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Prevalence and antimicrobial resistance of *Klebsiella* species isolated from clinically ill companion animals

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Conflict of Interest
The authors declare no conflict of interest.

ABSTRACT

Background: *Klebsiella* spp. is an important conditional pathogen in humans and animals. However, due to the indiscriminate use of antibiotics, the incidence of antimicrobial resistance has increased.
Objectives: The purpose of this study was to investigate antimicrobial resistance in strains of *Klebsiella* strains and the phylogenetic relatedness of extended-spectrum cephalosporin (ESC)-resistance among *Klebsiella* strains isolated from clinically ill companion animals.
Methods: A total of 336 clinical specimens were collected from animal hospitals. Identification of *Klebsiella* species, determination of minimum inhibitory concentrations, detection of ESC resistance genes, polymerase chain reaction-based replicon typing of plasmids by conjugation, and multilocus sequence typing were performed.
Results: Forty-three *Klebsiella* strains were isolated and, subsequently, 28 were identified as *K. pneumoniae*, 11 as *K. oxytoca*, and 4 as *K. aerogenes*. Eleven strains were isolated from feces, followed by 10 from ear, 7 from the nasal cavity, 6 from urine, 5 from genitals, and 4 from skin. *Klebsiella* isolates showed more than 40% resistance to penicillin, cephalosporin, fluoroquinolone, and aminoglycoside. ESCresistance genes, CTX-M groups (CTX-M-3, CTX-M-15, and CTX-M-65), and AmpC (CMY-2 and DHA-1) were most common in the *K. pneumoniae* strains. Some *K. pneumoniae* carrying CTX-M or AmpC were transferred via IncFII plasmids. Two sequence types, ST709 and ST307, from *K. pneumoniae* were most common.
Conclusions: In conclusion, this is the first report on the prevalence, ESCresistance genotypes, and sequence types of *Klebsiella* strains isolated from clinically ill companion animals. The combination of infectious diseases and antimicrobial resistance by *Klebsiella* in companion animals suggest that, in clinical veterinary, antibiotic selection should be made carefully and in conjunction with the disease diagnosis.

Keywords: Companion animals; *Klebsiella* species; extended-spectrum cephalosporins; multilocus sequence type (MLST)

INTRODUCTION

Klebsiella spp. is the second most common member of the *Enterobacteriaceae* and is present on the mucosal surfaces of mammals, such as humans and dogs, as well as in water,

Author Contributions

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food, and soil environments [1]. They cause severe hospital-acquired or community-onset bacterial infections of the cardiovascular, respiratory, gastrointestinal, pancreatic, renal, and coagulation systems in humans and animals [2-5]. The β-lactam drugs are the most commonly prescribed and widely used antimicrobial class for treating bacterial infections caused by *Enterobacteriaceae*, including *Klebsiella* spp. [6,7]. However, as a result of the indiscriminate use of those antibiotics, the emergence of antimicrobial resistance in *Klebsiella*-producing broad spectrum β-lactamases, such as the extended-spectrum β-lactamases (ESBL) and AmpC β-lactamases, is threatening the future of the application of β-lactam drugs in both humans and animals [6,7]. Antimicrobial resistance increases the risk of antimicrobial treatment failure in humans and animals. In addition, the emergence of antibiotic-resistant bacteria in companion animals may affect human public health if such bacteria are transmitted to humans [8]. Therefore, identification of antibiotic susceptibilities and genetic characteristics of ESBL and AmpC β-lactamases-producing *Klebsiella* spp. has an important role in the treatment of pathogenic infections. Although there have been several studies on infection, antibiotic resistance, and the possibility of transmission of *Klebsiella* spp. in animals in Germany, Italy, France, Spain, Switzerland, China, and Taiwan [9-16], similar studies of clinically ill companion animals have been insufficient in South Korea. This study aimed to investigate the prevalence, antimicrobial resistance mechanisms, and phylogenetic relatedness of *Klebsiella* strains isolated from clinically ill companion animals.

MATERIALS AND METHODS

Bacterial isolation and identification

Between May and October 2019, 336 clinical samples were collected from clinically ill dogs (n = 277) and cats (n = 59) that had not been prescribed antibiotics at animal hospitals in Seoul, with each sample obtained after obtaining owner's prior consent for the use of the samples. Samples were collected via sterile swabs from the ear canal, nasal cavity, urine, skin, genitalia, feces, ascites, pericardial effusion, or blood. To isolate *Klebsiella* spp., clinical sample swabs were suspended in 2 mL of Mueller Hinton Broth (Difco, USA) and inoculated onto MacConkey agar (Difco) with disposable sterile loops (SPL Co., Korea). The viscous red colonies were re-inoculated on MacConkey agar (Difco), and single cultured colonies were further identified as *Klebsiella pneumoniae*, *Klebsiella oxytoca*, or *Klebsiella aerogenes* by using a Matrix-Assisted Laser Desorption Ionization-Time-of-Flight (MALDI-TOF) mass Spectrometer (VITEK MS; bioMérieux, France).

Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) were determined by broth microdilution using Sensititre ESBL 96-well plates (ESB1F; Trek Diagnostic Systems, United Kingdom); The MIC determination for *Klebsiella* strains followed the Clinical and Laboratory Standards Institute (CLSI) guideline [17]. The following antibiotics were used: ampicillin, cefazolin, cephalothin, cefoxitin, cefpodoxime, ceftriaxone, cefotaxime, ceftazidime, cefepime, cefotaxime/clavulanic acid, ceftazidime/clavulanic acid, imipenem, meropenem, ciprofloxacin, and gentamicin. *Escherichia coli* ATCC 25922 was used as a quality-control strain.

Detection of extended-spectrum cephalosporin (ESC) resistance genes

ESC resistance genes, $bla_{\text{CTX-M}}$, bla_{SHV}, bla_{TEM}, bla_{CMY}, and bla_{DHA}, from the 21 *Klebsiella* strains resistant (≥ 64 ug/mL) to cefotaxime or cefoxitin were detected by performing multiplex polymerase chain reaction (PCR) and sequencing analysis [18,19]. To evaluate the genotype

of ESC, amplified PCR products were sequenced and subsequently analyzed using a BLAST search engine (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Conjugation and genotyping of ESC-resistant *Klebsiella* strains

Conjugation was conducted by broth mating to confirm the transmissibility of plasmids carrying ESC resistance genes from the 21 *Klebsiella* strains harboring ESBLs and AmpC β-lactamase genes as donors to a recipient *E. coli* J53 resistant to sodium-azide [20]. The conjugation culture broth was streaked on Mueller Hinton medium and MacConkey medium containing cefotaxime (30 μg/mL) or cefoxitin (30 μg/mL) and sodium-azide (200 μg/mL), and the transconjugants were tested for antimicrobial susceptibility and genotyping. The following commercial antibiotic disks (Oxoid, United Kingdom) were used: amoxicillin-clavulanic acid (AMC, 30/10 μg); ampicillin (AMP, 10 μg); amikacin (AMK, 30 μg); azithromycin (AZM, 15 μg); ampicillin-sulbactam (SAM, 10/10 μg); cefazolin (FAZ, 30 μg); cefaclor (CEC, 30 μg); cefixime (CFM, 30 μg); cefoxitin (FOX, 30 μg); cefotaxime (FOT, 30 μg); ceftriaxone (AXO, 30 μg); cephalothin (CEP, 30 μg); ceftazidime (TAZ, 30 μg); cefepime (FEP, 30 μg); cefpodoxime (POD, 10 μg); norfloxacin (NOR, 10 μg); ciprofloxacin (CIP, 5 μg); clindamycin (CLN 10 μg); doxycycline (DOX, 30 μg); tobramycin (TOB, 10 μg); gentamicin (GEN, 10 μg); imipenem (IMI, 30 μg); polymyxin B (PB, 300 μg); and trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 μg).

PCR-based plasmid replicon typing

PCR-based plasmid replicon typing (PBRT) was performed to evaluate incompatibility (Inc) groups and replicase (rep) genes of plasmids harboring ESC resistance gene from *Klebsiella* strains and transconjugants by using a PBRT kit (Diatheva, Italy) in accordance with the manufacturer's protocol [21].

MLST and phylogenic analysis of ESC-resistant *Klebsiella* strains

Sequence types (STs) were determined by multilocus sequence typing (MLST) analysis involving the amplification of seven housekeeping genes. PCR amplification was performed with 0.5 μL of DNA templates, 4 μL of Hifi Super premix (ELPIS Co., Korea), and 10 pmol of each primer [22]. The analyzed nucleotide sequences were compared with the nucleotide sequences of allele types for each gene downloaded from the *Klebsiella* spp. MLST database (http://bigsdb.web.pasteur.fr) to determine the allelic type of each isolate. The sequence type for each isolate was confirmed by searching the existing database for the identified allele type profile.

Statistical analysis

The data are presented as a number or percentage of the distribution of the values. Fisher's exact test and Pearson's χ^2 test were applied to compare antimicrobial susceptibilities among *Klebsiella* species. All statistical analyses were done using SPSS 20.0 software (IBM SPSS, Inc., USA). The results were considered statistically significant if p values were less than 0.05.

RESULTS

Prevalence of Klebsiella species

A total of 43 *Klebsiella* species (12.8%) within 336 clinical samples of companion dogs (42/277, 15.2%) and cats (1/59, 1.7%) were identified by MALDI-TOF MS. Of the total isolates, *K. pneumoniae* accounted for 8.3% (n = 28), *K. oxytoca* for 3.3% (n = 11), and *K. aerogenes* for 1.2% (n = 4) (**Table 1**). *Klebsiella* strains were most common from feces (11/43, 25.6%), followed by

Table 1. Prevalence of *Klebsiella* strains isolated from the clinical specimens of companion dogs and cats (n = 336)

Clinical samples	No.	Diagnosis	No. (%) of *Klebsiella* isolates by specimens			
			KPN	KOX	KAE	Total
Feces	35	Enteritis	10 (3.0)	1 (0.3)	0 (0.0)	11 (3.3)
Ear canal	112	Otitis externa	7 (2.1)	1 (0.3)	2 (0.6)	10 (3.0)
Nasal cavit	42	Pneumonia, Bronchitis	5 (1.5)	1 (0.3)	1 (0.3)	7 (2.1)
Urine	79	Cystitis, Urolithiasis	2 (0.6)	4 (1.2)	0 (0.0)	6 (1.8)
Genitalia	24	Pyometra, Endometriosis	2 (0.6)	2 (0.6)	1 (0.3)	5 (1.5)
Skin	26	Dermatitis	2 (0.6)	2 (0.6)	0 (0.0)	4 (1.2)
Others*	18	Trauma, Sepsis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No. (%) of isolates			28 (8.3)	11 (3.3)	4 (1.2)	43 (12.8)

KPN, *Klebsiella pneumoniae*; KOX, *Klebsiella oxytoca*; KAE, *Klebsiella aerogenes*.
*Others include ascites, pericardial effusion, and blood.

