents three or four patients. A $\log_{10}$ (AUC_{0-24} /MIC) of 2.1 is equivalent to an AUC_{0-24} /MIC of 125, and $\log_{10}$ (AUC_{0-24} /MIC) of 2.4 is equivalent to 250 (Modified from Forrest et al., 1993).

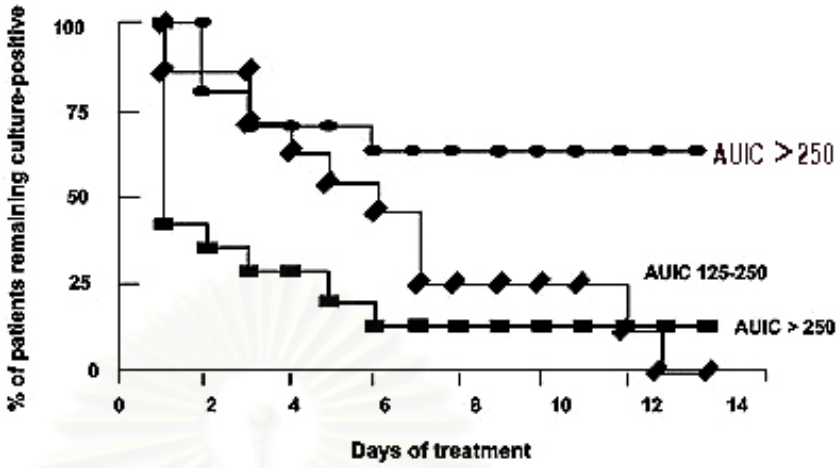


Figure 2-4 Time (days of therapy) to bacterial eradication versus AUC_{0-24}/MIC illustrated by a time-to-event plot. Show is the day of therapy versus patients remaining culture positive on the day (Modified from Forrest et al., 1993).

Andes et al. study about dose effective relations at several dosing intervals for quinolones against gram-negative bacilli in murine thigh infection and pneumonitis model demonstrated that AUC_{0-24}/MIC of 100 or grater and Peak/MIC above 8 and serum levels above MIC 100% of time predicted efficacy of fluoroquinolones in treating animal models by AUC_{0-24}/MIC show the best linear correlation (Andes et al., 1998).

The study of Preston et al were conducted in humans when treatment of respiratory, urinary, or skin and soft tissue infections with levofloxacin resulted in a high frequency of clinical and microbiologic cure when Peak/MIC above 12.2 (Preston et al., 1998). In this study Peak/MIC ratio and AUC_{0-24}/MIC ratio were highly correlated, with a mean AUC_{0-24}/MIC least than 65 show unsuccessful outcome in the contrast when AUC_{0-24}/MIC greater than 100 in group with successful outcome. Thomas et al investigated pharmacodynamic evaluation of factors associated with the development of bacterial resistance in acutely ill patient demonstrated that 82% of patient who had AUC_{0-24}/MIC below 100 were developed an infection with a resistant organism. However, when AUC_{0-24}/MIC above 100, only 9% of patients developed a resistant infection

(Figure 2-5) (Thomas et al., 1998). This study suggest that the resistance can be avoid with attention to dosing regimen to provide an AUC_{0-24}/MIC not least than 100.

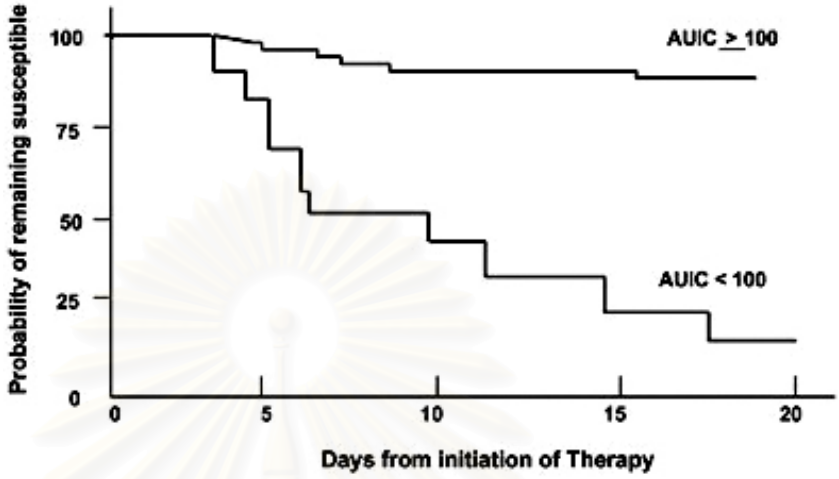


Figure 2-5 Relationship between the probability of developing resistance and treatment duration (days). When the AUC_{0-24}/MIC ratio was 100 or grater, only 8% of organisms developed resistance, whereas only 7% remained susceptible when the AUC_{0-24}/MIC ratio was less than 100. (Modified from Thomas et al., 1998).

Gram-positive microorganisms, *S.pneumoniae* have been studied *in vitro* and animal models of bacterial killing rate. Klepser et al evaluated activity of 6 fluoroquinolones against 3 strains of *S.pneumoniae* for found activity in 24 hours. In this study using an *in vitro* pharmacodynamic model exposed to microorganisms to 0.1, 1 and 10 times the pharmacokinetic profile observed in human of ciprofloxacin, clinafloxacin, grepafloxacin, levofloxacin, moxifloxacin, and trovafloxacin demonstrated that AUC_{0-24}/MIC between 54 and 175 was achieve. Ciprofloxacin was the least activity for all 3 isolate at simulated 24 hours about levofloxacin resulted in variable bacteriostatic and bactericidal activity (Klepser et al., 2001).

Lister et al study demonstrated in vitro bactericidal rates to compare the pharmacodynamic of levofloxacin and ciprofloxacin against 4 penicillin-susceptible and 4 penicillin-resistant strain of *S.pneumoniae*. When simulated concentration of levofloxacin after oral dose 500 mg and ciprofloxacin 750 mg. Levofloxacin was slowly

bactericidal in 36 hours for all 8 strain, 6 strain at 24 hours were eradication. AUC_{0-24}/MIC this drug was 32 to 64. About ciprofloxacin had a slower rate of organism killing than levofloxacin, but managed to eradicate 5 of 8 strains in 36 hours for AUC_{0-24}/MIC only 44. In this study when AUC_{0-24}/MIC approximately 37 for 24 hours there was emergence of nonsusceptible single-step mutants to ciprofloxacin (Lister et al., 1999).

Experiment of animal model about AUC/MIC target in gram-negative microorganisms. One study demonstrated the relationship between the 24 hours AUC/MIC ratio and mortality in murine pneumonitis and thigh-infection model were treated for $\geq$ 2 day, survival result when finish were report with pharmacokinetic data as show in figure 2-6. This study demonstrated two break points versus mortality, first when AUC/MIC least than 30 animal survival was < 50% second when AUC/MIC $\geq$ 100 no motality.

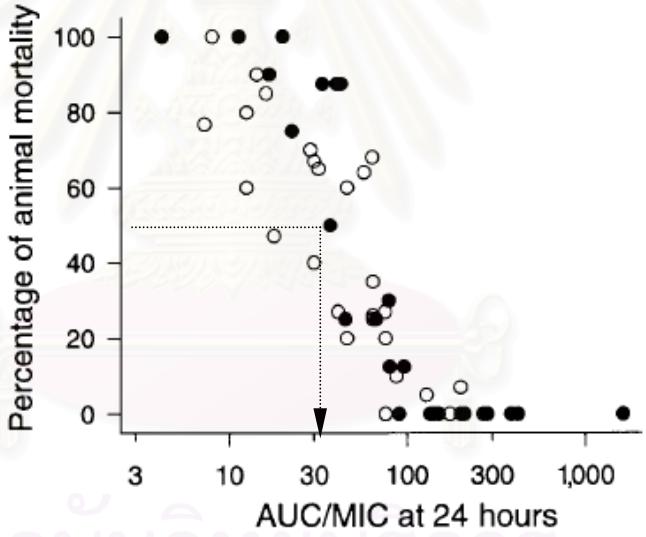


Figure 2-6 The relationship between murine infection mortality and AUC/MIC ratio in gram-negative infections of the thigh and lung. Mice were inoculated with pathogens such as *Klebsiella pneumoniae* and then treated to the resulting AUC/MIC by varying the dosage. The predictive dose that produces half of the maximum effect attributable to the drug occurred at an AUC/MIC of 30, at which point animal survival was 50%. In order to achieve 100% murine survival, the AUC/MIC ratio need to exceed 100 (Craig , 1998).

The studies of Peak/MIC ratio which studies in neutropenic rat model with Pseudomonase sepsis suggest that Peak/MIC ratio or AUC/MIC was significant to predict survivorship (Drusano et al., 1993). This result shows that AUC/MIC was held

constant and vary Peak/MIC ratio, the outcome significantly improved. Moreover the investigator suggest that it may reflect effective radication of bacterial subpopulations which remain viable and are selected resistance by lower exposure. At lower, physiologically achievable doses, which produce Peak/MIC ratio < 10:1, AUC/MIC ratio 125 was require to achieve at least 90% survivorship.

In gram-positive have been reported in experiments with murine model. There are two drived AUC/MIC targets. First bacteriostatic point that in general defined as the dose to produce half of the maximum effects [PD_{50}] occurs at AUC/MIC ratio approximately 30-40. Second bactericidal effect (3-4 log killing) requires higher AUC/MIC ratio approximately 100-125 (Schentag et al., 2003).

For the past report of Fukuda et al, *S.pneumoniae* resists ciprofloxacin and levofloxacin by selected *parC* muattion, while moxifloxacin and gatifloxacin select *gyrA* mutants with this microorganism (Fukuda et al., 2001). When the first-step mutation was appearing in target site above. The next mutation is the one not already selected.

Coyle et al demonstrated that fluoroquinolones against mutated strain of both *S.pneumoniae*. Both strains were resist to ciprofloxacin one strain was *gyrA* mutation and another strain was efflux-mediated mutation. MIC of ciprofloxacin against were 4.0, 8.0 μg/ml. In contrast these strain had low MIC (≤ 0.5 μg/ml) against moxifloxacin, gatifloxacin, trovafloxacin and grepafloxacin. For levofloxacin MIC were 2.0 and 4.0 μg/ml respectively. In this study the investigator design to match AUC exposed microorganisms for 24-48 hours. The result demonstrated that when AUC/MIC of fluoroquinolones above 100, there are no microorganisms regrowth within 48 hours. When AUC/MIC of all fluoroquinolones ≤ 75 microorganisms show regrowth at 48 hours (Coyle et al., 2001). It can predict AUC/MIC to prevent resistance must be not least than 100.

The suitable target AUC/MIC ratio of fluoroquinolones against microorganisms in summary. When AUC/MIC in the range of 30-60 or Peak/MIC ratio in the range of 5:1 are related to bacteriostatic (Forrest et al., 1993). In the middle of the AUC/MIC range about 100 to 250 are related slow killing rate of microorganisms within 7 day of treatment. At an AUC/MIC above 250 or Peak/MIC of 25:1are related rapid killing and bacterial eradication within 24 hours (Schentag et al., 2003).

6. In vitro pharmacodynamic model (IVPM)

The development of *in vitro* pharmacodynamic models for evaluation of antimicrobial activity has aroused interest since they offer the possibility of producing *in vitro* drug levels of the same pharmacodynamics as found *in vivo*. This would permit investigation into how certain pharmacokinetic parameters influence antimicrobial activity.

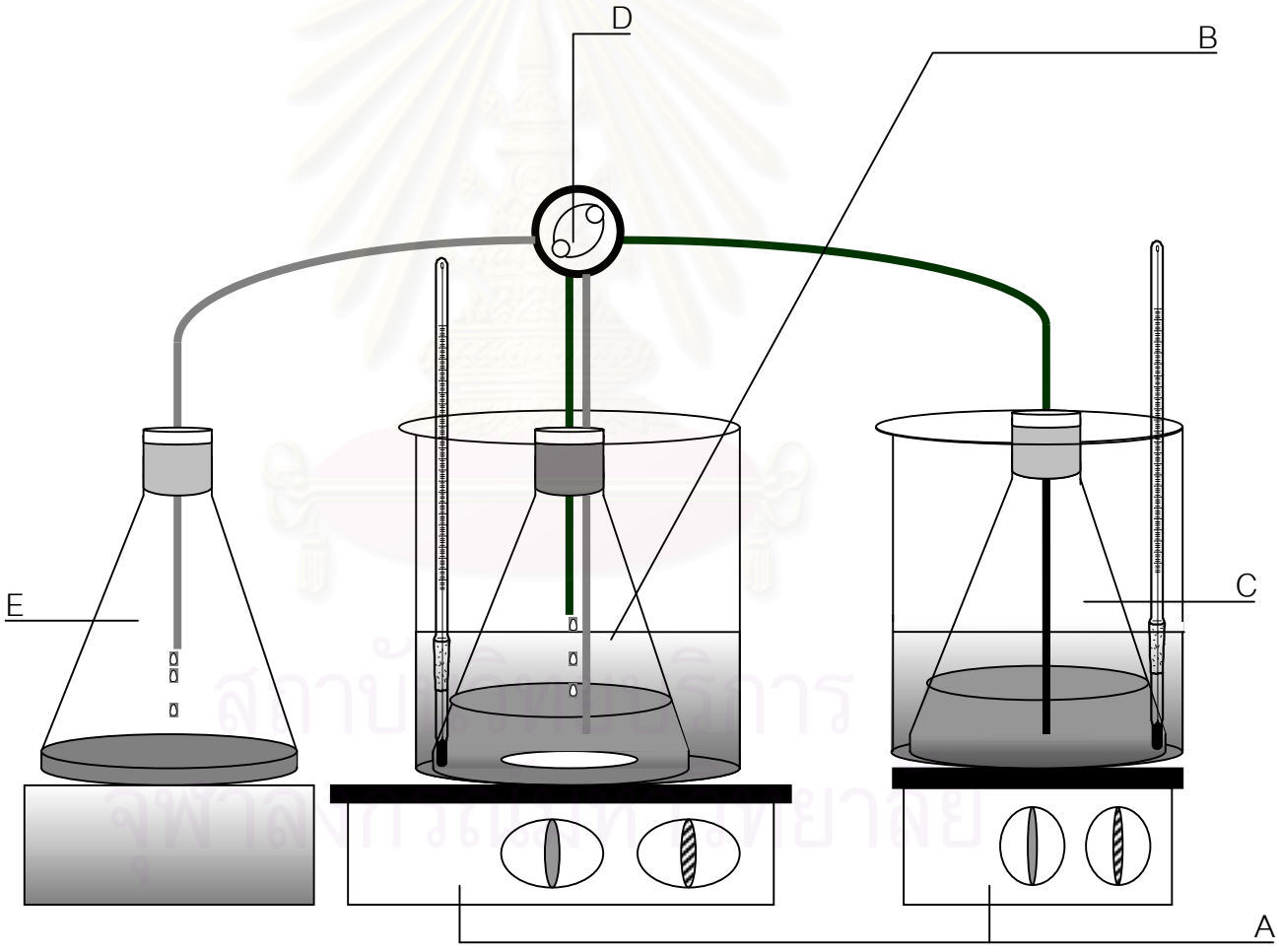
The original version of in vitro pharmacodynamic model to be described in the literature was that of the urinary bladder, developed to simulate conditions of uncomplicated cystitis. This system was use to study the effect of cycles of 'dilution' and 'micturition' on bacterial growth. The model consisted of a glass vessel was a tubular prolongation at the base. The bacterial culture in the vessel was diluted over time with fresh broth, using a metering pump (O'grady & Pennington, 1966).

Sanfilippo & Morvill were developing the first *in vitro* pharmacodynamic model that reproduced plasma levels of antimicrobial agents similar to that observed in vivo Sanfilippo & Morvill, 1968). The apparatus consisted of a series of flasks containing a bacterial culture with antimicrobial agents added. Each flask was connected by rubber tubing to another series of reservoir flasks, containing sterile broth with or without antimicrobial agents, depending on whether constant levels or concentration decreasing with time were to be simulated. The sterile diluent broth was pumped into the flasks holding bacterial culture by peristaltic pump with a timer to control the flow. Bacterial culture samples were taken at intervals for turbidimetric or reading, or for viable cell counts. Since this model was not based on dilution at a constant rate, drug concentration did not vary exponentially. Additionally, as the volume of culture broth increased when concentration-time curves were simulated for rapidly eliminated drugs, large amounts of diluent broth were needed.

Since 1978, Grasso et al was developing *in vitro* pharmacodynamic for study the antibacterial activity of antimicrobial agents (Gasso et al., 1978). The apparatus operated by first-order dilution techniques, and reproduced concentration-time curves with mono- or biexponential pattern. In this version that simulated the pharmacokinetic one-compartment open model with iv administration, the apparatus consisted through a two-hole rubber stopper with glass and Tygon tubing to a reservoir containing sterile

broth and to a vessel to collect outgoing fluid from flask. The diluent was pumped from the reservoir into the flask by peristaltic pump at a constant flow-rate; since the flask was tightly stoppered, the fluid was forced out of it at an equal flow-rate. The fluid coming out of the flask constituted a continuous sample of the culture, on which it was possible to determine the bacterial count and the antibiotic concentration as function of time.

The model described by Bergan, Carlsen & Fuglesang (1980) was used in this study show in figure 2-7. This model was base on the same dilution process, but differed from the above method in that it used two peristaltic pumps working at the constant flow-rate, and culture flask was used both for collecting samples and for taking turbidimetric or viable count reading.



A: heater and magnetic stirrer, B: central compartment, C: fresh media reservoir,

D: peristaltic pump, E: collecting reservoir.

Figure 2-7 Diagram of In vitro pharmacodynamic model.

In vitro pharmacodynamic systems are usually designed to emulate the serum concentration-time curve of a human antibiotic exposure and to study the effect of changing antibiotic concentrations on bacteria over time. Experiments can be run in a controlled, reproducible environment where antibiotic exposures can be repeated to simulate conditions not possible in a human host. These experiments can be done relatively quickly and at fraction of the cost of a clinical trial. Efficacy and toxicity in these systems are not a concern as patient outcome is not an issue. Data gleaned from appropriately designed in vitro experiments can quickly depict an antibiotic as a time- or concentration-dependent killer of a specific microorganism. However, in vitro pharmacodynamic models have limitations. They do not account for the effects of the immune system and can not assess toxicity. Because of in vitro pharmacodynamic model have limitations. Therefore work done with in vitro model is intended to and should be complementary to work done with in vivo systems.

In vitro models to play a valuable role in the antibiotic development process. In vitro pharmacodynamic testing represents a complementary step in the development process from benchtop to animal model and eventually to human trials. Demonstrating that pharmacodynamic data are reproducible in vitro, in animals, and through clinical trials should expedite the optimization of anti biotic dosing and should result in the ongoing validation of pharmacodynamic parameter. The in vitro pharmacodynamic model is a cost effective and rapid means for gathering preliminary in formation on antibiotic activity and establishing pharmacodynamic parameters under the influence of a variety of clinical variables.

Identifying optimal pharmacodynamic parameters along with other variables influencing activity and designing theoretically effective regimens in preclinical testing will give a new antibiotic the best possible chance of establishing clinical efficacy in phase III and IV trials and beyond (Gunderson et al., 2001).

CHAPTER III

MATERIALS & METHODS

MATERIALS

1. Microorganisms, Chemicals and Reagents

1.1 Microorganisms

The bacterial strains used thoughout this study was *Moraxella (Branhamella) catarrhalis*, *Haemophilus influenzae* and *Streptococcus pneumoniae*. These bacteria were selected from clinically isolated from patients in Siriraj Hospital by randomization during year 2001-2002. In this study was categorized the microorganism into six strains by patterns of susceptibility to ampicillin and penicillin show in table 3-1

Table 3-1 Strains of microorganisms used in this study.

Microorganisms	Strains
M.catarrhalis	
Ampicillin-resistance	Bc 312
H.influenzae	
Ampicillin-susceptible	Bc 38
Ampicillin-resistance	Bc 255
S.pneumoniae	
Penicillin-susceptible	94
Penicillin-intermediate resistance	38
Penicillin-resistance	14

1.2 Chemicals

- Standard powders

Two fluoroquinolone standard powders were tested: levofloxacin was supplied by Daiichi pharmaceutical and moxifloxacin was supplied by Bayer Corporation. One fluoroquinolone solution for injection was tested: Gatifloxacin was purchased from Bristal-Mayer Squib for potency of this agent was quality control by test

with standard microorganisms (*E.coli* ATCC 25922) follows by NCCLS and adjust the strenge of this drug follow by result of their MIC.

1.3 Reagents

- Mueller-Hinton Agar (MHA) purchased from BBL (Becton, Dickinson, USA) was used as the test medium for bioassay method.

- Mueller-Hinton Broth (MHB) purchased from BBL (Becton, Dickinson, USA) was used as the test medium for *M.catarrhalis*.

- 0.5%Haemophilus test medium (HTM) supplement purchased from Oxiod (Oxoid chemical, England) were used as the supplement in MHB for *H.influenzae*.

- 5% Lyse horse blood purchased from Sigma (Sigma Aldrich Co., USA) was used as the supplement in MHB for *S.pneumoniae*.

- Chocolate agar has been specifically combination of GC medium base purchased from Oxoid (Oxoid chemical, England), haemoglobin powder and Iso-Viterex purchased from BBL (BBL, USA) used as the media for culture *H.influenzae*.

- Blood agar was prepared from blood agar base purchased from from Oxiod (Oxoid chemical, England) and 5% human whole blood by used as the media to culture *M.catarrhalis* and *S.pneumoniae*.

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

- 9% Sterile normal saline (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 follows:

- 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 0.08 to 0.10 for the 0.5 McFarland 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 stored in the dark at room temperature.
- The barium sulfate turbidity standard should be vigorously agitated on a mechanical vortex mixer before each use and 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 verified monthly.

2. Laboratory Equipment

2.1 Disposable Equipment

- Cotton plugs.
- Aluminum foil.
- Blank disk (Whatman, England).

2.2 Sterile Glass Equipment

- Petri dishes.
- Erlenmeyer flasks 250ml.
- Cylinders 100ml.
- Glass tubes.
- Pipettes were used in experiment divided into 2 types
  - Glass pipettes.
  - Micropipette.

2.3 General Equipment

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

3. Laboratory Instruments

3.1 *In vitro* pharmacodynamic model

The *In vitro* pharmacodynamic model (IVPM) are tools used to evaluate the killing kinetics of antimicrobial under controlled condition that allow the simulation of the pharmacokinetic parameter.

IVPM was used to investigate the antimicrobial activity in this experiment to simulate the pharmacokinetic one-compartment open model which consist of a central culture compartment, medium and waste reservoir, and a peristaltic pump was used to control flow rate. By using a central compartment model, bacteria were exposed to concentration of antimicrobial agents to simulate human pharmacokinetic parameters. Free medium was continuously pumped into each of the model by a peristaltic pump at the rate which simulated the elimination half-life of the test antimicrobial agents (for levofloxacin, gatifloxacin and moxifloxacin, the elimination half-life are 6.8, 7.5 and 12 hours, respectively). The flow rate of medium was adjusted based on the targeted half-life of the individual experiment and the volume of the test culture (V) was determined according to the formula $0.693\ V/T_{1/2}$.

- Peristaltic pump (model ISM 838, Ismatic) used for control the flow rate of solution in an *in vitro* pharmacodynamic model. In this study, the peristaltic pump has two channels, one control fluid that pumped from fresh media reservoir, another one control fluid that pumped from central compartment outgoing to collect reservoir.

- Tygon tube has diameter about 0.19 mm. used for transfer the media from fresh media reservoir to central compartment reservoir and transfer culture from central compartment reservoir to waste reservoir of *in vitro* pharmacodynamic model.

- Hot plate with magnetic stirrer used for produce the temperature of central compartment of model to 37 ^{0}C and magnetic stirrer used for mixes the culture in central compartment.

3.2 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 maintain antibiotic activity between 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 the Tygon tube and sterilize all glass equipment before using.

3.3 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^{TM} 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. Broth Macrodilution Method to determine minimum inhibitory concentration (MIC) of levofloxacin, gatifloxacin and moxifloxacin to selected microorganisms.

2. Time Kill Method to investigate bactericidal activity of levofloxacin, gatifloxacin and moxifloxacin against to selected microorganisms by collected samples from *in vitro* pharmacodynamic model and construct time killing curves.

3. Bioassay Method to determine concentration of levofloxacin, gatifloxacin and moxifloxacin during 0 to 24 hours by collected samples from *in vitro* pharmacodynamic model and construct concentration-time curve for determine area under the curve (AUC_{0-24}).

4. Determine ratio of area under the curve (AUC_{0-24}) of levofloxacin, gatifloxacin and moxifloxacin divided by MIC of each antimicrobial agent against selected microorganisms.

5. Determine antibacterial activity of levofloxacin, gatifloxacin and moxifloxacin by compare pharmacodynamic parameter with their ratio of AUC_{0-24}/MIC.

1. Broth Macrodilution Procedures (Susceptibility testing)

1.1 Test Broth

1.1.1 MHB was recommended as the medium of choice for the susceptibility testing of commonly isolated such as *M.catarrhalis*.

1.1.2 MHB supplement with 0.5% HTM were recommended as the medium of choice for the susceptibility testing of fastidious organisms such as *H.influenzae*.

1.1.3 MHB supplement with 5% Lyse horse blood was recommended as the medium of choice for the susceptibility testing for *S.pneumoniae*.

1.2 Diluted Antimicrobial Agents preparation

1.2.1 Standard powder of levofloxacin and moxifloxacin were dissolved in sterile water for injection to final concentration approximately 1,000 μg/ml. The concentration of gatifloxacin solution about 10,000 μg/ml was diluted by sterile water for injection to final concentration approximately 1,000 μg/ml. All stock solution were aliquot to 1.5 ml and stored in the refrigerator at -80 ^{O}C before used. *S.aureus* ATCC 29213 and

E.coli ATCC 25922 were also included in the study as the control strains as recommended by NCCLS 2000. The MICs of the control strains were show in table 3-1

Table 3-2 Susceptibility of levofloxacin, gatifloxacin and moxifloxacin against *S.aureus* ATCC 29213 and *E.coli* ATCC 25922 (Data from NCCLS, 2000).

Antimicrobial agents	MIC (μg/ml)	
	S.aureus ATCC 29213	*E.coli* ATCC 25922
Levofloxacin	0.125 (0.06-0.5)	0.03 (0.008-0.03)
Gatifloxacin	0.03 (0.03-0.12)	0.015 (0.008-0.03)
Moxifloxacin	0.06 (0.016-0.12)	0.03 (0.008-0.06)

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

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

1.2.4 The tube can be closed with cotton plugs.

1.2.5 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 0.5 ml of broth containing antimicrobial and 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.

1.3 Broth Dilution Testing

1.3.1 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.

1.3.1.1 Growth Method

- At least three to five well-isolated colonies of the same morphological type of *M.catarrhalis* 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 McFaland 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.

1.3.1.2 Direct Colony Suspension Method

- As a convenient alternative to the growth method, the inoculum can be prepared by making a direct broth or saline suspension of isolated colonies selected from an 18- to 24-hour agar plate (chocolate agar for *H.influenzae* and blood agar for *S.pneumoniae*). The suspension was adjusted to match the 0.5 McFaland turbidity standard. This suspension will contain approximately 1 to 2X10^{8} CFU/ml.
- This approach is the recommended method for testing the fastidious organisms such as *H.influenzae*, and streptococci such as *S.pneumoniae*.

1.3.1.3 Optimally, within 15 minutes the adjusted inoculum suspension should be diluted in broth so that, after inoculation, each tube contained approximately 5X10^{5} CFU/ml.

1.3.1.4 *H.influenzae*, a suspension of test organism is prepared in Mueller-Hilton broth using colonies taken directly from an overnight chocolate agar culture. This suspension should be adjusted to a turbidity equivalent to 0.5 McFaland standard and verified using a photometer. Suspending the adjusted inoculum into Mueller-Hinton broth supplement with 4% Haemophilus Test Medium for adjusted inoculum concentration to 5X10^{5} CFU/ml.

1.3.1.5 *S.pneumoniae*, a suspension of test organism was prepared in Mueller-Hinton broth using colonies taken directly from an overnight (18- to 20-hour)

blood agar culture. This suspension should be adjusted carefully to a turbidity equivalent to a 0.5 McFaland standard. Suspending the adjusted inoculum into Mueller-Hinton broth supplement with 4% lyses horse blood for adjusted inoculum concentration to $5X10^5$ CFU/ml.

1.3.1.6 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 result in a 1:2 dilution of each antimicrobial concentration and 1:2 dilution of the inoculum.

1.3.1.7 The inoculated macrodilution tubes should be incubated at 37 ^{O}C for 16 to 24 hours in an ambient air incubator. When testing in *H.influenzae*, incubation should proceed for 20 to 24 hours in ambient air before interpreting result.

1.4 Determining MIC End Points

The MIC is the lowest concentration of antimicrobial agents that completely inhibits 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 tests when determining the growth end points.

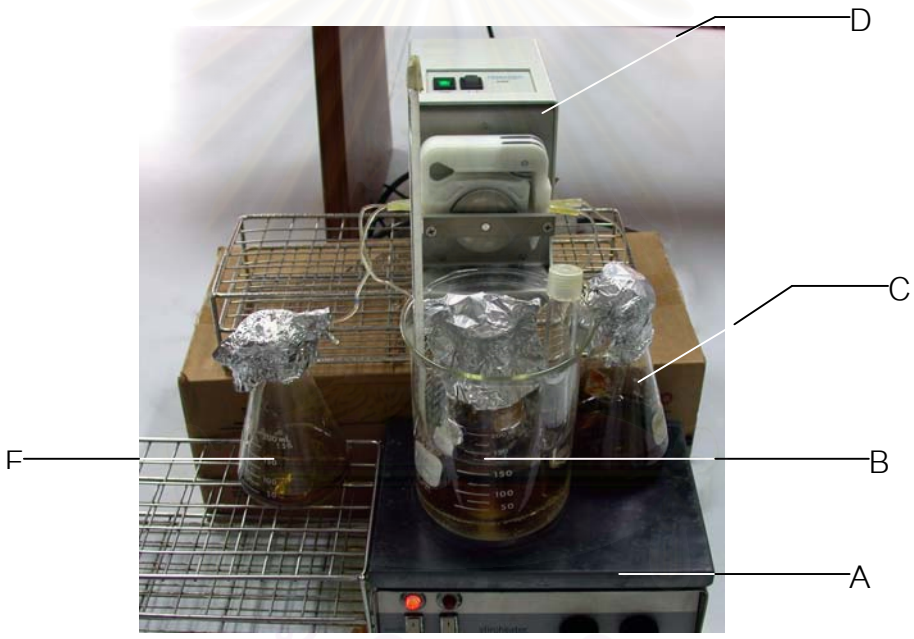
2. Determine of antimicrobial activity (Time Kill Method)

The *in vitro* pharmacodynamic model was used for determine antimicrobial activity in this study which consist of three part such as central compartment, fresh media reservoir and collecting reservoir. The central compartment was the flask containing bacterial culture in contact with antimicrobial agents at maximum concentration when the agents distributed into maxillary sinus. The central compartment connected through two-hold stopper and Tygon tubing (diameter was 0.19 mm.) to other part such as fresh media reservoir which containing sterile broth without antimicrobial agents and collecting reservoir which collect outgoing fluid from the flask of central compartment. The constant flow rate of fluid in this model was controlled by peristaltic pump and depend upon the size of Tygon tube's diameter in this experiment the

diameter of Tygon tube was 0.19 mm. should be generated flow rate of fluid approximately 0.185 ml/min.

