Evaluation of antibacterial activities of flomoxef against ESBL producing Enterobacteriaceae analyzed by Monte Carlo Simulation

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The growing number of infection caused by extended-spectrum *β*-lactamase (ESBL) producing pathogens has prompted a more rational use of available antibiotics because of the paucity of new, effective agents. Flomoxef (FMOX) is one of the *β*-lactam antibiotic which is stable against *β*-lactamase. In this study, the antibacterial activity of FMOX was investigated, and Monte Carlo Simulation was conducted to determine the appropriate dosing regimens of FMOX based on the probability of target attainment (TA%) at the critical drug exposure metric of time that drug concentrations remain above 40% (showing bacteriostatic effect) or 70% (showing bactericidal effect) of time during which plasma concentration above minimum inhibitory concentration (MIC) of the drug ($T_{>MIC}$) against the ESBL producing Enterobacteriaceae. The effective regimens to achieve 80% of TA% at 70% of $T_{>MIC}$ were 1 g every 8 hours with 2–4 hours infusion, and 1 g every 6 hours with 1–4 hours infusion. Moreover, all the tested regimens were effective to achieve 80% of TA% at 40% of $T_{>MIC}$. These results of pharmacokinetics/pharmacodynamics (PK/PD) modeling showed the potential efficacy of FMOX against bacterial infections caused by ESBL producing Enterobacteriaceae.

Introduction

Extended-spectrum *β*-lactamase (ESBL) is spreading among Enterobacteriaceae. Associated infectious syndromes include the infections following a surgical procedure, urinary tract infections, bloodstream and intra-abdominal infections. The rates of ESBL-expression among nosocomial Enterobacteriaceae isolates, particularly *Klebsiella pneumoniae*, have risen substantially in several countries[1–3]. In a recent study based on the Tigecycline Evaluation and Surveillance Trial

(TEST) global surveillance database, the rate of ESBL production among the *K. pneumoniae* and the *Escherichia coli* isolates collected in Latin America were 44.0% and 13.5%, respectively[4]. The increasing rate of ESBL producing Enterobacteriaceae in Japan has been also reported[5–8].

Available therapeutic options for the treatment of ESBL-associated infections are limited by drug resistance conferred by the ESBL[9]. Therefore, it is important to verify whether the dosage and administration schedule of the currently available antibiotics are appropriate to cure infection, minimize safety risks, and curb the emergence of antibiotic resistance[10].

Pharmacokinetics/pharmacodynamics (PK/PD) has been investigated to maximize clinical response and to minimize the emergence of antibiotic resistance and exposure-related toxicities[11,12]. For cephalosporin antibiotics, the fraction of time during the dosing interval that drug concentration remains above its MIC for the infecting pathogen ($T_{>MIC}$) is the target that best relates to patient outcomes[11,12]. Although $T_{>MIC}$ is decided by PK of the antibiotics and MIC of the bacteria, both of them are variable among cases, and it is not always possible to obtain the information at the time of administration. Therefore, Monte Carlo Simulation has been applied to predict the outcome of the antibiotic treatment[13–19]. With the Monte Carlo Simulation, distribution of PK and MIC are processed to obtain a set of virtual population, and the various regimens are applied to the set of virtual population to calculate the probability of target attainment (TA%) at $T_{>MIC}$ for the dosage interval. This simulation can predict the outcome of various dosage and administration schedule, which helps us to choose the appropriate regimens for the treatment.

Flomoxef (FMOX) is one of the cephamycins, characterized by its 7-methoxy, and also called 1-oxacephem antibiotic different structurally from the cephalosporins and other cephamycins, in which sulfur molecule in the cephem nucleus is substituted with oxygen molecule. It has potent *in vitro* activity against Gram-negative organisms with broad spectrum, and was stable in the presence of *β*-lactamase and active against cephalosporin-resistant strains. Although FMOX has a potential to be effective against *β*-lactamase producing strains, there is little information for prediction of efficacy against ESBL producing Enterobacteriaceae in the current clinical use. Therefore, it is important to understand the characteristics of FMOX with PK/PD analysis as well as *in vitro* antibacterial activities against emerging ESBL such as SHV, TEM, and CTX-M. The objective of this study was to investigate the potency of FMOX for the treatment of the infection caused by ESBL producing Enterobacteriaceae in the clinical use by *in vitro* activities and Monte-Carlo Simulation.

