Pharmacokinetic/Pharmacodynamic-based Assessment on the Efficacy of Cephamycins for Extended-Spectrum β-Lactamase-Producing Enterobacterales Infections in Dogs

（犬の基質特異性拡張型 β-ラクタマーゼ産生菌感染症に対するセファマイシン系抗菌薬の薬物動態学・薬力学解析に基づく有効性評価）

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General Introduction

Extended spectrum β-lactamases (ESBLs) are β-lactamases classified as class A or D in the Ambler system and group 2be in the Bush-Medeiros-Jacoby system (Ambler, 1980; Bush & Jacoby, 2010). They have been found in many Enterobacterales, and are associated with both chromosomes and plasmids (Castanheira *et al*., 2021). A notable feature of ESBLs is to hydrolyze third-generation cephalosporins, in addition to penicillins, first- and second-generation cephalosporins, and monobactams, although they can be inhibited by β-lactamase inhibitors (Pitout & Laupland, 2008; Castanheira *et al.*, 2021). Thus, Enterobacterales that produce extended-spectrum β-lactamases (ESBL-E) are essentially resistant to a wide range of β-lactams. In addition, most ESBL-E exhibit resistance to the other classes of antimicrobials for the following reasons. One reason is that the plasmids encoding ESBL genes often carry resistance genes for different classes of antimicrobials. Another reason is that ESBL-E are frequently subjected to selective pressure with other classes of antimicrobials. These circumstances can limit the number of effective antimicrobial drugs available for the treatment of infections caused by ESBL-E (ESBL infections) (Pitout & Laupland, 2008).

ESBL-E are widely distributed in both the veterinary and human domains worldwide (Castanheira *et al.*, 2021; Tseng *et al.*, 2023). A meta-analysis by Salgado-Caxito *et al.* (2021) revealed that ESBL-producing *Escherichia coli* are widely distributed in companion animals across all continents, with a prevalence of 6.87% in dogs and 5.04% in cats. The previous studies identified ESBLs in 15.5% of *E. coli*, 34% of *Klebsiella pneumoniae*, 3.6% of *Proteus mirabilis*, and 36.4% of *Enterobacter cloacae* isolates from companion animals in Japan (Harada *et al.*, 2017; Maeyama *et al.*, 2018). These ESBL-E have been the most frequently isolated from companion animals with urinary tract infections, followed by skin and respiratory tract infections (Shimizu *et al.*, 2017; Woerde *et al.*, 2023). Most of ESBL-E in companion

animals have multidrug-resistance phenotypes including resistance to almost all veterinary antimicrobial products, making their treatment complex (Woerde *et al.*, 2023). However, therapeutic guidelines for ESBL infections in companion animals have not yet been established.

Carbapenems are primarily used for the treatment of ESBL infections in human medicine because of the high *in vitro* and clinical efficacy of ESBL-E (Perezt *et al.*, 2007; Karaiskos & Giamarellou, 2020). However, the use of carbapenems has a risk of selecting and increasing carbapenem-resistant Enterobacterales (CRE), which exhibits a more serious multidrug-resistance phenotype than ESBL-E (Perezt *et al.*, 2007). In CRE, carbapenem resistance can be brought about by the production of carbapenemase, β-lactamases classified as class A, B, or D in the Ambler system, mutation/deletion of porins, and/or efflux of antimicrobial substances by efflux pumps (Ma *et al.*, 2023). The World Health Organization has listed CRE as the most important priority pathogen for new antimicrobial product development (Tacconelli *et al.*, 2017; Tompkins *et al.*, 2021). Although the true prevalence of CRE in companion animals is unknown, there have been several reports on CRE isolation in dogs and cats worldwide (Köck *et al.*, 2018; KuKanich *et al.*, 2023). Such prevalence of CRE in companion animals represents not only a serious concern in veterinary medicine but also potential public health threats by transmitting to surrounding people through close contact (Roscetto *et al.*, 2021). These findings emphasize the need to explore alternatives to carbapenems for the treatment of ESBL infections in companion animal medicine.

In recent years, the development of new antimicrobial product has become more difficult in veterinary medicine (Prescott, 2017). Such a background can promote the effective utilization of human antimicrobial products in companion animals. Cephamycins, including cefmetazole (CMZ), flomoxef (FMX), and latamoxef (moxalactam, LMX), are hardly hydrolyzed by ESBLs due to the presence of 7α methoxy constituents (Neu, 1986; Jacoby & Carreras, 1990). These cephamycins have a high *in vitro* susceptibility rate of > 90% against human-origin ESBL-E

(Sato *et al.*, 2015; Yang *et al.*, 2015; Jung *et al.*, 2019). These cephamycins are also highly safe for dogs: no observed adverse effect levels of CMZ, FMX, and LMX in dogs have been reported to be 1,200, 200, and 400 mg/kg/day, respectively (Masuda *et al.*, 1978; Kobayashi *et al.*, 1980; Mitsuzono *et al.*, 1987), which is extremely high compared to human dosage (i.e., a maximum of 37.5 mg/kg four times per day, respectively). These findings raise the possibility that CMZ, FMX, and LMX may be alternatives to carbapenems for the treatment of dogs with ESBL infections. However, the *in vitro* activity of these cephamycins against ESBL-E from companion animals remains to be fully investigated. In addition, there is little knowledge of the pharmacokinetics (PK) of these cephamycins in dogs, because these drugs are approved for use in humans but not in dogs. Therefore, appropriate dosage regimens of cephamycins for canine ESBL infections remain to be investigated.

In the last decade, PK/pharmacodynamics (PD) analysis using Monte Carlo simulation (MCS) has been practically used in many researches to consider appropriate dosage regimens of antimicrobial products (Trang *et al.*, 2017; Toutain *et al.*, 2020). MCS can create a large virtual population via randomization of PK/PD indices (PDIs), which allows estimation of the probability of target attainment (PTA) by dosage regimen (Trang *et al.*, 2017). The measurement of PTAs contributes to determining the following values essential to estimate the appropriateness of dosage regimens: nonclinical PK/PD cutoff and cumulative fraction of response (CFR). The nonclinical PK/PD cutoff values are defined as the highest minimum inhibitory concentration (MIC) of those achieve a PTA of 90% or higher (Toutain *et al.*, 2020), and are utilized as one of the valuable indices when the Clinical and Laboratory Standards Institute (CLSI) establishes susceptibility breakpoints for each antimicrobial substance (Toutain *et al.*, 2020; CLSI, 2023). The CFR, which represents the expected population of PTA for a specific dosage of antimicrobial product for a specific MIC distribution of microorganisms, is calculated by multiplying the PTA calculated for each MIC by the percentage of the microbial

population (Drusano *et al.*, 2001; Bradley *et al.*, 2003; Wang *et al.*, 2021). Such MCS-based PK/PD approaches have been frequently used to assess the efficacy of antibiotics in human medicine. MCS has been increasingly used in veterinary medicine in recent years (Toutain *et al.*, 2020), and has thereby contributed to the establishment of veterinary-specific breakpoints, as described in the CLSI guidelines (CLSI guideline M23, 2023).

This thesis consists of four chapters. In Chapter 1, the author determined MICs of CMZ, FMX, and LMX in wild-type ESBL-producing *K. pneumoniae*, *P. mirabilis*, and *E. cloacae* isolates from companion animals to investigate the PD index in Enterobacterales other than *E. coli*.

In Chapters 2, 3, and 4, the author performed intravenous administration studies of CMZ, FMX, and LMX, respectively, using healthy beagle dogs, and calculated PK parameters based on blood concentrations of these cephamycins. In addition, the author determined the nonclinical PK/PD cutoff values of these cephamycins in dogs by using PK parameters. Furthermore, CFRs were estimated based on wild-type MIC distributions in ESBL-E isolated from companion animals. Considering these findings, the author finally proposed clinically effective dosage regimens of cephamycins for ESBL infections in dogs.

Chapter 1

In vitro efficacy of cephamycins against multiple extended spectrum β-lactamase-producing *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterobacter cloacae* isolates from dogs and cats

1. Introduction

The cephamycins CMZ, FMX, and LMX are hardly hydrolyzed by ESBLs (Neu, 1986; Jacoby & Carreras, 1990). In human medicine, the primary antimicrobial products of choice for the treatment of ESBL infections is carbapenems, but CMZ, FMX, and LMX are considered carbapenem-sparing antibiotics (Huang *et al.*, 2019; Karaiskos & Giamarellou, 2020). Shimizu *et al.* (2017) have demonstrated the high *in vitro* efficacy of these cephamycins against ESBL-producing *E. coli* isolates from companion animals. However, the efficacy of these cephamycins against other ESBL-E has not yet been investigated.

AmpC β-lactamases (ABLs) are β-lactamases classified as class C in the Ambler system and group 1e in the Bush-Medeiros-Jacoby system (Ambler, 1980; Bush & Jacoby, 2010), and can hydrolyze third-generation or extended-spectrum cephalosporins (Paterson, 2006; Jacoby, 2009; Bush & Jacoby, 2010). Unlike ESBLs, ABLs also hydrolyze cephamycins, in addition to clavulanate and other β-lactamase inhibitors (Jacoby, 2009; Bush & Jacoby, 2010). The previous studies identified ABLs in ESBL-E from companion animals (Harada *et al.*, 2016; Harada *et al.*, 2017; Shimizu *et al.*, 2017). Therefore, it is important to investigate the prevalence of ABLs among ESBL-E to assess the efficacy of cephamycins.

The objective of this chapter was to compare the effects of CMZ, FMX, and LMX with those of meropenem (MEM), one of the carbapenems, on *K. pneumoniae*, *P. mirabilis*, and *E. cloacae* isolated from companion animals that produce ESBLs with or without ABLs *in vitro*.

2. Materials and Methods

1) Bacterial strains

A total of 218 ESBL-producing strains of *K. pneumoniae* (n = 120), *P. mirabilis* (n = 29), and *E. cloacae* (n = 69) were collected from 136 dogs and 82 cats treated at Japanese veterinary clinics between 2019 and 2022. These isolates were obtained from collections ordered to

commercial labs for clinical purposes. The isolates were obtained from multiple anatomical sites evaluated by clinical veterinarians as sites of bacterial infection. *K. pneumoniae* strains were isolated from the urinary tract (n = 83), pus (n = 13), respiratory organs (n = 6), skin (n = 4), ear (n = 3), and others (n = 11); *P. mirabilis* strains were isolated from urinary tract (n = 23) and others (n = 6); and *E. cloacae* strains were isolated from urinary tract (n = 45), skin (n = 9), respiratory organs (n = 3), and others (n = 12). Further details of the isolates in this study are given in Table 1. No information was available regarding previous antimicrobial treatment of the dogs and cats.

Bacterial species were identified by monitoring their growth on deoxycholate-hydrogen sulfide-lactose agar (Eiken Chemical Co., Ltd., Tochigi, Japan) and using the Crystal Enteric/Non-Fermenter ID kit (Bruker Daltonik, Bremen, Germany), API 20E kit (SYSMEX bioMérieux, Tokyo, Japan), and/or MALDI-TOF MS was evaluated with the Bruker MALDI Biotyper system (Bruker Daltonik, Bremen, Germany). After the identification of bacterial species, all isolates were stored at -80°C in 10% skim milk. ESBL and ABL production was confirmed for all isolates using the ABL and ESBL Detection Set (MAST Group Ltd., Merseyside, UK). This set contained cefpodoxime disks, alone and in combination with ABL and/or ESBL inhibitors (Rodríguez-Guerrero *et al.*, 2022). Such phenotypic tests cannot detect inducible chromosomal ABLs (Rodríguez-Guerrero *et al.*, 2022), which are widely prevalent in *E. cloacae* but not in *K. pneumoniae* and *P. mirabilis* (Jacoby, 2009); therefore, these tests can mainly help in detecting plasmid-mediated ABLs. The results were interpreted as previously described (Rodríguez-Guerrero *et al.*, 2022; Shimizu *et al.*, 2017).

2) Antimicrobial susceptibility testing

All isolates were tested for susceptibility to the following antimicrobial products: CMZ (Nichi-Iko Pharmaceutical Co. Ltd, Tokyo, Japan), FMX (Shionogi Co. Ltd, Osaka, Japan),

LMX (Shionogi Co. Ltd, Osaka, Japan), and MEM (Wako Pure Chemical Industries, Ltd., Osaka, Japan). MICs were determined using the agar dilution method according to CLSI guidelines (CLSI standard VET01, 2018) and were interpreted based on the CLSI breakpoints (CLSI supplement M100, 2022) for CMZ, LMX, and MEM except FMX, for which the breakpoint of LMX was used instead, according to the previous study (Yang *et al.*, 2015; Matsumura *et al.*, 2016; Shimizu *et al.*, 2017). Strains of *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality controls.

3) Statical analysis

The antimicrobial susceptibility rates between the two groups were compared using Fisher's exact test. A P value < 0.05 was considered significant.

3. Results and Discussion

Research into antimicrobial products of carbapenem-sparing candidates is an urgent issue for the treatment of ESBL infections without the risk of developing CRE. To solve this problem, the author first investigated the *in vitro* efficacy of cephamycins against a large collection of representative ESBL-E other than *E. coli* isolated from dogs and cats and compared the susceptibility of these cephamycins with that of MEM.

It is important to determine the distribution of ABLs in ESBL-E when considering the use of cephamycin, since ABLs, unlike ESBLs, can hydrolyze cephamycins (Jacoby, 2009; Rodríguez-Guerrero *et al.*, 2018). Confirmatory phenotypic testing found only ESBLs in 112 *K. pneumoniae* (93.3%), 29 *P. mirabilis* (100%), and 54 *E. cloacae* (78.3%) isolates, while both ESBL and ABL (mainly, plasmid-mediated ABLs) were detected in only 8 *K. pneumoniae* (6.7%) and 15 *E. cloacae* (21.7%) isolates. A similar prevalence of ABLs was confirmed in previous studies with a smaller number of ESBL-producing *K. pneumoniae* and *E. cloacae*

(Harada *et al.*, 2016; Harada *et al.*, 2017). It appears that the prevalence of ABLs is likely to differ among ESBL-E species, and this should be taken into account when using cephamycins for ESBL infections.

The results of the susceptibility testing in this study are summarized in Table 2 and the distribution of MIC for the tested substances is shown in Figs. 1–3. All isolates tested, with or without ABLs, were highly susceptible to MEM, indicating that carbapenems have high efficacy against ESBL-producing isolates of *K. pneumoniae*, *P. mirabilis*, and *E. cloacae*, as well as *E. coli* (Shimizu *et al.*, 2018), from companion animals. However, previous studies showed the prevalence of CRE in pets (Guerra *et al.*, 2014). Therefore, the use of carbapenems for pets infected with ESBL-E should be carefully considered due to the potential public health risk of CRE (Roscetto *et al.*, 2021).

Cefmetazole is an antimicrobial candidate for infections caused by ESBL-E in humans (Doi *et al.*, 2013; Fukuchi *et al.*, 2016). In this study, there were no significant differences in susceptibility rates between CMZ and MEM in *K. pneumoniae* isolates producing only ESBL ($P > 0.05$), indicating that CMZ has non-inferior *in vitro* efficacy against ESBL-producing *K. pneumoniae* isolates, as well as *E. coli* isolates (Shimizu *et al.*, 2017), when compared to carbapenems. These findings were supported by previous *in vitro* studies using human isolates (Yang *et al.*, 2015; Matsumura *et al.*, 2016). However, most *K. pneumoniae* isolates producing both ESBL and ABL demonstrated extremely high MICs (≥ 16 μg/mL) which resulted in significantly decreased rates of CMZ susceptibility ($P < 0.05$). This decreased CMZ susceptibility in ESBL and ABL-producing *K. pneumoniae* was previously confirmed in isolates from both animals and humans (Matsumura *et al.*, 2016; Shimizu *et al.*, 2017). This was due to the high stability of ABLs to CMZ (Jacoby, 2009). ESBL-producing *P. mirabilis* isolates exhibited a CMZ susceptibility rate ≥ 80%, but this was somewhat lower than the rate in previous studies (95.5–100%) (Yang *et al.*, 2015; Matsumura *et al.*, 2016). One possible

reason is that the *P. mirabilis* isolates in the current study had ABL-independent resistance mechanisms, such as less production of porin (Ito *et al.*, 2022) and expression of multidrug efflux pump (Wang *et al.*, 2021b); however, further studies are warranted to confirm the role of these resistance mechanisms. Additionally, *E. cloacae* isolates had higher MICs than other bacterial species, resulting in their significantly lower susceptibility rates ($P < 0.05$). This may be explained by the fact that most *E. cloacae* have inducible chromosomal ABLs (Jacoby *et al.*, 2009), in addition to plasmid-mediated ABLs, which were mainly detected in this study. A previous study demonstrated that most *E. cloacae* isolates exhibited significant resistance to CMZ regardless of the presence of ESBLs (Harada *et al.*, 2017). The results of the current study indicate that CMZ should be limited to the treatment of infection with ESBL- and non-ABL-producing isolates of *K. pneumoniae* and *P. mirabilis*, as well as *E. coli*.