The process of *in vitro* pharmacodynamic model was started by peristaltic pump that pumping the sterile broth from fresh media reservoir thought the tube into the flask of central compartment. At the same time, the fluid in this flask should be pumped at the same flow rate outgoing to the collecting reservoir. The *in vitro* pharmacodynamic model used in this study was shown in Figure 3-1

The equation of the flow rate of the *in vitro* pharmacodynamic model was $F = 0.693xV/T_{1/2}$ where F= flow rate (ml/min), V= volume of fluid in the flask of central compartment (ml) and $T_{1/2}$: half-life of the antimicrobial agents (hours) (Grasso, 1978).



A: heater and magnetic stirrer, B: central compartment, C: fresh media reservoir, D: peristaltic pump, E: collecting reservoir.

Figure 3-1 *In vitro* pharmacodynamic model used in this study.

For this study flow rate of the fluid was constant at 0.185 ml/min in all experiments. The volume of fluid in the central compartment was varied which depend on $T_{1/2}$ of each antimicrobial agent. From the above equation, the volume in the central compartment of levofloxacin ($T_{1/2}$ = 6.8 hours), gatifloxacin ($T_{1/2}$ = 7.5 hours) and moxifloxacin ($T_{1/2}$ = 12 hours) approximately 108, 160 and 190 ml, respectively.

Because of the regimen of levofloxacin, gatifloxacin and moxifloxacin for the treatment of sinusitis were multiple doses as shown in table 3-2. Therefore, the determination of antimicrobial activity in this study was design in two phases, single dose regiment and multiple doses regiment for the treatment maxillary sinusitis.

Table 3-3 Dosage regimen and duration used in the treatment of sinusitis by levofloxacin, gatifloxacin and moxifloxacin.

Antimicrobial agents	Dosage regimen	Duration (day)
Levofloxacin	500 mg oral every day	7
Gatifloxacin	400 mg oral every day	10
Moxifloxacin	400 mg oral every day	5

2.1 Preparation of the initial concentration

2.1.1 Phase I; the initial concentration of antimicrobial agents in central compartment were designed to the maximal concentration (C_{max}) of each drug by diluting the stock solutions of levofloxacin, gatifloxacin and moxifloxacin to their final concentrations approximately 6.9, 6.7 and 6 μg/ml, respectively. These are the concentrations of antimicrobial when they distributed into the maxillary sinus after single dose administration.

2.1.2 Phase II; the initial concentration of antimicrobial agents in central compartment were designed to the maximal concentration (C_{max}) of each drug by diluting the stock solutions of levofloxacin, gatifloxacin and moxifloxacin to their final concentrations approximately 7.0, 8.1 and 6.4 μg/ml, respectively. These are the concentrations of antimicrobial when they distributed into the maxillary sinus after multiple dose administration.

2.2 Dilute the standardized inoculum to obtain the final bacterial quantity 1 $X10^6$ to $2X10^6$ CFU/ml into working media in central compartment of *in vitro* pharmacodynamic model and into working media in control tube and then incubate on water bath at 37 ^{O}C.

2.3 When antimicrobial agents exposed to microorganisms in an *in vitro* pharmacodynamic model should be collect the samples from central compartment and control tube to detect colony forming unit at the time 0, 0.25, 0.5, 0.75, 1, 2, 4 and 6

hours. And then, for time 10.5, 15.0, 19.5 and 24 hours should be determine bacterial activity by macrodilution method. By time 6 hours, the antimicrobial agents of central compartment fluid were diluted to the concentration of each agents at time 10.5, 15, 19.5 and 24 hours respectively and incubated at 37 ^{O}C until 24 hour before collect the sample to detect colony forming unit.

2.4 Inoculate the samples on appropriate solid media for 24 to 48 hours at 37 ^{O}C in an atmosphere of 5% CO_2 to detect for colony forming units.

2.5 Calculate the quantity of survival bacteria to obtain the killing curves data.

2.6 Time-kill curves were constructed by SigmaPlot 8.0. The criteria to define the bactericidal property is the decreasing in colony forming unit from the original point $\geq$ 3 logCFU/ml at 24 hours of exposure (Amsterdam, 1996; Pankuch, Jacobs and Appelbaum, 1994; Satta, et al., 1995). The quantitative evaluation of antimicrobial effect was calculated as in the published article (Firsov, et al., 1997).

The Quantitative Evaluation of Antimicrobial Effect

The parameters were estimated by extrapolation of the Time-killing curves are

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

$T_{eradication}$ = Time required to decrease viable counts below the 10 CFU/ml (1logCFU/ml) limit of detection.

3. Determination of Antimicrobials concentration (Bioassay Method)

After collected sample form *in vitro* pharmacodynamic model and control tube at the time 0, 0.25, 0.5, 0.75, 1, 2, 4, 6, 10.5, 15.0, 19.5 and 24 hours all sample should be determine concentration of antimicrobial agents by bioassay method.

3.1 Preparation of standardized inoculum

3.1.1 The initial inoculum of *E.coli* ATCC 25922 was subculture on blood agar incubate overnight before used.

3.1.2 Suspend at least three to five well-isolated colonies of the same morphological type that selected from an agar plate culture into 3 to 4 ml of MHB.

3.1.3 The broth culture was incubated at 37 ^{O}C for 1 to 2 hours until the inoculum was excess over 0.5 McFaland standard.

3.1.4 Adjust the turbidity of the culture by suspending broth culture into MHB to equivalent 0.5 McFaland standard which approximately 1 to 2X10^{8} CFU/ml.

3.1.5 Transfer the adjusted turbidity of culture into melted MHA and mixed well for final inoculates approximately 1 to 2X10^{6} CFU/ml.

3.1.6 Transfer 10 ml of the agar culture to peti dished.

3.2 Preparation of Antimicrobial Agents concentration.

3.2.1 Prepare standardize concentration disk of antimicrobial agents.

- Each stock solution of antimicrobial agents were varied concentration about 8, 7, 6, 5, 4, 3, 2, 1.75, 1.50, 1.25 μg/ml
- Transfer 10 μl of each concentration of antimicrobial agents into blank disk.
- The predetermine battery of each antimicrobial agent's disk were dispensed onto the surface of the agar culture plate. Each disk should press down to ensure completed contact with the agar surface. In addition the both difference concentration disk should press onto same agar surface and press by duplicate.
- The plates were inverted and placed in an incubator set at 37 ^{O}C for 24 hours.

3.2.2 Preparation of the sample disk of the samples.

- Transfer 10 μl of each sample that collected from the *in vitro* pharmacodynamic model into the blank disk.
- In the same way as the standard disks, the sample disks were placed on the agar surface culture. Each disk should be pressed down to ensure the complete contact with the agar surface.
- The plates are inverted and placed in an incubator set at 37 ^{O}C for 24 hours.

3.2.3 Plates reading and Construction Standard Curve.

- After 24 hours of incubation, each plate appears inhibition zone around disks depend on concentration of each disk.
- The inhibition zone of each standardizes disks measuring by vernier caliper in scale millimeter.
- To average the inhibition zone of standardizes disks and used these data constructed standard curve by in x-axis was represented in inhibition zone and y-axis was represented in concentration.
- Transform the data in y-axis was be logarithm.
- Add trend line on the graph and calculated equation of line and determine R^2 (R^2 should be not least than 0.90).

3.2.4 Reading plates and Construction Concentration-Time curves.

- After 24 hours of incubation, each plate appears inhibition zone around disks depend on concentration of each disk.
- The inhibition zone of each standardizes disks measuring by vernier caliper in scale millimeter.
- To average inhibition zone of each sample disk and convert inhibition zone to concentration by replace these data into the equation of standard curve for determine concentration of each sampling time.
- Constructed concentration-time curves by in X-axial represent in concentration and in Y-axial represent in time (hours).
- Determine area under concentration-time curves in 24 hours (AUC_{0-24}) by the trapezoidal rule.

4. <u>Determination ratio of the area under concentration-time curves and MIC (AUC_{0-24}/MIC) of levofloxacin, gatifloxacin and moxifloxacin</u>

The AUC_{0-24}/ MIC ratio for levofloxacin, gatifloxacin and moxifloxacin were calculated.

5. Determination of the antimicrobial activity of levofloxacin, gatifloxacin and moxifloxacin

Evaluation of the antimicrobial activity of levofloxacin, gatifloxacin and moxifloxacin was done by comparing the parameter of antimicrobial effect such as $T_{99.9\%}$, $T_{eradicate}$, with the ratio between area under concentration-time curve (AUC_{0-24}) and minimal inhibitory concentration of levofloxacin, gatifloxacin and moxifloxacin (AUC_{0-24}/MIC).

CHAPTER IV

RESULT

Susceptibility testing

All of the isolates were susceptible to levofloxacin, gatifloxacin and moxifloxacin. The MIC of levofloxacin, gatifloxacin and moxifloxacin for *M.catarrhalis*, *H.influenzae* and *S.pneumoniae* isolate are shown in table 4-1.

The MIC of levofloxacin against *M.catarrhalis* (Bc.312), *H.influenzae* (Bc. 255) and *S.pneumoniae* (94 and 38) are lower than those of gatifloxacin and moxifloxacin. *H.influenzae* (Bc.38) and *S.pneumoniae* (14) was the most sensitive to moxifloxacin and gatifloxacin, respectively.

Table4-1 Susceptibility of microorganism to levofloxacin, gatifloxacin and moxifloxacin.

Bacterial strain	MIC (μg/ml)		
	Levofloxacin	Gatifloxacin	Moxifloxacin
M.catarrhalis			
-Ampicillin resistant (Bc 312)	0.015	0.03	0.05
H.influenzae			
-Ampicillin susceptible (Bc38)	0.015	0.0035	0.0015
-Ampicillin resistant (Bc255)	0.0078	0.015	0.025
S.pneumoniae			
-Penicillin susceptible (94)	0.05	0.25	0.5
-Penicillin intermediate resistant (38)	0.1	0.5	0.5
-Penicillin resistant (14)	1.0	0.25	0.4

Pharmacokinetic and Bacterial killing curve after single dose.

- Pharmacokinetic of levofloxacin, gatifloxacin and moxifloxacin within the IVPM.

Pharmacokinetic curve: The measured levofloxacin, gatifloxacin and moxifloxacin concentrations from IVPM in all experiments were in the agreement with the expected concentration, calculated from the first order pharmacokinetic equation ($P<0.05$).

Pharmacokinetic profiles of levofloxacin against each microbial strain shown in table 4-2. Peak concentration (mean ± S.D.) and trough concentration (mean ± S.D.) in IVPM are 7.35 ± 0.60 and 0.82 ± 0.09 μg/ml, respectively. Calculated $T_{1/2}$ (mean ± S.D.) and AUC_{0-24} (mean ± S.D.) are 7.61±0.33 hours and 68.81±3.81 μg.hr/ml, respectively. Concentration-time curve of levofloxacin against each strain of microbial in IVPM show in Figure 4-1, 4-3, 4-5, 4-7, 4-9 and 4-11.

Table 4-2 Pharmacokinetic parameter of levofloxacin 500 mg single dose against *M.catarrhalis* (Bc.312), *H.influenzae* (Bc.38), *H.influenzae* (Bc.255), *S.pneumoniae* (38), *S.pneumoniae* (94) and *S.pneumoniae* (14).

Microorganisms	Peak concentration (μg/ml)	Trough concentration (μg/ml)	$T_{1/2}$ (hours)	AUC_{0-24} (μg.hr/ml)
M.catarrhalis Bc.312	7.25	0.94	8.14	67.73
H.influenzae Bc.38	8.44	0.90	7.43	75.01
H.influenzae Bc.255	7.17	0.80	7.58	70.47
S.pneumoniae 94	7.26	0.73	7.24	63.54
S.pneumoniae 38	7.40	0.90	7.89	68.93
S.pneumoniae 14	6.60	0.70	7.41	67.21
Mean±S.D.	7.35 ± 0.60	0.82 ± 0.09	7.61 ± 0.33	68.81 ± 3.81

Pharmacokinetic profiles of gatifloxacin against each microbial strain were shown in table 4-3. Peak concentration (mean ± S.D.) and trough concentration (mean ± S.D.) in IVPM are 6.96 ± 0.16 and 0.82 ± 0.09 μg/ml, respectively. Calculated $T_{1/2}$ (mean ± S.D.) and AUC_{0-24} (mean ± S.D.) are 7.80 ± 0.45 hours and 65.41± 2.50 μg.hr/ml, respectively. Concentration-time curve of gatifloxacin against each microbial strain in IVPM was shown in Figure 4-13, 4-15, 4-17, 4-19, 4-21 and 4-23.

Table 4-3 Pharmacokinetic parameter of gatifloxacin 400 mg single dose against *M.catarrhalis* (Bc.312), *H.influenzae* (Bc.38), *H.influenzae* (Bc.255), *S.pneumoniae* (38), *S.pneumoniae* (94) and *S.pneumoniae* (14).

Microorganisms	Peak concentration (μg/ml)	Trough concentration (μg/ml)	$T_{1/2}$ (hours)	AUC_{0-24} (μg.hr/ml)
M.catarrhalis Bc.312	7.13	0.93	8.16	65.63
H.influenzae Bc.38	6.93	0.74	7.43	69.52
H.influenzae Bc.255	6.77	0.97	8.56	66.71
S.pneumoniae 94	7.11	0.82	7.70	63.19
S.pneumoniae 38	7.07	0.77	7.50	62.68
S.pneumoniae 14	6.78	0.74	7.50	64.78
Mean±S.D.	6.96 ± 0.16	0.82 ± 0.09	7.80 ± 0.45	65.41 ± 2.50

Pharmacokinetic profiles of gatifloxacin against each microbial strain were shown in table 4-4. Peak concentration (mean ± S.D.) and trough concentration (mean ± S.D.) in IVPM are 6.20 ± 0.30 and 1.51 ± 0.14 μg/ml, respectively. Calculated $T_{1/2}$ (mean ± S.D.) and AUC_{0-24} (mean ± S.D.) are 11.84±1.02 hours and 78.22±2.22 μg.hr/ml, respectively. Concentration-time curve of gatifloxacin against each microbial strain in IVPM was shown in Figure 4-25, 4-27, 4-29, 4-31, 4-33 and 4-35.

Table 4-4 Pharmacokinetic parameter of moxifloxacin 400 mg single dose against *M.catarrhalis* (Bc.312), *H.influenzae* (Bc.38), *H.influenzae* (Bc.255), *S.pneumoniae* (38), *S.pneumoniae* (94) and *S.pneumoniae* (14).

Microorganisms	Peak concentration (μg/ml)	Trough concentration (μg/ml)	$T_{1/2}$ (hours)	AUC_{0-24} (μg.hr/ml)
M.catarrhalis Bc.312	5.81	1.67	13.34	80.39
H.influenzae Bc.38	6.65	1.30	10.18	75.63
H.influenzae Bc.255	6.48	1.67	12.26	80.76
S.pneumoniae 94	6.16	1.52	11.88	77.68
S.pneumoniae 38	6.07	1.45	11.61	79.09
S.pneumoniae 14	6.04	1.48	11.82	75.80
Mean±S.D.	6.20 ± 0.30	1.51 ± 0.14	11.84 ± 1.02	78.22 ± 2.22

■ Pharmacodynamic of levofloxacin, gatifloxacin and moxifloxacin single dose against each microorganism within the IVPM.

Time-kill curve: The viable count of all microorganisms which sampling from screw cap tube that are growth control in all experiment were agreement with viable count of all microorganisms which sampling from IVPM ($P<0.05$).

Pharmacodynamic data for all microorganisms that were exposed to 500 mg single dose of levofloxacin were shown in table 4-5. The Peak/MIC of levofloxacin and AUC_{0-24}/MIC were in the range of 6.60 to 919.23 and 67.21 to 9034.61, respectively. The time for 99.9% kill ranged from 1.22-3.77 hours, and the time required to decrease viable counts below the 10 cfu/ml limit of detection ranged from 6-10.5 hours.

The pharmacodynamics of levofloxacin against *M.catarrhalis* required the maximum time (3.71 hours) to achieve 99.9% kill and eradication (10.5 hours).

Levofloxacin was rapidly bactericidal against all six strains of the microorganisms evaluated in this study. Time-kill curves of levofloxacin against *M.catarrhalis* (Bc.312), *H.influenzae* (Bc.38), *H.influenzae* (Bc.255), *S.pneumoniae* (38), *S.pneumoniae* (94) and *S.pneumoniae* (14) were shown in figure 4-2, 4-4, 4-6, 4-8, 4-10 and 4-12, accordingly.

Table 4-5 Pharmacodynamics of levofloxacin 500 mg single dose against *M.catarrhalis* (Bc.312), *H.influenzae* (Bc.38), *H.influenzae* (Bc.255), *S.pneumoniae* (38), *S.pneumoniae* (94) and *S.pneumoniae* (14).

Microorganism	MIC	Peak/MIC	AUC_{0-24}/MIC	$T_{99.9\%}$ (hr.)	$T_{eradication}$ (hr.)
M.catarrhalis (Bc.312)	0.015	483.33	4515.33	3.77	10.5
H. influenzae (Bc.38)	0.015	562.66	5000.66	1.92	10.5
H. influenzae (Bc.255)	0.0078	919.23	9034.61	1.22	10.5
S.pneumoniae (94)	0.05	145.20	1270.80	1.45	6.00
S pneumoniae (38)	0.1	74.00	689.30	2.00	6.00
S.pneumoniae (14)	1.0	6.60	67.21	2.41	6.00

Pharmacodynamic data for all microorganisms that were exposed to 400 mg single dose of gatifloxacin were shown in table 4-6. The Peak/MIC of gatifloxacin and AUC_{0-24}/MIC were in the range of 14.14 to 1980.00 and 125.36 to 19862.85, respectively. The time for 99.9% kill ranged from 1.32-3.66 hours and the time required to decrease viable counts below the 10 cfu/ml limit of detection ranged from 4-10.5 hours.

The pharmacodynamics of gatifloxacin against *M.catarrhalis* required the maximum time (3.66 hours) to achieve 99.9% kill and eradication (10.5 hours).

Gatifloxacin was rapidly bactericidal against all six strains of the microorganisms evaluated in this study. Time-kill curves of gatifloxacin against *M.catarrhalis* (Bc.312), *H.influenzae* (Bc.38), *H.influenzae* (Bc.255), *S.pneumoniae* (38), *S.pneumoniae* (94) and *S.pneumoniae* (14) were shown in figure 4-14, 4-16, 4-18, 4-20, 4-22 and 4-24, accordingly.

Table 4-6 Pharmacodynamics of gatifloxacin 400 mg single dose against *M.catarrhalis* (Bc.312), *H.influenzae* (Bc.38), *H.influenzae* (Bc.255), *S.pneumoniae* (38), *S.pneumoniae* (94) and *S.pneumoniae* (14).

Microorganism	MIC	Peak/MIC	AUC_{0-24}/MIC	$T_{99.9\%}$ (hr.)	$T_{eradication}$ (hr.)
M.catarrhalis (Bc.312)	0.03	237.86	2187.66	3.66	10.5
H. influenzae (Bc.38)	0.0035	1980.00	19862.85	1.73	4.00
H. influenzae (Bc.255)	0.015	451.33	4447.33	1.32	4.00
S.pneumoniae (94)	0.25	28.44	252.76	1.48	4.00
S pneumoniae (38)	0.5	14.14	125.36	1.92	6.00
S.pneumoniae (14)	0.25	27.12	259.12	2.12	6.00

Pharmacodynamic data for all microorganisms that were exposed to 400 mg single dose of moxifloxacin were shown in table 4-7. The Peak/MIC of moxifloxacin and AUC_{0-24}/MIC in the range of 12.14 to 4433.33 and 155.36 to 50420.00, respectively. The time for 99.9% kill ranged from 1.29-3.53 hours and the time required to decrease viable counts below the 10 cfu/ml limit of detection ranged from 4-10.5 hours.

The pharmacodynamics of moxifloxacin against *M.catarrhalis* required the maximum time (3.53 hours) to achieve 99.9% kill and eradication (10.5 hours).

Moxifloxacin was rapidly bactericidal against all six strains of the microorganisms evaluated in this study. Time-kill curves of moxifloxacin against *M.catarrhalis* (Bc.312), *H.influenzae* (Bc.38), *H.influenzae* (Bc.255), *S.pneumoniae* (38), *S.pneumoniae* (94) and *S.pneumoniae* (14) were shown in figure 4-26, 4-28, 4-30, 4-32, 4-34 and 4-36, accordingly.

Table 4-7 Pharmacodynamics of moxifloxacin 400 mg single dose against *M.catarrhalis* (Bc.312), *H.influenzae* (Bc.38), *H.influenzae* (Bc.255), *S.pneumoniae* (38), *S.pneumoniae* (94) and *S.pneumoniae* (14).

Microorganism	MIC	Peak/MIC	AUC_{0-24}/MIC	$T_{99.9\%}$ (hr.)	$T_{eradication}$ (hr.)
M.catarrhalis (Bc.312)	0.05	116.20	1607.80	3.53	10.5
H. influenzae (Bc.38)	0.0015	4433.33	50420.00	1.29	4.00
H. influenzae (Bc.255)	0.025	259.20	3230.40	1.31	4.00
S.pneumoniae (94)	0.5	12.32	155.36	1.42	4.00
S pneumoniae (38)	0.5	12.14	158.18	1.78	4.00
S.pneumoniae (14)	0.4	15.10	189.50	1.86	4.00

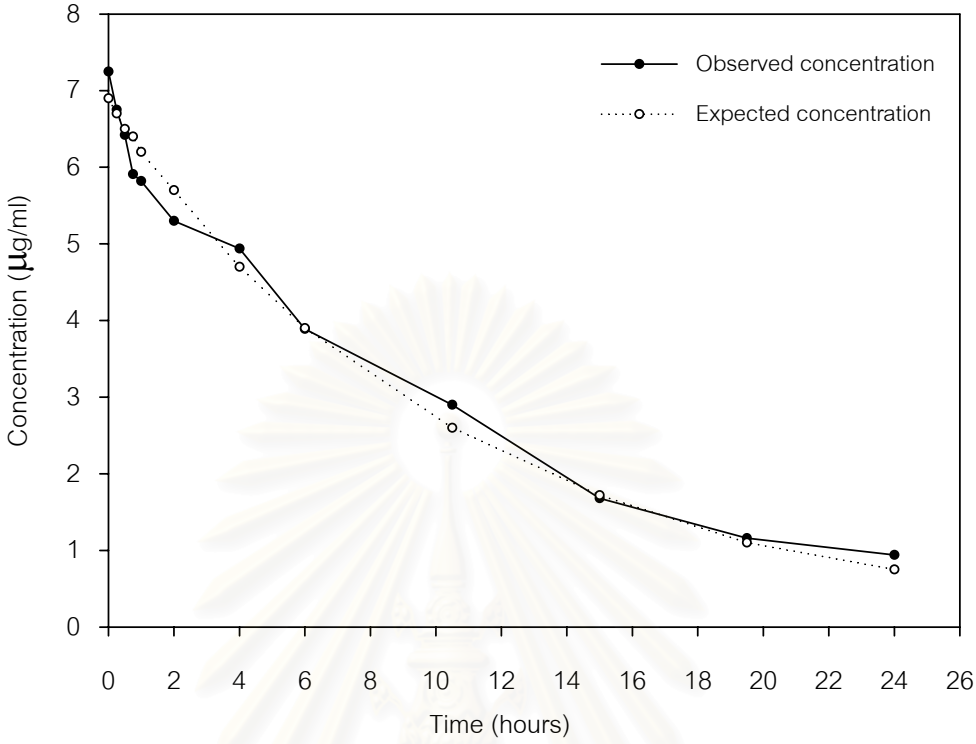


Figure 4-1 Concentration-time curve of levofloxacin 500 mg (single dose) against *M.catarrhalis* (Bc.312).

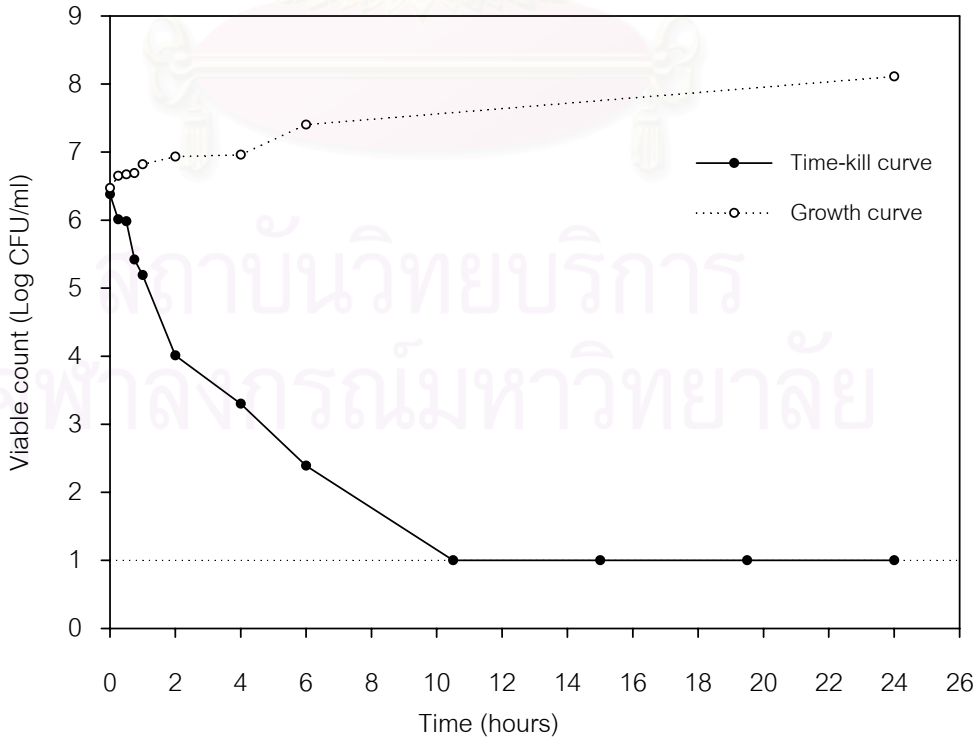


Figure 4-2 Time-kill curve of levofloxacin 500 mg (single dose) against *M.catarrhalis* (Bc.312).

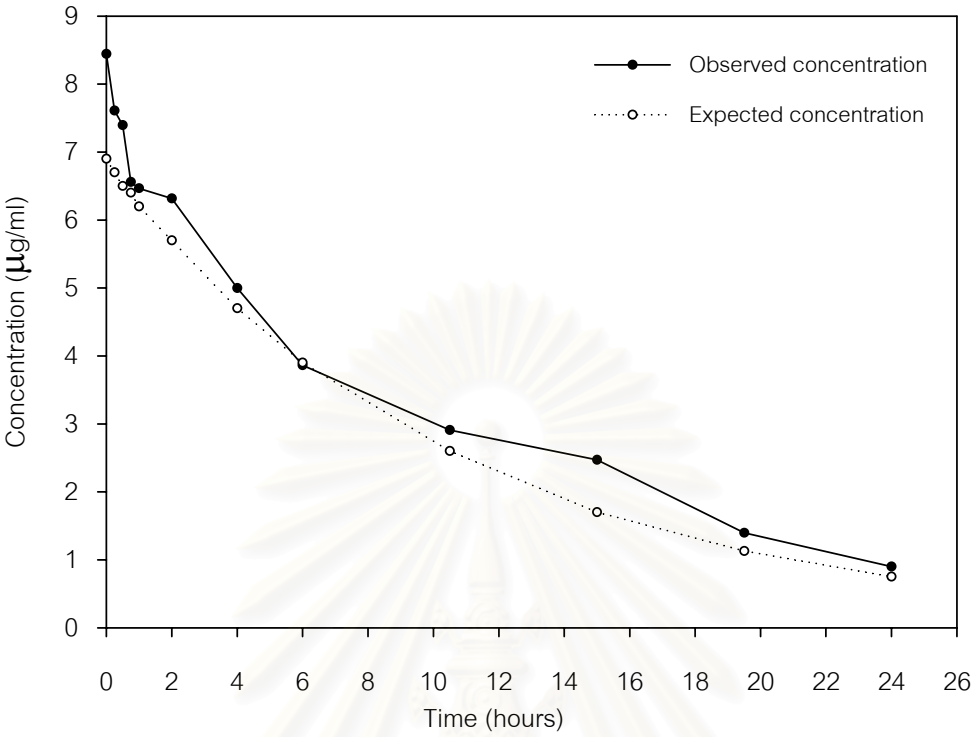


Figure 4-3 Concentration-time curve of levofloxacin (single dose) against *H.influenzae* (Bc.38)

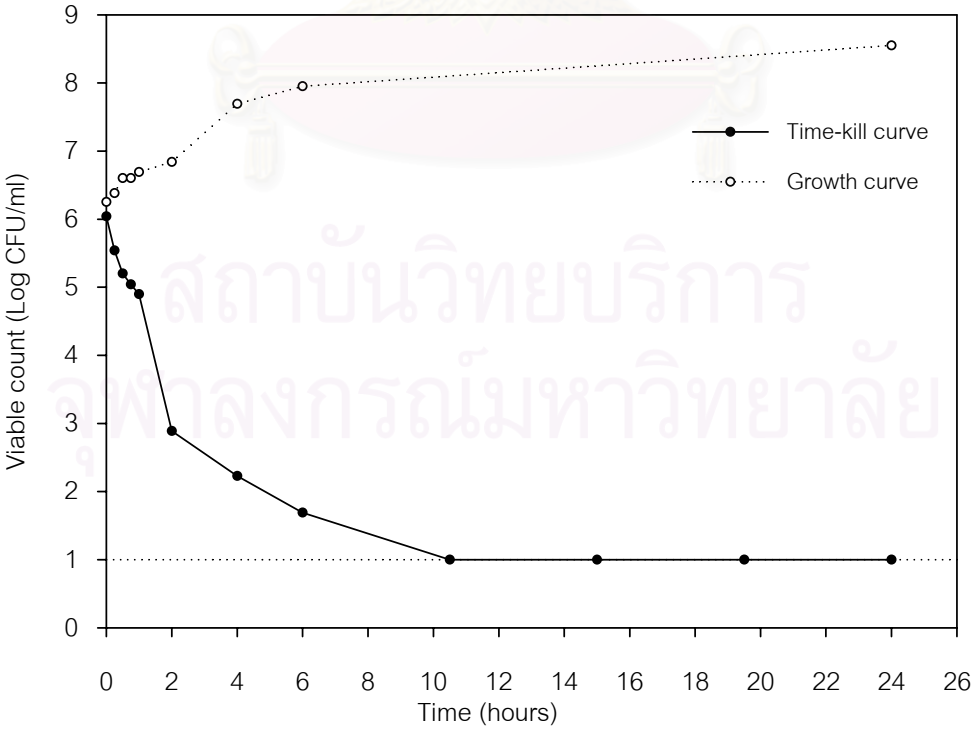


Figure 4-4 Time-kill curve of levofloxacin 500 mg (single dose) against *H.influenzae* (Bc. 38).

