

# Aetiology and local antimicrobial resistance patterns of bacterial pathogens causing dogs urinary tract infections from January 2019 to July 2024 in Split, Croatia

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## Abstract

Urinary tract infections in dogs are common in veterinary practice and one of the main reasons for the use of antimicrobial drugs. They are mostly caused by bacterial infections, while viral, fungal and parasitic infections account for less than 1% of cases. In practice, treatment usually starts with antimicrobial drugs selected based on existing clinical experience, without knowing the specific pathogen or its sensitivity to a particular drug. The aim of this study was to present the local prevalence and antimicrobial resistance of the most common bacterial pathogens of dog urinary tract infections isolated from samples obtained in the city Split, Croatia and its surrounding areas. From January 2019 to July 2024, 897 urine samples were analysed bacteriologically, of which 307

were positive. Of the total number of bacterial isolates, 194 (62.8%) were Gram-negative bacteria and 115 (37.2%) were Gram-positive. The most frequently isolated bacterial pathogens were *E. coli* (45%), coagulase-positive *Staphylococcus* sp. (13.9%), *Proteus* sp. (10%), beta-haemolytic *Streptococcus* sp. (9.7%), coagulase-negative *Staphylococcus* sp. (7.4%), *Enterococcus* sp. (5.5%), *Pseudomonas* sp. (4.2%) and *Klebsiella* sp. (2.6%). Data on local susceptibility and resistance patterns of the most common uropathogens can help clinicians in the selection of antimicrobial drugs and can serve as a basis for antimicrobial resistance monitoring in the coming years.

**Key words:** UTI; dog; uropathogen; antimicrobial resistance

## Introduction

Urinary tract infections (UTI) in dogs are common in veterinary practice and one of the main reasons for the use of antimicrobial drugs (Sykes and Westropp, 2014; Hernando et al., 2021). Mostly caused by bacterial infections, while viral, fungal and parasitic infections

account for less than 1% of UTI cases (Kogika and Waki, 2015; Dorsch et al., 2019). Fungal infections, including *Candida* spp., have been described in cases of immunosuppression, chronic urinary tract disease and in animals receiving concurrent antibiotic therapy (Reagan et al., 2019; Dowling, 2023).

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## Aetiology of UTIs

The urinary system is sterile, with the exception of distal urethra and external genitalia, where a physiological (commensal) microflora is present (Lanzi et al., 2022). Infections are most frequently caused by members of this microflora or the commensal flora of the intestine (ascending infection) (Rodriguez, 2016). Less frequently, pathogens can enter the lower urinary tract via the descending route from the kidneys or can be spread haematogenously (Littman, 2011; Sykes and Westropp, 2014). Essentially an infection, there are two conditions for developing infection; temporary or permanent decrease in host immunity, and presence of a sufficient quantity of virulent pathogens (Kogika and Waki, 2015; Kocúreková et al., 2021). In general, the bacterial pathogens causing UTIs are similar in dogs and cats (Labato, 2009). *Escherichia coli* is the most commonly isolated pathogen in dogs, cats and humans (Kocúreková et al., 2021; Weese et al., 2019), accounting for 33–55% of isolated pathogens (Lima et al., 2021; DiBartola and Westropp, 2023). Other frequently isolated bacteria include *Enterococcus* spp., *Staphylococcus* spp., *Streptococcus* spp., *Enterobacter* spp., *Proteus* spp., *Klebsiella* spp., *Pseudomonas* spp., *Pasteurella* spp., and *Corynebacterium* spp. (Sykes and Westropp, 2014; Byron, 2019; Lanzi et al., 2022). UTIs are most commonly caused by a single bacterial species, while concurrent infections involving two or more microorganisms are less common (Nelson and Couto, 2009). According to Smee (2020), in 75% of canine UTI cases, the infection is caused by a single bacterial species, 20% are caused by a combination of two species and only 5% involve three or more bacterial species. Mixed infections are more common in cases of prolonged use of urinary catheters or the presence of other concurrent diseases (Dorsch et al., 2016).

## Clinical forms of UTIs

Clinical signs depend on the location of the infection, duration of the disease, presence or absence of predisposing factors, the animal's immune response, as well as the virulence and quantity of the uropathogen (Smee, 2020). The most common clinical forms of lower urinary tract bacterial infections are sporadic and recurrent bacterial cystitis. These usually result in polyuria, stranguria, dysuria, inappropriate urination, or haematuria (Dorsch et al., 2019; Smee, 2020). Animals with upper UTIs may exhibit polyuria, polydipsia, pain in the lumbodorsal region, vomiting, hyperthermia, lethargy or anorexia (Rodriguez, 2016).

## Diagnosis

Diagnosis is based on the clinical signs of urinary tract inflammation, urinalysis (physical and chemical examination, as well as microscopic examination of urinary sediment), and the results of bacteriological examination. Physical examination of urine includes macroscopic assessment of colour and turbidity through inspection, and specific gravity testing with a refractometer. Chemical analysis is carried out using urine test strips, which are read with a spectrophotometer. This enables the semi-quantitative determination of values for pH, glucose, protein, ketones, erythrocytes/haemoglobin, leukocytes, urobilinogen and nitrates. Microscopic examination of urinary sediment determines the average number of cellular elements per field of view (erythrocytes, leukocytes, crystals, epithelial cells, hyaline casts, non-hyaline casts, microorganisms and other possible elements).