I. Materials and Methods

1. Antimicrobial agents

Susceptibilities of the bacterial strains were tested with the following antimicrobial agents: flomoxef (FMOX), cefmetazole (CMZ), ceftriaxone (CTRX), cefotiam (CTM), cefazolin (CEZ),

cefepime (CFPM), piperacillin-tazobactam (piperacillin with 4μg/mL of tazobactam, PIPC/TAZ), imipenem (IPM), and doripenem (DRPM).

2. Bacterial strains

Thirty-one isolates were used in this study. Ten isolates obtained from ATCC include 4 isolates of TEM producing *E. coli*, 4 isolates of SHV producing *E. coli*, an isolate of SHV producing *K. pneumoniae*, and an isolate of SHV producing *K. oxytoca.* Twenty-one clinical isolates were collected from 1992 to 2002 in 12 medical facilities in Japan. They include 3 isolates of CTX-M producing *E. coli*, 6 isolates of CTX-M producing *K. pneumoniae*, an isolate of SHV producing *K. pneumoniae*, 6 isolates of CTX-M producing *K. oxytoca*, 4 isolates of CTX-M producing *Proteus mirabilis*, and an isolate of TEM producing *P. mirabilis*.

3. MIC determination

The antimicrobial susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) standards M07-A8 for the micro-broth dilution method[20,21]. Briefly, 5×10^4 CFU/well was inoculated in cation-adjusted Mueller Hinton Broth containing drugs, and incubated at 35℃ for 16 to 20 hours. The MIC was defined as the lowest concentration of antimicrobial agent that completely inhibited bacterial growth as detected by the unaided eye.

4. Monte Carlo Simulation

Monte Carlo Simulation was employed to investigate the probability of target attainment (TA%) against the clinical isolates of ESBL producing strains. Virtual populations of 5,000 patients were created for each dosage regimen against each bacterial strain. Pharmacokinetic parameters of FMOX were taken from the Phase 1 studies in Japan[22]. Pharmacokinetic parameters of FMOX for typical values of two-compartment model were as follows: Vc＝7.14 L, k_{10}＝2.12 hr^{-1}, k_{12}＝2.45 hr^{-1}, and k_{21}＝2.57 hr^{-1}. A log-normal distribution was assumed for the inter-individual variability for pharmacokinetic parameters with 20% of coefficient of variation. It was assumed that free plasma concentration of FMOX would correlate with the bactericidal effect, and free drug concentrations were used for the simulation based on a plasma protein binding ratio of 35%[22]. MIC values were generated from the discrete MIC distribution obtained from 31 isolates. The regimens used for the simulation were 1 g every 6, 8, or 12 hours (q6h, q8h, or q12h, respectively) with 1 to 4 hours infusion, and 2 g q12h with 1 to 4 hours infusion (Table 1). TA% was calculated based on the simulated plasma concentrations at 1-minute interval by using two-compartment model. The TA% at various $T_{>MIC}$ for the dosage interval was calculated by using 5,000 virtual patients for each dosing regimen against each bacteria strain.

Table 1. Dosing regimen of flomoxef for Monte Carlo Simulation.

Total dosage (g/day)	Dosage (g)	Number of dosage	Infusion time (h)
2	1	2	1
			2
			3
			4
4	2	2	1
			2
			3
			4
3	1	3	1
			2
			3
			4
4	1	4	1
			2
			3
			4

II. Results

1. *In vitro* activity of FMOX against ESBL producing strains

Table 2 shows *in vitro* activity of antimicrobial agents including FMOX. FMOX showed potent *in vitro* activity against ESBL producing strains whose MIC_{90} was 0.5 μg/mL. FMOX was potent against all the tested type of ESBL such as TEM, SHV, and CTX-M. DRPM also showed potent *in vitro* activity whose MIC_{90} was 0.25 μg/mL. MIC_{90} of CFPM, CTRX, CTM, CEZ, and PIPC/TAZ against ESBL producing strains were 128 or $>$128 μg/mL. MIC of FMOX against most of the tested strains were 0.5 or $<$0.5 μg/mL. There was only a strain whose MIC was 2 μg/mL which was SHV-2 producing *E. coli* (Table 3).