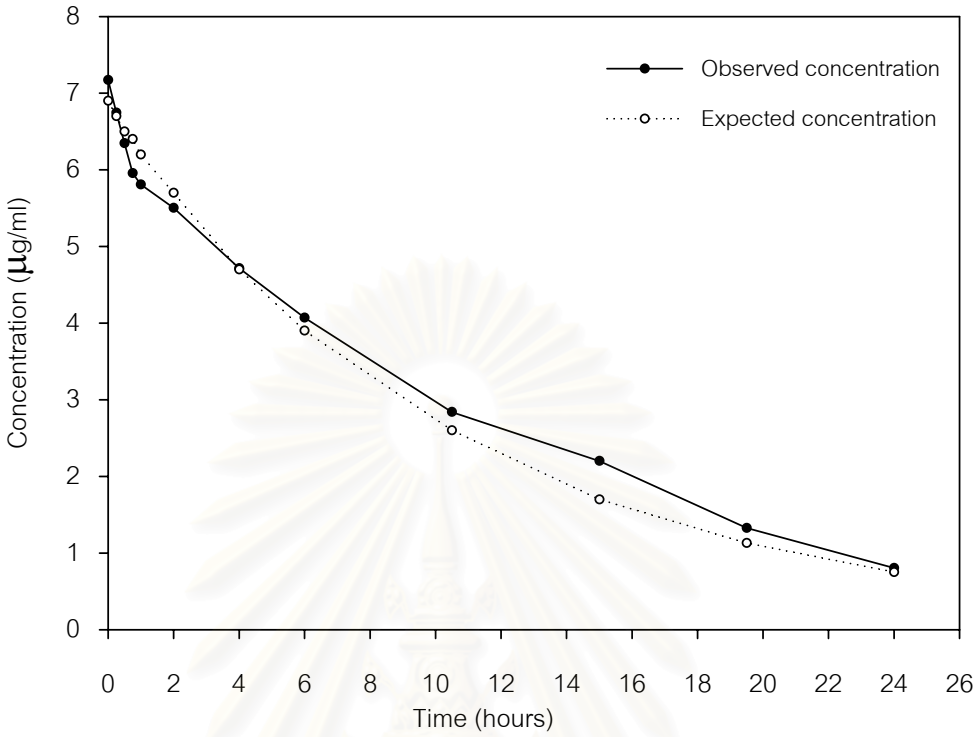


Figure 4-5 Concentration-time curve of levofloxacin 500 mg (single dose) against *H.influenzea* (Bc.255).

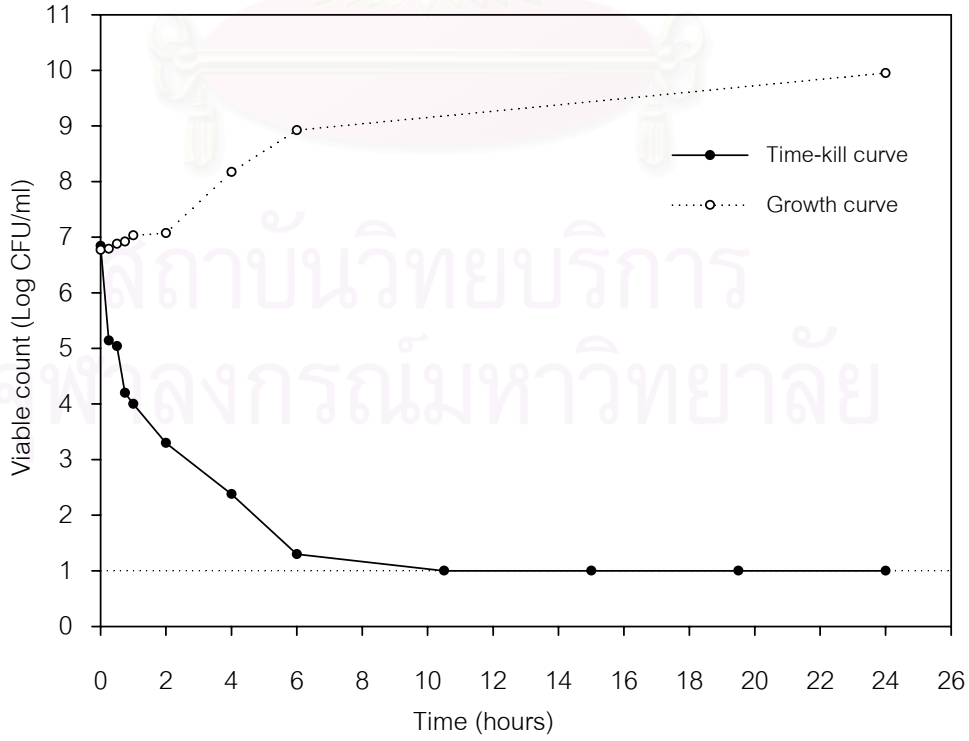


Figure 4-6 Time-kill curve of levofloxacin 500 mg (single dose) against *H.influenzea* (Bc.255).

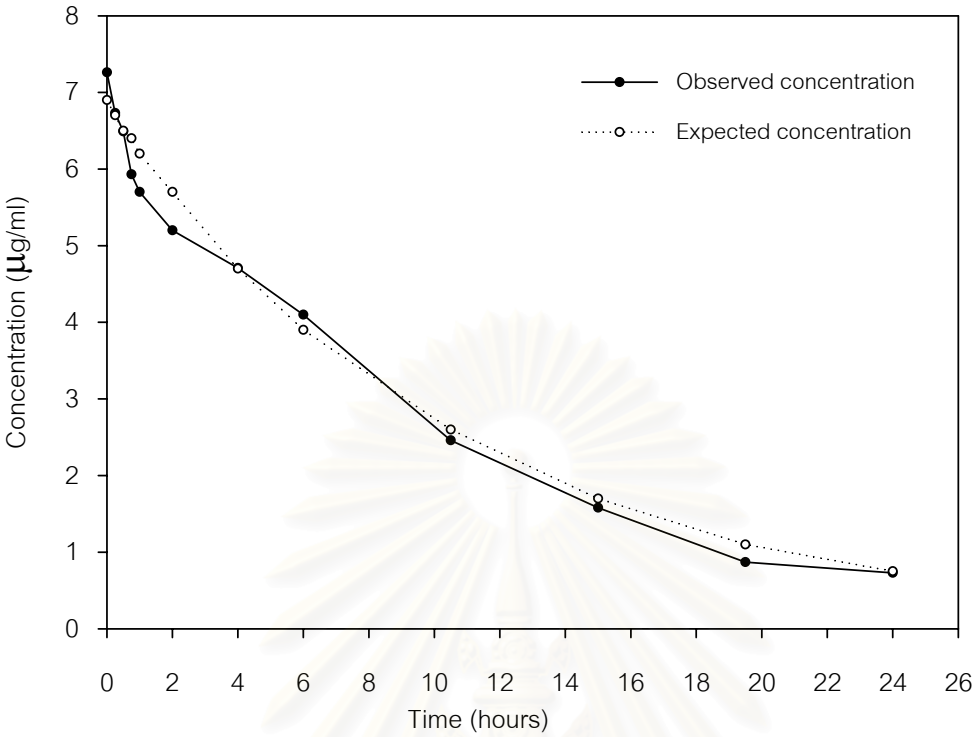


Figure 4-7 Concentration-time curve of levofloxacin 500 mg (single dose) against *S.pneumoniae* (94).

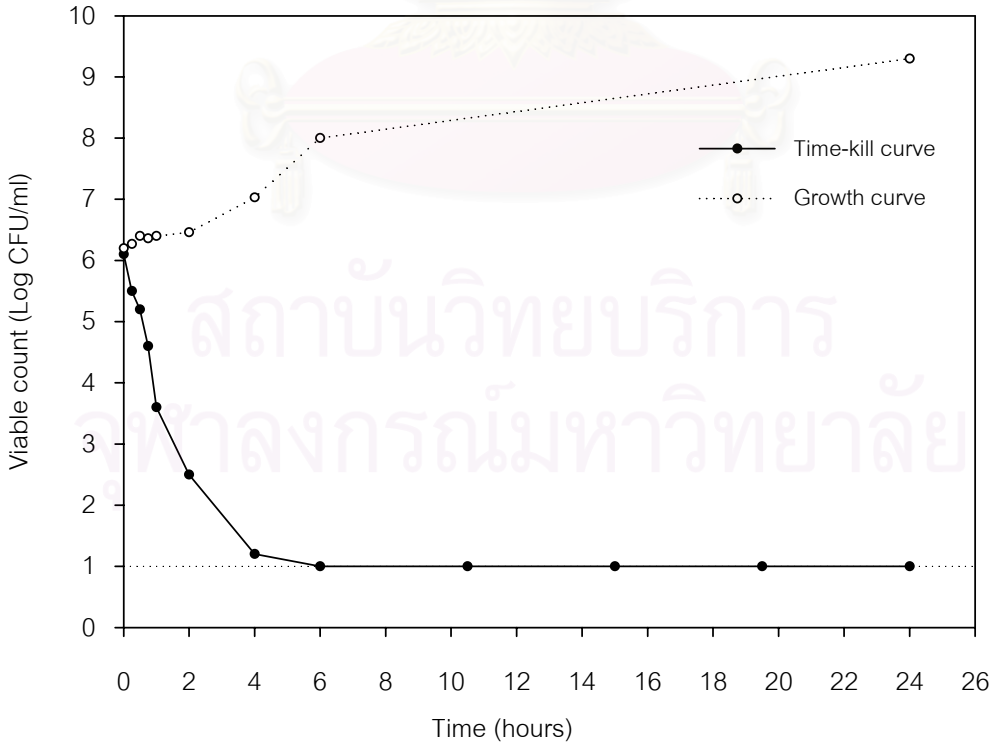


Figure 4-8 Time-kill curve of levofloxacin 500 mg (single dose) against *S.pneumoniae* (94).

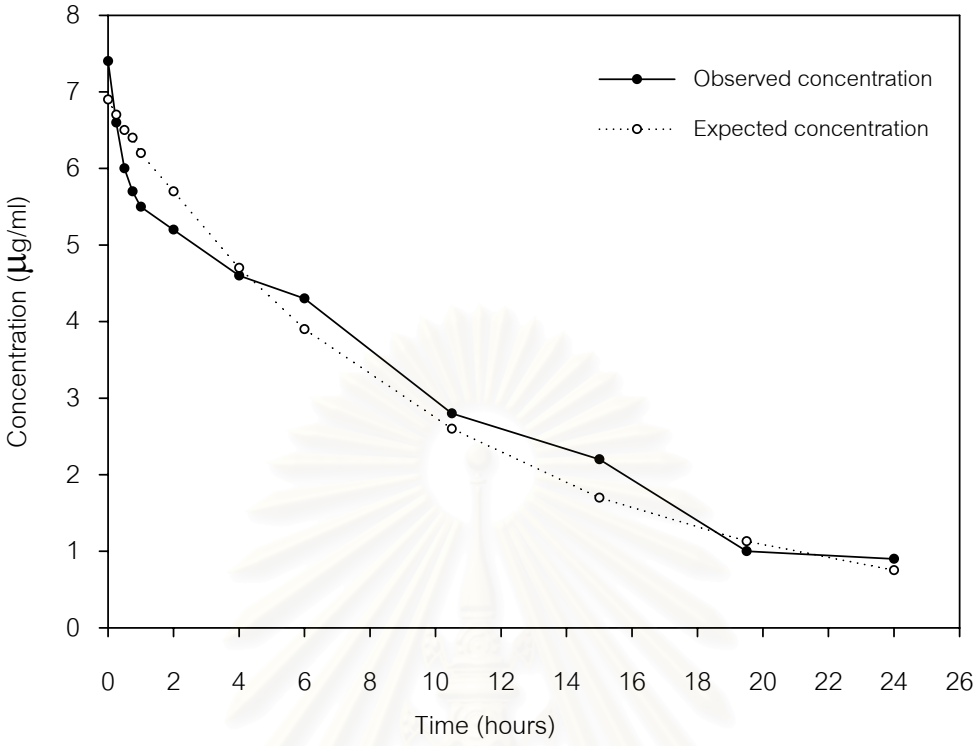


Figure 4-9 Concentration-time curve of levofloxacin 500 mg (single dose) against *S.pneumoniae* (38).

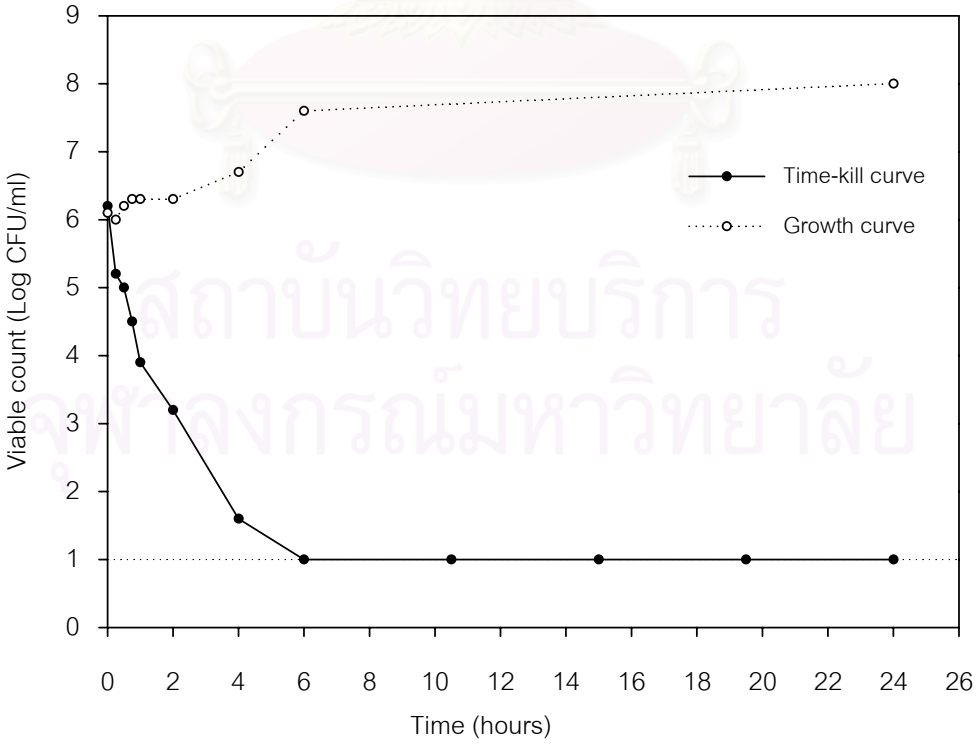


Figure 4-10 Time-kill curve of levofloxacin 500 mg (single dose) against *S.pneumoniae* (38).

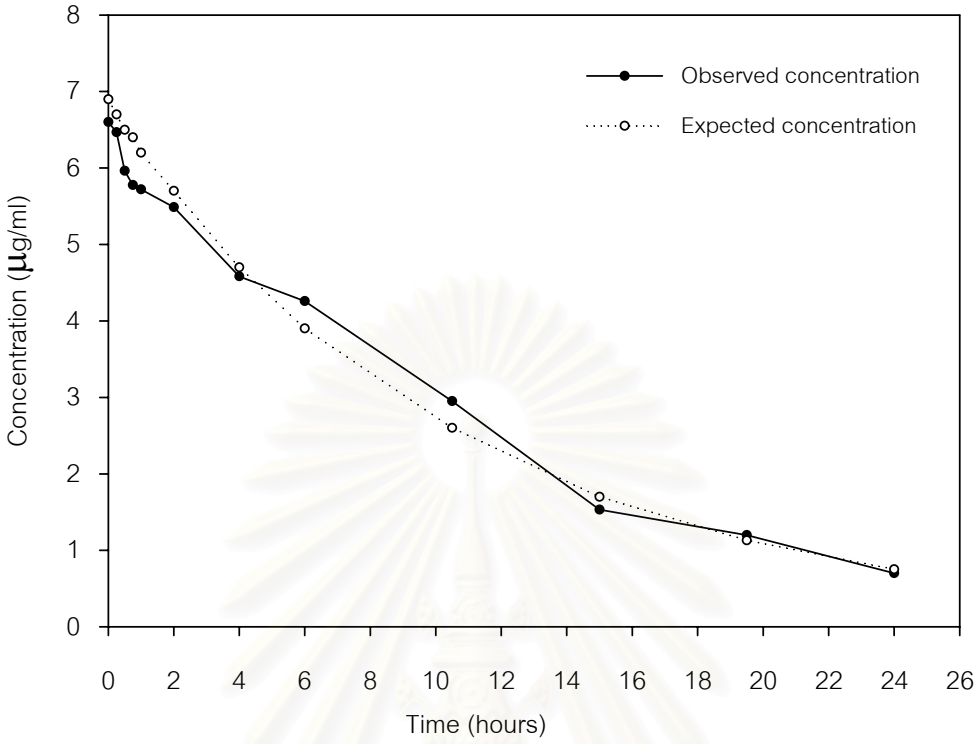


Figure 4-11 Concentration-time curve of levofloxacin 500 mg (single dose) against *S.pneumoniae* (14).

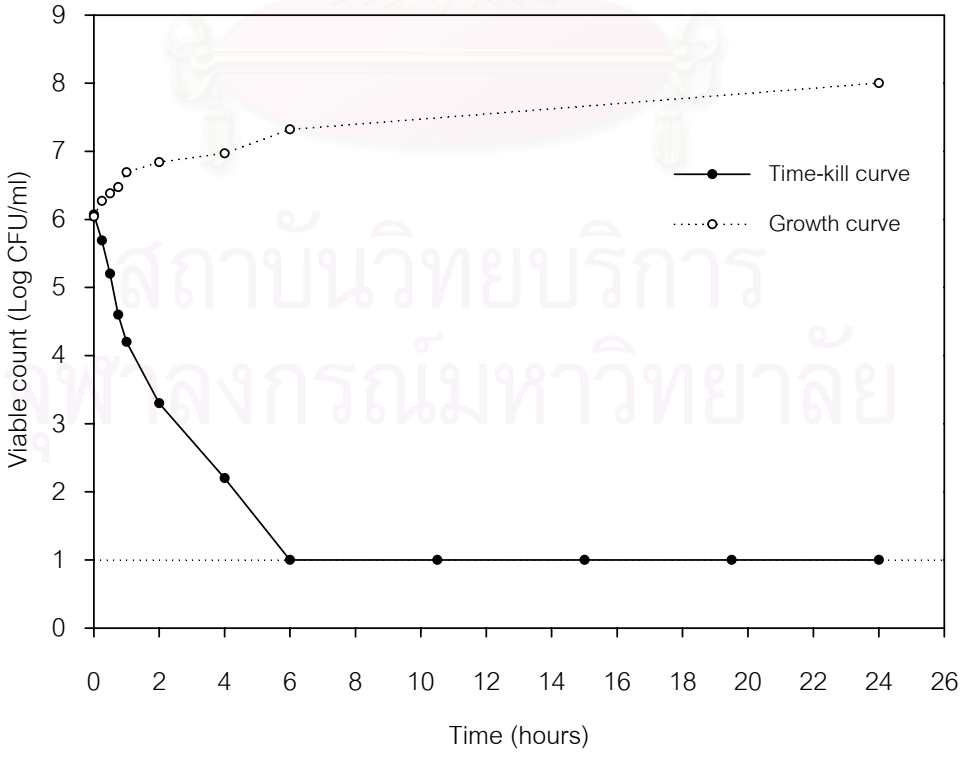


Figure 4-12 Time-kill curve of levofloxacin 500 mg (single dose) against *S.pneumoniae* (14).

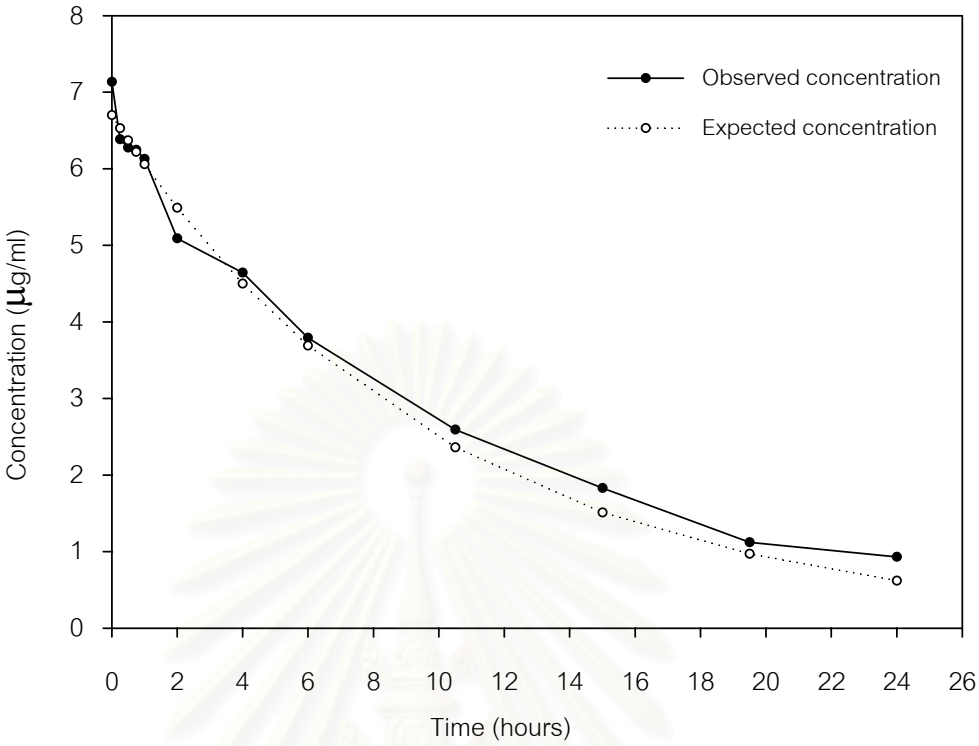


Figure 4-13 Concentration-time curve of gatifloxacin 400 mg (single dose) against *M.catarrhalis* (Bc.312).

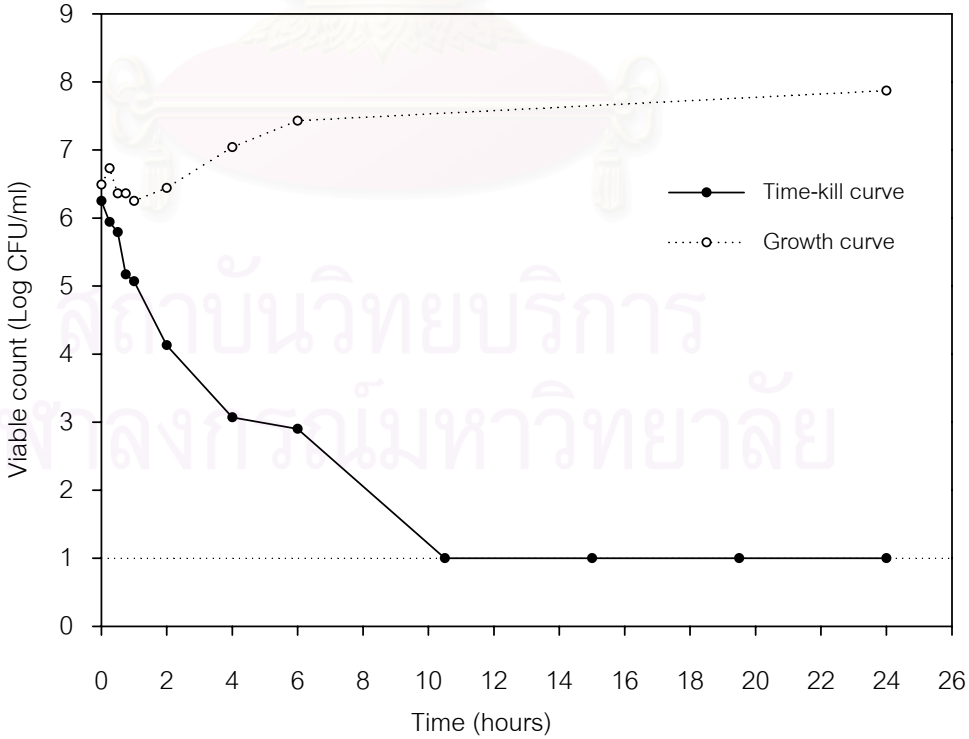


Figure 4-14 Time-kill curve of gatifloxacin 400 mg (single dose) against *M.catarrhalis* (Bc.312).

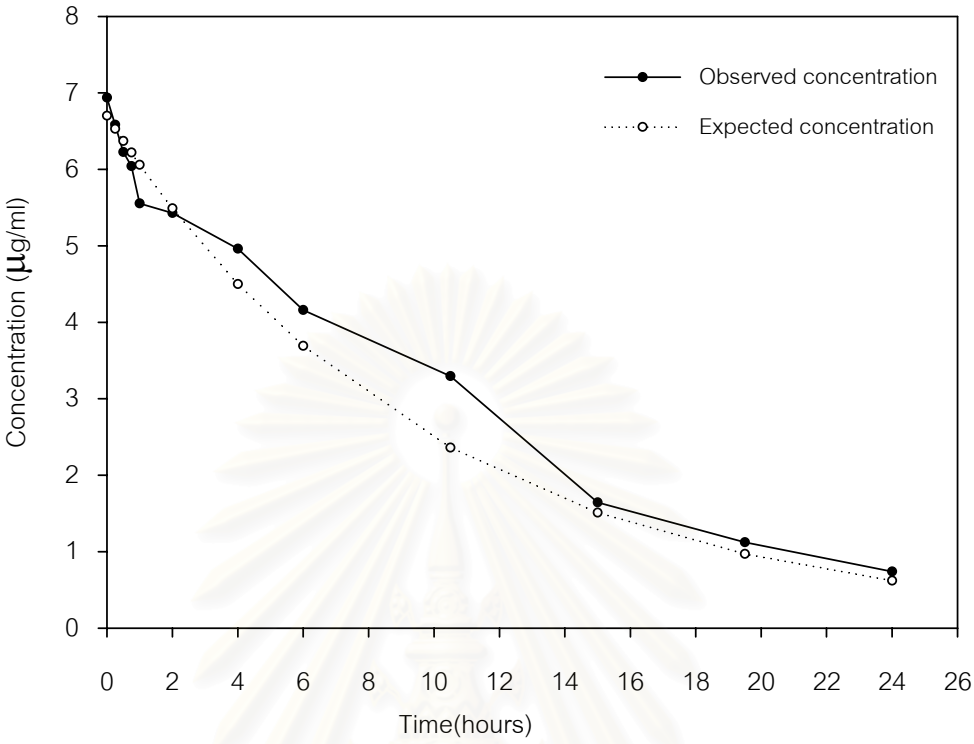


Figure 4-15 Concentration-time curve of gatifloxacin 400 mg (single dose) against *H.influenzae* (Bc.38)

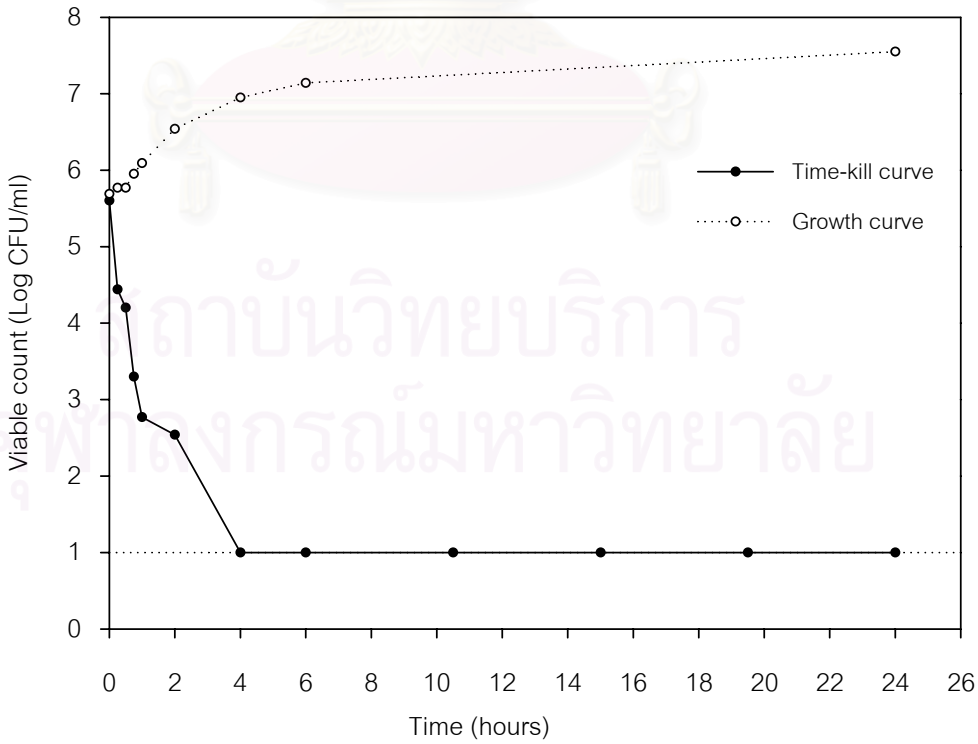


Figure 4-16 Time-kill curve of gatifloxacin 400 mg (single dose) against *H.influenzae* (Bc.38)

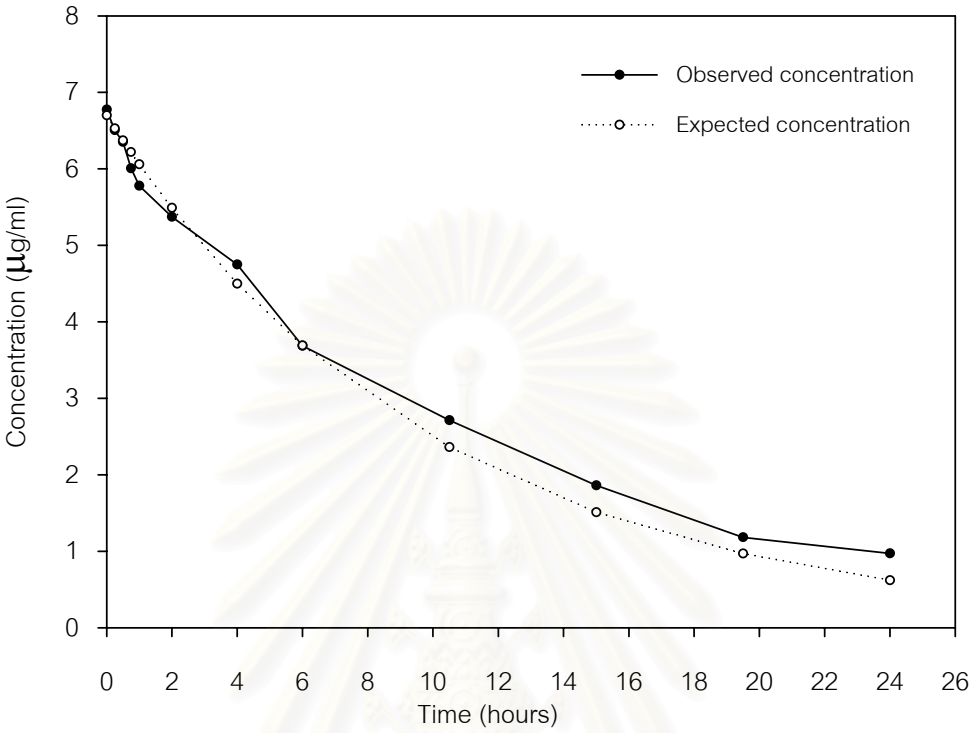


Figure 4-17 Concentration-time curve of gatifloxacin 400 mg (single dose) against *H.influenzae* (Bc.255).

