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REVIEW ON THE AETIOLOGY OF URINARY TRACT INFECTIONS IN DOMESTIC CARNIVORES AND THEIR ANTIBIOTICS-RESISTANCE

RECENZIE PRIVIND ETIOLOGIA INFECȚIILOR TRACTULUI URINAR LA CARNIVORELE DOMESTICE ȘI REZISTENȚA LOR LA ANTIBIOTICE

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ABSTRACT | REZUMAT

Antibiotic resistance is the natural or acquired ability of microorganisms to adapt and multiply in the presence of antibiotics. In veterinary medicine, empirical treatments are often used for lower urinary tract infections. These practices lead to an increase in antibiotic resistance, which poses a threat for both animals and humans. Investigations were carried out on 102 urine samples collected from 64 cats and 38 dogs diagnosed with infectious uropathy. Bacterial isolates obtained from urinary tract infections were purified and tested by the Kirby-Bauer diffusimetric method. In the tested canine patients (n=38), 94.73% (n=36) had positive microbiological test results and 5.26% (n=2) of the urocultures were negative. Of the 64 feline patients tested, 75% (n=48) had positive uroculture results and 25% (n=16) had negative results. Gram-positive strains isolated from cats showed the highest resistance to chloramphenicol (50%) and a significantly lower incidence than the other antibiotics: gentamicin (28.94%), enrofloxacin (20%), trimethoprim + sulfamethoxazole (20%), amoxicillin + clavulanic acid (10%), cephalexin (10%), and cefadroxil (10%). In dogs with urinary tract infections, the highest resistance was reported for florfenicol (20.27%), followed by cephalexin (18.18%), amoxicillin + clavulanic acid, gentamicin, chloramphenicol, trimethoprim+sulfamethoxazole, and doxycycline (9.09%). Gram-negative strains isolated from urinary tract infections in cats showed distinct resistance to the whole panel of antibiotics: enrofloxacin (34.21%), amoxicillin + clavulanic acid (31.57%), oxytetracycline (31.57%), gentamicin (28.94 %), cephalexin (28.94%), doxycycline (26.31%), lincomycin (21.05%), florfenicol (18.42%), chloramphenicol (18.42%), marbofloxacin (13.15%), trimethoprim+sulfamethoxazole (18.42%), and cefadroxil (7.89%). Gram-negative strains isolated from dogs showed the same pattern of extensive resistance to all tested antibiotics. The highest resistance was to chloramphenicol (40%), followed by: amoxicillin + clavulanic acid and trimethoprim+sulfamethoxazole (36%), florfenicol (24%), enrofloxacin (24%), lincomycin (20%), oxytetracycline (20%), cefadroxil (20%), cephalexin (16%), marbofloxacin (12%), doxycycline (12%), and gentamicin (9.09%).

Keywords: dog, cat, urinary tract infections, antibiotic resistance

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Antibiorezistența este capacitatea naturală sau dobândită a microorganismelor de a se adapta și de a se multiplica în prezența antibioticelor. În medicina veterinară, pentru infecțiile tractului urinar inferior sunt deseori utilizate tratamente empirice. Aceste practici duc la o creștere a antibiorezistenței, ceea ce reprezintă o amenințare atât pentru animale, cât și pentru oameni. Investigațiile au fost efectuate pe 102 de probe de urină recoltate de la 64 de pisici și 38 de câini a căror diagnostic a fost de uropatie de natură infecțioasă.Izolatele bacteriene obținute din infecțiile tractului urinar au fost purificate și testate prin antibiogramă - metoda difuzimetrică Kirby-Bauer. La pacienții canini testați (n=38), 94,73% uroculturi (n=36) au fost pozitive microbiologic, iar 5,26% (n=2) au fost negative. Dintre pacienții felini testați (n=64), 75% (n=48) au avut rezultate pozitive la urocultură și 25% (n=16) au avut rezultate negative. Tulpinile Gram-pozitive izolate de la pisici au prezentat cea mai ridicată rezistență față de cloramfenicol (50%) și rezistență semnificativ mai mică față de celelalte antibiotice: gentamicină (28,94%), enrofloxacină (20%), trimetoprim+sulfametoxazol (20%),amoxicilină+acid clavulanic (10%), cefalexină (10%) și cefadroxil (10%). La câinii cu infecții ale tractului urinar, cea mai ridicată rezistență a fost semnalată la florfenicol (27,27%), urmat de cefalexină (18,18%), amoxicilină+acid clavulanic, gentamicină, cloramfenicol, trimetoprim+sulfametoxazol și doxiciclină (9,09%). Tulpinile Gram-negative izolate din infecțiile tractului urinar la pisici au manifestat rezistență cu o pondere diferită, față de tot panelul de antibiotice: enrofloxacină (34,21%), amoxicilină+acidclavulanic (31,57%), oxitetraciclină (31,57%), gentamicină (28,94%), cefalexină (28,94 %), doxiciclină (26,31%), lincomicină (21,05%), florfenicol (18,42%), cloramfenicol (18,42%), marbofloxacină (13,15%), trimetoprim+sulfametoxazol (18,42%) și cefadroxil (7,89%). Tulpinile Gram negative izolate de la câini au manifestat același model de rezistență extinsă față de toate antibioticele testate. Cea mai ridicată rezistență a fost manifestată față de cloramfenicol (40%), urmat de: amoxicilină+acid clavulanic și trimetoprim+sulfametoxazol (36%), florfenicol (24%), enrofloxacină (24%), lincomicină (20%), oxitetraciclină (20%), cefadroxil (20%), cefalexină (16%), marbofloxacină (12%), doxiciclină (12%) și gentamicină (9,09%).

