수의학석사학위논문

Characterization of Extended-Spectrum β-Lactamase-Producing and AmpC β-Lactamase-Producing *Enterobacterales* Isolated from Companion Animals in South Korea

국내 반려동물에서 분리된 Extended-Spectrum β-Lactamases 및 AmpC β-Lactamases 생성 장내세균총에 대한 특성 분석

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Abstract

The emergence of extended-spectrum cephalosporin (ESC)-resistant *Enterobacterales* is of great concern in both human and veterinary medicine. The aim of this study was to compare ESC-resistant *Enterobacterales* isolated from companion animals in South Korea between 2017-2019. Isolates with ESC resistance genes, which were identified by PCR, were assessed for genetic relatedness by multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE). A total of 91 ESC-resistant *Enterobacterales* (*Escherichia coli, Klebsiella* spp*., Serratia* spp. and *Enterobacter cloacae*) harbored the bla_{TEM} gene. Among other ESC resistance genes, *CTX-M-15*, *CITM*, and *CTX-M-55* were predominantly detected in *E. coli* isolates, whereas bla_{SHV} and *DHAM* were more frequently detected

in *K. pneumoniae* isolates. In addition, all of the *EBCM*-positive isolates were classified as *E. cloacae*. From the MLST results, *CTX-M-9*-carrying ST131, *CITM*-carrying ST405, and *CTX-M-1*-carrying ST3285 strains were dominant in *E. coli* isolates. ST273 and ST275 strains harboring bla_{SHV} were frequently detected in *K. pneumoniae* isolates. Various sequence types were obtained in *E. cloacae* and *K. oxytoca* isolates. All isolates demonstrated unique PFGE profiles (< 57~98% similarity) and were unlikely to be derived from a single clone. The present study indicates the presence and wide genetic distribution of ESC-resistant *Enterobacterales* in South Korean companion animals.

Keywords: *Enterobacterales,* extended-spectrum cephalosporins (ESCs), extended-spectrum-β-lactamases (ESBLs), AmpC β-lactamases (AmpCs), antimicrobial resistance (AMR), companion animal, South Korea

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Introduction

The emergence and prevalence of β-lactam-resistant *Enterobacterales* has increased consistently over the past few decades [1, 2]. Resistance to β-lactam is mostly caused by bacterially produced β-lactamases that hydrolyze and inactivate extended-spectrum cephalosporins (ESCs), such as 3rd and 4th generation cephalosporins [1]. ESC resistance is mainly caused by the expression of extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase (AmpC) genes that are normally encoded on mobile genetic elements, mostly plasmids [1].

Since 2000, CTX-M-type enzymes have comprised the largest portion within ESBLs, among which the presence of $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-14}}$ has increasingly been reported in most areas of the world, including South Korea [3, 4]. Among AmpCs, CITM- and DHAM-type enzymes are the most prevalent [2]. Especially, *DHAM*-carrying *Klebsiella* spp. isolates and *CITM*-carrying *Escherichia coli* isolates have been repeatedly reported for *Enterobacterales* from South Korea [2, 5]. Despite many studies on ESBL- and AmpC-producing bacteria from human or livestock isolates, studies concerning antimicrobial resistance (AMR) bacteria associated with companion animals are lacking, especially *Serratia* spp. and *Enterobacter* spp. [6-8].

The popularity of companion animals in South Korea has grown, which provides a potential reservoir of AMR bacteria as pets are closely associated with humans, living in their homes and near their food [5]. Thus, the importance of profiling AMR bacteria was emphasized in the “One Health” initiative, which integrates veterinary medicine, human health, animal-production systems, and the environment [9]. Systematic control and prevention, through implementation of a national AMR surveillance program, is greatly needed and should be applied in both human and veterinary clinical medicine.

The goal of the current study was to investigate AMR among *Enterobacterales* in companion animals within the province of South Korea, with an emphasis on ESC resistance genes in *E. coli*, *Klebsiella* spp., *Enterobacter cloacae,* and *Serratia* spp.

Materials and methods

1. Bacterial characterization

Sampling, isolation, identification, and phenotypic characterization of *Enterobacterales* were previously described [10, 11]. A total of 91 *Enterobacterales* isolates carrying ESC resistance genes (51 *E. coli,* 17 *K. pneumoniae*, five *K. oxytoca,* four *Serratia marcescens,* seven *S. liquefaciens* and seven *E. cloacae*) were collected from companion animals (56 hospital-admitted dogs, 23 stray dogs, 11 hospital-admitted cats, and one stray cat). ESC resistance gene-carrying *Enterobacterales* were not isolated from human samples.

