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Effects of ciprofloxacin concentrations on the resistance of uropathogen *Escherichia coli*: *in vitro* kinetics and dynamics simulation model

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ABSTRACT

Ciprofloxacin is recommended for complicated urinary tract infection (UTIs) caused by multidrug-resistant pathogens included *Escherichia coli.* However, its optimum dose for UTIs remains uncertain that may cause the bacterial resistance. This study was conducted to evaluate the effects of ciprofloxacin concentrations on the resistance of *E. coli.* The *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) models of ciprofloxacin 750 mg oral dose twice a day for one daywas compared to that dose of 500 mg twice a day for three days.Pharmacokinetic parameters i.e.$AUC_{0\text{-}24}$ and C_{max}. and pharmacodynamic parameter i.e. MIC of ciprofloxacin against *E. coli* which previously had MIC of 0.5 µg/mL were determined. The PK/PD parameters combination of ciprofloxacin included $AUC_{0\text{-}24}$/MIC, C_{max}/MIC, and T>MIC ratio were used to evaluate its antimicrobial activities which was measured based on kill and re-growth rates of bacterial colony after the ciprofloxacin administration. The result showed that MIC value against *E. coli* increase to 8-16 and 32-64 µg/mL after ciprofloxacin 750 and 500 mg administration, respectively, indicating the emergence of resistance. Both doses of ciprofloxacin were able to reduce the number of bacterial colony in the first two hours administration. However, after two hours administration, those both doses could make re-growth of bacterial colony. The value of $AUC_{0\text{-}24}$/MIC (120.42±1.27 vs.92.62±9.36), C_{max}/MIC (4.75±0.21 vs. 3.26±0.30), and (T>MIC 89.58±7.22 vs. 76.39±9.39) after ciprofloxacin administration at dose of 750 mg were higher than those at dose of 500 mg. The increase of $AUC_{0\text{-}24}$/MIC and C_{max}/MIC values could reduce the number of bacteria colony, however could not for T>MIC value. In conclusion, the $AUC_{0\text{-}24}$/MIC and C_{max}/MIC parameters of ciprofloxacincan be used to evaluate its activity. In addition, ciprofloxacin twice per day at dose 500 mg for three days and 750 mg for one day are not different in the inhibition of *E. coli* resistance emergence.

ABSTRAK

Siprofloksasin direkomendasikan untuk infeksi saluran kemih dengan kompliaksi (ISK) yang disebabkan pathogen resistensi multipel obat termasuk *Escherichia coli.* Namun demikian, dosis optimumnya untuk ISK masih belum pasti sehingga kemungkinan dapat menyebabkan resistensi bakteri. Penelitian ini bertujuan untuk mengkaji efek kadar siprofloksasin pada resistensi *E. coli.* Model farmakokinetik dan farmakodinamik (PK/PD)*in vitro*siprofloksasin 750 mg dosis oral dua kali sehari satu hari dibandingkan dengan dosis dosis 500 mg dua kali sehari tiga hari. Parameter farmakokinetik yaitu $AUC_{0\text{-}24}$ dan C_{maks} dan parameter farmakodinamik yaitu MIC siprofloksasin terhadap *E. coli,* yang sebelumnyatelah diukur mempunyai nilai MIC 0,5 µg/mL. Kombinasi parameter PK/PD siprofloksasin yaitu rasio $AUC_{0\text{-}24}$/MIC, C_{max}/MIC, dan T>MIC digunakan untuk mengevaluasi aktivitas antimikrobialnya yang ditetapkan berdasarkan daya bunuh koloni bakteri setelah pemberian siprofloksasin. Hasil penelitian menunjukkan bahwa nilai MIC terhadap *E. coli* meningkat menjadi 8-16 dan 32-64 µg/mL setelah pemberian berturut-turut siprofloksasin dosis 750 dan 500 mg yang mengindikasikan munculnya resistensi. Kedua dosis siprofloksasin dapat menurunkan jumlah koloni bakteri pada dua jam pertama pemberian. Namun demikian, setelah dua jam pemberian kedua dosis siprofloksasin dapat membuat koloni bakteri tumbuh kembali. Nilai $AUC_{0\text{-}24}$/MIC (120,42±1,27 vs. 92,62±9,36), C_{max}/MIC (4,75±0,21 vs. 3,26±0,30), dan (T>MIC 89,58±7,22 vs. 76,39±9,39)setelah pemberian siprofloksasin dosis 750 mg lebih tinggi dari dosis 500 mg. Kenaikan nilai $AUC_{0\text{-}24}$/MIC dan C_{max}/MIC dapat menurunkan jumlah koloni bakteri, namun tidak untuk kenaikan nilai T>MIC. Dapat disimpulkan, siprofloksasin dua kali sehari dosis 500 mg selama tiga hari atau 750 mg sehari tidak berbeda dalam menghambat munculnya resistensi *E. coli.*

Keywords:
ciprofloxacin;
pharmacokinetic;
pharmacodynamics-*E. coli;*
resistance;

