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Original Article

Identification of Extended – Spectrum β - Lactamases and Metallo - Beta-Lactamases Producing *Escherichia Coli* and *Klebsiella Pneumoniae* Strains Isolated from Stool Samples of Dogs and Cats

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Abstract

Background and Aim: Bacterial infections are the most frequently occurring infections in pets. *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) have been recognized as two opportunistic pathogens that are prevalent in pets. The aforementioned organisms play a vital role in the development and propagation of infections that affect the urinary tract, respiratory system, and gastrointestinal tract. The growing emergence of multidrug resistance among the bacteria is a global concern. The investigation of antibiotic resistance and genotypic characterization of Extended- spectrum β-lactamases (ESBLs) and Metallo - β - lactamase (MBL) - producing *Enterobacteriaceae* (CPE) in companion animals in Iran has been infrequently documented. The aim of this study was to identify the phenotypic and genotypic characterization of ESBL and MBL - producing *Escherichia coli* and *Klebsiella pneumoniae* strains that were isolated from stool samples of dogs and cats.

Methods: A total of 65 stool samples of dogs and cats were collected from five veterinary hospitals between February to August 2022. The antimicrobial susceptibility test (AST) was evaluated by using disk diffusion according to The Clinical and Laboratory Standards Institute guidelines (CLSI). The detection of ESBLs and MBLs producing isolates was performed by Combination Disk Diffusion Test (CDDT). The presence of bla_{CTXM}, bla_{TEM}, bla_{SHV}, bla_{NDM}, bla_{VIM}, bla_{IMP} genes was detected by PCR technique.

Results: Among 65 samples, 24 *E. coli* and 6 *K. pneumoniae* strains were identified. According to our findings, the most effective antibiotics against bacterial isolates were piperacillin - tazobactam imipenem and meropenem, respectively. The prevalence of ESBL and MBL was found to be 66.66% and 0%, respectively. The PCR assay revealed the presence of $bla_{\text{CTXM-15}}$, bla_{TEM}, bla_{SHV}, bla_{IMP} genes 28, 28, 18, 1 number, respectively. Whereas bla_{NDM}, and bla_{VIM} genes were not detected.

Conclusion: The increasing prevalence of antibiotic resistance genes is a significant concern in the field of medicine. The excessive utilization of antibiotics may lead to the acquisition of genes that contribute to resistance.

Keywords: *Klebsiella pneumonia; Escherichia coli*; Antibiotic Resistance; ESBLs; MBLs; Dogs; Cats.

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Introduction

The global emergence of antimicrobial resistance (AMR) has arisen as a significant apprehension, posing a threat to the public healthcare system. Owing to their extensive exposure to humans and administration of broad - spectrum antimicrobial agents, dogs, and cats have been predominantly recognized as potential reservoirs of AMR. Enterobacteriaceae are naturally found in the intestinal tracts of mammals, moreover, they have the potential to cause a variety of infections in dogs and cats, including those affecting the skin, urinary tract, soft tissue, ears, and respiratory system (1). *E. coli and K. pneumoniae*, two significant members of Enterobacteriaceae, possess the capability to develop