2. Prediction of clinical efficacy by Monte Carlo Simulation with the regimen of 1 g q12h with 1 to 4 hours infusion

Table 4 and Figure 1 show TA% at each $T_{>MIC}$ calculated by Monte Carlo Simulation with the regimen of 1 g q12h with 1 to 4 hours infusion against ESBL producing strains. The TA% achieved more than 80% with 1 to 4 hours infusion, and achieved more than 90% with 2 to 4 hours infusion at $T_{>MIC}$=40%. At $T_{>MIC}$=70%, even though TA% increased with the increase of

Table 2. MIC of flomoxef and other antimicrobial agents against clinical isolates of ESBL producing Enterobacteriaceae.

			MIC (μg/mL)								
Species	Isolate	β-lactamase	Flomoxef	Cefmetazole	Ceftriaxone	Cefotiam	Cefazolin	Cefepime	Piperacillin/ Tazobactam	Imipenem	Doripenem
E. coli	BAA-201	TEM-3	0.125	2	16	4	64	2	4	0.25	0.063
E. coli	BAA-196	TEM-10	0.25	2	2	2	16	2	8	0.25	0.063
E. coli	BAA-197	TEM-12	0.5	4	0.5	1	16	4	8	0.5	0.063
E. coli	BAA-198	TEM-26	0.125	2	2	1	8	2	4	0.25	0.063
E. coli	BAA-204	SHV-2	2	16	128	64	>128	64	>128	0.25	0.063
E. coli	BAA-199	SHV-3	0.125	1	128	32	>128	4	>128	0.25	0.031
E. coli	BAA-200	SHV-4	0.063	1	128	32	>128	8	4	0.063	0.016
E. coli	BAA-203	SHV-5	0.25	2	64	32	>128	8	32	0.5	0.125
E. coli	SR21003	CTX-M-14	0.5	8	>128	>128	>128	16	8	0.063	0.016
E. coli	SR21182	CTX-M-14	0.5	8	>128	>128	>128	>128	32	0.125	0.031
E. coli	SR21266	CTX-M-14	0.063	4	>128	>128	>128	8	1	0.5	0.063

Tazobactam was added with 4 μg/mL

Table 2. MIC of flomoxef and other antimicrobial agents against clinical isolates of ESBL producing Enterobacteriaceae. (Continued)

			MIC (μg/mL)								
Species	Isolate	β-lactamase	Flomoxef	Cefmetazole	Ceftriaxone	Cefotiam	Cefazolin	Cefepime	Piperacillin/ Tazobactam	Imipenem	Doripenem
K. pneumoniae	ATCC700603	SHV-18	0.5	8	16	32	128	1	8	0.125	0.063
K. pneumoniae	SR13708	CTX-M-3	0.063	1	>128	>128	>128	16	2	0.25	0.063
K. pneumoniae	SR13740	CTX-M-3	0.063	1	>128	>128	>128	>128	2	0.5	0.063
K. pneumoniae	SR14915	CTX-M-3 & -44 like	0.063	1	>128	64	>128	1	2	0.125	0.031
K. pneumoniae	SR14933	SHV-3	0.125	1	32	16	64	2	4	0.125	0.063
K. pneumoniae	SR14935	CTX-M-3	0.125	1	>128	>128	>128	>128	2	0.25	0.031
K. pneumoniae	SR22015	CTX-M type	0.25	4	>128	>128	>128	128	16	0.125	0.031
K. pneumoniae	SR22138	CTX-M type	0.063	1	>128	>128	>128	1	>128	0.25	0.063
K. oxytoca	ATCC51983	SHV-5	0.063	1	32	16	128	1	2	0.25	0.031
K. oxytoca	SR13552	CTX-M type	0.125	4	>128	>128	>128	128	>128	0.25	0.063
K. oxytoca	SR18750	CTX-M type	0.5	4	8	16	>128	0.5	>128	0.25	0.063
K. oxytoca	SR18754	CTX-M type	0.063	2	>128	>128	>128	4	>128	0.25	0.063
K. oxytoca	SR21502	CTX-M-3	0.063	1	>128	>128	>128	128	1	0.25	0.063
K. oxytoca	SR21583	CTX-M type	0.063	1	>128	>128	>128	2	>128	0.25	0.063
K. oxytoca	SR21592	CTX-M type	0.125	2	>128	>128	>128	32	>128	0.5	0.125