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INTRODUCTION

New antibiotic discovery and development have slowed alarmingly in recent decades. Otherwise, bacterial resistance to antimicrobial agents is a growing problem due to not optimal of the dose regimensof the antimicrobial agents. Therefore, effort to optimize the dose regimen of antimicrobial agentsis urgentlyneededinordertoobtainoptimal clinical benefit and minimize the risk of multidrug resistant bacterial pathogens.[1] To obtain the optimal of the dose regimen, linking the concentration-time course at the site of action and the antibiotic susceptibility (pharmacokinetic and pharmacodynamic relationship) should be considered.[2,3]

In vivo pharmacokinetic and pharmacodynamic relationship (PK/PD) models of an antibiotic could be conducted in animal. The *in vivo* PK/PD models provide similar growing conditions for bacteria, closely imitatingthe characteristics of a human infection, and clearly defining the endpoint of aninfection (cure or death) and comparable tothat in humans.[4] A significant disadvantage of animal modelsis differences in the PK, which limit sophisticated scaling methods for transferring data from animals to humans.[5]

In vitro PK/PD models have its own advantages. This models can be applied without influenced by bacterial phenotype at the site of infection, *in vivo* bacterial growth phase, host immunity, infection site, and pharmacokinetics of the antibiotics. In addition, this method allows resistance analyses, determination of time-kill behaviour, and identification and optimization of PK/PD indices and breakpoints.[3,6] Therefore, the *in vitro* PK/PD models are often applied before the *in vivo* PK/PD models conducted.

Urinary tract infection (UTI) is an infection that affects part of the unrinary tract. The UTIs occur more commonly in women than men, with half of women having at least one infectionin their lifetimes. The most common cause of UTIs is *Escherichia coli* which reaches 80% of UTI cases.[7] Amoxicillin has traditionally been a first-line antibiotic for UTI, however increased rates of *E. coli* resistance have made it a less acceptable choice. Fluoroquinolones is useful for UTIs caused by multidrug-resistantpathogens.[8] Ciprofloxacin has been recommended for complicated UTIs and pyelonephritis caused by *E. coli* in patients one to 17 years old.[9]

The dose regimens of ciprofloxacin for UTIs were investigated by some authors. However the optimum dose regimen of ciprofloxacin for UTIs remains uncertain. Ciprofloxacin can be administered in doses of 500 mg twice per day or 1000 mg once per day for seven days.[10] In addition, ciprofloxacin dose of 400-500 mg twice per day for 10-14 days has been recommended. Although in short-course therapy for seven days with the same dose of the ciprofloxacin is effective for the treatment of uncomplicated UTIs.[11,12]

Milo *et al.*[13] reported no difference in outcome regarding symptoms between 3-day and 5-10 day antibiotic regimens for uncomplicated UTI in women. However, the longer regimen was more common in adverse effects although it was more effective at eradicating bacteriuria. Futhermore, Lutters and Vogt-Ferrier[14] reported that resolution of short-term bacteriuria of UTI in older women was better in the longer course treatment group compared with the shorter term treatment group. However, there was no difference in long-term bacteriuria or clinical cure rate.

This study was conducted to evaluate the effects of ciprofloxacin

concentrations on the resistance of uropathogen *E. coli*. The *in vitro* PK/PD models of ciprofloxacin oral dose of 750 mg twice per day for one day was compared to thatdose of 500 mg twice per day for three days in this study.

MATERIALS AND METHODS

Strains and antibiotic

Escherichia coli strain isolated from urinary tract infection patients was used in this study. The uropathogenic *E. Coli* isolate was identified and cultured in Laboratory of Department of Microbiology, Faculty of Medicine, Universitas Muhammadiyah Semarang. Furthermore, susceptibility of the *E. coli* strain to ciprofloxacin was determined using broth dilution method. The *E. coli* strain was ciprofloxacin-sensitive strain with MIC value of 0.5mg/mL according to EUCAST. Ciprofloxacin was obtained from pharmaceutical companies PT Phapros Tbk Semarang, Indonesia. Ciprofloxacin stock solution was prepared with 4% NaOH in sterile distilled water and stored at 4°C before use.

MIC determination

The MIC of ciprofloxacin against uropathogenic *E. Coli* isolate was determined using the microdilution method according to the laboratory standard guideline. Bacteria from clones werepicked from freshly culture plates and then diluted with Meuller-Hinton Broth (MHB) liquidmedia to final concentration of 10^8CFU/mL. Serial dilutions of ciprofloxacin ranged from 0.008 to 32 µg/mLwere then prepared by diluting the stock solution with MHB media. One hundred uL of the diluted bacterial suspension was added to tube containing 1900 uL of the serial dilutions of ciprofloxacin to yield the appropriate density ($5x10^6$ CFU/mL) and the incubated for 18 to 24 h at 37°C. As control was a tube containing the diluted bacterial suspensions without ciprofloxacin. MICs were defined as the lowest concentration of ciprofloxacin that completely inhibits the growth of the organism as detected by the unaided eye.

In vitro kineticmodel

An *in vitro* kinetic one-compartmentmodel as described previously was used to simulate the serum ciprofloxacin-time curve in humans.[15,16] The model consists offresh medium reservoir, central compartment, andliquid waste storage compartment (FIGURE 1). The medium is pumped from the fresh medium reservoir to the central compartment by a peristaltic pump. A filter membrane with pore size of 0.45 µmis placed at the bottom of central compartment to prevent bacteria from flowing out with the medium. A magnetic stirrer is attached to the central compartment, ensuring homogenous mixing of the culture and preventing the membrane poresbeing blocked by bacteria. The central compartment has also sampling port to facilitate repeated sampling.During the experiments, the apparatuswas placed in a thermostatic room at 35°C. Fresh MHB medium pumped intothe central compartment displaces an equal volume of liquid, which flows out through and enters the waste medium container.

