Potent antibacterial activities of latamoxef (moxalactam) against ESBL producing Enterobacteriaceae analyzed by Monte Carlo simulation

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Latamoxef (LMOX, Moxalactam) is one of the *β*-lactam antibiotics which is stable against *β*-lactamase. In this study, the antibacterial activity of LMOX was investigated, and Monte Carlo simulation was conducted to determine the appropriate dosing regimens of LMOX against extended-spectrum *β*-lactamase (ESBL) producing Enterobacteriaceae. The probability of target attainment (PTA) was analyzed at 40% and 70% of time above minimum inhibitory concentration (MIC) (time above MIC, $T_{>MIC}$) for bacteriostatic and bactericidal effect respectively. All the tested regimens achieved 85% of PTA at 40% of $T_{>MIC}$ against ESBL producing *Escherichia coli*, and all the tested regimens except 1g q12h with 1 hour infusion achieved 85% of PTA at 40% of $T_{>MIC}$ against ESBL producing *Klebsiella pneumoniae*. The effective regimens to achieve 85% of PTA at 70% of $T_{>MIC}$ against *E. coli* were 1g q12h with 4 hours infusion, 1g q8h with 1–4 hours infusion, 2g q12h with 2–4 hours infusion, and 1g q6h with 1–4 hours infusion. The effective regimens to achieve 85% of PTA at 70% of $T_{>MIC}$ against *K. pneumoniae* were 1g q8h with 3–4 hours infusion and 1g q6h with 1–4 hours infusion. These results of pharmacokinetics/pharmacodynamics (PK/PD) modeling showed the potent efficacy of LMOX against bacterial infections caused by ESBL producing Enterobacteriaceae.

Introduction

Extended-spectrum *β*-lactamase (ESBL) is spreading among Enterobacteriaceae. Associated infections include urinary tract infections, bloodstream infections, 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].

Available therapeutic options for the treatment of ESBL associated infections are limited by drug resistance conferred by the ESBL[5]. 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[6, 7].

Pharmacokinetics/pharmacodynamics (PK/PD) has been investigated to maximize clinical response and to minimize the emergence of antibiotic resistance and exposure-related toxicities[8~10]. For cephalosporin antibiotics, the time above minimum inhibitory concentration (MIC) (time above MIC, $T_{>MIC}$) is the target that best relates to patient outcomes[8, 9]. Although $T_{>MIC}$ is decided by PK of the antibiotics and MIC against 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[11~15]. 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 (PTA) at $T_{>MIC}$. This simulation can predict the outcome of various dosage and administration schedule, which helps us to choose the appropriate regimens for the treatment.

Latamoxef (LMOX, Moxalactam) is one of the cephamycins, characterized by its 7-alpha-methoxy, and also called 1-oxa-β-lactam antibiotic which is different structurally from the cephalosporins and other cephamycins, in which sulfur atom in the cephem nucleus is substituted with oxygen atom[16, 17]. 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 LMOX 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 LMOX with PK/PD analysis as well as *in vitro* antibacterial activities against emerging ESBL such as variants of SHV, TEM, and CTX-M families. The objective of this study was to investigate the potency of LMOX 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. Bacterial strains

The clinical isolates used in this study were isolates collected from all over the world from

2001 to 2009, isolates collected from various medical facilities in Japan from 1992 to 2002, and ATCC strains. They include various variants of SHV, TEM, and CTX-M families.

2. 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[18]. 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 drug that completely inhibited bacterial growth as detected by the unaided eye.

3. Antibacterial agents

Susceptibilities of the bacterial strains were tested for the following antimicrobial agents: latamoxef (LMOX, Moxalactam), flomoxef (FMOX), cefepime (CFPM), and tazobactam/piperacillin (piperacillin with 4 μg/mL of tazobactam, TAZ/PIPC). Clavulanic acid (CVA) was used as β-lactamase inhibitor with 4 μg/mL.

4. Monte Carlo simulation

Monte Carlo simulation was employed to investigate the probability of target attainment (PTA) against the clinical isolates of ESBL producing *E. coli* and *K. pneumoniae* by using the simulation software Crystal Ball® 7 (KOZO KEIKAKU ENGINEERING Inc., Japan). Virtual populations of 5,000 patients were generated for each dosage regimen against each bacterial strain. Pharmacokinetic parameters of LMOX (2 g over 2 hours infusion) were taken from the Phase 1 study in Japan[19]. Pharmacokinetic parameters of LMOX for typical values of 2-compartment model were as follows: Vc＝6.50 L, $k_{10}=0.72\,hr^{-1}$, $k_{12}=0.76\,hr^{-1}$, and $k_{21}=1.33\,hr^{-1}$. A log-normal distribution was arbitrarily assumed for the inter-individual variability for pharmacokinetic parameters with 20% of coefficient of variation as a conservative value. It was assumed that free plasma concentrations of LMOX would correlate with the bactericidal effect, and free drug concentrations were used for the simulation based on a plasma protein binding ratio of 60%[20, 21]. MIC values were generated from the discrete MIC distributions obtained from 50 isolates of ESBL producing *E. coli* and 28 isolates of ESBL producing *K. pneumoniae*. The regimens used for the simulation were 1 g every 6, 8, or 12 hours (q6h, q8h, or q12h respectively) with 1–4 hours infusion, which corresponded to 4, 3, and 2 g/day respectively, and 2g q12h with 1–4 hours infusion which corresponded to 4 g/day. The PTA was calculated based on the simulated plasma concentrations at 1-minute interval by using two-compartment model. The PTA at various $T_{>MIC}$ was calculated by using 5,000 virtual patients for each dosing regimen against each bacteria strain.