## Sample collection methods

Urine samples for urine culture should, whenever possible, be collected via cystocentesis to prevent contamination by bacteria present in the distal urethra, prepuce or vulva (DiBartola and Westropp, 2023). Other

collection methods (catheterisation or free-catch) are justified in cases where cystocentesis is contraindicated (e.g., suspected transitional cell carcinoma of the bladder or pyoderma of the ventral abdomen) (Smee et al., 2013) or when it is not feasible (animals with severe clinical signs of UTIs) (Pressler and Bartges, 2010). Van Duijkeren et al. (2004) found a negative result for urine culture in 79% of samples collected by cystocentesis. This value decreased to 55% when samples were collected via catheterisation and to only 17% when the same samples were collected by free-catch.

## Treatment

Treatment should always be based on the identified susceptibility of the bacterial pathogen to selected antimicrobial drugs, which often is not the case. For various reasons (financial constraints of the owner, efforts to improve treatment outcomes by early intervention, etc.), an etiologic diagnosis may be omitted, leading to the initiation of empirical antibiotic treatment.

Empirical treatment involves the use of antimicrobial drugs before the specific pathogen or its sensitivity to a particular drug is known, relying on existing clinical experience with a particular disease. Each empirical treatment carries risks, including selecting an ineffective antimicrobial drug, encountering side effects, and applying selective pressure on antimicrobials. This can ultimately lead to the proliferation of resistant bacterial strains, increased antimicrobial resistance, the emergence of multidrug-resistant strains, and disruption of the physiological microflora (Wong et al., 2015). Empirical treatment is approved in dogs with limited prior exposure to antimicrobials and in cases where the likely pathogens and their susceptibility are predictable. If the clinical response is favourable, empirical treatment is continued until an etiologic diagnosis is made. If there is no clinical improvement, the antibiotic is

replaced by another. If there is a suspicion of infection with bacteria such as *Escherichia coli* and *Staphylococcus* spp., whose susceptibility cannot be predicted, it is necessary to determine their sensitivity to antimicrobial drugs before treatment begins (Šeol et al., 2010).

## Selection of antimicrobial drugs

Amoxicillin, amoxicillin/clavulanic acid and trimethoprim/sulfonamides are considered first-line agents for the empirical treatment of UTI in dogs. Nitrofurantoin, fluoroquinolones and third-generation cephalosporins are only recommended in cases where there is resistance to first-line drugs (Weese et al., 2019). The World Health Organization (WHO) has published a list of critically important antimicrobial drugs that should not be used in veterinary medicine. These include third-, fourth- and fifth-generation cephalosporins, as well as quinolones (WHO, 2018). However, numerous studies have indicated an increasing use of fluoroquinolones and third-generation cephalosporins in the treatment of animals, particularly for UTIs (Buckland et al., 2016; Burke et al., 2017; Singleton et al., 2018; Van Cleven et al., 2018).

## Antimicrobial resistance

Antimicrobial resistance (AMR) is the ability of a microorganism to resist an antimicrobial treatment, making it less effective or completely ineffective. Although it is often mentioned in a negative context, AMR is actually a natural phenomenon in which microorganisms adapt to external influences. In many cases, it is the result of irrational use of antimicrobial drugs, during which microorganisms mutate and acquire resistance genes (WHO, 2016). The emergence of multidrug-resistant isolates, as a cause of infections, is particularly concerning. Based on documents and studies by the Clinical

and Laboratory Standards Institute (CLSI), the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the U.S. Food and Drug Administration (FDA), a standardised international terminology has been formulated to define bacterial resistance of *Staphylococcus aureus*, *Enterococcus* spp., *Enterobacteriaceae* (except *Salmonella* and *Shigella*), *Pseudomonas aeruginosa* and *Acinetobacter* spp. to antibiotics. Multidrug-resistance (MDR) is defined as bacterial resistance to at least one antibiotic from three or more antibiotic classes. Extensively drug-resistance (XDR) is defined as bacterial resistance to at least one antibiotic in all antibiotic classes, although resistance may not be present in one or two classes (i.e., the bacterial isolate is sensitive to only one or two antibiotic classes). Pandrug-resistance (PDR) is defined as bacterial resistance to all antibiotics in all antibiotic classes (Magiorakos et al., 2012).

The aim of this study was to present the local prevalence and antimicrobial resistance of the most common bacterial pathogens causing UTIs in dogs, isolated from samples obtained in Split area and its surroundings. The results will contribute to the understanding of local resistance patterns, thereby optimising treatment, promoting the rational use of antimicrobials, and reducing the emergence and spread of antimicrobial resistance.

## Materials and methods

Samples were received for analysis and processed at the Croatian Veterinary Institute, Split branch, Diagnostics laboratory. From January 2019 to July 2024, 897 urine samples from dogs with suspected UTIs were bacteriologically analysed. A total of 309 bacterial isolates were obtained from 307 bacteriologically positive samples. Isolation and identification of bacteria was performed according to standard laboratory operating procedures. The susceptibility of each isolated

pathogen to the appropriate antibiotics was tested using the disk diffusion method. Antimicrobial susceptibility results are shown for the bacterial species that were isolated and tested for a specific antimicrobial agent in at least five isolates.

