



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

NAAS Rating (2025): 4.61

VET 2025; 10(6): 132-135

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www.veterinarypaper.com

Received: 16-04-2025

Accepted: 19-05-2025

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Prevalence of extended-spectrum beta-lactamase producing enterobacteriaceae in companion animal clinical specimens

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DOI: <https://www.doi.org/10.22271/veterinary.2025.v10.i6b.3460>

Abstract

Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae in companion animals pose a growing concern for veterinary therapeutics and public health. This research determined the prevalence, species distribution, and genotypic profiles of ESBL producers from clinical specimens of companion animals in Busan, South Korea, between March 2022 and February 2024. Of 648 non-duplicate specimens screened on cefotaxime-supplemented MacConkey agar, 142 (21.9%) yielded ESBL producers. Dogs accounted for 61.3% of positive isolates, followed by cats (28.9%). *Escherichia coli* was predominant (68.3%), followed by *Klebsiella pneumoniae* (18.3%). blaCTX-M was the most common gene family (82.4%), with CTX-M-15 and CTX-M-14 predominating. Multi-gene isolates had significantly higher cefotaxime MICs (geometric mean 78.4 vs. 12.3 µg/mL; $p < 0.001$). These findings confirm a substantial ESBL burden in companion animals in South Korea.

Keywords: ESBL, Enterobacteriaceae, companion animals, antimicrobial resistance, CTX-M, canine, feline, South Korea

Introduction

While antimicrobial resistance surveillance in food-producing animals has advanced considerably, companion animals have received comparatively little attention despite their close contact with human household members^[1]. ESBL enzymes hydrolyse oxanion cephalosporins and their genes are carried on mobile genetic elements capable of horizontal transfer between species^[2, 3]. In South Korea, companion animal ownership has risen sharply, with 6.4 million dogs registered in 2023. Veterinary antibiotic prescribing creates selective pressure favouring resistant organisms^[4]. Reports from Seoul documented ESBL prevalence of 15-25% in canine urinary isolates, but data from other regions are scarce^[5, 6]. This research aimed to determine the ESBL prevalence, species distribution, and gene profiles in companion animal specimens from the Busan metropolitan area^[7].

Material and methods

Reagents and chemicals

MacConkey agar supplemented with cefotaxime (2 µg/mL) was prepared using MacConkey agar base (BD Difco) and cefotaxime sodium (Sigma-Aldrich). PCR reagents included GoTaq Green Master Mix (Promega) and published primer sets targeting blaCTX-M, blaTEM, and blaSHV^[3, 8].

Instrumentation and analytical equipment

Species identification used MALDI-TOF MS (Bruker MALDI Biotyper). MICs were determined by broth microdilution on Sensititre plates (Thermo Fisher Scientific). PCR amplicons were confirmed by Sanger sequencing on an ABI 3500 Genetic Analyzer^[5, 8].

Material

A total of 648 non-duplicate clinical specimens (281 urine, 187 wound swabs, 112 ear swabs, and 68 blood cultures) were collected from 424 dogs, 163 cats, 38 rabbits, and 23 other small mammals at four veterinary referral hospitals in Busan. All procedures were approved by the Ethics committee (Protocol BIVM-2022-014)^[4].

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Quality control

ATCC reference strains *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were included in every batch. Reproducibility was 98.6% for phenotype and 100% for genotype [3, 7].

Methods

Specimens were inoculated onto cefotaxime-supplemented MacConkey agar (37 °C, 18-24 h). ESBL was confirmed by

DDST per CLSI 2023 guidelines. All confirmed producers underwent MALDI-TOF MS, broth microdilution MIC determination, and multiplex PCR. Statistical analyses used chi-square, Mann-Whitney U, and Kruskal-Wallis tests in R v4.3.2 [8, 9].