II. Results

1. Antimicrobial activities of latamoxef against clinical isolates of ESBL producing Enterobacteriaceae

Table 1 shows MIC of LMOX against clinical isolates of ESBL producing Enterobacteriaceae such as *E. coli*, *K. pneumoniae*, *Enterobacter cloacae*, *Citrobacter freundii*, *Serratia marcescens*, and Indole-positive *Proteus* spp. collected from all over the world. MIC_{90} of CFPM and TAZ/PIPC against ESBL non-producing bacteria were 2–8 μg/mL and 0.06–1 μg/mL, respectively, and MIC_{90} of CFPM and TAZ/PIPC against ESBL producing bacteria were 8–＞64 and 16–＞256 μg/mL, respectively. MIC_{90} of LMOX against ESBL non-producing and ESBL producing bacteria were 0.12–0.5 and 1–32 μg/mL, respectively, which indicated potent antimicrobial activities of LMOX against ESBL producing strains. When LMOX were supplemented with 4 μg/mL of CVA, MIC_{90} of LMOX against ESBL non-producing and ESBL producing bacteria were 0.12–0.5 and 1–16 μg/mL, respectively, which were similar with those without CVA. These results indicated that LMOX was stable against ESBL.

Among the tested isolates, there were three isolates showing higher MIC of LMOX such as *E. cloacae* 920, *E. cloacae* 2144, and *K. pneumoniae* 13731, which possesses SHV-12, SHV-30, and SHV-5, respectively. MICs of LMOX against these isolates were 32, 128, and 32 μg/mL, respectively. MIC ranges of LMOX against the isolates producing SHV-12, SHV-30, and SHV-5 except these isolates were 0.12–1, 0.12–8, and 0.25–4 μg/mL, respectively. These results demonstrated that even though there were some isolates whose MICs of LMOX were high, LMOX was potent against most of the isolates producing SHV-12, SHV-30, and SHV-5. Moreover, MIC distribution of LMOX against *E. cloacae* and *K. pneumoniae* other than these three resistant isolates were 0.25–8 and 0.12–8 μg/mL, respectively, which showed potent activity of LMOX against most of the clinical isolates of these species.

2. Frequency distribution of MIC of LMOX against clinical isolates of ESBL producing Enterobacteriaceae

Table 2 shows frequency distribution of MIC of LMOX against ESBL producing *E. coli* and *K. pneumoniae* which are same isolates on Table 1. MICs of CFPM and TAZ/PIPC against the most of the isolates of *E. coli* were more than 32 and 8 μg/mL, respectively, and MICs of CFPM and TAZ/PIPC against the most of the isolates of *K. pneumoniae* were more than 8 and 16 μg/mL, respectively. On the other hand, MICs of LMOX and LMOX supplemented with CVA against the most of the isolates of *E. coli* were less than 0.5 and 0.25 μg/mL, respectively, and MICs of LMOX and LMOX supplemented with CVA against the most of the isolates of *K. pneumoniae* were less than 0.5 and 0.25 μg/mL. These results indicated that LMOX had a potent *in vitro* activity against ESBL producing *E. coli* and *K. pneumoniae* that were resistant to CFPM and TAZ/PIPC.

Table 1. *In vitro* activities against ESBL producing Enterobacteriaceae

LMOX: latamoxef (moxalactam), CVA: clavulanic acid, CFPM: cefepime, TAZ/PIPC: tazobactam/piperacillin. CVA and TAZ were added with 4 μg/mL.

		MIC (μg/mL)											
		LMOX			CVA/LMOX			CFPM			TAZ/PIPC		
		Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
E. coli	ESBL non-producer (n = 10)	≤0.06 - 0.25	0.12	0.25	≤0.06 - 0.25	<0.06	0.25	1 - 4	1	2	≤0.015 - 0.06	≤0.015	0.06
	ESBL producer (n = 50)	0.12 - 16	0.5	2	0.06 - 4	0.25	1	0.06 - >64	32	>64	1 - >256	8	128
K. pneumoniae	ESBL non-producer (n = 10)	≤0.06 - 0.25	≤0.06	0.12	≤0.06 - 0.25	≤0.06	0.12	1 - 4	2	4	≤0.015 - 0.25	≤0.015	0.25
	ESBL producer (n = 28)	0.12 - 32	0.5	8	0.06 - 8	0.25	1	0.25 - >64	8	>64	2 - >256	16	>256
E. cloacae	ESBL non-producer (n = 9)	≤0.06 - 0.5	0.12	0.5	≤0.06 - 0.5	0.12	0.5	1 - 8	2	8	≤0.015 - 1	0.12	1
	ESBL producer (n = 10)	0.25 - 128	4	32	0.06 - 16	2	16	1 - >64	4	>64	2 - >256	16	256
C. freundii	ESBL non-producer (n = 10)	≤0.06 - 0.5	≤0.06	0.5	≤0.06 - 0.5	≤0.06	0.25	1 - 2	1	2	≤0.015 - 1	0.06	0.25
	ESBL producer (n = 7)	0.12 - 2	0.5	2	0.06 - 2	0.25	2	8 - >64	32	>64	4 - >256	4	>256
S. marcescens	ESBL non-producer (n = 30)	0.12 - 0.5	0.25	0.5	0.12 - 0.5	0.25	0.5	0.5 - 32	2	8	≤0.015 - 0.5	0.06	0.12
	ESBL producer (n = 9)	0.5 - 4	1	4	0.25 - 16	1	16	1 - >64	4	>64	2 - 16	8	16
Indole-positive *Proteus* spp.	ESBL non-producer (n = 30)	≤0.06 - 0.25	0.12	0.25	≤0.06 - 0.25	0.12	0.25	≤0.12 - 4	0.5	2	≤0.015 - 0.5	0.12	0.25
	AmpC derepressed/ ESBL producer (n = 10)	0.06 - 1	0.12	1	0.06 - 2	0.25	1	≤0.03 - 16	0.25	8	0.25 - 256	1	64

