Antimicrobial Resistance Profile of Urinary Bacterial Isolates from Hospitalized Companion Animals Reveals a Potential Public Health Risk in South Korea

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Article

Antimicrobial Resistance Profile of Urinary Bacterial Isolates from Hospitalized Companion Animals Reveals a Potential Public Health Risk in South Korea

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Simple Summary

Antibiotic resistance in companion animals is becoming a serious public health concern. When bacteria develop resistance to multiple antibiotics, they can spread from animals to humans, making infections harder to treat. This study examined urine samples from companion animals hospitalized with urinary tract infections over two years. Three types of bacteria were most commonly found: *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Alarmingly, nearly three-quarters of these bacteria were resistant to multiple antibiotics, including some "last-resort" antibiotics called carbapenems. Additionally, one *E. coli* strain carried a dangerous resistance gene called *bla*$_{NDM-1}$, which makes bacteria extremely difficult to treat. These findings highlight why veterinarians must use antibiotics carefully and why companion animal owners should follow treatment instructions accurately. The health of companion animals and human families is connected, as what affects one can affect the other. Better infection control in veterinary hospitals and smarter antibiotic use can help protect both animal and human health.

Abstract

Emerging antimicrobial resistance (AMR) in companion animals represents a global health concern as they serve as potential reservoirs for multidrug-resistant (MDR) bacteria, which can be transmitted to humans. Herein, we provide comprehensive surveillance data on resistance patterns in veterinary hospital settings, focusing on urinary tract infection. A total of 23 bacterial strains were isolated from urine specimens of hospitalized companion animals suspected of UTI between 2022 and 2024. 16S rRNA sequencing analysis revealed that *Escherichia coli* (47.8%), *Klebsiella pneumoniae* (21.7%), and *Pseudomonas aeruginosa* (8.7%) were predominant uropathogens. Minimum inhibitory concentration and minimum bactericidal concentration tests were employed to analyze AMR patterns across different classes of antibiotics. Moreover, antimicrobial susceptibility test exhibited 73.91% MDR according to the standard definition given by the Clinical and Laboratory Standards Institute (CLSI) M100 guidelines. Most Gram-negative bacteria have been shown to be resistant to beta-lactam antibiotics, especially carbapenems. Notably, an *E. coli* strain was confirmed to possess the *bla*$_{NDM-1}$ gene encoding the carbapenemase New Delhi metallo-β-lactamase. These findings support the implementation of targeted infection control measures and evidence-based treatment protocols to preserve antimicrobial efficacy in companion animal medicine to minimize potential public health risks through the One Health approach.

Keywords: antimicrobial resistance; multidrug-resistant pathogens; urinary tract infection

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1. Introduction

Antimicrobial resistance (AMR) represents one of the most urgent global threats to both human and veterinary public health. In 2021, bacterial AMR was associated with approximately 4.71 million deaths worldwide, of which 1.14 million were directly attributable to drug-resistant infections [1]. To counter this escalating challenge, the World Health Organization (WHO) updated the Bacterial Priority Pathogens List (BPPL) in 2024 to guide research prioritization, policy development, and the discovery of novel antimicrobials. Within this framework, third-generation cephalosporin-resistant (3GCRE) and carbapenem-resistant Enterobacterales (CRE) were classified as "critical" priority pathogens due to their limited therapeutic options and significant threat to global health [2].

Growing evidence indicates that companion animals can serve as reservoirs and potential disseminators of multidrug-resistant (MDR) bacteria, posing risks of zoonotic transmission to humans. Frequently reported MDR species in companion animals include *Escherichia coli, Staphylococcus aureus, Staphylococcus pseudintermedius, Klebsiella pneumoniae, and Acinetobacter baumannii* [3–7]. Many of these belong to the ESKAPE group—pathogens renowned for their remarkable ability to evade antimicrobial action and acquire resistance through genetic mutations and mobile genetic elements, including plasmids, transposons, and integrons [8]. Such mechanisms enable the rapid spread of resistance traits across bacterial populations and host species.

Urinary tract infections (UTIs) are among the most common bacterial infections in companion animals, representing one of the leading indications for antimicrobial prescription in veterinary practice and accounting for approximately 12% of all antibiotic use in dogs [9,10]. Inappropriate or empirical antibiotic use can lead to therapeutic failure, resistance development, and increased public health risks [11]. Gram-negative bacteria dominate as the causative agents of canine and feline UTIs, with uropathogenic *E. coli* (UPEC) being the most prevalent pathogen in both uncomplicated and complicated infections. Other frequently isolated uropathogens include *K. pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, and S. aureus* [12,13].