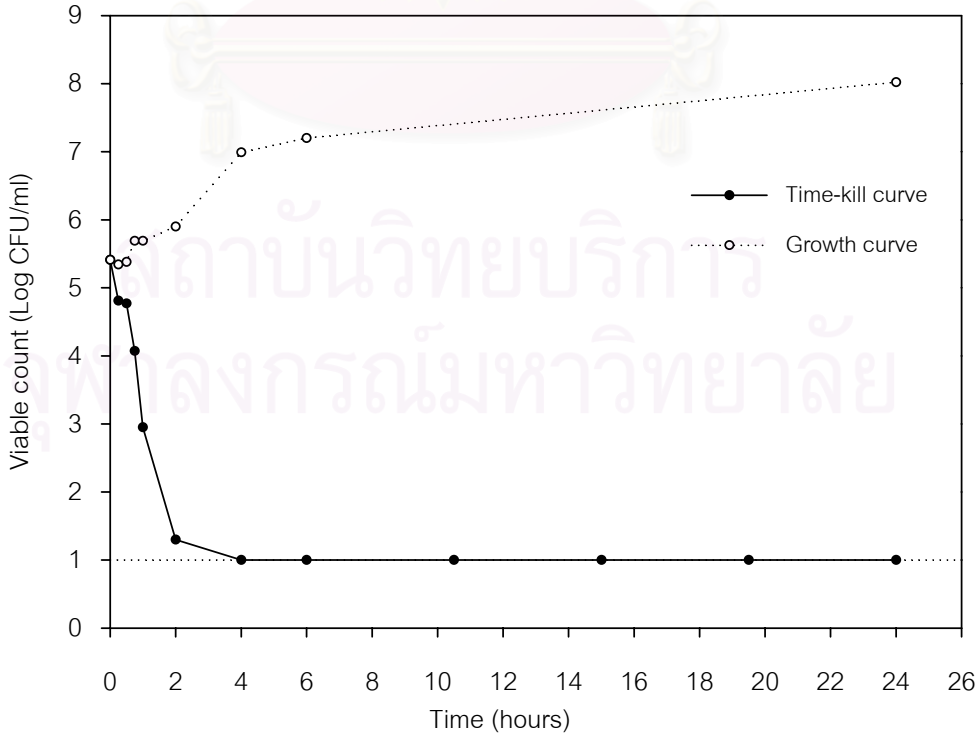


Figure 4-18 Time-kill curve of gatifloxacin 400 mg (single dose) against *H.influenzae* (Bc.255).

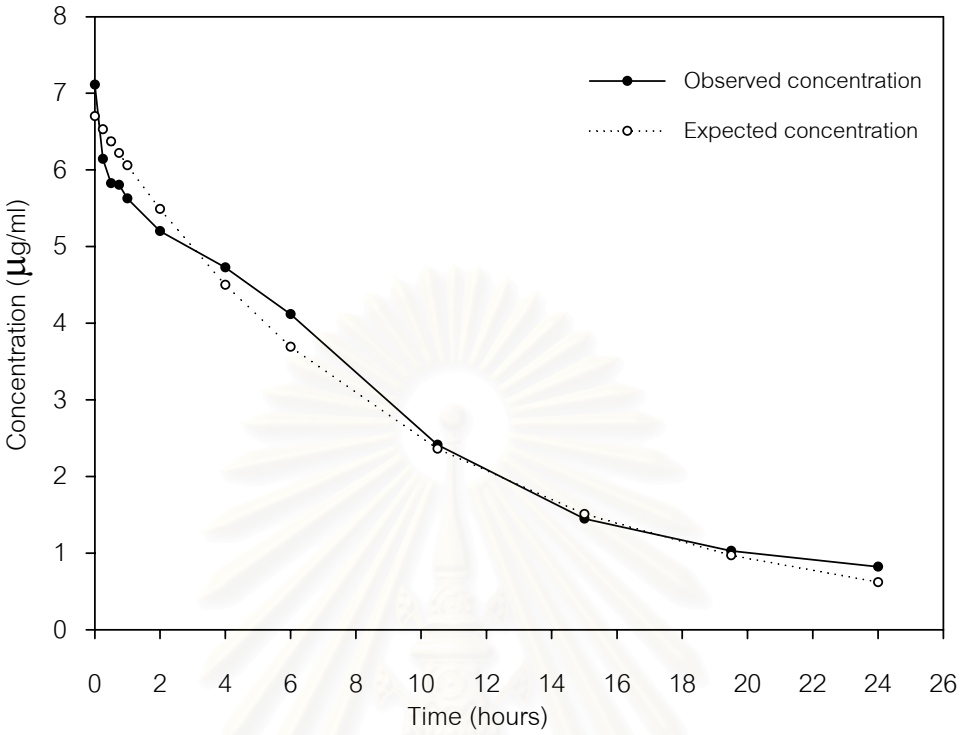


Figure 4-19 Concentration-time curve of gatifloxacin 400 mg (single dose) against *S.pneumoniae* (94).

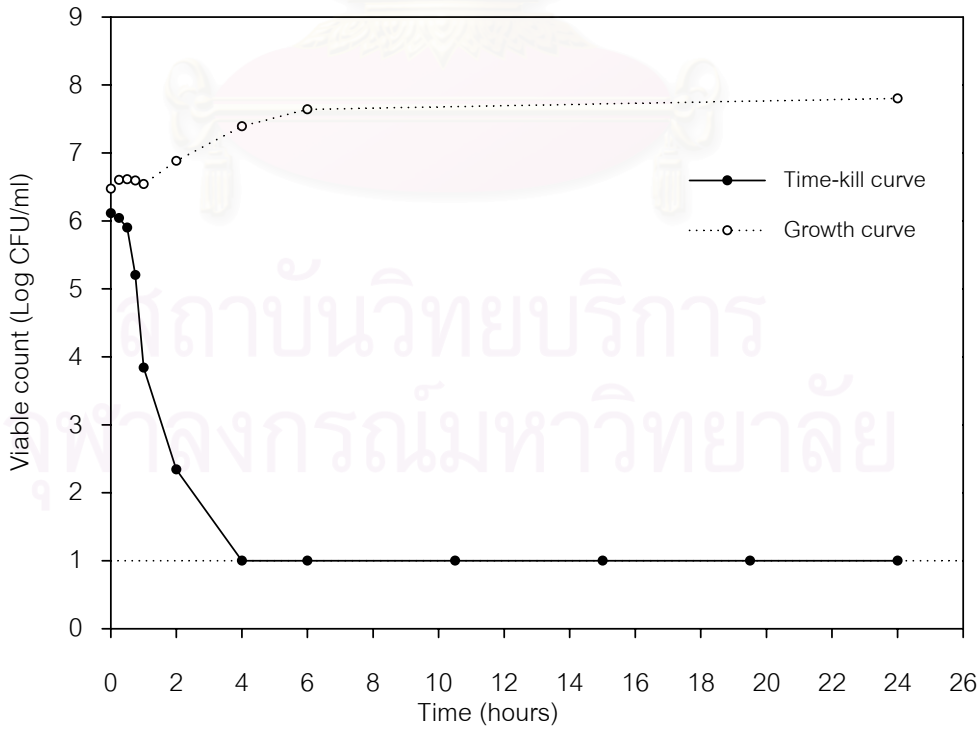


Figure 4-20 Time-kill curve of gatifloxacin 400 mg (single dose) against *S.pneumoniae* (94).

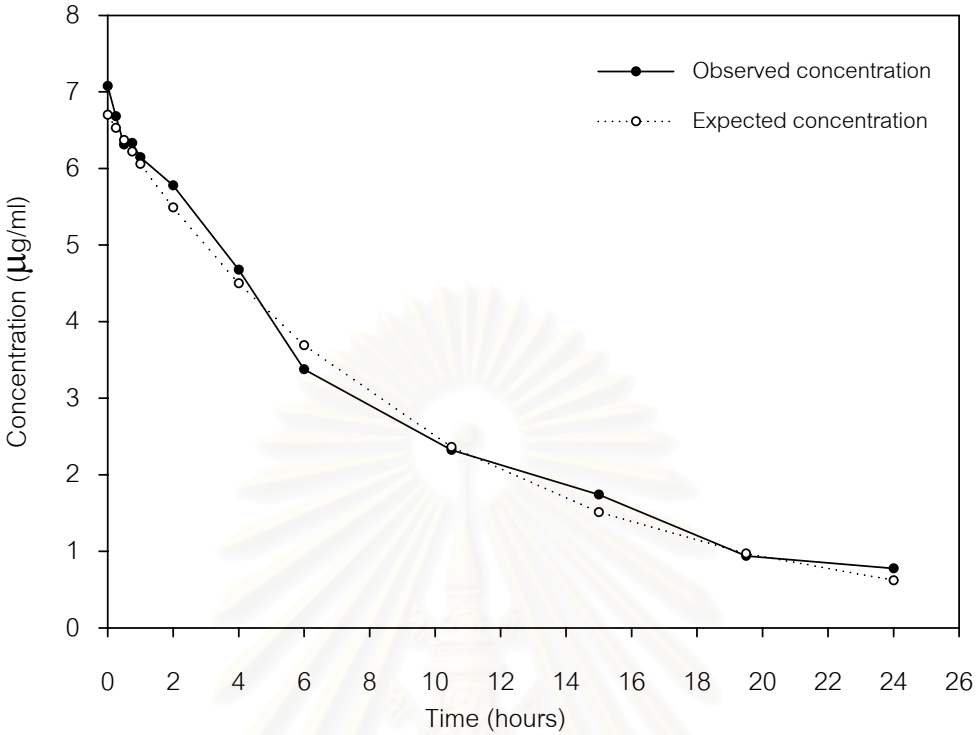


Figure 4-21 Concentration-time curve of gatifloxacin 400 mg (single dose) against *S.pneumoniae* (38).

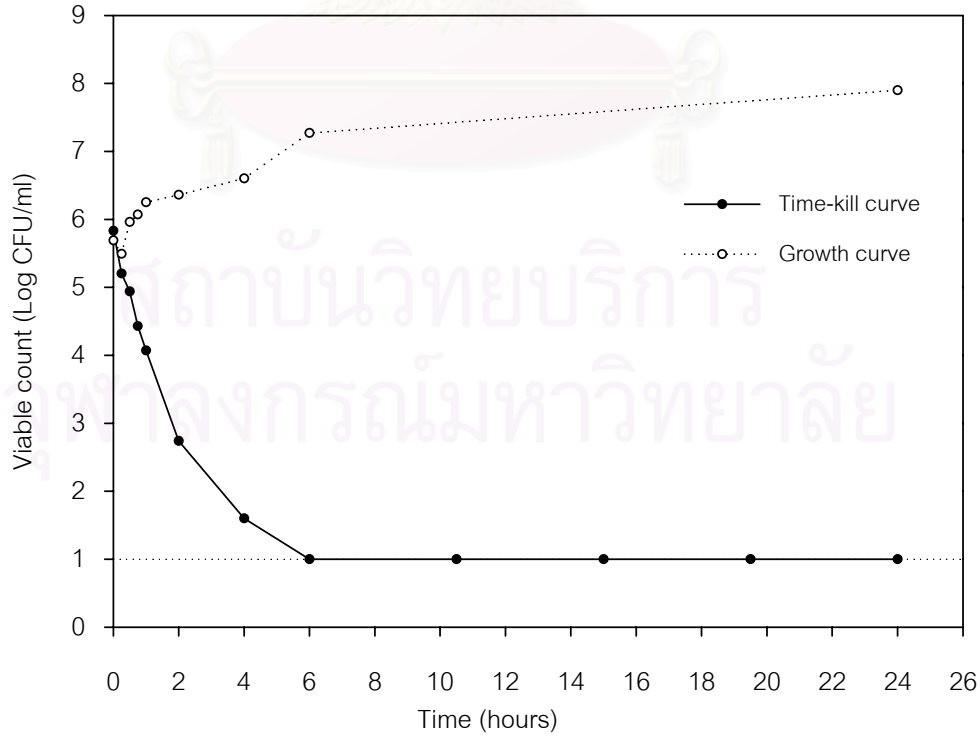


Figure 4-22 Time-kill curve of gatifloxacin 400 mg (single dose) against *S.pneumoniae* (38).

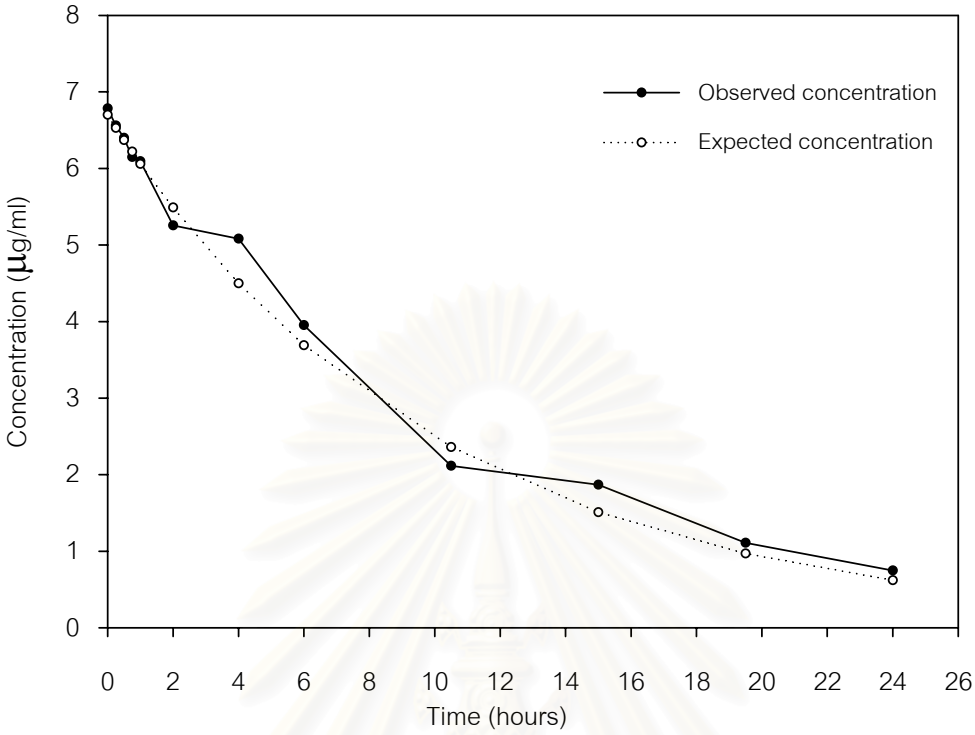


Figure 4-23 Concentration-time curve of gatifloxacin 400 mg (single dose) against *S.pneumoniae* (14).

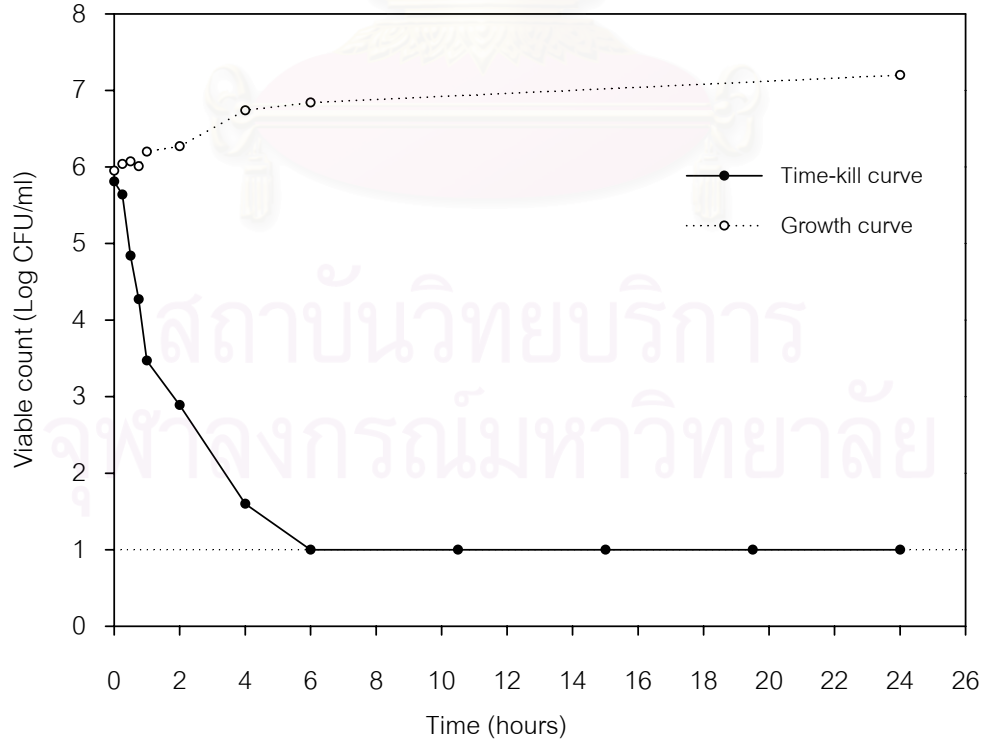


Figure 4-24 Time-kill curve of gatifloxacin 400 mg (single dose) against *S.pneumoniae* (14).

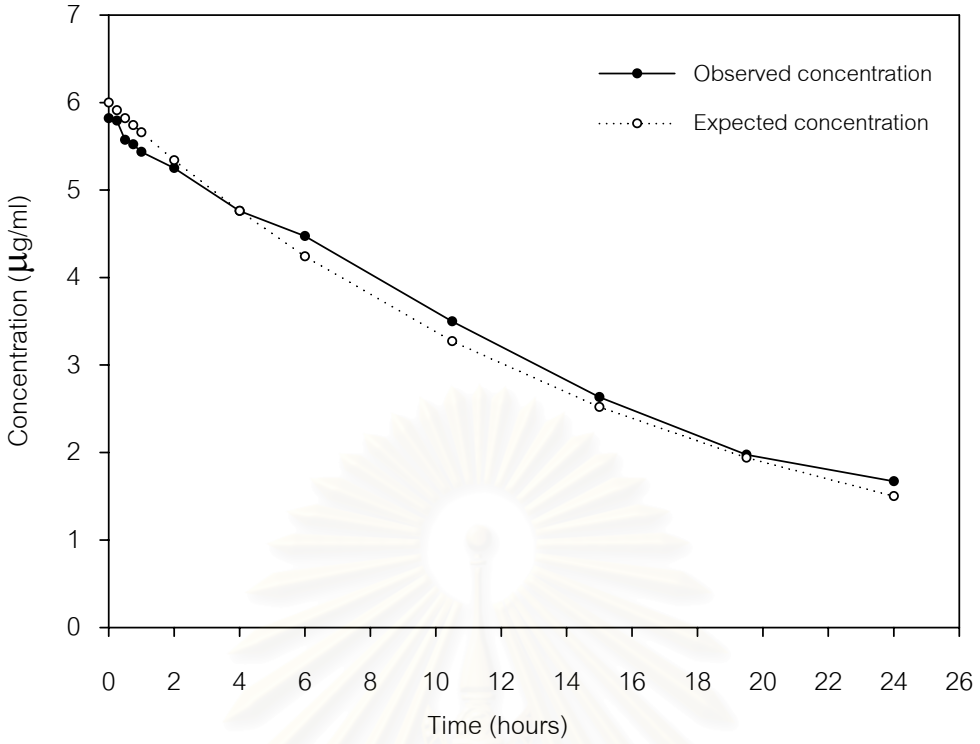


Figure 4-25 Concentration-time curve of moxifloxacin 400 mg (single dose) against *M.catarrhalis* (Bc.312).

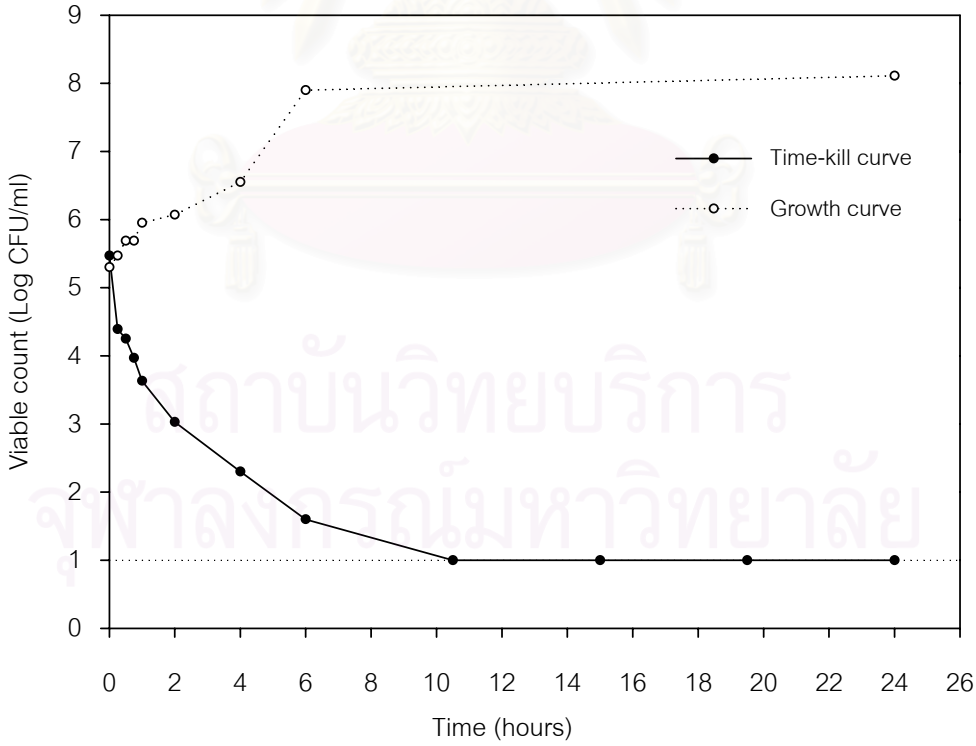


Figure 4-26 Time-kill curve of moxifloxacin 400 mg (single dose) against *M.catarrhalis* (Bc.312).

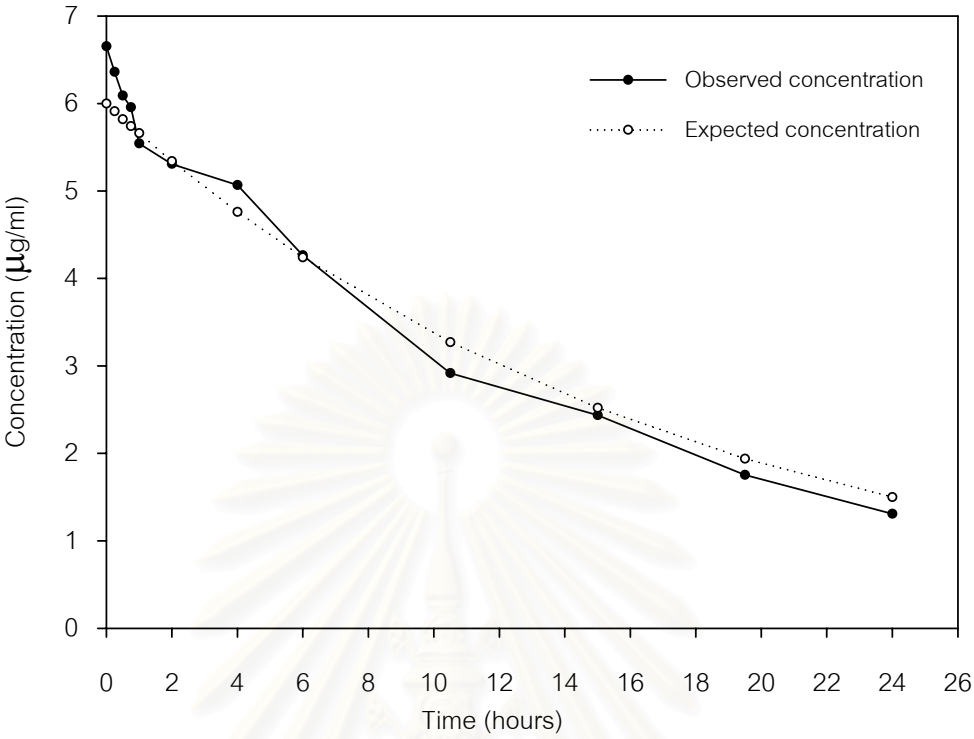


Figure 4-27 Concentration-time curve of moxifloxacin 400 mg (single dose) against *H.influenzae* (Bc.38).

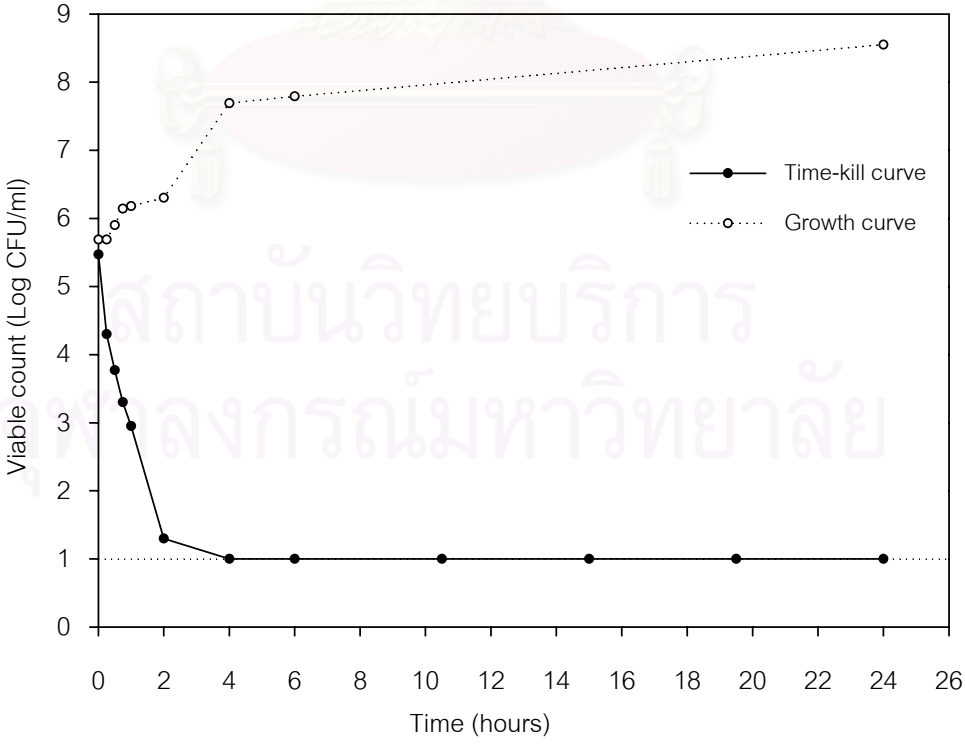


Figure 4-28 Time-kill curve of moxifloxacin 400 mg (single dose) against *H.influenzae* (Bc.38).

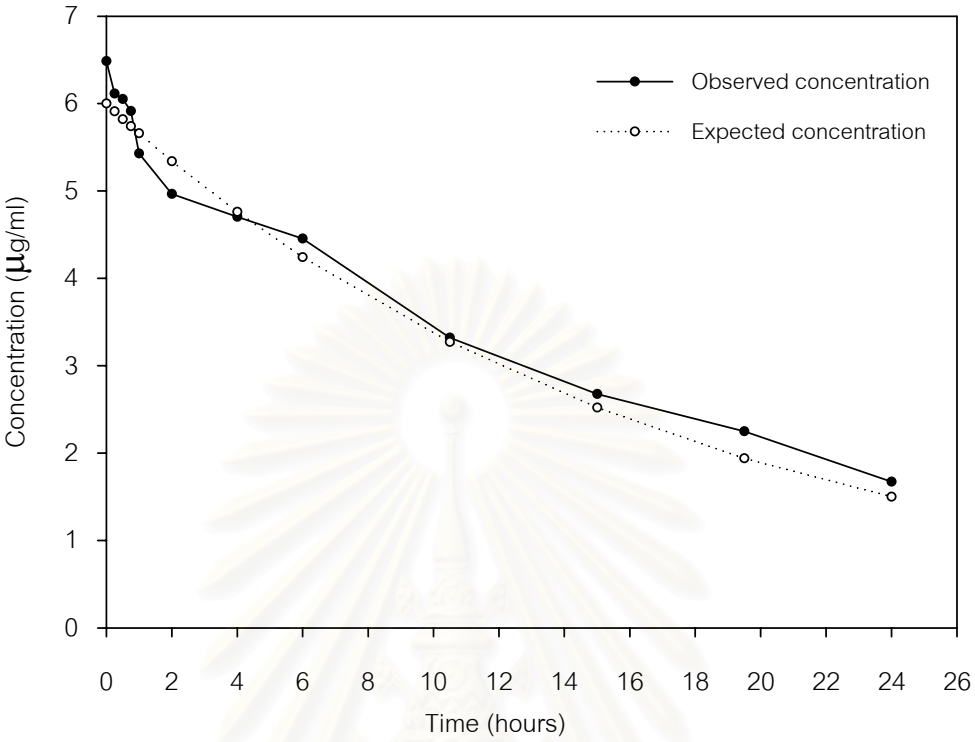


Figure 4-29 Concentration-Time curve of moxifloxacin 400 mg (single dose) against *H.influenzae* (Bc.255).

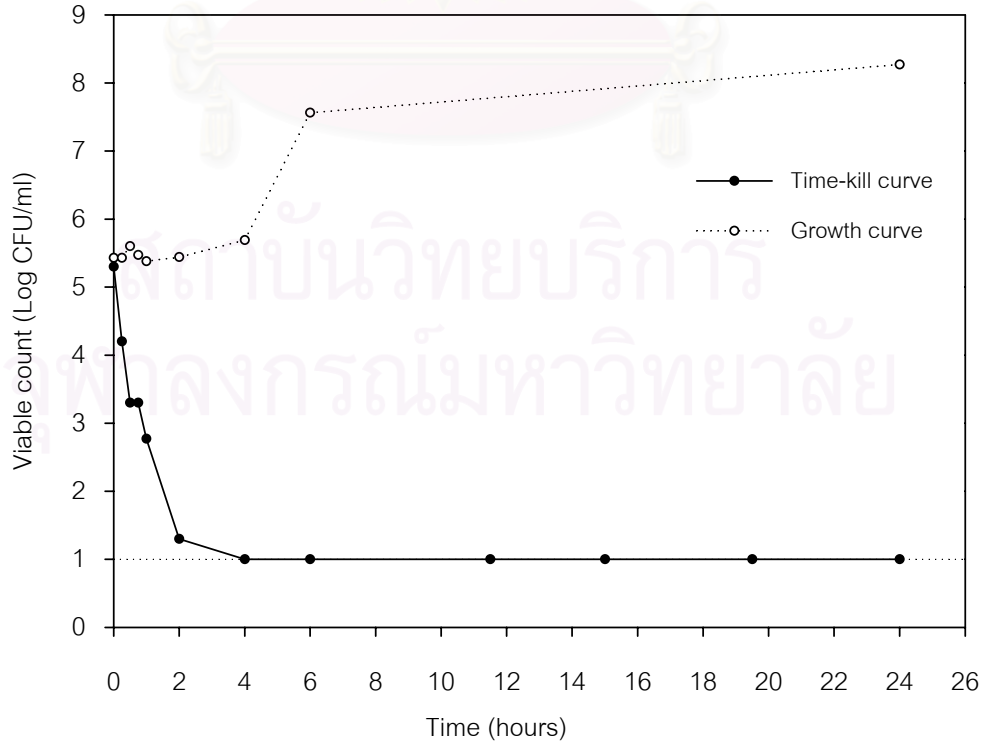


Figure 4-30 Time-kill curve of moxifloxacin 400 mg (single dose) against *H.influenzae* (Bc.255).