resistance against various classes of antibiotics. The identification of antibiotic resistance determinants at the genomic level holds a crucial role in comprehending and potentially managing the proliferation of multi-drug resistant (MDR) pathogens. The propagation of MDR strains of Enterobacteriaceae, gram - negative opportunistic pathogens and a prevailing source of nosocomial infections, are of particular apprehension (2). Cephalosporins represent a frequently prescribed class of antibiotics for the management of Enterobacteriaceae infections. Nevertheless, the emergence of antibiotic resistance, which may be attributed to the production of AmpC type β - lactamase or ESBLs by Enterobacteriaceae, has resulted in inadequate therapeutic outcomes in the management of infectious diseases caused by this bacterial species (3). In recent years, the prevalence of CTX-M enzymes in clinical gram - negative isolates, particularly in ESBL - producing Enterobacteriaceae, has surpassed that of all other ESBL enzymes. Conversely, ESBL enzymes (TEM and SHV types) are predominantly detected in *E. coli* and ESBL - producing *K. pneumoniae* clinical isolates (4). Carbapenem - hydrolyzing β - lactamases belonging to Ambler classes A, B, and D have been detected worldwide in gram - negative bacterial populations. Among these types, NDM, VIM, and IMP variants are the most clinically relevant, with *K. pneumoniae* serving as a primary source of nosocomial outbreaks, many of which are multidrug - resistant (5). Most reported ESBL and MBL - producing bacteria have been isolated from humans in Iran. In a pioneering effort, we have succeeded in recognizing ESBL - and MBL - generating *Enterobacteriaceae* gathered from animals. As such, the aims of our study were focused on identifying the phenotypic and genotypic properties of ESBL and MBL in *Escherichia coli* and *Klebsiella pneumoniae* strains that were obtained from Stool Samples of dogs and cats during the year 2022.

Methods

Bacterial isolates

From February to August 2022, a total of 65 stool samples of dogs and cats were collected from five veterinary hospitals located in Tehran, Iran. Among these samples we have acquired 24 *E. coli* and 6 *K. pneumoniae* strains. These isolates were subsequently preserved at a temperature of -70°C in trypticase soy broth, which was supplemented with 20% glycerol.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of every isolate was evaluated on Mueller Hinton agar (HiMedia Company) and analyzed according to the guidelines of The Clinical and Laboratory Standards Institute (CLSI, 2022) (6). The study employed a range of antibiotics, specifically Ampicillin, Piperacillin, Piperacillin/Tazobactam, Ceftazidime, Cefotaxime, Ceftriaxone, Doripenem, Imipenem, Meropenem, Aztreonam, Tetracycline, Amikacin, Ciprofloxacin, Trimethoprim-sulfamethoxazole, and Tigecycline (Mast, Company). Additionally, *Escherichia coli* ATCC25922 were used as a control strain.

Phenotypic detection of β-lactamases

The Combination Disk Diffusion Test (CDDT) was conducted to identify Metallo - beta - lactamases (MBLs) through the utilization of Imipenem and Meropenem, both separately and in conjunction with 0.5 M EDTA (Sigma) (7). ESBL production was assessed for all the isolates by the CDDT method. To determine ESBL production, *E. coli* ATCC 25922 and *K. pneumoniae* ATCC700603 were employed as negative and positive controls, respectively.

Detection of *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$, *bla*$_{IMP}$, *bla*$_{VIM}$ and *bla*$_{NDM}$ Genes by the Polymerase chain reaction (PCR)

Total DNAs of the different bacterial isolates were extracted by the boiling method. Genomic DNA obtained earlier was utilized for the detection of resistance genes through the Polymerase Chain Reaction (PCR) technique. PCR was performed in 25-µl reaction volumes using specific primers listed in Table 1. The PCR reaction mixture included 1 µl (20 ng) of DNA template, 1 × PCR buffer, 12.5 µl of 2 × Master Mix (SinaClon - Iran), 3 mmol/L MgCl2, 0.4 mmol/L dNTPs, 9.5 µl of sterile distilled water, 1 µl of 10 pmol of each primer, and 0.08 IU of Taq DNA polymerase. The PCR conditions comprised an initial denaturation at 94°C for 5 min, followed by 36 cycles of denaturation at 94°C for 45 s, annealing at 59-62°C (depending on the gene - specific primers) for 45 s, and extension at 72°C for 45 s. A final extension step was performed at 72°C for 5 min. The resulting PCR products were then analyzed using agarose gel electrophoresis (1.5% agarose gel), stained with DNA Safe, and visualized under UV light.

Statistical Analysis

Data were analyzed by Excel software and using descriptive statistics.