10/43 (23.3%) from the ear canal, 7/43 (16.3%) from the nasal cavity, 6/43 (14.0%) from urine, 5/43 (11.6%) from genitalia, and 4/43 (9.3%) from skin.

Antimicrobial resistance of *Klebsiella* species

Antimicrobial resistance to three *Klebsiella* species is summarized in **Table 2** and **Fig. 1**. All strains showed more than 40% resistance to nine antibiotics from four antimicrobial classes, including penicillins, cephems, fluoroquinolone, and aminoglycoside. The resistance rate of the *K. pneumoniae* strains (n = 28) to the antimicrobials tested was as follows; ampicillin 85.7%, cefazolin 57.1%, cephalothin 64.3%, cefoxitin 42.9%, cefpodoxime 53.6%, ceftriaxone 50.0%, cefotaxime 50.0%, ceftazidime 39.3%, cefepime 10.7%, cefotaxime/clavulanic acid 35.7%, ceftazidime/clavulanic acid 32.1%, imipenem 3.6%, meropenem 0.0%, ciprofloxacin 53.6%, and gentamicin 50.0% (**Table 2**, **Fig. 2**). The resistance rates of *K. oxytoca* (n = 11) to ampicillin were 81.8%, cefazolin 54.5%, cephalothin 54.5%, cefoxitin 27.3%, cefpodoxime 27.3%, ceftriaxone 18.2%, cefotaxime 18.2%, ceftazidime 18.2%, cefepime 0.0%, cefotaxime/clavulanic acid 0.0%, ceftazidime/clavulanic acid 27.3%, imipenem 0.0%, meropenem 0.0%, ciprofloxacin 36.4%, and gentamicin 36.4%. All *K. aerogenes* isolates (n = 4) were resistant to ampicillin, cefazolin, cephalothin, and cefoxitin. The resistance rate to cefpodoxime, ceftriaxone, cefotaxime, cefotaxime/clavulanic acid, and ciprofloxacin was 75% and to ceftazidime and ceftazidime/clavulanic acid was 50%. In addition, the resistance rate to imipenem and gentamicin was 25.0%. Of all tested antibiotics, the resistance rate to

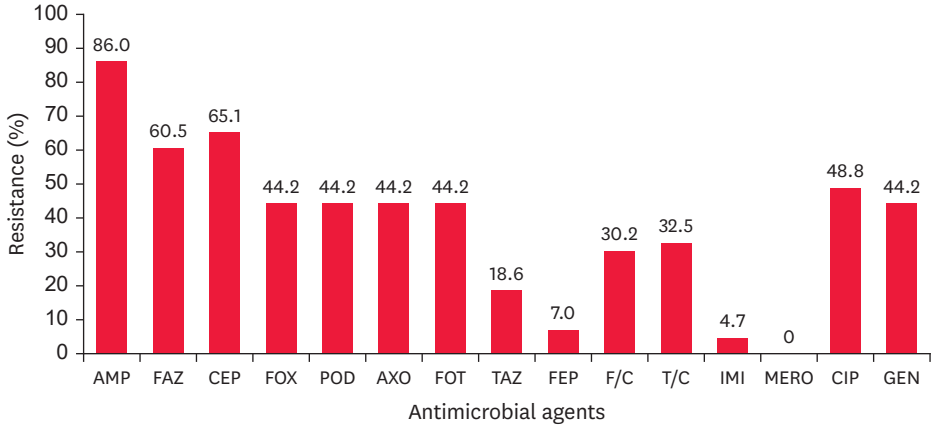

Fig. 1. Comparative antibiotic resistance patterns of *Klebsiella* strains isolated from clinically ill companion animals. AMP, ampicillin; FAZ, cefazolin; CEP, cephalothin; FOX, cefoxitin; POD, cefpodoxime; AXO, ceftriaxone; FOT, cefotaxime; TAZ, ceftazidime; FEP, cefepime; F/C, cefotaxime/clavulanic acid; T/C, ceftazidime/clavulanic acid; IMI, imipenem; MERO, meropenem; CIP, ciprofloxacin; GEN, gentamicin.