Of the cephamycins, FMX and LMX are subclassified as oxacephems due to the S-O replacement in the 7β-difluoromethylthioacetamido derivative (Tsuji *et al.*, 1985). There are several clinical studies on the efficacy of FMX, as well as CMZ, for treating people with ESBL infections (Lee *et al.*, 2015; Matsumura *et al.*, 2015). The results of this study showed clear differences of *in vitro* activity against ESBL-E between the two substances. FMX was found to be non-inferior to MEM in *K. pneumoniae* and *P. mirabilis* isolates that only produced ESBL. Similar findings were observed in animal-origin ESBL-producing *E. coli* (Shimizu *et al.*, 2017) and human-origin ESBL-E isolates (Yang *et al.*, 2015; Matsumura *et al.*, 2016). Furthermore, this study found significantly reduced rates of susceptibility in *E. cloacae* isolates alongside the *K. pneumoniae* isolates that produced both ESBL and ABL, although they were not comparable to CMZ. However, LMX had the same high *in vitro* potency as MEM in all *K. pneumoniae* and *P. mirabilis* isolates producing ESBL with or without ABL. Surprisingly, LMX was also non-inferior to MEM against *E. cloacae* isolates producing only ESBL with an MIC ≤ 0.25 μg/mL. However, *E. cloacae* isolates producing both ESBLs and ABLs had lower susceptibility rates

to LMX as well as CMZ and FMX. To the best of her knowledge, this is the first report on LMX susceptibility of ESBL-producing *P. mirabilis* and *E. cloacae* but not ESBL-producing *E. coli* and *K. pneumoniae* (Sato *et al.*, 2015; Salgado-Caxito *et al.*, 2021). These results imply that of the three cephamycins, LMX is the least likely to be hydrolyzed by ABLs and/or induce chromosomal ABLs.

There were no significant differences in the susceptibility rates in dogs and cats, except for CMZ susceptibility rates in *K. pneumoniae*, which was significantly lower in dogs than in cats. This finding can be explained by the higher prevalence of ABL-producing *K. pneumoniae* in dogs. However, this finding cannot be necessarily generalized because the insufficient number of *K. pneumoniae* isolates were used in this study. Thus, differences in cephamycin susceptibility rates between animal species should be further considered by more extensive investigations.

In conclusion, the author evaluated the *in vitro* activity of three cephamycins against representative ESBL-E isolates from companion animals. Results indicated that all three cephamycins can be carbapenem-sparing antibiotics against infection with *K. pneumoniae* and *P. mirabilis* isolates producing only ESBL. However, of the cephamycins tested, LMX is the best option for ESBL-E producing any ABLs.

Table 1a. The details of *K. pneumoniae* isolates used in this study

Isolates No.[a)]	Isolation years	Animals	Isolation sites	Production of: [a)]		MICs (μg/mL) for: [a)]			
				ESBL	ABL	CMZ	FMX	LMX	MEM
KP1	2021	Cat	Urinary tract	+	-	1	0.125	0.5	0.031
KP2	2021	Dog	Skin	+	-	1	0.125	0.5	0.031
KP3	2021	Dog	Urinary tract	+	-	1	0.125	0.25	0.031
KP4	2021	Dog	Urinary tract	+	-	2	0.125	0.25	0.031
KP5	2021	Dog	Urinary tract	+	-	1	0.125	0.125	0.031
KP6	2021	Cat	Urinary tract	+	-	1	0.125	0.5	0.031
KP7	2021	Dog	Urinary tract	+	-	32	2	8	0.5
KP8	2021	Dog	Respiratory organs	+	-	2	0.125	0.25	0.031
KP9	2021	Dog	Urinary tract	+	-	4	0.25	0.5	0.031
KP10	2021	Cat	Urinary tract	+	-	4	0.25	1	0.031
KP11	2021	Dog	Urinary tract	+	-	1	0.125	0.125	0.031
KP12	2021	Dog	Urinary tract	+	-	1	0.125	0.25	0.063
KP13	2021	Cat	Urinary tract	+	-	1	0.125	0.25	0.031
KP14	2021	Dog	Urinary tract	+	-	2	0.125	0.25	0.031
KP15	2021	Dog	Respiratory organs	+	-	2	0.125	1	0.063
KP16	2021	Dog	Eye	+	-	1	0.125	0.25	0.063
KP17	2021	Dog	Urinary tract	+	-	2	0.125	1	0.063
KP18	2021	Dog	Unknown	+	-	1	0.125	0.25	0.031
KP19	2021	Dog	Urinary tract	+	-	1	0.125	0.25	0.031
KP20	2021	Dog	Urinary tract	+	-	4	0.125	0.5	0.063
KP21	2021	Dog	Unknown	+	-	8	0.25	0.5	0.031
KP22	2021	Dog	Genitals	+	-	1	0.125	0.25	0.031
KP23	2021	Dog	Urinary tract	+	-	1	0.125	0.5	0.031
KP24	2021	Dog	Urinary tract	+	-	8	0.5	1	0.031
KP25	2021	Cat	Skin	+	-	4	0.25	0.5	0.031
KP26	2021	Cat	Urinary tract	+	-	2	0.125	0.5	0.031
KP27	2021	Cat	Urinary tract	+	-	2	0.125	0.5	0.031
KP28	2021	Cat	Urinary tract	+	-	1	0.125	0.25	0.031
KP29	2021	Dog	Urinary tract	+	-	2	0.125	0.5	0.031
KP30	2021	Dog	Ear	+	-	1	0.125	0.5	0.031
KP31	2021	Cat	Urinary tract	+	-	1	0.125	0.25	0.031
KP32	2021	Cat	Urinary tract	+	-	1	0.125	0.25	0.031

KP33	2021	Cat	Urinary tract	+	-	4	0.125	0.25	0.031
KP34	2021	Dog	Urinary tract	+	-	2	0.125	0.5	0.063
KP35	2021	Dog	Bile	+	-	1	0.063	0.25	0.031
KP36	2021	Dog	Urinary tract	+	-	8	1	2	0.063
KP37	2021	Cat	Urinary tract	+	-	2	0.125	1	0.031
KP38	2021	Cat	Urinary tract	+	-	1	0.125	0.5	0.063
KP39	2021	Dog	Urinary tract	+	-	128	8	32	0.125
KP40	2021	Cat	Urinary tract	+	-	4	0.25	0.5	0.031
KP41	2021	Dog	Unknown	+	-	16	0.5	1	0.031
KP42	2021	Cat	Urinary tract	+	-	2	0.125	1	0.125
KP43	2021	Dog	Pus	+	-	2	0.125	0.25	0.031
KP44	2021	Dog	Urinary tract	+	-	2	0.125	0.5	0.063
KP45	2021	Dog	Urinary tract	+	-	8	0.5	2	0.063
KP46	2021	Dog	Ascites	+	-	1	0.125	0.5	0.063
KP47	2021	Dog	Urinary tract	+	-	2	0.5	0.5	0.063
KP48	2021	Dog	Urinary tract	+	-	4	0.25	0.5	0.031
KP49	2021	Dog	Pus	+	-	8	0.5	1	0.063
KP50	2021	Cat	Urinary tract	+	-	0.5	0.063	0.125	0.031
KP51	2021	Cat	Urinary tract	+	-	2	0.125	0.5	0.063
KP52	2021	Cat	Urinary tract	+	-	4	0.25	1	0.063
KP53	2021	Dog	Urinary tract	+	-	4	0.125	0.25	0.063
KP54	2021	Dog	Urinary tract	+	-	1	0.063	0.25	0.031
KP55	2021	Dog	Bile	+	-	4	0.25	1	0.063
KP56	2021	Dog	Urinary tract	+	-	2	0.125	0.5	0.031
KP57	2021	Cat	Urinary tract	+	-	1	0.063	0.25	0.031
KP58	2021	Dog	Urinary tract	+	-	1	0.063	0.5	0.031
KP59	2021	Cat	Urinary tract	+	-	2	0.125	0.5	0.063
KP60	2021	Dog	Urinary tract	+	-	1	0.063	0.25	0.031
KP61	2021	Cat	Urinary tract	+	-	1	0.125	0.25	0.063
KP62	2021	Dog	Urinary tract	+	-	1	0.063	0.5	0.031
KP63	2021	Cat	Urinary tract	+	-	2	0.063	0.25	0.031
KP64	2021	Dog	Urinary tract	+	-	1	0.063	0.5	0.031
KP65	2021	Dog	Respiratory organs	+	-	4	0.125	1	0.031
KP66	2021	Dog	Urinary tract	+	-	32	4	8	1
KP67	2021	Cat	Urinary tract	+	-	1	0.063	0.125	0.031
KP68	2021	Dog	Urinary tract	+	-	8	0.25	1	0.031

KP69	2021	Dog	Urinary tract	+	-	1	0.125	0.25	0.031
KP70	2021	Dog	Urinary tract	+	-	2	0.125	0.5	0.063
KP71	2021	Dog	Urinary tract	+	-	32	16	2	0.063
KP72	2021	Dog	Urinary tract	+	-	16	0.5	1	0.031
KP73	2021	Dog	Urinary tract	+	-	2	0.25	1	0.063
KP74	2021	Cat	Urinary tract	+	-	1	0.125	0.5	0.031
KP75	2021	Dog	Respiratory organs	+	-	4	0.25	0.5	0.031
KP76	2021	Cat	Pus	+	-	2	0.125	0.5	0.063
KP77	2021	Dog	Pus	+	-	16	1	2	0.031
KP78	2021	Dog	Urinary tract	+	-	4	0.25	1	0.031
KP79	2021	Dog	Respiratory organs	+	-	4	0.25	0.5	0.031
KP80	2021	Dog	Urinary tract	+	-	16	0.5	1	0.031
KP81	2021	Dog	Pus	+	-	16	1	2	0.031
KP82	2021	Dog	Urinary tract	+	+	>256	128	4	0.063
KP83	2021	Dog	Urinary tract	+	-	4	0.25	1	0.031
KP84	2021	Dog	Urinary tract	+	-	4	0.25	1	0.031
KP85	2021	Cat	Urinary tract	+	-	2	0.125	1	0.063
KP86	2021	Dog	Urinary tract	+	-	2	0.25	2	0.031
KP87	2021	Cat	Urinary tract	+	-	1	0.125	0.25	0.031
KP88	2021	Dog	Urinary tract	+	-	32	1	8	0.125
KP89	2021	Dog	Urinary tract	+	-	1	0.125	0.25	0.031
KP90	2021	Dog	Urinary tract	+	+	>256	128	16	0.063
KP91	2021	Cat	Pus	+	-	1	0.125	0.25	0.031
KP92	2021	Dog	Urinary tract	+	+	16	8	1	0.031
KP93	2021	Dog	Pus	+	-	2	0.125	0.5	0.031
KP94	2021	Dog	Genitals	+	+	32	32	2	0.063
KP95	2021	Dog	Urinary tract	+	-	2	0.125	1	0.031
KP96	2021	Dog	Pus	+	-	1	0.125	0.5	0.031
KP97	2021	Dog	Urinary tract	+	-	1	0.063	0.125	0.031
KP98	2021	Dog	Urinary tract	+	-	4	0.125	0.25	0.031
KP99	2021	Cat	Urinary tract	+	-	4	0.25	1	0.031
KP100	2021	Cat	Urinary tract	+	-	4	0.25	0.5	0.031
KP101	2021	Dog	Pus	+	-	2	0.125	1	0.031
KP102	2021	Dog	Unknown	+	-	1	0.125	0.25	0.031
KP103	2021	Dog	Pus	+	-	2	0.125	1	0.031

KP104	2021	Dog	Ascites	+	+	>256	64	8	0.063
KP105	2021	Dog	Pus	+	-	1	0.125	0.25	0.031
KP106	2021	Dog	Urinary tract	+	-	1	0.063	0.125	0.015
KP107	2021	Dog	Skin	+	+	>256	128	8	0.063
KP108	2021	Dog	Urinary tract	+	-	1	0.25	0.125	0.031
KP109	2021	Dog	Ear	+	-	1	0.125	0.25	0.031
KP110	2021	Dog	Skin	+	-	16	1	4	0.063
KP111	2021	Dog	Urinary tract	+	+	>256	64	2	0.063
KP112	2021	Dog	Urinary tract	+	-	4	0.25	0.5	0.031
KP113	2021	Dog	Pus	+	-	1	0.125	0.25	0.063
KP114	2021	Dog	Respiratory organs	+	+	128	64	4	0.063
KP115	2021	Dog	Urinary tract	+	-	1	0.125	0.25	0.063
KP116	2021	Dog	Urinary tract	+	-	4	0.25	0.5	0.031
KP117	2021	Cat	Pus	+	-	4	0.25	0.5	0.031
KP118	2021	Dog	Urinary tract	+	-	2	0.125	0.5	0.063
KP119	2021	Dog	Ear	+	-	1	0.125	0.125	0.031
KP120	2021	Dog	Urinary tract	+	-	1	0.125	0.5	0.031

a) KP, *K. pneumoniae*; ESBL, extended spectrum β-lactamase; ABL, AmpC β-lactamase; MIC, minimum inhibitory concentration; MEM, meropenem; CMZ, cefmetazole; FMX, flomoxef; LMX, latamoxef.

Table 1b. The details of *P. mirabilis* isolates used in this study

Isolates No.[a)]	Isolation years	Animals	Isolation sites	Production of: [a)]		MICs (μg/mL) for: [a)]			
				ESBL	ABL	CMZ	FMX	LMX	MEM
PM1	2021	Cat	Urinary tract	+	-	32	1	0.25	0.125
PM2	2021	Cat	Urinary tract	+	-	2	0.125	0.25	0.063
PM3	2021	Dog	Urinary tract	+	-	16	0.25	0.125	0.063
PM4	2021	Cat	Urinary tract	+	-	2	0.125	0.125	0.063
PM5	2020	Dog	Urinary tract	+	-	16	16	8	1
PM6	2020	Dog	Urinary tract	+	-	2	0.5	0.125	0.063
PM7	2020	Dog	Urinary tract	+	-	2	0.25	0.25	0.063
PM8	2020	Dog	Respiratory organs	+	-	2	0.25	0.25	0.063
PM9	2020	Dog	Urinary tract	+	-	2	0.25	0.25	0.063
PM10	2021	Cat	Urinary tract	+	-	2	0.25	0.063	0.063
PM11	2021	Dog	Ear	+	-	2	0.125	0.063	0.063
PM12	2021	Cat	Urinary tract	+	-	8	0.5	0.125	0.063
PM13	2021	Dog	Urinary tract	+	-	4	1	0.25	0.063
PM14	2021	Cat	Urinary tract	+	-	8	0.25	0.25	0.063
PM15	2021	Dog	Urinary tract	+	-	32	0.25	1	0.063
PM16	2021	Cat	Urinary tract	+	-	64	2	1	0.125
PM17	2021	Dog	Urinary tract	+	-	8	0.25	0.25	0.063
PM18	2021	Dog	Urinary tract	+	-	8	0.125	0.063	0.063
PM19	2022	Dog	Urinary tract	+	-	2	0.5	0.063	0.063
PM20	2021	Cat	Urinary tract	+	-	2	0.125	0.063	0.063
PM21	2022	Cat	Urinary tract	+	-	4	0.25	0.125	0.063
PM22	2021	Dog	Skin	+	-	32	0.25	0.25	0.063
PM23	2021	Cat	Urinary tract	+	-	4	0.25	0.125	0.063
PM24	2021	Dog	Urinary tract	+	-	16	0.5	0.25	0.125
PM25	2022	Dog	Urinary tract	+	-	32	0.5	0.25	0.063
PM26	2022	Cat	Urinary tract	+	-	16	0.5	0.063	0.063
PM27	2022	Cat	Respiratory organs	+	-	4	1	0.125	0.063
PM28	2021	Dog	Unknown	+	-	4	0.25	0.125	0.063
PM29	2021	Dog	Unknown	+	-	4	0.25	0.25	0.125

a) PM, *P. mirabilis*; ESBL, extended spectrum β-lactamase; ABL, AmpC β-lactamase; MIC, minimum inhibitory concentration; MEM, meropenem; CMZ, cefmetazole; FMX, flomoxef; LMX, latamoxef.