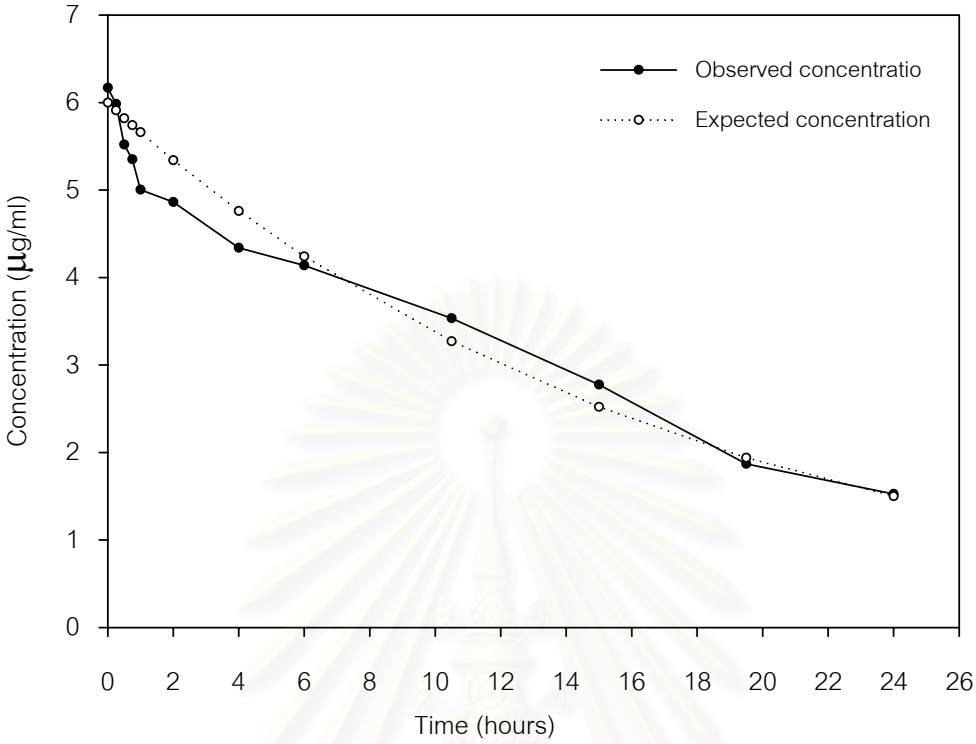


Figure 4-31 Concentration-time curve of moxifloxacin 400 mg (single dose) against *S.pneumoniae* (94).

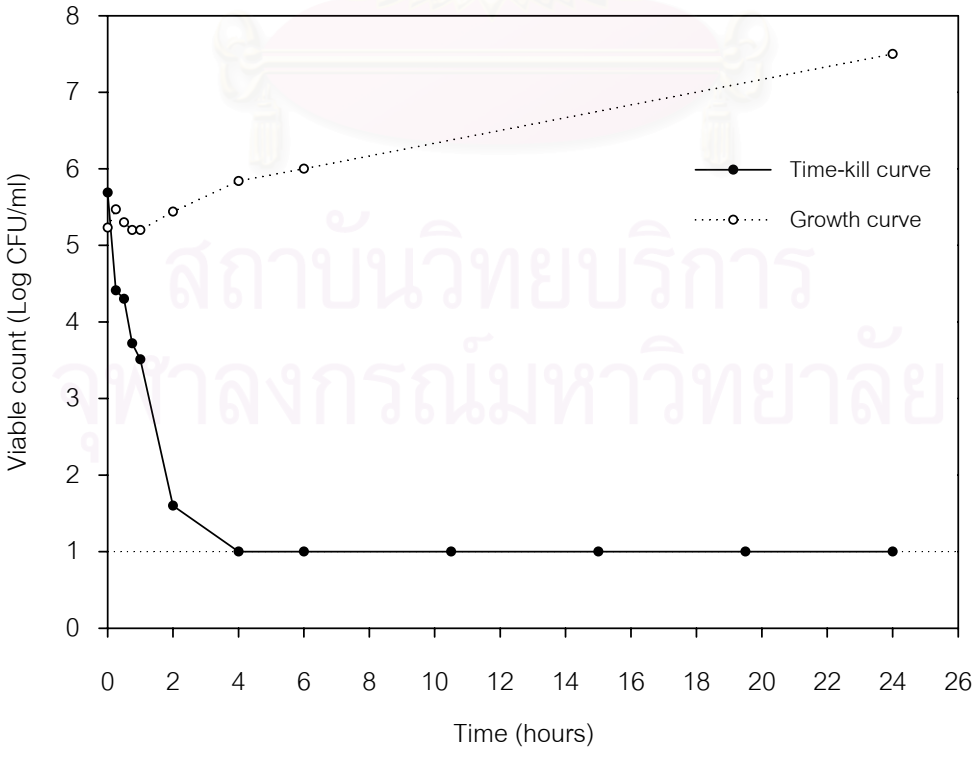


Figure 4-32 Time-kill curve of moxifloxacin 400 mg (single dose) against *S.pneumoniae* (94).

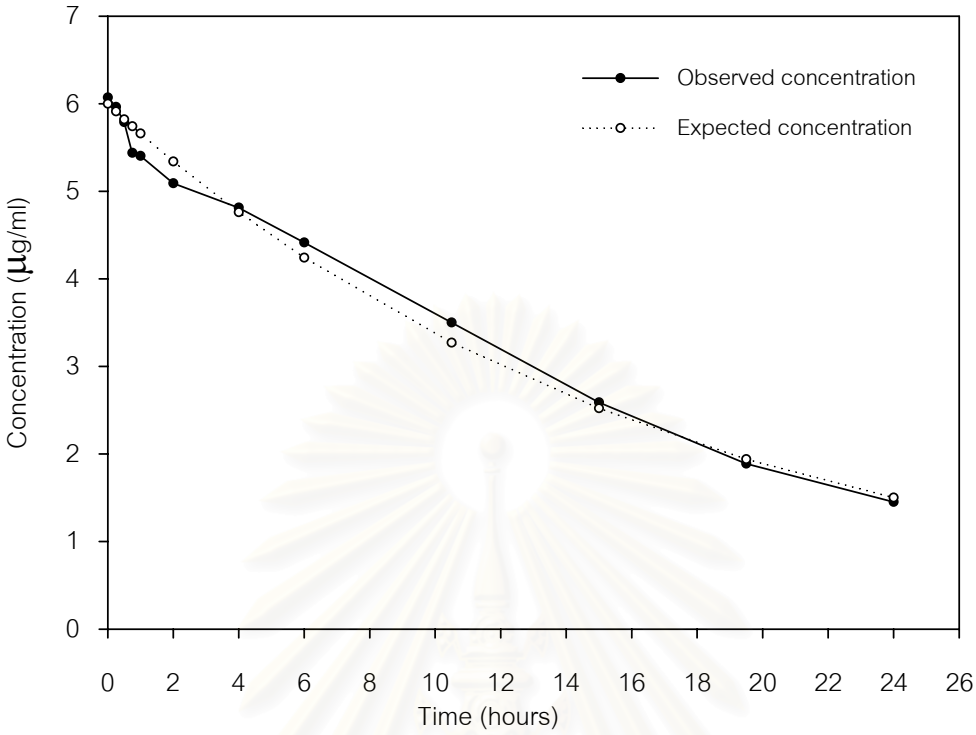


Figure 4-33 Concentration-time curve of moxifloxacin 400 mg (single dose) against *S.pneumoniae* (38).

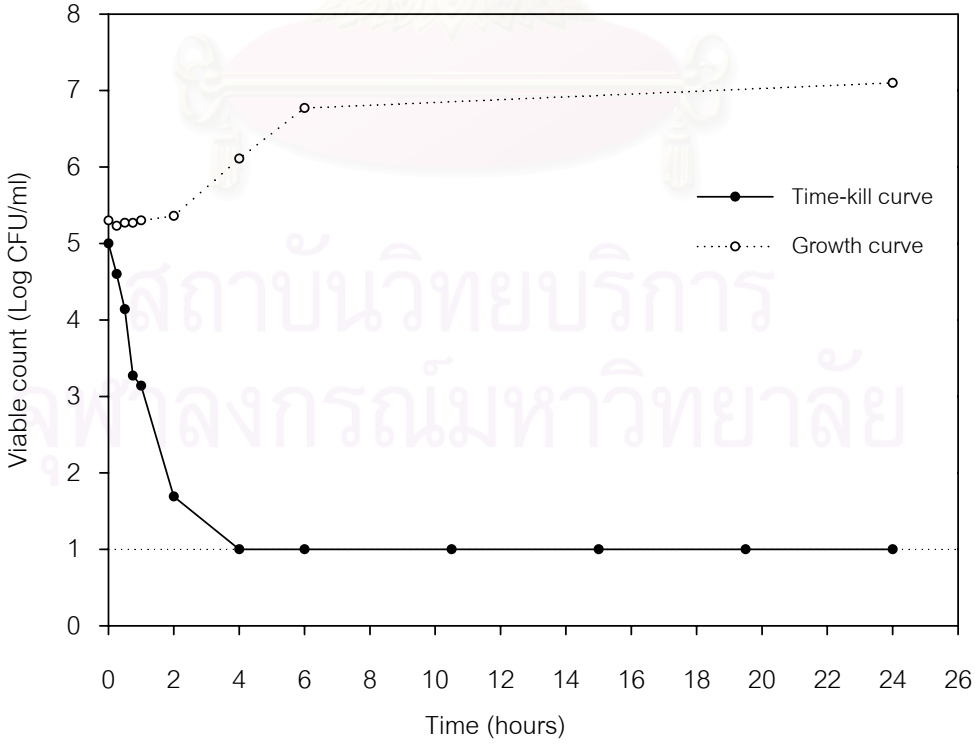


Figure 4-34 Time-kill curve of moxifloxacin 400 mg (single dose) against *S.pneumoniae* (38).

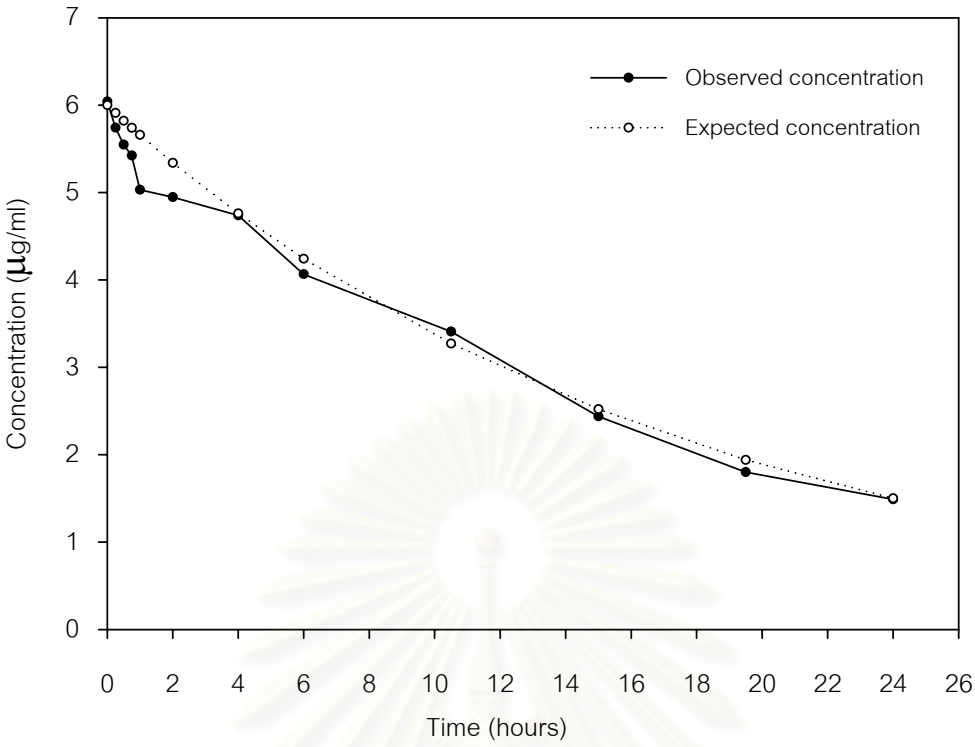


Figure 4-35 Concentration-time curve of moxifloxacin 400 mg (single dose) against *S.pneumoniae* (14).

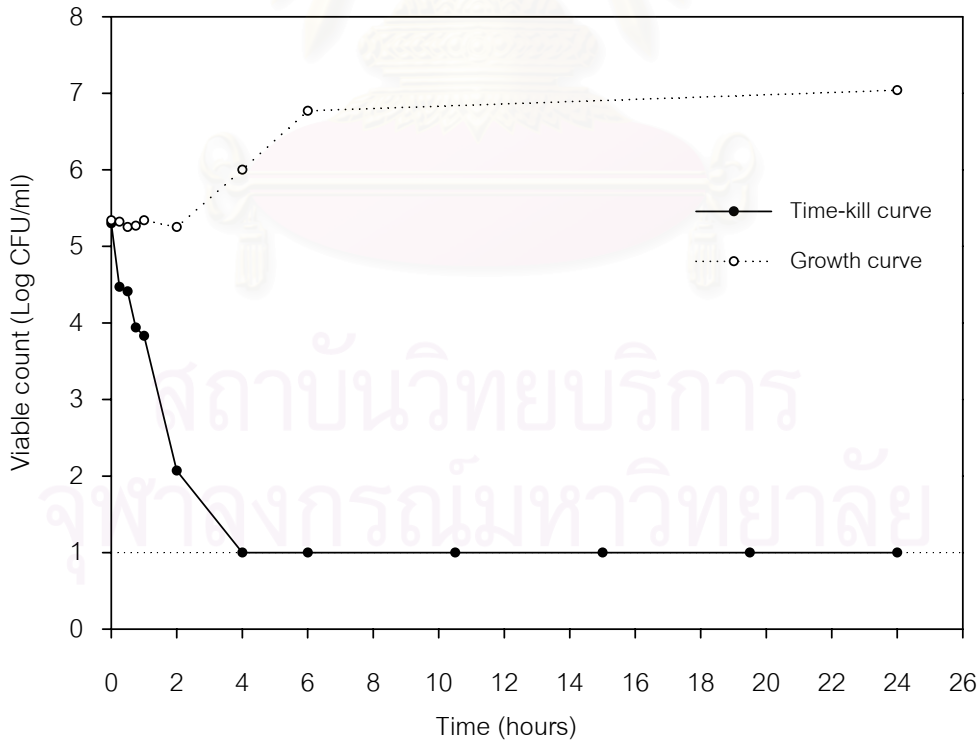


Figure 4-36 Time-kill curve of moxifloxacin 400 mg (single dose) against *S.pneumoniae* (14).

Pharmacokinetic and Bacterial killing curve after multiple doses.

- Pharmacokinetic of levofloxacin, gatifloxacin and moxifloxacin within the IVPM

Pharmacokinetic curve: The measured levofloxacin, gatifloxacin and moxifloxacin concentrations from IVPM in all experiments were agreement with expected concentration that calculated from first order pharmacokinetic equation ($P<0.05$).

Pharmacokinetic profiles of levofloxacin against each strain of bacteria were shown in table 4-8. Peak concentration (mean $\pm$ S.D.) and trough concentration (mean $\pm$ S.D.) in IVPM were 7.31 $\pm$ 0.46 and 0.92 $\pm$ 0.04 μg/ml, respectively. Calculated $T_{1/2}$ (mean $\pm$ S.D.) and AUC_{0-24} (mean $\pm$ S.D.) were 8.03 $\pm$ 0.20 hours and 70.74 $\pm$ 3.37 μg.hr/ml respectively. Concentration-time curve of levofloxacin against each strain of microbial in IVPM were shown in Figure 4-37, 4-39, 4-41, 4-43, 4-45 and 4-47, accordingly.

Table 4-8 Pharmacokinetic parameter of levofloxacin 500 mg multiple doses against *M.catarrhalis* (Bc.312), *H. influenzae* (Bc.38), *H. influenzae* (Bc.255), *S.pneumoniae* (38), *S.pneumoniae* (94) and *S.pneumoniae* (14).

Microorganisms	Peak concentration (μg/ml)	Trough concentration (μg/ml)	$T_{1/2}$ (hours)	AUC_{0-24} (μg.hr/ml)
M.catarrhalis Bc.312	6.95	0.90	8.13	65.11
H.influenzae Bc.38	8.08	0.95	7.76	75.18
H.influenzae Bc.255	7.55	0.97	8.10	72.99
S.pneumoniae 94	6.89	0.92	8.26	69.97
S.pneumoniae 38	7.40	0.96	8.14	70.54
S.pneumoniae 14	6.99	0.84	7.80	70.70
Mean$\pm$S.D.	7.31 $\pm$ 0.46	0.92 $\pm$ 0.04	8.03 $\pm$ 0.20	70.74 $\pm$ 3.37

Pharmacokinetic profiles of gatifloxacin against each strain of bacteria were shown in table 4-9. Peak concentration (mean ± S.D.) and trough concentration (mean ± S.D.) in IVPM were 8.39 ± 0.31 and 1.11 ± 0.09 μg/ml respectively. Calculated $T_{1/2}$ (mean ± S.D.) and AUC_{0-24} (mean ± S.D.) were 8.24 ± 0.36 hours and 72.07±2.47 μg.hr/ml respectively. Concentration-time curve of gatifloxacin against each strain of microbial in IVPM were shown in Figure 4-49, 4-51, 4-53, 4-55, 4-57 and 4-59, accordingly.

Table 4-9 Pharmacokinetic parameter of gatifloxacin 400 mg multiple doses against *M.catarrhalis* (Bc.312), *H. influenzae* (Bc.38), *H. influenzae* (Bc.255), *S.pneumoniae* (38), *S.pneumoniae* (94) and *S.pneumoniae* (14).

Microorganisms	Peak concentration (μg/ml)	Trough concentration (μg/ml)	$T_{1/2}$ (hours)	AUC_{0-24} (μg.hr/ml)
M.catarrhalis Bc.312	8.77	1.17	8.25	77.51
H.influenzae Bc.38	8.54	1.09	8.07	77.83
H.influenzae Bc.255	8.67	1.04	7.84	79.47
S.pneumoniae 94	8.24	1.27	8.89	82.70
S.pneumoniae 38	8.12	1.02	8.01	75.33
S.pneumoniae 14	8.00	1.10	8.38	79.49
Mean±S.D.	8.39 ± 0.31	1.11 ± 0.09	8.24 ± 0.36	72.07±2.47

Pharmacokinetic profiles of moxifloxacin against each strain of bacteria were shown in table 4-10. Peak concentration (mean ± S.D.) and trough concentration (mean ± S.D.) in IVPM were 6.67 ± 0.21 and 1.66 ± 0.11 μg/ml respectively. Calculated $T_{1/2}$ (mean ± S.D.) and AUC_{0-24} (mean ± S.D.) were 12.01 ± 0.69 hours and 83.77 ± 1.67 μg.hr/ml respectively. Concentration-time curve of moxifloxacin against each strain of microbial in IVPM show in Figure 4-61, 4-63, 4-65, 4-67, 4-69 and 4-71, accordingly.

Table 4-10 Pharmacokinetic parameter of moxifloxacin400 mg multiple doses against *M.catarrhalis* (Bc.312), *H. influenzae* (Bc.38), *H. influenzae* (Bc.255), *S.pneumoniae* (38), *S.pneumoniae* (94) and *S.pneumoniae* (14).

Microorganisms	Peak concentration (μg/ml)	Trough concentration (μg/ml)	$T_{1/2}$ (hours)	AUC_{0-24} (μg.hr/ml)
M.catarrhalis Bc.312	6.86	1.55	11.18	82.69
H.influenzae Bc.38	6.83	1.83	12.62	86.52
H.influenzae Bc.255	6.65	1.75	12.45	82.63
S.pneumoniae 94	6.84	1.55	11.20	84.80
S.pneumoniae 38	6.55	1.62	11.90	82.07
S.pneumoniae 14	6.31	1.71	12.73	83.96
Mean±SD	6.67 ± 0.21	1.66 ± 0.11	12.01 ± 0.69	83.77 ± 1.67

- Pharmacodynamic of levofloxacin, gatifloxacin and moxifloxacin multiple doses against each microbial within the IVPM.

Time-kill curve: The viable count of all microorganisms which sampling from screw cap tube that are growth control in all experiment were agreement with viable count of all microorganisms which sampling from IVPM ($P<0.05$).

Pharmacodynamic data for all microorganisms that were exposed to 500 mg multiple doses of levofloxacin were shown in table 4-11. The Peak/MIC of levofloxacin and AUC_{0-24}/MIC were in the range of 6.99 to 967.94 and 70.70 to 9357.69, respectively. The time for 99.9% kill ranged from 1.23-3.68 hours, and the time required to decrease viable counts below the 10 cfu/ml limit of detection ranged from 6-10.5 hours.

The pharmacodynamics of levofloxacin against *M.catarrhalis* required the maximum time (3.68 hours) to achieve 99.9% kill and eradication (10.5 hours).

Levofloxacin was rapidly bactericidal against all six strains of the microorganisms evaluated in this study. Time-kill curves of levofloxacin against *M.catarrhalis* (Bc.312), *H.influenzae* (Bc.38), *H.influenzae* (Bc.255), *S.pneumoniae* (38), *S.pneumoniae* (94) and *S.pneumoniae* (14) show in figure 4-2, 4-4, 4-6, 4-8 and 4-12. Time-kill curve of levofloxacin against *M.catarrhalis* (Bc.312), *H.influenzae* (Bc.38), *H.influenzae* (Bc.255), *S.pneumoniae* (38), *S.pneumoniae* (94) and *S.pneumoniae* (14) were shown in figure 4-38, 4-40, 4-42, 4-44, 4-46 and 4-48, accordingly.

Table 4-11 Pharmacodynamics of levofloxacin 500 mg multiple doses against *M.catarrhalis* (Bc.312), *H.influenzae* (Bc.38), *H.influenzae* (Bc.255), *S.pneumoniae* (38), *S.pneumoniae* (94) and *S.pneumoniae* (14).

Microorganism	MIC	Peak/MIC	AUC_{0-24}/MIC	$T_{99.9\%}$ (hr.)	$T_{eradication}$ (hr.)
M.catarrhalis (Bc.312)	0.015	463.33	4340.66	3.68	10.5
H. influenzae (Bc.38)	0.015	538.66	5012.00	1.96	10.5
H. influenzae (Bc.255)	0.0078	967.94	9357.69	1.23	10.5
S.pneumoniae (94)	0.05	137.80	1399.40	1.33	6.00
S pneumoniae (38)	0.1	74.00	705.40	2.01	6.00
S.pneumoniae (14)	1.0	6.99	70.70	2.09	6.00

Pharmacodynamic data for all microorganisms that were exposed to 400 mg multiple doses of gatifloxacin were shown in table 4-12. The Peak/MIC of gatifloxacin and AUC_{0-24}/MIC were in the range of 16.24 to 2440.00 and 150.66 to 22237.14, respectively. The time for 99.9% kill ranged from 1.20-3.42 hours and the time required to decrease viable counts below the 10 cfu/ml limit of detection ranged from 4-10.5 hours.

The pharmacodynamics of gatifloxacin against *M.catarrhalis* required the maximum time (3.42 hours) to achieve 99.9% kill and eradication (10.5 hours).

Gatifloxacin was rapidly bactericidal against all six strains of the microorganisms evaluated in this study. Time-kill curves of gatifloxacin against *M.catarrhalis* (Bc.312), *H.influenzae* (Bc.38), *H.influenzae* (Bc.255), *S.pneumoniae* (38), *S.pneumoniae* (94) and *S.pneumoniae* (14) were shown in figure 4-50, 4-52, 4-54, 4-56, 4-58 and 4-60, accordingly.

Table 4-12 Pharmacodynamics of gatifloxacin 400 mg multiple doses against *M.catarrhalis* (Bc.312), *H.influenzae* (Bc.38), *H.influenzae* (Bc.255), *S.pneumoniae* (38), *S.pneumoniae* (94) and *S.pneumoniae* (14).

Microorganism	MIC	Peak/MIC	AUC_{0-24}/MIC	$T_{99.9\%}$ (hr.)	$T_{eradication}$ (hr.)
M.catarrhalis (Bc.312)	0.03	292.33	2583.66	3.42	10.5
H. influenzae (Bc.38)	0.0035	2440.00	22237.14	1.37	4.00
H. influenzae (Bc.255)	0.015	578.00	5298.00	1.20	4.00
S.pneumoniae (94)	0.25	32.96	330.80	1.15	4.00
S pneumoniae (38)	0.5	16.24	150.66	1.71	6.00
S.pneumoniae (14)	0.25	32.00	317.96	1.86	6.00

Pharmacodynamic data for all microorganisms that were exposed to 400 mg multiple doses of moxifloxacin show in table 4-13. The Peak/MIC of moxifloxacin and AUC_{0-24}/MIC in the range of 13.10 to 4553.33 and 164.14 to 57680.00, respectively. The time for 99.9% kill ranged from 0.96-2.42 hours and the time required to decrease viable counts below the 10 cfu/ml limit of detection ranged from 4-10.5 hours.

The pharmacodynamics of moxifloxacin against *M.catarrhalis* required the maximum time (2.42 hours) to achieve 99.9% kill and eradication (10.5 hours).

Moxifloxacin was rapidly bactericidal against all six strains of the microorganisms evaluated in this study. Time-kill curves of moxifloxacin against *M.catarrhalis* (Bc.312), *H.influenzae* (Bc.38), *H.influenzae* (Bc.255), *S.pneumoniae* (38), *S.pneumoniae* (94) and *S.pneumoniae* (14) were shown in figure 4-62, 4-64, 4-66, 4-68, 4-70 and 4-72, accordingly.

Table 4-13 Pharmacodynamics of moxifloxacin 400 mg multiple doses against *M.catarrhalis* (Bc.312), *H. influenzae* (Bc.38), *H. influenzae* (Bc.255), *S.pneumoniae* (38), *S.pneumoniae* (94) and *S.pneumoniae* (14).

Microorganism	MIC	Peak/MIC	AUC_{0-24}/MIC	$T_{99.9\%}$ (hr.)	$T_{eradication}$ (hr.)
M.catarrhalis (Bc.312)	0.05	137.20	1653.80	2.42	10.5
H. influenzae (Bc.38)	0.0015	4553.33	57680.00	0.96	4.00
H. influenzae (Bc.255)	0.025	266.00	3305.20	1.12	4.00
S.pneumoniae (94)	0.5	13.68	169.60	1.04	4.00
S pneumoniae (38)	0.5	13.10	164.14	1.00	4.00
S.pneumoniae (14)	0.4	15.77	209.90	1.30	4.00

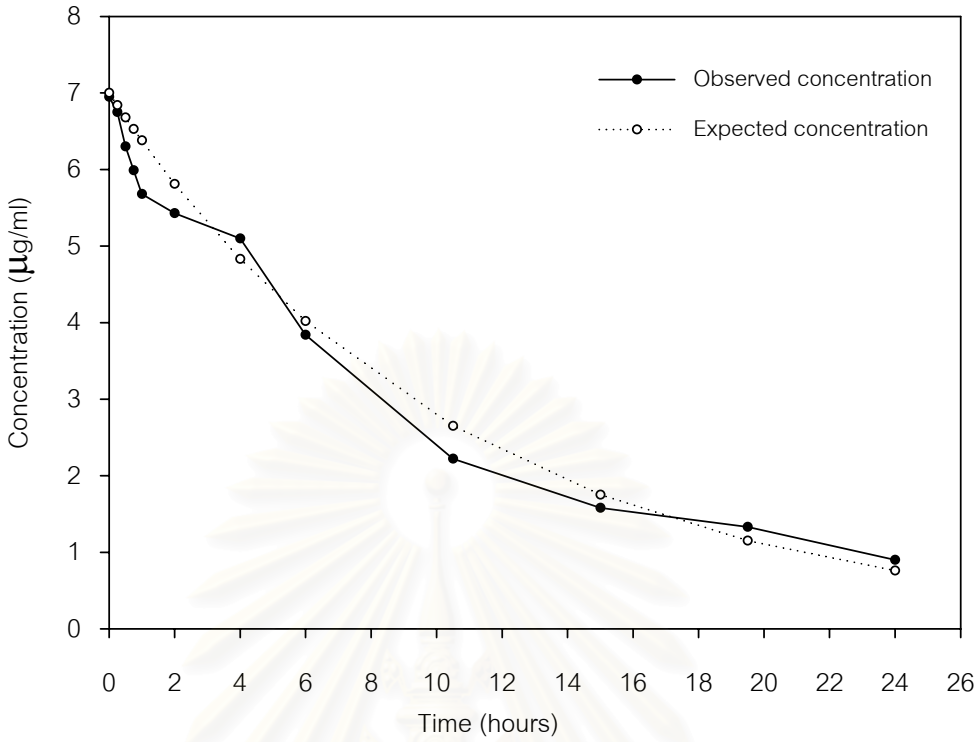


Figure 4-37 Concentration-time curve of levofloxacin 500 mg (multiple dose) against *M.catarrhalis* (Bc.312).

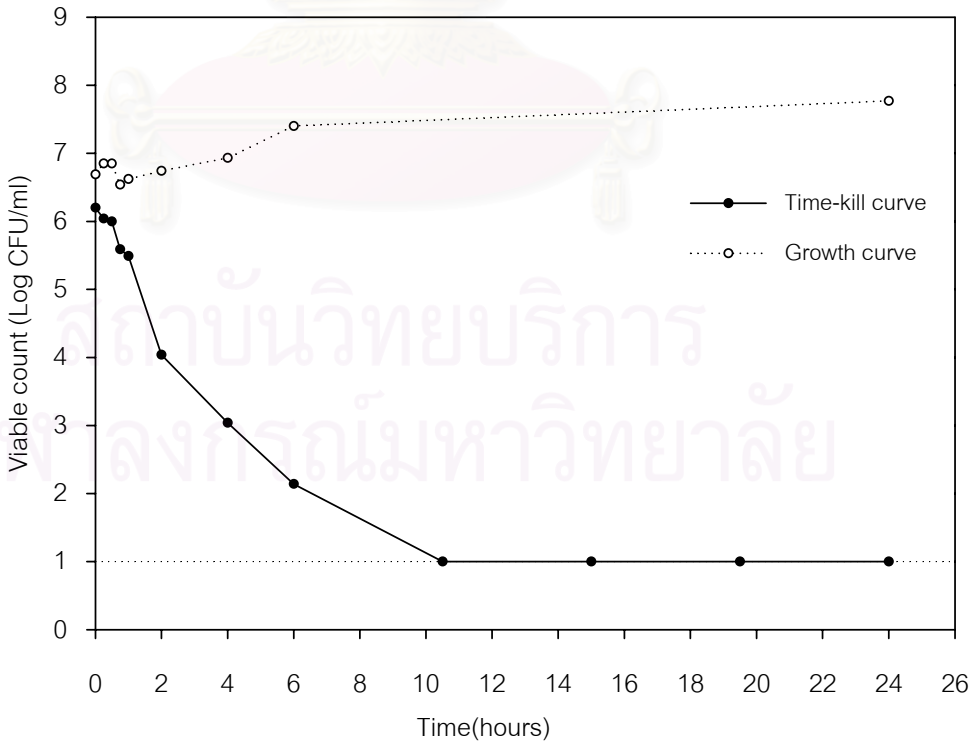


Figure 4-38 Time-kill curve of levofloxacin 500 mg (multiple dose) against *M.catarrhalis* (Bc.312).