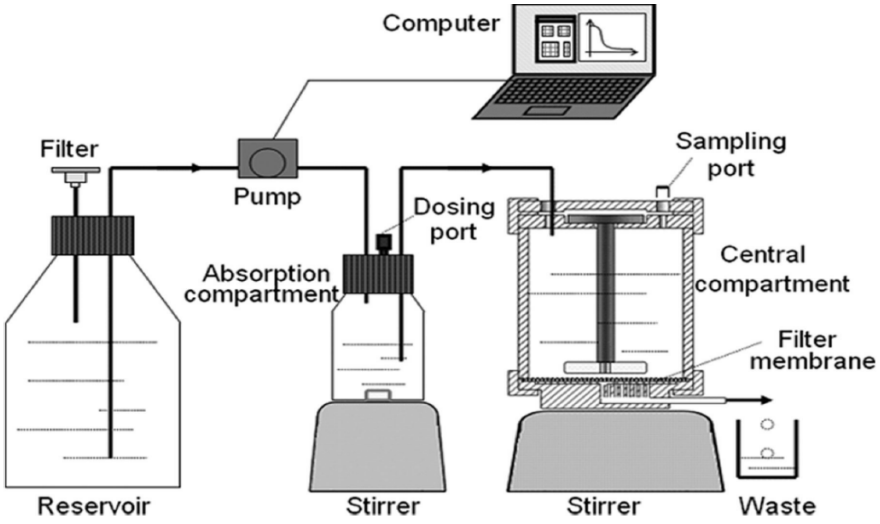


FIGURE 1. Equipment for *in vitro* kinetic model study

Ciprofloxacin added to the central compartment was diluted according to the first order kinetics $C = C_{max} \times e^{-2kt}$, where C was the ciprofloxacin concentration at time t, C_{max} was the initial antibiotic concentration, k was the rate of elimination and t was the time elapsed since cirpofloxacin addition. Pharmacokinetics in healthy volunteers receiving oral dose of ciprofloxacin (750mg twice per day for one day and 500mg twice per day for three days) were simulated in this study.

In vitro PK/PD study of ciprofloxacin

The culture of *E. coli* isolates in concentration of 5 x 10^8 CFU was added to the central compartment containing fresh MHB medium and then incubated at 35°C for three days. Followed after this incubation period, the ciprofloxacin at concentration corresponding to a dose of 750mg twice per day for one day or 500mg twice per day for three days were added into the central compartment and the pump was turned on.

For the PD analysis, a 200µL sample was then taken through the sampling port at the following time points: 0, 2,7,12,15, and 24h for the dose of ciprofloxacin of 750mg and 0, 2, 7, 12, 24, 36, 48, and 72 h for the dose of ciprofloxacin of 500mg. Samples were properly diluted with MHB, and then 10µL aliquots of the diluted samples were poured on to Meuller-Histon agar (MHA) plates and incubated at 35°C for 24 h. After incubation, colonies of the samples were counted and the log-transformed colony counts (y axis) obtained were plotted against time (x axis) for the time-kill curve.

For PK analysis, a 200µL sample was taken at the following time points: 0, 0.5, 1, 1.5, 2, 4, 7, 11, 12, 12.5, 13, 13.5, 15, 23, 24, 26 and 28 h for the dose of ciprofloxacin of 750 mg and 0, 0.5, 1, 1.5, 2, 4, 7, 11, 12, 12.5, 13, 13.5, 15, 23, 24, 24.5, 25, 25.5, 36, 36.5, 37, 37.5, 48, 48.5, 49, 49.5, 60, 60.5, 61, 61.5 and 72 h for the dose of ciprofloxacin of 500mg. The sample was stored at 2-8°C until the ciprofloxacin concentration was determined.

Determination of ciprofloxacin concentration

The determination of ciprofloxacin concentration was performed using a high performance liquid chromathography (HPLC) method. The HPLC instrument (Shimadzu, Kyoto, Japan) was equipped with a model series LC-10 AD VP, Rheodyne 7725i injector with a 100µL loop and SPD-10A UV-

Visible detector. A reversed-phase C_{18} column (Eurospher; 4.6µm, 250mm x 4.6mm i.d) was used as the stationary phase for separation and quantitation. The mobile phase consisted of phosphate buffer-acetonitrile-triethylamine (65:35:0.6 v/v/v) pH adjusted to 3.0 ± 0.05 with phosphoric acid. A flow rate of 0.8mL/min was maintained. The injection volume was 20µL. The detector was set at 275nm. A 200µL sample was precipitated with 200µL acetonotrile and then was vortexed for one min. After centrifugation at 5000 rpm for 10 min, the supernatant was transferred to a 2-mL polyethylene tube and 20µL of the supernatant was injected into the HPLC system. All assays were conducted in triplicate and the correlation coefficient for standard curves was always ≥0.99. The lower limit of quantitation was 4.12µg/mL and the coefficient of variation was 7.06%.

