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Prevalence and molecular characteristics of carbapenem-resistant *Escherichia coli* isolated from dogs in South Korea

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ABSTRACT

Importance: Carbapenem-resistant *Enterobacteriaceae* are emerging as a global public health risk. Therefore, assessing the prevalence of carbapenem-resistant *Escherichia coli* (CRE) in both humans and animals is important.
Objective: We aimed to ascertain the occurrence and characteristics of CRE isolated from companion animals, dogs and cats.
Methods: *E. coli* strains were tested for antimicrobial susceptibility using the broth microdilution technique. Antimicrobial resistance genes were detected by polymerase chain reaction and sequencing analysis. The molecular characteristics of CRE were determined using multi-locus sequence typing, replicon typing, and pulsed-field gel electrophoresis (PFGE).
Results: In total, 13 CRE isolates (0.13%) were identified from dogs possessing *bla*$_{\text{NDM-5}}$ along with β-lactamase genes, mostly *bla*$_{\text{CMY-2}}$ (92.2%) and *bla*$_{\text{TEM-1}}$ (53.8%). The commonly observed mutations were S83L and D87N in *gyrA*, S80I in *parC*, and S458A in *parE*. CRE carried non-beta-lactam resistance genes, with the majority being *tet*(B) (100%), *sul* (84.6%), and *aac(3)-II* (53.8%). Nine different PFGE patterns (P1–P9), IncX3-type plasmids (69.2%), and ST410 (84.6%) were predominantly detected.
Conclusions and Relevance: This investigation provides significant insight into the prevalence and molecular characteristics of *bla*$_{\text{NDM-5}}$-carrying *E. coli* in dogs. The co-existence of *bla*$_{\text{NDM-5}}$ and other antimicrobial resistance genes in *E. coli* potentially poses severe health hazards to humans.

Keywords: *bla*$_{\text{NDM-5}}$; *Escherichia coli*; dogs; ST410; IncX3 plasmid

INTRODUCTION

Escherichia coli, belonging to the *Enterobacteriaceae* family, is widely known as a common opportunistic pathogen. It causes various infections, such as gastroenteritis, urinary tract infections, and septicemia [1]. Carbapenems, a class of β-lactam antimicrobials known for their broad-spectrum antibacterial activity, are used as a last resort in treating severe bacterial infections [2].

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Conflict of Interest
The authors declare no conflicts of interest.

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Enterobacteriaceae primarily develop carbapenem resistance by producing carbapenemase. The New Delhi metallo-β-lactamase (NDM) is the most common carbapenemase type, conferring resistance to nearly all β-lactam antimicrobials and often identified in *E. coli* [3]. Moreover, *E. coli* strains that can produce carbapenemases frequently exhibit elevated resistance to other non-β-lactam antimicrobials, significantly restricting the available treatment options [4].

Although carbapenems are not frequently used in veterinary practice, the *bla*$_{NDM-5}$ gene has been found globally in animals, including dogs and cats [3,5]. In Korea, the carbapenem-resistant gene identified as *bla*$_{NDM-5}$ in *E. coli* among companion animals was first described in 2019 [6]. Since then, several studies have reported *bla*$_{NDM-5}$-carrying *E. coli* recovered from companion animals [7-9]. Regarding the emergent threat posed by carbapenem-resistant *E. coli* (CRE), it is crucial to continue surveillance at the national level and genetic characterization of CRE strains in order to create effective strategies for preventing the hazards to both humans and animals. Thus, our aim was to ascertain the prevalence and molecular characterization of CRE isolated from companion animals nationwide between 2018 and 2022 in South Korea.