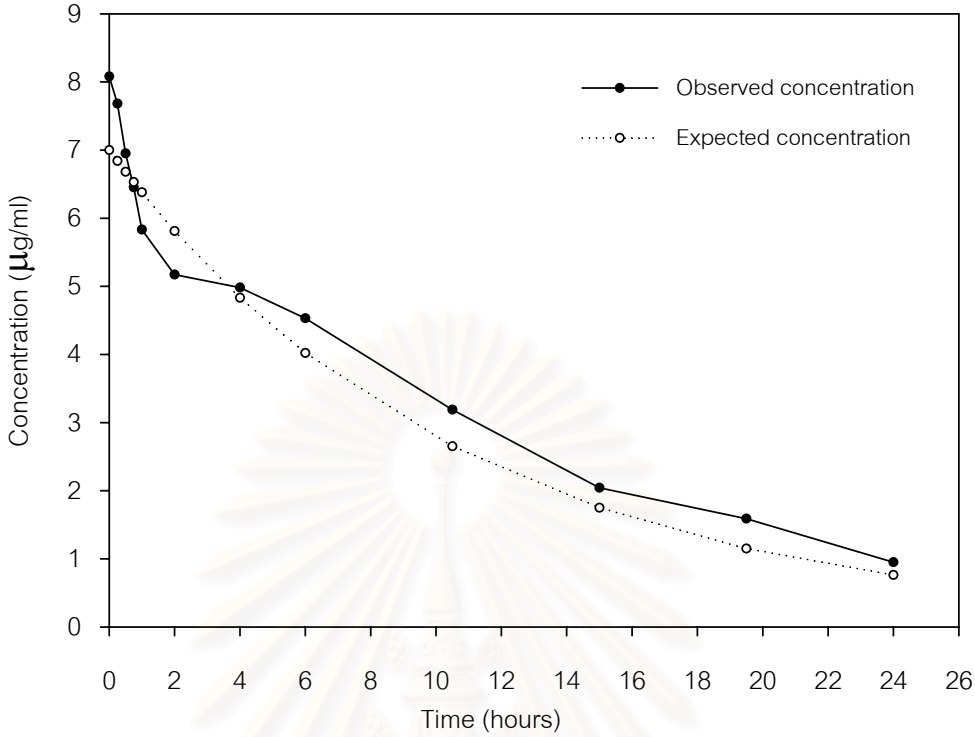


Figure 4-39 Concentration-time curve of levofloxacin 500 mg (multiple dose) against *H.influenzea* (Bc.38).

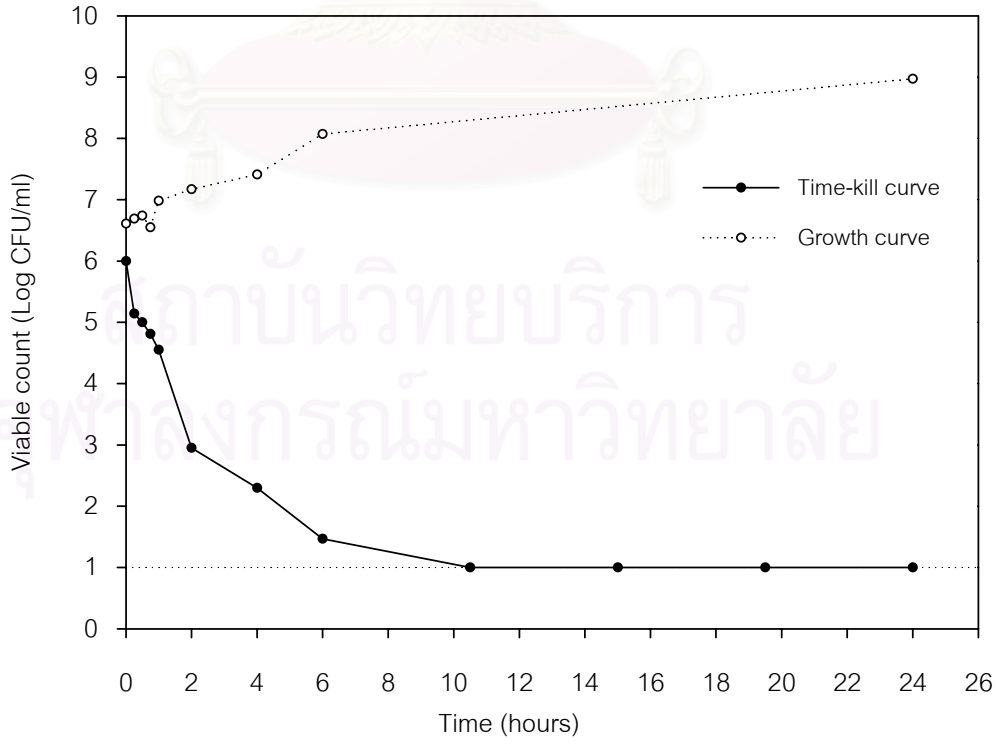


Figure 4-40 Time-kill curve of levofloxacin 500 mg (multiple dose) against *H.influenzea* (Bc.38).

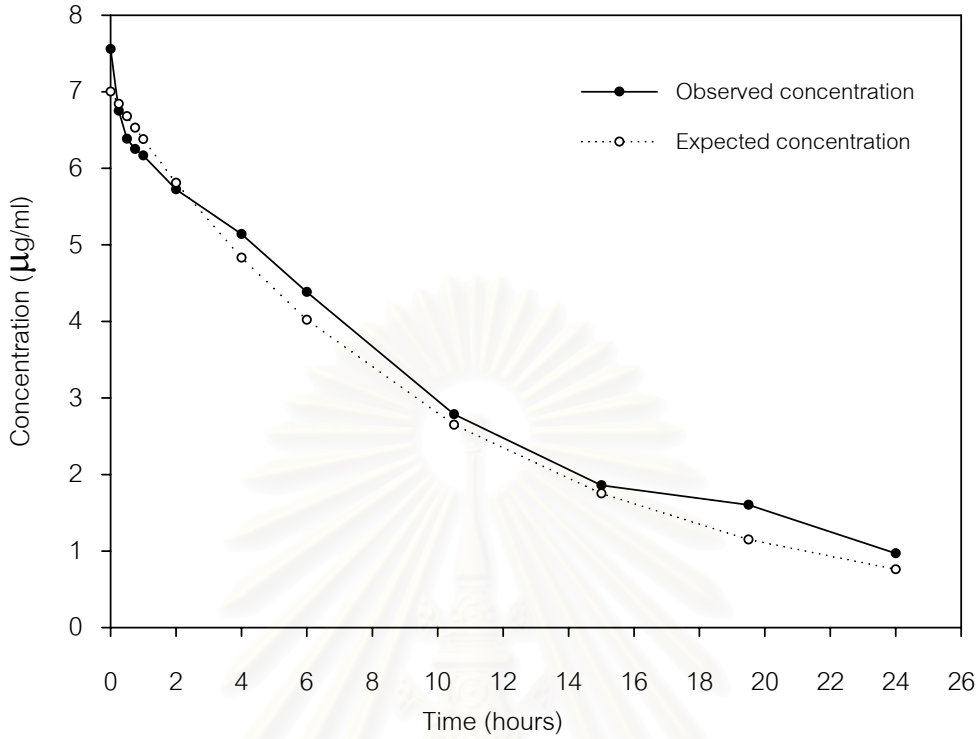


Figure 4-41 Concentration-time curve of levofloxacin 500 mg (multiple dose) against *H.influenzea* (Bc.255).

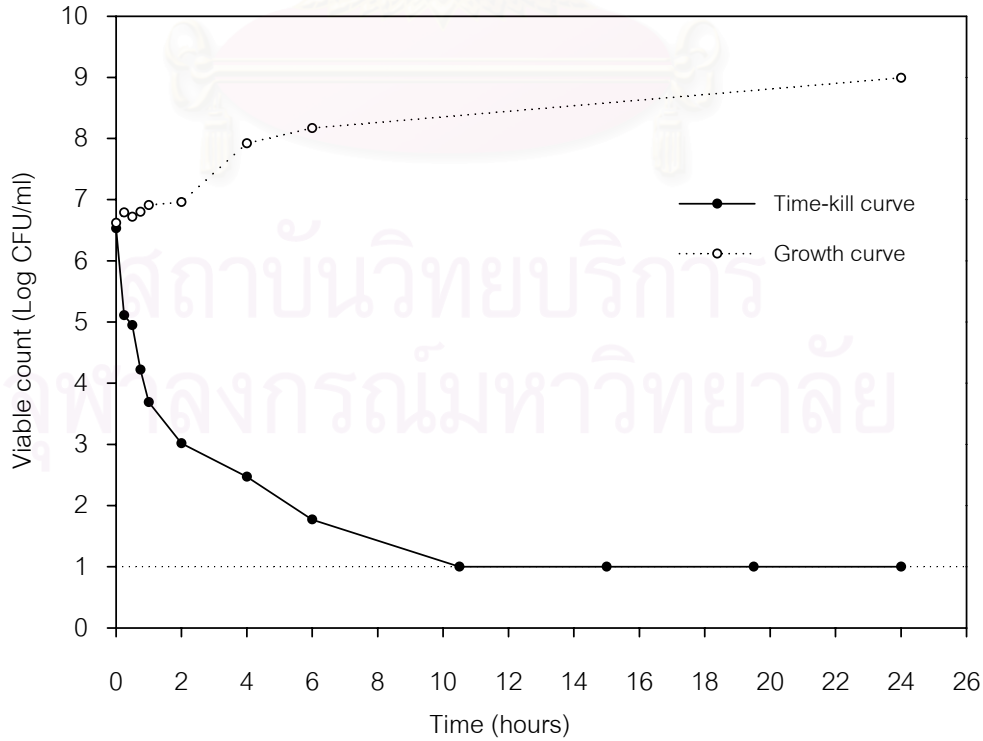


Figure 4-42 Time-kill curve of levofloxacin 500 mg (multiple dose) against *H.influenzea* (Bc.255).

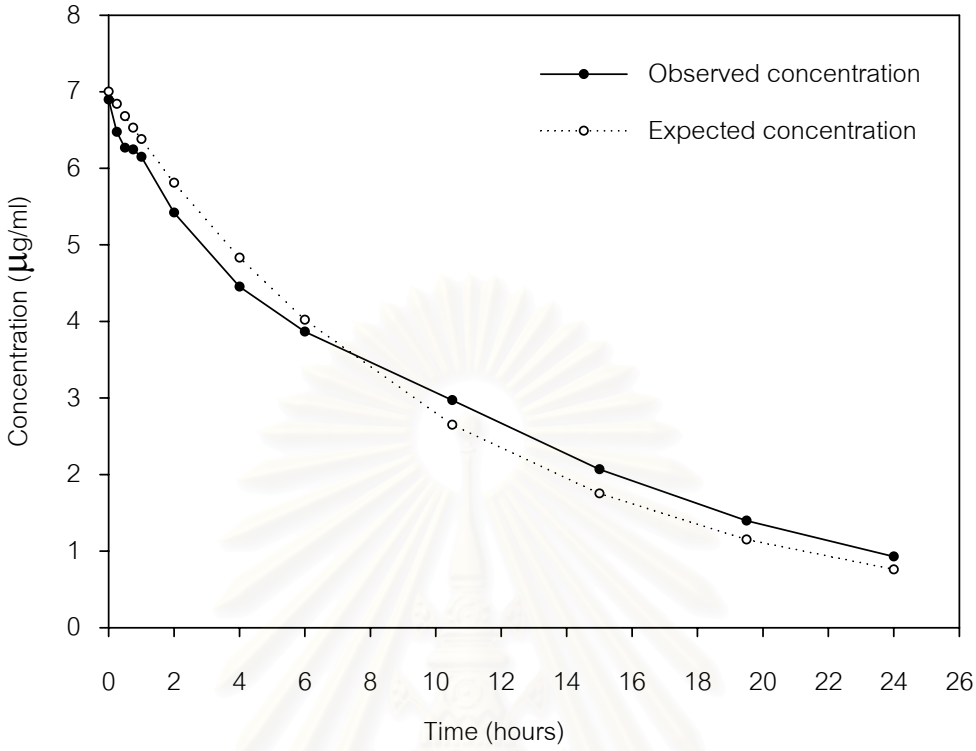


Figure 4-43 Concentration-time curve of levofloxacin 500 mg (multiple dose) against *S.pneumoniae* (94).

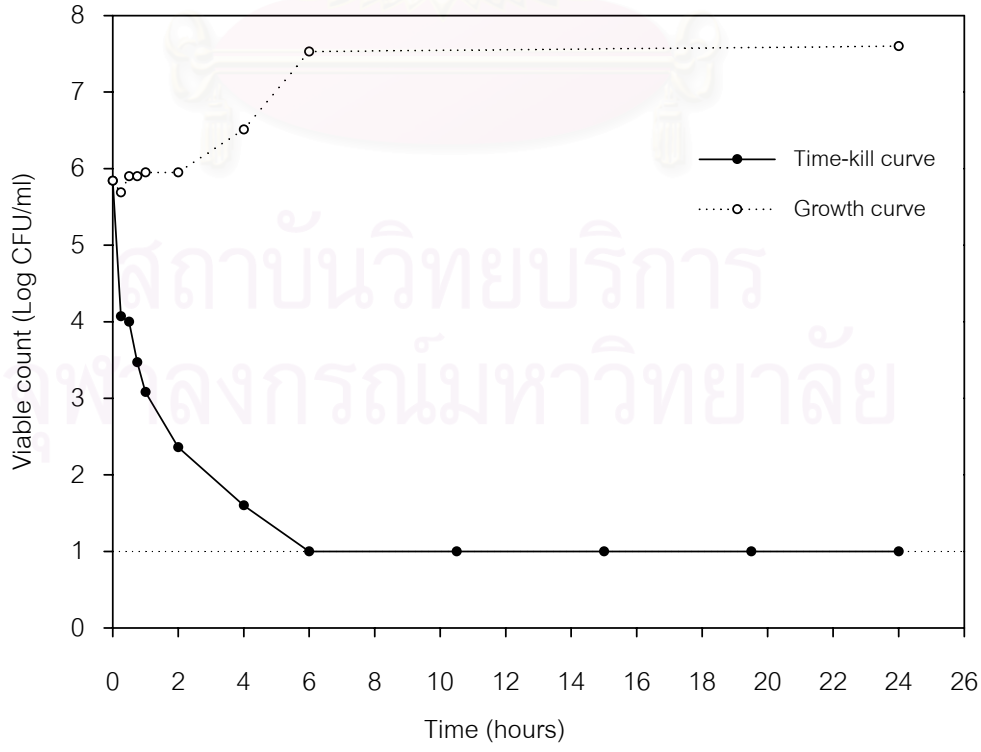
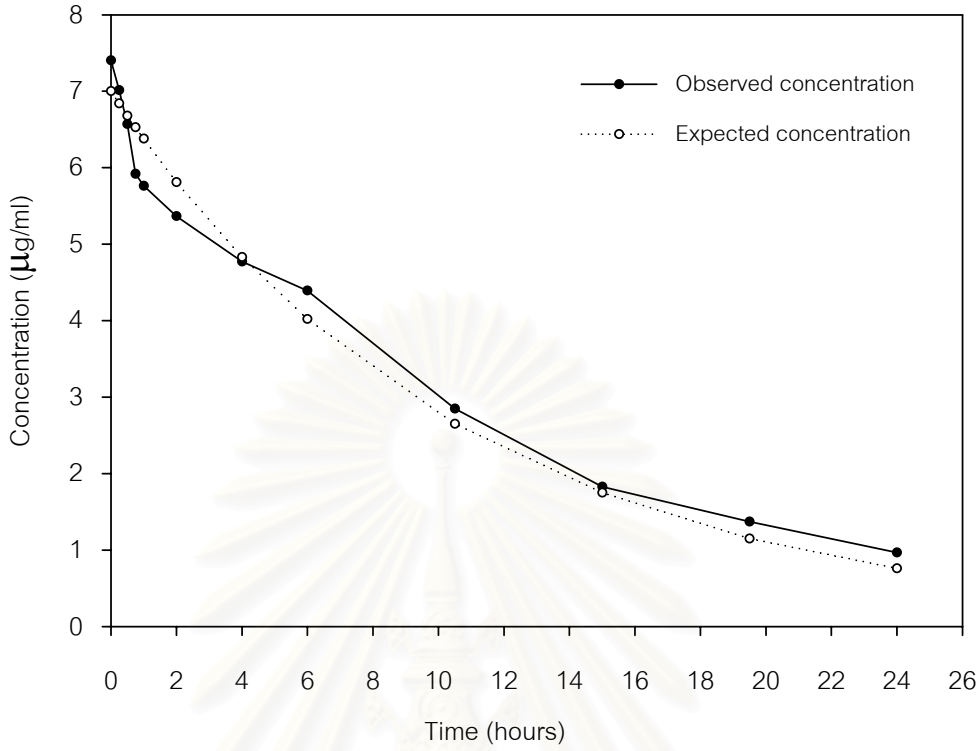
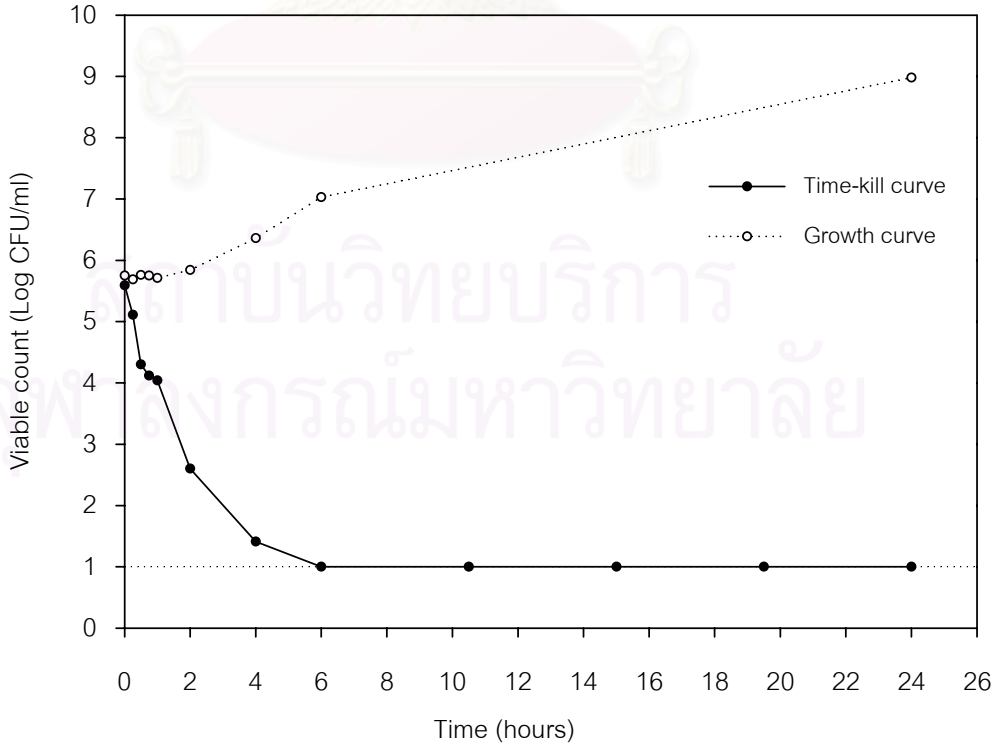


Figure 4-44 Time-kill curve of levofloxacin 500 mg (multiple dose) against *S.pneumoniae* (94).



Fiureg 4-45 Concentration-time curve of levofloxacin 500 mg (multiple dose) against *S.pneumoniae* (Bc.38).



Fiureg 4-46 Time-kill curve of levofloxacin 500 mg (multiple dose) against *S.pneumoniae* (Bc.38).

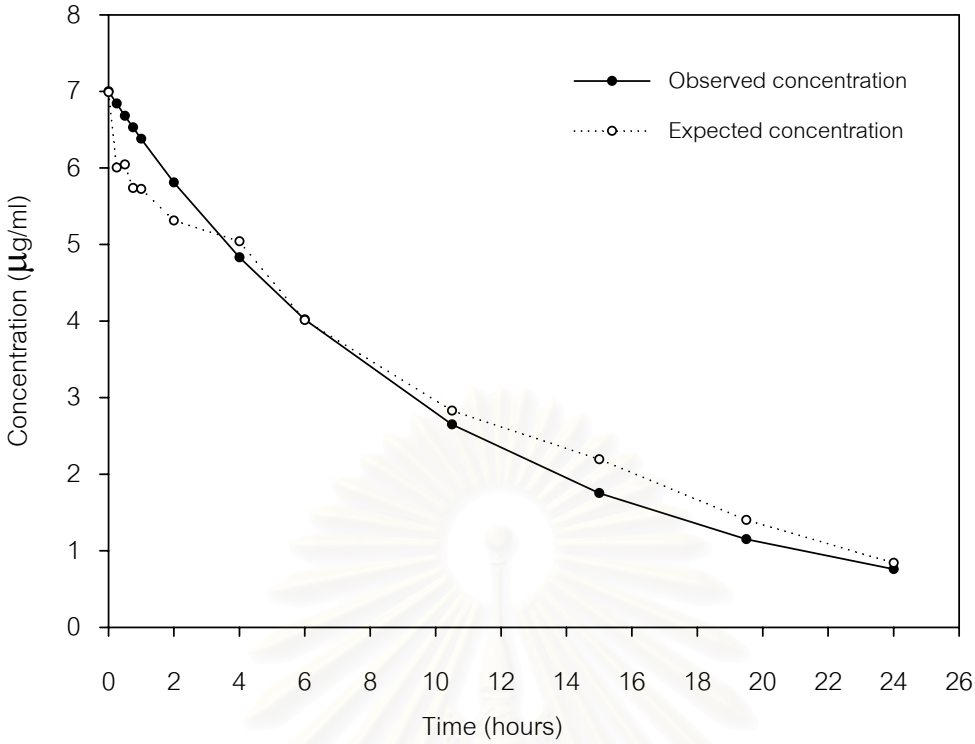


Figure 4-47 Concentration-time curve of levofloxacin 500 mg (multiple dose) against *S.pneumoniae* (14).

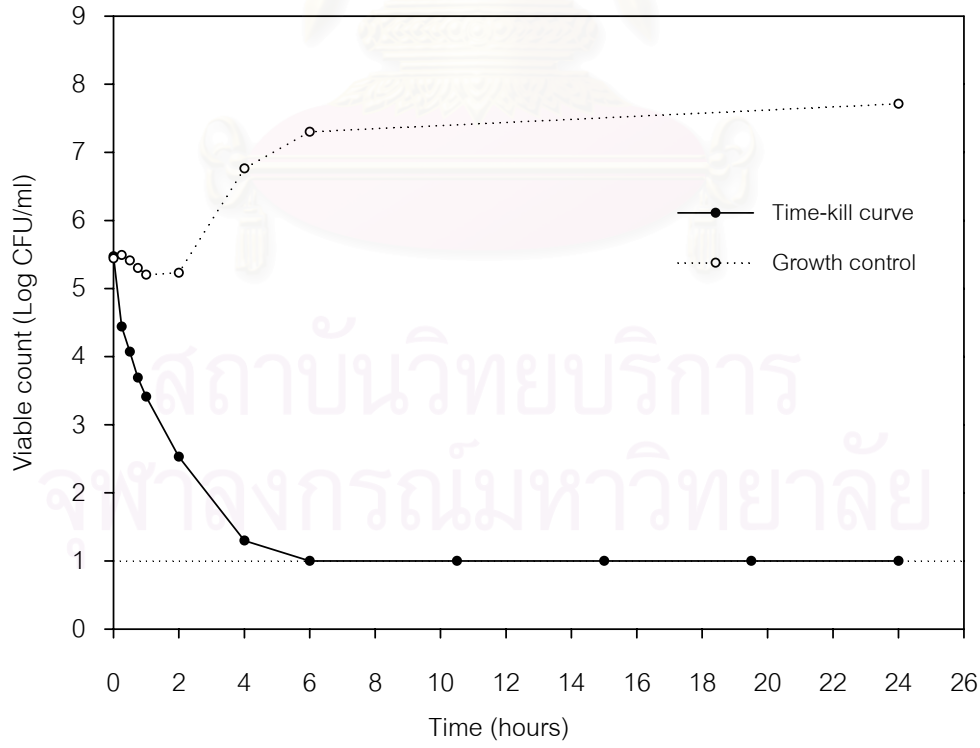


Figure 4-48 Time-kill curve of levofloxacin 500 mg (multiple dose) against *S.pneumoniae* (14).

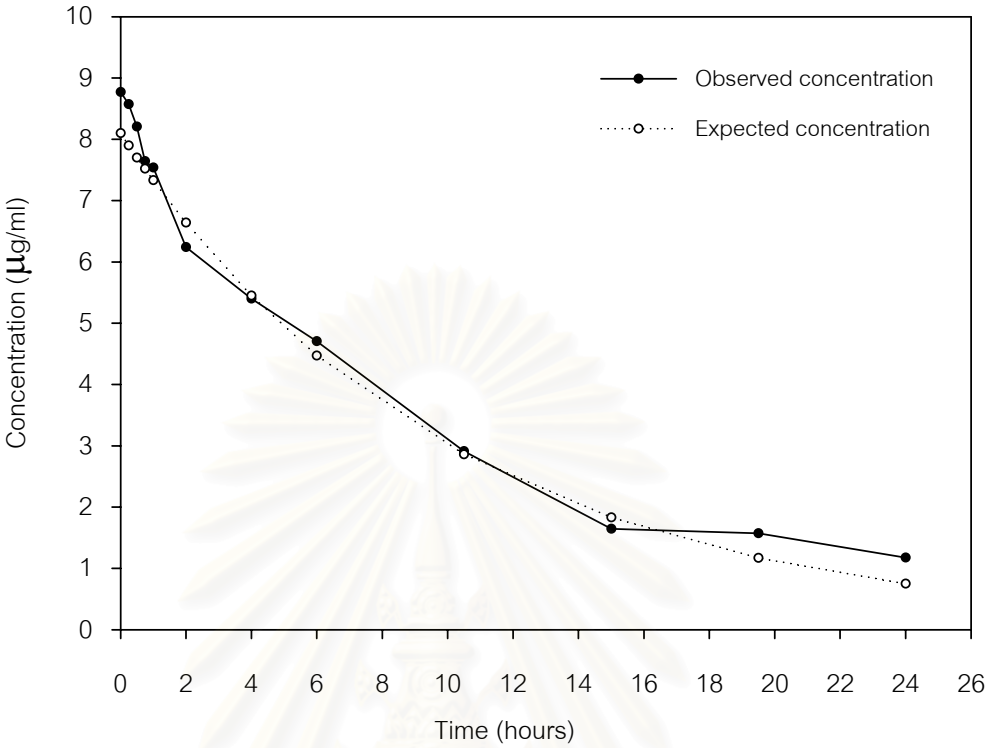


Figure 4-49 Concentration-time curve of gatifloxacin 400 mg (multiple dose) against *M.catarrahalis* (Bc.312).

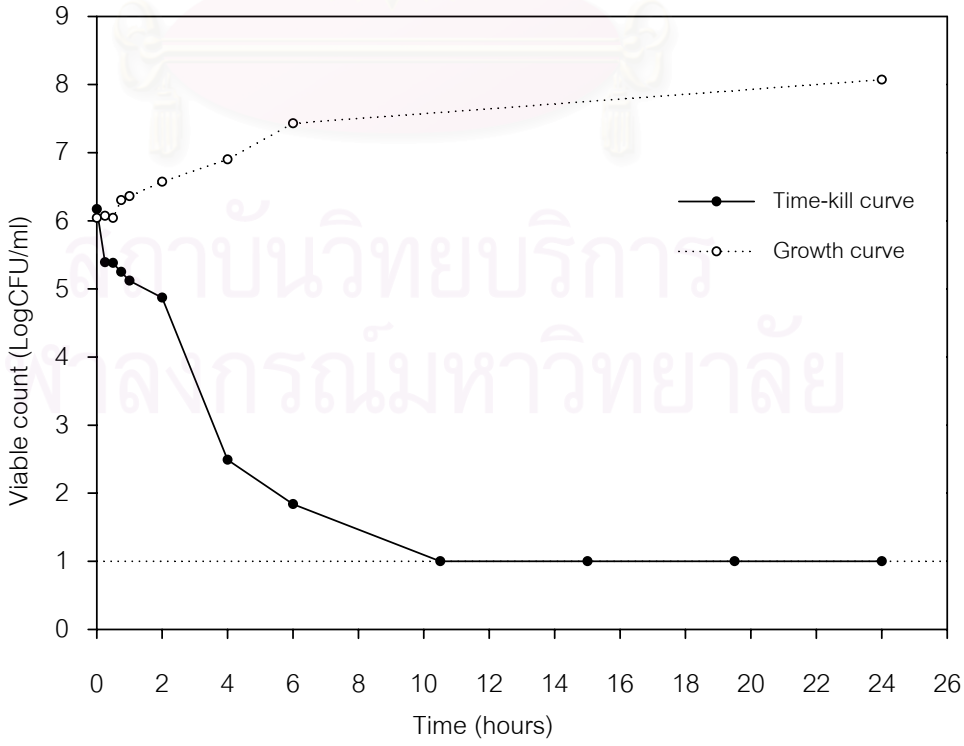


Figure 4-50 Time-kill curve of gatifloxacin 400 mg (multiple dose) against *M.catarrahalis* (Bc.312).

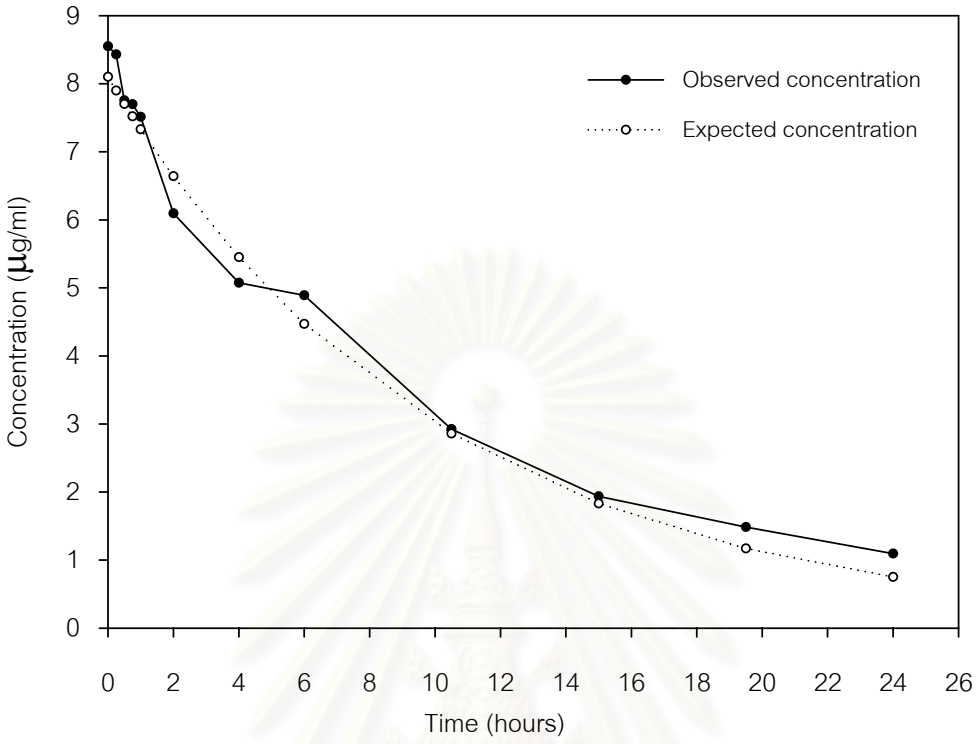


Figure 4-51 Concentration-time curve of gatifloxacin 400 mg (multiple dose) against *H.influenzae* (Bc.38).

