Antimicrobial susceptibility of bacteria isolated from urine samples in dogs

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Abstract
Urinary tract infections are very common in dogs, while the number of antimicrobials available for therapy has decreased due to the adaptation and evolution of bacterial populations. The aim of the study was to establish the prevalence of the main etiological agents involved in the pathogenesis of urinary tract infections, antimicrobial susceptibility and treatment alternatives.For this study, 50 dogs of different breeds, both females and males, aged between 2 years and 13 years were considered. The experimental part of this study took place between February 2018 and May 2019 in the Department of Microbiology, Faculty of Veterinary Medicine, Cluj-Napoca. Samples were processed using microscopic and cultural examinations. The Petri dishes were inoculated and incubated for 24 hours at 37°C, and interpreted, analyzing the cultural and morphological characteristics of the bacterial colonies as well as the presence or absence of haemolysis areas. The identification of bacterial species was performed by microscopic examination of cells from isolated colonies and biochemical examinations. Antibiotic susceptibility testing was performed by Mueller-Hinton agar disk diffusion technique. The most frequently isolated bacterial strain was E. coli, present in 21 samples of 54 (38.8%), followed by Staphylococcus spp. in 16 (29.6%), Streptococcus spp., present in 10 samples (18.5%), Proteus spp. with 4 samples (7.4%), and Klebsiella spp. and Pseudomonas spp., each present in 3 (5.5%). Bacterial associations were only observed in 7 samples, mainly involving E. coli and Proteus spp., while 4 samples were negative for bacterial growth. Increased susceptibility was observed for enrofloxacin, doxycycline and amoxicillin with clavulanic acid, while increased resistance was recorded for cefovecin, cephalexin and trimethoprim.
Keywords: *urinary tract infections, dogs, antimicrobial susceptibility.*

Introduction

Urinary tract infections in dogs are common, with about 15% of the dogs experiencing at least one bacterial infection during their lifetime (7). Cystitis caused by bacterial infections is usually accompanied by hematuria, dysuria, pollakiuria, stranguria and discomfort. The main source of bacteria invading the lower urinary tract is the colon and skin. The proximity of the rectum to the vulva makes this more common in female dogs than in males. The most frequently bacteria isolated from urinary tract infections the dog is *E coli*, followed by *Staphylococcus* species, *Proteus*, and *Klebsiella* (2). The presence of bacterial species in the lower urinary tract is in the typical form (planktonic bacteria) and biofilm, which is also represented by bacterial cells embedded into their own gel-like secretions. The main difference is that planktonic bacteria do not easily attach to the bladder and urinary tract walls, are more susceptible to antimicrobials, while biofilm is formed on inert surfaces such as implants, stents and urinary catheters, with major importance in antimicrobial resistance and recurrent infections (2,7).

The number of antimicrobials available for therapy has decreased due to the adaptation and evolution of bacterial populations (6,8). Bacterial species have evolved and responded to antimicrobial exposure and selective pressure, exhibiting new antimicrobial resistance mechanisms (3,5). Antimicrobial resistance is important in animal care management, treatment alternatives and health complications (1,4,5). The increased resistance to antimicrobials of urinary tract pathogens

is related to amoxicillin and clavulanic acid, quinolones, and third generation cephalosporins. These are all important veterinary antimicrobial agents (6).

The aim of the study was to establish the prevalence of the main etiological agents involved in the pathogenesis of urinary tract infections, antimicrobial susceptibility and treatment alternatives.

Materials and methods

The study was carried out in Cluj-Napoca, Faculty of Veterinary Medicine Cluj-Napoca, Department of Microbiology. Dogs from different breeds (n=50), 28 females and 22 males, aged between 2 years and 13 years were evaluated between February 2018 and May 2019. The urine samples collected were initially evaluated by microscopic examination, from urine sediment, followed by the inoculation of 1 ml urine onto the surface of a blood agar and MacConkey agar Petri plates.

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The microscopic examination of the bacteria was carried out using Gram staining technique. Biochemical identification was based on API 20 Biomerieux system (Bio Mérieux, France) and Vitek 2 technique. Susceptibility testing was performed using Kirby Bauer disk diffusion method. The antibiotics included in this study were represented by amoxicillin and clavulanic acid, doxycycline, ceftiofur, enrofloxacin, penicillin, cefovecin, trimethoprim and cephalexin.

Results and discussions

The most frequently isolated bacterial strain was *E. coli*, present in 21 samples of 54 (38.8%), followed by *Staphylococcus* spp. in 16 (29.6%), *Streptococcus* spp., present in 10 samples (18.5%), *Proteus* spp. with 4 samples (7.4%), and *Klebsiella* spp. and *Pseudomonas* spp., each present in 3 (5.5%). Bacterial associations were only observed in 7 samples, mainly involving *E. coli* and *Proteus* spp., while 4 samples were negative for bacterial growth.