PK/PD analysis

The pharmacokinetic data included C_{max} (the maximum concentration of ciprofloxacin in central compartment), AUC_{0-24} (the area under the concentration-time curve for ciprofloxacin from h 0 to 24), T>MIC (the percentage of a 24-h period in which the ciprofloxacin concentration exceeds the MIC) were calculated using standard method. Furthermore, the PK/PD parameters included C_{max}/MIC, AUC_{0-24}/MIC and T>MIC were determined using the pharmacokinetic values and MIC data in each experiment. The relationship between the PK/PD parameters and a log *E. coli* colony at final phase of growth rate as well as of final phase of the kill rate were evaluated.

Data were expressed as the mean ± standard deviation (SD). Statistical comparisons were conducted using Student's t-test. The differences between the parameters after ciprofloxacin oral dose of 750mg and 500mg were considered significant at a value of <0.05.

RESULTS

MIC of ciprofloxacin against *E. coli* isolates

The original MIC of ciprofloxacin against uropathogenic *E. coli* isolates was 0.5µg/mL. With this MIC value, the isolates was considered susceptible to ciprofloxacin. After ciprofloxacin addition at concentration corresponding to a doseof 750mg twice per day for one dayor 500mg twice per day for three days, the isolates developed loss of susceptibility with MICs ranging from 8.0 to 16.0µg/mL and from 32.0 to 256.0µg/mL, respectively.

***In vitro* pharmacokinetic profile**

In vitro pharmacokinetic profile of ciprofloxacin at dose of 750mg twice per day for one day and at dose of 500mg twice per day for three days is presented in FIGURE 2, where as their pharmacokinetic values are presented in TABLE 1.

TABLE 1. Pharmacokinetic parameters (mean ± SD) of ciprofloxacin at dose of 750 and 500mg

Parameter	Dose 750 mg	Dose 250 mg
C_{max} (µg/mL)	2.25 ± 0.150	1.84 ± 0.106
T_{max} (h)	1.58 ± 0.200	1.53 ± 0.128
$t_{1/2}$ (h)	4.69 ± 0.625	5.41 ± 0.625
AUC_{0-24} (µg/mL. h)	59.25 ± 1.72	43.29 ± 6.19