Fluoroquinolones and cephalosporins remain among the most frequently prescribed antimicrobials for UTIs; however, their extensive use accelerates the selection and dissemination of resistant strains [12,14]. Horizontal gene transfer (HGT) of extended-spectrum β-lactamase (ESBL) and carbapenemase genes plays a central role in conferring resistance to β-lactams and other antimicrobial classes [15]. Although carbapenems are rarely used in veterinary settings, carbapenemase-producing Enterobacterales have been increasingly reported in companion animals [14]. According to the Ambler classification system, β-lactamases are categorized into classes A, B, and D. The carbapenemases KPC (class A) [16,17], NDM (class B) [16,18,19], and OXA-48-like (class D) [16,20,21] β-lactamases have been detected in animal isolates, raising new challenges for treatment, infection control, and interspecies transmission.

Close human–animal interactions facilitate bidirectional exchange of bacterial pathogens, increasing the risk of zoonotic transmission through both direct contact and indirect exposure [5]. Therefore, continuous monitoring of AMR in companion animals, and human surveillance, is essential for an effective One Health approach, particularly within veterinary hospitals. Despite growing global attention to AMR in veterinary contexts, much of the existing research remains geographically limited, hindering a comprehensive understanding of resistance dynamics in companion animals.

In this study, bacterial isolates were obtained from urine specimens of hospitalized companion animals to characterize AMR patterns and assess the prevalence of resistant uropathogens. Understanding these AMR profiles is crucial for guiding evidence-based therapeutic decisions, supporting antimicrobial stewardship in veterinary medicine, underscoring the potential risk for zoonotic dissemination of resistant bacteria.

2. Materials and Methods

2.1. Antibiotic Discs

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A total of 29 antibiotic discs (BD BBL™ Sensi-Disc™, BD, USA) were used as follows: Amikacin (30 μg), Gentamicin (10 μg), Kanamycin (30 μg), Streptomycin (10 μg), Tobramycin (10 μg), Ampicillin (10 μg), Amoxicillin/Clavulanic acid (20/10 μg), Ampicillin/Sulbactam (10/10 μg), Cefazolin (30 μg), Cefaclor (30 μg), Cefoxitin (30 μg), Cefuroxime (30 μg), Cefixime (5 μg), Cefotaxime (30 μg), Ceftazidime (30 μg), Ceftriaxone (30 μg), Cefepime (30 μg); Ertapenem (10 μg), Imipenem (10 μg), Meropenem (10 μg), Aztreonam (30 μg), Chloramphenicol (30 μg), Ciprofloxacin (5 μg), Nalidixic acid (30 μg), Colistin (10 μg), Doxycycline (30 μg), Tetracycline (30 μg), Tigecycline (15 μg), Azithromycin (15 μg).

2.2. Sample Collection and Clinical Information

Urine samples were collected by cystocentesis from hospitalized dogs suspected of urinary tract infection, with careful attention to prevent cross-contamination. The samples were then cultured in LB broth at 37 °C overnight. From 51 specimens, 23 bacterial isolates were obtained and identified by 16S rRNA gene sequencing. All isolates were stored at −80 °C for future research. Each bacterial isolate was assigned a unique code consisting of the institutional abbreviation, the bacterial species name, and a sequential isolation number (Table 1).

Table 1. Pathogenic bacterial isolates and the corresponding origins of their isolation are summarized.

Strains	Species	Specimen	Breeds	Age	Sex
CNUAH-EC002	*Escherichia coli*	Urine	Maltese	16y	SF
CNUAH-EC003	*Escherichia coli*	Urine	Jindo	9y	SF
CNUAH-EC004	*Escherichia coli*	Urine	Mixed	9y	SF
CNUAH-EC005	*Escherichia coli*	Urine	Labrador retriever	11y	SF
CNUAH-EC019	*Escherichia coli*	Urine	Jindo	12y	SF
CNUAH-EC023	*Escherichia coli*	Urine	Mixed	7y	SF
CNUAH-EC025	*Escherichia coli*	Urine	Yorkshire terrier	12y	SF
CNUAH-EC026	*Escherichia coli*	Urine	Shiba Inu	6y	SF
CNUAH-EC029	*Escherichia coli*	Urine	Welsh Corgi	8y	CM
CNUAH-EC030	*Escherichia coli*	Urine	Spitz	7y	CM
CNUAH-EC031	*Escherichia coli*	Urine	Maltese	15y	SF
CNUAH-KP014	*Klebsiella pneumoniae*	Urine	Mixed	12y	M
CNUAH-KP015	*Klebsiella pneumoniae*	Urine	Mixed	12y	M
CNUAH-KP020	*Klebsiella pneumoniae*	Urine	Mixed	5y	SF
CNUAH-KP021	*Klebsiella pneumoniae*	Urine	Mixed	14y	SF

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CNUAH-KP022	*Klebsiella pneumoniae*	Urine	Mixed	14y	SF
CNUAH-PA009	*Pseudomonas aeruginosa*	Urine	Mixed	13y	SF
CNUAH-PA010	*Pseudomonas aeruginosa*	Urine	Mixed	13y	CM
CNUAH-PM028	*Proteus mirabilis*	Urine	Maltese	13y	SF
CNUAH-SF024	*Shigella flexneri*	Urine	Beagle	7y	SF
CNUAH-EH032	*Enterobacter hormaechei*	Urine	Yorkshire terrier	8y	M
CNUAH-SI017	*Staphylococcus pseudintermedius*	Urine	Jindo	12y	SF
CNUAH-SI027	*Staphylococcus pseudintermedius*	Urine	Maltese	13y	SF

CM, Castrated male; SF, spayed female; M, Male.