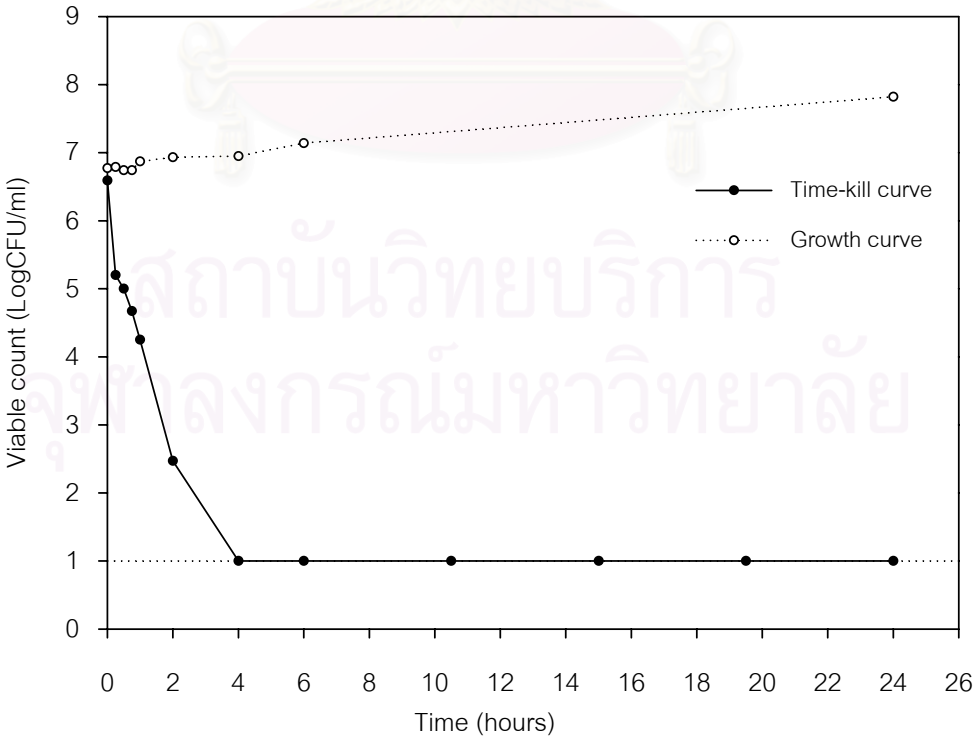


Figure 4-52 Time-kill curve of gatifloxacin 400 mg (multiple dose) against *H.influenzae* (Bc.38).

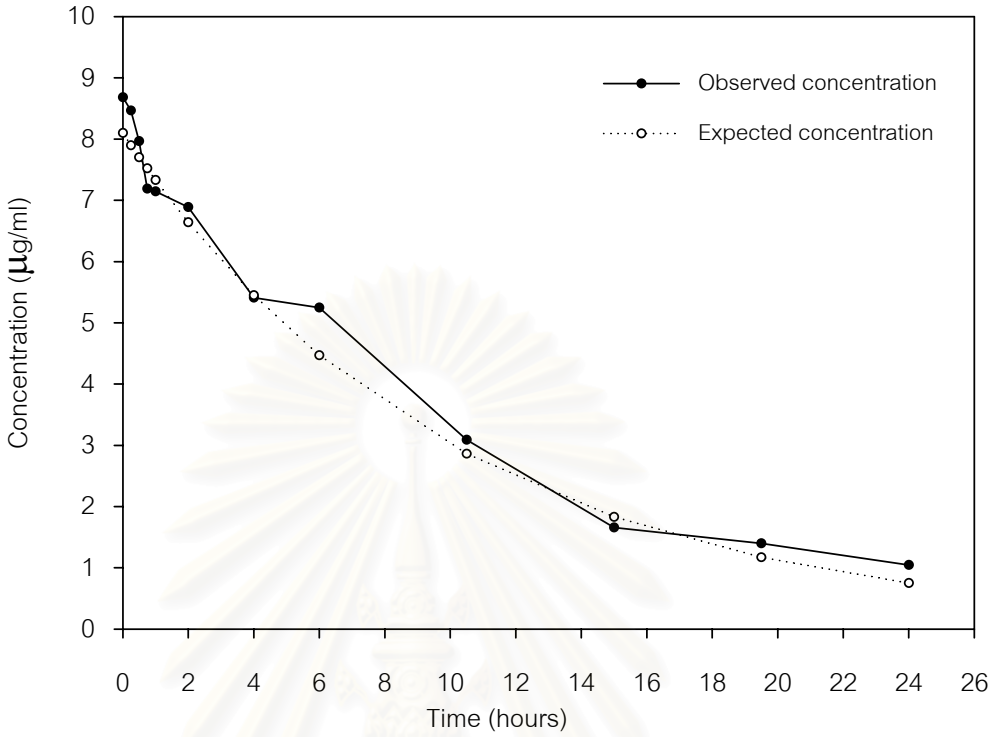


Figure 4-53 Concentration-time curve of gatifloxacin 400 mg (multiple dose) against *H.influenzae* (Bc.255).

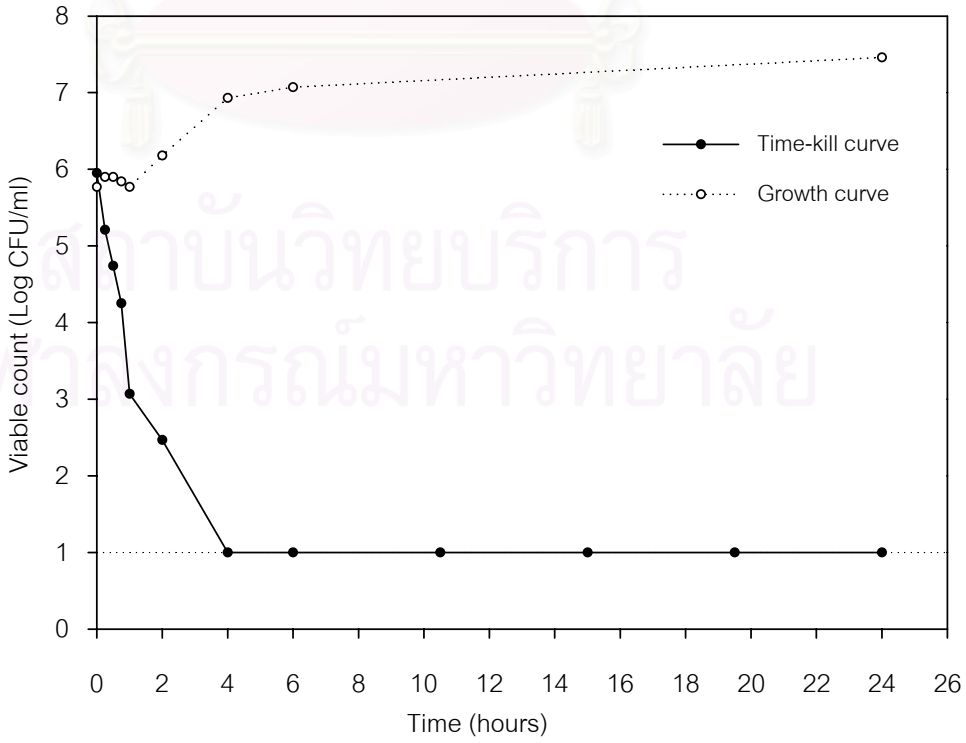


Figure 4-54 Time-kill curve of gatifloxacin 400 mg (multiple dose) against *H.influenzae* (Bc.255).

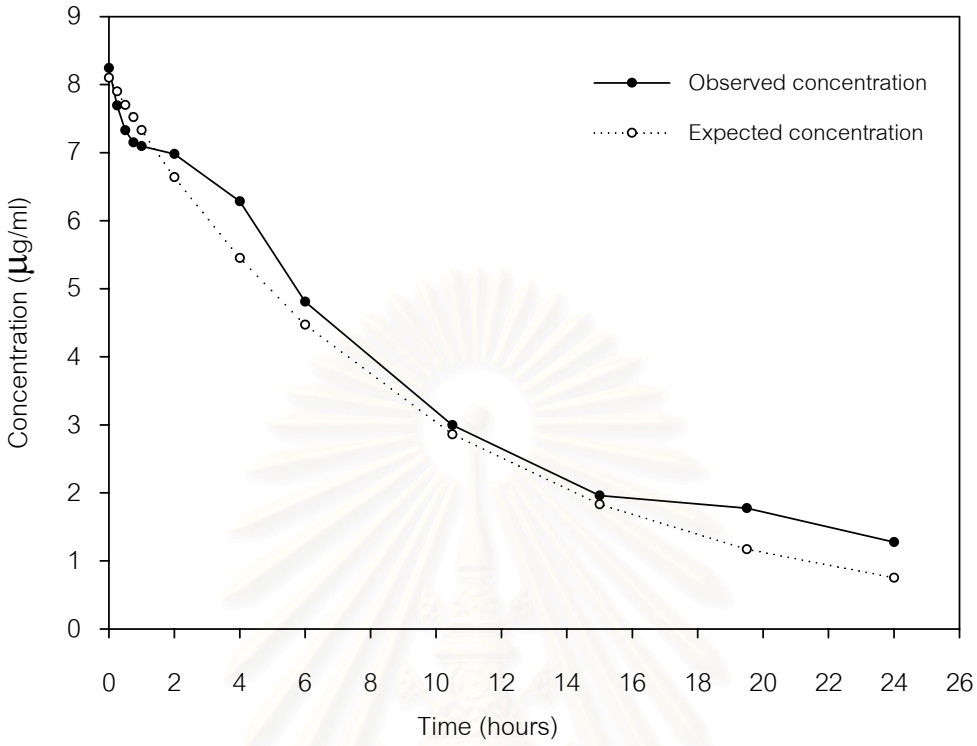


Figure 4-55 Concentration-time curve of gatifloxacin 400 mg (multiple dose) against *S.pneumoniae* (94).

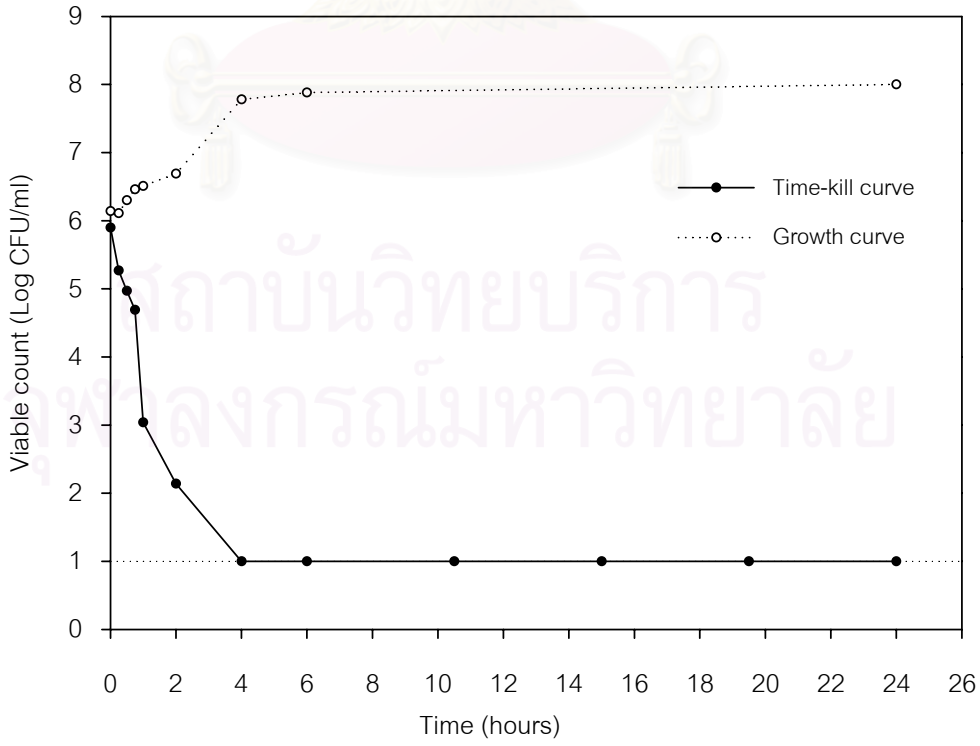


Figure 4-56 Time-kill curve of gatifloxacin 400 mg (multiple dose) against *S.pneumoniae* (94).

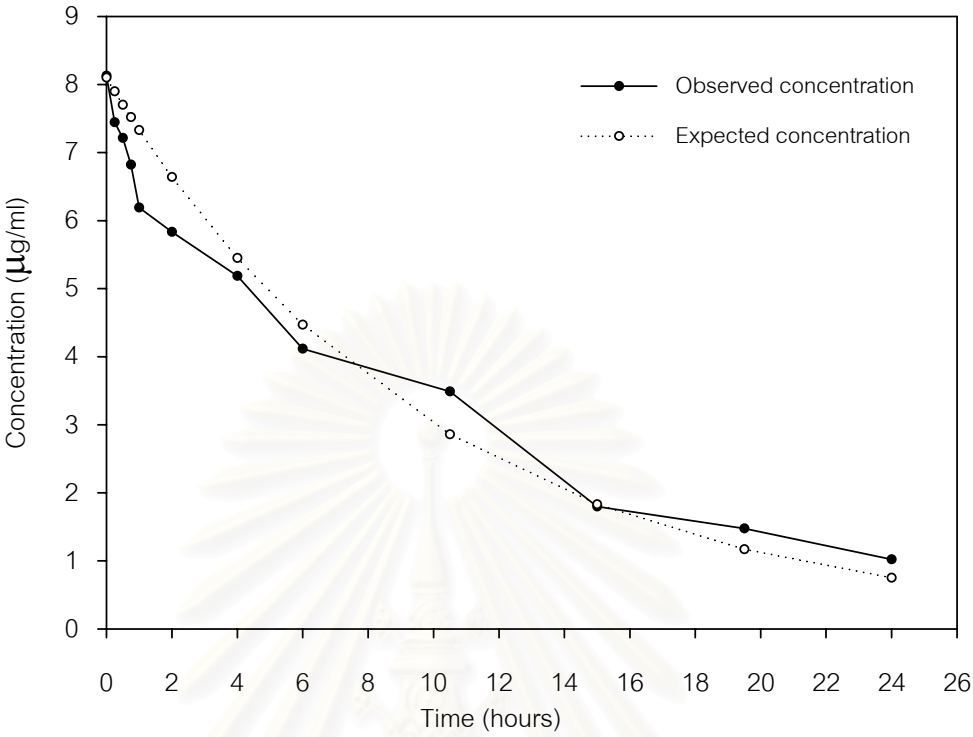


Figure 4-57 Concentration-time curve of gatifloxacin 400 mg (multiple dose) against *S.pneumoniae* (38).

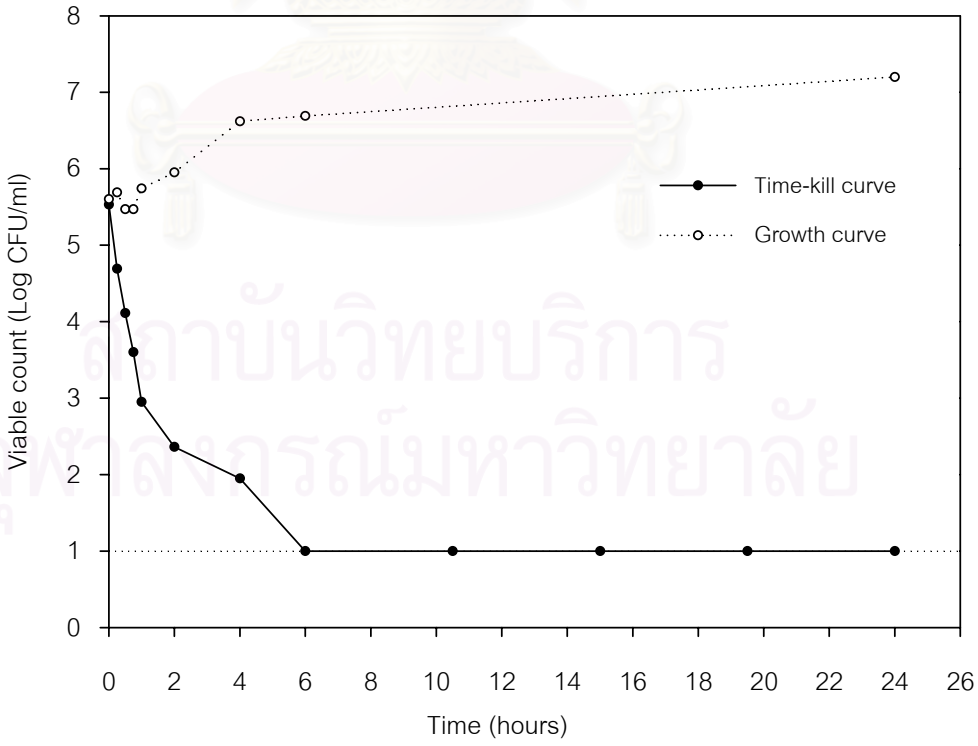


Figure 4-58 Time-kill curve of gatifloxacin 400 mg (multiple dose) against *S.pneumoniae* (38).

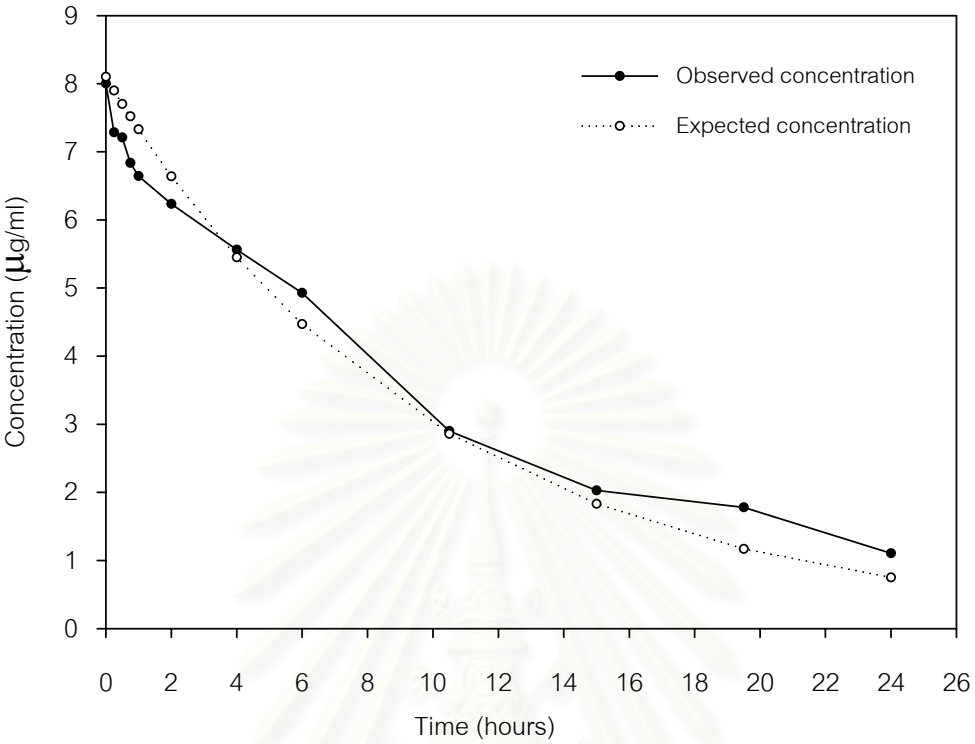


Figure 4-59 Concentration-time curve of gatifloxacin 400 mg (multiple dose) against *S.pneumoniae* (14).

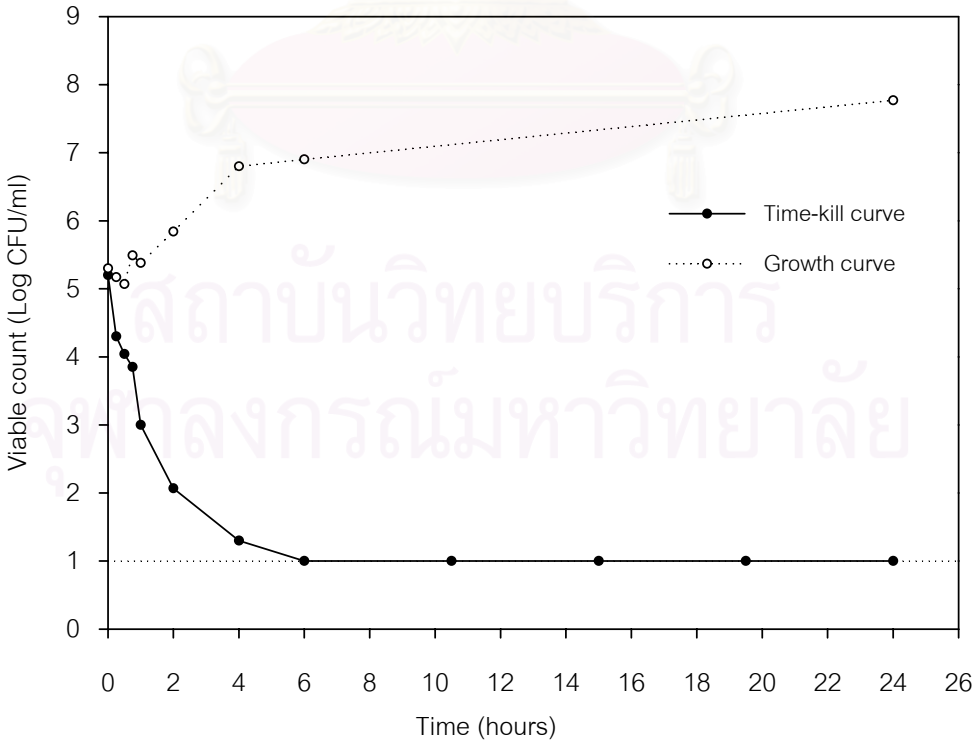


Figure 4-60 Time-kill curve of gatifloxacin 400 mg (multiple dose) against *S.pneumoniae* (14).

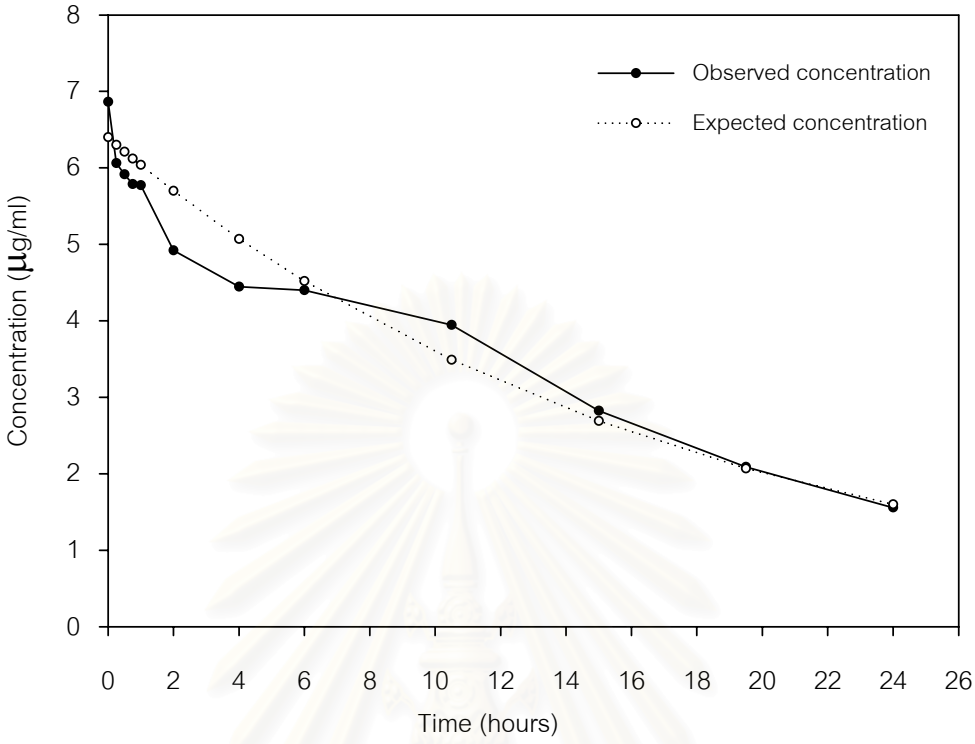


Figure 4-61 Concentration-time curve of moxifloxacin 400 mg (multiple dose) against *M.catarrhalis (Bc.*312).

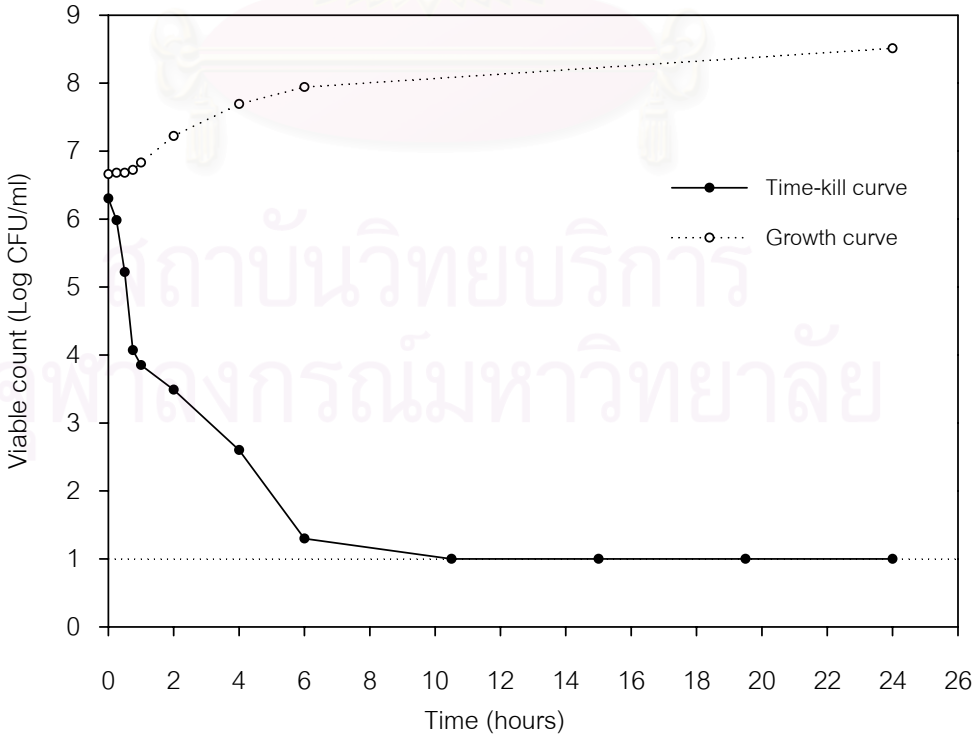


Figure 4-62 Time-kill curve of moxifloxacin 400 mg (multiple dose) against *M.catarrhalis (Bc.*312).

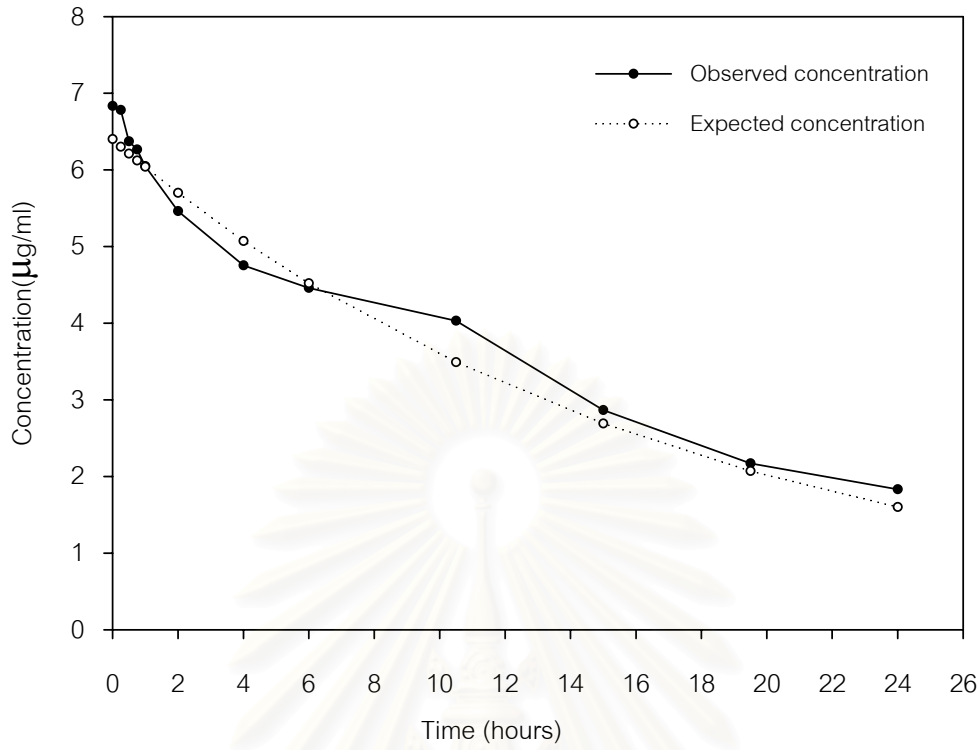


Figure 4-63Concentration-time curve of moxifloxacin 400 mg (multiple dose) against *H.influenzae* (38).

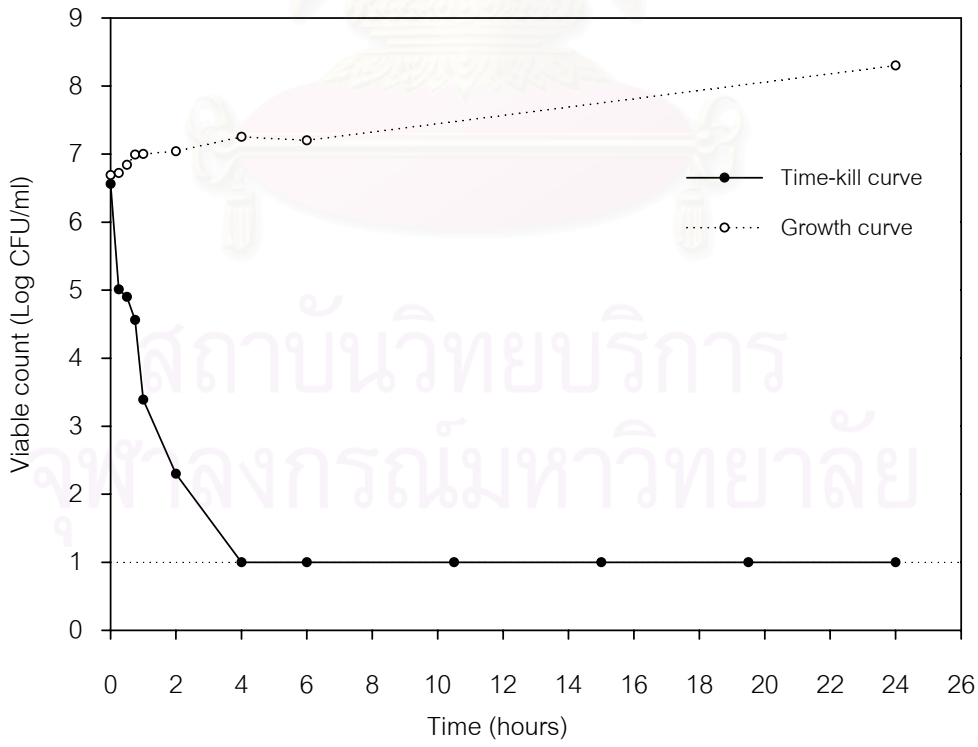


Figure 4-64 Time-kill curve of moxifloxacin 400 mg (multiple dose) against *H.influenzae* (38).