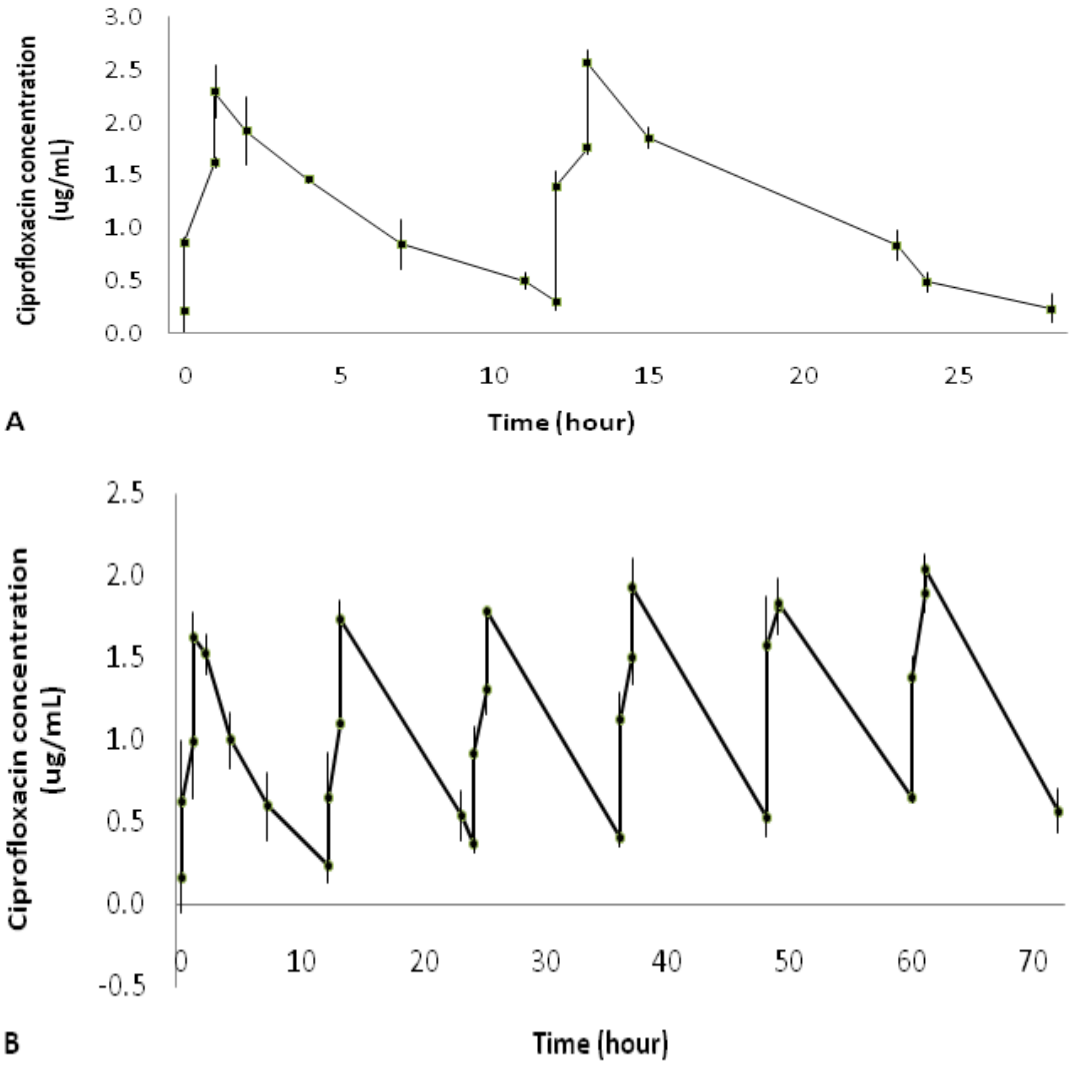


FIGURE 2. *In vitro* pharmacokinetic profile of ciprofloxacin at A) dose of 750mg twice per day for one day and B) dose of 500mg twice per day for three days

In vitro pharmacodynamic profile

In vitro pharmacodynamic profile of ciprofloxacin at dose of 750mg twice per day for one day and at dose of 500mg twice per day for three days as well as control is presented in FIGURE 3. The number of bacterial colonies significantly decreased at the first two hours after ciprofloxacin administration at doses of 750 and 500mg and then significantly increased two hours after ciprofloxacin administration ($p<0.05$). Moreover, the *E. coli* growth rate after ciprofloxacin at dose of 750 mg was significantly lower than that at dose of 500mg ($p<0.05$). In contrast, the number of bacterial colonies significantly increased during control administration. It was indicated that the *E. coli* kill rate phase is observed at the first two hours, where as the *E. coli* re-growth rate phase is observed after two hours of ciprofloxacin administration.

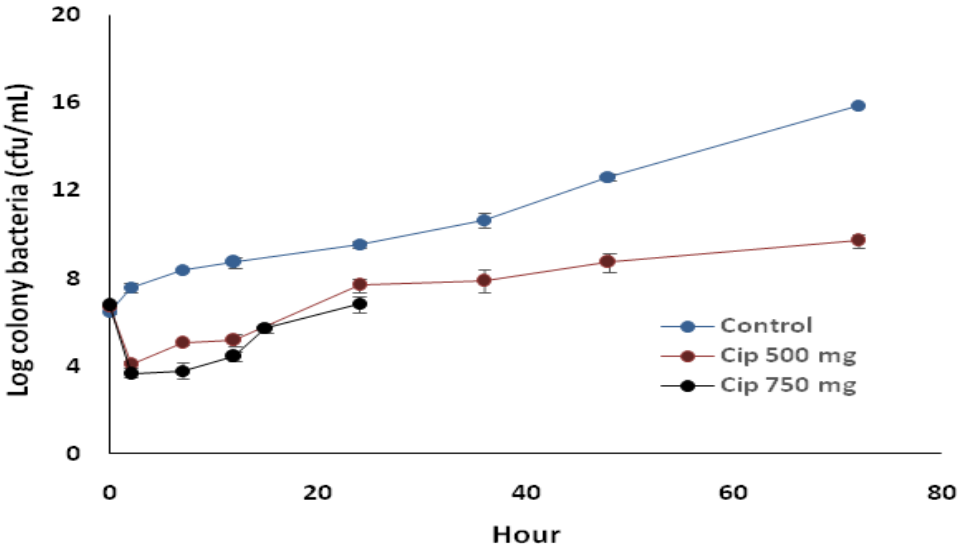


FIGURE 3. Changes in the number of log *E. coli* colony vs. time in the control treatment, and after the ciprofloxacin administration at doses of 750 and 500mg.

TABLE 2. Kill rate and growth rate slope (mean ± SD) after the control or the ciprofloxacin administration at dose of 500mg twice day for 3 days, or 750mg twice day for 1 day

Control	Dose of 500 mg		Dose of 750 mg	
	Kill rate slope 2 hours	Regrowth rate slope on 3 days	Kill rate slope 2 hours	Regrowth rate slope on 1 day
0.098	-1.31	0.063	-1.51	0.034
0.104	-1.32	0.079	-1.60	0.045
0.104	-1.36	0.076	-1.60	0.035
0.102±0.003	-1.33±0.026	0.073±0.009	-1.57±0.05	0.038±0.006