Tazobactam was added with 4 μg/mL

Table 2. MIC of flomoxef and other antimicrobial agents against clinical isolates of ESBL producing Enterobacteriaceae. (Continued)

			MIC (μg/mL)								
Species	Isolate	β-lactamase	Flomoxef	Cefmetazole	Ceftriaxone	Cefotiam	Cefazolin	Cefepime	Piperacillin/ Tazobactam	Imipenem	Doripenem
P. mirabilis	SR22765	TEM-19 like	0.25	2	8	64	>128	2	1	2	0.25
P. mirabilis	SR22790	CTX-M type	0.5	2	>128	>128	>128	16	0.25	2	0.5
P. mirabilis	SR22798	CTX-M type	0.5	2	>128	>128	>128	16	0.25	2	0.25
P. mirabilis	SR13993	CTX-M type	0.5	2	>128	>128	>128	16	0.5	2	0.25
P. mirabilis	SR22754	CTX-M type	0.5	2	>128	>128	>128	16	0.5	2	0.25
MIC Range			0.063 - 2	1 - 16	0.5 - >128	1 - >128	8 - >128	0.5 - >128	0.25 - >128	0.063 - 2	0.016 - 0.5
MIC_{50}			0.125	2	>128	>128	>128	8	4	0.25	0.063
MIC_{90}			0.5	8	>128	>128	>128	128	>128	2	0.25

Tazobactam was added with 4 μg/mL

Table 3. MIC distribution of flomoxef against ESBL producing Enterobacteriaceae.

	MIC (μg/mL)								MIC_{50}	MIC_{90}
	0.031	0.063	0.125	0.25	0.5	1	2	4		
E. coli		2	3	2	3		1			
K. pneumoniae		4	2	1	1					
K. oxytoca		4	2		1					
P. mirabilis				1	4					
Total		10	7	4	9		1		0.125	0.5

Table 4. Probability of target attainment at each dosing regimen.

			Probability of target attainment (%)	
Dosage amount (g)	Number of dosage	Infusion time (h)	$T_{>MIC}$ = 40%	$T_{>MIC}$ = 70%
1	2	1	84.8	15.0
		2	92.8	22.2
		3	98.0	32.0
		4	99.6	49.4
2	2	1	93.6	26.4
		2	98.0	37.1
		3	99.5	53.6
		4	100.0	67.6
1	3	1	98.8	67.3
		2	99.7	81.6
		3	100.0	92.3
		4	100.0	97.3
1	4	1	99.9	93.1
		2	100.0	97.9
		3	100.0	99.4
		4	100.0	100.0

infusion time, the highest TA% was 49.4% with 4 hours infusion.

3. Prediction of clinical efficacy by Monte Carlo Simulation with the regimen of 2 g q12h with 1 to 4 hours infusion

Table 4 and Figure 2 show TA% at each $T_{>MIC}$ calculated by Monte Carlo Simulation with the regimen of 2 g q12h with 1 to 4 hours infusion against ESBL producing strains. The TA%

Fig. 1. Probability of target attainment of flomoxef with 1 g q12h at different free drug $T_{>MIC}$ targets against ESBL producing strains.