## Bacteriological examination

The criteria for evaluating the results of bacteriological examination included growth density (10,000 colony-forming units (CFU)/mL), bacterial species (i.e., isolation of common uropathogens such as *Enterobacteriales* or coagulase-positive staphylococci), and growth in pure culture. Each sample was inoculated onto blood agar, MacConkey agar, *Pseudomonas* agar and Uriselect 4 chromogenic agar. After incubation under aerobic conditions at 37°C for 18–24 hours, the inoculated media were visually inspected to determine bacterial growth, morphology and approximate number of CFU/mL of urine. The number of CFU was determined separately. Identification of the genus was based on morphological and biochemical characteristics. All samples with  $\geq 10^4$  CFU/mL were considered positive. Growth in pure culture is of particular diagnostic importance as approximately 80% of urinary tract infections are caused by a single bacterial species, 17% by two species and only 3% by three species (Allen et al., 1987; Ling, 1995). The growth of several bacterial species usually indicates that one or more species are contaminants. If no or only slight bacterial growth was observed, the media were incubated for a further 24 hours. If no bacterial growth was detected after this period or if the growth was below the significance threshold ( $<10^4$  CFU/mL), the samples were declared bacteriologically negative.

## Antimicrobial susceptibility testing

The antimicrobial susceptibility of the bacteria was tested using the Kirby-Bauer

disk diffusion method. In this method, a suspension of a bacterial culture with a specific density is applied to an agar medium followed by placing paper disks impregnated with a specific concentration of antimicrobial agents on the surface. The plates are then incubated at 37°C for 16–18 hours, and the diameters of the inhibition zones surrounding the disks are measured. Interpretation of the bacterial growth inhibition zones was classified as: sensitive (S), intermediate (I) and resistant (R). Not all isolates were tested for every antimicrobial agent. The selection was based on clinical indications, animal species and intrinsic resistance of certain bacterial species. The entire antimicrobial susceptibility testing process was conducted and the results interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2018, 2019, 2020, 2021, 2022, 2023, 2024).

## Results

During the period from January 2019 to July 2024, a total of 897 dog urine samples were bacteriologically analysed and 307 samples (34.2%) tested positive, with 309 UTI pathogens isolated. A single bacterial species was isolated in 305 samples (99.3%), while two bacterial species were isolated from only two samples (0.65%). Of the total number of bacterial isolates, 194 (62.8%) were Gram-negative bacteria and 115 (37.2%) were Gram-positive. The most frequently isolated bacterial pathogens were *E. coli* (45%, 139/309), coagulase-positive *Staphylococcus* sp. (13.9%, 43/309), *Proteus* sp. (10%, 31/309), beta-haemolytic *Streptococcus* sp. (9.7%, 30/309), coagulase-negative *Staphylococcus* sp. (7.4%, 23/309), *Enterococcus* sp. (5.5%, 17/309), *Pseudomonas* sp. (4.2%, 13/309) and *Klebsiella*

**Table 1.** Bacterial isolates from urine samples testing positive (>10<sup>4</sup> CFU/mL).

	n	Percentage of total isolates recovered (n=309)
<b>Gram-negative bacteria</b>	194	62.8
<b>Enterobacterales</b>	178	57.6
<i>Escherichia coli</i>	139	45
<i>Proteus spp.</i>	31	10
<i>Pseudomonas spp.</i>	13	4.2
<i>Klebsiella spp.</i>	8	2.6
<i>Acinetobacter</i>	2	0.65
<i>Pasteurella</i>	1	0.32
<b>Gram-positive bacteria</b>	115	37.2
<i>Staphylococcus CPS</i>	43	13.9
<i>Streptococcus spp.</i>	30	9.7
<i>Staphylococcus spp.</i>	23	7.4
<i>Enterococcus spp.</i>	17	5.5
<i>Bacillus</i>	1	0.32
<i>Corynebacterium</i>	1	0.32



sp. (2.6%, 8/309). Bacterial pathogens present in less than 1% of cases included *Acinetobacter*, *Bacillus*, *Corynebacterium* and *Pasteurella* sp., and their antimicrobial resistances were not analysed in this study.

## Antimicrobial resistance profiles of bacterial isolates

### *Escherichia coli* isolates

Resistance to amoxicillin/clavulanic acid was detected in 34.3% (46/134) of isolates. Resistance to first-generation (cephalexin) and second-generation cephalosporins (cefoxitin) was 34.3% (47/137) and 39.1% (9/23), respectively. For the third-generation cephalosporins tested (cefotaxime, ceftazidime and ceftriaxone), resistance ranged from 8 to 11.8%. For the only tested fourth-generation cephalosporin, ceftazidime, resistance was observed in 13.5% (18/133) of isolates. Resistance to the tested fluoroquinolone antibiotics (enrofloxacin and marbofloxacin) amounted to 20.7% (28/135) and 19% (26/137), respectively. Resistance to meropenem from the carbapenem group was low, at 4.1% (2/49). The proportion of strains resistant to oxytetracycline was 36% (9/25). Resistance to sulfamethoxazole/trimethoprim was found in 21.4% (24/112) of the isolates. Resistance to gentamicin, the only antibiotic tested from the aminoglycoside group, was low at 4.4% (6/135).