Results

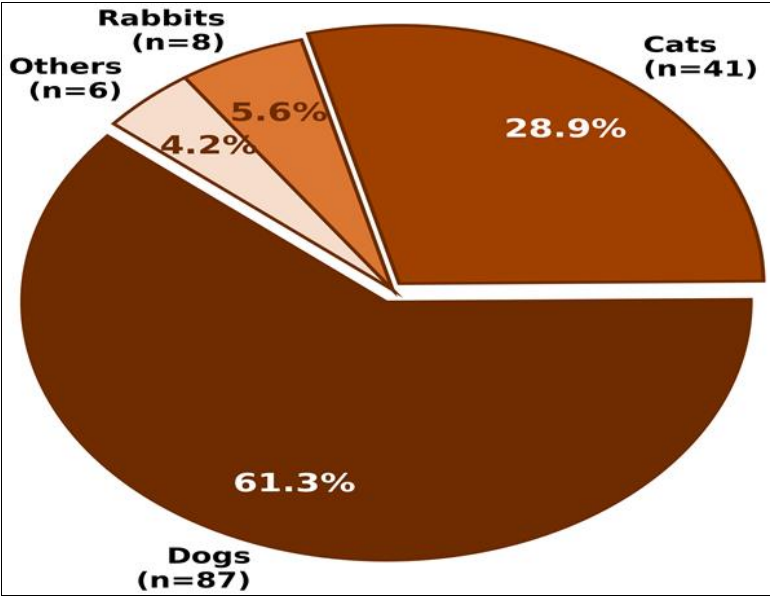


Fig 1: Host species distribution of 142 ESBL-producing isolates. Dogs 61.3%, cats 28.9%, rabbits 5.6%, others 4.2%. Of 648 specimens, 142 (21.9%) yielded ESBL producers. Prevalence was highest in urine (28.1%) and lowest in blood cultures (8.8%). *Escherichia coli* was the most frequent species (97/142, 68.3%), followed by *Klebsiella pneumoniae* (26/142, 18.3%), *Proteus mirabilis* (11/142, 7.7%), and *Enterobacter cloacae* (8/142, 5.6%)

Table 1: Ranking of ESBL gene families by prevalence

Gene family	Positive (n)	Prevalence (%)	Rank
blaCTX-M	117	82.4	1
blaTEM	63	44.4	2
blaSHV	29	20.4	3
≥ 2 families	48	33.8	-

Percentages exceed 100% because 33.8% carried genes from two or more families.

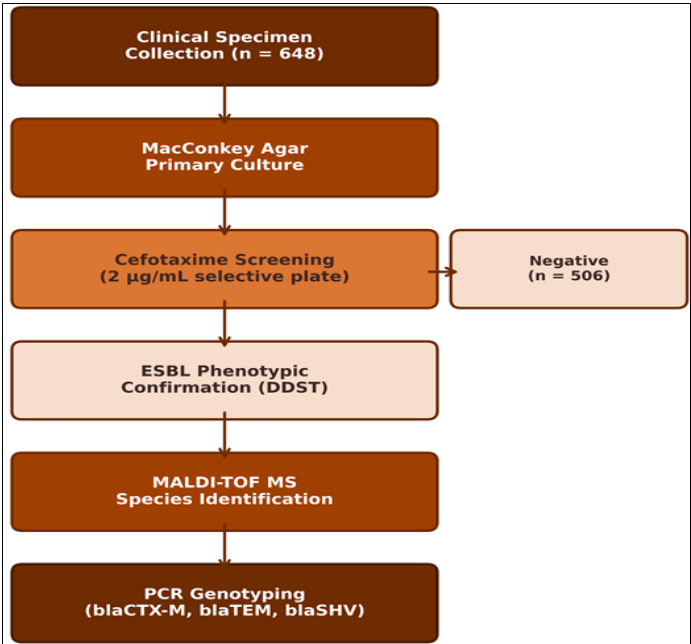


Fig 2: Flowchart of the specimen processing and ESBL identification pipeline from primary culture through multiplex PCR genotyping. Among CTX-M-positive isolates, CTX-M-15 was the most common variant (54/117, 46.2%), followed by CTX-M-14 (38/117, 32.5%). Co-resistance to fluoroquinolones was observed in 58.5% of ESBL producers, and 12.7% exhibited carbapenem non-susceptibility

Table 2: Antimicrobial susceptibility: ESBL-producing vs. non-ESBL isolates

Antimicrobial	ESBL+ (%)	ESBL- (%)	p value
Amoxicillin-clavulanate	43.0	91.3	< 0.001
Cefotaxime	0.0	94.7	< 0.001
Ciprofloxacin	41.5	87.2	< 0.001
Gentamicin	62.0	93.5	< 0.001
Meropenem	87.3	99.6	< 0.001
Nitrofurantoin	78.2	95.1	< 0.001