Table 1c. The details of *E. cloacae* isolates used in this study

Isolates No.[a)]	Isolation years	Animals	Isolation sites	Production of: [a)]		MICs (μg/mL) for: [a)]			
				ESBL	ABL	CMZ	FMX	LMX	MEM
EC1	2022	Dog	Ascites	+	-	128	8	0.5	0.063
EC2	2022	Dog	Urinary tract	+	+	>256	>256	16	0.5
EC3	2021	Cat	Urinary tract	+	-	64	2	0.25	0.063
EC4	2021	Dog	Urinary tract	+	-	64	4	0.125	0.063
EC5	2021	Cat	Urinary tract	+	-	256	4	0.25	0.063
EC6	2021	Dog	Urinary tract	+	-	256	1	0.5	0.031
EC7	2021	Cat	Skin	+	-	>256	16	2	0.125
EC8	2021	Cat	Urinary tract	+	-	128	16	4	0.063
EC9	2021	Cat	Urinary tract	+	-	128	4	0.25	0.063
EC10	2021	Cat	Urinary tract	+	-	>256	8	0.125	0.063
EC11	2021	Cat	Urinary tract	+	+	256	128	0.25	0.125
EC12	2021	Cat	Urinary tract	+	-	256	64	0.5	0.063
EC13	2021	Cat	Urinary tract	+	-	128	1	0.063	0.031
EC14	2021	Cat	Urinary tract	+	-	64	0.25	0.125	0.031
EC15	2021	Dog	Urinary tract	+	-	256	16	0.125	0.063
EC16	2021	Dog	Urinary tract	+	-	256	4	2	0.125
EC17	2021	Cat	Urinary tract	+	-	>256	16	2	0.063
EC18	2021	Cat	Urinary tract	+	+	>256	>256	256	0.25
EC19	2021	Dog	Skin	+	-	128	4	0.125	0.031
EC20	2021	Cat	Urinary tract	+	+	>256	>256	>256	0.25
EC21	2021	Cat	Urinary tract	+	-	1	0.063	0.25	0.031
EC22	2021	Dog	Genitals	+	-	1	0.063	0.25	0.063
EC23	2021	Cat	Urinary tract	+	-	256	2	0.25	0.063
EC24	2021	Dog	Respiratory organs	+	-	256	16	0.5	0.063
EC25	2021	Dog	Urinary tract	+	-	>256	8	1	0.063
EC26	2021	Cat	Urinary tract	+	-	128	8	0.25	0.063
EC27	2021	Cat	Urinary tract	+	-	>256	16	2	0.063
EC28	2021	Cat	Urinary tract	+	-	128	4	0.25	0.031
EC29	2021	Dog	Skin	+	+	>256	>256	128	0.25
EC30	2021	Cat	Urinary tract	+	-	256	2	0.5	0.031
EC31	2021	Cat	Urinary tract	+	-	64	0.25	0.25	0.015
EC32	2021	Cat	Urinary tract	+	-	64	8	0.125	0.031

EC33	2021	Cat	Urinary tract	+	-	64	2	0.125	0.031
EC34	2021	Dog	Respiratory organs	+	-	256	16	0.25	0.063
EC35	2021	Dog	Urinary tract	+	-	256	16	0.5	0.063
EC36	2021	Dog	Ear	+	-	128	8	0.125	0.031
EC37	2020	Cat	Urinary tract	+	-	>256	>256	2	0.063
EC38	2020	Dog	Skin	+	-	256	8	0.125	0.031
EC39	2020	Dog	Skin	+	-	256	16	0.25	0.031
EC40	2020	Dog	Ascites	+	-	64	0.5	0.125	0.031
EC41	2020	Cat	Skin	+	-	64	4	0.125	0.063
EC42	2020	Cat	Skin	+	+	256	256	8	0.031
EC43	2020	Dog	Urinary tract	+	+	32	2	0.5	0.015
EC44	2020	Cat	Urinary tract	+	+	>256	>256	32	0.063
EC45	2020	Cat	Unknown	+	-	>256	64	1	0.125
EC46	2020	Dog	Urinary tract	+	+	256	256	128	0.125
EC47	2020	Cat	Urinary tract	+	+	>256	>256	64	0.125
EC48	2020	Dog	Genitals	+	-	>256	8	0.125	0.063
EC49	2020	Cat	Urinary tract	+	+	256	256	0.125	0.063
EC50	2020	Dog	Ear	+	-	2	0.125	0.5	0.031
EC51	2020	Dog	Bile	+	-	64	0.5	0.063	0.031
EC52	2020	Dog	Urinary tract	+	+	64	256	32	0.25
EC53	2020	Cat	Urinary tract	+	-	64	1	0.063	0.031
EC54	2020	Dog	Joint	+	-	64	4	0.063	0.063
EC55	2020	Dog	Urinary tract	+	-	64	4	0.5	0.063
EC56	2020	Cat	Eye	+	-	2	0.125	0.25	0.031
EC57	2020	Cat	Urinary tract	+	-	128	4	0.5	0.063
EC58	2020	Dog	Urinary tract	+	+	>256	128	1	0.063
EC59	2019	Dog	Unknown	+	-	256	2	0.25	0.031
EC60	2019	Cat	Urinary tract	+	+	256	>256	32	0.125
EC61	2019	Cat	Urinary tract	+	-	4	0.125	0.5	0.063
EC62	2019	Dog	Respiratory organs	+	-	256	16	1	0.063
EC63	2019	Dog	Urinary tract	+	+	>256	>256	128	0.5
EC64	2022	Cat	Urinary tract	+	-	>256	128	1	0.25
EC65	2022	Dog	Skin	+	-	>256	8	0.5	0.063
EC66	2022	Cat	Pus	+	-	>256	8	0.5	0.063
EC67	2022	Cat	Urinary tract	+	-	256	4	1	0.25

EC68	2022	Dog	Skin	+	-	256	4	0.125	0.063
EC69	2022	Cat	Urinary tract	+	-	128	1	0.25	0.031

a) EC, *E. cloacae*; ESBL, extended spectrum β-lactamase; ABL, AmpC β-lactamase; MIC, minimum inhibitory concentration; MEM, meropenem; CMZ, cefmetazole; FMX, flomoxef; LMX, latamoxef.

Table 2a. The number of susceptible isolates (%) to cephamycins in 120 ESBL-producing *K. pneumoniae* isolates from dogs (n = 88) and cats (n = 32).

Antimicrobials (Breakpoint (μg/ml)) [a)]		MIC_{50}[b)] (μg/ml)	MIC_{90}[b)] (μg/ml)	Total	Only ESBL	Both ESBL and ABL
MEM (≤1)						
	Total	0.031	0.063	120 (100)	112 (100)	8 (100)
	Dogs	0.031	0.063	88 (100)	80 (100)	8 (100)
	Cats	0.002	0.004	32 (100)	32 (100)	NA
CMZ (≤16)						
	Total	2	32	108 (90.0)[c)]	107 (95.5)	1 (12.5)[c,d)]
	Dogs	2	32	76 (86.4)[c,e)]	75 (93.8)	1 (12.5)[c,d)]
	Cats	2	4	32 (100)	32 (100)	NA
FMX (≤8)						
	Total	0.125	1	112 (93.3)[c)]	111 (99.1)	1 (12.5)[c,d)]
	Dogs	0.125	8	80 (90.9)[c)]	79 (98.8)	1 (12.5)[c,d)]
	Cats	0.125	0.25	32 (100)	32 (100)	NA
LMX (≤8)						
	Total	0.5	2	118 (98.3)	111 (99.1)	7 (87.5)
	Dogs	0.5	4	86 (97.7)	79 (98.8)	7 (87.5)
	Cats	0.5	1	32 (100)	32 (100)	NA

a) MEM, meropenem; CMZ, cefmetazole; FMX, flomoxef; LMX, latamoxef.

b) MIC_{50}, MIC that can inhibit the growth of 50% of the isolates; MIC_{90}, MIC that can inhibit the growth of 90% of the isolates.

c) Significantly lower compared with meropenem susceptibility rates ($P < 0.05$).

d) Significantly lower compared with susceptibility rates against the same substance in isolates producing only ESBL ($P < 0.05$).

e) Significantly different compared with susceptibility rates in feline isolates.

Table 2b. The number of susceptible isolates (%) to cephamycins in 29 ESBL-producing *P. mirabilis* isolates from dogs (n = 17) and cats (n = 12).

Antimicrobials (Breakpoint (μg/ml)) [a]	MIC_{50} [b] (μg/ml)	MIC_{90} [b] (μg/ml)	Total
MEM (≤1)			
Total	0.063	0.125	29 (100)
Dogs	0.063	0.125	17 (100)
Cats	0.063	0.125	12 (100)
CMZ (≤16)			
Total	4	32	24 (82.7)
Dogs	4	32	14 (82.4)
Cats	4	32	10 (83.3)
FMX (≤8)			
Total	0.25	1	28 (96.6)
Dogs	0.25	1	16 (94.1)
Cats	0.25	1	12 (100)
LMX (≤8)			
Total	0.25	1	29 (100)
Dogs	0.25	1	17 (100)
Cats	0.125	0.25	12 (100)

a) MEM, meropenem; CMZ, cefmetazole; FMX, flomoxef; LMX, latamoxef.

b) MIC_{50}, MIC that can inhibit the growth of 50% of the isolates; MIC_{90}, MIC that can inhibit the growth of 90% of the isolates.

Table 2c. The number of susceptible isolates (%) to cephamycins in 69 ESBL-producing *E. cloacae* isolates from dogs (n = 31) and cats (n = 38).

Antimicrobials (Breakpoint (μg/ml))[a]		MIC_{50}[b] (μg/ml)	MIC_{90}[b] (μg/ml)	Total	Only ESBL	Both ESBL and ABL
MEM (≤1)						
	Total	0.063	0.25	69 (100)	54 (100)	15 (100)
	Dogs	0.063	0.25	31 (100)	24 (100)	7 (100)
	Cats	0.063	0.25	38 (100)	30 (100)	8 (100)
CMZ (≤16)						
	Total	256	>256	5 (7.2)[c]	5 (9.3)[c]	0 (0)[c]
	Dogs	256	>256	2 (6.5)[c]	2 (6.5)[c]	0 (0)[c]
	Cats	256	>256	3 (7.9)[c]	3 (7.9)[c]	0 (0)[c]
FMX (≤8)						
	Total	8	>256	41 (59.4)[c]	40 (74.0)[c]	1 (6.7)[c,d]
	Dogs	8	256	19 (61.3)[c]	18 (75.0)[c]	1 (14.3)[c,d]
	Cats	8	>256	22 (57.9)[c]	22 (73.3)[c]	0 (0)[c,d]
LMX (≤8)						
	Total	0.5	32	59 (85.5)[c]	54 (100)	5 (33.3)[c,d]
	Dogs	0.5	32	26 (83.9)[c]	24 (100)	2 (28.6)[c,d]
	Cats	0.5	32	33 (86.8)[c]	30 (100)	3 (37.5)[c,d]

a) MEM, meropenem; CMZ, cefmetazole; FMX, flomoxef; LMX, latamoxef.

b) MIC_{50}, MIC that can inhibit the growth of 50% of the isolates; MIC_{90}, MIC that can inhibit the growth of 90% of the isolates.

c) Significantly lower compared with meropenem susceptibility rates ($P < 0.05$).

d) Significantly lower compared with susceptibility rates against the same substance in isolates producing only ESBL ($P < 0.05$).

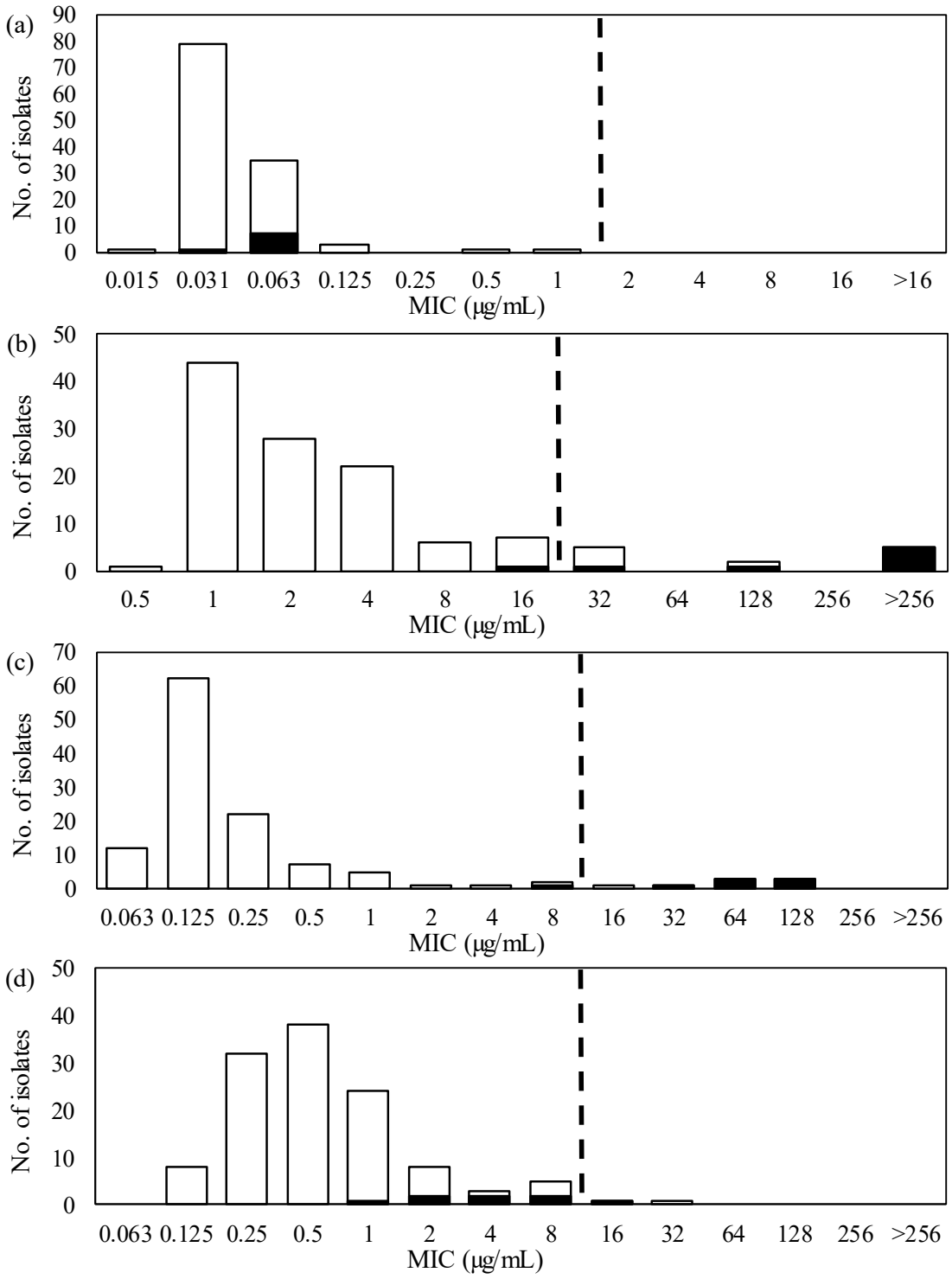

Fig. 1. MIC distribution of ESBL-producing *K. pneumoniae* isolates (n = 120) against MEM (a), CMZ (b), FMX (c), and LMX (d).

The blackened bar indicates the number of ABL-producing isolates.

Vertical broken lines mean the breakpoints of each substance.

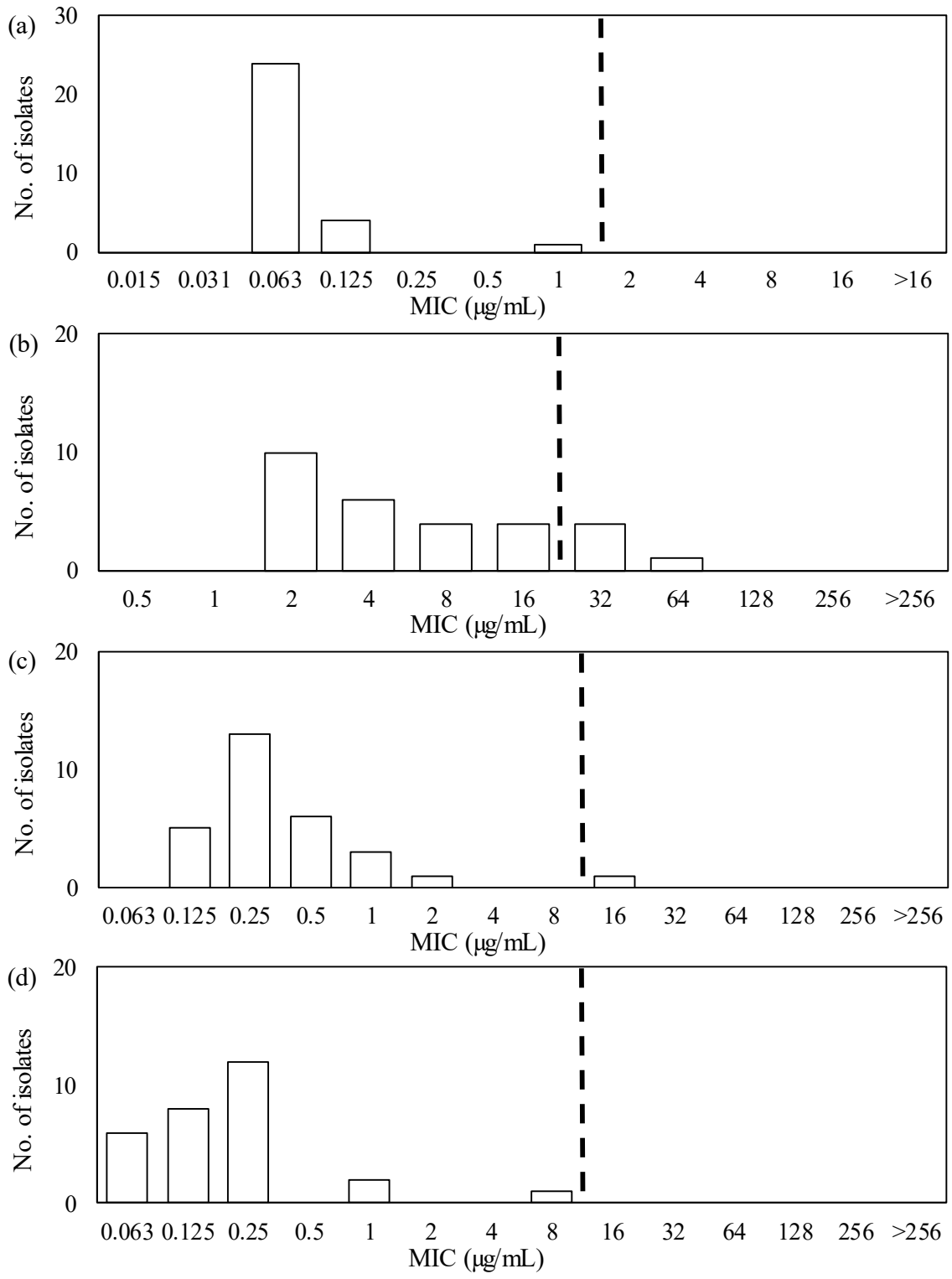

Fig. 2. MIC distribution of ESBL-producing *P. mirabilis* isolates (n = 29) against MEM (a), CMZ (b), FMX (c), and LMX (d).