2.3. Identification of Bacterial Strains

Bacterial strains were identified by 16S rRNA gene sequencing conducted by Macrogen Inc. (Korea). PCR amplification of the 16S rRNA gene was performed using primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) [22]. Approximately 1,400 base pairs of the amplified products were analyzed using the NCBI BLAST database to determine bacterial species.

2.4. Antimicrobial Susceptibility Test

The Kirby-Bauer disk diffusion method was used to determine the antibiotic susceptibility profile, employing Mueller-Hinton agar (MHA; #M1033, MB-cell, Korea). Clinical isolates from animals were first cultured in nutrient broth (NB; #234000, BD, USA) and incubated overnight at 37 °C in a shaking incubator. The bacterial suspension was adjusted to a 0.5 McFarland standard, then diluted in 11 mL of Mueller-Hinton broth (MHB) to reach approximately 1.0×10^5 CFU/mL. The appropriate volume of inoculum was swabbed evenly onto the surface of MHA plates, following the CLSI guidelines. Antibiotic discs were placed on the inoculated plates, which were then incubated at 37 °C for 18 hours. Zones of inhibition were measured with a ruler or calipers, and susceptibility interpretations (resistant, intermediate, or susceptible) were made according to CLSI M100 breakpoint criteria. Isolates resistant to at least one agent in three or more antibiotic classes were classified as multidrug-resistant (MDR), based on criteria proposed by Magiorakos et al.[1].

2.5. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) was determined using Sensititre™ Companion Animal Vet AST Plates (#COMPGN1F, #COMPGP1F; Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Briefly, single colonies grown on agar were suspended in phosphate-buffered saline (PBS), and the turbidity was adjusted to a 0.5 McFarland standard. This suspension was further diluted in 11 mL of MHB to achieve an approximate final concentration of 1.0 × 105 CFU/mL. Antimicrobial susceptibility testing was performed against 19 antibiotics using a standardized dilution range as specified by the test plate. Plates were sealed with adhesive film and incubated at 37 °C for 18 hours. The tested antibiotic concentrations are listed in Table 3. To determine the minimum bactericidal concentration (MBC), 40 µL of broth from each well corresponding to the MIC value and the next higher concentration was inoculated onto MHA plates and incubated at 37 °C for 18 hours. The MBC was defined as the lowest concentration at which no visible bacterial growth was observed.

2.6. Polymerase Chain Reaction

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Isolates exhibiting carbapenem resistance were subjected to PCR to detect the presence of the *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{IMP}$, *and bla*$_{VIM}$ genes. Individual colonies from each plate were suspended in 100 µL of distilled water and boiled at 100 °C for 10 minutes to obtain each template DNA. PCR amplification was conducted using AccuPower™ PCR PreMix (#K-2016; Bioneer, Korea). The 20 µL reaction mixture contained 10 ng of template DNA, 2 µL of forward primer (20 µM), 2 µL of reverse primer (20 µM), and 14 µL of RNase-free water. The thermal cycling conditions consisted of an initial denaturation at 95 °C for 5 minutes; followed by 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 60 °C for 45 seconds, and extension at 72 °C for 1 minute; with a final extension at 72 °C for 10 minutes. PCR products were stained using RedSafe™ Nucleic Acid Staining Solution (#21141, iNtRON Biotechnology, Korea) and visualized on a 1.5% agarose gel under UV light. The specific primers of targeted genes are listed in Table S1 [23].

3. Results

3.1. Isolation of Bacterial Strains in Urine Specimens

A total of 51 urinary specimens were collected between 2022 and 2024 from hospitalized companion dogs and cats, and 23 bacterial strains were isolated. The species with the highest prevalence were *Escherichia coli* (47.8%) and *Klebsiella pneumoniae* (21.7%), both of which are Gram-negative bacteria. Moreover, *Pseudomonas aeruginosa, Proteus mirabilis, Shigella flexneri, and Enterobacter hormaechei*, which are also Gram-negative bacteria, were also isolated. In contrast, *Staphylococcus pseudintermedius*, the Gram-positive bacterium, was isolated from only two specimens (8.6%).