2. Characterization of β-lactamases genes

PCR amplification of entire $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} genes was performed as previously described [10, 11]. For $bla_{\text{CTX-M}}$-positive isolates, PCR and DNA sequencing were carried out for CTX-M subtype detection. $bla_{\text{CTX-M}}$ group-specific primers for five clusters (CTX-M-1,-2,-8,-9,and -25) were used following Kor-GLASS (Korea Global Antimicrobial Resistance Surveillance System) guidelines and previously published protocols [12, 13]. DNA sequencing was performed by Intron Biotechnology (Seongnam, South Korea) and homologous sequences were searched against the GenBank database using the BLAST tool of the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST). In ESBL-positive strains, six groups of AmpC genes (*MOXM, CITM, DHAM, ACCM, EBCM,* and *FOXM*) were screened by PCR amplification [14].

3. Multi-locus sequence typing (MLST)

For *E. coli*, *K. pneumoniae, K. oxytoca* and *E. cloacae* isolates, MLST with seven housekeeping genes for each bacterial strain was carried out in reference to previous studies [15-17]. PCR was performed using primers for *adk, fumC, gyrB, icd, purA mdh* and *recA* (*E. coli); gapA, infB, mdh, pgi, phoE, rpoB* and *tonB* (*K. pneumoniae* and *K. oxytoca)*; and *danA, fusA, gyrB, leuS, pyrG, rplB* and *rpoB (E. cloacae).* Allelic profile and ST

determinations were performed according to web-based MLST databases (https://pubmlst.org/databases/ and https://bigsdb.pasteur.fr/klebsiella/klebsiella.html).

3. Pulsed-field gel electrophoresis (PFGE)

PFGE of *XbaI* (Takara Bio Inc., Shiga, Japan)-digested genomic DNA was carried out for *E. coli, K. pneumoniae, K. oxytoca*, and *E. cloacae* isolates according to the CDC PulseNet standardized procedure using the Chef Mapper system (Bio-Rad Laboratories, Hercules, CA, USA) [18]. PFGE analysis for *Serratia* spp. was ignored because different digested genomic DNAs were employed for *Serratia* spp. Similarities between restriction fragment length polymorphisms were analyzed using GelCompar II software v. 6.5 (Applied Maths NV, St-Martens-Latem, Belgium) to produce a dendrogram. Unweighted-pair group method using average linkages (UPGMA) cluster analysis was conducted based on a 85% similarity cut-off with 0.5% optimization and 2.0% band tolerance.

Result

1. ESCs resistance gene detection

A total of 91 ESC-resistant *Enterobacterales* isolates were identified, each of which harbored the *bla*$_{TEM}$ gene. *bla*$_{CTX-M}$ (n=42, 82.4%) was abundantly detected in *E. coli* isolates, while *bla*$_{SHV}$ (n=16, 94.1%) was mainly detected in *K. pneumoniae* isolates (Table 1). None of *Serratia* spp. and *E. cloacae* isolates, except one *S. marcescens* isolate from *Serratia* spp. that harbored *bla*$_{SHV,}$ were positive for *bla*$_{SHV}$ or *bla*$_{CTX-M}$ (Table 1). Among 55 *bla*$_{CTX-M}$-positive *Enterobacterales* isolates, *CTX-M-15* (n=23, 41.8%) and *CTX-M-55* (n=12, 21.8%) were the most common subtypes, followed by *CTX-M-14* (n=7, 12.7%) (Table 2). *Enterobacterales* isolates carrying *CTX-M-3, CTX-M-61, CTX-M-27,* and *CTX-M-65* were also identified (Table 2*). CTX-M-2, CTX-M-8,* and *CTX-M-25* clusters were not detected in the total isolates. Among AmpC genes, *CITM* was prevalent in *E. coli* isolates, *DHAM* was predominantly detected in *Klebsiella* spp. isolates, and *EBCM* was common in *E. cloacae* isolates (Table 3). No isolates carrying *MOXM* or *ACCM* genes were detected.

2. MLST

Various STs were revealed among *E. coli*, *K. pneumoniae, K. oxytoca*, and *E. cloacae* isolates. STs of *Serratia* spp. isolates were not defined due to a lack of MLST scheme for *Serratia* spp. Among *E. coli* isolates, five ST131 and two ST3285 strains were detected from hospital-admitted dogs, with one of each of the following STs: ST372, ST457, ST648, ST1981, ST2179, ST2505, ST4616, ST5150, ST5667, ST8451, ST8885, ST8908, ST10207, ST10220, and ST11000. Accordingly, five ST405, three ST354, two of each ST3285, ST410, ST448 and ST457, and one of each ST68, ST38, ST648, ST1193, ST2541, ST7644 and ST10459 strains were detected in stray dogs. Among six *E. coli* isolates from hospital-admitted cats, two ST131, two ST156, and one of each ST1262 and ST6105 strains were obtained. In a comprehensive analysis of 19 *Klebsiella* spp. isolates from hospital-admitted dogs, one ST285, eight ST275, six ST273 stains were identified in *K. pneumoniae* isolates, whereas one of each ST34, ST145, ST273, and ST293 strains were identified in *K. oxytoca* isolates. All *Klebsiella* spp. isolates from hospital-admitted and stray cats were identified as ST273 strains. Among seven *E.*

cloacae isolates, two ST114 and one of each ST110, ST171, ST198, ST1252 and ST1303 strains were identified (Table 4).