Table 1. Oligonucleotide primers used in this study

Genes	Sequences (5'→3')	Size	References
TEM-F TEM-R	TCGGGGAAATGTGCGCG TGCTTAATCAGTGAGGCACC	972	**(8)**
SHV-F SHV-R	TTAGCGTTGCCAGTGCTC GGTTATGCGTTATATTCGCC	861	**(8)**
CTX-M-F CTX-M-R	TTTGCGATGTGCAGTACCAGTAA CGCTATCGTTGGTGGTGCCATA	544	**(8)**
IMP-F IMP=R	GAAGGCGTTTATGTTCATAC GTAAGTTTCAAGAGTGATGC	587	**(8)**
VIM-F VIM-R	GATGGTGTTTGGTCGCATA CGAATGCGCAGCACCAG	390	**(8)**
NDM-F NDM-R	GGTTTGGCGATCTGGTTTTC CGGAATGGCTCATCACGATC	621	**(8)**

Results

The process of isolating and identifying bacterial species

A complete 24 *E. coli* and 6 *K. pneumoniae* strains were extracted from a 65 stool samples of dogs and cats from five veterinary hospitals located in Tehran, Iran between February 2022 and August 2022 (Table 2).

Antimicrobial Susceptibility testing

The resistance patterns of 24 *E.coli* and 6 *K. pneumoniae* isolates against antimicrobial drugs have been displayed in Table 3. According to our findings, the most effective antibiotics against bacterial isolates were piperacillin-tazobactam imipenem and meropenem, respectively.

Table 2. The prevalence of *E. coli* and *K. pneumoniae* among Feline and Canine

Bacteria	Total = N	Feline	Canine
E.coli	24	15 (62.5%)	9 (37.5%)
K. pneumoniae	6	2 (33.3%)	4 (66.6%)

Table 3. The antimicrobial susceptibility profile of isolates

Antibiotics	*K. pneumoniae*			*E.coli*		
	Resistant No (%)	Intermediate No (%)	Sensitive No (%)	Resistant No (%)	Intermediate No (%)	Sensitive No (%)
Aztreonam (ATM,10 µg)	3 (50%)	0 (0%)	3 (50%)	10 (41.6%)	1 (4.1%)	13 (54.1%)
Ampicillin (AP,10 µg)	5 (83.3%)	0 (0%)	1 (16.6%)	22 (91.6%)	0 (0%)	2 (8.3%)
Ciprofloxacin (CIP, 30 µg)	5 (83.3%)	0 (0%)	1 (16.6%)	13 (54.1%)	1 (4.1%)	5 (20.8%)
Trimethoprim / Sulfamethoxazole (TS, 30 µg)	4 (66.6%)	0 (0%)	2 (33.3%)	13 (54.1%)	6 (25%)	10 (41.6%)
Amikacin (AK,30 µg)	3 (50%)	0 (0%)	3 (50%)	0(0%)	0 (0%)	24 (100%)
Cefotaxime (CTX, 30 µg)	6 (100%)	0 (0%)	0 (0%)	12 (50%)	0 (0%)	12 (50%)
Ceftazidime (CAZ,30 µg)	6 (100%)	0 (0%)	0 (0%)	16 (66.6%)	0 (0%)	8 (33.3%)
Ceftriaxone (CRO,30 µg)	4 (66.6%)	0 (0%)	2 (33.3%)	16 (66.6%)	0 (0%)	8 (33.3%)
Tetracycline (T, 30 µg)	5 (83.3%)	0 (0%)	1 (16.6%)	16 (66.6%)	0 (0%)	8 (33.3%)
Piperacillin (PIP, 100 µg)	6 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	24 (100%)
Tigecycline (TGC,15µg)	4 (66.6%)	0 (0%)	2 (33.3%)	0 (0%)	0 (0%)	24 (100%)
Doripenem (DOR,10 µg)	0 (0%)	0 (0%)	6 (100%)	17 (70.8)	0 (0%)	7 (29.1%)
Imipenem (IMI, 10 µg)	0 (9%)	1 (16.6%)	5 (83.3%)	0 (0%)	0 (0%)	24 (100%)
Meropenem (MEM, 10 µg)	1 (16.6%)	0 (0%)	5 (83.3%)	0 (0%)	0 (0%)	24 (100%)
Piperacillin/Tazobactam (PTZ,100/10µg)	0 (0%)	0 (0%)	6 (100%)	0 (0%)	0 (0%)	24 (100%)

The β- lactamases-producing *E. coli* and *K. pneumoniae* Clinical Isolates

In the present study, ESBL production was detected in 14 (58.3%) and 6 (100%) isolates of *E. coli* and *K. pneumoniae*, respectively. (Figure 1) Upon conducting the CDDT Test, it was observed that none of the isolates of positive for MBL production.