3.2. Characterization of the Antibiotic Resistance Profile of the Gram-Negative Strains

Among the Gram-negative bacterial isolates, the highest rates of antibiotic resistance were observed for ampicillin (90.4%) and amoxicillin/clavulanic acid (90.4%). According to the WHO, amoxicillin/clavulanic acid is classified as a Highly Important Antimicrobial used for treating a wide range of bacterial infections. In contrast, all Gram-negative isolates were susceptible to amikacin (100%), while high susceptibility was also observed for gentamicin (90.4%), imipenem (95.2%), and colistin (90.4%) (Table S2).

Escherichia coli isolates exhibited higher resistance to ceftazidime and ceftriaxone, whereas *Klebsiella pneumoniae* showed complete resistance (100%) to ampicillin, amoxicillin/clavulanic acid, ampicillin/sulbactam, doxycycline, and tetracycline (Table 2). Notably, all Gram-negative isolates remained susceptible to colistin, which serves as a last-resort therapeutic agent for infections caused by multidrug-resistant Gram-negative bacteria in both humans and animals.

Table 2. Antibiotic resistance profiles for each isolated species.

Antibiotics	Resistance (%)			
	Total (n = 21)	***E. coli* (n = 11)**	***K. pneumoniae* (n = 5)**	***P. aeruginosa* (n = 2)**
Amikacin	0.0	0.0	0.0	0.0
Gentamicin	9.5	18.2	0.0	0.0
Kanamycin	23.8	27.3	0.0	100
Streptomycin	47.6	36.4	60	100
Tobramycin	4.7	9.1	0.0	0.0
Ampicillin	90.4	90.9	100	100
Amoxicillin/Clavulanic Acid	90.4	90.9	100	100
Ampicillin/Sulbactam	80.9	81.8	100	100
Cefazolin	76.2	81.8	60	100
Cefaclor	76.2	81.8	60	100
Cefoxitin	47.6	36.4	60	100

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Cefuroxime	52.3	63.6	40	100
Cefixime	71.4	81.8	60	100
Cefotaxime	47.6	63.6	20	100
Ceftazidime	19.0	36.4	0.0	0.0
Ceftriaxone	28.5	54.5	0.0	0.0
Cefepime	9.5	18.2	0.0	0.0
Ertapenem	19.0	18.2	0.0	100
Imipenem	4.7	9.1	0.0	0.0
Meropenem	23.8	9.1	60	0.0
Aztreonam	4.7	9.1	0.0	0.0
Chloramphenicol	28.5	9.1	60	100
Ciprofloxacin	42.8	45.5	60	0
Colistin	0.0	0.0	0.0	0.0
Doxycycline	61.9	45.5	100	100
Tetracycline	57.1	36.4	100	100
Tigecycline	9.5	0.0	0.0	100
Nalidixic Acid	61.9	45.5	60	100
Azithromycin	19.0	18.2	0.0	50

Resistance rates (%) of *Escherichia coli* (n = 11), *Klebsiella pneumoniae* (n = 5), and *Pseudomonas aeruginosa* (n = 2) are presented, along with the overall resistance rate among all isolates (n = 21). Resistance was determined according to the CLSI M100 guidelines.

Additionally, several Gram-negative strains exhibited resistance to carbapenem antibiotics, including ertapenem (19%), imipenem (4.7%), and meropenem (23.8%). The emergence of carbapenem-resistant Enterobacterales (CRE) is of particular concern, as these bacteria can rapidly disseminate carbapenemase genes that confer broad resistance. Conversely, the two *Pseudomonas aeruginosa* isolates demonstrated similar resistance patterns to most Gram-negative antibiotics, except for azithromycin.

According to the definitions proposed, 71.43% of the 21 Gram-negative bacterial isolates were classified as MDR, with the remainder being 1-2 drug resistance (1-2DR) (Figure 1A). Notably, 81.81% of the 11 *E. coli* strains (Figure 1B) and 80% of the 5 *K. pneumoniae* strains (Figure 1C) were MDR. Most of the strains presented an MDR profile and did not show an extensive drug-resistance (XDR) profile.

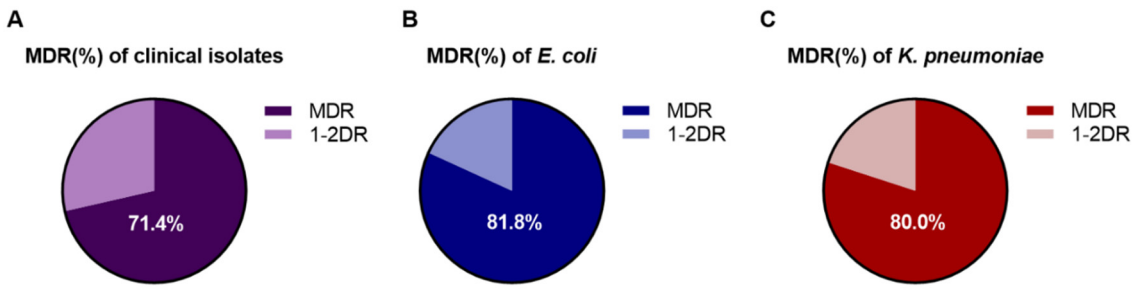

Figure 1. Proportion of MDR among Gram-negative isolates. **(a)** Gram-negative isolates (n = 21); (**b**) *E. coli* isolates (n = 11); (**c**) *K. pneumoniae* isolates (n = 5).