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Table 2. Minimum inhibitory concentrations and resistance rates of *Klebsiella* species isolated from clinically ill companion animals

Antibiotics	Species	No. of strains	MIC (μg/mL)*										No. (%) of resistant
			< 0.25	0.5	1	2	4	8	16	32	64	128	
AMP	KPN	28						4		24			24 (85.7)
	KOX	11						2		9			9 (81.8)
	KAE	4								4			4 (100.0)
bFAZ	KPN	28						12		16			16 (57.1)
	KOX	11						5		6			6 (54.5)
	KAE	4								4			4 (100.0)
CEP	KPN	28						10	18				18 (64.3)
	KOX	11						5	6				6 (54.5)
	KAE	4							4				4 (100.0)
FOX	KPN	28					15		1	1	11		12 (42.9)
	KOX	11					6	2			3		3 (27.3)
	KAE	4									4		4 (100.0)
POD	KPN	28	8	1	3	1	1	1		14			15 (53.6)
	KOX	11	7				1	1		2			3 (27.3)
	KAE	4			1					3			3 (75.0)
AXO	KPN	28			14		1	1		2		10	14 (50.0)
	KOX	11			9			1			1		2 (18.2)
	KAE	4			1					2	1		3 (75.0)
FOT	KPN	28	12	1	1				3	1	10		14 (50.0)
	KOX	11	8	1			1			1			2 (18.2)
	KAE	4	1							3			3 (75.0)
TAZ	KPN	28	10	1	1	1		4	3	2	2	4	11 (39.3)
	KOX	11	5	2		1		1	1	1			2 (18.2)
	KAE	4		1		1					1	1	2 (50.0)
FEP	KPN	28			16	2	4	3	3				3 (10.7)
	KOX	11			10			1					0 (0.0)
	KAE	4			1	2	1						0 (0.0)
F/C	KPN	28	17	3	1	1	3	1	3	1	2		10 (35.7)
	KOX	11	10			2							0 (0.0)
	KAE	4	1					1		1	1		3 (75.0)
T/C	KPN	28	10	6	1		1	1	1	1	2	5	9 (32.1)
	KOX	11	7	1					1	2			3 (27.3)
	KAE	4		1			1			1	1		2 (50.0)
IMI	KPN	28		18	8	1	1						1 (3.6)
	KOX	11		10	1								0 (0.0)
	KAE	4		1	2				1				1 (25.0)
MEM	KPN	28			18								0 (0.0)
	KOX	11			11								0 (0.0)
	KAE	4			4								0 (0.0)
CIP	KPN	28			13		15						15 (53.6)
	KOX	11			6	1	4						4 (36.4)
	KAE	4			1		3						3 (75.0)
GEN	KPN	28			1		12	1	14				14 (50.0)
	KOX	11			1		6		4				4 (36.4)
	KAE	4					3		1				1 (25.0)

AMP, ampicillin; FAZ, cefazolin; CEP, cephalothin; FOX, cefoxitin; POD, cefpodoxime; AXO, ceftriaxone; FOT, cefotaxime; TAZ, ceftazidime; FEP, cefepime; F/C, cefotaxime/clavulanic acid; T/C, ceftazidime/clavulanic acid; CIP, ciprofloxacin; GEN, gentamicin; IMI, imipenem; MEM, meropenem; KPN, *Klebsiella pneumoniae*; KOX, *Klebsiella oxytoca*; KAE, *Klebsiella aerogenes*.
*Vertical line indicates the breakpoint for each drug, according to the 2018 Clinical and Laboratory Standards Institute guideline.

cefoxitin and cefotaxime/clavulanic acid of the *Klebsiella* spp. were statistically significant (p = 0.041 and p = 0.008, respectively; **Fig. 2**).

Detection of ESC resistance genes

All subtypes of resistance genes detected in *Klebsiella* strains are shown in **Table 3**. Resistance genes associated with ESC were detected in *K. pneumoniae* and *K. oxytoca*. Genotyping patterns differed in the *K. pneumoniae* strains. Together with TEM type-1, SHV was classified into eight