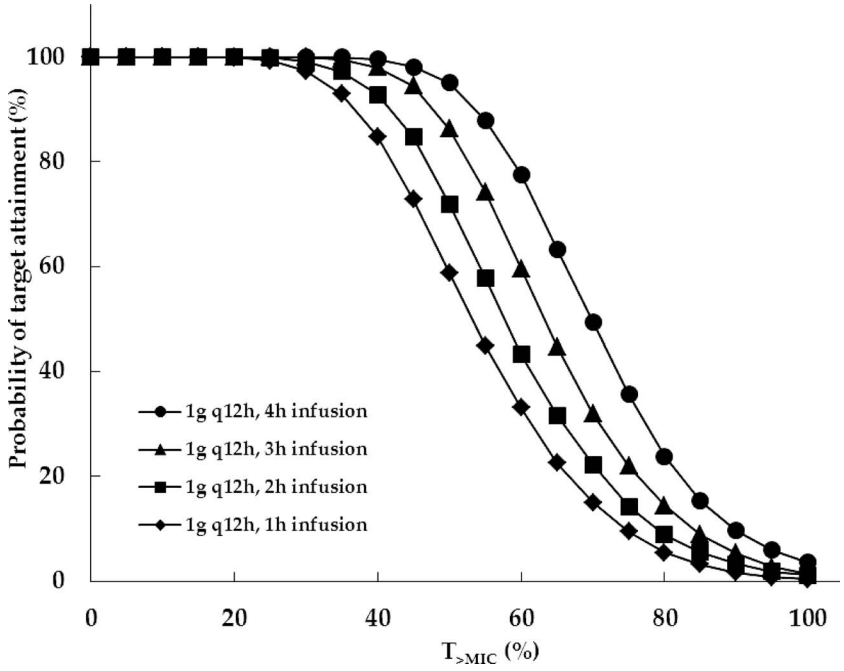
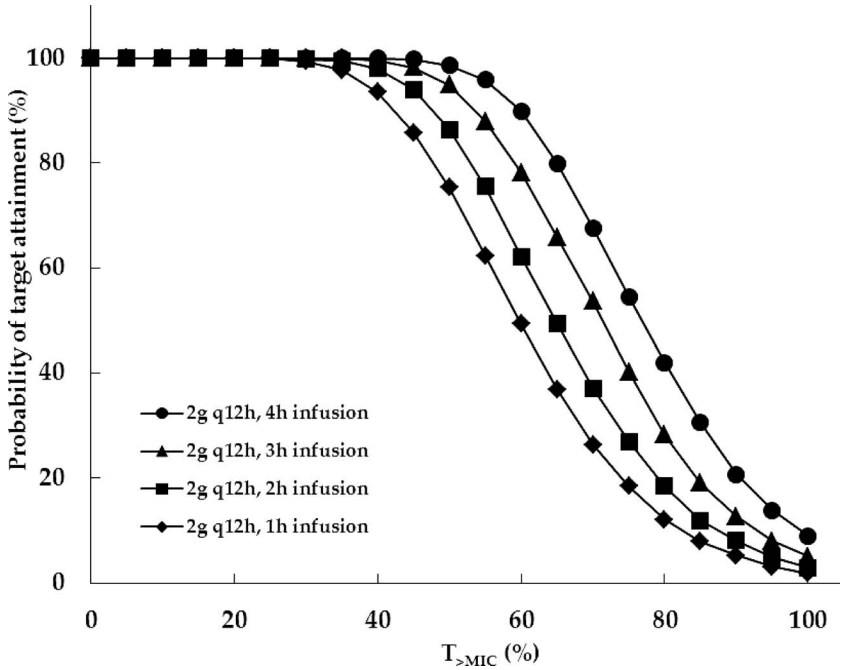


Fig. 2. Probability of target attainment of flomoxef with 2 g q12h at different free drug $T_{>MIC}$ targets against ESBL producing strains.



achieved more than 90% with 1 to 4 hours infusion at $T_{>MIC}$=40%. At $T_{>MIC}$=70%, even though TA% increased with the increase of infusion time, the highest TA% was 67.6% with 4 hours infusion.

4. Prediction of clinical efficacy by Monte Carlo Simulation with the regimen of 1 g q8h with 1 to 4 hours infusion

Table 4 and Figure 3 show the TA% at each $T_{>MIC}$ calculated by Monte Carlo Simulation with the regimen of 1 g q8h with 1 to 4 hours infusion against ESBL producing strains. The TA% achieved more than 95% with 1 to 4 hours infusion at $T_{>MIC}$=40%. At $T_{>MIC}$=70%, the TA% achieved more than 80% with 2 to 4 hours infusion, and achieved more than 90% with 3 to 4 hours infusion.

5. Prediction of clinical efficacy by Monte Carlo Simulation with the regimen of 1 g q6h with 1 to 4 hours infusion

Table 4 and Figure 4 show the TA% at each $T_{>MIC}$ calculated by Monte Carlo Simulation with the regimen of 1 g q6h with 1 to 4 hours infusion against ESBL producing strains. The TA% achieved almost 100% with 1 to 4 hours infusion at $T_{>MIC}$=40%. At $T_{>MIC}$=70%, TA% achieved more than 90% with 1 to 4 hours infusion.