3.3. Carbapenemase Gene-Harboring Isolates

To investigate the presence of specific resistance genes, nine clinical isolates initially identified as carbapenem-resistant by AMR disc diffusion testing were further analyzed using PCR. Unexpectedly, most isolates did not harbor known carbapenemase genes, including *bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{IMP}$, *and bla*$_{VIM}$. However, isolate EC019 carried the *bla*$_{NDM}$ gene and exhibited strong resistance to all three carbapenem antibiotics, ertapenem, imipenem, and meropenem (Figure 2).

 doi:10.20944/preprints202511.2149.v1

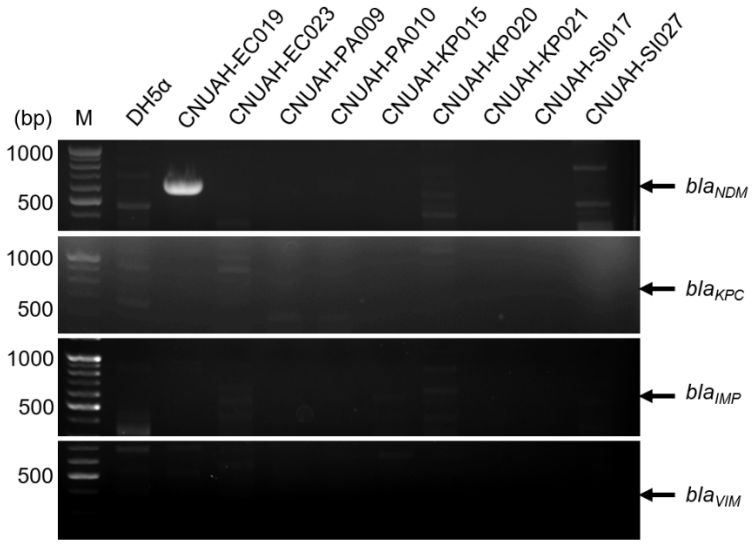

Figure 2. Detection of the *bla*$_{NDM}$ gene in carbapenem-resistant isolates by PCR. DNA templates from each isolate were amplified using specific primers by PCR. The DH5α strain was used as a negative control. The expected amplicon sizes and primer sequences for each target gene are provided in Table S1.

3.4. MIC and MBC of All Bacterial Strains

MIC results are presented in Tables 3 and 5, and MBC results in Tables 4 and 5. Consistent with the AMR profiles obtained from the disc diffusion test, many isolates exhibited resistance to multiple classes of antibiotics. Notably, a high prevalence of resistance was observed against β-lactam antibiotics, including β-lactam/β-lactamase inhibitor combinations and cephalosporins. Although fluoroquinolones were not included in the AMR disc test, MIC analysis revealed relatively high levels of resistance, with fewer than half of the isolates remaining susceptible. These findings collectively indicate that most isolates displayed broad-spectrum AMR.

Among the isolates, *Staphylococcus pseudintermedius* strains were identified to show resistance to oxacillin. Specifically, isolate SI027 exhibited resistance in both the AMR disc diffusion and MIC tests based on the CLSI M100 breakpoint criteria. In contrast, isolate SI017 was resistant only in the disc diffusion test but not in the MIC test. Cefoxitin is typically used as a surrogate marker for detecting oxacillin resistance in *Staphylococcus* spp., and isolates demonstrating resistance to either cefoxitin or oxacillin are categorized as methicillin (oxacillin)-resistant. However, both isolates were susceptible to penicillinase-labile penicillins, such as ampicillin, suggesting that they may behave similarly to methicillin-susceptible staphylococci, which are generally susceptible to most β-lactam antibiotics, β-lactam/β-lactamase inhibitor combinations, and cephalosporins. Given these inconsistent results, further molecular analyses, including PCR detection of *mecA* and *mecC* genes, are warranted to confirm methicillin resistance.

Table 3. MIC of antibiotics against Gram-negative isolates.

