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Notes

Dissemination of multidrug-resistant *Escherichia coli* in Korean veterinary hospitals ☆

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ABSTRACT

This study was performed to investigate the prevalence of rectal colonization with multidrug-resistant *Escherichia coli* in dogs hospitalized at veterinary hospitals in Korea and to assess the molecular epidemiologic traits of this organism. A total of 63 unique *E. coli* isolates obtained from the rectal swabs of hospitalized dogs were analyzed. Genes encoding CTX-M extended-spectrum β-lactamases (ESBLs) and AmpC enzymes were detected in 21 (33.3%) and 15 (23.8%) canine *E. coli* isolates, respectively. Twelve canine *E. coli* isolates harbored both the genes encoding the CTX-M and AmpC enzymes. Six ESBL-producing *E. coli* isolates also carried the *rmtB* gene. All 24 *E. coli* isolates producing CTX-M ESBL and/or CMY-2 were resistant to ciprofloxacin. Furthermore, mutations were found in the *gyrA* and the *parC* genes. In most cases, the *bla* genes of the CTX-M ESBL and AmpC enzymes and the *rmtB* gene were localized to incompatibility group F (IncF) plasmids. Possible small clonal outbreaks are suggested because some *E. coli* isolates recovered in the same veterinary hospital were identified as identical sequence types and showed identical banding patterns in repetitive sequence-based polymerase chain reaction. The horizontal transfer of IncF plasmids and the clonal transfer of *E. coli* strains are suggested to play a role in the dissemination of antimicrobial resistance genes, and this transfer may occur across host species (i.e., between humans and dogs).

The dissemination of multidrug-resistant (MDR) *Escherichia coli* constitutes a major threat in infection control. Some *E. coli* isolates are expanded-spectrum β-lactam–resistant primarily because they produce plasmid-mediated extended-spectrum β-lactamases (ESBLs) and/or AmpC enzymes (Pitout, 2008). Until the early 1990s, the major hospital-acquired ESBL types were TEM and SHV enzymes, which were most frequently found in *Klebsiella pneumoniae* (Cantón and Coque, 2006). However, during the late 1990s and 2000s, community-onset *E. coli* strains resistant to expanded-spectrum β-lactams were identified in the hospital setting and have since become the most common Enterobacteriaceae producing CTX-M–type ESBLs and/or AmpC enzymes (Pitout, 2008).

Enterobacteriaceae becomes fluoroquinolone resistant as a result of the accumulation of mutations in quinolone resistance–determining regions (DNA gyrase, and topoisomerase IV), loss of outer membrane protein, overexpression of efflux pumps, and acquisition of plasmid-mediated quinolone resistance (PMQR) determinants. Many types of PMQR genes, such as *qnrB6*, *qnrB10*, *qnrB19*, *qnrS1*, *qepA*, and *aac(6′)-Ib-cr*, have also been detected in *E. coli* isolated from animals (Gibson et al., 2010; Strahilevitz et al., 2009).

In Gram-negative rods, methylation of 16S rRNA can confer high-level resistance to clinically important aminoglycosides, including amikacin (Doi and Arakawa, 2007). The 16S rRNA methylase gene *rmtA* was first identified in a clinical isolate of *Pseudomonas aeruginosa* in 2003 (Yokoyama et al., 2003). Since then, several types of transferable 16S rRNA methylase genes, including *armA, rmtA, rmtB, rmtC*, and *rmtD*, have been detected worldwide in clinical isolates of Gram-negative rods (Yamane et al., 2005). The *armA* and *rmtB* genes have also been detected in *E. coli* isolates from the rectal swabs of animals (Du et al., 2009; Liu et al., 2008).

Companion animals, such as dogs and cats, not only share a common environment with humans but are also administered drugs similar to those prescribed to humans. It is postulated that companion animals may be an integral part of resistance transfer through close and direct contact (DeVincent and Reid-Smith, 2006). Indeed, Carattoli et al. (2005b) described *E. coli* isolates harboring CMY-2, CTX-M-1, or SHV-12 enzymes recovered from sick and healthy dogs and cats in Italy. Recently, Sun et al. (2010) described the high prevalence of CTX-M-14 and CTX-M-55 ESBLs, which are the frequently encountered types of ESBL in humans, in *E. coli* isolates from pets in China. In this study, we investigated the prevalence of MDR *E. coli* isolates colonizing the rectums of dogs

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Table 1
Primers used in this study.