3. PFGE

PFGE analysis was only conducted for 46 *E. coli,* 13 *K. pneumoniae,* five *K. oxytoca*, and six *E. cloacae* isolates; no or few banding patterns were obtained for five *E. coli*, four *K. pneumoniae*, and one *E. cloacae* isolates. Using a >85% similarity cut-off, thirty-one pulsotypes in *E. coli* (e1 to e31), nine pulsotypes in *K. pneumoniae* (kp1 to kp9), four pulsotypes in *K. oxytoca* (ko1 and ko4) and four pulsotypes in *E. cloacae* (ec1 to ec4) were identified. Generally, all isolates demonstrated unique PFGE profiles (57~95% similarity), indicating genetic heterogeneity in ESBL- or AmpC-producing genes (Figure 1).

4. Genetic relatedness

In *E. coli* PFGE analysis, e1 group consisted of three ST3285 strains which containing *CTX-M-55,* bla_{TEM} and *CITM* from hospital-admitted and stray dogs and showed high similarity (>90%) (Figure 1A). Seven ST131 strains of *E. coli* isolates from five hospital-admitted dogs and two hospital-admitted cats were identified. PFGE results involved only five hospital-admitted dogs and one hospital-admitted cat, because the banding pattern for one hospital-admitted cats isolate was not defined. Two of them which belonged to e18 group showed more than 93% similarity, compared with the remain isolates in which low similarity was observed (<85%) (Figure 1A). e3 and e25 group contained two ST448 strains with *CTX-M-15* and bla_{TEM} and two ST457 strains with bla_{TEM} and *CITM* from same shelter and showed high similarity (>87%) (Figure 1A).

All bla_{TEM}-positive *K. pneumoniae* isolates from hospital-admitted dogs harbored the bla_{SHV} gene and exhibited 60~95% similarity (Figure 1B). One *K. pneumoniae* ST273 strain from stray cats belonged to kp8 group and showed 87% similarity with hospital-admitted dogs isolate (Figure 1B). Two ST275 strains co-carrying bla_{SHV} and bla_{TEM} which belonged to kp5 group showed high similarity (>90%) (Figure 1B). The same pulsotype belonged to both ST273 and ST275 which were single-locus variants at the *tonB* allele, for example; kp1 and

kp8. The remaining strains were independent of the groups were obtained.

Various STs and PFGE profiles were obtained for *K. oxytoca* and *E. cloacae* isolates, and genetic relatedness was not revealed for those strains (Figure 1C and D).

Discussion

This study presents the characteristics of 91 *Enterobacterales* isolates harboring ESC resistance genes, including *E. coli*, *Klebsiella* spp., *Serratia* spp. and *Enterobacter cloacae*, collected from South Korean companion animals between 2017-2019. All isolates harbored the *bla*$_{TEM}$ gene and demonstrated unique PFGE profiles. Similarly, a study by Shin et al. revealed that all *E. coli* isolates from beef cattle harbored the *bla*$_{TEM}$ gene [8]. Recent reports have identified *bla*$_{CTX-M}$-type β-lactamases as the most widespread ESBL type, replacing classical *bla*$_{TEM}$- and *bla*$_{SHV}$-type ESBLs [19]; however, *bla*$_{TEM}$-type β-lactamases remained the most prevalent ESBL type identified in the current study. We observed varying predominant β-lactamase gene types in different *Enterobacterales* species: *bla*$_{CTX-M}$ and *CITM* in *E. coli* isolates, *bla*$_{SHV}$ and *DHAM* in *K. pneumoniae* isolates, and *EBCM* in *E. cloacae*. β-lactamase gene distribution for each *Enterobacterales* species was similar to that described in a previous study with human samples [2]. These findings indicate that ESC resistance gene variants are not limited to certain hosts, emphasizing the need for coordinated control in both human and animals.

In *E. coli* isolates in this study, *bla*$_{TEM}$ and *bla*$_{CTX-M}$ was most frequently detected, followed by *CITM*. Among *bla*$_{CTX-M}$-type genes, *CTX-M-15* was the most common genotype followed by *CTX-M-55* in both dogs and cats. A 2012 study investigating *E. coli* isolates from dogs reported that *CTX-M-15, CTX-M-14*, and *CITM* were the most prevalent genotypes, while *CTX-M-55* was rarely detected in South Korea [20]. However, *CTX-M-55*-carrying *E. coli* has become increasingly prevalent in dogs in South Korea [5]. The present study revealed *CTX-M-55* was predominantly detected rather than *CTX-M-14* in companion animals, which concurred with the results of the study by Hong et al. (2019). All five *E. coli* ST405 strains investigated in the current study harbored both *bla*$_{TEM}$ and *CITM* and were collected from stray dogs in the same shelter. There was no evidence of clonal spread between humans and companion animals, or transmission among populations in the present study. However, the spread of *CITM*-carrying *E. coli* ST405 was described in a previous study, which suggested the possibility of direct transmission between humans and companion animals [5]. The spread of *E. coli* ST405 was usually described in humans harboring *CTX-M-15* [21]. However, *E. coli* ST405 did not harbor *CTX-M-15* in the current study. The increasing prevalence of *E. coli* ST131 carrying *CTX-M-15* has been described in humans and animals [20, 22]. Unexpectedly, only one *CTX-M-15*-carrying *E. coli* ST131 strain was detected

from hospital-admitted cats in this study. From *E. coli* PFGE results, the two ST3285 strains showed same PFGE pattern were both from same shelter and isolated within the same date (Figure 1A). However, it could be the result of a random effect, indicating they were unlikely to be derived from a single clone.