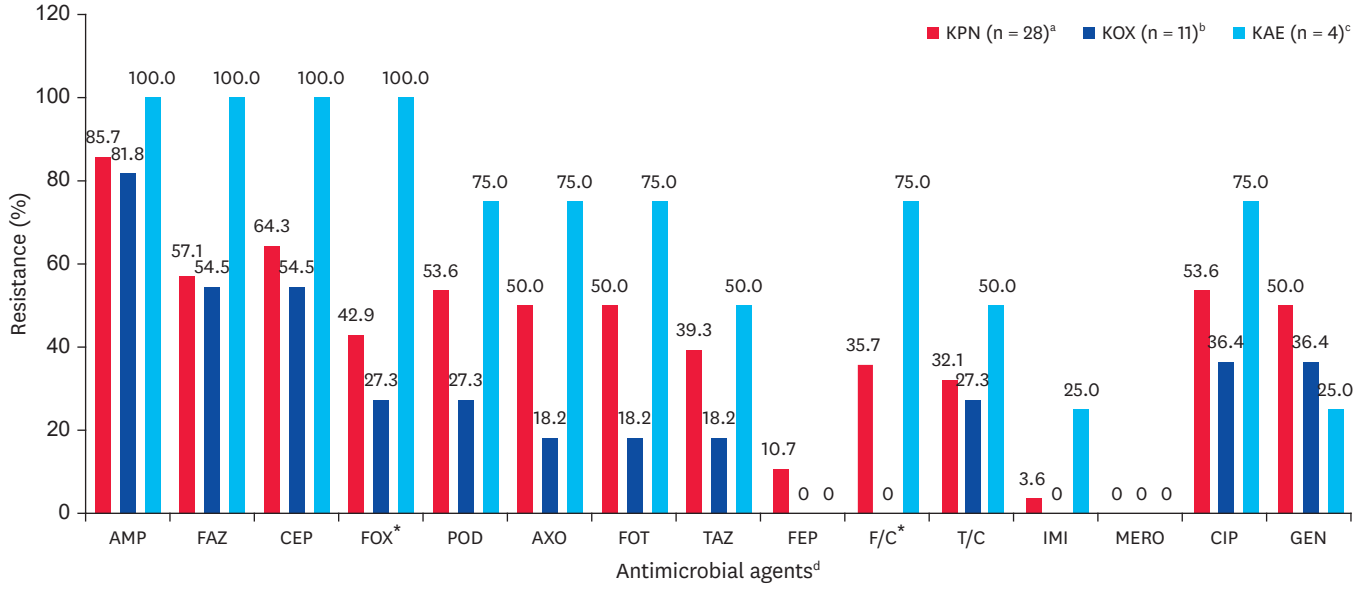

Fig. 2. Antimicrobial resistance patterns of *Klebsiella* species from clinically ill companion animals.
KPN, *Klebsiella pneumoniae*; KOX, *Klebsiella oxytoca*; KAE, *Klebsiella aerogenes*; AMP, ampicillin; FAZ, cefazolin; CEP, cephalothin; FOX, cefoxitin; POD, cefpodoxime; AXO, ceftriaxone; FOT, cefotaxime; TAZ, ceftazidime; FEP, cefepime; F/C, cefotaxime/clavulanic acid; T/C, ceftazidime/clavulanic acid; IMI, imipenem; MEM, meropenem; CIP, ciprofloxacin; GEN, gentamicin.
*Indicates the difference is statistically significant ($p < 0.05$).

different subtypes (SHV-1, -11, -12, -25, 26, -28, -79, and -148). Sixteen *K. pneumoniae* strains carried the ESBL or AmpC gene with TEM or SHV subtypes. The CTX-M and AmpC subtypes detected in 16 *K. pneumoniae* strains were classified into 16 genotype patterns (**Table 3**). Of these, CTX-M-15 patterns were the most frequent (n = 6, 14.0%), followed by DHA-1 patterns (n = 4, 8.3%), CMY-2 patterns (n = 2, 4.2%), and CTX-M-3, -9, and -65 genotype patterns (n = 1, 2.1%). Twelve genotype patterns with SHV alone or with TEM-1 were identified. On the other hand, five *K. oxytoca* strains were classified into three genotype patterns DHA-1, DHA1/TEM-1, and CTX-M-15/TEM-1. No resistance gene was detected in *K. aerogenes* strains.

Characterization of the transconjugants and PCR-based replicon typing

Four (9.3%) of the 43 *Klebsiella* spp. strains were transferred to a recipient strain, *E. coli* J53. The transconjugants were resistant to multiple antibiotics, including penicillins, cephalosporins, lincosamides, tetracyclines, aminoglycosides, co-trimoxazole, and β-lactam/β-lactamase inhibitor combinations; resistance to which was also shown in the donor strains. **Table 4** shows the resistance pattern and antibiotic resistance genes detected in each isolate. Four transconjugants contained different ESC resistance genes, CTX-M-3, -15, -65, and CMY-2. The PBRT results showed that those resistance genes were carried via the IncFII plasmid (FII and FIIK). The IncFII-type plasmid carrying CMY-2 from the *K. pneumoniae* LK278 strain was identified as plasmid-mediated AmpC β-lactamase (PABL).

Sequence type of *Klebsiella* spp.

Distribution and allelic profiles of STs and resistance genes of each *Klebsiella* strains are summarized in **Tables 5** and **6**. Twenty-two STs were identified in *K. pneumonia* strains. The ST307 (4/22, 18.2%) and ST709 (4/22, 18.2%) clones were dominant, followed by ST1114 (2/22, 9.1%) (**Table 5**). Several ESC resistance genes, CTX-M-15/-65, DHA-1, and TEM-1, appeared in those isolates (**Table 6**). Other STs, ST17 (1/22, 4.5%), ST39 (1/22, 4.5%), ST202 (1/22, 4.5%), ST378 (1/22, 4.5%), ST392 (1/22, 4.5%), ST655 (1/22, 4.5%), ST1530 (1/22,

Table 3. Distribution of extended-spectrum cephalosporine resistance genes from *Klebsiella. pneumoniae* and *Klebsiella oxytoca* strains