Name	Target gene	Sequence (5′ to 3′)	Reference
CTX-M1-F	$bla_{CTX-M-1}$ cluster	CCG TCA CGC TGT TGT TAG G	Carattoli et al., 2005
CTX-M1-R		ACG GCT TTC TGC CTT AGG TT	
CTX-M9-F	$bla_{CTX-M-9}$ cluster	CAA AGA GAG TGC AAC GGA TG	Carattoli et al., 2005
CTX-M9-R		CCT TCG GCG ATG ATT CTC	
CMY-2F	bla_{CMY-2} cluster	GCAGGCYATTCCGGGTATG	This study
CMY-2R		GCYACGTAGCTGCCAAAYCC	
rmtB-F	*rmtB*	AAC ATC AAC GAT GCC CTC AC	This study
rmtB-R		GAT GGT CTT TTT ATC CTC AAT CTC A	
gyrA-F	*gyrA*	CGA GAG AAA TTA CAC CGG TCA	This study
gyrA-R		AGC CCT TCA ATG CTG ATG TC	
gyrB-F	*gyrB*	CTG CTT TAC CAA CAA CAT TCC	This study
gyrB-R		TTG TCC GGG TTG TAC TCG TC	
parC-F	*parC*	ATG AGC GAT ATG GCA GAG C	This study
parC-R		GGT GGT TCA TCA CCT GAT CC	
parE-F	*Pare*	GCG GAA GAT ATC TGG GAT CG	This study
parE-R		CAG CAG CAT ATC CAT CAT CG	

hospitalized at veterinary hospitals in Korea and assessed the associated epidemiologic traits.

A total of 63 unique *E. coli* isolates were obtained from rectal swabs of dogs hospitalized at 3 reference veterinary hospitals (H, L, and K) in Seoul, Korea, from 2008 through 2009. The isolates were identified using a Vitek GNI card (bioMérieux, Marcy-l'Etoile, France).

Antibiotic discs (Becton Dickinson, Spark, MD, USA) were used for routine susceptibility testing on Mueller-Hinton (MH) agar (Difco, Cockeysville, MI, USA) according to the guidelines recommended by CLSI (2009). A phenotypic confirmatory test using clavulanic acid and aminophenyl boronic acid as β-lactamase inhibitors was performed for the detection of ESBL and AmpC enzyme production, as described previously (Song et al., 2007). MICs of clinically important antibiotics (cefotaxime, cefotaxime–clavulanic acid, ceftazidime, ceftazidime–clavulanic acid, cefoxitin, amikacin, and ciprofloxacin) were determined by the agar dilution method using MH agar with an inoculum of 10^4 CFU per spot (CLSI, 2009). MICs of ceftazidime and cefotaxime were determined alone or in combination with the concentration of clavulanic acid fixed at 4 mg/L. *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used as MIC reference strains.

Detection of the *bla* genes encoding the ESBLs and AmpC enzymes, the 16S rRNA methylase genes, the PMQR genes, and the genes (*gyrA* and *parC*) encoding DNA gyrase and topoisomerase IV was performed by polymerase chain reaction (PCR) amplification using the primers listed in Table 1 (Bae et al., 2011). The genomic DNA used as the template for PCR amplification of the clinical isolates was prepared using a DNA extraction kit (Qiagen, Hilden, Germany). All PCR products were sequenced twice with an automatic sequencer (model 3730*xl*; Applied Biosystems, Weiterstadt, Germany). Sequence alignment and analysis were performed using the BLAST program (http://www.ncbi.nlm.nih.gov/blast).

Conjugation experiments were performed between the donor strains and the azide-resistant recipient strain, *E. coli* J53 (Jacoby and Han, 1996), on MH agar plates. Transconjugants were selected on MH agar plates supplemented with cefotaxime (2 mg/L), cefoxitin (8 mg/L) or amikacin (30 mg/L), and sodium azide (100 mg/L). The locations of antimicrobial resistance genes were identified by hybridization of I-*Ceu*I–digested genomic DNA or S1 nuclease–treated linearized plasmids using probes specific for the antimicrobial resistance genes and 16S rRNA. The probes were prepared with the commercial DIG DNA Labeling and Detection Kit (Roche Applied Science, Mannheim, Germany). Electrophoresis was conducted using the CHEF-DRII system (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Pulsed-field gel electrophoresis (PFGE) conditions were 6 V/cm^2 for 20 h with pulse times ranging from 9 to 90 s at a temperature of 11 °C. The lambda ladder (Bio-Rad) was used as a DNA size marker.

Replicons of plasmids carrying antimicrobial resistance genes were identified by hybridization of S1 nuclease–treated linearized

Table 2
Characteristics of MDR canine *E. coli* isolates.