Cuvinte cheie: câine, pisică, infecții urinare, antibiorezistență

Urinary tract infections are common in both humans and pet carnivores. Among the cases with uropathy, bacteriuria can reach 18.9% in cats (13) and 34.02%–60.27% in dogs (31, 48). Urinary tract infections are generally caused by microorganisms that enter the urinary tract and overgrow. Bacteria are the main pathogens of urinary tract infections, and only 1% of urinary tract infections are produced by other microorganisms such as fungi, viruses, and parasites (14). The pathogenetic mechanism of urinary tract infections in dogs and cats involves colonisation, adherence, multiplication of bacteria, and the triggering of an inflammatory response, leading to specific clinical symptoms(14).

Recent studies have demonstrated that the urinary tract has a unique microbiota (5, 43). Thus, during the lifetime, episodes of bacteriuria without clinical manifestations may occur, which can be identified only by quantitative microbiological determinations (42). Urinary tract infections always alter the appearance of urine and trigger clinical manifestations that can vary in severity, frequency, and location. The infectious process is triggered when, with a background of comorbidities, host defence mechanisms are compromised and pathogenic or conditionally pathogenic bacteria, which have invasive capacity, adhere to the mucosa of the urinary tract, multiply, and persist (35).

Early diagnosis and treatment are essential for effective management of urinary tract infections and prevention of complications (14). The therapeutic approach to urinary tract infections differs depending on the type of infection present. Two types of infection are described in the literature:

1) Sporadic infectious cystitis is a sporadic bacterial bladder infection that occurs in (a) non-pregnant females or castrated males with no other signs of disease; (b) patients without anatomic or functional abnormalities of the urinary tract; and (c) patients who have had fewer than three episodes of suspected or proven bacterial cystitis in the past 12 months. Diagnosis is made on the basis of clinical signs (urinary symptoms: haematuria, pyuria, obvious bacteriuria on cytology) and bacterial culture results.

2) Recurrent infectious cystitis involves a diagnosis of three or more episodes of bacterial cystitis in the last 12 months or two or more episodes in the last six months. Recurrent infectious cystitis may result from relapsing (or persistent) infection or reinfection. This information is useful for determining the diagnostic plan. Recurrent cystitis is usually associated with an underlying cause, and identifying this, along with risk factors, is of particular importance forlong-term therapeutic success (41).The therapeutic protocol is established in accordance with the recommendations suggested by the International Society for Companion Animal Infectious Disease (ISCAID), which is based on publications in the field and is regularly updated with data on antimicrobial use and therapeutic efficacy, as well as antimicrobial resistance (40).

The spread of antibiotic resistance is a well-known problem worldwide and is increasingly occurring in veterinary medicine. Usually, the selection of the most appropriate antibiotic in veterinary medicine is initially empirical, and later, antibiotic therapy is administered based on a microbiological examination. This practice can deregulate the commensal microbiota and select bacteria that are multiresistant to antibiotics. Microorganisms can be transferred between humans and animals through close contact and social interactions between them. Resistant bacteria can circulate among humans and animals via food, water, and the environment, with transmission between individuals influenced by trade, travel, and human and animal migration (4, 19, 45, 46).Antimicrobial resistance varies by geographic area and underscores the importance of developing local antibiotic use surveillance programmes (17, 40). This study aimed to provide an update on the aetiology of urinary tract infections in pet carnivores registered as patients in veterinary practices in the North-Eastern region of Romania. The second objective was to test the isolated bacterial strains against a panel of antibiotics commonly used in first-line therapy for infections.