Table 2. Frequency distribution of MIC against *β*-lactamase producing *E. coli* and *K. pneumoniae*

LMOX: latamoxef (moxalactam), CVA: clavulanic acid, CFPM: cefepime, TAZ/PIPC: tazobactam/piperacillin. CVA and TAZ were added with 4*μ*g/mL. *: MICs of CFPM against these isolates were ＞64*μ*g/mL.

	MIC (μg/mL)														MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256		
E. coli (n = 50)																
LMOX		4	8	19	10	4	3	1	1						0.5	2
CVA/LMOX	1	11	17	15	2	2	2								0.25	1
CFPM	1		2		3	6	3	2	4	7	2	20*			32	>64
TAZ/PIPC					1	9	9	7	9	5	2	3	2	3	8	128
K. pneumoniae (n = 28)																
LMOX		4	3	7	5	2	3	3		1					0.5	8
CVA/LMOX	5	4	8	5	4			2							0.25	1
CFPM			1		2	4	4	4	1	2	1	9*			8	>64
TAZ/PIPC						3	3	4	5	2		1	4	6	16	>256

3. Effect of CVA on susceptibility of ESBL producing Enterobacteriaceae

Table 3 shows MICs of LMOX, CFPM, TAZ/PIPC, and FMOX against ESBL producing Enterobacteriaceae such as *E. coli*, *K. pneumoniae*, *K. oxytoca*, and *P. mirabilis* collected from various medical facilities in Japan and ATCC. These isolates possess various types of ESBL such as variants of SHV, TEM, and CTX-M families. MIC_{90} of LMOX against these isolates was 2*μ*g/mL, and MIC_{90} of LMOX supplemented with 4*μ*g/mL of CVA was 1*μ*g/mL. These results demonstrated that the *in vitro* activities of LMOX with the *β*-lactamase inhibitor against ESBL producing Enterobacteriaceae were similar with those without the *β*-lactamase inhibitor, which indicated that LMOX were stable against ESBL. Among these three types of ESBL, MICs of LMOX with the *β*-lactamase inhibitor against variants of SHV and TEM families were 2–8 times lower than those without the *β*-lactamase inhibitor, and MICs of LMOX with the *β*-lactamase inhibitor against variants of CTX-M families were almost same with those without the *β*-lactamase inhibitor. These results indicated that LMOX were stable especially against variants of CTX-M families.

MIC_{90} of CFPM against these isolates was ＞64*μ*g/mL, and MIC_{90} of CFPM supplemented with 4*μ*g/mL of CVA was 0.25*μ*g/mL. These results showed that the *in vitro* activities of CFPM against ESBL were dramatically increased by addition of the *β*-lactamase inhibitor. MICs of CFPM against most of the isolates possessing variants of SHV family were less than 8*μ*g/mL except isolates whose MICs of CFPM were 16*μ*g/mL and 64*μ*g/mL, and MICs of CFPM against all of the isolates possessing variants of TEM family were less than 8*μ*g/mL. On the other hand, MICs of CFPM against 11 out of 18 isolates harboring variants of CTX-M family was ＞64*μ*g/mL. When CFPM was supplemented with CVA, MICs against all of the isolates were less than

Table 3. MIC against Enterobacteriaceae producing various types of *β*-lactamase

LMOX: latamoxef (moxalactam), CFPM: cefepime, TAZ/PIPC: tazobactam/piperacillin, FMOX: flomoxef, CVA: clavulanic acid.
CVA and TAZ were added with 4 *μ*g/mL.