Vertical broken lines mean the breakpoints of each substance.

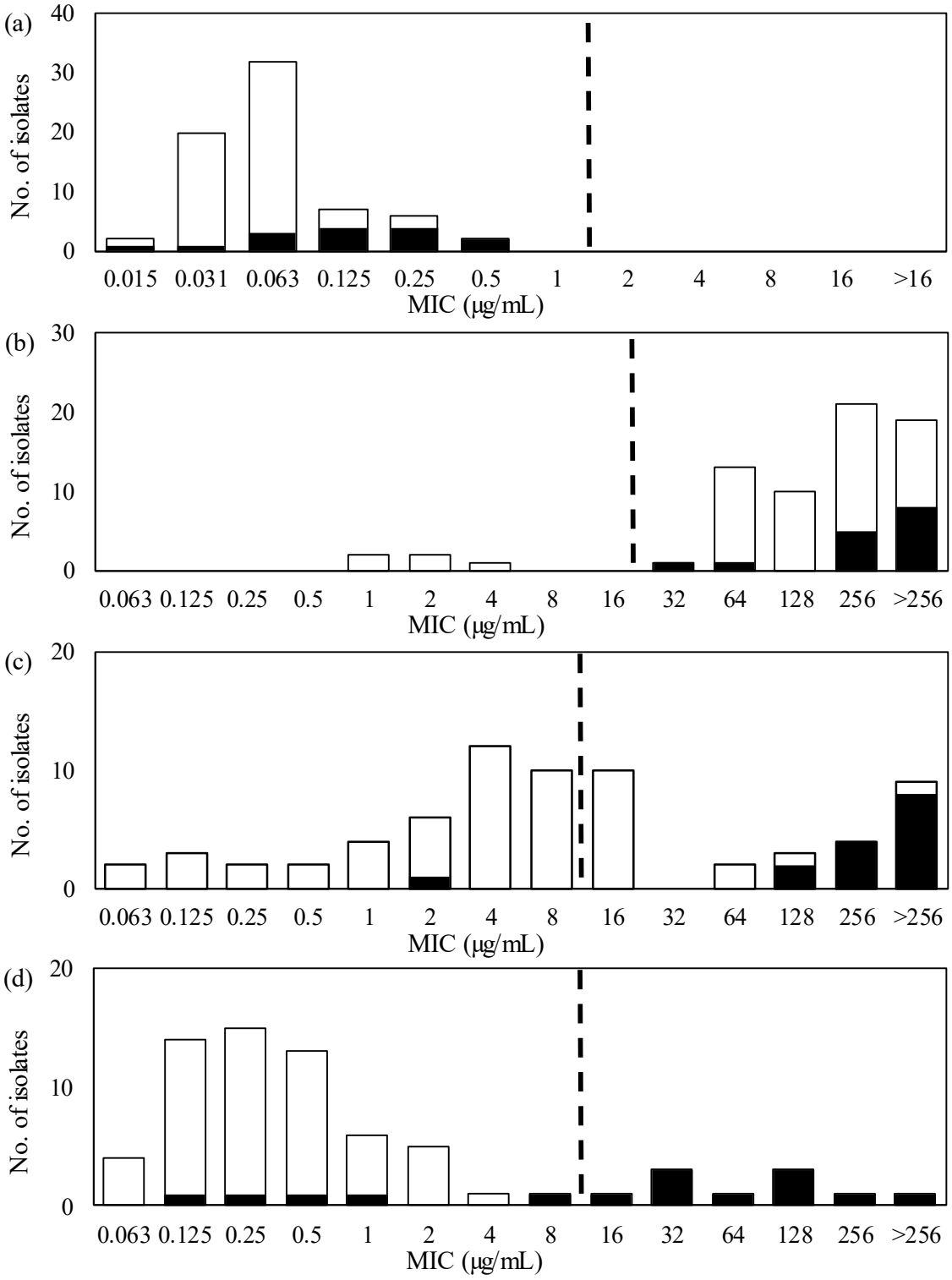

Fig. 3. MIC distribution of ESBL-producing *E. cloacae* isolates (n = 69) against MEM (a), CMZ (b), FMX (c), and LMX (d).

The blackened bar indicates the number of ABL-producing isolates.

Vertical broken lines mean the breakpoints of each substance.

Chapter 2

Pharmacokinetics/pharmacodynamics analysis of cefmetazole against extended spectrum β-lactamase-producing Enterobacterales in dogs using Monte Carlo simulation

1. Introduction

Cefmetazole belongs to the group of cephamycins and exhibits high stability against ESBLs due to the presence of the 7α methoxy constituent (Jones, 1989; Neu, 1986). A previous study in humans has reported that compared with carbapenems, CMZ exhibits non-inferior clinical efficacy for treating ESBL infections (Hamada *et al.*, 2021). Furthermore, Shimizu *et al.* (2017) and the author previously investigated the PD of CMZ and found that cephamycin exhibits high *in vitro* efficacy against ESBL-E isolates from companion animals (Chapter 1), as well as some essential oils (Sipahi *et al.*, 2022). These findings suggest that CMZ can be used as a carbapenem-sparing antibiotics in companion-animal medicine. However, CMZ dosage regimens for treating ESBL infections in dogs have not been established because the PK of CMZ in dogs is not known.

In this chapter, the author calculated the PK indices of CMZ in dogs after intravenous administration study. To the best of her knowledge, this is the first study to perform PK/PD analysis using MCS to determine the nonclinical PK/PD cutoff value and propose dosage regimens of CMZ which can be clinically effective for ESBL infections in dogs.

2. Materials and Methods

1) Animals

In total, six healthy beagles (4 males, and 2 females, aged 5.3 ± 2.0 years and weighing 12.5 ± 1.8 kg, SHIMIZU Laboratory Supplies Co., Ltd., Kyoto, Japan) were used in this study. The dogs were clinically healthy based on their condition, physical examination, complete blood counts, and blood biochemical tests, and did not receive any drugs in the 6 months before the study. They were fed the same commercial food (Aiken Genki, Unicharm Corporation, Tokyo, Japan) and were individually housed in separate cages in the same room at the experiment animal facility. This study was conducted under an ethics committee-approved

protocol in accordance with the Tottori University Animal Use Committee (approval number No. 19-T-17).

2) Cefmetazole administration and serum sampling

The day before administration of antimicrobial product, a central venous catheter (Covidien Japan, Inc., Tokyo, Japan) was placed in the jugular vein of the dog under general anesthesia. Anesthesia was induced by intravenously administering propofol (4 mg/kg body weight, Propofol, DS Pharma Animal Health Co., Ltd., Osaka, Japan), and subsequently intubated with a cuffed endotracheal tube. The vaporizer was adjusted to deliver 2% isoflurane (ISOFLURANE Inhalation Solution, Mylan EPD G.K., Tokyo, Japan) at an oxygen flow rate of 2 L/min. CMZ sodium (Nichi-Iko Pharmaceutical Co., Ltd., Tokyo, Japan) was dissolved in water for a bolus injection (Nissin Pharmaceutical Co., Ltd., Yamagata) at a final concentration of 100 mg/mL, which was injected in the radial cutaneous vein at 40 mg/kg body weight. For CMZ quantification, venous blood samples (2 mL each) were collected from each participant via a central venous catheter at predetermined time points (0, 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, 240, 360, 480, 600, and 720 min). Serum samples were collected after coagulation by centrifugation at 1,300 × g for 10 min and stored at -80°C until assayed.

3) Sample preparation

Cefmetazole was extracted from each serum sample using solid-phase extraction (SPE) according to a previously described protocol (Ohmori *et al.*, 2011). Ethylparaben (Sigma-Aldrich Japan, Tokyo, Japan) was used as an internal standard (IS), which was dissolved in methanol to 10 μg/mL because the compound is stable and shows a retention time (6.36 min) close to that of CMZ (3.6 min). The prepared sample was stored frozen at −80°C until analysis.

4) Determination of cefmetazole concentrations

The serum concentration of CMZ was determined according to a previously described protocol with slight modifications (Ohmori *et al.*, 2011). High-performance liquid chromatography (HPLC) separations were performed with a 10A HPLC system (Shimadzu, Kyoto, Japan) under 50:50 isocratic conditions using the following two solutions: 0.1% (v/v) formic acid in 10 mM ammonium formate water as mobile phase A and 0.1% (v/v) formic acid-acetonitrile as mobile phase B at a flow rate of 0.2 mL/min. A 20 μL sample of serum was injected and target molecules were separated using TSKgel Reversed Phase Chromatography (TSKgel ODS-100Z 3 μm, 2.0 mm i.d. x 150 mm, TOSOH, Tokyo, Japan), with the temperature controlled at 40°C. Mass spectra (MS) were measured using an Exactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA, United States) under electrospray ionization (ESI) with a tube lens voltage of 80 V and a skimmer voltage of 30 V. CMZ was detected by $[M + H]^+$ precursor ion m/z = 472.1, and IS was detected by $[M-H]^-$ precursor ion m/z = 165.1. The area under the peak was determined using commercial analytical software (Xcalibur QualBrowser, Thermo Fisher Scientific Inc., MA, United States). The concentration of CMZ in each sample was calculated based on the calibration curve (0.1, 1, 5, 10, 50, 100, and 200 μg/mL) constructed by mixing canine serum with the known substance concentrations. The reliability of the analytical method was based on the guidelines of the Ministry of Health, Labor, and Welfare (MHLW) (Ministry of Health, Labour and Welfare, 2013).

5) Monte Carlo simulation

MCS was performed to calculate PTA based on the PK/PD parameters of CMZ when administered at 40 mg/kg body weight every 12, 8, and 6 h, using commercial software (Oracle Crystal Ball version 11.1.2.4.850, Kozo Keikaku Engineering Inc., Tokyo, Japan). The PK parameters in the non-compartment model (Ikawa & Tanaka, 2015) were calculated based on

the serum CMZ concentrations in the six dogs by computing 10,000 bootstrap replicates using the PK package (ver. 4.0.3) of R software (ver. 4.2.1) (Jaki & Wolfsegger, 2010). Assuming that PK parameters were distributed lognormally, 10,000 virtual patients were generated for each dosage regimen to build the serum CMZ concentration-time profile. The percentage of time during a dosing interval that the CMZ concentration of the unbound fraction (f) remains above the MIC for the pathogen ($f\%T_{>MIC}$) was estimated as the PDI to determine the optimal dosage regimen (Craig, 1998; Papich, 2014). In this study, a serum protein-binding rate of 26% in dogs was applied according to a previous study (Murakawa *et al.*, 1980). The PTA for each dosage regimen was calculated at each MIC to determine the percentage of subjects achieving the PDI that could achieve bacteriostatic activity (i.e., $f\%T_{>MIC} \geq 40\%$) (Craig, 1998; Papich, 2014). The nonclinical PK/PD cutoff was calculated as the highest MIC that achieved the target of PTA ≥ 90% (Papich, 2014). The CFR was calculated as the proportion of %PTA of each MIC based on the wild-type MIC distribution (Jitaree *et al.*, 2019), of which CMZ in ESBL-E (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *E. cloacae*) isolates from companion animals were determined in the previous studies (Shimizu *et al.*, 2017; Chapter 1).

3. Results

1) Animals

None of the dogs exhibited clinical signs of adverse reactions or abnormal blood test results throughout the experiment.

2) Pharmacokinetics parameters of cefmetazole in dogs

The coefficient of variation (CV) of CMZ, IS, and CMS peak area value corrected by IS (CMZ/IS) was 4.01, 12.1, and 9.50%, respectively. The blood concentration-time curves and PK parameters in dogs after intravenous bolus administration of CMZ at 40 mg/kg are presented

in Fig. 4 and Table 3, respectively. Serum CMZ concentration at 5 min was 154.32 ± 14.01 μg/mL, which decreased gradually.

3) Nonclinical pharmacokinetics/pharmacodynamics cutoff and cumulative fraction of response of cefmetazole for extended spectrum β-lactamase-producing Enterobacterales in dogs

The PTA results at each MIC with regimens of 40 mg/kg every 12, 8, and 6 h are shown in Fig. 5 All dosage regimens achieved a PTA of ≥ 90%, with a MIC of ≤ 0.5 μg/mL but not with a MIC of ≥ 8 μg/mL. Based on the calculated PTA, the nonclinical PK/PD cutoff values for every 12, 8, and 6 h were ≤ 0.5, ≤ 2, and ≤ 4 μg/mL, respectively.

Table 4 shows the CFR results that were calculated based on the wild-type MIC distribution of ESBL-E. Overall, higher CFRs were observed for *E. coli* and *K. pneumoniae* than for *P. mirabilis*, and extremely low CFRs (< 10%) were confirmed for *E. cloacae*, regardless of the regimen. When using $\%fT_{>MIC} \geq 40\%$ as the target of the PDI, none of the regimens achieved a CFR ≥ 90% in canines infected with all bacterial species. Regimens of 40 mg/kg every 6 and 8 h were estimated to achieve CFR of 80–90% and 70–80%, respectively, for patients infected with *E. coli* and *K. pneumoniae*. In contrast, all regimens were estimated to have a CFR of ≤ 70% for patients infected with *P. mirabilis* and *E. cloacae*.

4. Discussion

Cefmetazole is one of the candidate antibiotics to spare carbapenems because it is highly stable against ESBLs (Karaiskos & Giamarellou, 2020). However, the PK/PD properties of CMZ, which are essential to consider optimal dosage regimens (Cagnardi *et al.*, 2018; Vegas Cómitre *et al.*, 2021), are still unknown.

The author investigated the PK parameters of CMZ in dogs based on the results of blood concentration transition after bolus administration. All of which fulfilled the criteria (< 15%) according to the MHLW guideline (Ministry of Health, Labour and Welfare, 2013). Borin *et al.* (1990) determined the corresponding PK data in young adult humans, ranging in age from 21 to 40 years when intravenously administered, as follows: the mean residence time (MRT) was 1.78 h, the elimination half-life (T1/2) was 1.34 h, total body clearance (CL) was 0.10 L/h/kg, and the volume of distribution (Vd) was 0.17 L/kg. This present study indicates the shorter MRT and T1/2, higher CL, and larger Vd in dogs administered with CMZ, compared with the PK data in humans. These results may be explained by the fact that the protein-binding rate of CMZ is significantly lower in dogs than in humans (66%) (Murakawa *et al.*, 1980), which can result in wider tissue distribution and faster renal excretion. These PK traits of CMZ in dogs may negatively affect its clinical efficacy because its bactericidal activity is time-dependent (Shah *et al.*, 2015).

Breakpoints of antimicrobial susceptibility are essential for appropriate antimicrobial treatment. The CLSI (CLSI Supplement M100, 2023) provided the human-specific breakpoint of the CMZ but not the canine-specific breakpoint. Therefore, the author studied the canine-specific nonclinical PK/PD cutoff values of CMZ for practical application in dogs with bacterial infections. The finding revealed that nonclinical PK/PD cutoff values can be increased by shortening dose interval; however, nonclinical PK/PD cutoff values of CMZ based on all dosage regimens were lower than the CLSI susceptibility breakpoint for Enterobacterales (≤ 16 μg/mL), probably due to the higher clearance in dogs. Therefore, the application of the CLSI breakpoint may overestimate CMZ susceptibility in canine pathogens, resulting in treatment failure.

The present nonclinical PK/PD cutoff values with all regimens were also lower than the previously reported MIC_{90} values for ESBL-producing *E. coli* (8 μg/mL), for *K. pneumoniae* (32 μg/mL), *P. mirabilis* (32 μg/mL), and *E. cloacae* (> 256 μg/mL) (Shimizu *et al.*, 2017;

Chapter 1). Such lower nonclinical PK/PD cutoff values were supported by other findings in this study that CMZ exhibited a relatively short time during which the blood concentration remained above the MIC_{90} of these bacteria. When administered with the regimens of every 6 and 8 h, the nonclinical PK/PD cutoff induced by CMZ exceeded the MIC_{50} values of ESBL-producing *E. coli* (1 μg/mL) and *K. pneumoniae* (2 μg/mL), but not *P. mirabilis* (4 μg/mL) and *E. cloacae* (256 μg/mL). Furthermore, the author calculated the CFRs based on the wild-type MIC distribution of ESBL-E and thereby clarified that the every 6 h regimens exhibited more than 80% CFRs for ESBL-producing *E. coli* and *K. pneumoniae*. Generally, regimens with ≥ 90% CFR are optimal, whereas those with 80–90% CFR are associated with moderate probabilities of success (Wang *et al.*, 2021a). Therefore, at least the regimens of CMZ 40 mg/kg every 6 h may have moderate clinical efficacy in canine patients infected with ESBL-producing *E. coli* and *K. pneumoniae*. However, all regimens in this study exhibited lower CFRs against ESBL-producing *P. mirabilis* and *E. cloacae* and thus may have less efficacy for these bacteria. Such differences in the putative clinical efficacy of CMZ between bacteria should be considered when administering CMZ in dogs.

Unfortunately, the optimal dose of CMZ in dogs has not yet been determined. In previous reports (Katayama *et al.*, 2017; Kanno *et al.*, 2019; Mochizuki *et al.*, 2021), CMZ was administered to canine patients at 20–25 mg/kg body weight per dose. However, these usages were intended for the perioperative prevention of infection but not for the treatment of bacterial infection. In the preliminary study, nonclinical PK/PD cutoff and CFR at a dose of 20 mg/kg were extremely low (data not shown); thus, this dose is unlikely to be suitable for treating dogs with ESBL infections. Therefore, the author used 40 mg/kg per dose in this study, referencing the human dosage (i.e., a maximum of 37.5 mg/kg four times per day). This dose is considered acceptable from a safety perspective because CMZ has a wide margin of safety (Moe *et al.*,

1989) and an extremely high level of no observed effect in dogs (i.e., 1,200 mg/kg) (Masuda *et al.*, 1978).