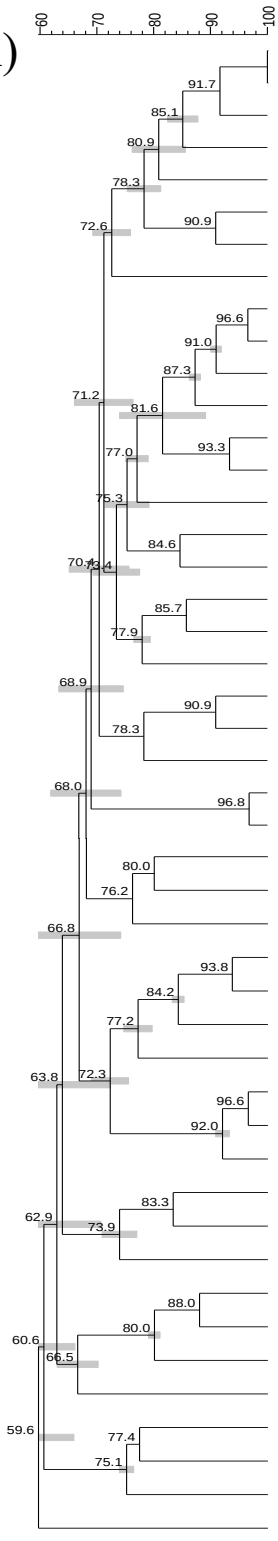
Among the seven *K. pneumoniae* and four *K. oxytoca* isolates harboring $bla_{\text{CTX-M}}$ from hospital-admitted dogs in this study, the *CTX-M-15* genotype accounted for a large proportion. A recent study also reported that *K. pneumoniae* isolates from companion animals including *CTX-M-15* either alone or in combination with *DHAM* were frequently detected in South Korea [5]. The present MLST results revealed that ST275 and ST273 strains carrying both bla_{SHV} and bla_{TEM} were most commonly identified among *K. pneumoniae* isolates from hospital-admitted dogs. Recently, the bla_{SHV}- and bla_{TEM}-co-carrying *K. pneumoniae* ST273 strain has emerged in human patients in Italy and is being disseminated, while ST273 and ST275 *Klebsiella* spp. isolates carrying both bla_{SHV} and bla_{TEM} have not yet been reported in South Korea [23, 24]. Moreover, ST11, ST15, ST307, and ST392 strains have been world-widely identified as β-lactamase-producing *Klebsiella* spp. [5, 25, 26]. β-lactamase-producing ST275 or ST273 strains in *Klebsiella* spp. isolates were newly discovered in South Korean companion animals in the current study.

All *E. cloacae* isolates in this study harbored bla_{TEM} and some carried *EBCM*, while none carried $bla_{\text{CTX-M}}$ and bla_{SHV}. *E. cloacae* isolates from companion animals combined with more than two ESBL-type genes were described in Germany [27]. While *E. cloacae* isolates investigated in the present study harbored only one type of ESBL gene. In *Serratia* spp. isolates, only bla_{TEM} was detected, except one *S. marcescens* isolate that harbored both bla_{TEM} and bla_{SHV}. A *S. marcescens* isolate carrying bla_{TEM} was previously identified in South Korea that caused urinary infections in humans [28]. However, in many countries including South Korea, the status of emerging AMR among *Serratia* spp. and *E. cloacae* in companion animals remains unknown. To our knowledge, this is the first report of ESC-resistant *Serratia* spp. and *E. cloacae* isolates from companion animals in South Korea.

In conclusion, we illustrate the presence and genetic heterogeneity of ESC-resistant *Enterobacterales* in companion animals in South Korea, providing a potential reservoir of ESC-resistant bacteria and transmission pathway. More organized surveillance is required to prevent and control the spread of ESC-resistant bacteria between companion animals and humans, in accordance with the “One Health” initiative.