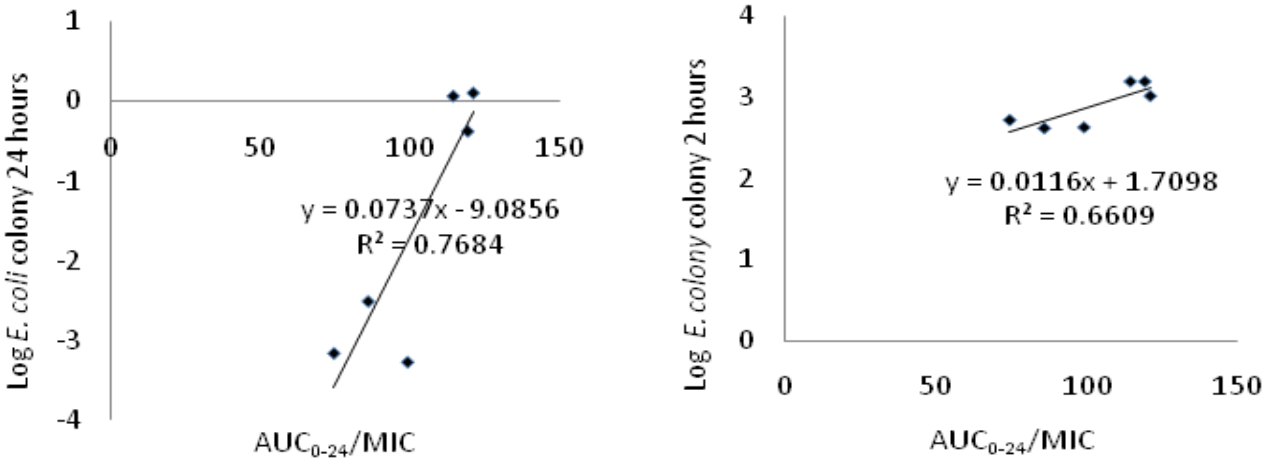
The kill rate slope 2 hours and re-growth rate slope on 1 day were calculated and used as *in vitro* of pharmacodynamic parameters to describe antimicrobial activities. The results are presented in TABLE 2.

To investigate the PK/PD relationship, the PK/PD parameters combination of ciprofloxacin included AUC_{0-24}/MIC, C_{max}/MIC, and T>MIC ratio and its antimicrobial activities included the kill rate slope 2 hours and re-growth rate slope on 1 day were analyzed. The results are presented in TABLE 3-5 and FIGURE 4-6.

A positive relationship between AUC_{0-24}/MIC with the number of log *E. coli* colony at the final phases of growth rateand kill rate was observed (TABLE 3 and FIGURE 4). The increase of the $AUC_{0-24/}$MIC ratio might increase the antimicrobial activity of ciprofloxacin as indicated by greater decreased in the number of log *E. coli* colony.

TABLE 3. AUC_{0-24}/MIC parameters of dose of ciprofloxacin 500 and 750mg and the decrease or increase in the number of log *E. coli* colony kill rate and growth rate phases

Ciprofloxacin	AUC_{0-24}/MIC	Log colony decrease in kill ratephase	Log colony increase/ decrease in growth ratephase
Dose 500mg			
1	86.00	2.62	-2.51
2	99.24	2.63	-3.27
3	74.48	2.72	-3.16
Mean ± SD	92.62±9.36	2.625±0.007	-2.89±0.537
Dose 750mg			
1	121.32	3.02	0.10
2	119.52	3.20	-0.38
3	114.66	3.20	0.06
Mean ± SD	120.42±1.27	3.11±0.13	-0.14±0.34



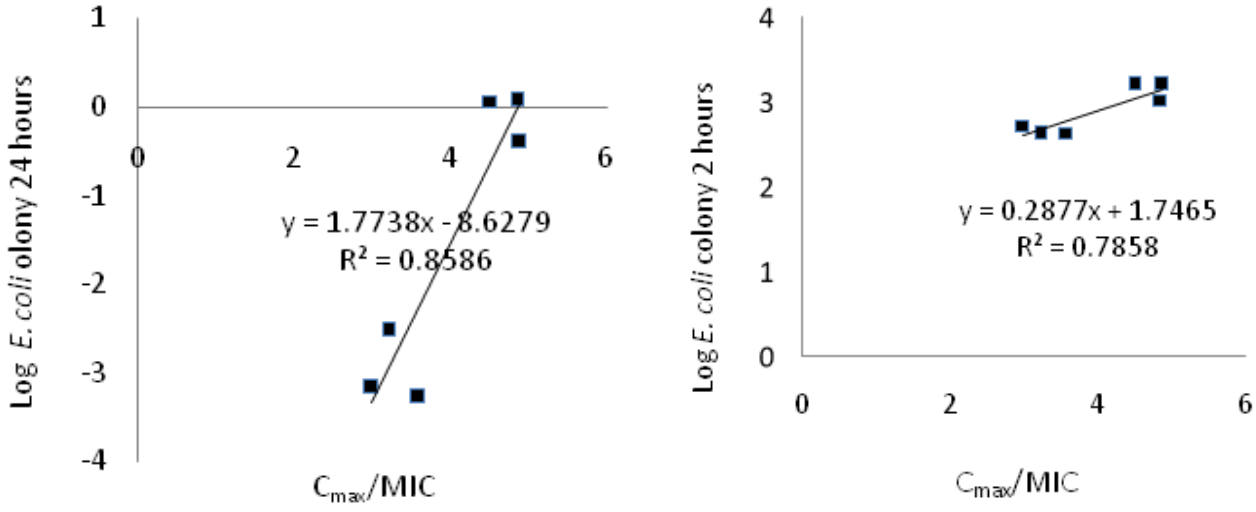
(A) (B)

FIGURE 4. Linear regression relationship between AUC_{0-24}/MIC and the number of log *E. coli* colony at final phase of the growth rate (A) Linear regression relationship between AUC_{0-24}/MIC and the number of log *E. coli* colony at final phase of the kill rate (B)

A positive relationship between Cmax/MIC with the number of log *E. coli* colony at the final phases of growth rate and kill rate was also observed (TABLE 4 and FIGURE 5). However, significantly different in the phase of kill rate at 2 hours after ciprofloxacin administration at dose of 750mg and that of 500mg was observed. The Cmax at dose of 750mg was significantly higher than that at dose of 500mg resulting higher Cmax/ MIC ratio and higher antimicrobial activity as demonstrated by the greater decrease in the number of log E. coli colony in the first 2 hours ciprofloxacin administration.