ESBL and AmpC genes	No. (%) of *Klebsiella* strains	
	K. pneumoniae (n = 28)	*K. oxytoca* (n = 11)
CMY-2/SHV-79	1 (3.5)	0 (0.0)
CMY-2/SHV-148/TEM-1	1 (3.5)	0 (0.0)
DHA-1	0 (0.0)	2 (18.1)
DHA-1/SHV-12/TEM-1	1 (3.5)	0 (0.0)
DHA-1/SHV-26/TEM-1	1 (3.5)	0 (0.0)
DHA-1/SHV-28/TEM-1	1 (3.5)	0 (0.0)
DHA-1/TEM-1	1 (3.5)	2 (18.1)
CTX-M-3/SHV-1/TEM-1	1 (3.5)	0 (0.0)
CTX-M-9/DHA-1/SHV-1/TEM-1	1 (3.5)	0 (0.0)
CTX-M-14/DHA-1	1 (3.5)	0 (0.0)
CTX-M-15/SHV-11	1 (3.5)	0 (0.0)
CTX-M-15/TEM-1	0 (0.0)	1 (9.1)
CTX-M-15/DHA-1	1 (3.5)	0 (0.0)
CTX-M-15/SHV-1/TEM-1	1 (3.5)	0 (0.0)
CTX-M-15/SHV-11/TEM-1	1 (3.5)	0 (0.0)
CTX-M-15/SHV-28/TEM-1	1 (3.5)	0 (0.0)
CTX-M-15/CTX-M-65/DHA-1/SHV-26/TEM-1	1 (3.5)	0 (0.0)
CTX-M-65/DHA-1/SHV-26/TEM-1	1 (3.5)	0 (0.0)
SHV-1	3 (10.7)	0 (0.0)
SHV-11	3 (10.7)	0 (0.0)
SHV-25	1 (3.5)	0 (0.0)
TEM-1	0 (0.0)	3 (27.2)
SHV-1/ TEM-1	2 (7.1)	0 (0.0)
SHV-11/ TEM-1	2 (7.1)	0 (0.0)
SHV-28/TEM-1	1 (3.5)	0 (0.0)

ESBL, extended-spectrum β-lactamases.

Table 4. Resistance pattern, antibiotic resistance genes, and replicon type of wild strain and their transconjugants isolated from clinically ill companion animals of *Klebsiella pneumoniae*

No.	Wild			Transconjugant		
	Resistance pattern	Beta-lactamase	Replicon type	Resistance pattern	Beta-lactamase	Replicon type
LK044	AMC-AMP-AMK-SAM-FAZ-CEC-CFM-FOT-AXO-TAZ-NOR-CIP-CLN-DOX-TOB-GEN-STX	CTX-M-15	FIIK, FIB	AMP-FAZ-CEC-CFM-FOT-AXO-CLNDOX	CTX-M-15	FIIK
LK318	AMC-AMP-AZM-SAM-FAZ-CEC-CFM-FOT-AXO-FOX-TAZ-NOR-CIP-CLN-DOX-GEN-STX	CTX-M-3, SHV-1, TEM-1	FIIK, R	AMP-AZM-SAM-FAZ-CEC-FOT-CLN-STX	CTX-M-3	FIIK
LK334	AMC-AMP-AMK-AZM-SAM-FAZ-CEC-CFM-FOT-AXO-FOX-NOR-CIP-CLN-DOX-TOB-GEN-STX	CTX-M-15, -65, DHA-1	FIIK, FII	AMP-AMK-SAM-FAZ-CEC-FOT-TOB-GEN	CTX-M-65	FIIK
LK278	AMC-AMP-SAM-FAZ-CEC-CFM-FOT-AXO-FOX-TAZ-CLN-DOX-STX	CMY-2, TEM-1, SHV-148	FIA, FII, R	AMC-AMP-SAM-FAZ-CEC-CFM-FOT-AXO-FOX-TAZ-CLN-DOX	CMY-2	FII

AMC, amoxicillin-clavulanic acid; AMP, ampicillin; AMK, amikacin; AZM, azithromycin; SAM, ampicillin-sulbactam; FAZ, cefazolin; CEC, cefaclor; CFM, cefixime; FOX, cefoxitin; FOT, cefotaxime; AXO, ceftriaxone; CEP, cephalothin; TAZ, ceftazidime; FEP, cefepime; POD, cefpodoxime; NOR, norfloxacin; CIP, ciprofloxacin; CLN, clindamycin; DOX, doxycycline; TOB, tobramycin; GEN, gentamicin; IMI, imipenem; PB, polymyxin B; STX, trimethoprim-sulfamethoxazole.

4.5%), ST2459 (1/22, 4.5%) and ST3833 (1/22, 4.5%), were also identified (**Table 5**). In *K. oxytoca*, eight STs were identified, with two ST clones, ST88 (2/8, 25.0%) and ST145 (2/8, 25.0%) being the most frequent. Furthermore, ST108 (1/8, 12.5%), ST2 (1/8, 12.5%), ST95 (1/8, 12.5%), ST34 (1/8, 12.5%) and ST108 (1/8, 12.5%) were also identified (**Table 5**). The eBURST analyses confirmed that STs of *K. pneumoniae* and *K. oxytoca* strains belong to human-related sequence types, based on the MLST database. In *K. pneumoniae,* between ST709 and ST655, a single locus variant suggestive of a close clonal relationship was identified (**Fig. 3**).