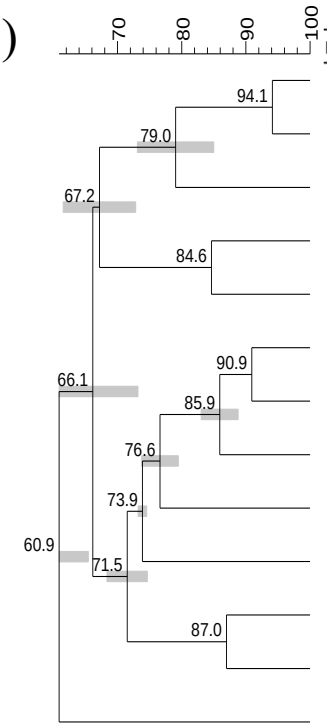
Figure 1. Dendrogram of PFGE patterns showing the genetic relatedness of ESBL-producing *Enterobacterales* isolates. (A) *Escherichia coli*, (B) *Klebsiella pneumoniae*, (C) *Klebsiella oxytoca* and (D) *Enterobacter cloacae*. ESBL; extended-spectrum β-lactamase, PFGE; pulsed-field gel electrophoresis

(A)



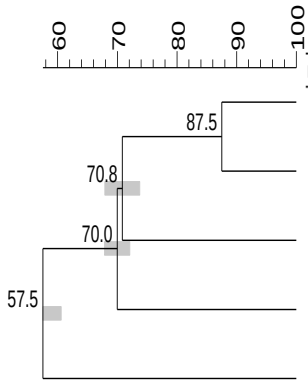
Isolates	Group	Species	Sequence type	ESBL gene	AmpC gene	Sample origin
E29		*Escherichia coli*	3285	CTX-M-55, TEM	CITM	Stray dogs
E28	e1	*Escherichia coli*	3285	CTX-M-55, TEM	CITM	Stray dogs
E25		*Escherichia coli*	3285	CTX-M-55, TEM	CITM	Hospital-admitted dogs
E6		*Escherichia coli*	8451	CTX-M-55, TEM		Hospital-admitted dogs
E23	e2	*Escherichia coli*	8908	CTX-M-55, TEM		Hospital-admitted dogs
E36	e3	*Escherichia coli*	448	CTX-M-15, TEM		Stray dogs
E35		*Escherichia coli*	448	CTX-M-15, TEM		Stray dogs
E39	e4	*Escherichia coli*	2541	TEM		Stray dogs
E46		*Escherichia coli*	156	TEM		Hospital-admitted cats
E20	e5	*Escherichia coli*	10459	CTX-M-15, TEM		Stray dogs
E4		*Escherichia coli*	10220	CTX-M-14, TEM		Hospital-admitted dogs
E15		*Escherichia coli*	1193	CTX-M-27, TEM	FOXM	Stray dogs
E10	e6	*Escherichia coli*	2179	CTX-M-15, TEM		Hospital-admitted dogs
E50		*Escherichia coli*	6105	TEM		Hospital-admitted cats
E27	e7	*Escherichia coli*	354	CTX-M-15, TEM	CITM	Stray dogs
E24	e8	*Escherichia coli*	3285	CTX-M-61, TEM		Hospital-admitted dogs
E47	e9	*Escherichia coli*	156	CTX-M-55, TEM		Hospital-admitted cats
E26	e10	*Escherichia coli*	354	CTX-M-15, TEM		Stray dogs
E48		*Escherichia coli*	1262	CTX-M-15, TEM		Hospital-admitted cats
E44	e11	*Escherichia coli*	405	TEM	CITM	Stray dogs
E14	e12	*Escherichia coli*	38	CTX-M-14, TEM		Stray dogs
E19		*Escherichia coli*	8885	CTX-M-3, TEM		Hospital-admitted dogs
E16	e13	*Escherichia coli*	4616	CTX-M-55, TEM		Hospital- admitted dogs
E33	e14	*Escherichia coli*	354	CTX-M-15, TEM	CITM	Stray dogs
E31		*Escherichia coli*	2505	CTX-M-55, TEM	CITM	Hospital-admitted dogs
E17	e15	*Escherichia coli*	11000	CTX-M-15, TEM	CITM	Hospital-admitted dogs
E18	e16	*Escherichia coli*	5150	CTX-M-55, TEM		Hospital-admitted dogs
E1	e17	*Escherichia coli*	68	CTX-M-14, TEM		Stray dogs
E7	e18	*Escherichia coli*	131	CTX-M-14, TEM		Hospital-admitted dogs
E5		*Escherichia coli*	131	TEM		Hospital-admitted dogs
E8	e19	*Escherichia coli*	131	CTX-M-14, TEM		Hospital-admitted dogs
E3	e20	*Escherichia coli*	131	TEM		Hospital-admitted dogs
E13	e21	*Escherichia coli*	648	CTX-M-14, TEM		Stray dogs
E12		*Escherichia coli*	131	CTX-M-27, TEM	FOXM	Hospital-admitted dogs
E11		*Escherichia coli*	10207	CTX-M-15, TEM		Hospital-admitted dogs
E34	e22	*Escherichia coli*	7644	CTX-M-15, TEM		Stray dogs
E9	e23	*Escherichia coli*	648	CTX-M-15, TEM		Hospital-admitted dogs
E2	e24	*Escherichia coli*	1981	CTX-M-15, TEM		Hospital-admitted dogs
E45	e25	*Escherichia coli*	457	TEM	CITM	Stray dogs
E43		*Escherichia coli*	457	TEM	CITM	Stray dogs
E37	e26	*Escherichia coli*	372	TEM		Hospital-admitted dogs
E22	e27	*Escherichia coli*	457	CTX-M-15, TEM	CITM	Hospital-admitted dogs
E40	e28	*Escherichia coli*	405	TEM	CITM	Stray dogs
E51	e29	*Escherichia coli*	131	CTX-M-15, TEM		Hospital-admitted cats
E38	e30	*Escherichia coli*	405		CITM	Stray dogs
E21	e31	*Escherichia coli*	410	CTX-M-15, TEM	CITM	Stray dogs