			MIC (μg/mL)					
			LMOX		CFPM		TAZ/PIPC	FMOX
Species	Isolate	β-lactamase	-	CVA	-	CVA	-	-
E. coli	BAA-201	TEM-3	1	0.25	2	0.063	4	0.125
E. coli	BAA-196	TEM-10	2	0.5	2	0.063	8	0.25
E. coli	BAA-197	TEM-12	2	1	4	0.125	8	0.25
E. coli	BAA-198	TEM-26	1	0.25	2	0.031	4	0.125
E. coli	BAA-204	SHV-2	16	8	64	0.25	>64	2
E. coli	BAA-199	SHV-3	1	0.25	4	0.063	>64	0.125
E. coli	BAA-200	SHV-4	0.5	0.125	4	0.031	4	0.063
E. coli	BAA-203	SHV-5	4	0.5	8	0.063	32	0.25
E. coli	SR21003	CTX-M-14	0.5	0.5	16	0.25	8	0.5
E. coli	SR21182	CTX-M-14	2	0.5	>64	0.125	32	0.25
E. coli	SR21266	CTX-M-14	0.25	0.125	8	0.031	1	0.063
K. pneumoniae	ATCC 700603	SHV-18	1	1	1	0.125	8	0.25
K. pneumoniae	SR13708	SHV-18	0.125	0.125	16	0.031	1	0.063
K. pneumoniae	SR13740	CTX-M-3	0.25	0.125	>64	0.031	2	0.063
K. pneumoniae	SR14915	CTX-M-3 & -44 like	0.125	0.125	1	0.031	2	0.063
K. pneumoniae	SR14933	SHV-3	0.25	0.125	2	0.063	4	0.063
K. pneumoniae	SR14935	CTX-M-3	0.25	0.125	>64	0.031	2	0.063
K. pneumoniae	SR22015	CTX-M type	1	0.5	>64	0.25	16	0.25
K. pneumoniae	SR22138	CTX-M type	0.125	0.063	1	0.031	>64	0.063
K. oxytoca	ATCC 51983	SHV-5	0.25	0.125	0.5	0.031	2	0.125
K. oxytoca	SR13552	CTX-M type	2	1	>64	0.125	>64	0.25
K. oxytoca	SR18750	CTX-M type	1	0.5	0.25	0.063	>64	0.5
K. oxytoca	SR18754	CTX-M type	0.25	0.125	4	0.125	>64	0.063
K. oxytoca	SR21502	CTX-M-3	0.125	0.063	>64	0.031	1	0.125
K. oxytoca	SR21583	CTX-M type	0.25	0.25	4	0.125	>64	0.063
K. oxytoca	SR21592	CTX-M type	0.5	0.25	>64	1	>64	0.125
P. mirabilis	SR22765	TEM-19 like	0.125	0.125	2	0.063	1	0.125
P. mirabilis	SR22790	CTX-M type	0.5	0.5	>64	0.031	0.25	0.5
P. mirabilis	SR22798	CTX-M type	0.5	0.5	>64	0.031	0.25	0.5
P. mirabilis	SR13993	CTX-M type	0.5	0.5	>64	0.031	0.5	0.5
P. mirabilis	SR22754	CTX-M type	0.5	0.5	>64	0.031	0.5	0.25
Range			0.125 - 16	0.063 - 8	1 - >64	0.031 - 1	0.25 - >64	0.063 - 2
MIC_{50}			0.5	0.25	8	0.063	4	0.125
MIC_{90}			2	1	>64	0.25	>64	0.5

2 *μ*g/mL, and there was no significant difference among MIC of CFPM supplemented with CVA against these three types of ESBL such as SHV, TEM, and CTX-M families. These results demonstrated that CFPM was less stable against variants of CTX-M family than that against variants of SHV and TEM families.

4. Probabilities of target attainment against clinical isolates of ESBL producing *E. coli* and *K. pneumoniae*

Figures 1 and 2 show probability of target attainment (PTA) at different time above MIC

($T_{>MIC}$) in various regimens of LMOX against ESBL producing *E. coli* and *K. pneumoniae*, respectively. In the case of *E. coli*, all the tested regimens achieved 85% of PTA at 40% of $T_{>MIC}$ (Figure 1 (a) and (b)). The effective regimens of 2- to 3-g dose/day to achieve 85% of PTA at 70% of $T_{>MIC}$ were 1g q12h with 4 hours infusion and 1g q8h with 1 to 4 hours infusion (Figure 1 (a)). When the regimen was increased to 4-g dose/day, the effective regimens to achieve 85% of PTA at 70% of $T_{>MIC}$ were 2g q12h with 2 to 4 hours infusion and 1g q6h with 1 to 4 hours infusion (Figure 1 (b)).

In the case of *K. pneumoniae*, all the tested regimens except 1g q12h with 1 hour infusion achieved 85% of PTA at 40% of $T_{>MIC}$ (Figure 2 (a) and (b)). The effective regimens of 2- to 3-g dose/day to achieve 85% of PTA at 70% of $T_{>MIC}$ were 1g q8h with 3 to 4 hours infusion (Figure 2 (a)). When the regimen was increased to 4-g dose/day, the effective regimens to achieve 85% of PTA at 70% of $T_{>MIC}$ were 1g q6h with 1 to 4 hours infusion (Figure 2 (b)).

