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ANTIMICROBIAL RESISTANCE PROFILE IN CANINE UROPATHOGENIC *ESCHERICHIA COLI*

PROFILUL DE REZISTENȚĂ ANTIMICROBIANĂ AL TULPINILOR UROPATOGENE DE *ESCHERICHIA COLI* IZOLATE DE LA CAINE

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ABSTRACT

The elevated level of antimicrobial resistance in both human and veterinary pathogens represent a worldwide concern, with *Escherichia coli* as one of the most relevant prototypes of multidrug-resistant bacteria. This retrospective study aimed to investigate the percentage and patterns of antimicrobial resistance in *E. coli* strains isolated from dogs with urinary tract infections (UTI) referred to the Veterinary Medical Teaching Hospital and local clinics from Cluj-Napoca. Urine samples collected with cystocentesis from animals with confirmed UTI were subjected to microbiological processing involving culture on enrichment and selective media and *in vitro* antimicrobial susceptibility testing. Overall, the determined rates of antimicrobial susceptibility towards the antimicrobials most commonly used in small animal practice were relatively low, and resistance to at least two antimicrobial agents was noted. Several distinct resistance patterns were established, notably involving multidrug resistance (M DR). The highest rates of resistance were recorded towards penicillin G, kanamycin and enrofloxacin (66.27 %), followed by chloramphenicol (60.24%), amoxicillin/clavulanic acid (59.04%), ampicillin (51.81%), and tetracycline and marbofloxacin (48.19%). These results emphasise the importance of monitoring antibiotic usage and resistance patterns in the management of E. coli-associated UTI in dogs.

Keywords: *Escherichia coli*, dogs, urinary infection, multidrug resistance, antimicrobial therapy

REZUMAT

Nivelul ridicat de rezistență antimicrobiană înregistrat atât la agenții patogeni umani, cât și animali reprezintă o preocupare la nivel mondial, *Escherichia coli* fiind unul dintre cele mai relevante prototipuri de bacterii multirezistente. Acest studiu retrospectiv și-a propus să investigheze procentul și tiparele rezistenței antimicrobiene la tulpinile de *E. coli* izolate de la câini cu infecții ale tractului urinar (ITU) de la un spital și clinici veterinare locale din Cluj-Napoca. Probele de urină recoltate prin cistocenteză de la animale cu ITU confirmate au fost supuse prelucrării microbiologice care implică cultivare pe medii de îmbogățire și selective, dar și efectuarea de antibiograme. În general, ratele de susceptibilitate determinate față de antibioticele cel mai frecvent utilizate în practica animalelor mici au fost relativ scăzute și a fost observată rezistența la cel puțin doi agenți antimicrobieni. Au fost stabilite mai multe modele distincte de rezistență care implică în special rezistența la mai multe medicamente (MDR). Cele mai mari rate de rezistență au fost înregistrate față de penicilină G, kanamicină si enrofloxacină (66.27%), urmate de cloramfenicol (60.24%), amoxicilină/acid clavulanic (59.04 %), ampicilină (51.81%), tetraciclină și marbofloxacină (48.19%). Aceste rezultate subliniază importanța monitorizării utilizării antibioticelor și a modelelor de rezistență în gestionarea ITU asociate cu *E. coli* la câini.

Cuvinte cheie: *Escherichia coli*, câini, infecții urinare, rezistență antimicrobiană, antibioterapie