(B)



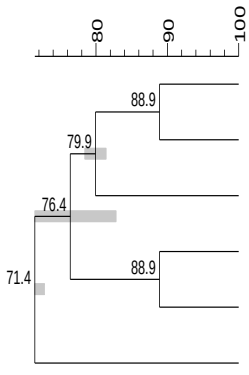
Isolates	Group	Species	Sequence type	ESBL gene	AmpC gene	Sample origin
K15	kp1	*Klebsiella pneumoniae*	273	CTX-M-27, SHV, TEM	DHAM	Hospital- admitted dogs
K16		*Klebsiella pneumoniae*	275	CTX-M-55, SHV, TEM	DHAM	Hospital- admitted dogs
K14	kp2	*Klebsiella pneumoniae*	285	CTX-M-15, SHV, TEM	EBCM	Hospital- admitted dogs
K11	kp3	*Klebsiella pneumoniae*	273	SHV, TEM		Hospital- admitted dogs
K9	kp4	*Klebsiella pneumoniae*	273	SHV, TEM	CITM	Hospital- admitted dogs
K8	kp5	*Klebsiella pneumoniae*	275	SHV, TEM		Hospital- admitted dogs
K1		*Klebsiella pneumoniae*	275	SHV,TEM		Hospital- admitted dogs
K2		*Klebsiella pneumoniae*	273	CTX-M-15, SHV, TEM		Hospital- admitted dogs
K12	kp6	*Klebsiella pneumoniae*	275	SHV, TEM	DHAM	Hospital- admitted dogs
K5	kp7	*Klebsiella pneumoniae*	275	SHV, TEM		Hospital- admitted dogs
K4	kp8	*Klebsiella pneumoniae*	275	CTX-M-65, SHV, TEM	DHAM	Hospital- admitted dogs
K6		*Klebsiella pneumoniae*	273	TEM		Stray cats
K13	kp9	*Klebsiella pneumoniae*	275	SHV, TEM		Hospital- admitted dogs

(C)



Isolates	Group	Species	Sequence type	ESBL gene	AmpC gene	Sample origin
K7	ko1	*Klebsiella oxytoca*	145	CTX-M-14, TEM		Hospital- admitted dogs
K18		*Klebsiella oxytoca*	273	SHV, TEM		Hospital- admitted cats
K3	ko2	*Klebsiella oxytoca*	34	TEM	DHAM	Hospital- admitted dogs
K17	ko3	*Klebsiella oxytoca*	293	CTX-M-15, TEM		Hospital- admitted dogs
K10	ko4	*Klebsiella oxytoca*	273	CTX-M-15, SHV, TEM	DHAM	Hospital- admitted dogs

(D)



Isolates	Group	Species	Sequence type	ESBL gene	AmpC gene	Sample origin
En4	ec1	*Enterobacter cloacae*	114	TEM	EBCM	Hospital-admitted dogs
En5		*Enterobacter cloacae*	198	TEM	DHAM	Hospital-admitted dogs
En1	ec2	*Enterobacter cloacae*	110	TEM	EBCM	Hospital-admitted dogs
En2	ec3	*Enterobacter cloacae*	1303	TEM	DHAM+EBCM	Hospital-admitted cats
En3		*Enterobacter cloacae*	114	TEM		Hospital-admitted dogs
En6	ec4	*Enterobacter cloacae*	171	TEM	CITM+EBCM	Hospital-admitted cats

Table 1. Distribution of extended-spectrum β-lactamases genes among 91 *Enterobacterales* isolated from companion animals

	Hospital-admitted dogs (n=56)			Stray dogs (n=23)			Hospital-admitted cats (n=11)			Stray cats (n=1)			Total		
	bla$_{\text{CTX-M}}$	*bla*$_{\text{SHV}}$	*bla*$_{\text{TEM}}$	*bla*$_{\text{CTX-M}}$	*bla*$_{\text{SHV}}$	*bla*$_{\text{TEM}}$	*bla*$_{\text{CTX-M}}$	*bla*$_{\text{SHV}}$	*bla*$_{\text{TEM}}$	*bla*$_{\text{CTX-M}}$	*bla*$_{\text{SHV}}$	*bla*$_{\text{TEM}}$	*bla*$_{\text{CTX-M}}$	*bla*$_{\text{SHV}}$	*bla*$_{\text{TEM}}$
E. coli (n=51)	21	-	22	15	-	23	6	-	6	-	-	-	42 (82.4%)	-	51 (100%)
K. pneumoniae (n=17)	7	15	15	-	-	-	1	1	1	1	-	1	9 (17.6%)	16 (94.1%)	17 (100%)
K. oxytoca (n=5)	4	1	4	-	-	-	-	1	1	-	-	-	4 (80.0%)	2 (40.0%)	5 (100%)
S. marcescens (n=4)	-	1	3	-	-	-	-	-	1	-	-	-	-	1 (45.0%)	4 (100%)
S. liquefaciens (n=7)	-	-	7	-	-	-	-	-	-	-	-	-	-	-	7 (100%)
E. cloacae (n=7)	-	-	5	-	-	-	-	-	2	-	-	-	-	-	7 (100%)