III. Discussion

In this study, antibacterial activity and clinical efficacy of LMOX against ESBL producing Enterobacteriaceae was investigated by MIC determination and Monte Carlo simulation. In the Monte Carlo simulation, PK parameter obtained in Phase 1 studies in Japan were used, and the PTA at $T_{>MIC}$=40% and $T_{>MIC}$=70% were determined for bacteriostatic and bactericidal activity, respectively, according to Craig[8, 9]. Our study had three major findings. First, LMOX had potent *in vitro* activities against ESBL producing Enterobacteriaceae. Second, LMOX was stable against clinical isolates producing ESBL such as variants of SHV, TEM, and CTX-M families. Third, pharmacokinetics/pharmacodynamics (PK/PD) modeling by Monte Carlo simulation showed the potent efficacy of LMOX against bacterial infections caused by *E. coli* and *K. pneumoniae* producing ESBL such as variants of SHV, TEM, and CTX-M families.

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. Currently, carbapenems are regarded as the drug of choice for infections caused by ESBL producing pathogens[22~24]. 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[25]. Therefore, there are increasing demands for effective antimicrobial agents other than carbapenems or quinolones to treat the ESBL associated infections.

Sader, *et al.* reported the susceptibility of ESBL producing *E. coli* and *Klebsiella* spp. to CFPM and TAZ/PIPC according to CLSI susceptible breakpoints[26]. The report demonstrated that CFPM and TAZ/PIPC were not active against many of the isolates of ESBL producing *E. coli* and *Klebsiella* spp. Yoshida, *et al.* reported the increase of ESBL producing strains in Japan[27~30]. LMOX has maintained potent antibacterial activity against ESBL producing strains even though

Fig. 1(a). Probability of target attainment of 2 or 3 g (1g q12h or 1g q8h, respectively) of latamoxef at different free drug $T_{>MIC}$ targets against ESBL producing *E. coli*

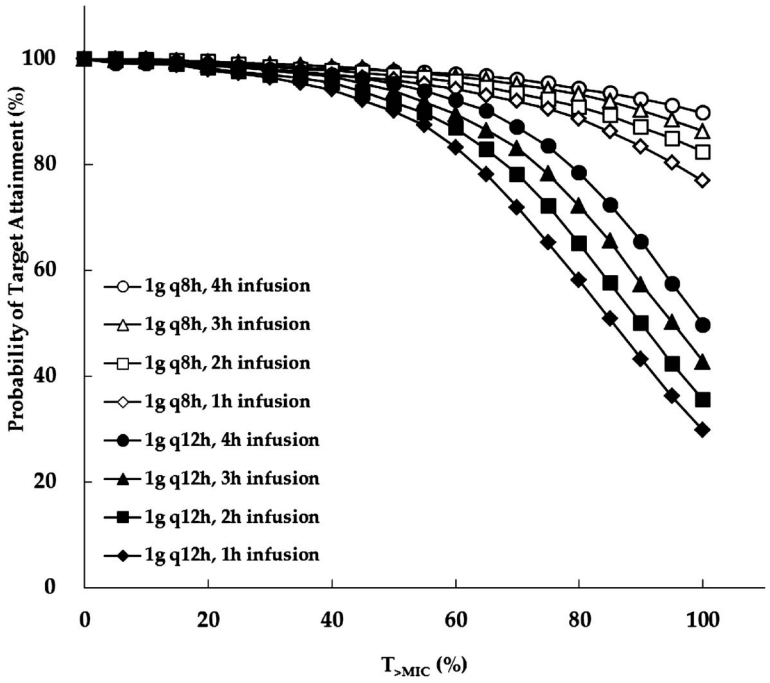


Fig. 1(b). Probability of target attainment of 4 g (1g q6h or 2g q12h) of latamoxef at different free drug $T_{>MIC}$ targets against ESBL producing *E. coli*

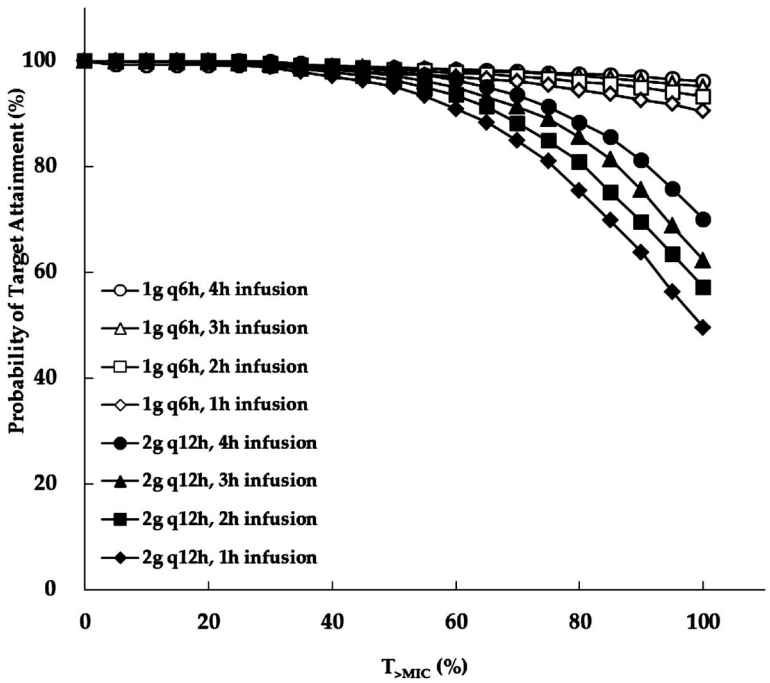


Fig. 2(a). Probability of target attainment of 2 or 3 g (1g q12h or 1g q8h, respectively) of latamoxef at different free drug $T_{>MIC}$ targets against ESBL producing *K. pneumoniae*