Urinary tract infections (UTIs) represent a top-ranked reason for consultation in canine medicine (2, 10, 16, 24). This pathology generally occurs when host defence mechanisms (normal micturition, anatomic structures, the mucosal barrier, properties of urine, and systemic immunocompetence) are compromised or altered, allowing virulent and potentially pathogenic organisms to adhere, multiply, and persist at the urinary tract level (14). Microorganisms, including bacteria, mycoplasma, fungi, and viruses, are the principal causes of canine UTIs (4,12). According to the currently available literature, canine UTI aetiology commonly includes bacteria, and one of the most important etiological agents associated with UTI is represented by *Escherichia coli* (*E. coli*) (21,27). While this bacterium is described as the organism most frequently cultured in both simple and complicated UTIs, *Enterococcus spp*. and Pseudomonas spp. are increasingly associated with the recurrent ones (21,23). Given this etiopathology background, UTI therapeutical protocol includes the use of antibiotics, one or even two antimicrobial agents' association (2,12,18,24). As this method of therapy is worldwide recognised, antibiotic treatment guidelines were established and updated by the International Society for Companion Animal Infectious Diseases (ISCAID) (24). Similar to the situation of other diseases that require the use of anti-

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biotics, in the case of urinary tract infections in dogs and cats, the problem of the emergence of antibiotic resistance is raised, and the mechanisms underlying the occurrence and spread of this phenomenon are suggested or demonstrated (8,15,19,21,22). Practically, the current antibiotic therapy guidelines advocate the importance of the rational use of antibiotics and contraindicate their abuse and unjustified use (2, 10, 22, 24). Increased levels of antibiotic resistance have been reported for canine uropathogenic *E. coli* (1, 6, 13, 16, 22, 25, 27).

In this context, the present study aimed to evaluate and characterise the level of antibiotic resistance in *E. coli* strains isolated from dogs and cats with urinary tract infections (UTI), representing the case of the Emergency Hospital, FMV Cluj-Napoca, and local veterinary clinics from Cluj-Napoca.

MATERIALS AND METHODS

The research was conducted between 2022 and 2024 and included a total number of 83 *E. coli* isolates originating from dogs diagnosed with UTI. These strains were previously isolated using referred urine samples aseptically collected by cystocentesis. As detailed data regarding the canine patients (breed, sex, age, reproductive status) and also the UTI, namely the course (acute, chronic, reinfection/relapse) and the antibiotic treatment (antimicrobial product, duration of therapy, response to therapy) were not provided for all cases, such data were not included in this research. The owner signed consent for using the microbiological data, which was obtained in all cases. The criteria used by distinct veterinary practitioners to diagnose UTI included clinical signs of pollakiuria, pain during urination, fever or vomiting, and the urine analyses with haematuria, pyuria, or cytologically evident bacteriuria (24).

Escherichia coli isolation and identification involved a preliminary culture on sheep blood agar under aerobic conditions at 37°C for 24 hours, followed by isolation on selective chromogenic agar (Brilliance™ E. coli / coliform Selective Agar, Oxoid Ltd., Cambridge/UK) and evaluation of biochemical properties using the API 20E system (BioMerieux SA, Marcy L'Etoile, France).

The antibiotic susceptibility testing of these isolates was evaluated *in vitro* using the standard Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI) (3). The tested antibiotics included aminoglycosides - kanamycin (30 µg), amikacin (30 µg), gentamicin (10 µg), tetracyclines – tetracycline (30 µg), doxycycline (30 µg), fluoroquinolones - enrofloxacin (5 µg), orbifloxacin (5 µg), marbofloxacin (5 µg), cephalosporins - cephalothin (30 µg), cefovecin (30 µg), cefaclor (30 µg), cefoxitin (30 µg), small and broad-spectrum penicillins - penicillin G (10 µg), ampicillin (10 µg), (amoxicillin/clavulanic acid) (20/10 µg), trimethoprim-sulphamethoxazole (25 µg), and chloramphenicol (30 µg). Based on the growth inhibition zone diameters (mm), compared to CLSI criteria, the strains were recorded as susceptible (S), intermediate (I) or resistant (R) (3). *Escherichia coli* ATCC® 25922 (Oxoid Ltd., Cambridge/UK) was also tested as a quality control organism. Statistical analysis was performed using Epi InfoTM7(CDC, Atlanta, GA, USA) software. The chi-square test was applied to compare the antimicrobial resistance rates (%) to antimicrobials. A p-value of ≤ 0.05 was considered to indicate statistically significant differences.