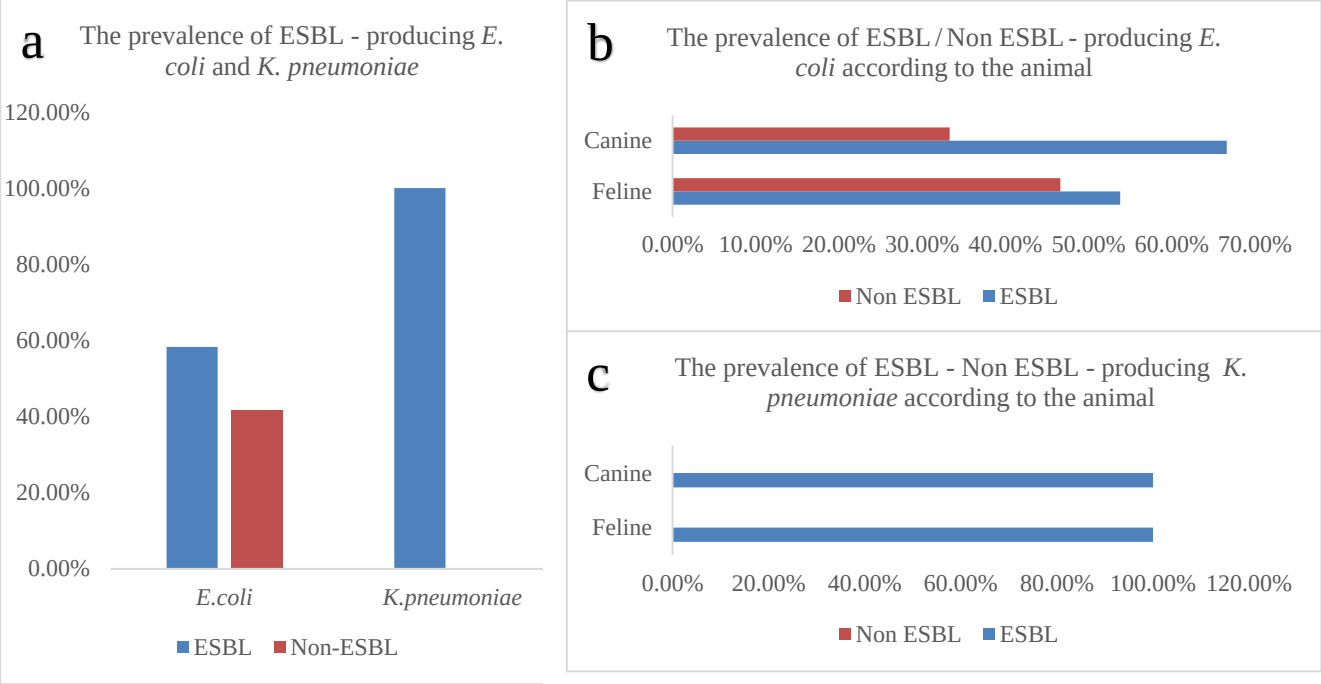

Figure 1. a) The prevalence of ESBL – producing *E. coli* and *K. pneumoniae;* b) The prevalence of ESBL - producing *E. coli* according to the animal*;* c) The prevalence of ESBL - producing *K. pneumoniae* according to the animal.