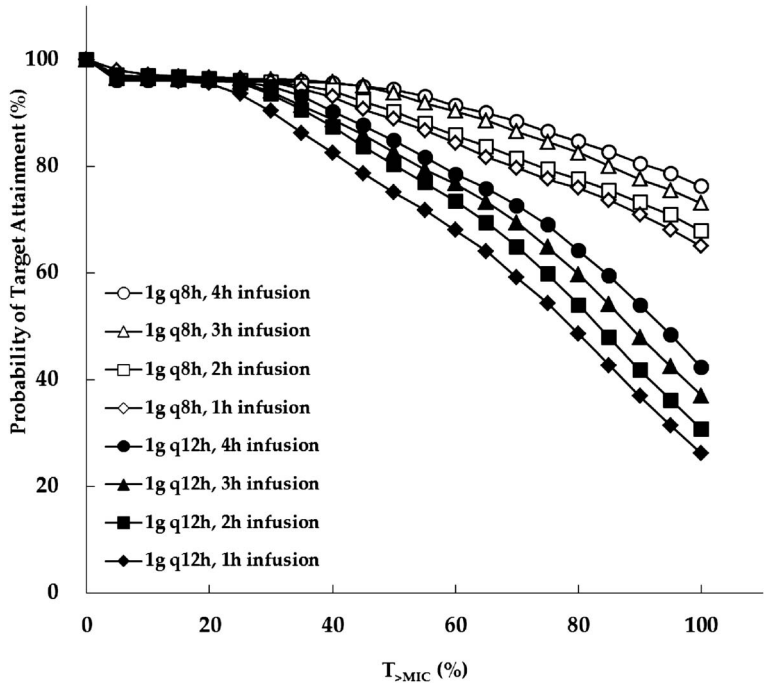
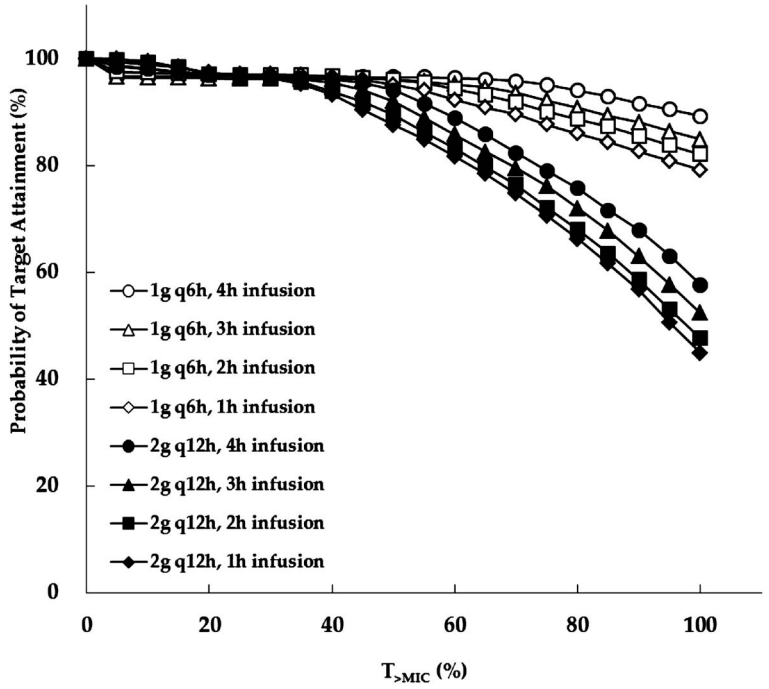


Fig. 2(b). Probability of target attainment of 4 g (1g q6h or 2g q12h) of latamoxef at different free drug $T_{>MIC}$ targets against ESBL producing *K. pneumoniae*



the rate of ESBL producing strains have increased among *E. coli*, *K. pneumoniae*, and *K. oxytoca*. In the present study, MIC_{90} of LMOX against *E. coli*, and *K. pneumoniae* used in Monte Carlo simulation were 2μg/mL and 8μg/mL, respectively. MIC_{90} of LMOX against 11 isolates of *E. coli* and 15 isolates of *Klebsiella* spp. were 2μg/mL. Even though MIC_{90} of LMOX were higher than those reported by Yoshida, *et al.*, the differences were not significant and were seemed to be appropriate for the analysis. Moreover, LMOX was reported to show a potent antibacterial activity against CTX-M-15 producing strains where MICs of LMOX were 2–4μg/mL[30].