RESULTS AND DISCUSSIONS

Results obtained for the *in vitro* antimicrobial susceptibility testing of the urinary infections associated with the canine-origin *E. coli* isolates are displayed in Table 1 and Figure 1. Overall, the determined rates of antimicrobial susceptibility towards the antimicrobials most commonly used in dogs were relatively low, and resistance to at least two antimicrobial agents was noticed in canine isolates. The highest rates of resistance were recorded towards penicillin G, kanamycin and enrofloxacin (66.27%), followed by chloramphenicol (60.24%), amoxicillin/clavulanic acid (59.04%), ampicillin (51.81%), tetracycline and marbofloxacin (48.19%). The highest susceptibility was found for cefoxitin (85.54%), trimethoprim-sulphamethoxazole (78.31%), cephalothin (59.04%), gentamicin (54.22 %), cefaclor (51.81%), cefovecin and doxycycline (49.40%). Significantly different rates ($p<0.05$) among antibiotic groups were determined (cephalosporins versus fluoroquinolones, cephalosporins versus broad-spectrum penicillin) (Fig. 1).

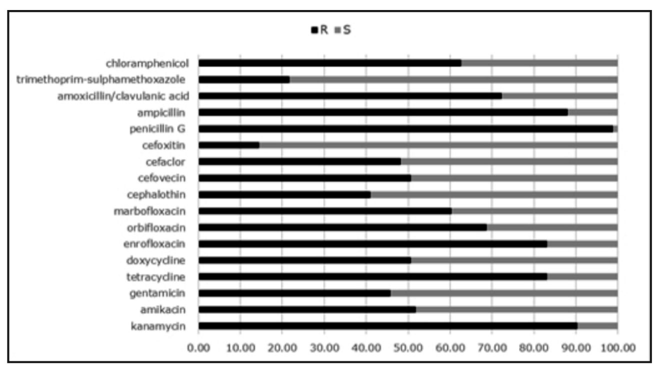

Fig. 1. Antimicrobial resistance rates of canine *E. coli* isolates

Nine distinct resistance patterns were established for canine isolates, notably involving multidrug resistance (MDR) and pandrug-resistance (PDR) (Table 2).

E. coli is the most frequent bacteria causing urinary tract infection in dogs (5,17). In the last few decades an increasing number of reports underlined the multidrug - resistant (MDR) profile of canine *E. coli* isolates (20). A rising concern regarding this aspect is further justified since the ownership of companion animals could be a risk factor in the spread of pathogenic *E. coli* strains between humans and pets, also favouring the dissemination of antimicrobial resistance in the community (19). Characteristics of AMR in uropathogenic strains of

Table 1

The *in vitro* antibiotic susceptibility for *E. coli* strains isolated from canine UTI

Antibiotic	Code	R		I		S	
		number	%	number	%	number	%
Kanamycin	K	55	66.27	20	24.10	8	9.64
Amikacin	AK	33	39.76	10	12.05	40	48.19
Gentamicin	CN	6	7.23	32	38.55	45	54.22
Tetracycline	T	40	48.19	29	34.94	14	16.87
Doxycycline	DO	32	38.55	10	12.05	41	49.40
Enrofloxacin	ENF	55	66.27	14	16.87	14	16.87
Orbifloxacin	OR	20	24.10	37	44.58	26	31.33
Marbofloxacin	MAR	40	48.19	10	12.05	33	39.76
Cephalothin	CF	21	25.30	13	15.66	49	59.04
Cefovecin	CVN	12	14.46	30	36.14	41	49.40
Cefaclor	CEC	30	36.14	10	12.05	43	51.81
Cefoxitin	FOX	5	6.02	7	8.43	71	85.54
Penicillin G	P	55	66.27	27	32.53	1	1.20
Ampicillin	AM	43	51.81	30	36.14	10	12.05
Amoxicillin/clavulanic acid	AMC	49	59.04	11	13.25	23	27.71
Trimethoprim-	SXT	12	14.46	6	7.23	65	78.31
Chloramphenicol	C	50	60.24	2	2.41	31	37.35