The author applied a $\%fT_{>MIC}$ of ≥ 40% as the PDI values (Craig, 1998; Papich, 2014), but McKinnon *et al*. (2008) reported that a $\%fT_{>MIC}$ of 100% is recommended as the target value of cefepime and ceftazidime for serious infection. Furthermore, in recent years, Takemura *et al*. (2021) demonstrated that a $\%fT_{>MIC}$ of > 57.6% was preferable as the target value of CMZ for static effects on ESBL-producing *E. coli* infections in a murine infection model. These studies emphasize the need to consider higher PK/PD target values for CMZ in dogs. More than half of dog-origin ESBL-E strains have been isolated from urinary tract infections (Shimizu *et al.*, 2017; Chapter 1). In addition, the peak CMZ concentration is approximately 20 times higher in the urine than in the blood of dogs after administration of CMZ (Komiya *et al.*, 1982), implying that CMZ may have high efficacy against urinary tract infection in dogs with ESBL-E, even if the blood concentration during repeated administration cannot achieve the target value. Further clinical studies are required to confirm this hypothesis.

In this chapter, the author estimated the nonclinical PK/PD cutoff of CMZ when administered with regimens of 40 mg/kg every 6, 8, and 12 h using MCS, and calculated the CFR based on the MIC distribution of wild-type ESBL-E. The results indicated that a CMZ breakpoint lower than the CLSI for humans is preferable for dogs. Furthermore, a CMZ regimen of 40 mg/kg every 6 h could be a treatment option for dogs infected with ESBL-producing *E. coli* and *K. pneumoniae*. The author believe that these data provide a basis for the use of CMZ in dogs with ESBL infections.

Table 3. Pharmacokinetics parameters were determined after intravenous administration of CMZ at the dose of 40 mg/kg body weight in dogs.

Parameters (unit) [a)]	Values (SD) [b)]
AUC (mg·h/L)	103.36 (7.49)
MRT (h)	1.21 (0.11)
T1/2 (h)	0.84 (0.07)
CL (L/h)	4.93 (0.36)
Vd (L)	5.97 (0.55)

a) AUC, the area under the concentration-time curve; MRT, mean residence time; T1/2, half-life; CL, total body clearance; Vd, the volume of distribution.

b) Values are the mean (SD) of six dogs after intravenous administration.

Table 4. CFR following regimens of 40 m/kg CMZ in dogs against wild-type MIC distribution of ESBL-E

Regimens	CFR (%)			
	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae*
every 12 h	31.91	26.86	1.76	2.38
every 8 h	78.12	72.91	48.29	6.57
every 6 h	87.27	82.29	63.73	7.24

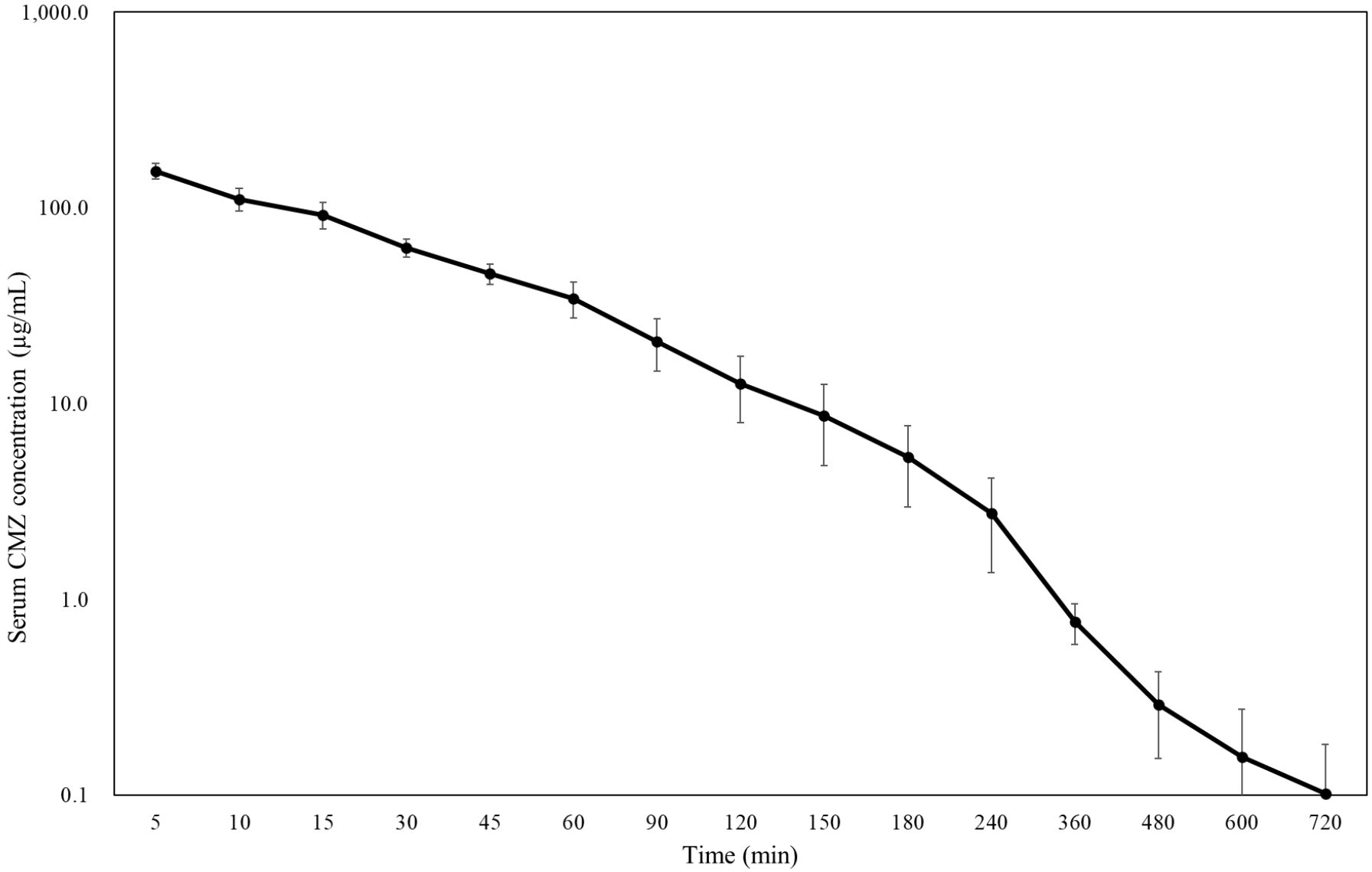

Fig. 4. Semilogarithmic plot of serum CMZ concentration in dogs administered a dose of 40 mg/kg body weight (Mean ± SD, n = 6).

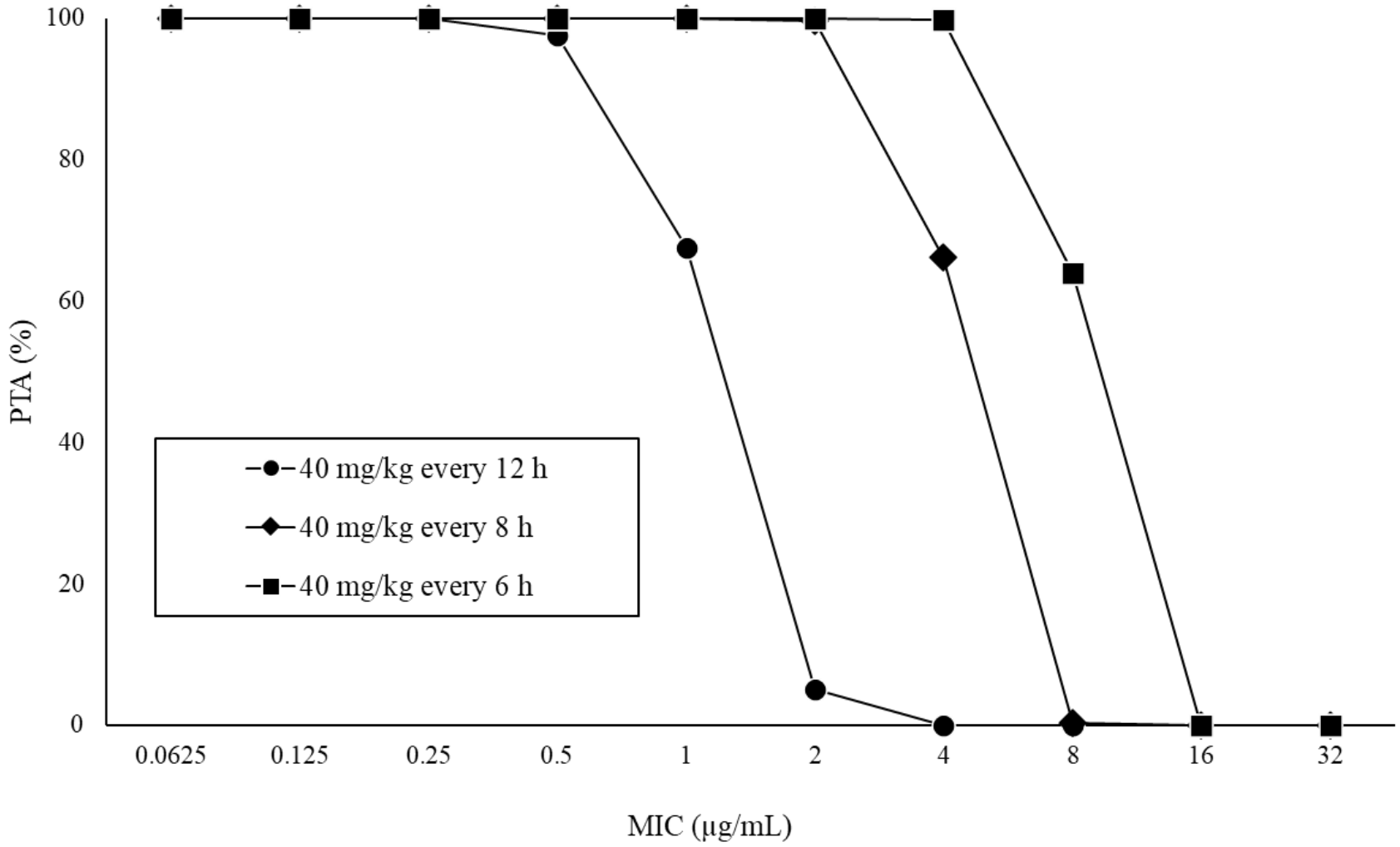

Fig. 5. PTA (%) at each MIC following intravenous administration of CMZ.

Chapter 3

Pharmacokinetics/pharmacodynamics analysis using Monte Carlo simulation of the oxacephem antibiotic flomoxef against extended spectrum β-lactamase-producing Enterobacterales

1. Introduction

FMX is an oxacephem that is resistant to degradation by ESBLs because of its characteristic structure, with a methoxy group at the 7S position (Jacoby & Carreras, 1990). In human medicine, FMX is an effective alternative to carbapenems for the treatment of ESBL infections (Lee *et al.*, 2006; Horie *et al.*, 2019; Darlow & Hope, 2022). Furthermore, Shimizu *et al.* (2017) and the author previously reported the high *in vitro* efficacy of FMX against ESBL-E derived from companion animals (Chapter 1). These findings suggest that FMX may be a potential alternative to carbapenems in companion animal medicine. However, there are insufficient reports on the PK of FMX in dogs, and a regimen of FMX for ESBL infections in dogs has yet to be established.

In this chapter, the author first determined PK parameters of FMX by administration experiments in healthy dogs, determined a canine nonclinical PK/PD cutoff based on PK/PD relationships analyzed by MCS, and proposed dosage regimens of FMX which can be clinically effective for ESBL infections in dogs.

2. Materials and Methods

1) Animals

The animal experiments in this study were conducted under an ethics committee-approved protocol in accordance with the Tottori University Animal Use Committee (Approval No. 19-T-17). Five beagle dogs were used in this study (four males and one female, aged 6.2±1.8 years and weighing 13.6±1.7 kg, SHIMIZU Laboratory Supplies Co., Ltd., Kyoto, Japan). The dogs were individually housed in each cage and confirmed to be clinically healthy based on physical tests, blood tests, and image examination prior to the study. They did not receive any medications in the 6 months prior to the examination. They were fed the same commercial food

(Aiken Genki, Unicharm Corporation, Tokyo, Japan) and were individually housed in separate cages in the same room at the experiment animal facility.

2) Flomoxef administration and serum sampling

A central venous catheter (Covidien Japan, Inc., Tokyo) was placed as described in Chapter 2. Flomoxef (Shionogi Co. Ltd, Osaka, Japan) was dissolved in water for injection (Nissin Pharmaceutical Co., Ltd., Yamagata, Japan) and was bolus administered at 40 mg/kg through the radial skin vein. Three mL blood samples were collected from a central venous catheter before administration and 2 mL at 5, 10, 30, 45, 60, 90, 120, 150, 180, 240, 300, and 360 min after administration. Serum samples were collected after coagulation by centrifugation at 1,300 × g for 10 min and stored at -80°C until analysis.

3) Determination of serum flomoxef concentrations in dogs

Calculation of FMX concentration in serum samples was outsourced to NDTS, Inc. (Hokkaido, Japan). Briefly, as an IS, 200 μL of LMX sodium (Shionogi, Osaka, Japan) solution (1 μg/mL) was added to the same volume of serum. After 100 μL of 20% sulfosalicylic acid was added, the IS was mixed vigorously for 30 sec and centrifuged at 12,000× g for 5 min. Then 250 μL of supernatant was collected and mixed with 250 μL of 100 mM acetic acid solution. The mixture was subjected to solid-phase extraction using Oasis HLB (1 cc, 30 mg; Waters, USA). After loading, each sample was washed with 1 ml of 20 mM aqueous acetic acid solution, followed by elution with 1 mL of methanol. The eluted solution was dried at 35°C under a stream of nitrogen and then dissolved into 100 μL of methanol. High-performance liquid chromatograph-tandem mass spectrometry (LC-MS/MS) was performed on a high-performance liquid chromatography–mass spectrometer (Prominence and LCMS-8045 tandem mass spectrometer, Shimadzu Corporation, Kyoto, Japan). Separation by high-performance

liquid chromatography was performed using two solutions: mobile phase A, 10 mM ammonium formate solution, and mobile phase B, 10 mM ammonium formate plus methanol, with the following gradient conditions: 5% (0 min)–40% (6 min)–100% (8 min)–100% (10 min)–5% (10.5 min). After 5 μL of sample was injected, target molecules were separated on a C18 reversed-phase column (Cadenza CD-C18, 3.0 mm i.d. × 150 mm, intact, Kyoto, Japan), which was controlled at a temperature under 40°C. Mass spectrometry was performed in electrospray ionization (positive) and multiple monitoring reaction mode at a capillary voltage of 4.5 Kv, source (DL) temperature of 250°C, nebulization gas 180 L/hr, and drench gas 10 L/min. LMX was detected at monitor ion m/z = 521 > 137, collision energy 27 V, and FMX at m/z = 497 > 137, collision energy 26 V. The area under the peak was determined by the analytical software LCMS solution (Shimadzu Corporation, Kyoto, Japan). The FMX concentration in each sample was calculated using a calibration curve with the serum obtained before antimicrobial product administration, to which a known concentration of FMX sodium (Shionogi Co., Ltd.) had been added.

4) Calculation of pharmacokinetics parameters

MCS was performed using commercial software (Oracle Crystal Ball version 11.1.2.4.850, Kozo Keikaku Engineering Inc., Tokyo, Japan) to calculate PTA based on the PK/PD parameters of FMX at a 40 mg/kg bolus dose at every 12, 8, and 6 h. The PK parameters from the non-compartment model were calculated using the package PK (ver. 4.0.3) of R software (Jaki & Wolfsegger, 2010) based on serum FMX concentrations in five dogs.

5) Monte Carlo simulation

Based on log-normally distributed PK parameters, 10,000 virtual patients were generated for each dosage regimen to construct serum concentration-time profiles of FMX. The

percentage of time that the f%$T_{>MIC}$, based on the serum protein binding rate of 8% (Kimura *et al.*, 1987), was employed as the PDI to determine the optimal dosage regimen. The PDI target value was set as ≥ 40% according to a previous study (Tashiro *et al.*, 2021). The nonclinical PK/PD cutoff was calculated as the highest MIC that achieved a PTA of ≥ 90% (Papich, 2014; CLSI guideline M23, 2023). The CFR was calculated based on the wild-type MIC distribution, of which FMX in ESBL-E (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *E. cloacae*) isolates from companion animals were determined in the previous studies (Shimizu *et al.*, 2017; Chapter 1). A regimen with a CFR of ≥ 90% is defined as optimal, and a regimen with a CFR of 80–90% is defined as moderately successful (Wang *et al.*, 2021).

3. Results

1) Animals

None of the dogs had any adverse effects or abnormal blood test results during the study.

2) Pharmacokinetics parameters of flomoxef in dogs

The blood concentration-time curve and PK parameters of FMX when bolus intravenous administrated at 40 mg/kg are shown in Fig. 6 and Table 5, respectively. Serum FMX concentration at 5 min was 111.82 ± 19.60 μg/mL, which decreased gradually.