In the present study, MIC_{90} of LMOX against *E. cloacae* was relatively higher than those against other bacteria. One of the reasons could be that the number of ESBL producing *E. cloacae* used in this study was small, and existence of two resistant isolates increased MIC_{90} significantly. MIC distribution other than 2 resistant isolates was 0.25–8μg/mL which was comparable to that of *K. pneumoniae* whose MIC distribution was 0.12–32μg/mL, indicating potent activity of LMOX against most of the clinical isolates in both species. According to the results of Monte Carlo simulation with *K. pneumoniae* which most of the clinical isolates showed MIC≤8μg/mL, LMOX was effective with all the tested regimens except 1g q12h with 1 hour infusion based on 40% of $T_{>MIC}$ target, and LMOX was effective with 1g q8h with 3 to 4 hours infusion and 1g q6h with 1 to 4 hours infusion based on 70% of $T_{>MIC}$ target. Because MIC distribution of *E. cloacae* other than 2 resistant isolates was similar to that of *K. pneumoniae*, LMOX should be effective against *E. cloacae* by same regimens with those against *K. pneumoniae.* Moreover, because most of the ESBL producing isolates were *E. coli* and *K. pneumoniae*[2, 31], the results revealed by Monte Carlo simulation is important findings which showed that LMOX is effective against ESBL producing *E. coli* and *K. pneumoniae* with dosing regimens currently approved and available in Japan.

There were a few PK/PD studies of CFPM and TAZ/PIPC against ESBL producing *E. coli* and *K. pneumoniae*. Ambrose, *et al.* demonstrated that the probability for attaining PK/PD target measures with 30 to 40% $T_{>MIC}$ against ESBL producing *E. coli* exceeded 0.86 by the regimen of 3.375 g of TAZ/PIPC i.v. every 4 or 6 hours, and the probability of meeting PK/PD targets with 50 to 60% $T_{>MIC}$ for CFPM was equal to or higher than that with TAZ/PIPC, which was an acceptable probability of the target attainment[32]. However, most of the isolates used for the pharmacodynamic models reported by Ambrose, *et al.* were susceptible to CFPM and TAZ/PIPC, which was not consistent with the report by Sader, *et al.*[26]. Reese, *et al.* also reported the PTA of TAZ/PIPC and CFPM against ESBL producing isolates[33]. They demonstrated that the TAZ/PIPC achieved 43% of the PTA with the regimen of 3.375 g every 4 h, and CFPM achieved 77% of the PTA with 4 g of continuous infusion. Reese, *et al.* concluded that neither TAZ/PIPC nor CFPM achieved a high PTA and should not be used routinely for the treatment of ESBL infections.

The present study is the first report to evaluate the pharmacodynamics of LMOX against ESBL producing *E. coli* and *K. pneumoniae*. The PK/PD findings as presented in this study may

facilitate customized dosing to optimize the PD behavior of LMOX. The simulations in the present study demonstrated that LMOX achieved a high likelihood of bactericidal activity against ESBL producing Enterobacteriaceae including *E. coli* and *K. pneumoniae* with currently available dosing regimen approved and available in Japan. Therefore, LMOX could be an alternative to carbapenems for the treatment of patients with ESBL producing Enterobacteriaceae.

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References

1) Falagas, M. E. & D. E. Karageorgopoulos: Extended-spectrum *β*-lactamase-producing organisms. J. Hosp. Infect. 73: 345～354, 2009
2) Pitout, J. D. D. & K. B. Laupland: Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect. Dis. 8: 159～166, 2008
3) Cantón, R. & T. M. Coque: The CTX-M beta-lactamase pandemic. Curr. Opin. Microbiol. 9: 466～475, 2006
4) Reinert, R. R.; D. E. Low, F. Rossi, *et al.*: Antimicrobial susceptibility among organisms from the Asia/Pacific Rim, Europe and Latin and North America collected as part of TEST and the *in vitro* activity of tigecycline. J. Antimicrob. Chemother. 60: 1018～1029, 2007
5) Talbot, G. H.; J. Bradley, J. E. Edwards Jr., *et al.*: Bad bugs need drugs: An update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. Clin. Infect. Dis. 42: 657～668, 2006
6) Nicolau, D. P.: Pharmacodynamic optimization of beta-lactams in the patient care setting. Crit. Care 12 (Sppl. 4): S2, 2008
7) Samtani, M. N.; R. Flamm, K. Kaniga, *et al.*: Pharmacokinetic-pharmacodynamic-model-guided doripenem dosing in critically ill patients. Antimicrob. Agents Chemother. 54: 2360～2364, 2010
8) Craig, W. A.: Pharmacokinetic/pharmacodynamic parameters: Rationale for antibacterial dosing of mice and men. Clin. Infect. Dis. 26: 1～12, 1998
9) Craig, W. A.: Dose the dose matter? Clin. Infect. Dis. 33: 233～237, 2001
10) Drusano, G. L.: Antimicrobial pharmacodynamics: critical interactions of “bug and drug”. Nat. Rev. Microbiol. 2: 289～300, 2004
11) Kuti, J. L.; C. H. Nightingale & D. P. Nicolau: Optimizing pharmacodynamic target attainment using the MYSTIC antibiogram: data collected in North America in 2002. Antimicrob. Agents Chemother. 48: 2464～2470, 2004
12) Kuti, J. L.; P. D. Dandekar & C. H. Nightingale: Use of Monte Carlo Simulation to design an optimized pharmacodynamic dosing strategy for meropenem. J. Clin. Pharmacol. 43: 1116～1123, 2003
13) Lomaestro, B. M. & G. L. Drusano: Pharmacodynamic evaluation of extending the administration time of meropenem using Monte Carlo Simulation pharmacodynamic. Antimicrob. Agents Chemother. 49: 461～463, 2005
14) Sun, H. K.; J. L. Kuti & D. P. Nicolau: Pharmacodynamics of antimicrobial for the empirical

treatment of nosocomial pneumonia: A report from the OPTAMA program. Crit. Care Med. 33: 2222～2227, 2005