Antibiotics	EC002	EC003	EC004	EC005	EC019	EC023	EC025	EC026	EC029	EC030	EC031	KP014	KP014	KP020	KP021	KP022	KP029	PA010	PA010	PM028	SF024	EH032
	MIC (μg/mL)																					
AMI	<4	<4	<4	<4	8	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4
AUG2	>8/4	>8/4	8/4	8/4	>8/4	>8/4	2/1	>8/4	>8/4	>8/4	2/1	8/4	4/2	>8/4	>8/4	>8/4	>8/4	>8/4	1/0.5	2/1	>8/4	
AMP	>8	>8	>8	>8	>8	>8	2	>8	>8	>8	2	>8	>8	>8	>8	>8	>8	>8	>8	2	>8	
FAZ	>32	>32	>32	>32	32	>32	2	>32	>32	>32	2	4	4	>32	>32	>32	>32	>8	2	2	>32	
FOV	>8	>8	>8	>8	>8	>8	<0.25	8	>8	>8	<0.25	>8	<0.25	>8	8	>8	>8	>8	>8	<0.25	2	
POD	>8	>8	>8	>8	>8	>8	<1	>8	>8	>8	<1	>8	<1	>8	>8	>8	>8	>8	>8	<1	4	
TAZ	>16	>16	<4	<4	>16	16	<4	<4	>16	16	<4	<4	<4	<4	<4	<4	<4	<4	>16	>16	>16	
LEX	<0.25	<0.25	>2	2	>2	<0.25	<0.25	>16	>16	>16	8	>2	8	>16	>16	>16	<0.25	>16	8	8	>16	
CHL	4	<2	4	<2	8	4	4	<2	32	4	4	4	4	>32	>32	>32	16	32	32	<2	4	

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DOX	1	1	>8	8	>8	1	0.5	0.5	8	1	1	>8	>8	>8	8	8	1	8	>8	0.5	>8
ENRO	0.25	<0.12	>4	>4	>4	<0.12	<0.12	0.25	>4	0.25	<0.12	<0.12	<0.12	<0.12	>4	>4	0.25	0.25	>4	>4	4
GEN	1	1	>8	>8	>8	2	1	4	1	1	1	0.5	0.5	0.5	0.5	0.5	<0.25	<0.25	4	1	0.5
IMI	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
MAR	0.25	0.25	>4	>4	>4	0.25	<0.12	0.5	>4	0.25	<0.12	<0.12	<0.25	4	4	4	<0.25	<0.12	>4	<0.25	2
ORB	<1	<1	>8	>8	>8	<1	<1	2	>8	<1	<1	<1	<1	>8	>8	>8	<1	<1	>8	>8	8
P/T4	<8/4	<8/4	<8/4	<8/4	>64/4	<8/4	<8/4	<8/4	16/4	<8/4	<8/4	<8/4	<8/4	<8/4	<8/4	16/4	<8/4	<8/4	<8/4	<8/4	<8/4
PRA	<0.25	<0.25	>2	2	>2	>2	<0.25	<0.25	>2	>2	<0.25	<0.25	<0.25	2	2	2	<0.25	<0.25	2	2	1
TET	<4	<4	>16	>16	>16	<4	<4	<4	>16	<4	<4	>16	>16	>16	>16	>16	<4	8	>16	<4	>16
SXT	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	2/38	>4/76	>4/76	>4/76

MIC (μg/ mL) of 19 antibiotics against the isolates was determined using the broth microdilution method. AMI, Amikacin; AUG2, Amoxicillin/clavulanic acid 2:1; AMP, Ampicillin; FAZ, Cefazolin; FOV, Cefovecin; POD, Cefpodoxime; TAZ, Ceftazidime; LEX, Cephalexin; CHL, Chloramphenicol; DOX, Doxycycline; ENRO, Enrofloxacin; GEN, Gentamicin; IMI, Imipenem; MAR, Marbofloxacin; ORB, Orbifloxacin; P/T4, Piperacillin/tazobactam constant 4; PRA, Pradofloxacin; TET, Tetracycline; SXT, Trimethoprim / sulfamethoxazole.

Table 4. MBC of antibiotics against Gram-negative isolates.