3) Nonclinical pharmacokinetics/pharmacodynamics cutoff and cumulative fraction of response of flomoxef for extended spectrum β-lactamase-producing Enterobacterales in dogs

The PTA results of FMX at each MIC, when administered at 40 mg/kg every 12, 8, and 6 h, are shown in Fig. 7. All regimens achieved a PTA of more than 90% at MIC of ≤ 0.5 μg/mL but

not at an MIC of ≥ 16 μg/mL. Based on the calculated PTA, the nonclinical PK/PD cutoff values at 40 mg/kg at 12, 8, and 6 h were ≤ 0.5, ≤ 2, and ≤ 8 μg /mL, respectively.

Table 6 shows the results of CFR calculated based on the wild-type MIC distribution of ESBL-E. Considering the estimated CFR, the regimens of 40 mg/kg every 8 and 6 h were optimal, and that of 40 mg/kg every 12 h was moderately successful for dogs infected with ESBL-producing *E. coli*, *K. pneumoniae*, and *P. mirabilis*. In contrast, none of the regimens achieved CFR < 80% for ESBL-producing *E. cloacae*-infected dogs.

4. Discussion

This study is the first to report the usefulness of FMX, an oxacephem used in humans, against ESBL infections in dogs, based on a PK/PD approach.

Although antimicrobial susceptibility breakpoints are essential indicators for appropriate antimicrobial therapy, the breakpoint for FMX has not yet been established in both humans and animals. In this study, the author attempted to establish canine-specific nonclinical PK/PD cutoff values by using MCS analysis. The results showed that nonclinical PK/PD cutoff values for FMX increase with shorter dosing intervals, as previously reported in humans (Hirano *et al.*, 2023). In addition, these nonclinical PK/PD cutoff values are higher than the MIC_{90} of ESBL-producing *K. pneumoniae* and *P. mirabilis* (1 μg/mL each) and that of ESBL-producing *E. coli* (4 μg/mL) (Shimizu *et al.*, 2017; Chapter 1) when administered at every 8 and 6 h, respectively. In addition, the CFR simulated in this study suggests that every 6 and 8 h regimens of FMX are appropriate for the treatment of ESBL-producing *E. coli*, *K. pneumoniae,* and *P. mirabilis* infections in dogs. Similar dosing intervals were proposed to achieve bactericidal concentrations against ESBL infections as found in human patients based on PK/PD simulation (Hirano *et al.*, 2021). These findings in this present study indicate that FMX administration at

shorter dose intervals can be an alternative treatment for ESBL-producing *E. coli*, *K. pneumoniae*, and *P. mirabilis* infections in dogs.

In contrast, all of the nonclinical PK/PD cutoff values calculated in this study were lower than FMX MIC_{90} for ESBL-producing *E. cloacae* (> 256 μg/mL) (Chapter 1). This finding supports that the CFR for ESBL-producing *E. cloacae* was not even moderately successful. It is known that *E. cloacae* has an inducible chromosomal ABL, which can be induced by cephamycins, including oxacephems (Neu & Chin, 1986; Jacoby, 2009). Therefore, FMX is unlikely to be a candidate antimicrobial product for ESBL-producing *E. cloacae* infections in dogs. However, infection with ESBL-producing *E. cloacae* is less prevalent in companion animals (Zogg *et al.*, 2018).

The optimal dose of FMX In dogs has not yet been established. In this study, the author used 40 mg/kg per dose, referencing to the human dosage (i.e., a maximum of 37.5 mg/kg four times per day), and investigated the blood PK of FMX in dogs when bolus administered at this dose. The results revealed similar values for T1/2 and CL, compared with those in healthy human subjects, 44.2–46.2 min and 15.14 L/h, respectively (Yasunaga *et al.*, 1987; Hamada *et al.*, 2022). This implies that the elimination rate of FMX in dogs is comparable to that in humans, although the protein binding rate is much lower in dogs (8%) than in humans (36.2%) (Hamada *et al.*, 2022). Mitsuzono *et al.* (1987) estimated that the no-observed effect level of FMX in dogs is 200 mg/kg/day based on a 6-month intravenous toxicity study. Therefore, the author believes that the dosage regimens in this study (40 mg/kg every 12, 8, and 6 h) are fully acceptable from the viewpoint of safety.

In this chapter, the author calculated nonclinical PK/PD cutoff values at 40 mg/kg FMX every 12, 8, and 6 h by MCS and estimate CFR based on the MIC distribution of wild-type ESBL-E. These results indicated that every 8 and 6 h dosage regimens of 40 mg/kg FMX are effective non-carbapenem treatment options for infections with ESBL-producing *E. coli*, *P.*

mirabilis, and *K. pneumoniae*. However, ESBL-producing *E. cloacae* infection in dogs cannot be treated with FMX. The author believes that these results provide a basis for the use of FMX in dogs with ESBL infections.

Table 5. Pharmacokinetics parameters were determined after intravenous administration of FMX at the dose of 40 mg/kg body weight in dogs.

Parameters (unit) [a)]	Values (SD) [b)]
AUC (mg·h/L)	134.61 (6.79)
MRT (h)	1.10 (0.09)
T1/2 (h)	0.76 (0.06)
CL (L/h)	2.97 (0.15)
Vd (L)	3.27 (0.27)

a) AUC, area under the concentration-time curve; MRT, mean residence time; T1/2, elimination half-life; CL, total body clearance; Vd, volume of distribution.

b) Values are the mean (SD) of five dogs after intravenous administration.

Table 6. CFR following regimens of 40 mg/kg FMX in dogs against wild-type MIC distribution of ESBL-E

Regimens	CFR (%)			
	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae*
every 12 h	83.63	87.12	87.85	23.12
every 8 h	91.32	92.40	96.22	50.17
every 6 h	93.26	94.13	98.57	65.28

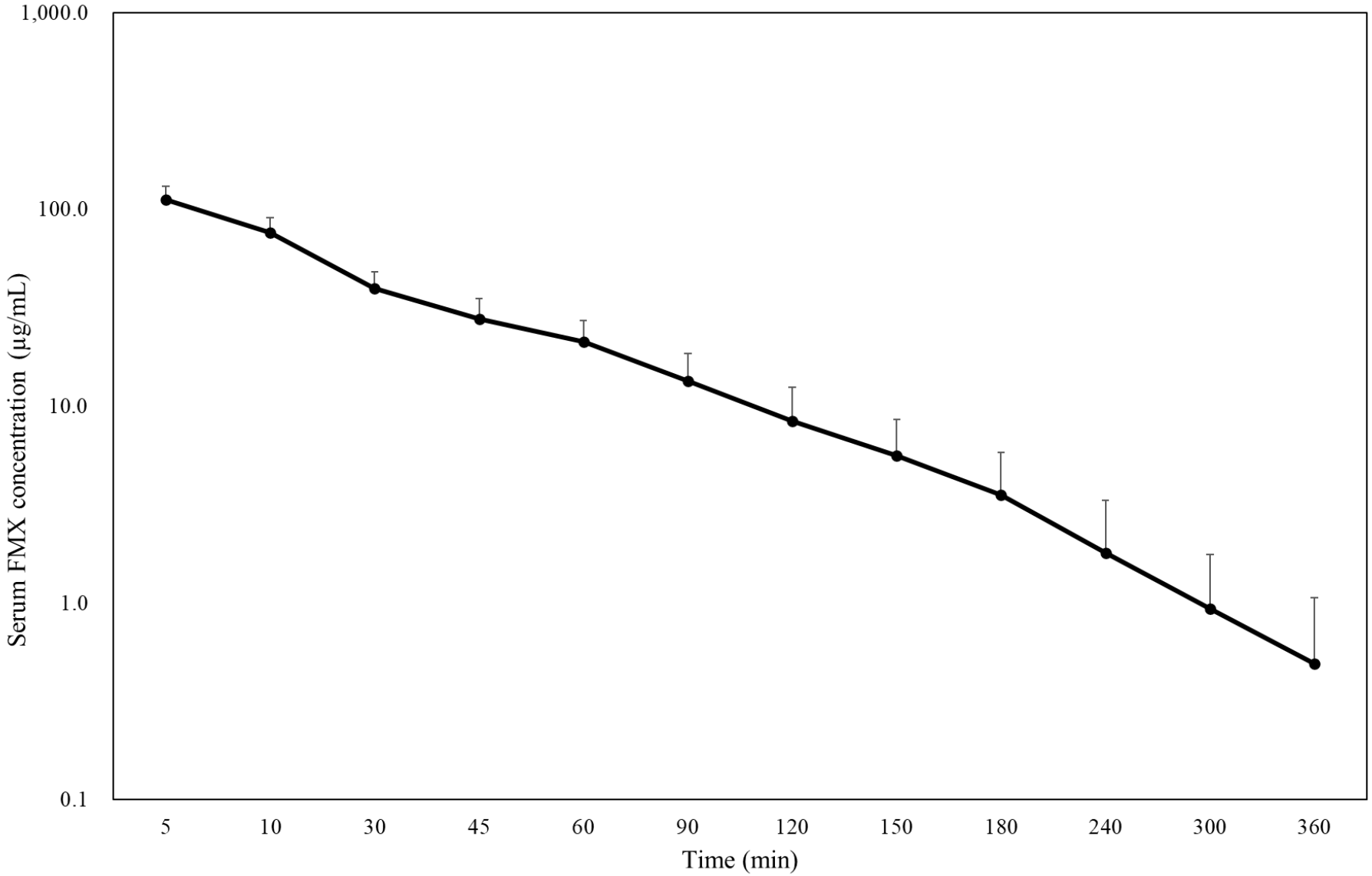

Fig 6. The semilogarithmic plot of serum FMX concentration in dogs administered a dose of 40 mg/kg body weight (mean ± SD, n = 5).

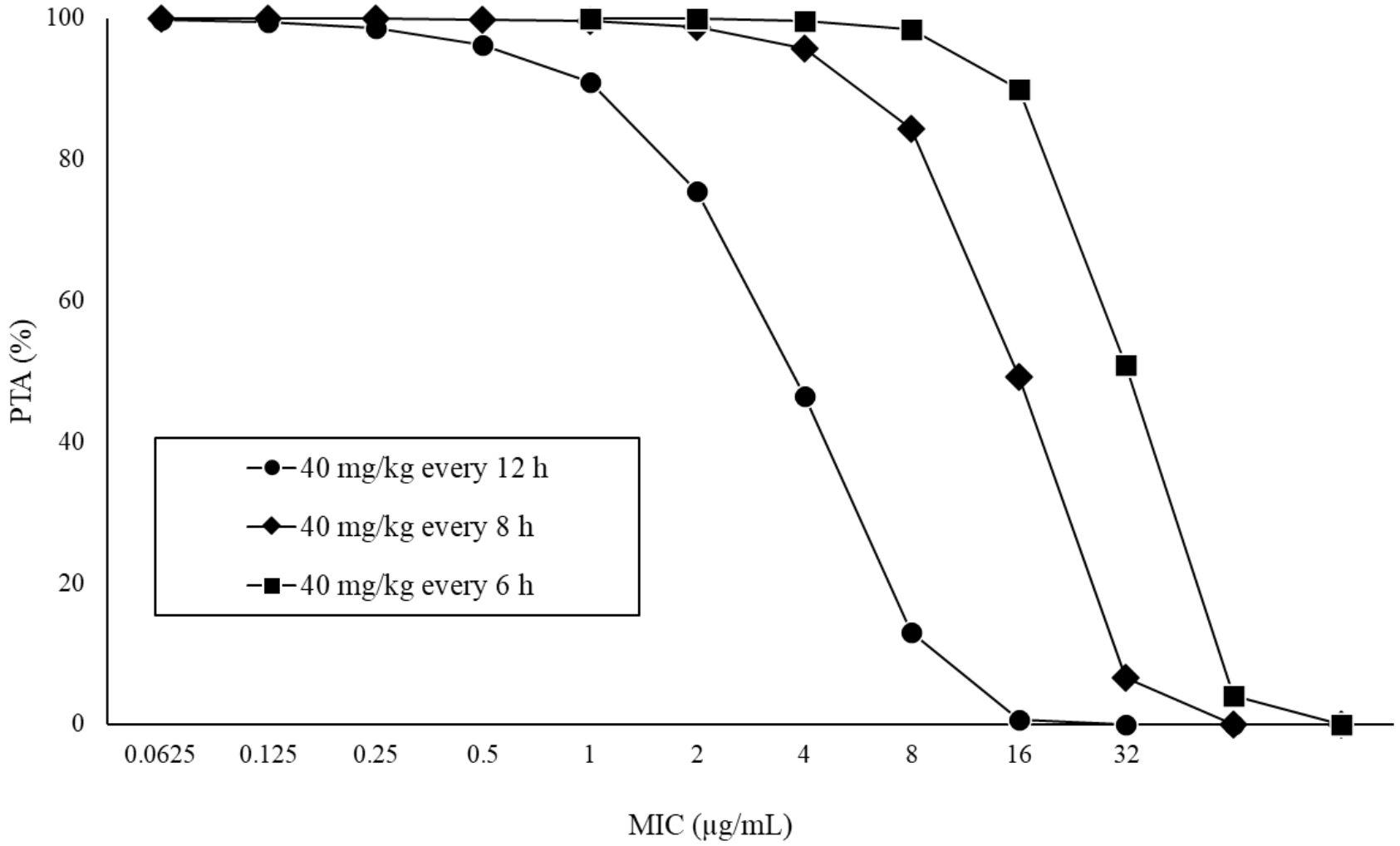

Fig. 7. PTA (%) at each MIC following intravenous administration of FMX.

Chapter 4

Application of Monte Carlo simulation to examine the pharmacokinetics and pharmacodynamics of latamoxef against extended spectrum β-lactamase infections in dogs

1. Introduction

Latamoxef is an oxacephem that exhibits stable properties against ESBL due to a methoxy group at position 7 in its structure (Jacoby & Carreras, 1990). LMX is considered an alternative to carbapenems against ESBL infections in humans (Ito *et al.*, 2014; Huang *et al.*, 2019). Shimizu *et al.* (2017) and the author have also previously demonstrated that ESBL-E from dogs and cats are more susceptible to LMX than CMZ and FMX (Chapter 1), which indicates that LMX might be a more effective alternative antibiotic. However, the LMX dosage regimen for dogs with ESBL infection has not been fully investigated because of a lack of PK data.

In the present investigation, canine-specific nonclinical PK/PD cutoff values were established by MCS; LMX PK profiles were obtained through administration studies in dogs; and the efficacy of LMX dosage regimens for canine ESBL infections was investigated.

2. Materials and Methods

1) Animals

Three male and two female beagle dogs, weighing 13.7 ± 1.9 kg and aged 7.1 ± 1.0 years, were purchased from SHIMIZU Laboratory Supplies Co., Ltd. (Kyoto, Japan) and evaluated. Assessments of the dogs' physical state, blood test outcomes, and visual presentation were used to confirm that they were clinically healthy. They were not given any medicine in the 6 months before the trial. They were given the same commercial food (Aiken Genki, Unicharm Corporation, Tokyo, Japan) and were individually housed in cages placed in one room at the experimental animal facility. This study was conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of Tottori University and ethically approved by the Committee (approval number no. 22-T-12).

2) Latamoxef administration and blood sampling

A central venous catheter (Covidien Japan, Inc., Tokyo) was placed as described in Chapter 2. After dissolution in sterile water, LMX (Shionogi Co. Ltd., Osaka, Japan) was bolus-administered via the radial cutaneous vein at a dose of 40 mg/kg body weight. Approximately 3 mL of blood was drawn from the CVC prior to administration, and 2 mL was drawn at 5, 10, 15, 30, 60, 90, 120, 150, 180, 240, 300, 360, 540, 720, and 1,440 min following the injection. After clotting, each blood sample was centrifuged at 1,300 × g for 10 min to collect the serum and, which was then stored at -80°C until analysis.

3) Serum latamoxef concentration measurement

Measurement of serum LMX concentrations was outsourced to NDTS, Inc. (Hokkaido, Japan). Briefly, 200 μL FMX sodium (Shionogi, Osaka, Japan) solution (1 μg/mL) was added to the same volume of serum as an IS. Following centrifugation at 12,000 × g for 5 min, the IS solution was mixed with 100 μL of 20% sulfosalicylic acid and stirred thoroughly for 30 seconds. Solid-phase extraction was performed with Oasis HLB (1 cc, 30 mg; Waters, MA, USA), using 250 μL of the supernatant combined with the same volume of 100 mM acetic acid solution. Each sample was loaded, rinsed with 1 mL acetic acid solution, and eluted with 1 mL methanol. An HPLC–mass spectrometer (Prominence and LCMS-8045 tandem mass spectrometer, Shimadzu Corporation, Kyoto, Japan) was used to perform HPLC–tandem mass spectrometry (LC/MS/MS). Mobile phases A (10 mM ammonium formate solution) and B (10 mM ammonium formate plus methanol solution) were employed under the following gradient conditions: 5%, 0 min; 40%, 6 min; 100%, 8 min; 100%, 10 min; and 5%, 10.5 min. On a C18 reversed-phase column (Cadenza CD-C18, 3.0 mm i.d. × 150 mm, undamaged, Kyoto, Japan), regulated at 40°C, target molecules were separated after injecting 5 μL of each sample. Mass spectrometry was performed under a capillary voltage of 4.5 kV, source temperature of 250°C,

nebulization gas flow rate of 180 L/h, drench gas flow rate of 10 L/min, positive electrospray ionization, and multiple monitoring reaction mode. LMX was detected at monitor ion m/z = 521 > 137 and collision energy 27 V, whereas FMX was detected at m/z = 497 > 137 and collision energy 26 V. Using analytical software (LCMS solution, Shimadzu Corporation, Kyoto, Japan), the area under the curve was determined. A calibration curve was prepared by spiking known concentrations of LMX into antibiotic-free serum and was used to calculate the amount of LMX in each sample.