MATERIALS AND METHODS

Sample collection

The collection of animal tissue samples for this study was approved as part of the study protocol. Investigations were performed on 102 urine samples collected from 64 cats and 38 dogs with clinical signs of cystitis or obstructive uropathy. Urine collection was performed by catheterization or cystocentesis. Patients selected for cystocentesis were approached indorsal recumbency under ultrasound guidance. After collection, urine was evaluated physically (odour, colour, appearance, turbidity, and specific gravity) and chemically by a semi-quantitative method using Abaxis urine strips for: pH, leukocytes, ketones, nitrites, urobilinogen, bilirubin, glucose, protein, ascorbic acid, microalbuminuria, calcium, creatinine, and protein/creatinine ratio. Subsequently, the urine was centrifuged for five minutes at 1500 rpm, and the urine sediment was microscopically examined (Leica microscope, Leica Microsystems, Germany)both directly and afterGram staining. The presence of red blood cells, white blood cells, epithelial cells, cylinders, crystals, and microorganisms was observed using 10 to 15 microscopic fields to count the urinary components (18). Urine sediment samples in which leukociteswere accompanied by microorganisms were further investigated microbiologically. Samples were processed within 24 hours of collection.

Bacterial isolation and identification

In order to obtain accurate and reliable results, the microbiological examination of urine samples underwent certain standard microbial identification procedures.

The Gram-stainedsmears were examined microscopically using immersion oil (x1000). Depending on the bacterial morphology identified in the sediment, microscopically positive samples (bacterial cells > 1000 germs/ml urine) were seeded on various culture media: Mueller Hinton Nutrient Broth (Oxoid), Mueller Hinton Nutrient Agar (Oxoid), and Nutrient Agar enriched with defibrinated horse blood (Bio-Rad) and selective chromogenic media such asUriSelect Agar (Uri Select™4 Medium; Bio-Rad Laboratories, California), CLED Agar (Cystine-Lactose-Electrolyte Deficient, Bio -Rad), Chapmann Agar (Oxoid), and Levine Agar (Eosin Methylene Blue Agar Levine, Bio-Rad).

Incubation was performed at 37 °C for 24–48 hours. Cultures obtained were purified and culturally, morphologically, and biochemically examined for identification of bacterial species and genera (6).

Phenotypic characteristics of purified bacterial strains were correlated with the results of bacterial metabolism testing determined by miniAPI galleries for aerobic bacteria - API 20 NE, API 20 E, rapid ID 32 E, API 3.2 GN, or RapID™ STAPH PLUS System (Bio Mérieux, France). Biochemical tests do not always lead to a satisfactory identification of a bacterium; the diagnosis iscompleted by antigen-antibody serological reactions (ProlexStreptococcal Grouping Latex Kit; Pro-Lab Diagnostics, USA).

Antimicrobial susceptibility

Microbial suspensions were adjusted to a density equivalent to 0.5 McFarland Standard ($1.5x10^{8}$ cfu/ml),and then Mueller-Hinton agar plates were seeded. Bacterial isolates were purified and tested by the Kirby -Bauer diffusimetric method, using a standardised procedure to determine the susceptibility or resistance of bacteria to different antibiotics.

The panel of antibiotics included 12 agents: Amoxicillin+clavulanic acid (AMC, 20+10µg), Gentamicin (GN, 10µg), Florfenicol (FFC, 30µg), Chloramphenicol (C,30µg), Enrofloxacin (ENR, 30µg), Marbofloxacin (MAR,30µg), Trimethoprim+sulfamethoxazole (STX, 1.25+23.75µg), Lincomycin (LNC,15 µg), Doxycycline (DOX, 30µg), Oxytetracycline (OT,30 µg), Cephalexin (CL, 30µg), and Cefadroxil (CFR, 30µg).

Results were obtained after incubation at 37 C for 24 h. The working protocol and interpretation were carried out according to the techniques described in the main recommended standards (10, 20).

The results are expressed as sensitive, resistant, or intermediate (moderately sensitive).

RESULTS AND DISCUSSIONS

Patients were examined for clinical signs consistent with lower urinary tract disease, including pollakiuria, dysuria, periuria, stranguria, and absence of urination prior to the clinical examination.Pyuria (>3-5 white blood cells/high power field) and haematuria (>3-5 red blood cells/high power field) were present in all analysedurine samples.