Fig. 3. Probability of target attainment of flomoxef with 1 g q8h at different free drug $T_{>MIC}$ targets against ESBL producing strains.

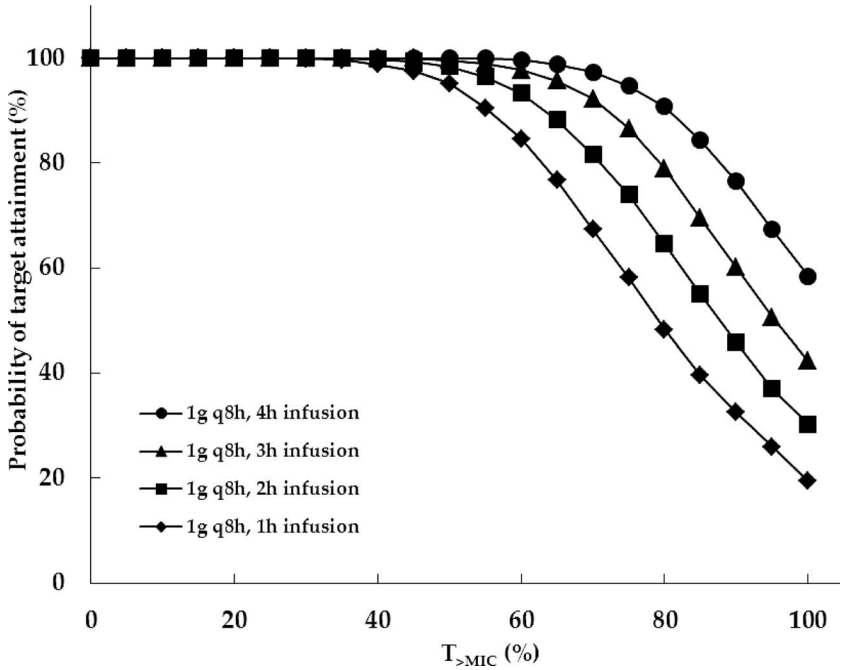
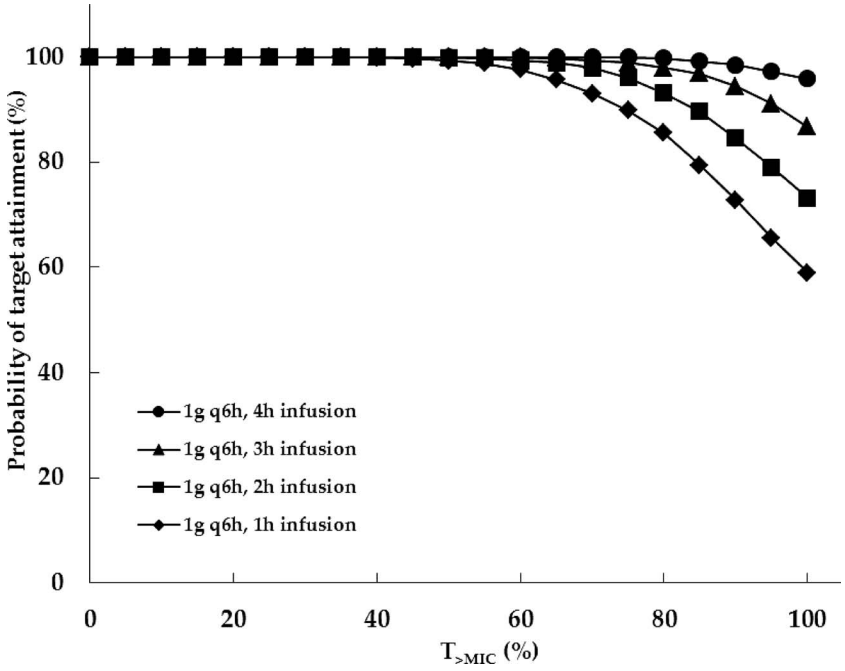


Fig. 4. Probability of target attainment of flomoxef with 1 g q6h at different free drug $T_{>MIC}$ targets against ESBL producing strains.



III. Discussion

It has been becoming difficult to treat the ESBL-associated infections because of the increase of resistant pathogens. *K. pneumoniae* producing SHV or TEM has been reported to be cause of nosocomial infections from 1980s. Moreover, *E. coli* producing CTX-M has been increasing in the community from 1990s, and has been reported to be a cause of community acquired infections including urinary tract infections [2,23–25]. The reports included patients who do not have record to be hospitalized, which indicated the spread of ESBL producing strains in the community.