4) Pharmacokinetics parameter computation

To estimate PTA based on the PK and PD characteristics of 40 mg/kg LMX bolus administration at every 12, 8, and 6 h, MCS was conducted using commercial software (Oracle Crystal Ball version 11.1.2.4.850, Kozo Keikaku Engineering Inc., Tokyo, Japan). Based on the serum LMX concentrations obtained from five dogs, the PK profiles from the non-compartment model were calculated using the package PK (ver. 4.0.3) of R software (Jaki & Wolfsegger, 2010).

5) Monte Carlo simulation

To develop serum LMX concentration-time profiles, 10,000 virtual patients were created for each dosage regimen of LMX, based on PK profiles presumed to be log-normally distributed. The PDI, which measures the $f\%T_{>MIC}$, was used to calculate the clinical effectiveness of LMX. We adopted 45% as the canine-specific serum protein-binding rate of LMX (Yoshida *et al.*, 1980) and $f\%T_{>MIC} \geq 40\%$ as the PDI (Craig, 1998; Papich, 2014). The highest MIC to attain a PTA ≥ 90% was used to determine the nonclinical PK/PD cutoff values (Mouton *et al.*, 2012; Xiao *et al.*, 2015). CFR was determined using the wild-type MIC distribution of LMX in ESBL-E isolates from companion animals, including *K. pneumoniae*, *E. coli*, *P. mirabilis*, and *E.*

cloacae (Shimizu *et al.*, 2017; Chapter 1). A regimen was characterized as optimal if its CFR ≥ 90%, and considered moderately successful if it was between 80–90% (Wang *et al.*, 2021).

3. Results

1) Animals

Over the course of the dosing regimen studies, none of the dogs showed any negative side effects or unusual blood test findings.

2) Pharmacokinetics profiles of latamoxef in dogs

Fig. 8 and Table 7 depict the blood concentration-time curve and PK profiles of the LMX bolus administered intravenously at a dose of 40 mg/kg. Serum LMX concentration at 5 min was 215.17 ± 35.43 μg/mL, which decreased gradually.

3) Nonclinical pharmacokinetics/pharmacodynamics cutoff and cumulative fraction of response of latamoxef for canine extended spectrum β-lactamase infections

Fig. 9 shows the PTA results for LMX at each MIC at 40 mg/kg every 12, 8, and 6 h. PTA ≥ 90% was attained in all regimens at MICs ≤ 2 μg/mL but not at MICs ≥ 32 μg/mL. Based on these PTA, the nonclinical PK/PD cutoff values determined at 40 mg/kg every 12, 8, and 6 h were ≤ 2, ≤ 8, and ≤ 16 μg/mL, respectively.

The CFR derived from the wild-type MIC distribution of ESBL-E is presented in Table 8. Dogs infected with ESBL-producing isolates of *E. coli*, *K. pneumoniae*, and *P. mirabilis* responded best to 40 mg/kg every 12, 8, and 6 h (CFR ≥ 90%). Conversely, for dogs infected with ESBL-producing *E. cloacae*, all regimens were only modestly successful (CFR of 80–90%).

4. Discussion

Breakpoints for LMX have been established by the CLSI (CLSI supplement M100, 2023) for humans but not for dogs. The present results showed that the canine-specific nonclinical PK/PD cutoff value increased with a shorter dosing interval and that dosing at 40 mg/kg every 8 h was comparable to the CLSI susceptibility breakpoint (≤ 8 μg/mL) (CLSI supplement M100, 2023). Furthermore, nonclinical PK/PD cutoff values in the present study were higher than the MIC_{90} values for ESBL-producing *P. mirabilis*, *K. pneumoniae*, and *E. coli* isolated from cats and dogs (Shimizu *et al.*, 2017; Chapter 2). Based on the calculation of CFRs, LMX protocols at 40 mg/kg every 12 h, in addition to every 8 and 6 h, appear to be optimally effective (CFR ≥ 90%) for the management of ESBL infections in dogs. For ESBL-producing *E. cloacae*, the nonclinical PK/PD cutoff values were lower than the MIC_{90} (32 μg/mL), but the CFRs with all dosing intervals indicated a moderate therapeutic effect.

No LMX dosage has been approved in dogs. A reference dose of 40 mg/kg for humans (each dose, maximum 37.5 mg/kg four times a day) was evaluated. LMX showed a higher clearance rate and a shorter T1/2 than those reported for humans (1.9–2.2 h and 0.08–0.104 L/min, respectively) (Carmine *et al.*, 1983). These differences may be due to the lower protein-binding rate in dogs (45%) than in humans (60%) (Yoshida *et al.*, 1980). LMX has a risk of hemorrhagic adverse events due to hypoprothrombinemia in humans (Park *et al.*, 2019). The highest dose of LMX that has no impact on dogs is 400 mg/kg/day, as reported by Kobayashi *et al.* (1980) based on a 32-day safety investigation. In addition, they confirmed that dogs exhibited hypoprothrombinemia when administered at a dose of 1,600 mg/kg/day, which is 10 times the human dose administered 6 h apart at 40 mg/kg. Based on the differences between humans and dogs, the dosage regimens in the present study (40 mg/kg every 12, 8, and 6 h) would be entirely satisfactory from a safety perspective.

In this chapter, the author established the nonclinical PK/PD cutoff values of 40 mg/kg LMX delivered at every 12, 8, and 6 h via MCS and determined CFRs using the wild-type MIC distribution of ESBL-E isolated from companion animals. The results indicate that 40 mg/kg LMX every 12 h, in addition to every 8 and 6 h, the administration is an effective non-carbapenem therapy for ESBL infections in dogs.

Table 7. Pharmacokinetics characteristics of dogs (n = 5) intravenously administered with LMX at a dose of 40 mg/kg body weight.

Parameters (unit) [a)]	Values (SD) [b)]
AUC (mg·h/L)	266.05 (31.01)
MRT (h)	1.80 (0.26)
T1/2 (h)	1.24 (0.18)
CL (L/h)	1.50 (0.18)
Vd (L)	2.70 (0.34)

a) MRT, mean residence time; CL, total body clearance; T1/2, elimination half-life; Vd, volume of distribution; AUC, area under the concentration-time curve; SD, standard deviation.

b) After intravenous administration, values are expressed as mean (SD).

Table 8. CFR in dogs treated with 40 mg/kg LMX against the wild-type MIC distribution of ESBL-E.

Regimens	CFR (%)			
	E. coli	*K. pneumoniae*	*P. mirabilis*	*E. cloacae*
every 12 h	97.36	95.41	97.86	84.93
every 8 h	99.31	98.80	99.94	86.45
every 6 h	99.89	99.36	100	87.82

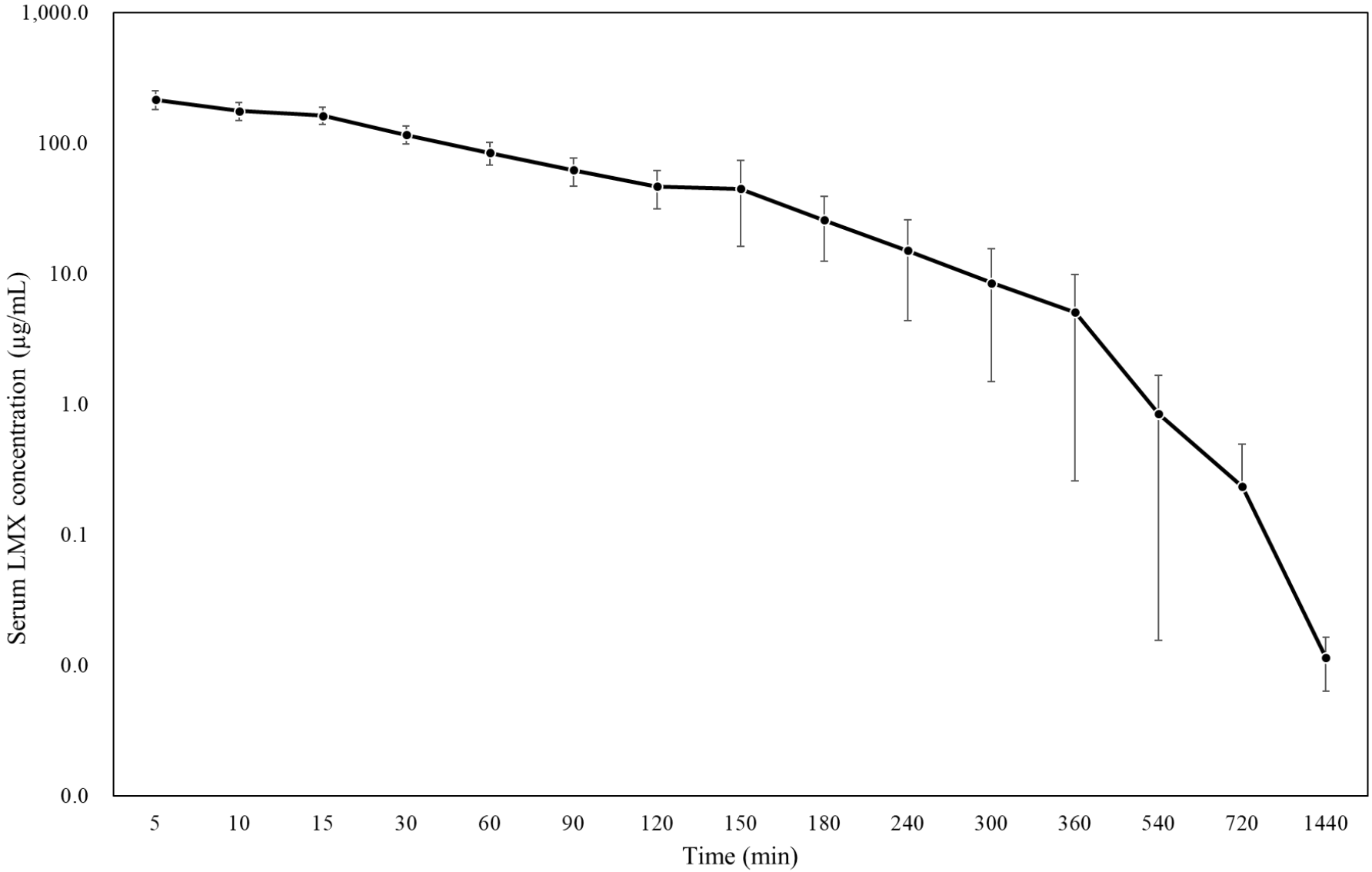

Fig 8. The semilogarithmic plot of serum LMX concentration in dogs administered a dose of 40 mg/kg body weight (mean ± SD, n = 5).

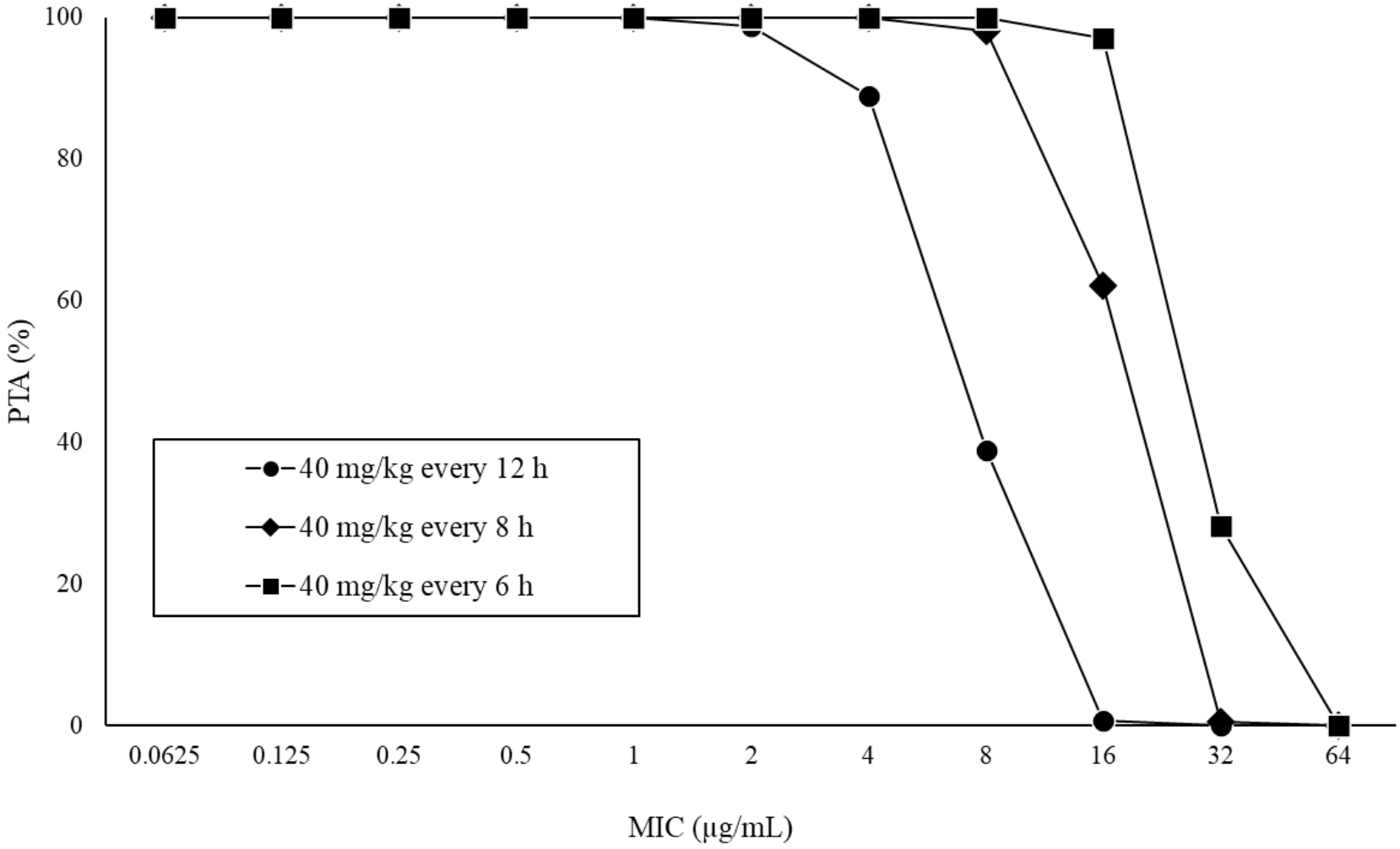

Fig. 9. PTA (%) at each MIC following intravenous administration of LMX.

General Conclusion

There are no established treatment guidelines for ESBL infections in veterinary medicine. In addition, effective therapeutic antimicrobial products for the infection have not been fully investigated in companion animals. Carbapenems, which are used as first-line antibiotics in humans, have not been approved for use in companion animals due to the risk of selecting CRE. Cephamycins, including CMZ, FMX, and LMX, have high stability against ESBLs and high safety for dogs, indicating these cephamycins are carbapenem-sparing candidates for ESBL infections in dogs. However, these cephamycins are not approved for veterinary use, and their *in vitro* and *in vivo* efficacies have not yet been fully investigated in companion animals. The present studies were carried out to obtain knowledge of the efficacies and practicalities of cephamycins and to establish appropriate dosing regimens of some cephamycins for the treatment of canine ESBL infections by the MCS-based PK/PD approach.

In Chapter 1, the author examined the *in vitro* efficacy of CMZ, FMX, and LMX in ESBL-producing isolates of *K. pneumoniae*, *P. mirabilis*, and *E. cloacae* isolated from companion animals. As a result, *K. pneumoniae* and *P. mirabilis* isolates producing only ESBLs exhibited high susceptibility rates to all of the tested cephamycins (95.5–99.1% and 82.7–100%, respectively), which were comparable to that of MEM. In contrast, *K. pneumoniae* isolates producing both ESBLs and ABLs exhibited low susceptibility rates to CMZ and FMX (both 12.5%), although these bacteria exhibited higher susceptibility rates to LMX, as well as MEM, irrespective of ABL production status. The author further found the low susceptibility rates of all cephamycins in ESBL-producing *E. cloacae* isolates, compared with those of MEM: however, there are differences in the susceptibility rates among the three cephamycins [i.e., extremely lower (7.2%), moderate (59.4%), and higher susceptibility rates (85.5%) to CMZ, FMX, and LMX, respectively]. These results imply that the *in vitro* efficacy of cephamycins on

ESBL-E can be greatly affected by antimicrobial substance, ABL production status, and bacterial species, and of these drugs, LMX has the highest *in vitro* efficacy on ESBL-E isolates from companion animals.

In Chapters 2, 3, and 4, the author investigated the PKs of CMZ, FMX, and LMX, respectively, in dogs. The administration experiments revealed that LMX had a higher area under the concentration-time curve (266.05 mg·h/L) and longer elimination half-life (1.24 h) in dogs, compared with those of CMZ (103.36 mg·h/L and 0.84 h) and FMX (134.61 mg·h/L and 0.76 h), due to the lowest total body clearance. Subsequently, the author established canine-specific nonclinical PK/PD cutoff values of these cephamycins by the MCS using PK indices. As a result, nonclinical PK/PD cutoff values of all the cephamycins increased by shortening dosing intervals, reflecting the characteristics of time-dependent antibiotics. A comparison between the three cephamycins revealed that the nonclinical PK/PD cutoff values of LMX at every 12, 8, and 6 h (≤ 2, ≤ 8, and ≤ 16 μg/mL) were greatly higher than those of CMZ (≤ 0.5, ≤ 2, and ≤ 4 μg/mL) and FMX (≤ 0.5, ≤ 2, and ≤ 8 μg/mL), respectively. Thus, it is likely that LMX has more advantageous PK characteristics, thereby resulting in higher nonclinical PK/PD cutoff values, compared with CMZ and FMX.