Table 2. *bla*$_{\text{CTX-M}}$ subtype detection of 55 *bla*$_{\text{CTX-M}}$ positive *Enterobacterales.*

Sample Origin	Organism	Subtype group									
		CTX-M-1					CTX-M-9				Unidentified
		CTX-M-15	CTX-M-55	CTX-M-3	CTX-M-61	Total	CTX-M-14	CTX-M-27	CTX-M-65	Total	
E. coli (n=42)	Hospital-admitted dogs (n=21)	7	6	1	1	15	3	1	-	4	2
	Stray dogs (n=15)	8	3	-	-	11	3	1	-	4	0
	Hospital-admitted cats (n=6)	2	2	-	-	4	-	-	-	0	2
K. pneumoniae (n=9)	Hospital-admitted dogs (n=7)	4	1	-	-	5	-	1	1	2	0
	Hospital-admitted cats (n=1)	-	-	1	-	1	-	-	-	0	0
	Stray cats (n=1)	-	-	-	-	0	-	-	-	0	1
K. oxytoca (n=4)	Hospital-admitted dogs (n=4)	2	-	-	-	2	1	-	-	1	1
Total		23 (41.8%)	12 (21.8%)	2 (3.6%)	1 (1.8%)	38 (69.1%)	7 (12.7%)	3 (5.5%)	1 (1.8%)	11 (20.0%)	6 (10.9%)

Table 3. Distribution of AmpC β-lactamases genes for extended-spectrum β-lactamases-producing *Enterobacterales* isolates.

Organism	Sample origin	AmpC β-lactamases gene								
		MOXM	CITM	DHAM	ACCM	EBCM	FOXM	EBCM+CITM	EBCM+DHAM	Total
E. coli	Hospital-admitted dogs (n=22)	-	5	-	-	-	-	-	-	5
	Stray dogs (n=23)	-	13	-	-	-	1	-	-	14
K. pneumoniae	Hospital-admitted dogs (n=15)	-	1	4	-	1	-	-	-	6
	Hospital-admitted cats (n=1)	-	-	1	-	-	-	-	-	1
K. oxytoca	Hospital-admitted dogs (n=4)	-	-	2	-	-	-	-	-	2
E. cloacae	Hospital-admitted dogs (n=5)	-	-	1	-	2	-	-	-	3
	Hospital-admitted cats (n=2)	-	-	-	-	-	-	1	1	2
Total		-	19	8	-	3	1	1	1	33

Table 4. Multi-locus sequencing typing result based on the spread of β-lactamases resistance genes.

Organism	*bla*CTX-M Cluster	[a]ESC resistance gene				No. isolation			
		[b]ESBLs			[c]AmpCs	[d]ST type			
		*bla*CTX-M	*bla*SHV	*bla*TEM		Hospital-admitted dogs	Stray dogs	Hospital-admitted cats	Stray cats
E. coli	CTX-M-1	CTX-M-15	-	+	-	648 (n=1)	354 (n=1)	131 (n=1)	-
						1981 (n=1)	448 (n=2)	1262 (n=1)	-
						2179 (n=1)	7644 (n=1)	-	-
						10207 (n=1)	10459 (n=1)	-	-
			-	+	CITM	457 (n=1)	354 (n=2)	-	-
						5667 (n=1)	410 (n=1)	-	-
						11000 (n=1)	-	-	-
		CTX-M-55	-	+	-	4616 (n=1)	-	131 (n=1)	-
						5150 (n=1)	-	156 (n=1)	-
						8451 (n=1)	-	-	-
						8908 (n=1)	-	-	-
			-	+	CITM	2505 (n=1)	410 (n=1)	-	-
						3285 (n=1)	3285 (n=2)	-	-
	CTX-M-9	CTX-M-61	-	+	-	3285 (n=1)	-	-	-
		CTX-M-3	-	+	-	8885 (n=1)	-	-	-
		CTX-M-14	-	+	-	10220 (n=1)	38 (n=1)	-	-
						131 (n=2)	68 (n=1)	-	-
						-	648 (n=1)	-	-