TABLE 4. Cmax/MIC parameters of dose of ciprofloxacin 500 and 750mg and the value respectively decrease or increase in the number of log *E. coli* colony kill rate and growth rate phases

Ciprofloxacin	C_{max}/MIC	Log colony decrease in kill ratephase	Log colony increase/ decrease in growth rate phase
Dose 500mg			
1	3.22	2.62	-2.51
2	3.58	2.63	-3.27
3	2.98	2.72	-3.16
Mean ± SD	3.26±0.30	2.66±0.05	-2.98±0.41
Dose 750mg			
1	4.86	3.02	0.10
2	4.88	3.20	-0.38
3	4.50	3.20	0.06
Mean ± SD	4.75±0.21	3.14±0.10	-0.07±0.27

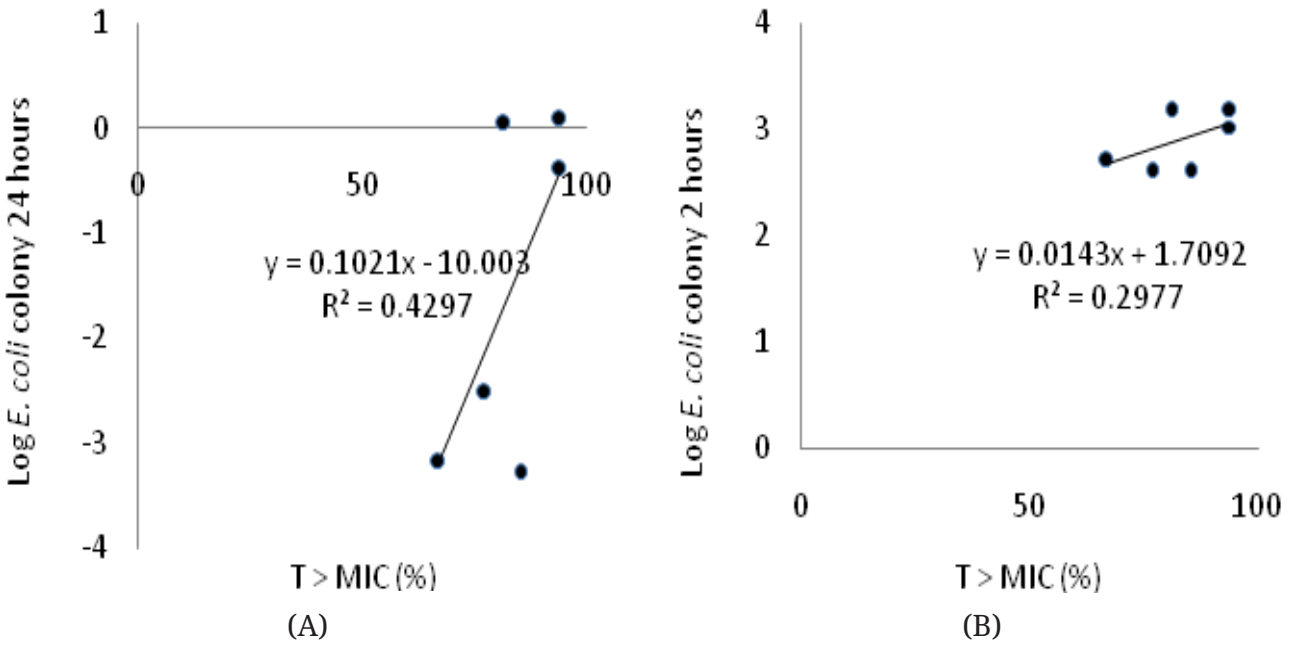


(A) (B)

FIGURE 5. Linear regression relationship between C_{max}/MIC and the number of log *E. coli* colony at final phase of the growth rate (A) Linear regression relationship between AUC_{0-24}/MIC and the number of log *E. coli* colony at final phase of the kill rate (B)

TABLE 5. T>MIC parameters of dose of ciprofloxacin 500 and 750mg and the value respectively decrease or increase in the number of log *E. coli* colony kill rate and growth rate phases

Ciprofloxacin	T>MIC	Log colony decrease in kill ratephase	Log colony increase/ decrease in growth rate phase
Dose 500mg			
1	77.08	2.62	-2.51
2	85.41	2.63	-3.27
3	66.67	2.72	-3.16
Mean ± SD	76.39±9.39	2.66±0.05	-2.98±0.41
Dose 750mg			
1	93.75	3.02	0.10
2	93.75	3.20	-0.38
3	81.25	3.20	0.06
Mean ± SD	89.58±7.22	3.14±0.10	-0.07±0.27



(A) (B)

FIGURE 6. Linear regression relationship between T>MIC and the number of log *E. coli* colony at final phase of the growth rate (A) Linear regression relationship between AUC0-24/ MIC and the number of log *E. coli* colony at final phase of the kill rate (B)

DISCUSSION

The result of the PK/PD parameters relationship analysis showed the emergence of *E. coli* resistance after ciprofloxacin at dose of 750mg twice a day for one day or at dose of 500mg twice a day for three days. It was demonstrated by the increase of MIC value against *E. Coli* to be 8-16 and 32-64µg/mL after ciprofloxacin 750 and 500mg administration, respectively. The both doses of ciprofloxacin administration could notinhibit the emergence of *E. coli* resistance. Although, the increase of MIC value ciprofloxacin after administration at dose 750mg was lower that at dose 500mg.