### *Coagulase-positive Staphylococcus* spp. strains

Resistance to amoxicillin/clavulanic acid was found in 7% (3/39) of isolates. A high proportion of strains showed resistance to ampicillin, at 73.9% (17/23). Resistance to the first-generation cephalosporin, cephalexin, was detected in 7.7% of strains, while all isolates were sensitive to the second-generation cephalosporin, cefoxitin. For the third-generation cephalosporins tested (cefepime, cefotaxime and ceftazidime),

resistance ranged from 4.8 to 8%. Isolates showed a slightly higher resistance to ceftriaxone at 11% (1/9). Resistance to tested macrolide antibiotics (azithromycin and erythromycin) was observed in 35.1% (13/37) and 36.8% (14/38) of isolates respectively. Only 7.9% (3/38) of isolates were resistant to oxacillin. A total of 40.5% (15/37) of isolates were resistant to clindamycin. The highest resistance was recorded against penicillin, with 82.1% (23/28) of isolates showing resistance. Resistance to tested the fluoroquinolone antibiotics (enrofloxacin and marbofloxacin) was 8.7% (2/23) and 7.1% (3/42), respectively. Resistance to meropenem from the carbapenem group was low at 5% (2/40). Resistance to sulfamethoxazole/trimethoprim was found in 20.8% (5/24) of isolates.

### *Proteus* spp. strains

Resistance to amoxicillin/clavulanic acid was found in 30% (9/30) of isolates. Resistance to the first-generation cephalosporin, cephalexin, was observed in 48.3% (14/29) of isolates. For the third-generation cephalosporins tested (cefotaxime, ceftazidime and ceftazidime), resistance rates were 25% (5/20), 36.7% (11/30) and 30% (9/30), respectively. For the only tested representative of the fourth-generation cephalosporin, ceftazidime, resistance was found in 27.6% (8/29) of isolates. Resistance to fluoroquinolone antibiotics (enrofloxacin and marbofloxacin) was 32.3% (10/31) and 19.4% (6/31) respectively. Resistance to meropenem from the carbapenem group was found in 35.7% (5/14) of isolates. All tested isolates were resistant to oxytetracycline (10/10). Almost half of isolates, 46.2% (12/26), were resistant to sulfamethoxazole/trimethoprim. Resistance to gentamicin, an antibiotic from the aminoglycoside group, was found in 16.7% (5/30) of isolates.

### *Streptococcus* spp. strains

All isolates were 100% (30/30) susceptible to amoxicillin/clavulanic acid. Resistance

to ampicillin was found in 9.1% (1/11) of isolates. Resistance to the first-generation cephalosporin, cephalexin, was found in 6.7% (2/30) of strains, while 11.8% (2/17) showed resistance to the second-generation cephalosporin, cefoxitin. For the third-generation cephalosporins (cefpodoxime, cefotaxime and ceftiofur), the resistance rates were 13.3% (2/15), 4.5% (1/22) and 10% (3/30), respectively. Among the tested macrolide antibiotics (azithromycin and erythromycin), resistance was recorded in 20.7% (6/29) and 16.7% (5/30) of the isolates, respectively. Nearly half of the isolates, 48.1% (13/27), were resistant to clindamycin. Penicillin resistance was detected in 25% (5/20) of isolates. Resistance to the fluoroquinolones (enrofloxacin and marbofloxacin) amounted to 40% (6/15) and 13.3% (4/30), respectively. Resistance to meropenem, a carbapenem, was low at 3.3% (1/30). Sulfamethoxazole/trimethoprim resistance was found in 45.4 % (5/11) of the isolates.

### **Coagulase-negative *Staphylococcus* spp. strains**

Resistance to amoxicillin/clavulanic acid was detected in 8.7% (2/23) of isolates. Resistance to the first-generation cephalosporin, cephalexin, was found in 13% (3/23) of strains. Among the tested third-generation cephalosporins (cefotaxime, ceftiofur and ceftazidime), resistance rates were 12.5% (2/16), 13% (3/23) and 12.5% (1/8) of the isolates, respectively. For macrolides (azithromycin and erythromycin), resistance was observed in 50% (11/22) and 41% (9/22) of isolates, respectively. Oxacillin resistance was found in 30% (6/20) of isolates. More than half of the isolates, 52.4% (11/21), were resistant to clindamycin. All isolates were 100% (15/15) resistant to penicillin. Resistance to fluoroquinolones (enrofloxacin and marbofloxacin) was 10% (2/20) and 8.7% (2/23), respectively. Meropenem resistance was detected in 8.7% (2/23) of isolates. All tested

isolates were susceptible to sulfamethoxazole/trimethoprim (8/8).