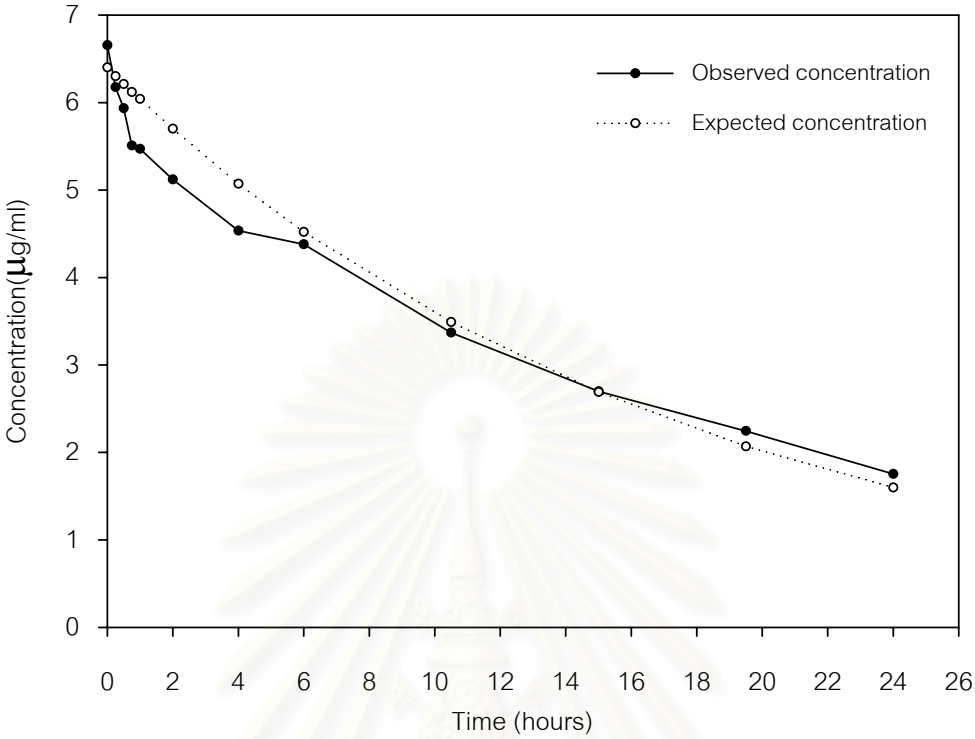


Figure 4-65 Concentration-time curve of moxifloxacin (multiple dose) against *H.influenzae* (Bc.255).

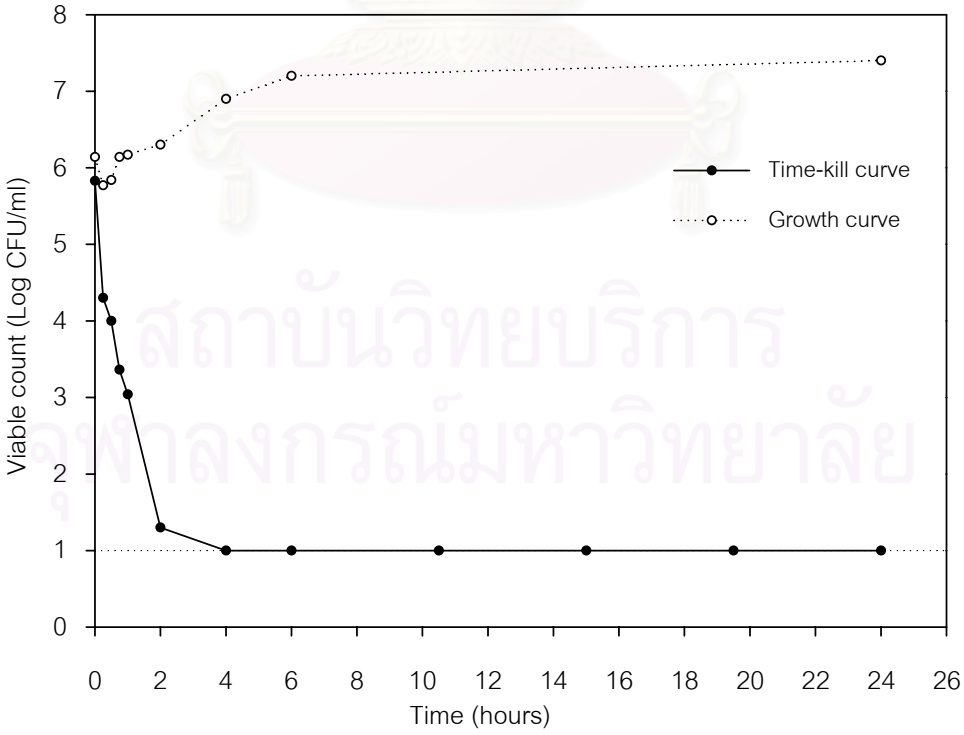


Figure 4-66 Time-kill curve of moxifloxacin 400 mg (multiple dose) against *H.influenzae* (Bc.255).

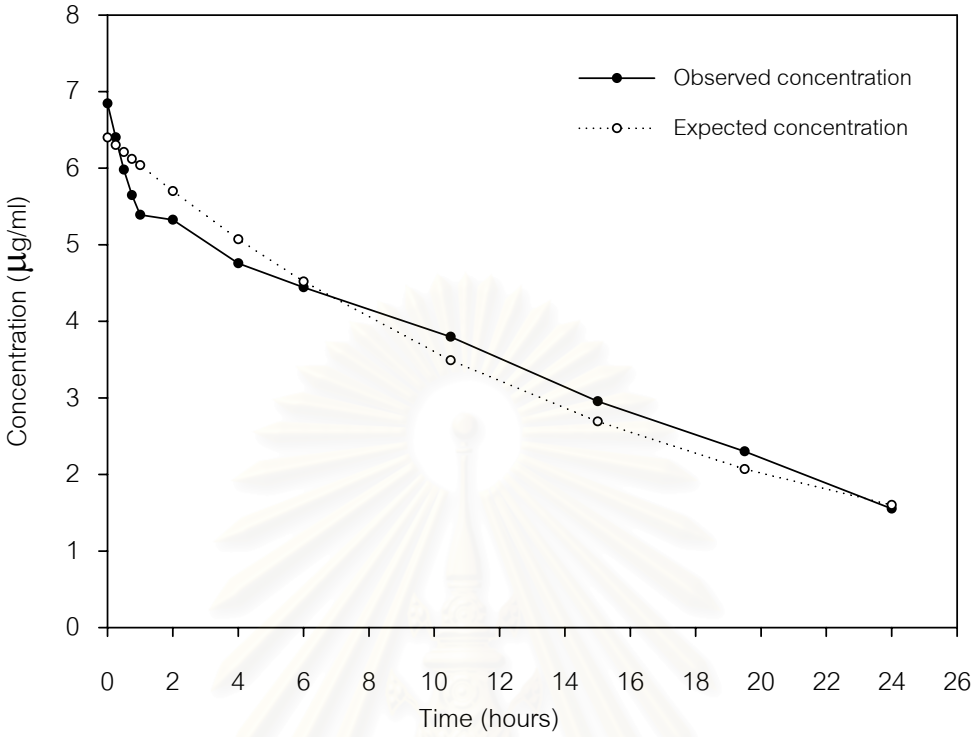


Figure 4-67 Concentration-time curve of moxifloxacin 400 mg (multiple dose) against *S.pneumoniae* (94).

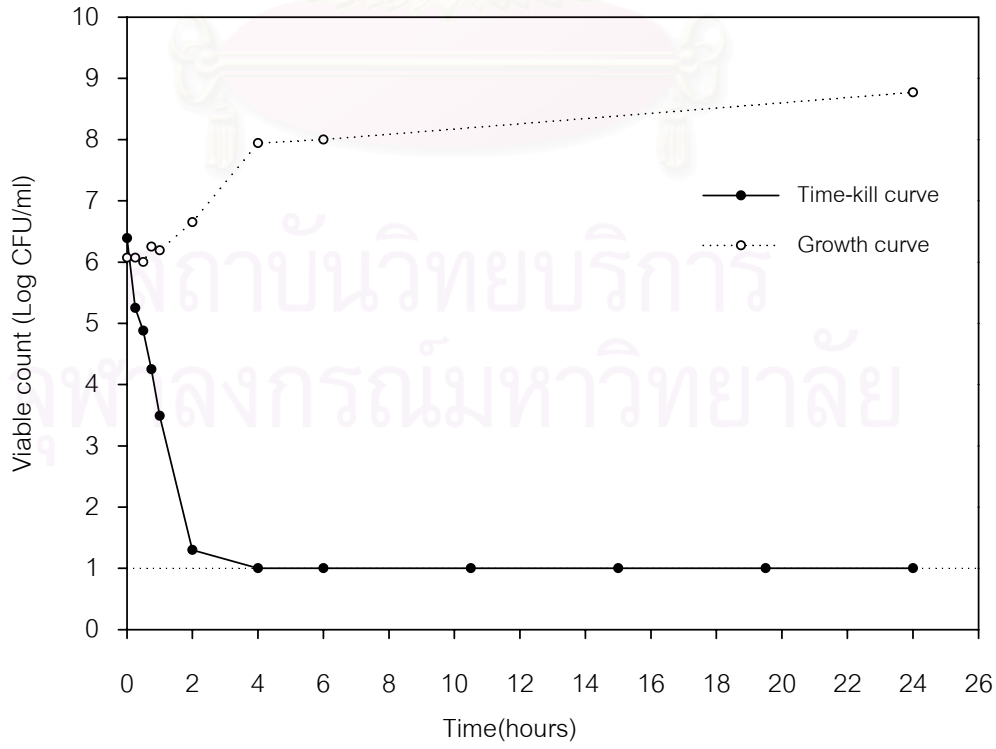


Figure 4-68 Time-kill curve of moxifloxacin 400 mg (multiple dose) against *S.pneumoniae* (94).

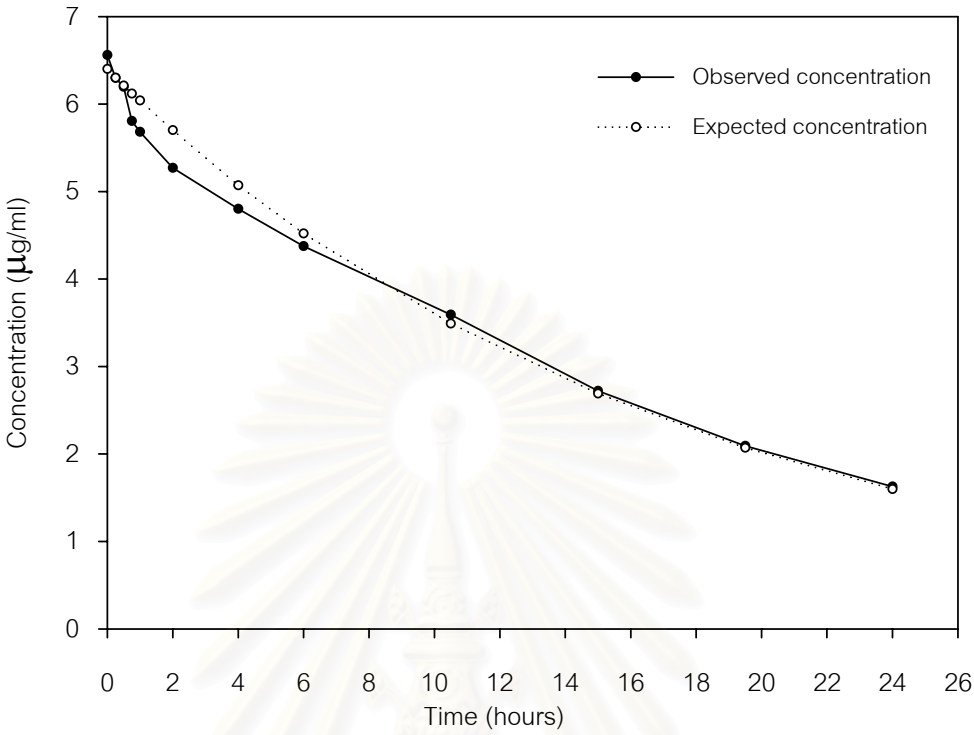


Figure 4-69 Concentration-time curve of moxifloxacin 400 mg (multiple dose) against *S.pneumoniae(*38).

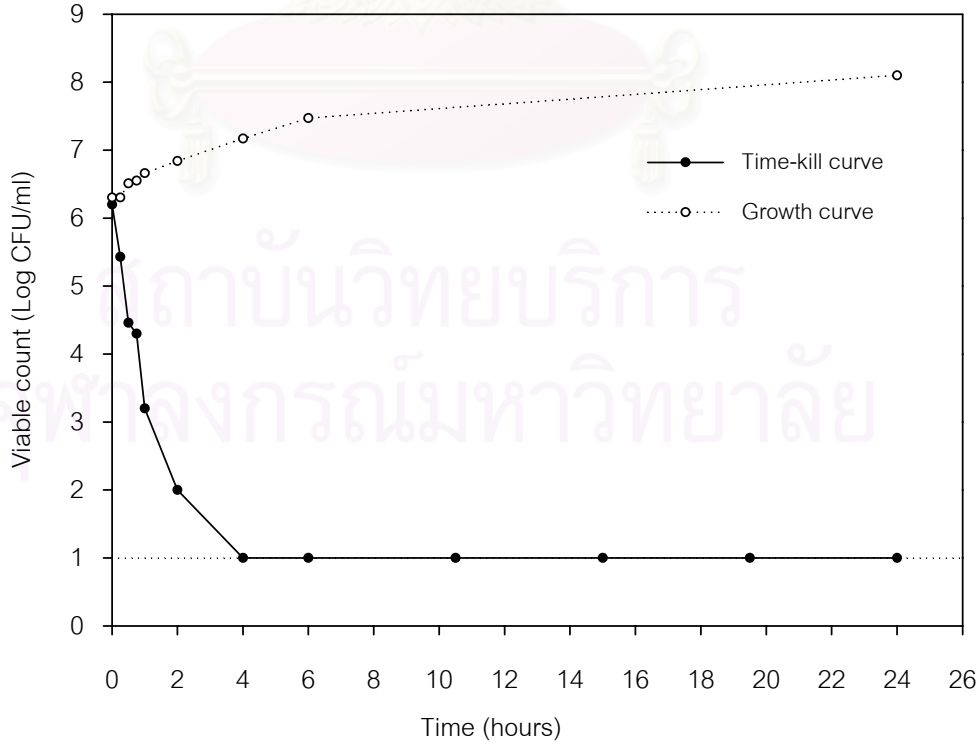


Figure 4-70 Time-kill curve of moxifloxacin 400 mg (multiple dose) against *S.pneumoniae(*38).

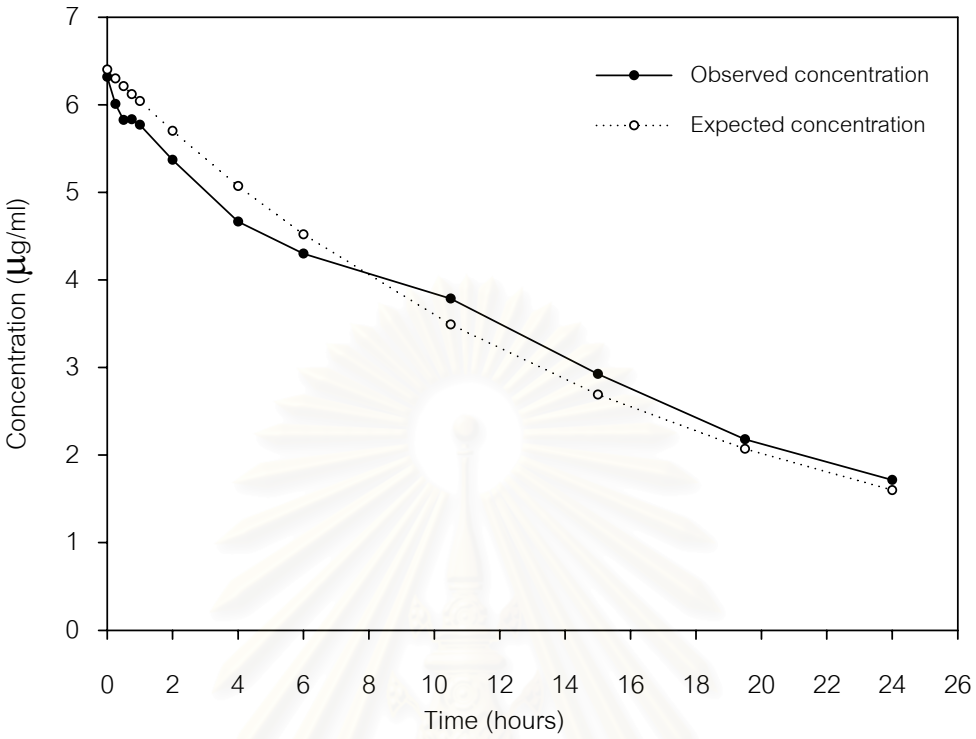


Figure 4-71 Concentration-time curve of moxifloxacin 400 mg (multiple dose) against *S.pneumoniae* (14).

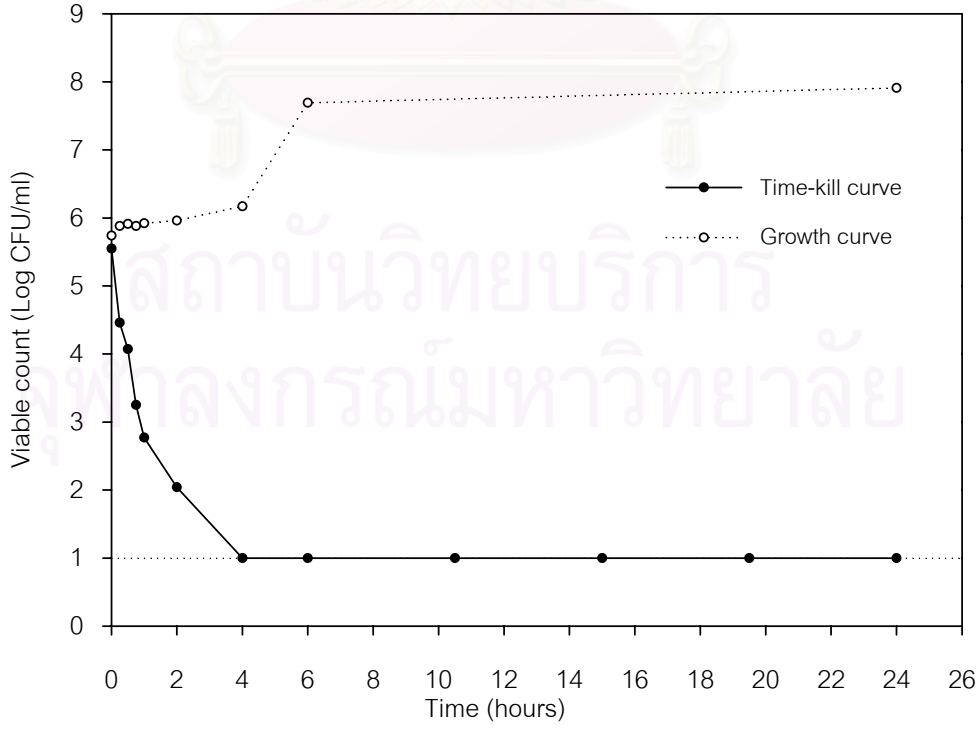


Figure 4-72 Time-kill curve of moxifloxacin 400 mg (multiple dose) against *S.pneumoniae* (14).

CHAPTER V

Discussion & Conclusion

IVPM was used to simulate the maxillary pharmacokinetic of levofloxacin, gatifloxacin and moxifloxacin in the study by Gehanno et al. which suggested that in maxillary sinus the fluoroquinolones was declined at the similar rate as the concentrations in the plasma (Gehanno et al., 2002). Therefore, in this study we adjusted the elimination half-life of all fluoroquinolones in IVPM to the same elimination rate in plasma concentrations.

In this study, IVPM is one compartment, which demonstrated the elimination of drug when its complete distributed into maxillary sinus after oral administration. Thus, the pharmacokinetic parameters of each drug in this study are represent pharmacokinetic of each drug after complete by distributed into maxillary sinus.

During the running process of IVPM in this study, the waste product from killing microorganisms obstructed the Tygon tube that has a very small diameter (0.19 mm.) after 6 hours. Thus, the model was only operated 6 hours. Then the macrodilution method was used to evaluate the antimicrobial activity at the 4 point of time (10.5, 15.0, 19.5 and 24 hours).

The viable count of microorganisms that lower than limit of detection (10 cfu/ml) can not detected. Therefore, the result of $T_{eradication}$ may be in accurate time for eradication.

An IVPM was used to simulate the maxillary pharmacokinetic of oral doses of levofloxacin, gatifloxacin and moxifloxacin and to compare their pharmacodynamics against 6 clinical isolates of *M.catarrhalis*, *H.influenzae* and *S.pneumoniae*. All of the strains selected for this study were susceptible to levofloxacin, gatifloxacin and moxifloxacin. The strains of *S.pneumoniae* 14 was shown lack of susceptibility to levofloxacin (MIC = 1 μg/ml). Although fluoroquinolone resistance among *S.pneumoniae* is not currently a serious problem, remaining below 1% in large surveillance studies (Odland et al., 1999), there are some reports suggesting that it may be increasing (Chen et al., 1999). Therefore, in addition to evaluating new fluoroquinolones against random

clinical isolates, it is important to evaluate them against isolates that are not susceptible to the older fluoroquinolones.

Moxifloxacin and gatifloxacin were rapidly bactericidal in less than 2 hours against 3 strains of *S.pneumoniae* in this study, and could eradicate most of the strains from IVPM within 4 to 6 hours of the first dose. These data are supported by Zinner and colleagues, who observed similar rates of killing and eradication of six *S.pneumoniae* isolates from the same IVPM (Zinner et al., 1998).

Levofloxacin in comparison with moxifloxacin and gatifloxacin exhibited rapidly bactericidal effect on the only 1 strain of *S.pneumoniae* (PSSP) within 2 hours and eradicated all strains of *S.pneumoniae* within 6 hours. The largest differences in the initial kill rates were observed in 1 strain with MICs of 1 μg/ml. Against these strains, levofloxacin required more than 2 hours to produce a 99.9% kill of these strains compare with moxifloxacin and gatifloxacin. Although in the study with gram-negative microorganisms, levofloxacin, gatifloxacin and moxifloxacin have similar killing rate to 99.9%. Moxifloxacin and gatifloxacin exhibited the eradication of 2 strains of *H.influenzae* within 4 hours while levofloxacin exhibited slower eradication of these microorganisms (within 6 hours).

The multiple doses regimen of gatifloxacin and moxifloxacin exhibit improved efficacy to kill 99.9% of all *S.pneumoniae* within 2 hours, eradicating most strains similar to single dose regimen are within 4-6 hours.

Levofloxacin that simulated with the concentration of multiple doses exhibited the same 99.9% kill rate with that of the single dose regimen. This drug could eradicate all strains of *S.pneumoniae* within 6 hours. Although levofloxacin, gatifloxacin and moxifloxacin have similar killing 99.9% in all most gram-negative bacteria but moxifloxacin also exhibited the rapid kill of one strain of *H.influenzae* within least than 1 hour. Levofloxacin, gatifloxacin and moxifloxacin in a single dose regimen could eradicate all of gram-negative microorganisms in the similar rate.

Fluoroquinolones have been shown to exhibit a dose-response relationship in their bactericidal activity. In this study, the concentration of levofloxacin when administration in single dose regimen were not different from the maximum concentration of the multiple doses regimen. Therefore, the activity of levofloxacin in both regiment

were not different. The enhancement of gatifloxacin and moxifloxacin activity against *S.pneumoniae* compared with the activity of levofloxacin have been reported (Zhanel et al., 2002). Because gatifloxacin and moxifloxacin are methoxyfluoroquinolones, which have affinity binding to both enzyme DNA gyrase and topoisomerase IV. The available C-8-OMe fluoroquinolones moxifloxacin and gatifloxacin are potential alternatives to older fluoroquinolones such as levofloxacin, which possess a single topoisomerase IV target (Allen et al., 2003).

AUC_{0-24}/MIC ratio of levofloxacin, gatifloxacin and moxifloxacin of single dose regimen against gram-negative microorganisms are in the range of 4515.33 to 9034.61, 2187.66 to 19862.85 and 1607.80 to 50420.00, respectively. These ratios were much higher than the AUC_{0-24}/MIC break point of fluoroquinolones against gram-negative microorganisms that was 125 (Madaras-Kelly et al., 1996). In addition, AUC_{0-24}/MIC of levofloxacin, gatifloxacin and moxifloxacin against gram-positive microorganisms were in the range of 67.21 to 1270.80, 125.36 to 259.12 and 155.36 to 189.50 which were also higher than the 30-50 ranged of AUC_{0-24}/MIC recommended by Lister and Sander in their study on the eradication of *Streptococcus pneumoniae* with various fluoroquinolones (Lister and Sander., 1999).

AUC_{0-24}/MIC ratio of levofloxacin, gatifloxacin and moxifloxacin in the multiple doses regimen were higher than those obtained from the study on the single dose regimen by AUC_{0-24}/MIC ratio of three agents against gram-negative microorganisms were in the range of 4340.66 to 9357.69, 2583.66 to 22237.14 and 1653.80 to 57680.00, respectively. AUC_{0-24}/MIC of these agents against gram-positive microorganisms were in the range of 70.70 to 1399.40, 150.66 to 330.80 and 164.14 to 209.90, which were enough to eradicate all of strains in this study.

The data from this experiment, levofloxacin, gatifloxacin and moxifloxacin against gram-negative microorganisms such as *M.catarrhalis* and *H.influenzae* were exquisitely susceptible to all fluoroquinolones, thus it was nearly impossible to find an AUC_{0-24}/MIC ratio < 250 and all of three fluoroquinolones demonstrated the bacterial eradication within 10.4 hours. There are data support about AUC_{0-24}/MIC ratio > 250 or Peak/MIC > 25:1 of fluoroquinolones demonstrated the rapid concentration-dependent killing, and bacterial eradication occurred within 24 hours.

Vesga and Craig studied the activity of levofloxacin against 7 strains of penicillin-resistant *S.pneumoniae* in normal and neutropenic mice. A sigmoid dose-response model was used to estimate the dose required to achieve a net bacteriostatic effect over 24 hours. The data yielded static (i.e., no net change in the numbers of surviving organisms) break point AUC_{0-24}/MIC of 59 in neutropenic animals and 23 in normal, non-neutropenic mice. From this data the AUC_{0-24}/MIC >100-125 was necessary to achieve a 3-log killing of the organism. In summary, it was relatively easy to derive 2 AUC_{0-24}/MIC targets from this mouse model, typically 20-60 for bacteriostatic (with contribution of white blood cells clearly apparent) and >100-250 for maximal effect (Vesga and Craig, 1996).

The higher value of AUC_{0-24}/MIC ratio of levofloxacin, gatifloxacin and moxifloxacin against gram-negative microorganisms in this study may be related to the inaccuracy of MIC measurements at very low concentrations. It is also possible that pharmacokinetic estimates at concentrations (lowest MIC, 0.006 μg/ml) more than 30-flod lower than the lower limits of assay detection (0.2 μg/ml) are inaccurate. This problem is difficult to account for in studies with compounds of this potency (Andes et al., 2003).

Although all three fluoroquinolones bind to serum proteins (30% protein binding for levofloxacin, 20% for gatifloxacin and up to 40% for moxifloxacin) (Wise et al., 1999), these pharmacodynamic was performed in the absence of serum proteins. While the presence of serum proteins may have altered the pharmacodynamics observed the impact should have been similar for all three fluoroquinolones. In contrast, one trial examined the effects of purulent material and protein binding on *S.pneumoniae* activity in the presence of moxifloxacin. The albumin content 50%, and on measure able impact was found in *S.pneumoniae* killing rates by moxifloxacin (Rubinstein et al., 2000). From this study, it may be hypothesized that any antibiotic whose MIC is not changed in vitro by albumin should not require correction of the AUC for the effect of serum protein binding. Study data of Ernst et al. also showed no evidence of any impact of serum protein binding of levofloxacin and moxifloxacin on the outcomes of *S.pneumoniae* in a murine pneumonia model (Ernst et al., 2002). However, in this study free AUC_{0-24} /MIC could be calculated as shown in table 5-1. The free AUC_{0-24} /MIC of these agents are still

higher than 125 for against gram-negative and still higher than 30 for against gram-positive microorganisms.

Table 5-1 The free AUC_{0-24} /MIC of levofloxacin, gatifloxacin and moxifloxacin against six strains of microorganisms.

Microorganisms	Free AUC_{0-24}/MIC					
	Single dose			Multiple doses		
	levofloxacin	gatifloxacin	moxifloxacin	levofloxacin	gatifloxacin	moxifloxacin
M.catarrhalis Bc.312	3160.73	1750.13	964.68	3038.46	2066.93	992.28
H.influenzae (Bc.38)	3500.46	15890.30	30252.0	3508.40	17789.70	34608.0
H.influenzae (Bc.38)	6324.23	3557.86	1938.24	6550.38	4328.40	1983.12
S.pneumoniae (94)	889.56	202.20	93.21	979.53	264.64	101.76
S.pneumoniae (38)	482.51	1200.28	94.90	493.78	120.52	98.48
S.pneumoniae (14)	47.04	207.29	1123.70	49.49	254.36	125.94

The previous data suggested that AUC_{0-24}/MIC ratio which was less than 100, might be able to select mutation in microorganisms (Coyle et al., 2001). In this study, the AUC_{0-24}/MIC ratio of levofloxacin against *S.pneumoniae*, which were penicillin resistance, was less than 100. This result suggested that this agent was appropriate for the treatment of sinusitis caused by penicillin-resistant *S.pneumoniae*. Thomas et al. study demonstrated that in ill patients with nosocomial lower respiratory tract infections who were treated with an antimicrobial with an AUC_{0-24}/MIC<100, about 40% of the patients carried *S.pneumoniae* with stepwise increased in MIC by day 4, and by day 20 about 80% of the isolates showed reduce susceptibility, whereas with an AUC_{0-24}/MIC>100, only 8% of pathogens developed resistance by 20 days after initiation of therapy (Thomas et al., 1998). Therefore, in clinical use of levofloxacin in the treatment of sinusitis causing by penicillin resistant *S.pneumoniae*, it is necessary to increase the dose of levofloxacin to coverage AUC_{0-24}/MIC above 100.

Moreover, the resistant study of levofloxacin, gatifloxacin and moxifloxacin should be performed based on the minimum prevention resistance concentration (MPC). This value could display the suitable concentrations of levofloxacin, gatifloxacin and moxifloxacin in the treatment of susceptible microorganisms without select mutation for

resistance. The MPC is a new measure of antibiotic potency above which a microbe must acquire 2 concurrent resistance mutation for growth. Blondeau and colleague have begun to explore the MPC. The MPC was defined and measured for 5 different fluoroquinolones with clinical isolates of *S.pneumoniae*. Based on their potential for restricting the selection of the resistant mutants, the order was moxifloxacin> gatifloxacin>levofloxacin. The suitable MPC of levofloxacin, gatifloxacin and moxifloxacin against *S.pneumoniae* were 8, 4 and 2 mg/ml, respectively (Blondeau et al., 2001). In this study, peak concentration of moxifloxacin (6.0 μg/ml for single dose and 6.4 μg/ml for multiple doses) and gatifloxacin (6.7 μg/ml for single dose and 8.1 μg/ml for multiple doses) are higher than their MPC value (2 and 4 μg/ml, respectively). From this data, not only oral moxifloxacin and gatifloxacin 400 mg were effective against microorganisms but also they could prevent the mutation of all microorganisms included in the study. In contrast, the peak concentration of levofloxacin was 6.9 μg/ml for single dose and 7.0 μg/ml for multiple doses may not be able to prevent the mutation of microorganisms because of its peak concentration lower than MPC (8 μg/ml).

$T_{99.9\%}$ and $T_{eradication}$ of levofloxacin, gatifloxacin and moxifloxacin against microorganisms in this study could be used as the guideline for physician to choose the effective agents in the treatment of sinusitis. The moxifloxacin was the most effective in the treatment of sinusitis because these agents could rapidly kill the gram-positive microorganisms and the use time of eradication was less than 4 hours. Although gatifloxacin could rapidly kill strains of *S.pneumoniae* but the eradication time were longer in the gatifloxacin than moxifloxacin by 4-6 hours. Levofloxacin was shown to have the least efficacy because $T_{eradication}$ of this agent against all strains was 6-10.5 hours.

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