The *in vitro* PK/PD model to evaluate antimicrobial activity of ciprofloxacin against *E. coli* has been reported previously. Fantin *et al.*[15] reported that the resistant commensal *E. coli* from fecal and pharyngeal emerge during treatment with six dose of ciprofloxacin. However, the resistance emergence is not associated with the PK/PD parameters. The different of AUC/MIC, C_{max}/MIC, and T>MIC ratio values does not affect the emergence of *E. coli* resistance which not indicate significantly different across the different ciprofloxacin dosages. Khan *et al.*[16] developed an *in vitro* PK/PD model describing killing kinetics for *E. coli* following exposure to ciprofloxacin. This model successfully characterizes time-kill curve for both will type and the six *E. coli* mutants based on the MIC of ciprofloxacin.

Antibiotic resistance can occur due to three main mechanisms as follow 1) the transfer of resistant genes from resistant to susceptible micro organisms; 2) genetic adaptation (changing the drug target); and 3) phenotype adaptation, such as modification of efflux pumps. Resistance mechanisms of genetic and phenotypic adaptation can be triggered by de novo resistance that occurs quickly during therapy.[17,18]

Escherichia coli could be resistant to multiple antibiotics during minimal exposure of antibiotics levels due to horizontal gene transfer (HGT) of resistant strains to susceptible strains. Resistance is often associated with a reduction in 'fitness' of bacteria, which means the ability of a genotype or individuals of bacteria to survive and reproduce. Decreased use of antibiotics will reduce the environmental pressure on the bacteria and reduce the amount of acquired resistance. The speed of bacterial resistance is affected by the speed of de novo mutations and HGT from carrier of resistance determinants. The most frequent mutation is to change the target action of antibiotics and increasing antibiotic efflux. Gene amplification, decreased gene expression of the target antibiotics, and changes enzymes for the drugs modification are other mechanisms involving in microbial resistance. HGT-related mechanisms include modification of the drug itself, the target protection, bypass resistance (change in metabolic phase that inhibited by antibiotic), and the acquisition of efflux pumps. The increase MIC value to ≥ 32µg/mL against *E. coli* is associated with mutations of *gyr*A and *par*C or a combination mutation of *mar*R, *gyr*A, and *par*C. Where as, the MIC value < 1µg/mL is associated with *gyr*A gene mutation alone or mutation combination of *mar*R and *gyr*A.[19] In addition, it was reported that de novo *E. coli* resistance is associated with the sub optimal concentration due to enfloksasin use as demonstrated by the increase of its MIC value.[20] Some studies reported that the ratio of C_{max}/MIC and AUC_{0-24}/MIC should reach between 10-12 and 100-125, respectively to achieve therapeutic efficacy of fluoroquinolones against Gram – bacilli infections and to prevent emergence of resistance.[21] The emergence of Gram-bacilli resistance 80% to ciprofloxacin was reported when the ratio of C_{max}/MIC and AUC_{0-24}/MIC < 8 and < 100, respectively. This emergence of resistance decreased to be 10% (8 time

prevention), if the ratio of C_{max}/MIC and AUC_{0-24}/MIC > 8 and ≥ 100, respectively.[22] Other study reported that the ratio of AUC_{0-24}/MIC <100 cause 86% of developed patient resistance, however if the ratio ≥ 100, the incidence of resistance decrease to be 9%. The emergence of resistance is an important factor ofa clinical failure therapy.[23] It was reported that the ratio AUC_{0-24}/MIC should be >250 to provide the rapid Gram-bacilli elimination.[24]

In this study, the ratio of Cmax/MIC was < 8 after administration of ciprofloxacin at dose of 500 and 750mg. Although the ratioof AUC_{0-24}/MIC was >100 at dose of 750mg, it could not prevent the emergence of uropathogen *E. coli* resistance. Moreover, a regrowth rate phase of uropathogen *E. coli* was still observed at dose of 750mg.

Zelenitsky and Ariano[25] reported that among 178 *Enterobacteriacea* infection cases treated with ciprofloxacin, 8 cases had failed therapy with 3 cases of them (37.5%) were infection of uropathogen *E. coli.* The treatment failure might be due to low the ratio of AUC_{0-24}/MIC of ciprofloxacin. The ratio of AUC_{0-24}/MIC≥ 250 indicated the success of therapy (success rate 91.4%). If the ratio decreased to be< 250, the risk of treatment failure increased 27.8 times higher.

CONCLUSION

In conclusion, the AUC_{0-24}/MIC and C_{max}/MIC parameters of ciprofloxacin can be used to evaluate its antimicrobial activity against uropathogen *E. coli.* In addition, ciprofloxacin twice per day at dose 500mg for three days and 750mg for one day are not different in the inhibition of *E. coli* resistance emergence.

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