Sader reported the increased rate of resistant strains of *E. coli* and *K. pneumoniae* to CFPM and PIPC/TAZ according to the breakpoint by CLSI[26]. The rates of susceptible isolates to CFPM are 65.8% and 42.0% among ESBL producing *E. coli* isolated in North America and the rest of the world, respectively, and 89.4% and 51.2% among ESBL producing *K. pneumoniae* isolated in North America and the rest of the world, respectively. The rates of susceptible isolates to PIPC/TAZ are 72.5% and 69.1% among ESBL producing *E. coli* isolated in North America and the rest of the world, respectively, and 52.6% and 45.0% among ESBL producing *K. pneumoniae* isolated in North America and the rest of the world, respectively. As mentioned above, the rate of ESBL producing strains resistant to CFPM and PIPC/TAZ has been increasing in the region other than

North America, and causing serious problems in the healthcare settings. The further increase and spread of ESBL producing strains could cause decrease of effective antimicrobial agents.

In this study, antibacterial activity and clinical efficacy of FMOX against ESBL producing Enterobacteriaceae was investigated by MIC determination and Monte Carlo Simulation. In the Monte Carlo Simulation, MIC of FMOX against ESBL producing strains isolated in Japan and PK/PD parameter obtained in Phase 1 studies in Japan were used. The TA% at $T_{>MIC}$=40% and $T_{>MIC}$=70% were determined, which show bacteriostatic and bactericidal activity, respectively, according to Craig[11,12].

It was revealed in this study that FMOX and DRPM showed a potent *in vitro* antibacterial activity against ESBL producing strains. Although CMZ and IPM showed antibacterial activity against them, other antimicrobial agents tested in this study showed higher MIC whose MIC_{90} was 128 μg/mL or >128 μg/mL, which indicated the inadequate activity of these antimicrobial agents against ESBL producing strains. The increase of ESBL producing strains has been reported in Japan[5–8]. Yoshida *et al.* reported the increased rate of ESBL from 0.7% in 2002 to 3.8% in 2008 among *E. coli*, and from 1.3% in 2002 to 2.6% in 2008 among *K. pneumoniae*, and from 1.7% in 2002 to 6.8% in 2008 among *K. oxytoca*. However, MIC_{90} of FMOX against *E. coli* were 0.125 μg/mL in 2002 and 0.25 μg/mL in 2008, and MIC_{90} of FMOX against *K. pneumoniae* and *K. oxytoca* were 0.125 μg/mL in 2002 and 2008, and it was concluded that FMOX has maintained potent antibacterial activity against ESBL producing strains even though the rate of ESBL producing strains have increased among *E. coli* and *K. pneumoniae*. In the case of *P. mirabilis*, the rate of ESBL producing isolates decreased from 7.5% in 2002 to 5.5% in 2008, and MIC_{90} of FMOX were 0.5 μg/mL in 2002 and 0.25 μg/mL in 2008. In the present study, *in vitro* activities and probability of target attainment were investigated with the ESBL producing strains obtained by ATCC and those isolated in medical facilities in Japan. MIC ranges of FMOX against *E. coli, Klebsiella* spp., and *P. mirabilis* used in the present study were 0.063–2 μg/mL, 0.063–0.5 μg/mL, and 0.25–0.5 μg/mL, respectively. MIC_{90} of FMOX against 11 isolates of *E. coli*, 15 isolates of *Klebsiella* spp., and 5 isolates of *P. mirabilis* were 0.5 μg/mL. Even though MIC_{90} of FMOX were higher than those reported by Yoshida *et al.*, the differences were not significant and were seemed to be appropriate for the analysis. In 2008, 14 out of 16 ESBL producing strains possess CTX-M type, and 2 strains of them were identified to have CTX-M-15 whose increase has been reported recently in the world wide. FMOX was reported to show a potent antibacterial activity against CTX-M-15 producing strains where MIC of FMOX was 0.25–0.5 μg/mL[8]. One of the reasons of the potent antibacterial activity of FMOX against these ESBL producing strains could be its structural characteristic. FMOX known as one of the 7-methoxy β-lactam antibiotic is one of the 1-oxacephem antibiotic different structurally from the cephalosporins and other cephamycins. The 7-methoxy was reported to contribute to the stability against β-lactamases[27,28].