ESBL+ n=142; ESBL- n=506. Per CLSI 2023 breakpoints

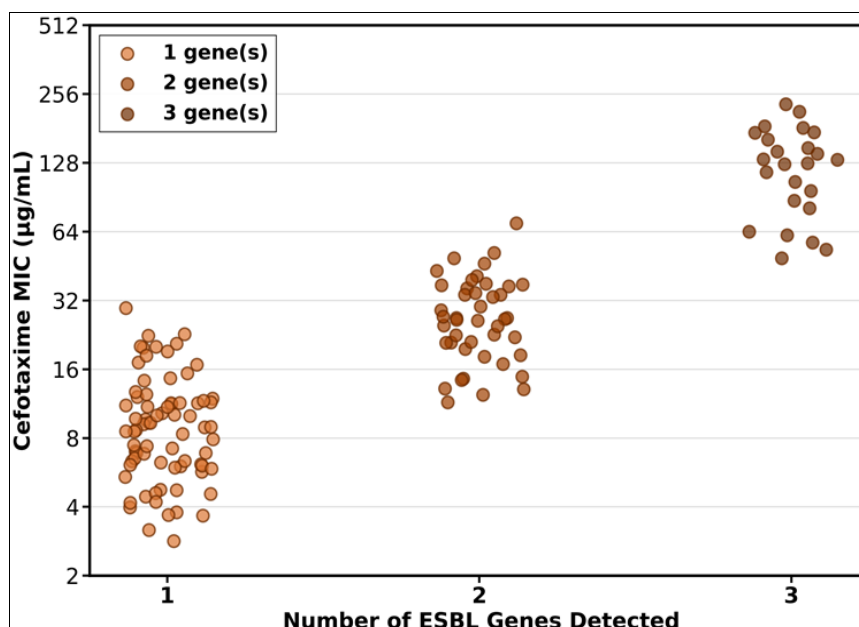


Fig 3: Relationship between ESBL gene count and cefotaxime MIC among 142 isolates. Multi-gene carriers had significantly higher MICs ($p < 0.001$)

Comprehensive interpretation

The species distribution, CTX-M preponderance, and high co-resistance confirm multidrug-resistant Enterobacteriaceae are established in Busan companion animals. Cumulative gene carriage correlates with stepwise MIC increases [6, 10].

Discussion

The 21.9% ESBL prevalence is consistent with 15-25% from Seoul and higher than 8-12% in European surveys [5, 11]. CTX-M-15 dominance mirrors global human ESBL epidemiology, suggesting zoonotic exchange supported by whole-genome sequencing identifying clonal *E. coli* ST131 in dogs and owners [12, 13].

Veterinarians should consider culture and susceptibility testing before prescribing cephalosporins. The 12.7% carbapenem non-susceptibility warrants monitoring, and 58.5% fluoroquinolone co-resistance underscores the need for stewardship [14, 15, 16].

Conclusion

This research found 21.9% ESBL prevalence among 648 companion animal clinical specimens in Busan, with *E. coli* carrying blaCTX-M-15 as the dominant genotype. One-third harbored multiple gene families with correspondingly higher MICs. Empiric cephalosporin therapy can no longer be assumed effective without susceptibility data. Culture-guided prescribing and antimicrobial stewardship should be prioritized. Future work should extend to community-acquired infections and incorporate whole-genome sequencing [17, 18, 19, 20].

Acknowledgements

Funding Sources

Supported by a grant from the Korean Foundation for Veterinary Antimicrobial Resistance Surveillance (KVARs-2022-008).

Institutional Support

The authors thank the Busan Institute of Veterinary Microbiology for laboratory infrastructure.

Contributions Not Qualifying for Authorship

The authors are grateful to Ms. Choi Min-Ji for specimen cataloguing.

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How to Cite This Article

Lee DW. Prevalence of extended-spectrum beta-lactamase producing enterobacteriaceae in companion animal clinical specimens. *International Journal of Veterinary Sciences and Animal Husbandry.* 2025; 10(6): 132-135.

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