Furthermore, the author estimated the CFRs of the three cephamycins based on each MIC distribution of wild-type ESBL-E isolates from companion animals. As the results, the dosage regimens of LMX 40 mg/kg every 12, 8, and 6 h and those of FMX 40 mg/kg every 8 and 6 h achieved CFR of ≥ 90% for infections of ESBL-producing *E. coli*, *K. pneumoniae*, and *P. mirabilis*, and thus the author supposes that these regimens are optimal regimens for these infections. In addition, the CMZ dosage regimen of 40 mg/kg every 6 h was estimated to be moderately effective for infections of ESBL-producing *E. coli* and *K. pneumoniae* due to the CFR of 80–90%. As for ESBL-producing *E. cloacae*, the LMX dosage regimens of 40 mg/kg every 12, 8, and 6 h achieved CFR of > 80%, which is expected to be moderately successful,

whereas the CMZ and FMX dosage regimens could not achieve satisfactory CFR irrespective of dosing intervals. These considerations of CFR suggest that regimens of CMZ, FMX and LMX are effective for infections of ESBL-producing *E. coli* and *K. pneumoniae*, FMX and LMX for that of *P. mirabilis*, and only LMX for that of *E. cloacae*.

This study has several limitations. First, the author used only a small number of dogs to calculate the PK parameters because of animal welfare concerns. However, the author increased the reliability of these parameters by using bootstrap replicates. Second, PK parameters were determined in healthy beagle dogs, which may differ from those in patients with renal dysfunction, as previously reported in humans (Bolton *et al.*, 1980; Ohkawa *et al.*, 1980; Aronoff *et al.*, 1982; Andrassy *et al.*, 1991). In dogs, Monaghan *et al*. (2021) demonstrated that the blood concentration of ampicillin, which is mainly excreted renally as well as cephamycins, can increase in dogs with renal diseases. Such increased concentrations of antibiotics may result in increased their efficacy. Third, MIC-based PK/PD analysis was performed in this study; therefore, the author did not consider other PD parameters, such as multiple PD parameters and kill rate (Zhang *et al.*, 2022). Further studies would be needed to overcome these limitations.

In conclusion, the author verified the efficacy of CMZ, FMX, and LMX for ESBL infections in dogs by PK/PD-based approaches. The author also found the higher efficacy of LMX dosage regimens for canine ESBL infections, compared with dosage regimens of CMZ and FMX, due to the preferable PK and PD indices. However, the author emphasizes the need to take into account bacterial species and ABL production status, which can affect their efficacy on the use of cephamycins. Further clinical research would provide a rationale for the practical use of these cephamycins as carbapenem-sparing antibiotics for ESBL infections in dogs.

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Summary

Enterobacterales that produce extended-spectrum β-lactamases (ESBL-E) have multidrug-resistance phenotypes including resistance to almost all veterinary drugs, making their treatment complex. However, therapeutic guidelines for infections by ESBL-E (ESBL infections) in companion animals have not yet been established. Carbapenems are primarily used for the treatment of ESBL infections in human medicine. However, the use of carbapenems has a risk of selecting and increasing carbapenem-resistant Enterobacterales (CRE), which exhibit a more serious multidrug-resistant phenotype than ESBL-E. Therefore, there is a need to explore alternatives to carbapenems for the treatment of ESBL infections in companion animal medicine.

Cephamycins, including cefmetazole (CMZ), flomoxef (FMX), and latamoxef (LMX), are hardly hydrolyzed by ESBLs, and the no observed adverse effect levels in dogs are extremely higher than the human dose (i.e., a maximum of 37.5 mg/kg four times per day, respectively). Thus, these cephamycins may be potential alternatives to carbapenems for the treatment of ESBL infections in dogs. In the last decade, pharmacokinetics (PK)/pharmacodynamics (PD) analysis has been practically used in many researches to consider appropriate dosage regimens of antimicrobial drugs. The *in vitro* activity of cephamycins against ESBL-E from companion animals is important to consider the PD parameters but has not yet been fully understood. In addition, there is little knowledge of the PK of cephamycins in dogs, because these drugs are approved for use in humans but not in dogs. The present studies were carried out to obtain knowledge of the efficacies and practicalities of cephamycins and to establish appropriate dosage regimens of some cephamycins for the treatment of canine ESBL infections based on PK/PD approach.

In Chapter 1, the author examined the *in vitro* efficacy of CMZ, FMX, and LMX and the prevalence of AmpC β-lactamases (ABLs), which can hydrolyze cephamycins, in ESBL-producing isolates of *Klebsiella pneumoniae* (n = 120), *Proteus mirabilis* (n = 29), and *Enterobacter cloacae* (n = 69) isolated from companion animals. They were collected from 136 dogs and 82 cats treated at Japanese veterinary hospitals between 2019 and 2022. The production of ESBL and ABL was confirmed for all isolates using the commercial kit. The minimum inhibitory concentrations (MICs) were determined using the agar dilution method. As a result, *K. pneumoniae* and *P. mirabilis* isolates producing only ESBLs exhibited high susceptibility rates to all of the tested cephamycins (95.5–99.1% and 82.7–100%, respectively). In contrast, *K. pneumoniae* isolates producing both ESBLs and ABLs exhibited low susceptibility rates to CMZ and FMX (both 12.5%), although these bacteria exhibited higher LMX susceptibility rates (87.5%), irrespective of ABL production status. The author further found the low susceptibility rates of all cephamycins in ESBL-producing *E. cloacae* isolates: however, there are differences in susceptibility rates among the three cephamycins [i.e., extremely lower (7.2%), moderate (59.4%), and higher susceptibility rates (85.5%) to CMZ, FMX, and LMX, respectively]. These results imply that the *in vitro* efficacy of cephamycins on ESBL-E can be greatly affected by antimicrobial substance, ABL production status, and bacterial species, and of these drugs, LMX has the highest *in vitro* efficacy on ESBL-E isolates from companion animals.

In Chapters 2, 3, and 4, the author investigated the PKs of CMZ, FMX, and LMX, respectively, in dogs. The day before administration of antimicrobial products, a central venous catheter was placed in the jugular vein of the dog under general anesthesia. Each antimicrobial product was bolus injected in the radial cutaneous vein at 40 mg/kg body weight, and venous blood samples were collected from each participant via a central venous catheter at predetermined time points. The concentration of cephamycins in each sample was determined

by high-performance liquid chromatography–mass spectrometry or –tandem mass spectrometry. Based on serum concentrations of antibiotics, the PK profiles from the non-compartment model were calculated. As a result, of the tested cephamycins, LMX had a higher area under the concentration-time curve (266.05 mg·h/L) and longer elimination half-life (1.24 h) in dogs, compared with those of CMZ (103.36 mg·h/L and 0.84 h) and FMX (134.61 mg·h/L and 0.76 h), due to the lowest total body clearance.

In these chapters, the author also conducted Monte Carlo simulation (MCS)-based PK/PD analysis to estimate the probability of target attainment (PTA) of 40 mg/kg CMZ, FMX, and LMX bolus administration at every 12, 8, 6 h. In these studies, the target value was set as ≥ 40% of the percentage of time during a dosing interval that the concentration of the unbound fraction of antimicrobial substance remains above the MIC for the pathogen, which is the PK/PD index associated with the clinical effectiveness of cephamycins. MCS allowed to creation of drug-serum concentration-time profiles of 10,000 virtual patients by each dosage regimen. The highest MIC to attain a PTA ≥ 90% was used to determine the nonclinical PK/PD cutoff values. The nonclinical PK/PD cutoff values of all the cephamycins increased by shortening dosing intervals, reflecting the characteristics of time-dependent drugs. A comparison between the three cephamycins revealed that the nonclinical PK/PD cutoff values of LMX at every 12, 8, and 6 h (≤ 2, ≤ 8, and ≤ 16 μg/mL) were greatly higher than those of CMZ (≤ 0.5, ≤ 2, and ≤ 4 μg/mL) and FMX (≤ 0.5, ≤ 2, and ≤ 8 μg/mL), respectively. Thus, it is likely that LMX has higher nonclinical PK/PD cutoff values, compared with CMZ and FMX.

Furthermore, the author estimated the cumulative fractions of response (CFRs) of the three cephamycins based on each MIC distribution of wild-type ESBL-E isolates from companion animals. A regimen with a CFR of ≥ 90% is defined as optimal, and a regimen with a CFR of 80–90% is defined as moderately successful. As the results, the dosage regimens of LMX 40 mg/kg every 12, 8, and 6 h and those of FMX 40 mg/kg every 8 and 6 h achieved CFR of ≥

90% for infections of ESBL-producing *E. coli*, *K. pneumoniae*, and *P. mirabilis*, and thus the author supposes that these regimens are optimal regimens for these infections. In addition, the CMZ dosage regimen of 40 mg/kg every 6 h was estimated to be moderately effective for infections of ESBL-producing *E. coli* and *K. pneumoniae* due to the CFR of 80–90%. As for ESBL-producing *E. cloacae*, the LMX dosage regimens of 40 mg/kg every 12, 8, and 6 h achieved CFR of > 80%, which is expected to be moderately successful, whereas the CMZ and FMX dosage regimens could not achieve satisfactory CFR irrespective of dosing intervals. These considerations of CFR suggest that dosage regimens of all cephamycins are effective for infections of ESBL-producing *E. coli* and *K. pneumoniae*, FMX, and LMX for that of *P. mirabilis*, and only LMX for that of *E. cloacae*.

In conclusion, the author verified the efficacy of CMZ, FMX, and LMX for ESBL infections in dogs through PK/PD-based approaches. The author also found the higher efficacy of LMX dosage regimens for canine ESBL infections, compared with dosage regimens of CMZ and FMX, due to the preferable PK and PD indices. However, the author emphasizes the need to take into account bacterial species and ABL production status, which can affect their efficacy on the use of cephamycins. Further clinical research would provide a rationale for the practical use of these cephamycins as carbapenem-sparing antibiotics for ESBL infections in dogs.

学 位 論 文 要 旨

基質特異性拡張型 β-ラクタマーゼ産生腸内細菌目細菌（ESBL-E）は，β-ラクタム系薬を含むほぼ全ての動物用抗菌薬に耐性を示す。そのため，伴侶動物における ESBL-E による感染症（ESBL 感染症）の治療は複雑化しているが，当該感染症に対する治療指針は未だ確立されていない。ヒト医療における ESBL 感染症の治療においては主にカルバペネム系抗菌薬が使用されているが，ESBL-E よりも重篤な多剤耐性を示すカルバペネム耐性腸内細菌目細菌を選択・増加させる危険性がある。従って，伴侶動物の ESBL 感染症の治療において，カルバペネム系抗菌薬の代替薬が必要とされている。

セファマイシン系抗菌薬（セフメタゾール（CMZ），フロモキセフ（FMX），ラタモキセフ（LMX））は，ESBL に安定的であり，かつ，犬に対する安全性が高いことが知られている。この背景から，セファマイシン系抗菌薬は犬の ESBL 感染症に対する治療薬となる可能性がある。近年，抗菌薬の適切な投与条件の検討において，薬物動態学（PK）/薬力学（PD）解析が行われている。しかし，セファマイシン系抗菌薬は動物用抗菌薬として承認されていないため，犬におけるこれら抗菌薬の PD パラメータや PK パラメータに関しては十分には明らかにされていない。本研究は，セファマイシン系抗菌薬の犬の ESBL 産生菌感染症に対する有効性と実用性に関する知見を得ること，また，PK/PD アプローチに基づきこれら抗菌薬の適切な投与条件を検討することを目的として実施された。

第 1 章では，伴侶動物由来 ESBL 産生 *Klebsiella pneumoniae*（120 株），*Proteus mirabilis*（29 株）及び *Enterobacter cloacae*（69 株）に対する CMZ，FMX 及び LMX の *in vitro* での有効性を検討及びセファマイシン系抗菌薬を加水分解する AmpC 型 β-ラクタマーゼ（ABL）産生率の調査を行った。供試株は 2019 年～2022 年にかけて日本の動物病院で治療を受けた犬 136 頭及び猫 82 頭から収集された。市販のキットを用いて ESBL 及び ABL 産生性を確認するとともに，最小発育阻止濃度（MIC）を寒天平板希釈法により測定した。その結果，ESBL のみを産生する *K. pneumoniae* および *P. mirabilis* は 3 薬剤に対して高い感受性を示した（それぞれ 95.5–99.1%及び 82.7–100%）。ESBL と ABL の両方を産生する *K. pneumoniae* は，CMZ と FMX に対する感受性は低かったが（ともに 12.5%），一方で ABL の産生状況にかかわらず LMX に対する感受性は高かった（87.5%）。さらに ESBL 産生 *E. cloacae* は 3 薬剤全ての感受性率が低かったが，薬剤間では感受性率に差が認められた（CMZ，FMX 及び LMX の感受性率はそれぞれ 7.2%，59.4%及び 85.5%）。これらの結果は，ESBL-E に対するセファマイシン系薬の *in vitro* の有効性が，成分，ABL 産生状態及び菌種により大き

く影響されること，その中でも LMX は最も高い *in vitro* の有効性を示すことが明らかとなった。

第 2 章，第 3 章及び第 4 章では，それぞれ CMZ，FMX 及び LMX の犬特異的 PK を調査した。各薬剤を 40 mg/kg 静脈内にボーラス投与し，経時的に中心静脈カテーテルを介して静脈血を採取した。各血清中の抗菌薬濃度を，高速液体クロマトグラフィー質量分析法またはタンデム質量分析法により測定し，ノンコンパートメントモデルにより PK パラメータを算出した。その結果，犬における LMX の濃度時間曲線下面積（266.05 mg-h/L）及び消失半減期（1.24 時間）は、CMZ（103.36 mg-h/L，0.84 時間）及び FMX（134.61 mg-h/L，0.76 時間）と比較して、それぞれ高値であった。

次に，CMZ，FMX 及び LMX（それぞれ一回投与量 40 mg/kg）を 12，8 及び 6 時間間隔にボーラス投与した場合の目標達成確率（PTA）をモンテカルロシミュレーション（MCS）により算出した。10,000 頭の仮想患者の薬物-血清濃度-時間プロファイルを作成し，PTA≧90％を達成する最も高い MIC を非臨床 PK/PD カットオフ値とした。3 薬剤の非臨床 PK/PD カットオフ値は，時間依存性薬物の特性を反映し，投与間隔が短くなるにつれて増加した。LMX の 12，8 及び 6 時間間隔投与時の非臨床 PK/PD カットオフ値（≦2，≦8 及び≦16 μg/mL）は，CMZ（≦0.5，≦2 及び≦4 μg/mL）及び FMX（≦0.5，≦2 及び≦8 μg/mL）よりもそれぞれ高い値を示した。さらに，伴侶動物由来 ESBL-E の MIC 分布に基づいて 3 薬剤の累積治療反応率（CFR）を算出し，投与方法が 90％以上の CFR を示した場合に最適，80～90％の CFR を示した場合に中等度有効と判断した。その結果，12，8 及び 6 時間間隔の LMX 投与並びに 8 及び 6 時間間隔の FMX 投与は，犬の ESBL 産生 *E. coli*，*K. pneumoniae* 及び *P. mirabilis* 感染症に対して最適と判断された。また，6 時間間隔の CMZ 投与は，犬の ESBL 産生 *E. coli* 及び *K. pneumoniae* 感染症に対して中等度有効と判断された。一方，ESBL 産生 *E. cloacae* 感染症に対しては，12，8 及び 6 時間間隔の LMX 投与で中等度有効と判断されたが，CMZ 又は FMX の投与は投与間隔に関わらず満足できる CFR は得られなかった。以上より，ESBL 産生 *E. coli* 及び *K. pneumoniae* 感染症には CMZ，FMX 及び LMX，*P. mirabilis* 感染症には FMX 及び LMX，*E. cloacae* 感染症には LMX 投与が中等度以上の有効性を示すと考えられた。

今回，犬の ESBL 感染症に対するセファマイシン系抗菌薬の有効性を PK/PD アプローチにより検討した。結果として，LMX は CMZ や FMX と比較して，PK 及び PD パラメータが優れており，犬の ESBL 感染症に対して高い有効性が期待される結果を示した。しかし，セファマイシン系抗菌薬使用の際には，その有効性に影響を及ぼす菌種や ABL 産生性を考慮する必要がある。今後，犬の ESBL 感染症に対する治療薬としての実用化に向けたセファマイシン系抗菌薬の臨床研究が求められる。

List of Publications

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This article covers Chapter 1.

Kusumoto, M., Motegi, T., Uno, H., Yokono, M. & Harada, K. (2023). Pharmacokinetic-pharmacodynamic analysis of cefmetazole against extended-spectrum β-lactamase-producing Enterobacteriaceae in dogs using Monte Carlo Simulation. *Frontiers in Veterinary Science*, 10, 1270137.

This article covers Chapter 2.

Kusumoto, M., Motegi, T., Jitsuiki, M., Yokono, M. & Harada, K. (2024). Pharmacokinetic and pharmacodynamic analysis of the oxacephem antibiotic flomoxef against extended-spectrum β-lactamase-producing Enterobacterales from dogs. *International Journal of Molecular Sciences*, 25, 1105.

This article covers Chapter 3.