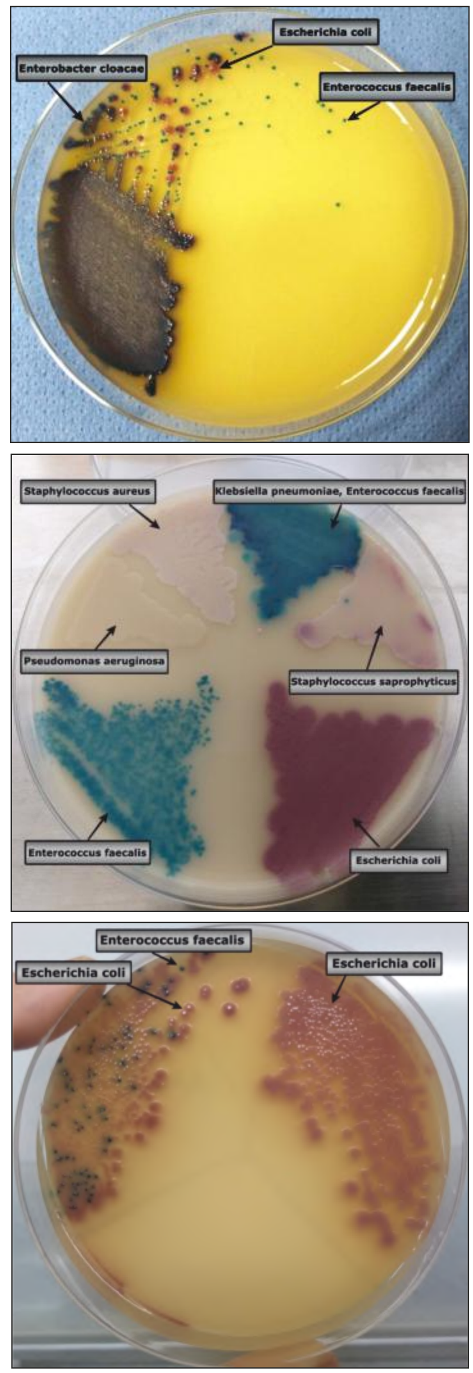

Fig. 1. Bacterial species isolated from urine cultures: cultural aspects on the UriSelect non-selective chromogenic medium (Bio-Rad)

Table 1

Bacterial species isolated from urine cultures from dogs and cats

Type of bacteria	Bacterial species	feline	canine
		Number of isolated (%)	
TOTAL= 84 (100%)		**48**(57.14%)	**36** (42.85%)
Gram(+)	*Staphylococcus pseudintermedius*	2/48 (4.16%)	3/36 (8.33%)
	Macrococcuscaseolyticus	1/48(2.08%)	1/36 (2.77%)
	Enterococcus faecalis	6/48(12.50%)	5/36(13.88%)
	Streptococcus canis	0	1/36(2.77%)
	Corynebacterium urealyticum	1/48(2.08%)	1/36(2.77%)
	Total	**10/48(20.83%)**	**11/36(30.55%)**
Gram (-)	*Escherichia coli*	20/48(41.66%)	13/36(36.11%)
	Klebsiella pneumoniae	2/48 (4.16%)	2/36(5.55%)
	Acinetobacter baumannii	2/48 (4.16%)	1/36(2.77%)
	Enterobacter cloacae	2/48 (4.16%)	1/36(2.77%)
	Citrobacterfreundii	2/48 (4.16%)	1/36(2.77%)
	Serratia marcescens	2/48 (4.16%)	1/36(2.77%)
	Proteus vulgaris	3/48 (6.25%)	4/36(11.11%)
	Proteus mirabillis	2/48 (4.16%)	0
	Pseudomonas aeruginosa	3/48 (6.25%)	2/36(5.55%)
	Total	**38/48**(79.16%)	**25/36**(69.44%)
TOTAL		**n=48(100%)**	**n=36(100%)**

Gram-stained urine smears also revealed the presence of bacteria, providing information about shape, mode, disposition, dye affinity. The use of nonselective chromogenic medium Uri Select proved to be very useful, allowing direct identification of *Escherichia coli, Proteus mirabilis, Enterococcus* spp. and presumptive identification of *Klebsiella pneumoniae, Enterobacter cloacae, Serratia marcescens, Streptococcus* spp, *Staphylococcus* spp. (Fig. 1)

Over the study period, the urine culture results were positive for 75% (n=48)of the feline patients. The risk factors noted for these patients were cats older than 10 years orhaving a previous history of urethral obstruction and catheterization. Previous research has also described the increased risk of urinary tract infections in cats in this category (7, 9, 25). Microbiological testing resulted in the isolation of 84 (100%) bacterial strains, of which 36 (42.85%) were isolated from dogs and 48 (57.14%) from cats. Based on cultural, morphological, and biochemical phenotypes, all isolates were classified up to the bacterial species level (Table 1).