METHODS

Isolation of *E. coli*

E. coli isolates were obtained from eight laboratories/centers and diagnostic laboratories located in seven metropolitan cities and one province that participated in the Korean Veterinary Antimicrobial Resistance Monitoring System during 2018–2022. The isolation of *E. coli* from the feces of apparently healthy animals and diarrhea, skin, ear canals, urine, genitalia, and respiratory systems of hospitalized dogs and cats was performed following the methods described in our previous study [10]. Briefly, *E. coli* was isolated using selective media: Eosin Methylene Blue agar and MacConkey agar plates. Suspected *E. coli* was confirmed by matrix-assisted laser desorption and ionization-time-of-flight mass spectrometry (bioMérieux, France). We have no information on the antimicrobial use history of dogs and cats in this study.

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was conducted by the broth microdilution method using the COMPGN1F Sensititre panel (Trek Diagnostic Systems, USA). The results were interpreted according to the guidelines provided by the Clinical and Laboratory Standard Institute [11]. *E. coli* ATCC25922 was used as a quality control strain.

Detection of β-lactamase genes and mutation in quinolone-resistance determining regions (QRDRs)

Polymerase chain reaction (PCR) and sequencing analyses were performed to identify genes conferring resistance to carbapenems according to the previously described methods [12]. The presence of genes encoding QRDRs was detected by PCR amplification using specific primers for *gyrA*, *gyrB*, *parC*, and *parE*. The PCR products were sequenced using an automated ABI Prism 3700 analyzer (Applied Biosystems, USA). We used the Basic Local Alignment Search Tool to identify gene mutations in QRDRs by comparing the sequences with those available in the GenBank nucleotide database at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST). The list of primers and PCR conditions are described in **Supplementary Table 1**.

Conjugation assay and replicon typing

The filter mating assay was performed in triplicate on Luria-Bertani plates with a 1:10 donor-to-recipient ratio. Transconjugants were selected on MacConkey agar plates containing sodium azide (100 μg/mL) and meropenem (2 μg/mL). The transfer frequencies were determined based on the number of transconjugants obtained for each donor. The replicon typing was performed using PCR of extracted DNA following the previously reported method [10].

Multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) analysis

MLST was carried out to determine the clonal relationship of the carbapenem-resistant isolates [10]. A standard set of primers was used to amplify and sequence the seven housekeeping genes: *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*. The determination of sequence types (STs) for *E. coli* was conducted using web-based MLST databases at https://pubmlst.org/databases/. In addition, the genetic diversity of the isolates was evaluated using PFGE of chromosomal DNA digested with *Xba*I (Takara, Japan). The unweighted pair group approach with the arithmetic average technique based on the dice similarity index (Bionumerics software, version 4.0; Applied Maths, Belgium) was used to determine the relatedness of the isolates.

RESULTS

In total, 9,898 *E. coli* isolates were recovered from apparently healthy and hospitalized companion animals (dogs, n = 7,800 and cats, n = 2,098) nationwide in seven metropolitan cities in South Korea during 2018–2022 (**Table 1**). Among them, 13 *E. coli* strains (0.13%), all obtained from dogs in nine animal hospitals in two cities and two from diagnostic laboratories, were identified as carbapenem-resistant. Moreover, all CRE was isolated from diseased dogs except one. However, no CRE isolate was detected in cats. In the sample levels, the CRE mainly recovered from cystocentesis of urine (0.54%), respiratory system (0.49%), diarrhea (0.39%), and normal feces (0.12%), while none was recovered from the genital organ and skin/ear. Regarding the age of the host, CRE was highly detected in about 69% (9/13) of seniors (6–10 years) and geriatric dogs (> 11 years).

All 13 CRE isolates possess *bla*$_{NDM-5}$ along with other β-lactamase genes, including *bla*$_{Oxa-1}$, *bla*$_{TEM-1}$, *bla*$_{CMY-2}$, and/or *bla*$_{CTX-M-65}$. Among them, the majority (38.5%, 5/13) of the isolates contained *bla*$_{CMY-2}$, and one isolate (7.7%) possessed *bla*$_{CTX-M-65}$. The presence of *bla*$_{CMY-2}$, *bla*$_{Oxa-1}$, *bla*$_{TEM-1}$, and *bla*$_{CTX-M-65}$ genes was found in one isolate.