The β- lactamase genes in ESBL and MBL-producing *E. coli* and *K. pneumoniae*

The results obtained indicate that the quantity of $bla_{\text{CTXM-15}}$ and bla_{TEM} genes was observed to be 28 while the quantity of bla_{SHV} genes was 18 isolate. However, it was not observed that any of the strains possessed the bla_{NDM} and bla_{VIM} genes, whereas merely one strain was identified to contain the bla_{IMP} gene (Table 4).

Table 4. The number of ESBL and MBL genes among *E. coli* and *K. pneumoniae* isolates

Dogs						
Bacteria	**Genes (Number)**					
	CTX-M	TEM	SHV	IMP	NDM	VIM
E. coli (9 isolates)	6	6	1	1	0	0
K. pneumoniae (4 isolates)	4	4	0	0	0	0
Cats						
Bacteria	**Genes (Number)**					
	CTX-M	TEM	SHV	IMP	NDM	VIM
E. coli (15 isolates)	8	8	4	0	0	0
K. pneumoniae (2 isolates)	2	2	1	0	0	0

Discussion

In the present investigation, we conducted a study on the antibiotic resistance profiles, ESBL and MBL, among *E. coli* and *K. pneumoniae* isolates procured from stool samples of dogs and cats. We reported that the *E. coli* and *K. pneumoniae* isolates had a higher level of resistance against ampicillin and a lower level of resistance against carbapenems. This result was consistent with beta-lactam antibiotics, mostly ampicillin and amoxicillin, being the most frequently used class of antimicrobial agents in animals. In

Shahrekord, Yousefi et al. demonstrated that *E. coli* strains exhibited the most elevated degrees of resistance towards gentamicin (95%), ampicillin (85%), amikacin (70%), amoxicillin (65%), as well as sulfamethoxazole - trimethoprim (65%) antibiotics (9). Hata et al. conducted a study in Japan in 2022, which found that *E. coli* strains from 16.4% and 26.0% of samples procured of dogs from Chiba and Kanagawa in Japan respectively, exhibited resistance towards at least one antibiotic (10). Chen et al. reported in 2019 that there were high prevalence rates of quinolone resistance (52.8-57.4%) and β - lactam resistance (58.3-77.9%) in pets. This finding contrasted with the data obtained from dog and cat *E. coli* isolates in the United States, which showed resistance to cephalexin at 98%, ampicillin at 40%, and doxycycline at 100% (11). Furthermore, a study conducted in Australia reported that clinical *E. coli* isolates from canines had low rates of resistance to quinolones (9.1-9.3%), and among the 392 canine UTI isolates, 9.9-10.2% were resistant to third - generation cephalosporins (12). Positive results for ESBL production were observed in 14 (58.3%) and 6 (100%) isolates of *E. coli* and *K. pneumoniae*, respectively. These isolates were obtained from fecal specimens of felines and canines that were gathered within the period of 2022-2023 from veterinary facilities and hospitals located in Tehran, Iran. The discovered rate is considerably greater than those observed in analogous investigations conducted on animals in France (3.7%), the United kingdom (7%), and the Netherlands (2%), and notably surpasses the incidence of 1.6% identified in a European assemblage of *Enterobacteriaceae* procured from afflicted domesticated animals in 2015 (1). Marchetti and colleagues conducted a study in Argentina wherein they observed that a mere one percent (2%) of the strains isolated from domestic dogs and twenty - two percent (22%) of those obtained from stray dogs were confirmed to be phenotypically classified as ESBL (13). Also, Bunt et.al conducted research in which they found out that ESBL-producing Enterobacteriaceae were present in dogs at 10.7%, humans at 3.8% and cats at 1.4%. The most abundant ESBL gene among animals was $bla_{\text{CTX-M-1}}$, followed by $bla_{\text{CTX-M15}}$ (14). In 2021, Formenti and colleagues conducted a study in Italy that revealed that a significant proportion (25.9%) of *E. coli* specimens was producing ESBL/AmpC. The detection of the $bla_{\text{CTX-M}}$ (79.7%), bla_{CMY} (13%), bla_{TEM} (47.8%), and bla_{SHV} (5.8%) genes confirmed this finding. Also, nearly 88.4% of the isolates were determined to be unyielding to cephalosporins, with 8.7% indicating unyieldingness to both cephalosporins and carbapenems, and 2.9% presenting unyieldingness to cephalosporins, carbapenems, and penicillins (15). In 2022, Ghenea et al. conducted a study in Romania whereby it was observed that the majority of *E. coli* strains contained the $bla_{\text{CTX-M15}}$ gene (13/14 strains), with only a single strain possessing the $bla_{\text{SHV-1}}$ gene, and 11 strains containing the $bla_{\text{TEM-1}}$ gene (16). The CDDT method was employed, and it was found that 0 (0%) of *E.coli* and *K. pneumoniae* isolates exhibited MBL production. MBL - producing strains are typically able to hydrolyze a wide range of antibiotics, except for aztreonam (17). Of the MBL genes, IMP is the most prevalent, particularly in Iran, and was first reported in Japan in 1980 (18). In the present investigation, a single isolate was found to harbor bla_{IMP}. The PCR analysis conducted in our study did not reveal the presence of bla_{NDM} and bla_{VIM} genes in the isolates. Furthermore, VIM-1 was detected in *K. pneumoniae* strains isolated from dogs in Spain. Notably, several instances of NDM - 5 - producing *E. coli* have been identified in dogs and cats, with six NDM - 1 - producing *E. coli* strains being isolated from feline and canine hosts in the United States. Two NDM - 1 - producing *E. coli* strains were also identified in a canine subject from China. Moreover, a solitary instance of NDM-9 was detected in a farm dog from China (19). NDM-5–producing *E. coli* have been reported in dogs from Finland, South Korea, and Algeria (20).