### ***Enterococcus* spp. strains**

Resistance to amoxicillin/clavulanic acid was observed in 29.4% (5/17) of the isolates. For the first-generation cephalosporin, cephalexin, resistance was particularly high at 93.3% (14/15) of strains. Resistance to the second-generation cephalosporin, cefoxitin, was found in 82% (9/11) of isolates. Among the third-generation cephalosporins tested (cefpodoxime, cefotaxime and ceftiofur), resistance rates were 88.9% (8/9), 100% (8/8) and 81.3% (13/16) of the isolates, respectively. Resistance to macrolide antibiotics (azithromycin and erythromycin) was observed in 71.4% (10/14) and 42.9% (6/14) of isolates respectively. A high percentage of isolates, 92.9% (13/14), were resistant to clindamycin. All isolates tested were resistant to penicillin (6/6). More than half of the isolated strains showed resistance to fluoroquinolones (enrofloxacin and marbofloxacin), with rates of 54.5% (6/11) and 58.8% (10/17), respectively. High resistance was recorded for meropenem, a carbapenem antibiotic, at 80% (12/15).

### ***Pseudomonas* spp. strains**

High resistance rates of 100% (8/8), 82% (9/11) and 77% (10/13) were observed in isolates tested with third-generation cephalosporins (cefpodoxime, ceftiofur and ceftazidime). For the fourth-generation cephalosporin, ceftazidime, resistance was found in 30.8% (4/13) of isolates. Resistance to fluoroquinolones (enrofloxacin and marbofloxacin) was recorded in 58.3% (7/12) and 36.4% (4/11) of isolates respectively. Meropenem resistance was observed in 63.6% (7/11) of the isolates. All isolates tested were resistant to oxytetracycline (10/10), sulfamethoxazole/trimethoprim (5/5) and the polymyxin class antibiotic, polymyxin B (5/5). Regarding gentamicin, the only aminoglycoside tested, resistance was noted in 7.7% (1/13) of the isolates.

### *Klebsiella* spp. strains

Resistance to amoxicillin/clavulanic acid was observed in 75% (6/8) of the isolates. For first-generation (cephalexin) and second-generation (cefoxitin) cephalosporins, resistance rates were 75% (6/8) and 100% (5/5) of isolates, respectively. Among third-generation cephalosporins (cefovecin and ceftazidime), resistance was found in 50% (4/8) and 37.5% (3/8) of isolates respectively. All isolates were susceptible to cefepime, the only fourth-generation cephalosporin tested. Resistance to both tested fluoroquinolones (enrofloxacin and marbofloxacin) was 25% (2/8). All tested isolates were susceptible to meropenem (7/7), sulfamethoxazole/trimethoprim (8/8) and gentamicin (8/8).

Isolates that were represented at a frequency of less than 1% were not included. Antimicrobial susceptibility results are presented for the isolated bacterial species that were tested each antimicrobial agent on at least five isolates. The results of antimicrobial resistance to antibiotics against which one third or less of the bacterial isolates were tested, were also not taken into account.

### Susceptibility of the most important isolates to antimicrobial agents

The highest susceptibility of *Escherichia coli* isolates was observed with meropenem (95.9%), gentamicin (94.8%), cefotaxime (92%), ceftriaxone (90%), cefovecin (79.6%), marbofloxacin (78.8%), ceftazidime (78.7%), sulfamethoxazole/trimethoprim (78.6%), cefepime (71.4%) and enrofloxacin (71.1%). Moderate susceptibility was noted for cephalexin (62%) and oxytetracycline (60%). The lowest susceptibility was found for cefoxitin (43.5%), amoxicillin/clavulanic acid (35.8%) and azithromycin (25%).

Coagulase-positive staphylococcal strains showed high susceptibility to  $\beta$ -lactam antibiotics: amoxicillin/clavulanic acid (90.7%), cephalexin (89.7%), cefoxitin (100%), cefpodoxime (90%), cefotaxime

(88%), cefovecin (90.5%), oxacillin (92.1%) and meropenem (95%). Exceptions were ampicillin and penicillin, for which only 26% and 17.9% of the isolates were susceptible, and ceftriaxone with a susceptibility of 55.6%. The isolates showed high susceptibility to fluoroquinolones: enrofloxacin (82.6%) and marbofloxacin (85.7%). Moderate susceptibility was observed for oxytetracycline (66.7%) and macrolides with azithromycin (56.8%) and erythromycin (57.9%). Half of the isolates tested were susceptible to sulfamethoxazole/trimethoprim (50%). A lower susceptibility was noted for the lincosamide antibiotic clindamycin (37.8%).

A total of 80% of *Proteus* spp. bacterial strains isolated from urine samples of dogs with significant bacteriuria were susceptible to gentamicin. A relatively high percentage of isolates was also susceptible to marbofloxacin (71%), cefotaxime (70%) and cefepime (69%). Moderate susceptibility was observed for enrofloxacin (64.5%), meropenem (64.3%), ceftazidime (63.3%), amoxicillin/clavulanic acid (60%), cefovecin (56.7%) and sulfamethoxazole/trimethoprim (53.8%). The lowest susceptibility was recorded for cephalexin (48.3%) and cefoxitin (33.3%).

The highest susceptibility of isolated *Streptococcus* spp. strains was recorded for  $\beta$ -lactam antibiotics: amoxicillin/clavulanic acid (100%), meropenem (96.7%), cephalexin (93.3%), cefotaxime (90.9%), cefoxitin (88.2%), cefpodoxime (86.7%), cefovecin (80%) and penicillin (75%). Additionally, susceptibility to the macrolide antibiotic azithromycin of 75.9% was noted. Moderate susceptibility was observed for ampicillin (54.5%) and erythromycin (50%).