In this study, all of the CRE also showed resistance to non-beta-lactam antimicrobials such as aminoglycosides, fluoroquinolones, phenicols, tetracyclines, and folate pathway inhibitors,

Table 1. Prevalence of carbapenem-resistant *Escherichia coli* isolated from healthy and hospitalized dogs and cats in South Korea from 2018 to 2022

Source	Prevalence % (No. of resistance/No. of isolate)		
	Dogs	Cats	Total
Normal feces	0.12 (1/862)	0 (0/326)	0.08 (1/1,188)
Diarrhea	0.39 (6/1,723)	0 (0/577)	0.26 (6/2,300)
Skin/ear	0 (0/3,401)	0 (0/498)	0 (0/3,899)
Urine (cystocentesis)	0.54 (4/738)	0 (0/144)	0.45 (4/882)
Genital organ	0 (0/666)	0 (0/92)	0 (0/758)
Respiratory system	0.49 (2/410)	0 (0/461)	0.23 (2/871)
Total	0.17 (13/7,800)	0 (0/2,098)	0.13 (13/9,898)

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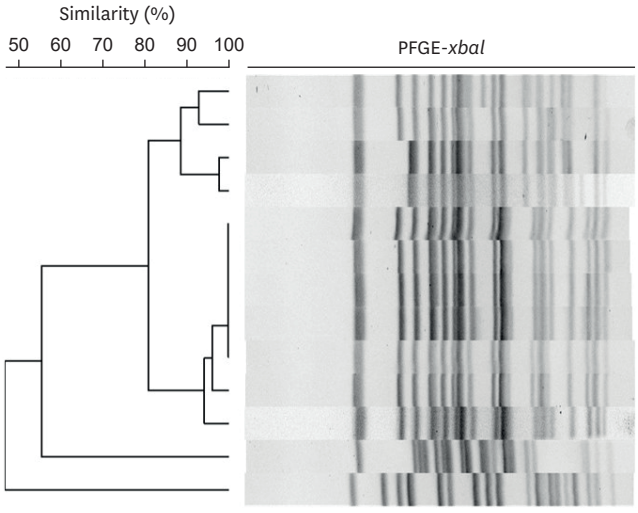
which are commonly used in companion animals. Moreover, all CRE isolates demonstrated resistance to fluoroquinolones (enrofloxacin, minimum inhibitory concentration [MIC] ≥ 4 μg/mL) except one. The sequencing analysis revealed that all isolates possessed more than one mutation in the QRDRs. The commonly observed mutations in the CRE isolates were S83L and D87N in *gyrA*, S80I in *parC*, and S458A in *parE*. The CRE isolates carried non-beta-lactam resistance genes, with the majority being *tet*(B) (13 isolates) and *sul* (11 isolates). In addition, three aminoglycoside resistance genes were detected, and the *aac(3)-II* (7 isolates) gene was most frequently identified, followed by *aac(3)-IV* (1 isolate) and *aph(3')-Ia* (1 isolate).

A total of nine *Xba*I-PFGE patterns (P1–P9) and three ST types (ST410, ST156, and ST70) were observed in the CRE isolates (**Table 2**, **Fig. 1**). PFGE could be differentiated from the same ST types. By combining the two methods, 9 different patterns were observed. Among them, the P5-ST410 pattern was detected in five isolates (38.5%) obtained from four hospitals (B, C, D, and F) in α in 2019 and 2021.

The conjugation assay showed that *bla*$_{NDM-5}$ was transferred to the recipient *E. coli* J53 by filter mating of 69.2% (9/13) isolates. Moreover, the replicon type IncX3 (69.2%, 9/13) was predominantly detected in the transconjugants. Additionally, non-β-lactam antimicrobial resistance, tetracycline, and trimethoprim/sulfamethoxazole were transferred along with the IncX3 plasmid.