In our study, the aetiology of urinary tract infections was bacterial, with predominant Gram-negative species isolated from both dogs (69.44%) and cats (79.16%). A significantly lower proportion of Gram-positive isolates were obtained from dogs (30.55%) and cats (20.83%). In cats, Gram-positive isolates were assigned to the following bacterial species: *Staphylococcus pseudintermedius* (2 strains, 4.16 %), *Macrococcuscaseolyticus* (2.08%), *Enterococcus faecalis* (12.5%), and *Corynebacterium urealyticum* (2.08%). Gram-negative isolates were assigned into 9 bacterial species, with the highest proportion attributed to *Escherichia coli* (41.66%), followed by *Proteus vulgaris* (6.25%), *Pseudomonas aeruginosa* (6.25%), *Klebsiella pneumoniae* (4.16%), *Acinetobacter baumannii* (4.16%), *Enterobacter cloacae* (4.16%), *Citrobacter freundii* (4.16%), and *Serratia marcescens* (4.16%). In dogs, Gram-positive bacterial isolates were classified as *Staphylococcus pseudintermedius* (8.33%),*Macrococcuscaseolyticus* (2.77%), *Enterococcus faecalis* (13.88%), *Streptococcus canis* (2.77 %), and *Corynebacterium urealyticum* (2.77%). Gram-negative isolates were also classified in the same bacterial species identified in cats, except for *Proteus mirabilis*, but with different frequencies: *Escherichia coli* (36.11%), *Proteus vulgaris* (11.11%), *Klebsiella pneumoniae* (5.55%), *Pseudomonas aeruginosa* (5.55%), *Acinetobacter baumannii* (2.77%), *Enterobacter cloacae* (2.77%), *Citrobacter freundii* (2.77%), and *Serratia marcescens* (2.77%). Similar results regarding bacterial aetiologywas also reported by Wong et al. (2015) (44). *Escherichia coli* is the most frequently isolated bacteria in both humans and animals. The results of our study are similar to those in previous studies (22, 23, 34, 39, 44).

In order to initiate an antimicrobial treatment, bacterial susceptibility to antibiotics commonly used in the therapy of urinary tract infections in dogs and cats was tested. Testing was performed for 12 antibiotics classified into 8 different classes: aminopenicillins and

Table 2

Susceptibility and resistance of bacterial isolates to the antibiotics used in the therapy of urinary tract infections

Antibiotic group	Antibiotics	Bacteria	Cats Susceptibility %	Cats Resistance %	Dogs Susceptibility %	Dogs Resistance %
Aminopenicillins + beta-lactamase inhibitors	AMC	G(+)	30	10	27.27	9.09
		G(-)	36.84	31.57	24	36
Aminoglycosides	GN	G(+)	60	10	36.36	9.09
		G(-)	26.31	28.94	52	12
Amphenicols	FFC	G(+)	50	0	27.27	27.27
		G(-)	42.1	18.42	36	24
	C	G(+)	10	50	18.18	9.09
		G(-)	36.84	18.42	32	40
Fluoroquinolones	ENR	G(+)	60	20	54.54	0
		G(-)	28.94	34.21	40	24
	MAR	G(+)	70	0	18.18	0
		G(-)	52.63	13.15	60	12
Sulfonamide+ diaminopyrimidine derivatives	STX	G(+)	30	20	36.36	9.09
		G(-)	39.47	18.42	32	36
Lincosamides	LNC	G(+)	40	0	63.63	0
		G(-)	34.21	21.05	36	20
Tetracyclines	DOX	G(+)	80	0	36.36	9.09
		G(-)	36.84	26.31	32	12
	OT	G(+)	30	0	81,81	0
		G(-)	31.57	31.57	36	20
Cephalosporin	CL	G(+)	50	10	54.54	18.18
		G(-)	34.21	28.94	24	16
	CFR	G(+)	10	10	54.54	0
		G(-)	31.57	7.89	52	20

Legend: Amoxicillin+clavulanic acid (AMC, 20+10 µg), Gentamicin (GN, 10 µg), Florfenicol (FFC, 30 µg), Chloramphenicol (C,30 µg), Enrofloxacin (ENR, 30 µg), Marbofloxacin (MAR,30 µg), Trimethoprim+sulfamethoxazole (STX, 1,25+23,75 µg), Lincomycin (LNC, 15µg), Doxycycline (DOX, 30µg), Oxytetracycline (OT, 30µg), Cephalexin (CL, 30µg), Cefadroxil (CFR, 30µg)

beta-lactamase inhibitors, aminoglycosides, amphenicols, fluoroquinolones, sulphonamides, lincosamides, tetracyclines, and cephalosporins (Table 2).

There were variations in susceptibility depending on the bacterial group to which the isolated species belonged (Gram-positive or Gram-negative). In dogs, the most effective antibiotics against Gram-positive species were oxytetracycline (81.81%), lincomycin (63.63 %), enrofloxacin, cephalexin, and cefadroxil (54.54%), and against the other antibiotics, the sus-ceptibility ratio was less than 50%. Gram-positive bac-terial species isolated from cats had a different sus-ceptibility profile than that shown in dogs, the most effective antibiotics being doxycycline (80%), marbofloxacin (70%), enrofloxacin and gentamicin (60%), and florfenicol and cephalexin (50%). Gram-negative bacterial strains isolated from both species were noted for reduced susceptibility to the panel of antibiotics tested. In cats, only marbofloxacin (52.63%) was effective in more than 50% of Gram-negative strains tested. In dogs, marbofloxacin (60%) was the most effective, followed by gentamicin and cefadaroxyl (52 %). The other antibiotics showed antimicrobial activity for less than 50% of Gram-negative strains tested (Fig. 2).