The lowest susceptibility was found for clindamycin (29.6%), sulfamethoxazole/trimethoprim (27.3%) and enrofloxacin (13.3%).

Isolated *Staphylococcus* spp. strains showed high susceptibility to  $\beta$ -lactam antibiotics: amoxicillin/clavulanic acid (91.3%), cephalexin



Table 2. Percentage of resistant bacterial strains in dogs to tested antimicrobial agents

	AMC	CL	CTX	CVN	CAZ	FEP	ATH	ENR	MAR	MEM	OT	SXT	GM	CD	PG
<i>Escherichia coli</i>	34.3	34.3	8	9.5	11.8	13.5	-	20.7	19	4.1	36	21.4	4.4	-	-
<i>Proteus</i> spp.	30	48.3	25	36.7	30	27.6	-	32.3	19.4	35.7	IR/ 100	46.2	16.7	-	-
<i>Pseudomonas</i> spp.	IR	IR	IR	IR/ [82]	77	30.8	-	58.3	36.4	63.6	IR	IR	7.7	-	-
<i>Klebsiella</i> spp.	75	75	-	50	37.5	0	-	25	25	0	0	0	0	-	-
<i>Staphylococcus</i> CPS	7	7.7	8	4.8	-	-	35.1	8.7	7.1	5	-	20.8	-	40.5	82.1
<i>Streptococcus</i> spp.	0	6.7	4.5	10	-	-	20.7	40	13.3	3.3	-	45.4	-	48.1	25
<i>Staphylococcus</i> spp.	8.7	13	12.5	13	-	-	50	10	8.7	8.7	-	0	-	52.4	100
<i>Enterococcus</i> spp.	29.4	IR	IR	IR	-	-	71.4	54.5	58.8	80	-	IR	IR	IR	100

AMC - amoxicillin/clavulanic acid, CL - cephalixin, CTX - cefotaxime, CVN - ceftazidime, FEP - cefepime, ATH - azithromycin, ENR - enrofloxacin, MAR - marbofloxacin, MEM - meropenem, OT - oxytetracycline, SXT - sulfamethoxazole/trimethoprim, GM - gentamicin, CD - clindamycin, PG - penicillin G  
--: Susceptibility not tested  
IR - Intrinsic resistance

(87%), cefpodoxime (83.3%), cefotaxime (87.5%), ceftazidime (82.6%) and meropenem (87%). Susceptibility to ceftriaxone was 75%, while 70% of isolates were susceptible to oxacillin. The isolates demonstrated high susceptibility to fluoroquinolones: enrofloxacin (85%) and marbofloxacin (82.6%). Moderate susceptibility was observed for the second-generation cephalosporin, cefoxitin (66.7%), as well as for azithromycin (50%) and sulfamethoxazole/trimethoprim (50%). Lower susceptibility was recorded for the lincosamide antibiotic clindamycin (42.9%) and the macrolide antibiotic erythromycin (41%). Susceptibility to ampicillin was very low (16.7%), while all tested isolates were resistant to penicillin.

The highest susceptibility of *Enterococcus* spp. bacterial isolates from urine samples with significant bacteriuria was observed for amoxicillin/clavulanic acid (70.6%) and sulfamethoxazole/trimethoprim (70%). Fifty percent of the isolates were susceptible to ampicillin. Susceptibility to other antimicrobials was low: enrofloxacin (27.3%), ceftriaxone (25%), ceftazidime (18.8%), marbofloxacin (17.6%), azithromycin (14.3%) and cefpodoxime (11%). Susceptibility to the remaining tested antibiotics was less than 10%: cefoxitin (9%), erythromycin (7.1%), clindamycin (7.1%) and meropenem (6.7%). All tested isolates were resistant to penicillin and cefotaxime.

High susceptibility of the bacterial isolates of *Pseudomonas* spp. was only found for gentamicin (92.3%). Significantly lower susceptibility was observed for ceftazidime (46.2%) and marbofloxacin (45.5%). The effect of other antibiotics tested on the isolates was low: enrofloxacin (16.7%), ceftazidime (9%), meropenem (9%) and ceftazidime (7.7%). All tested isolates were resistant to cefpodoxime and sulfamethoxazole/trimethoprim.

The highest susceptibility of bacterial isolates of *Klebsiella* spp. was recorded for meropenem (100%), gentamicin (100%) and

sulfamethoxazole/trimethoprim (100%). A relatively high susceptibility was noted for fluoroquinolones: enrofloxacin (75%) and marbofloxacin (75%). Moderate susceptibility was found for ceftazidime (62.5%). The efficacy of other antibiotics tested on the isolates was significantly lower: ceftazidime (37.5%), ceftriaxone (33.3%) and cephalexin (25%). Only 12.5% of the isolates showed susceptibility to amoxicillin/clavulanic acid.

## Discussion

UTIs in dogs are in most cases acute bacterial infections. One of their characteristics is the need to initiate empirical treatment before the pathogen has been identified and its sensitivity to various antimicrobial drugs has been determined. Understanding the local susceptibility patterns of the most common uropathogens is useful for ensuring appropriate empirical antibiotic treatment and adhering to the principles of justified and prudent use of antimicrobials.