15) Krueger, W. A.; J. Bulita, M. Kinzig-Schippers, *et al.*: Evaluation by Monte Carlo Simulation of the pharmacokinetics of two doses of meropenem administered intermittently or as a continuous infusion in healthy volunteers. Antimicrob. Agents Chemother. 49: 2464～2476, 2005

16) Narisada, M.; T. Yoshida, H. Onoue, *et al.*: Synthetic studies on beta-lactam antibiotics. Part 10. Synthesis of 7beta-[2-carboxy-2-(4-hydroxyphenyl)acetamido]-7-alpha-methoxy-3-[[1-methyl-1*H*-tetrazol-5-yl)thio]-methyl]-1-oxa-1-dethia-3-cephem-4-carboxylic acid disodium salt (6059-S) and its related 1-oxacephems. J. Med. Chem. 22: 757～759, 1979

17) Goto, S.: *In vitro* & *in vivo* antibacterial activity of moxalactam, an oxa-beta-lactam antibiotic. Rev. Infect. Dis. 4: S501～S510, 1982

18) Clinical & Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard-eighth edition (M07-A8). Wayne, CLSI, 2009

19) Yamada, H.; T. Yoshida, T. Oguma, *et al.*: Clinical pharmacology of 6059-S in healthy volunteers. Jpn. J. Chemother. 28(S-7): 251～262, 1980

20) Yoshida, T.; S. Matsuura, M. Mayama, *et al.*: Moxalactam (6059-S), a novel 1-oxa-*β*-lactam with an expanded antibacterial spectrum: Laboratory evaluation. Antimicrob. Agents Chemother. 17: 302～312, 1980

21) Yoshida, T.; Y. Kimura & Y. Tochino: Pharmacokinetics of 6059-S in experimental animals. Jpn. J. Chemother. 28(S-7): 194～206, 1980

22) Paterson, D. L. & R. A. Bonomo: Extended-spectrum beta-lactamase: A clinical update. Clin. Microbiol. Rev. 18: 657～686, 2005

23) Paterson, D. L.; W. C. Ko, A. Von Gottberg, *et al.*: Antibiotic therapy for *Klebsiella pneumoniae* bacteremia: Implications of production of extended-spectrum *β*-lactamases. Clin. Infect. Dis. 39: 31～37, 2004

24) Paterson, D. L; W. C. Co, A. Bon Gottberg, *et al.*: International prospective study of *Klebsiella pneumoniae* bacteremia: implications of extended-spectrum beta-lactamase production in nosocomial infections. Ann. Intern. Med. 140: 26～32, 2004

25) Nakamura, T.; C. Shimizu, M. Kasahara, *et al.*: Monte Carlo simulation for evaluation of the efficacy of carbapenems and new quinolones against ESBL-producing *Escherichia coli*. J. Infect. Chemother. 15: 13～17, 2009

26) Sader, H. S.; A. Hsiung, T. R. Fritsche, *et al.*: Comparative activities of cefepime and piperacillin/tazobactam tested against a global collection of *Escherichia coli* and *Klebsiella* spp. with an ESBL phenotype. Diagn. Microbiol. Infect. Dis. 57: 341～344, 2007

27) Yoshida, I.; T. Fujimura, Y. Jinushi, *et al.*: Antimicrobial susceptibility of clinical isolates of aerobic Gram-negative bacteria in 2002. Jpn. J. Chemother. 54: 355～377, 2006

28) Yoshida, I.; T. Fujimura, T. Itoh, *et al.*: Antimicrobial susceptibility of clinical isolates of aerobic Gram-negative bacteria in 2004. Jpn. J. Chemother. 56: 562～579, 2008

29) Yoshida, I.; T. Yamaguchi, Y. Itoh, *et al.*: Antimicrobial susceptibility of clinical isolates of aerobic Gram-negative bacteria in 2006. Jpn. J. Antibiotics 63: 457～479, 2010

30) Yoshida, I.; T. Yamaguchi, R. Kudo, *et al.*: Antimicrobial susceptibility of clinical isolates of aerobic Gram-negative bacteria in 2008. Jpn. J. Antibiotics 65: 73～96, 2012

31) Pitout, J. D. D.; P. Nordmann, K. B. Laupland, *et al.*: Emergence of enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs) in the community. J. Antimicrob. Chemother. 56:

52～59, 2005
32） Ambrose, P. G.; S. M. Bhavnani & R. N. Jonse: Pharmacokinetics-pharmacodynamics of cefepime and piperacillin-tazobactam against *Escherichia coli* and *Klebsiella pneumoniae* strains producing extended-spectrum beta-lactamases: Report from the ARREST program. Antimicrob. Agents Chemother. 47: 1643～1646, 2003
33） Reese, A. M.; C. R. Frei & D. S. Burgess: Pharmacodynamics of intermittent and continous infusion piperacillin-tazobactam and cefepime against extended-spectrum beta-lactamase-producing organisms. Int. J. Antimicrob. Agents 26: 114～119, 2005