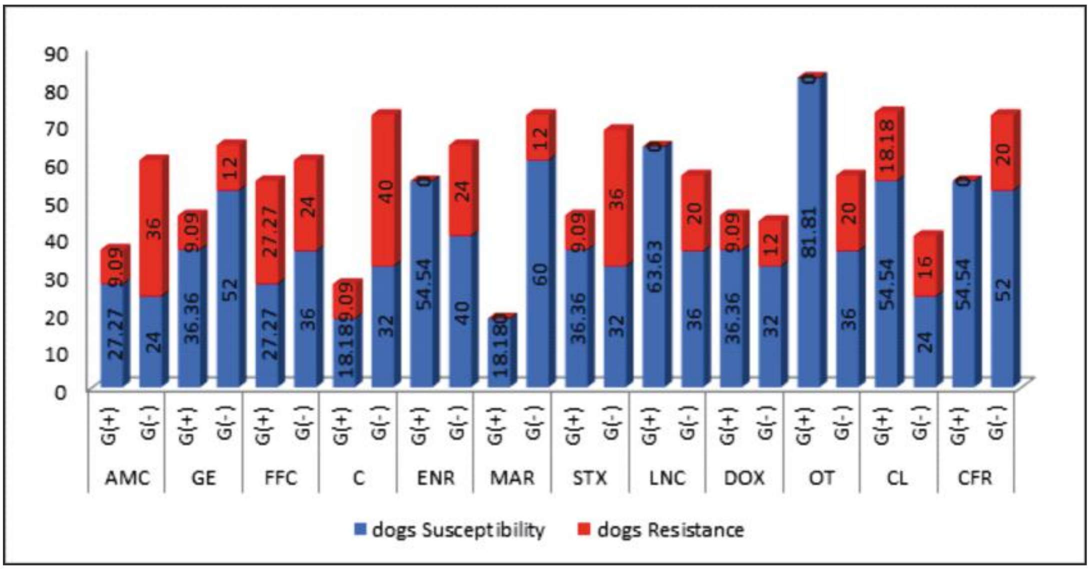

Fig. 2. Incidence of susceptibility and antimicrobial resistance of Gram-positive and Gram-negative strains isolated from urinary tract infections in dogs

Regarding bacterial resistance to the panel of antibiotics tested, a variable and obviously different incidence between Gram-positive and Gram-negative strains was noted. Antimicrobial resistance is a natural adaptive phenomenon that occurs over time and is based on a series of changes in bacterial genes under the selection pressure that antibiotics exert on microorganisms (8, 16).Gram-positive bacterial species isolated from cats had a different susceptibility profile than that shown in dogs, the most effective antibiotics being doxycycline (80%), marbofloxacin (70%), enrofloxacin and gentamicin (60%), florfenicol and cephalexin (50%). Gram-positive strains isolated from cats showed the highest resistance to chloramphenicol (50 %) and with a significantly lower incidence than the other antibiotics: gentamicin (28.94%), enrofloxacin (20%), trimethoprim+sulfamethoxazole (20%), amoxicillin + clavulanic acid (10%), cephalexin (10%), and cefadroxil (10%). There were no Gram-positive species resistant to florfenicol, marbofloxacin, lincomycin, doxycycline and oxytetracycline. The incidence of antimicrobial resistance in Gram-positive species isolated from dogs with urinary tract infections was lower, depending on the type of antibiotic. The highest proportion of resistance was reported for florfenicol (20.27%), followed by cephalexin (18.18 %), amoxicillin + clavulanic acid, gentamicin, chloramphenicol, trimethoprim+sulfamethoxazole, doxycycline (9.09 %). In isolates from dogs, no Gram-positive strains resistant to enrofloxacin, marbofloxacin, lincomycin,