The aim of justified and prudent antimicrobial use is to minimise their application in veterinary practice to the lowest possible level, i.e., only when treatment is warranted. In such cases, treatment should be targeted, clinically effective, and aimed at preventing the development and spread of antimicrobial resistance (Šeol et al., 2010). Recently, new resistance mechanisms have led to the development of bacteria that are resistant to antibiotics from several classes (MDR). Given the growing problem of bacterial antibiotic resistance worldwide, it is crucial to understand the susceptibility of specific pathogens in the local environment to ensure the most rational approach to empirical antibiotic therapy. Therefore, this study aimed to identify the most common bacterial pathogens of UTI in dogs in our region and to determine their susceptibility in order to establish an appropriate line of

treatment. The clinician's primary is the clinical care for dogs with a minimal risk of adverse effects (including antimicrobial resistance). A microbiological cure (elimination of the pathogen) is desirable, but not always achievable or necessary, in either short or long-term clinical treatment. Based on the obtained results during the observed period, it is evident that *Escherichia coli* and coagulase-positive staphylococci were the most common causes of UTI in dogs. Due to differences among bacterial isolates in their response to antibiotics, it is always necessary to determine the pathogen's susceptibility for effective treatment.

This study presents the results of bacteriological testing on canine urine samples collected from January 2019 to July 2024, along with an analysis of bacterial susceptibility and resistance patterns to various antimicrobial drugs. Significant bacteriuria was detected in 307 of 897 (34.2%) samples. This proportion of bacteriologically positive samples is slightly higher than the results reported in some previous studies, in which 29.9% and 30.4% of urine samples were bacteriologically positive (Fonseca et al., 2021; Garcês et al., 2022). However, an almost identical proportion was observed over a four-year period when testing similar samples in the laboratories of the Faculty of Veterinary Medicine, University of Zagreb (Caušević et al., 2023), where one third of the examined urine samples from dogs were bacteriologically positive. A higher proportion of bacteriologically positive samples (50.1%) was reported by Kompes et al. (2023). The most frequently isolated bacterial pathogen in this study was *Escherichia coli* (45%), which is consistent with numerous other reports (Norris et al., 2000; Ling et al., 2001; Prescott et al., 2002; Ball et al., 2008; Hall et al., 2013; Windahl et al., 2014; Wong et al., 2015; Marques et al., 2016; McMeekin et al., 2017; Moyaert et al., 2017; Fonseca et al., 2021; Aurich et al., 2022; Garcês et al., 2022). Following *E. coli*, the most common bacterial

pathogens were species from the genus *Staphylococcus* – coagulase-positive strains (13.9%), *Proteus* (10%), *Streptococcus* (9.7%), coagulase-negative *Staphylococcus* strains (7.4%), *Enterococcus* (5.5%), *Pseudomonas* (4.2%) and *Klebsiella* (2.6%). Other bacterial species were isolated only sporadically and accounted for less than 1% of samples. A similar distribution of species was noted by other authors (Wong et al., 2015; Fonseca et al., 2021; Garcês et al., 2022; Aurich et al., 2022; Ataya et al., 2023). In most samples (99.3%), only one bacterial pathogen was isolated, while two bacterial species were isolated from only two samples (0.65%).

In this study, the majority of isolates showed resistance to at least one class of antibiotics. None of the drugs tested was effective against all 139 *Escherichia coli* strains isolated. Approximately one third of the strains were resistant to amoxicillin/clavulanic acid, which is similar to data reported from some other Mediterranean countries (Marques et al., 2016). A slightly higher proportion of strains showed resistance to first- and second-generation cephalosporins, including cephalexin and cefoxitin, as well as oxytetracycline. Resistance to other antimicrobial drugs was significantly lower. The highest sensitivity among isolates was observed for meropenem (95.9%), gentamicin (94.8%), cefotaxime (92%), ceftriaxone (90%), cefovecin (79.6%), marbofloxacin (78.8%), ceftazidime (78.7%), sulfamethoxazole/trimethoprim (78.6%), cefepime (71.4%) and enrofloxacin (71.1%).

**Coagulase-positive *Staphylococcus* spp.** strains showed high resistance rates to penicillin (82.1%) and ampicillin (73.9%). More than one third of the isolates were resistant to clindamycin and the macrolide antibiotics azithromycin and erythromycin. This resistance pattern is similar to the results reported by Smoglica et al. (2022). The strains were highly sensitive to other  $\beta$ -lactam antibiotics, including amoxicillin/clavulanic

acid (90.7%), cephalexin (89.7%), cefoxitin (100%), cefpodoxime (90%), cefotaxime (88%), cefovecin (90.5%), oxacillin (92.1%) and meropenem (95%). They also showed a high sensitivity to fluoroquinolones: enrofloxacin (82.6%) and marbofloxacin (85.7%).

***Proteus* spp.** isolates demonstrated higher resistance to amoxicillin/clavulanic acid than reported by Kompes et al. (2023) and Caušević (2023). Nearly half of the isolates (46.2%) were resistant to sulfamethoxazole/trimethoprim, one of the first-line drugs for the treatment of UTIs in dogs. This percentage was higher than reported by Kompes et al. (2023), but slightly lower than by Caušević (2023). In general, resistance to cephalosporins, fluoroquinolones and gentamicin was higher compared to the results of Kompes et al. (2023) and Caušević (2023).