Antibiotics	EC002	EC003	EC004	EC005	EC019	EC023	EC025	EC026	EC029	EC030	EC031	KP014	KP014	KP020	KP021	KP022	PA000	PA010	PM028	SF024	EH032
	MBC (μg/mL)																				
AMI	<4	<4	<4	<4	8	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4
AUG2	>8/4	>8/4	>8/4	>8/4	>8/4	>8/4	2/1	>8/4	>8/4	>8/4	2/1	8/4	8/4	>8/4	>8/4	>8/4	>8/4	>8/4	1/0.5	2/1	>8/4
AMP	>8	>8	>8	>8	>8	>8	2	>8	>8	>8	2	>8	>8	>8	>8	>8	>8	>8	>8	2	>8
FAZ	>32	>32	>32	>32	32	>32	2	>32	>32	>32	2	4	4	>32	>32	>32	>32	<1	4	2	>32
FOV	>8	>8	>8	>8	>8	>8	<0.25	8	>8	>8	<0.25	0.5	<0.25	>8	>8	>8	>8	>8	>8	<0.25	2
POD	>8	>8	>8	>8	>8	>8	<1	>8	>8	>8	<1	<1	<1	>8	>8	>8	>8	>8	>8	<1	4
TAZ	>16	>16	<4	<4	>16	16	<4	<4	>16	16	<4	<4	<4	<4	<4	<4	<4	<4	>16	>16	>16
LEX	<0.25	<0.25	>2	2	>16	<0.25	<0.25	>16	>16	>16	8	8	8	>16	>16	>16	>16	>16	16	8	>16
CHL	8	<2	>8	<2	8	4	>8	<2	32	4	4	>8	>8	>32	>32	>32	16	32	>32	<2	8
DOX	2	1	>8	8	>8	1	>1	0.5	8	1	1	>8	>8	>8	>8	>8	1	8	>8	>1	>8
ENRO	0.5	<0.12	>4	>4	>4	<0.12	<0.12	0.25	>4	0.25	<0.12	<0.12	<0.12	<0.12	>4	>4	0.25	0.25	>4	>4	4
GEN	2	1	>8	>8	>8	2	1	4	1	1	1	0.5	0.5	0.5	0.5	0.5	<0.25	<0.25	4	1	0.5
IMI	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
MAR	0.25	0.25	>4	>4	>4	0.25	<0.12	0.5	>4	0.25	<0.12	<0.12	<0.12	4	4	4	<0.12	<0.12	>4	<0.12	2
ORB	<1	<1	>8	>8	>8	<1	<1	2	>8	<1	<1	<1	<1	>8	>8	>8	<1	<1	>8	>8	8
P/T4	<8/4	<8/4	<8/4	<8/4	>64/4	<8/4	<8/4	<8/4	32/4	<8/4	<8/4	<8/4	<8/4	<8/4	<8/4	4/16	<8/4	<8/4	<8/4	<8/4	<8/4
PRA	<0.25	<0.25	>2	2	>2	>2	<0.25	<0.25	>2	>2	<0.25	<0.25	<0.25	2	2	>2	<0.25	<0.25	>2	2	2
TET	<4	<4	>16	>16	>16	<4	<4	<4	>16	<4	<4	>16	>16	>16	>16	>16	<4	8	>16	<4	>16
SXT	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76	>4/76

MBC (μg/ mL) of 19 antibiotics against the isolates was determined by plating the contents from each well showing no detectable growth on MHA. AMI, Amikacin; AUG2, Amoxicillin/clavulanic acid 2:1; AMP, Ampicillin; FAZ, Cefazolin; FOV, Cefovecin; POD, Cefpodoxime; TAZ, Ceftazidime; LEX, Cephalexin; CHL, Chloramphenicol; DOX, Doxycycline; ENRO, Enrofloxacin; GEN, Gentamicin; IMI, Imipenem; MAR, Marbofloxacin; ORB, Orbifloxacin; P/T4, Piperacillin/tazobactam constant 4; PRA, Pradofloxacin; TET, Tetracycline; SXT, Trimethoprim / sulfamethoxazole.

Table 5. MIC and MBC of antibiotics against Gram-positive isolates.

Antibiotics	MIC (μg/mL)		MBC (μg/mL)	
	SI017	SI027	SI017	SI027
AMI	<16	<16	<16	<16
AUG2	<0.25/0.12	<0.25/0.12	<0.25/0.12	<0.25/0.12
AMP	<0.25	<0.25	<0.25	<0.25

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FAZ	<2	<2	<2	<2
FOV	0.12	0.12	0.24	0.24
POD	<2	<2	<2	<2
CEP	<2	<2	<2	<2
CHL	16	16	>32	>32
CLI	>4	>4	>4	>4
DOX	>0.5	>0.5	>0.5	>0.5
ENRO	0.5	0.5	0.5	1
ERY	>4	>4	>4	>4
GEN	<4	<4	<4	<4
IMI	<1	<1	<1	<1
MAR	<1	<1	<1	<1
MIN	2	>2	>2	>2
NIT	<16	64	<16	64
OXA+	<0.25	>2	<0.25	>2
PEN	<0.06	1	<0.06	1
PRA	<0.25	<0.25	<0.25	<0.25
RIF	<1	<1	<1	<1
TET	>1	>1	>1	>1
SXT	>4/76	<2/38	>4/76	<2/38
VAN	<1	>16	<1	>16

MIC and MBC were determined for Gram-positive isolates using the broth microdilution method. AMI, Amikacin; AUG2, Amoxicillin/clavulanic acid 2:1; AMP, Ampicillin; FAZ, Cefazolin; FOV, Cefovecin; POD, Cefpodoxime; CEP, Cephalothin; CHL, Chloramphenicol; CLI, Clindamycin; DOX, Doxycycline; ENRO, Enrofloxacin; ERY, Erythromycin; GEN, Gentamicin; IMI, Imipenem; MAR, Marbofloxacin; MIN, Minocycline; NIT, Nitrofurantoin; OXA+, Oxacillin+2%NaCl; PEN, Penicillin; PRA, Pradofloxacin; RIF, Rifampin; TET, Tetracycline; SXT, Trimethoprim / sulfamethoxazole; VAN, Vancomycin.