Conclusion

The increasing prevalence of antibiotic- resistance genes is a significant concern in the field of medicine. The excessive utilization of antibiotics may lead to the acquisition of genes that contribute to resistance.

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Conflict of Interest

The authors declared no conflicts of interest.

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Authors Contributions

Conceptualization: A.H., M.JD, and D.HA.; Methodology: A.H. and D.HA.; Validation: A.H., M.JD and D.HA.; Formal analysis: A.H. and D.HA.;

Investigation: A.H. and D.HA; Resources, A.H. and D.HA.; Writing - original draft preparation: A.H. and D.HA.; Writing - review and editing: A.H. and D.HA.; Visualization: A.H., M.JD, and D.HA.; Supervision: A.H., M.JD, and D.HA.; Project administration: A.H., M.JD, and D.HA.; Funding acquisition: A.H. and D.HA.

References

1. Zogg AL, Simmen S, Zurfluh K, Stephan R, Schmitt SN, Nüesch-Inderbinen M. High prevalence of extended-spectrum β-lactamase producing Enterobacteriaceae among clinical isolates from cats and dogs admitted to a veterinary hospital in Switzerland. Frontiers in veterinary science. 2018;5:62.

2. Kumar V, Sun P, Vamathevan J, Li Y, Ingraham K, Palmer L, et al. Comparative genomics of Klebsiella pneumoniae strains with different antibiotic resistance profiles. Antimicrobial agents and chemotherapy. 2011;55.76-4267:(9).

3. Tsai YK, Fung CP, Lin JC, Chen JH, Chang FY, Chen TL, et al. Klebsiella pneumoniae outer membrane porins OmpK35 and OmpK36 play roles in both antimicrobial resistance and virulence. Antimicrobial agents and chemotherapy. 2011;55(4):1485-93.

4. Wang G, Huang T, Surendraiah PK, Wang K, Komal R, Zhuge J, et al. CTX-M beta-lactamase-producing Klebsiella pneumoniae in suburban New York City, New York, USA. Emerging infectious diseases. 2013;19(11):1803-10.

5. Poirel L, Revathi G, Bernabeu S, Nordmann P. Detection of NDM-1-producing Klebsiella pneumoniae in Kenya. Antimicrobial agents and chemotherapy. 2011;55(2):934-6.