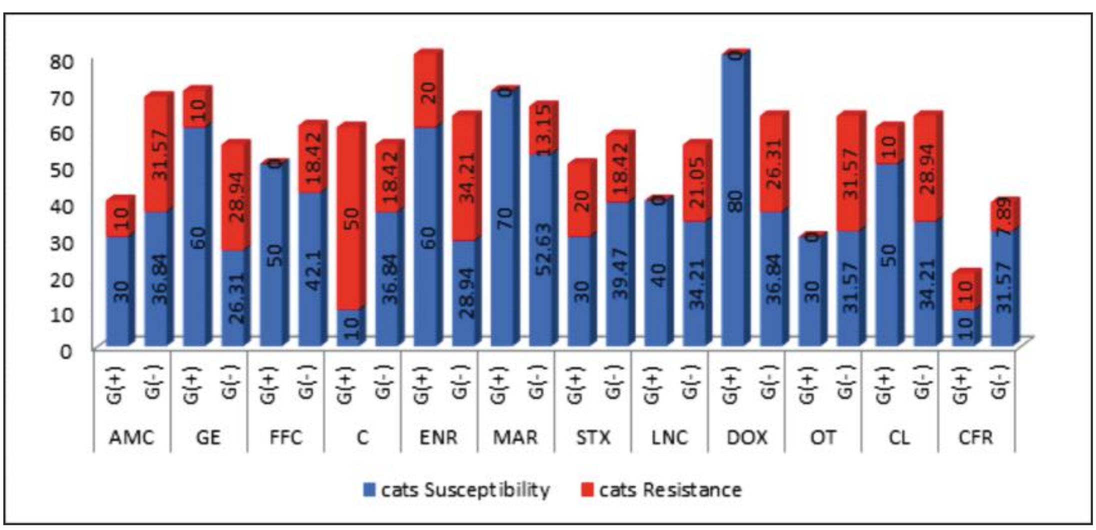

Fig. 3. Incidence of susceptibility and antimicrobial resistance of Gram-positive and Gram-negative strains isolated from urinary tract infections in cats

oxytetracycline, and cefadroxil were identified.

The resistance of Gram-negative species was increased and was more variable than those of Gram-positive species in both dogs and cats (Fig. 3).

Isolated strains from urinary tract infections in cats showed resistance at a different rate compared to the whole panel of antibiotics: enrofloxacin (34.21%), amoxicillin+clavulanic acid (31.57%), oxytetracycline (31.57%), gentamicin (28.94%), cephalexin (28.94 %), doxycycline (26.31%), lincomycin (21.05%), florfenicol (18.42%), chloramphenicol (18.42%), marbofloxacin (13.15%), trimethoprim+sulfamethoxazole (18.42%), and cefadroxil (7.89%). Gram-negative species isolated from dogs also showed the same pattern of extensive resistance to all antibiotics tested. The highest resistance was to chloramphenicol (40%), followed by: amoxicillin+clavulanic acid and trimethoprim + sulfamethoxazole (36%), florfenicol (24%), enrofloxacin (24%), lincomycin (20%), oxytetracycline (20%), cefadroxil (20%), cephalexin (16%), marbofloxacin (12%), doxycycline (12%), and gentamicin (9.09%). In pet carnivores with positive urine cultures, Gram-negative species (*Escherichia coli*, *Proteus* spp., *Klebsiella* spp.) are most commonly involved. In previous research, Dorschet al. (2015) and Punia et al.(2018) reached similar results (15, 36).

Analysing the data presented in Table 1, Gram-negative species showed resistance to all classes of antibiotics tested in different proportions. The prevalence of drug-resistant Gram-negative isolates was distinct between the two species of domestic carnivores. The highest resistance of Gram-negative species was observed for aminopenicillins + beta-lactamase inhibitors (36%) and sulphonamide + diaminopyrimidine derivatives (36%). The incidence of antimicrobial resistance of Gram-positive species isolated from urinary tract infections (*S.pseudintermedius*, *Macrococcus caseolyticus*, *Enterococcus faecalis*, *Streptococcus canis*, and *Corynebacterium urealyticum*) was only manifested with certain classes of antibiotics. Resistance of 10% (cat) and 9.09% (dog) to aminopenicillins + beta-lactamase inhibitors and aminoglycosides has been reported; resistance to amphenicols was 50% (cat) and 9.09-27.27% (dog); resistance to fluoroquinolones was 20% (cat); resistance to sulphonamide+ diaminopyrimidine derivatives was 20% (cat) and 9.09% (dog); resistance to tetracyclines was 9.09% (dog); and resistance to cephalosporins was 10% (cat). In our study, no Gram-positive strains resistant to florfenicol (amphenicols), marbofloxacin (fluoroquinolones), lincomycin (lincosamides), doxycycline, and oxytetracycline (tetracyclines) were isolated and identified from cat urinary infections. Similarly, to a significant extent, no Gram-positive isolates resistant to enrofloxacin and marbofloxacin (fluoroquinolones), lincomycin (lincosamides), oxytetracycline (tetracyclines), and cefadroxil (cephalosporins) were identified in dogs. Although the particular ability of bacteria to develop resistance mechanisms to antibiotics is demonstrated, we note in this study the sensitivity of pathogenic isolates to some classes of antibiotics that are used in the first-line therapy of urinary tract infections. It is also confirmed that, due to their distinct structure, Gram-negative species have intense resistance and a much higher capacity to develop new antibiotic resistance mechanisms compared to Gram-positive species (3). Gram-negative bacteria have a thin wall of peptidoglycans, located between the cytoplasmic and outer membranes, while Gram-positive bacteria have a thick cell wall of peptidoglycan, which is located outside the cytoplasmic membrane, a difference that gives them their dying character(33). The outer membrane is the main opponent to antimicrobials from different classes of antibiotics (beta-lactams, quinolones, and polymyxins) (21, 32). In Gram-positive bacteria, this membrane is missing, which makes them more vulnerable to the action of antibiotics, an aspect that also emerged from the testing of the isolates in our study.