Table 2

Antibiotic resistance patterns of *E. coli* strains

K+AM+AMC+P
K+ENF+MAR+P
K+ENF+DO+P
K+ENF+DO+T+OR+P
K+AM+AMC+MAR+P
K+AM+MAR+P
K+AM+ENF+MAR+P
K+AM+AMC+P
K+AM+AMC+C+P

E. coli may vary between different studies (20). A study conducted in Spain revealed an extremely high level of phenotypic resistance to 1st–3rd generation cephalosporins, followed by penicillins, fluoroquinolones and amphenicols. Apart from that, 13.46% of them were considered extended-spectrum beta-lactamase producers. An alarmingly high percentage (71.15%) of multidrug-resistant isolates were also detected (1). Another recent study presented an elevated prevalence of *E. coli* resistance towards ampicillin (68% in dogs, 100% in cats) and ampicillin with sulbactam (59% in dogs, 54% in cats); the most common antimicrobial resistance patterns of *E. coli* were ampicillin alone (12 isolates, 29.3 % in cats) and beta-lactams, including aztreonam (14 isolates, 21.2% in dogs) (7).

Based on results obtained by the microdilution method, 39% of *E. coli* was considered multidrug-resistant especially to ampicillin (40.5%), ceftazidime (59.5%), and florfenicol (42.9%), but with limited resistance against amikacin (2.38%), meropenem (7.14 %), and polymyxin E (7.14%) (27). Considerable levels of antimicrobial resistance were reported also for UPEC strains isolated from Iranian dogs. UPEC strains had the highest levels of resistance against gentamicin (95%), ampicillin (85%), amikacin (70%), amoxicillin (65%), and sulfamethoxazole-trimethoprim (65%) (26). High levels of AMR to penicillin-G (99%), clindamycin (100%), tylosin (95%), and cephalothin (84%) but relatively low levels of resistance to enrofloxacin (16%), and orbifloxacin (21%) were described by researchers from South Africa. Almost all (98%, 164/167) of the isolates exhibited multidrug resistance (MDR) (16). Similar data confirmed a large number of UPEC strains with a high level of resistance against several classes of antibiotics with respect to the prevalence of chloramphenicol resistance genes isolated from dogs' urine in India.

The UPEC isolates were found sensitive to chloramphenicol (84%), meropenem (64%), gentamicin (44 %) and amikacin (40%), while all the isolates showed resistance against ampicillin (25%), ciprofloxacin (25%) and tetracycline (25%) (11).

The multidrug-resistant *E. coli* strains recovered from the urinary samples in this study emerged most likely as a consequence of the antibiotics use. Unfortunately, detailed epidemiological data on the animals and antimicrobial regimen were not available to confirm this hypothesis. Still, our results are valuable in indicating the occurrence of MDR strains and the current susceptibility profiles.

The high prevalence of resistance to commonly used antibiotics in this study emphasises the need for the development and implementation of alternative therapeutic strategies for canine urinary tract infections. Further research should focus on exploring novel antimicrobial agents, bacteriophages, or other interventions to effectively treat infections caused by multidrug-resistant *E. coli*. This is particularly important given the limitations of current antibiotic therapies in addressing this growing problem.

CONCLUSIONS

The canine urinary isolates investigated in the current study displayed antimicrobial resistance, underlining the stringent need to evaluate and record the *in vitro* susceptibility patterns before the therapy. Given the chronic course of urinary infections, the rational use of antimicrobials is highly advisable to avoid further augmentation of the AM level. This study underscores the importance of ongoing surveillance of antimicrobial resistance patterns in canine uropathogens.Regular monitoring of resistance trends, coupled with detailed epidemiological data on antibiotic use in veterinary practice, is vital for informing evidence-based treatment strategies and developing novel approaches to combat antimicrobial resistance in both veterinary and human medicine.

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