		CTX-M-27	-	+	-	131 (n=1)	-	-	-
		CTX-M-27	-	+	FOXM	-	1193 (n=1)	-	-
						-	-	-	-
	Unidentified	-	-	+	-	131 (n=2)	-	156 (n=1)	-
						-	-	6105 (n=1)	-
	Negative	-	-	+	-	372 (n=1)	2541 (n=1)	-	-
		-	-	+	CITM	-	405 (n=5)	-	-
						-	457 (n=2)	-	-
	Total					22	23	6	0
K. pneumoniae	CTX-M-1	CTX-M-15	+	+	-	273 (n=2)	-	-	-
						275 (n=1)	-	-	-
			+	+	EBCM	285 (n=1)	-	-	-
		CTX-M-55	+	+	DHAM	275 (n=1)	-	-	-
		CTX-M-3	+	+	DHAM	-	-	273 (n=1)	-
	CTX-M-9	CTX-M-27	+	+	DHAM	273 (n=1)	-	-	-
		CTX-M-65	+	+	DHAM	275 (n=1)	-	-	-
	Unidentified	-	-	+	-	-	-		273 (n=1)
	Negative	-	+	+	-	273 (n=2)	-	-	-
						275 (n=4)	-	-	-
		-	+	+	CITM	273 (n=1)	-	-	-
		-	+	+	DHAM	275 (n=1)	-	-	-
	Total					15	0	1	1
K. oxytoca	CTX-M-1	CTX-M-15	-	+	-	293 (n=1)	-	-	-
			+	+	DHAM	273 (n=1)	-	-	-

	CTX-M-9	CTX-M-14	-	+	-	145 (n=1)	-	-	-
	Unidentified	-	-	+	DHAM	34 (n=1)	-	-	-
	Negative	-	+	+	-	-	-	273 (n=1)	-
	Total					4	0	1	0
S. liquefaciens	Negative	-	-	+	-	[e]ND (n=7)	-	-	-
S. marcescens	Negative	-	-	+	-	ND (n=2)	-	ND (n=1)	-
			+	+	-	ND (n=1)	-	-	-
	Total					10	0	1	0
E. cloacae	Negative	-	-	+	DHAM	198 (n=1)	-	-	-
		-	-	+	EBCM	114 (n=1)	-	-	-
						110 (n=1)	-	-	-
		-	-	+	CITM+EBCM	-	-	171 (n=1)	-
		-	-	+	DHAM+EBCM	-	-	1303 (n=1)	-
		-	-	+	-	1252 (n=1)	-	-	-
						114 (n=1)	-	-	-
	Total					5	0	2	0

[a]ESC, extended-spectrum cephalosporin. [b]ESBLs, extended-spectrum β-lactamases. [c]AmpCs, AmpC β-lactamases. [d]ST, sequence type. [e]ND, non-defined.

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국문초록

국내 반려동물에서 분리된 Extended-Spectrum β-Lactamases 및 AmpC β-Lactamases 생성 장내세균총에 대한 특성 분석

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Extended-spectrum β-lactamases (ESBLs)와 AmpC β-lactamases (AmpCs)를 부호화하는 extended-spectrum cephalosporins (ESCs) 내성 장내세균총의 출현은 의학 및 수의학 분야에서 큰 문제가 되고 있다. 이번 연구의 목표는 국내 반려동물에서 분리한 *Escherichia coli, Klebsiella* spp., *Enterobacter cloacae* 그리고 *Serratia* spp.를 포함하는 ESCs 내성 장내세균총의 분자학적 특성을 규명하는 것이다. PCR 증폭실험에 의해서 암호화된 ESCs 내성 유전자를 확인하였고 MLST 와 PFGE를 이용해서 유전적 다양성을 분석하였다. 2017년부터 2019년 까지 총 91개의 장내세균이 반려동물에서 분리되었고 모든 분리주는 bla_{TEM}에 양성이었다. 다른 ESCs 내성 유전자 중, *CTX-M-15*, *CITM* 그리고 *CTX-M-55*가 *E. coli* 에서, bla_{SHV} 그리고 *DHAM*이 *K. pneumoniae*에서 보편적인 유형이었다. 또한 *EBCM*을 포함하는 균주는 모두 *E. cloacae*였다. MLST 분석결과, *K. oxytoca*와 *E. cloacae*에서 주요 sequence type (ST)이 발견되지 않았지만 *E. coli*에서는 *CTX-M-9*를 동반하는 ST131, *CITM*을 동반하는 ST405 그리고 *CTX-M-1*을 동반하는 ST3285이 보편적이었고 *K. pneumoniae*에서는 bla_{SHV}를 동반하는 ST273과 ST275가 많은 비중을 차지했다. 분리된 모든 균주에서 57~90% 유사성을 가지는 다양한 PFGE결과를 보였으며 클론이 아닌 서로 다른 균주임을 확인하였다. 본 연구를 통해 국내 반려동물로부터 분리한 ESCs 내성 장내세균총이 광범위한 유전적 분포를 포함하고 있는 것을 확인하였다.

주요어 : 장내세균총, Extended-spectrum cephalosporins (ESCs), Extended-spectrum-β-

lactamases (ESBLs), AmpC β-lactamases (AmpCs), 항생제 내성, 반려동물, 한국

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