This study revealed that FMOX showed more than 80% of TA% at $T_{>MIC}$=40% with the

regimen of 1 g q12h with 1 to 4 hours infusion and 2 g q12h with 1 to 4 hours infusion, which indicated the bacteriostatic activity and efficacy of FMOX against immuno-competent patients. On the other hand, both of the regimen did not achive 80% of TA% at $T_{>MIC}$=70%. Even though increase of dosage from 1 to 2 g increased the TA%, both of them did not achive 80% of TA%, which indicated that regimens of q12h were not appropriate to obtain bactericidal activity, and was not effective against the ESBL-associated infections of compromized patients. It has been rerpoted that the increase of $T_{>MIC}$ helps for β-lactam antibiotics to achieve bactericidal activity[11,12], and the increase of $T_{>MIC}$ also increased the efficacy of FMOX predicted by Monte Carlo Simulation. The regimen of 1 g q8h and q6h with 1 to 4 hours infusion achieved more than 80% of TA% at $T_{>MIC}$=40%, and the regimens of 1 g q8h and q6h with 2 to 4 hours infusion achieved more than 80% of TA% at $T_{>MIC}$=70%. Therefore, the regimens of 1 g q8h with 2 to 4 hours infusion and 1 g q6h with 1 to 4 hours infusion are appropriate to obtain bactericidal activity against the ESBL-associated infections. These regimens are expected to be appropriate for the compromized patients.

Reese *et al.* reported the Monte Carlo Simulation of CFPM and PIPC/TAZ against the ESBL-associated infections[29]. PIPC/TAZ with the regimen of 3.375 g q6h achieved 43% of TA% at $T_{>MIC}$=40%, and CFPM with 4.0 g continuous infusion achieved 77% of TA% at $T_{>MIC}$=60%. Reese *et al.* concluded with these results that both of PIPC/TAZ and CFPM were not appropriate for the treatment of the ESBL-associated infections. There are only a few antibiotics to be used for the treatment of the ESBL-associated infections, and it is required to assess the existing antibiotics to investigate the appropriate regimens for the treatment. At the current situation, carbapenems and quinolones are the effective antibiotics against ESBL producing strains. However, there are many hospitals to limit the use of carbapenems, and the increase of resistance against quinolones by ESBL producing strains has been reported[30]. Therefore, there are increasing demand for effective antimicrobial agents other than carbapenems or quionolones to treat the ESBL-associated infections.

According to JAID/JSC Guide to Clinical Management of Infectious Diseases 2011, FMOX is one of the first-line agent for the treatment of sever infections such as urinary tract infections[31]. Although quinolones such as ciprofloxacin (CPFX) is the first-line agent for the treatment of mild or moderate cases of bladder inflammation and pyelonephritis, the resistance against quionolones has been increased among ESBL producing strains in the case of urinary tract infections, FMOX could be another option for the treatment of mild or moderate cases to treat promptly and prevent sever infections. The efficacy of FMOX to prevent the infections following surgical procedure has been reported[32–35]. Although these report did not verify whether ESBL producing strains were included in these cases or not, Monte Carlo Simulation in the present study showed the clinical efficacy of FMOX against ESBL producing strains, which indicated the effectiveness of FMOX to prevent the ESBL-associated infections.

This study investigated the efficacy of FMOX against the ESBL-associated infections caused by Enterobacteriaceae including *E. coli* and *K. pneumoniae* by using Monte Carlo Simulation. FMOX showed bacteriostatic activity against ESBL producing strains by the regimens of 1 g q12h with 1 to 4 hours infusion, which indicated potent efficacy for the treatment of the infections of competent patients. Moreover, FMOX showed bactericidal activity against ESBL producing strains with the regimens of 1 g q8h with 2 to 4 hours infusion and 1 g q6h with 1 to 4 hours infusion, which indicated potent efficacy for the treatment of the infections of the compromised patients. These regimens are approved regimens of FMOX in Japan, which indicated that FMOX can be used for the treatment of the ESBL-associated infections as well as for the prevention of severe infections following surgical procedure.

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