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Table 5. Distribution of sequence types of *Klebsiella* strains isolated from clinically ill companion animals

Sequence type	Allelic profile	*Klebsiella* species		
		KPN (n = 28)	KOX (n = 11)	KAE (n = 4)
307	4-1-2-52-1-1-7	4 (14.2)	-	-
709	1-1-1-1-1-1-4	4 (14.2)	-	-
1114	4-3-2-1-10-4-17	2 (7.1)	-	-
17	2-1-1-1-4-4-4	1 (3.6)	-	-
39	2-1-2-4-9-1-14	1 (3.6)	-	-
202	2-1-15-1-18-4-70	1 (3.6)	-	-
378	2-1-1-1-7-4-65	1 (3.6)	-	-
392	3-4-6-1-7-4-40	1 (3.6)	-	-
655	1-1-1-1-1-1-23	1 (3.6)	-	-
1530	1-1-1-3-27-1-39	1 (3.6)	-	-
2459	2-1-5-1-9-4-13	1 (3.6)	-	-
3833	4-18-5-1-26-8-13	1 (3.6)	-	-
88	3-8-24-33-20-6-23	-	2 (18.2)	-
145	1-3-2-34-16-34-1	-	2 (18.2)	-
2	1-2-2-1-2-1-2	-	1 (9.1)	-
34	2-2-2-17-2-1-2	-	1 (9.1)	-
95	3-24-15-4-43-6-4	-	1 (9.1)	-
108	3-5-21-20-24-6-30	-	1 (9.1)	-
ND	-	9 (32.1)	3 (27.3)	4 (100)

KPN, *Klebsiella pneumoniae*; KOX, *Klebsiella oxytoca*; KAE, *Klebsiella aerogenes*; ND, not determinded.

Table 6. Sequence types and resistance genes in *Klebsiella pneumoniae* and *Klebsiella oxytoca* isolated from clinically ill companion animals

Klebsiella species (No. of isolates)	Sequence type (No. of isolates)	Total (n = 30)	β-lacatmase type		
			AmpC (n = 9)	CTX-M (n = 9)	TEM/SHV
KPN (22)	307 (4)	1	-	CTX-M-15	TEM-1+SHV-1
		1	-	CTX-M-3	TEM-1+SHV-1
		1	-	CTX-M-15	TEM-1+SHV-28
		1	-	-	TEM-1+SHV-18
	709 (4)	1	DHA-1	CTX-M-65	TEM-1+SHV-26
		1	DHA-1	-	TEM-1+SHV-26
		1	DHA-1	CTX-M-15+65	TEM-1+SHV-26
		1	DHA-1	-	TEM-1
	1114 (2)	1	-	CTX-M-15	SHV-11
		1	-	CTX-M-15	TEM-1+SHV-11
	378 (1)	1	DHA-1	CTX-M-14	-
	655 (1)	1	DHA-1	-	TEM-1+SHV-28
	2459 (1)	1	CMY-2	-	TEM-1+SHV-148
	Others (9)	9	-	-	-
KOX (8)	2 (1)	1	-	CTX-M-15	TEM-1
	88 (2)	1	-	-	TEM-1
		1	-	-	TEM-1
	145 (2)	1	DHA-1	-	-
		1	DHA-1	-	TEM-1
	Others (3)	3	-	-	-

KPN, *Klebsiella pneumoniae*; KOX, *Klebsiella oxytoca*.

DISCUSSION

This is the first report describing the distribution rate of *Klebsiella* species and the associated antimicrobial resistance mechanisms in companion animals with clinical symptoms in South Korea. The study provides the distribution rate of *Klebsiella*s spp. isolated from various lesions of companion animals, as well as the antibiotic susceptibility patterns, and extended-spectrum cephalosporin resistance among them. Of the 3 *Klebsiella* species, *K. pneumoniae* and *K. oxytoca* appeared in almost all companion animals' lesions. In particular, *K. pneumoniae* was highly distributed in gastrointestinal diseases, otitis, and respiratory diseases. On the other

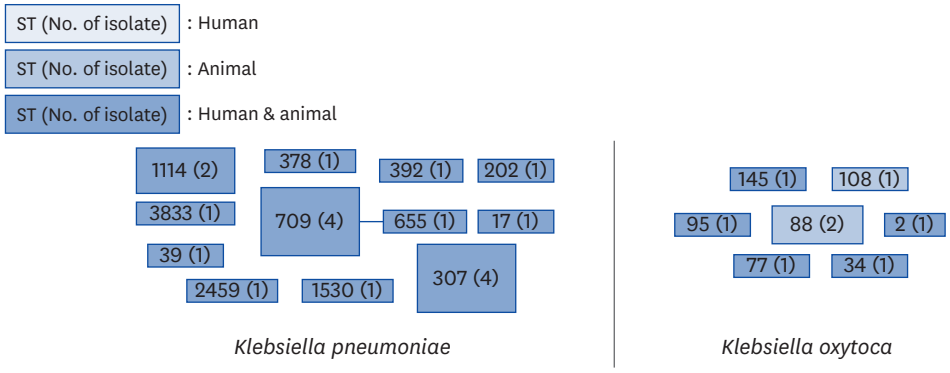

Fig. 3. Population structure obtained via eBURST analysis for ESCresistance *Klebsiella pneumoniae* and *Klebsiella oxytoca* against the entire MLST database.
Clusters of related STs from this study are displayed as a single eBURST diagram. Clusters of linked isolates, as single locus variants, correspond to clonal complexes.
ESC, extended-spectrum cephalosporin; ST, sequence type; MLST, multilocus sequence typing.