6. Clinical and Laboratory Standards Institute.Performance standards for antimicrobial susceptibility testing; Twenty-second informational supplement. Document M100-S22 Wayne, PA, CLSI. 2012;32.(3).

7. Fallah F, Borhan RS, Hashemi A. Detection of bla(IMP) and bla(VIM) metallo-beta-lactamases genes among Pseudomonas aeruginosa strains. International journal of burns and trauma. 2013;3.4-122:(2).

8. Shariati A, Azimi T, Ardebili A, Chirani AS, Bahramian A, Pormohammad A, et al. Insertional inactivation of oprD in carbapenem-resistant Pseudomonas aeruginosa strains isolated from burn patients in Tehran, Iran. New microbes and new infections. 2018;21:75-80.

9. Yousefi A, Torkan S. Uropathogenic Escherichia coli in the urine samples of Iranian dogs: Antimicrobial resistance pattern and distribution of antibiotic resistance genes. BioMed research international. 2017;2017.

10. Hata A, Fujitani N, Ono F, Yoshikawa Y. Surveillance of antimicrobial-resistant Escherichia coli in Sheltered dogs in the Kanto Region of Japan. Scientific Reports. 2022;12(1):773.

11. Chen Y, Liu Z, Zhang Y, Zhang Z, Lei L, Xia Z. Increasing prevalence of ESBL-producing multidrug resistance Escherichia coli from diseased pets in Beijing, China from 2012 to 2017. Frontiers in microbiology. 2019;10:2852.

12. Saputra S, Jordan D, Mitchell T, San Wong H, Abraham RJ, Kidsley A, et al. Antimicrobial resistance in clinical Escherichia coli isolated from companion animals in Australia. Veterinary microbiology. 2017;211:43-50.

13. Marchetti L, Buldain D, Gortari Castillo L, Buchamer A, Chirino-Trejo M, Mestorino N. Pet and stray dogs as reservoirs of antimicrobial-resistant Escherichia coli. International Journal of Microbiology. 2021;2021.

14. Van den Bunt G, Fluit A, Spaninks M, Timmerman A, Geurts Y, Kant A, et al. Faecal carriage, risk factors, acquisition and persistence of ESBL-producing Enterobacteriaceae in dogs and cats and co-carriage with humans belonging to the same household. Journal of Antimicrobial Chemotherapy. 2020;75(2):342-50.

15. Formenti N, Grassi A, Parisio G, Romeo C, Guarneri F, Birbes L, et al. Extended-Spectrum-β-Lactamase-and AmpC-Producing Escherichia coli in Domestic Dogs: Spread, Characterisation and Associated Risk Factors. Antibiotics. 2021;10(10):1251.

16. Ghenea AE, Zlatian OM, Cristea OM, Ungureanu A, Mititelu RR, Balasoiu AT, et al. TEM, CTX-M, SHV Genes in ESBL-Producing Escherichia coli and Klebsiella pneumoniae Isolated from Clinical Samples in a County Clinical Emergency Hospital Romania-Predominance of CTX-M-15. Antibiotics. 2022;11(4):503.

17. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-beta-lactamases: the quiet before the storm? Clinical microbiology reviews. 2005;18(2):306-25.

18. Fallah F, Noori M, Hashemi A, Goudarzi H, Karimi A, Erfanimanesh S, et al. Prevalence of blaNDM, blaPER, blaVEB, blaIMP, and blaVIM genes among Acinetobacter baumannii isolated from two hospitals of Tehran, Iran. Scientifica. 2014;2014.

19. Silva JMd, Menezes J, Marques C, Pomba CF. Companion Animals—An Overlooked and Misdiagnosed Reservoir of Carbapenem Resistance. Antibiotics. 2022;11(4):533.

20. Cole SD, Peak L, Tyson GH, Reimschuessel R, Ceric O, Rankin SC. New Delhi metallo-β-lactamase-5–producing Escherichia coli in companion animals, United States. Emerging Infectious Diseases. 2020;26(2):381 .