The resistance of Gram-negative isolates to aminopenicillins + beta-lactamase inhibitors (36%) was expected, since the production of beta-lactamases is one of the most important mechanisms of bacterial resistance. Those isolates havethe ability to inactivate aminopenicillins by cleaving the beta-lactam ring in the antibiotic structure. The presence of the beta-lactamase inhibitor (clavulanic acid) is intended to prevent this inactivation (3,37). However, Gram-negative bacteria have developed new bacterial strains that are less sensitive or even resistant to beta-lactamase inhibitors, an aspect also seen in isolates from urinary tract infections. Resistance of Gram-negative bacteria to sulphonamide+ diaminopyrimidine derivatives (trimethoprim + sulfamethoxazole) develops mainly through changes or mutations in the dihydrofolate reductase (DHFR) enzyme and changes in the flux of chemicals into the cell. Trimethoprim acts by inhibiting the enzyme DHFR, which is required for folic acid synthesis in bacteria. Folic acid is essential for DNA and protein synthesis, making disruption of this metabolic pathway lethal to bacteria (27). There are also other mechanisms by which Gram-negative bacteria protect themselves: inefficient transport of sulphonamide+ diaminopyrimidine derivatives into the bacterial cell (impermeability), overproduction of modified DHFR enzymes, or plasmid transfer of resistance genes between bacteria (47). Gram-negative bacteria have much more aggressive pathogenic characters than Gram-positive ones, with the morbidity and mortality caused by them being significant worldwide (1-3,11). Their involvement in urinary tract infections has an oscillating character (24, 26, 36). Gram-positive species isolated from urinary tract infectionsshowed good susceptibility to all classes of antibiotics tested, with the exception of the amphenicol antibiotic class (chloramphenicol and florfenicol). Resistance was observed in strains isolated from both cats (50%) and dogs (9.09–27.27%). This surprising resistance is difficult to explain given that no abuse of chloramphenicol and florfenicol was found. The first and most common mechanism of resistance is the inactivation of the antibiotic by acetylation, due to enzymes such as chloramphenicol acetyltransferases, which modify the antibiotic by adding acetyl groups, inactivating its activity (38). Other pathways are also mentioned: mutations affecting the structure of antibacterial targets; presence of modified ribosomal enzymes that are less sensitive to chloramphenicol and florfenicol; preventing or hindering the binding of the antibiotic to the ribosome(28). Long-term use (more than 10 days) of a single antibiotic may result in the selection of bacteria resistant not only to that antibiotic but also to other antibiotics such as ampicillin, streptomycin, chloramphenicol,and sulphonamides (12). Under continuous microbial selection, colonisation of the digestive tract with resistant bacteria occurs. Studies conducted on chickens showed that after a few days of administering tetracycline in their food, it was possible to isolate strains of *Escherichia coli* resistant to this class of antibiotics (29, 30). Pet carnivores may come into contact with multidrug-resistant bacteria from the environment or from contaminated chicken meat and subsequently develop urinary tract infections when imbalances in the body's defence mechanisms occur.

CONCLUSIONS

In our study on 102 urine samples collected from 64 cats and 38 dogs with urinary tract infections, the prevalence of drug-resistant Gram-negative isolates was distinct between the two species of domestic carnivores. The highest resistance of Gram-negative species was observed for aminopenicillins + beta-lactamase inhibitors (36%), and sulphonamide + diaminopyrimidine derivatives (36%). Gram-negative strains isolated showed a pattern of extensive resistance to all antibiotics tested. Gram-positive strains isolated from cats showed the highest resistance to chloramphenicol (50%), followed by gentamicin (28.94%), enrofloxacin (20%), trimethoprim+sulfamethoxazole (20%), amoxicillin+clavulanic acid (10%), cephalexin (10%), and cefadroxil (10%). In dogs, the highest resistance was obtained for florfenicol (20.27%), cephalexin (18.18%), amoxicillin + clavulanic acid, gentamicin, chloramphenicol, trimethoprim+sulfamethoxazole and doxycycline (9.09%). Previous studies also reported variable levels of resistance depending on the geographical area. This indicates a need for regular monitoring of antimicrobial susceptibility. Based on the findings of this study, information about antimicrobial resistance deserves iteration and updating in Romania.

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