DISCUSSION

A total of 13 *E. coli* isolates (0.13%) demonstrated resistance to carbapenem. Consistent with our study, relatively low CRE isolates have been identified in dogs in the UK (0.6%) [12]. In Korea, CRE was also detected to a lesser extent in *E. coli* isolates in dogs (0.6%) [8]. However, it was less than in previous reports in Algeria (2.6%) [13]. Although CRE was reported in rectal swabs, ear swabs, and urine samples of hospitalized dogs, it was identified in the normal feces of healthy dogs for the first time in this study in Korea. Thus, attention should be given to both diseased and healthy animals regarding antimicrobial resistance monitoring.



Isolates	Animal ID	Year	Hospital	Non-β-lactam antimicrobial resistance profile	Pulsotype	ST
21P11013	D12	2021	I	GEN ENR SXT TET	P8	156
21U11017	D10	2021	H	GEN ENR SXT TET	P2	410
21U11013	D9	2021	H	GEN ENR SXT TET	P3	410
21U11021	D11	2021	H	GEN ENR SXT TET	P4	410
19E11035	D2	2019	B	ENR SXT TET	P5	410
19E11038	D3	2019	C	ENR SXT TET	P5	410
19E11039	D4	2019	C	GEN ENR SXT TET	P5	410
20U11004	D5	2020	D	GEN ENR SXT TET	P5	410
21E11028	D8	2021	F	GEN AMK ENR TET	P5	410
21E11011	D7	2021	F	GEN AMK ENR TET	P6	410
19E11034	D1	2019	A	GEN ENR SXT TET	P7	410
21P11008	D13	2021	J	GEN ENR SXT TET	P1	410
20A11006	D6	2020	E	SXT TET	P9	70

Fig. 1. PFGE patterns of carbapenem-resistant *Escherichia coli* isolated from healthy and hospitalized dogs in South Korea during from 2018 to 2022.
PFGE, pulsed-field gel electrophoresis; ST, sequence type; GEN, gentamicin; ENR, enrofloxacin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; AMK, amikacin.

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Table 2. Characteristics of carbapenem-resistant *Escherichia coli* isolated from healthy and hospitalized dogs in South Korea from 2018 to 2022