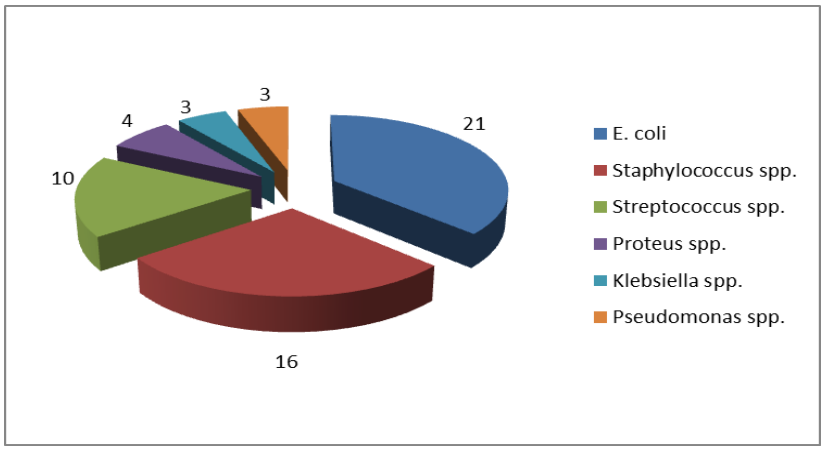

Fig. 1. Frequency of bacterial genera isolated from urine samples

Antimicrobial susceptibility performed by disk diffusion test was calculated as the average of the inhibition diameter area. The most efficient antibiotic was enrofloxacin with an average of inhibition diameter area of 15.22 mm, followed by doxycycline with 12.45 mm, amoxicillin and clavulanic acid (AMC) with 12.23 mm. For trimethoprim the inhibition diameter area was 10.9 mm, for ceftiofur 10.81 mm, for penicillin 10.5 mm, cephalexin 10.07 mm and for cefovecin 6.74 mm.

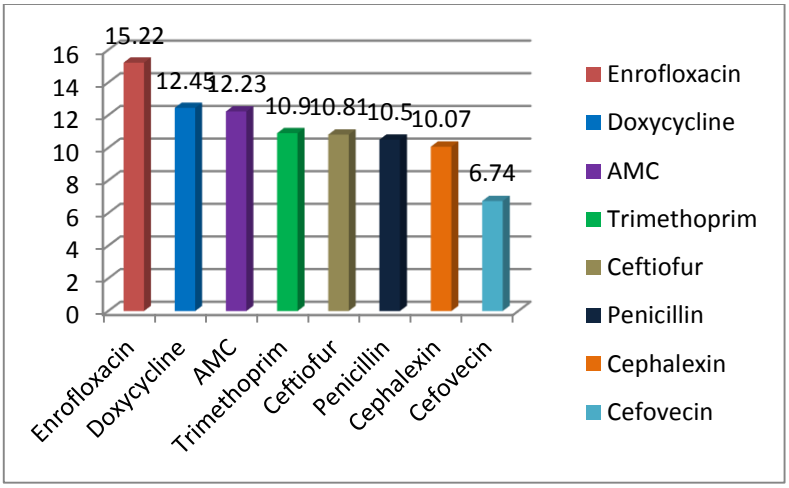

Fig. 2. The average of inhibition diameter area for the antimicrobials included in the study

The results of this study are consistent with the results of Hartmann and Thomson, with *E. coli* as the most frequently isolated pathogen, representing from 33-55% from the bacteria responsible for urinary tract, followed by *Staphylococcus* spp. and *Streptococcus* spp. (3,6). Regarding the antimicrobial susceptibility testing, past decade's trends describe an increase resistance to most antibiotics, from quinolones, third generation cephalosporins (7), doxycycline and amoxicillin-clavulanate (1).

Conclusions

The study regarding microbiological evaluation of urine samples in dogs and antimicrobial susceptibility testing of isolated strains concluded that:

- Both Gram positive and Gram negative bacteria were isolated, with a total percentage of 92.6, the rest of 7.4 samples were negative for bacterial growth;
- Gram negative species predominated, with a total of 62% from the total isolates;
- The antimicrobial profile showed moderate resistance to the antibiotics included in the study, with at least one antibiotic recommended in the treatment of each patient;
- Increased susceptibility was observed for enrofloxacin, doxycycline and amoxicillin with clavulanic acid, while increased resistance was recorded for cefovecin, cephalexin and trimethoprim.

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