4. Discussion

This study demonstrated that bacterial isolates obtained from companion animals with urinary tract infections exhibited diverse AMR profiles, with a substantial proportion meeting the criteria for MDR. These findings highlight not only the growing concern of MDR bacteria circulating among companion animals, which have been increasingly recognized as potential reservoirs, but also the importance of accurate diagnosis, continuous surveillance, and the implementation of effective control and prevention strategies, as essential factors to help mitigate the spread of AMR in both veterinary and public health sectors.

Such AMR poses serious challenges to both veterinary and human medicine, as pathogens exhibiting MDR are often associated with increased morbidity, prolonged hospitalization, treatment failure, and even mortality. The occurrence of MDR bacteria in animals was associated with the abuse of antibiotic prescriptions and antibiotic treatment [24–26]. Recently, there have been few antibiotic options for referral cases from primary animal hospitals; therefore, the initial antibiotic choice for the treatment of UTI has been amoxicillin/clavulanic acid. Indeed, AMR analysis exhibited MDR of more than 70% in all isolates, more than 80% in *E. coli* or *K. pneumoniae*. Although some carbapenem resistance genes were detected in resistant isolates, further research is needed to identify other types of carbapenem resistance genes and genes conferring resistance to the other classes of antibiotics. Furthermore, whole-genome sequencing of the isolates would provide a more precise and in-depth study of which resistance genes are responsible for resistance. However, this study did not address this issue due to cost constraints.

This study appears somewhat limited in that it analyzed only samples obtained from a single tertiary veterinary hospital. Furthermore, considering that only about 50 urine samples were

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collected from animals with suspected UTI over a two-year period, resulting in 23 strains, the results of the AMR profiling analysis are not generalizable. Nevertheless, the isolated strains and overall proportions are quite similar to previous reports conducted in clinical settings. *E. coli* was shown to be the predominant species that accounted for nearly half of all bacterial isolates, emphasizing its clinical significance as a major uropathogen in companion animals [3,27–29]. Moreover, despite the small sample size, the results demonstrate that AMR is a very critical crisis. Further studies involving larger sample sizes, diverse animal populations, and multicenter collaboration are warranted to obtain a more comprehensive understanding of AMR in companion animals. Monitoring antibiotic resistance profiles and the rational use of antibiotics in companion animals are essential for collecting data that aids in treating animal diseases. Furthermore, systematic testing of bacteria and periodic reporting of drug resistance patterns in companion animals continue to be needed for public health.

High prevalence of AMR, particularly multidrug resistance and ESBL production, among hospitalized companion animal uropathogens poses significant therapeutic challenges and potential zoonotic risks. The increasing trends in resistance to critically important antimicrobials underscore the urgent need for enhanced antimicrobial stewardship programs, diagnostic-guided therapy, and comprehensive surveillance systems in veterinary healthcare. These findings support the implementation of targeted infection control measures and evidence-based treatment protocols to preserve antimicrobial efficacy for the treatment of UTIs in companion animal medicine while minimizing public health risks through a One Health approach.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1: Primer sequences used are listed; Table S2: Antibiotic resistance profiles for total Gram-negative isolates.

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Institutional Review Board Statement: Informed consent was obtained from the owners of all animals involved in the study. This study primarily used urine samples collected from animals during routine diagnostic and therapeutic procedures at the hospital. As no additional samples were collected solely for research purposes, the study did not violate the basic principles outlined in the Institutional Animal Care and Use Committee (IACUC) standard operating guidelines. Therefore, the requirements for ethical review and approval were waived.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author(s).

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AMR	Antimicrobial resistance

 doi:10.20944/preprints202511.2149.v1

CLSI	Clinical and laboratory standards institute
CRE	Carbapenem-resistant Enterobacterales
ESBL	Extended-spectrum beta-lactamase
HGT	Horizontal gene transfer
MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
MDR	Multidrug-resistant
MHA	Mueller-Hinton agar
MHB	Mueller-Hinton broth
3GCRE	Third-generation cephalosporin-resistant
UTIs	Urinary tract infections
UPEC	Uropathogenic *E. coli*
WHO	World Health Organization
XDR	Extensive drug-resistance

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