Isolates	Animal ID	Sample	Year	Hospital	Age	City	MIC (μg/mL)			β-lactam resistance gene	Non-β-lactam antimicrobial resistance profile	Non-β-lactam antimicrobial resistance gene	Mutation of QRDR			Genotype		Conjugation efficacy	Replicon type
							IMI	MPN	ENR				*gyr*A	*par*C	*par*E	PFGE	ST		
19E11034	D1	Diarrhea	2019	A	13 yr	α	> 8	> 4	> 4	NDM-5, CMY-2, TEM-1	GEN ENR SXT TET	*aac(3)-II*, *sul*, *tet*(B)	S83L, D87N	S80I	S458A	P7	410	1.4×10^{-4}	IncX3
19E11035	D2	Diarrhea	2019	B	12 yr	α	8	> 4	> 4	NDM-5, CMY-2	ENR SXT TET	*sul*, *tet*(B)	S83L, D87N	S80I	S458A	P5	410	1.2×10^{-4}	IncX3
19E11038	D3	Diarrhea	2019	C	4 yr	α	8	> 4	> 4	NDM-5, CMY-2	ENR SXT TET	*sul*, *tet*(B)	S83L, D87N	S80I	S458A	P5	410	2.2×10^{-4}	IncX3
19E11039	D4	Diarrhea	2019	C	2 yr	α	8	> 4	> 4	NDM-5, CMY-2, TEM-1	GEN ENR SXT TET	*aac(3)-II*, *sul*, *tet*(B)	S83L, D87N	S80I	S458A	P5	410	9.0×10^{-3}	IncX3
20U11004	D5	Urine	2020	D	17 yr	α	> 8	> 4	4	NDM-5, CMY-2	GEN ENR SXT TET	*aph(3')-Ia*, *sul*, *tet*(B)	S83L, D87N	S80I	S458A	P5	410	2.6×10^{-4}	IncX3
20A11006	D6	Normal feces	2020	E	15 yr	Δ	8	> 4	1	NDM-5, CMY-2	SXT TET[a]	*sul*, *tet*(B)	ND	ND	ND	P9	70	3.0×10^{-4}	IncX3
21E11011	D7	Diarrhea	2021	F	6 yr	α	8	> 4	> 4	NDM-5, CMY-2, TEM-1, CTX-M-65	GEN AMK ENR TET	*aac*(3)-II, *tet*(B)	S83L, D87N	S80I	S458A	P6	410	1.2×10^{-4}	IncX3
21E11028	D8	Diarrhea	2021	F	11 yr	α	> 8	> 4	> 4	NDM-5, CMY-2, TEM-1, CTX-M-65	GEN AMK ENR TET	*aac(3)-II*, *tet*(B)	S83L, D87N	S80I	S458A	P5	410	6.7×10^{-3}	IncX3
21U11013	D9	Urine	2021	H	8 yr	α	4	> 4	> 4	NDM-5, CMY-2, OXA-1, TEM-1	GEN ENR SXT TET	*aac(3)-II*, *sul*, *tet*(B)	S83L, D87N	S80I	S458A	P3	410	Not transferred	NT
21U11017	D10	Urine	2021	H	11 yr	α	> 8	> 4	> 4	NDM-5, CMY-2, OXA-1, TEM-1	GEN ENR SXT TET	*aac(3)-II*, *sul*, *tet*(B)	S83L, D87N	S80I	S458A	P2	410	Not transferred	NT
21U11021	D11	Urine	2021	H	8 yr	α	8	> 4	> 4	NDM-5, CMY-2	GEN ENR SXT TET	*sul*, *tet*(B)	S83L, D87N	S80I	S458A	P4	410	Not transferred	NT
21P11013	D12	Lung	2021	I	2 mon	α	4	> 4	> 4	NDM-5, CMY-2, OXA-1, TEM-1, CTX-M-65	GEN ENR SXT TET	*aac(3)-II*, *sul*, *tet*(B)	S83L, D87Y	S80I	S458A	P8	156	1.4×10^{-4}	IncX3
21P11008	D13	Lung	2021	J	2 mon	α	8	> 4	> 4	NDM-5, CTX-M-65	GEN ENR SXT TET	*aac(3)-IV*, *sul*, *tet*(B)	S83L, D87N	S80I	S458A	P1	410	Not transferred	NT

MIC, minimum inhibitory concentration; IMP, imipenem; MPN, meropenem; ENR, enrofloxacin; AMK, amikacin; GEN, gentamicin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline; QRDR, quinolone-resistance determining region; PFGE, pulsed-field gel electrophoresis; ST, sequence type; ND, not detected; NT, not tested.
Underline and superscript lowercase letter 'a' mean transferred resistance.

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In our previous study, the prevalence of antimicrobial resistance differed by age group in dogs with frequently detected CRE in the geriatric group (> 11 years) [2]. Similarly, CRE was highly detected in about 69% (9/13) of seniors (6–10 years) and geriatric dogs (> 11 years) in this study. The higher incidence of antimicrobial resistance in older animals can be linked to their extended exposure to antimicrobials throughout their lifetimes [10].

In this study, all 13 CRE possess the *bla*$_{NDM-5}$ gene. Previous studies showed that CRE carrying *bla*$_{NDM-5}$ has been highly detected in dogs in Korea (100%) [6] and the USA (83.3%) [3]. Moreover, several outbreaks of *bla*$_{NDM-5}$-harboring *E. coli* infections in humans occurred globally [14,15]. Other β-lactamase genes, such as *bla*$_{Oxa-1}$, *bla*$_{TEM-1}$, *bla*$_{CMY-2}$, and/or *bla*$_{CTX-M-65}$, are present in the *bla*$_{NDM-5}$-carrying CRE. The *E. coli* strains co-harboring *bla*$_{NDM-5}$ and extended-spectrum/AmpC β-lactamase genes were found in companion animals globally. It was observed that *E. coli* obtained from dogs in Korea [6,7] and Switzerland [5] could simultaneously produce *bla*$_{NDM-5}$ and AmpC enzymes, especially *bla*$_{CMY-2}$.