Isolate	Hospital	Gene encoding			Transconjugation			MIC (mg/L) of the donors							Replacement in QRDR	
		CTX-M	CMY	Methylase	CTX	FOX	AN	CTX	CTX/CA	CAZ	CAZ/CA	FOX	AN	CIP	*gyrA*	*parC*
C9SNU02	K	CTX-M-24	CMY-2		+	+	NT	>128	16	64	32	>128	2	16	Ser83Leu, Asp87Asn	Ser80Ile
C9SNU05	H		CMY-2		NT	+	NT	6	16	32	32	128	2	32	Ser83Leu, Asp87Asn	Ser80Ile
C9SNU10	H		CMY-2		NT	−	NT	32	32	64	32	>128	1	64	Ser83Leu, Asp87Try	Ser80Ile
C9SNU11	H	CTX-M-27			−	NT	NT	128	0.125	8	0.25	128	1	32	Ser83Leu, Asp87Try	Ser80Ile
C9SNU27	H	CTX-M-57	CMY-2		+	+	NT	>128	8	64	16	128	2	32	Ser83Leu, Asp87Asn	Ser80Ile
C9SNU28	H	CTX-M-14	CMY-2		+	+	NT	128	16	16	16	128	2	64	Ser83Leu, Asp87Try	Ser80Ile
C9SNU29	H	CTX-M-14	CMY-2		+	+	NT	64	32	64	32	>128	2	128	Ser83Leu, Asp87Asn	Ser80Ile
C9SNU30	H	CTX-M-14	CMY-2		+	+	NT	128	32	64	16	>128	2	64	Ser83Leu, Asp87Asn	Ser80Ile
C9SNU31	H		CMY-2		NT	+	NT	64	32	32	32	>128	2	128	Ser83Leu, Asp87Try	Ser80Ile
C9SNU32	H	CTX-M-65	CMY-2	RmtB	+	+	+	128	16	32	16	>128	>128	64	Ser83Leu, Asp87Asn	Ser80Ile
C9SNU33	H	CTX-M-57	CMY-2		+	+	NT	>128	8	64	16	128	2	32	Ser83Leu, Asp87Asn	Ser80Ile, Glu84Gly
C9SNU37	H	CTX-M-57			+	NT	NT	>128	0.25	64	0.5	32	2	128	Ser83Leu, Asp87Asn	Ser80Ile, Glu84Val
C9SNU38	H	CTX-M-14			−	NT	NT	64	0.125	1	0.125	4	2	64	Ser83Leu, Asp87Asn	Ser80Ile
C9SNU39	H	CTX-M-14	CMY-2		+	+	NT	128	32	64	32	>128	2	128	Ser83Leu, Asp87Asn	Ser80Ile
C9SNU40	H	CTX-M-14	CMY-2		+	+	NT	64	32	64	32	>128	2	128	Ser83Leu, Asp87Asn	Ser80Ile
C9SNU41	H	CTX-M-65	CMY-2	RmtB	+	+	NT	64	16	64	32	128	>128	64	Ser83Leu, Asp87Asn	Ser80Ile
C9SNU47	L	CTX-M-14		RmtB	+	NT	+	64	2	8	4	64	>128	128	Ser83Leu, Asp87Asn	Ser80Ile
C9SNU50	L	CTX-M-14	CMY-2		+	+	NT	128	8	32	32	128	4	64	Ser83Leu, Asp87Asn	Ser80Ile
C9SNU51	L	CTX-M-14		RmtB	+	NT	+	128	4	16	8	128	>128	128	Ser83Leu, Asp87Asn	Ser80Ile
C9SNU52	L	CTX-M-14		RmtB	+	NT	+	64	2	8	4	64	>128	128	Ser83Leu, Asp87Asn	Ser80Ile
C9SNU54	L	CTX-M-14		RmtB	+	NT	+	64	2	8	2	64	>128	128	Ser83Leu, Asp87Asn	Ser80Ile
C9SNU56	H	CTX-M-14			−	NT	NT	128	0.125	2	0.25	16	1	32	Ser83Leu, Asp87Asn	Ser80Ile
C9SNU57	H	CTX-M-15	CMY-2		+	−	NT	>128	8	128	32	128	2	32	Ser83Leu, Asp87Asn	Ser80Ile
C9SNU58	H	CTX-M-14			−	NT	NT	64	0.125	1	0.125	8	4	32	Ser83Leu, Asp87Asn	Ser80Ile

QRDR = Quinolone resistance–determining regions; K, H, and L = the 3 animal hospitals located in Seoul, Korea, that were included in this study; ND = not detected; + or − = β-lactam resistance determinants were transferred or not to the recipient *E. coli* J53 by conjugation; NT = not tested; CTX = cefotaxime; CAZ = ceftazidime; FOX = cefoxitin; AN = amikacin; CIP = ciprofloxacin; CA = clavulanic acid at a fixed concentration of 4 mg/L.