hand, *K. oxytoca* was high in the urogenital system. The distribution of *Klebsiella* for each lesion type was highest in the feces of diarrhea, followed by ear canal, nasal cavity, urine, genitalia, and skin. However, these results are somewhat different from those presented in other reports [23], in which only urine and wound areas were common sources of isolates. These results suggest that *Klebsiella* infection is not limited to only local sites but also occurs in various lesion sites in companion animals. In addition, among the clinical isolates collected, *K. pneumoniae* strains (65.1%, 28/43) were commoner than those of Japan (34.8%, 31/89), Italy (21.4%, 15/70), Germany, and other European countries (7.6%, 84/1,112) [10,23,24]. It was observed that *K. pneumoniae* infection was relatively higher in companion animals in South Korea than in those in other countries. These data suggest that the risk of ESBL carriage is relatively high in *K. pneumoniae* clinical isolates from companion animals in South Korea.

In the present study, all *Klebsiella* strains showed high resistances (over 40%) against the β-lactams, including third-generation cephalosporin (3GC) antibiotics, fluoroquinolone, and aminoglycoside. Of the 3 species, *K. pneumoniae* strains, which are more than 50% resistant to third-generation cephalosporin antibiotics, were shown to have a high association with the presence of genes conferring resistance to ESCs. In addition, one case of resistance to imipenem was detected in *K. pneumoniae* and *K. aerogenes*. Fortunately, the NDM-1 gene (New Delhi metallo-β-lactamase-1), which is a carbapenemase-producing carbapenem-resistant Enterobacteriaceae (CP-CRE) that causes four times higher mortality than non-CP-CRE species [25], was not detected in those isolates.

Recently, studies related to antibiotic resistance in companion animals living with humans and based on the One Health concept have been reported in *Enterobacteriaceae* strains, such as *E. coli* and *K. pneumoniae*. In addition, *E. coli* and *K. pneumoniae* strains carrying CTX-M-1, -9 groups, CMY, and DHA genes from feces of healthy dogs have been reported in South Korea [26]. In particular, the CTX-M-15 and CMY-2 genes were most frequently detected in *E. coli*, whereas the DHA-1 gene was commonly distributed along with CTX-M-14, -15, -55 in *K. pneumoniae* strains with ESC resistance in healthy companion animals. In China, 44.1% (n = 15) out of the 34 *K. pneumoniae* strains isolated from ill dogs were CTX-M types (CTX-M-1 group and CTX-M-9 group), while 14.7% (n = 5) were AmpC types (DHA-1) in 2017 [15]. In Japan, 47.6% (n = 10) of 21 *K. pneumoniae* isolated from pet urine samples in 2018 were reported as CTX-M types (CTX-M-2, -14, and -15) [27]. CTX-M-15 was reported in 56.3% (n

= 9) of 16 *K. pneumoniae* isolated from ill dogs in Portugal in 2019 [28]. However, in a 2018 Canadian report, 84.6% (n = 11) was high in CMY in 13 *K. pneumoniae* isolated from dog feces, whereas CTX-M was low in 15.4% (n = 2) [29]. In summary, 35.7% (n = 10) of 28 *K. pneumoniae* strains in this study were observed to carry various CTX-M types, and 5 (21.4%) were CTX-M/DHA-1 types. AmpC (DHA-1 and CMY-2) was also detected in 21.4% of *K. pneumoniae* strains (n = 6). Unlike reports from other countries, ESC-resistant *K. pneumoniae* strains carrying CTX-M/AmpC were observed in this study, with AmpC (DHA-1) being more prevalent than the CTX-M type in *K. oxytoca* strains.

In addition, IncFIIK plasmids carrying CTX-M-3, -15, and -65 in the three transformants shown in this study were also reported in *K. pneumoniae* isolated from companion animals in Italy [10]. On the other hand, studies in Tunisia and Norway reported that the IncFIIK plasmid carrying CTX-M-15 was present in *K. pneumoniae* isolated from hospital- and community-acquired human infections [30,31]. An IncFII carrying CMY-2 was first identified in this study, although it has been reported in humans [32] but not detected in dogs and cats in other countries. As a result, it was confirmed that plasmids carrying ESC resistance genes that are commonly detected in humans were well distributed in companion animals.

Recently, CTX-M-15 producing *K. pneumoniae* ST11 and ST15 have emerged in human patients and are being further disseminated [23,33,34]; however, there was no previous report describing a *K. pneumoniae* isolate carrying CTX-M-15 from clinically ill dogs and cats in South Korea. Moreover, our identification of the ST for *K. oxytoca* is the first in both domestic and foreign studies. In this study, CTX-M-15 was essentially associated with the ST15, ST307, and ST392 clones in *K. pneumoniae*, which have been frequently detected in South Korea, Japan, and Italy. [23,24,26,35]. Especially, ST307, a CTX-15-producing *K. pneumoniae* that was predominant in this study, is also frequently reported from humans in South Korea, suggesting wider dissemination in our country than in other settings [36,37].

In conclusion, this is the first study to identify the prevalence, antimicrobial resistance mechanisms, and molecular genotypes of strains of *Klebsiella* from clinically ill dogs and cats in South Korea. As ESC-resistant *Klebsiella* genotypes, common in humans, have been isolated from companion animals, epidemiological studies may be needed to determine whether these bacteria are shared between humans and animals.

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