In our investigation, the CRE isolates also showed resistance to non-beta-lactam antimicrobials such as aminoglycosides, fluoroquinolones, phenicols, tetracyclines, and folate pathway inhibitors, which are commonly used in companion animals. These findings are consistent with other investigations conducted in Korea [6,8,14] and China [4]. It's interesting to note that all but one of the CRE isolates showed fluoroquinolone resistance. Moreover, the isolates possessed several mutations in QRDRs, including S83L and D87N in *gyrA*, S80I in *parC*, and S458A in *parE* was associated with resistance to enrofloxacin (MIC ≥ 4 μg/mL). High levels of fluoroquinolone resistance have been found to be connected with mutations in *gyrA*, *parC*, and *parE* [16]. The CRE isolates carried non-beta-lactam resistance genes, such as *tet*(B), and *sul*, *aac(3)-II*, *aac(3)-IV* and *aph(3′)-Ia*. The *tet*(B) gene was commonly identified among the tetracycline-resistant *E. coli* isolates, and *sul* was frequently detected in the sulfonamide-resistant determinant in *E. coli* isolated from companion animals [17]. The resistance genes *aac(3)-II*, *aac(3)-IV*, and *aph(3′)-Ia* frequently detected in *E. coli* isolated from dogs trigger aminoglycoside resistance [18].

We found nine *Xba*I-PFGE patterns (P1–P9) and three STs (ST410, ST156, and ST70) in the CRE isolates. In combination, the P5-ST410 pattern was mainly detected (38.5%) distributed in four hospitals in α. This predominant presence might be due to epidemic clones in a city or clonal dissemination. Previous studies showed that CRE ST410 has been identified in companion animals in different countries [1,12], including Korea [9]. However, ST70 was first reported in CRE strains in dogs in this study. The ST70, a new emerging ST, was identified in carbapenem-resistant *Enterobacteriaceae* in a hospital in China [19]. Moreover, CRE ST156 has also been detected in fecal samples from outpatient children [20].

In this investigation, we found that 69.2% of CRE isolates were transferred to the recipients by conjugation, and the replicon type IncX3 (69.2%, 9/13) was the most commonly detected in the transconjugants. The IncX3-type plasmids, which have a wide range of compatible hosts, are frequently associated with the uptake and dissemination of antimicrobial-resistant genes [21]. Recent outbreaks of the IncX3 plasmid-carrying and *bla*$_{NDM-5}$-producing *E. coli* in hospitals were reported from China, the United Arab Emirates, and the Czech Republic [22].

In conclusion, our study demonstrated that all CRE produce *bla*$_{NDM-5}$, which is responsible for the main mechanisms of carbapenem resistance. Moreover, the CRE isolates exhibit resistance to non-β-lactam antimicrobials and contain their resistance genes. In addition, the

CRE strains possess important mutations in the QRDRs. The majority of the isolates were identified as ST410, contributing to the clonal dissemination of *bla*$_{NDM-5}$-harboring *E. coli*. Moreover, the *bla*$_{NDM-5}$ genes are located on the conjugative plasmids, and the plasmid IncX3 was most prevalent, playing a crucial role in the horizontal transfer of *bla*$_{NDM-5}$. Thus, this investigation indicates that companion animal dogs can act as a reservoir of carbapenem-resistant genes, which can potentially be spread to humans.

SUPPLEMENTARY MATERIAL

Supplementary Table 1

List of primer sequences and PCR conditions

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