All ***Streptococcus* spp.** strains tested were sensitive to amoxicillin/clavulanic acid, which is completely consistent with the results reported by Kompes et al. (2023). Resistance to beta-lactam antibiotics was generally low, with the exception of clindamycin (48.1%) and penicillin (25%). Nearly half of the isolates were resistant to sulfamethoxazole/trimethoprim, significantly higher than the resistance values reported by Kompes et al. (2023).

Most coagulase-negative ***Staphylococcus* spp.** strains were sensitive to amoxicillin/clavulanic acid, first- and third-generation cephalosporins, meropenem and fluoroquinolones. All isolates were resistant to penicillin, with resistance to macrolide antibiotics ranging from 41 to 50%. Slightly more than half of the isolates (52.4%) were resistant to clindamycin and 30% were resistant to oxacillin.

Isolates of ***Enterococcus* spp.** exhibit intrinsic resistance to first-, second-, and third-generation cephalosporins and clindamycin, which was confirmed in this study, as the isolates showed high rates of resistance to these antibiotics, as well as to

meropenem. All isolates were resistant to penicillin, and more than half were resistant to fluoroquinolones. In the study by Gómez-Beltrán et al. (2020), an even higher resistance rate to enrofloxacin (74.3%) was found. Fluoroquinolones are not recommended for the treatment of enterococcal UTI due to their low efficacy against *Enterococcus* spp. *in vivo* (CLSI, 2020). The strains showed moderate to high resistance rates to macrolides. The highest susceptibility was observed with first-line drugs for canine UTIs, especially amoxicillin/clavulanic acid (70.6%), as reported by Aurich et al. (2022) and Kompes et al. (2023).

Most ***Pseudomonas* spp.** strains were resistant to first-, second- and third-generation cephalosporins. Intrinsic resistance was confirmed as all strains tested were resistant to oxytetracycline and sulfamethoxazole/trimethoprim. All tested isolates were resistant to polymyxin B. Moderate to high resistance was observed for fluoroquinolones and meropenem. However, high susceptibility was observed with gentamicin (92.3%), similar to the results reported by Smoglica et al. (2022) and Kompes et al. (2023).

***Klebsiella* spp.** isolates showed high resistance rates to amoxicillin/clavulanic acid and first- and second-generation cephalosporins (75%). Moderate to high resistance was observed with third-generation cephalosporins (cefovecin and ceftazidime), while a quarter of isolates were resistant to fluoroquinolones. All tested isolates were sensitive to cefepime, gentamicin, meropenem and sulfamethoxazole/trimethoprim. In the study by Yudhanto et al. (2022), resistance was found to be 28% for sulfamethoxazole/trimethoprim, 36% for enrofloxacin and marbofloxacin and 60% for gentamicin.

The aim of this study was to provide an overview of bacterial pathogens causing UTIs in dogs and their resistance patterns to specific antimicrobial drugs. The results contribute to the understanding of local resistance

patterns and can help clinicians select empirical treatment options. This supports the optimisation of treatment, rational use of antimicrobials and the reduction of the development and spread of antimicrobial resistance. The data obtained can also assist in monitoring antimicrobial resistance in Croatia at both the national and local levels.

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## Etiologija i lokalni obrazac antimikrobne rezistencije bakterijskih uzročnika infekcija mokraćnog sustava pasa s područja Splita u razdoblju od siječnja 2019. do srpnja 2024. godine

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Infekcije mokraćnog sustava (IMS) u pasa su česta pojava u veterinarskoj praksi i jedan od glavnih razloga za primjenu antimikrobnih lijekova. Uglavnom su posljedica bakterijskih infekcija, dok virusne, gljivične i nametničke infekcije predstavljaju manje od 1 % slučajeva IMS. U praksi se obično započinje liječenje s antimikrobnim lijekovima odabranim temeljem postojećeg kliničkog iskustva bez poznavanja uzročnika ili njegove osjetljivosti na određeni lijek. Cilj je ovog rada bio prikazati lokalnu prevalenciju i antimikrobnu rezistenciju najčešćih bakterijskih uzročnika IMS pasa izdvojenih iz uzoraka dobivenih s područja grada Splita i okolice. Od siječnja 2019. do srpnja 2024., bakteriološki je pretraženo 897 uzoraka urina od čega je 307 bilo

pozitivno. Od ukupnog broja bakterijskih izolata, gram-negativnih bakterija je bilo 194 (62,8 %), a gram-pozitivnih 115 (37,2 %). Najčešće izdvojeni bakterijski uzročnici bili su *E. coli* (45 %), koagulaza - pozitivni *Staphylococcus* sp. (13,9 %), *Proteus* sp. (10 %), beta hemolitični *Streptococcus* sp. (9,7 %), koagulaza - negativni *Staphylococcus* sp. (7,4 %), *Enterococcus* sp. (5,5 %), *Pseudomonas* sp. (4,2 %) te *Klebsiella* sp. (2,6 %). Podatci o obrascima lokalne osjetljivosti i otpornosti najčešćih uropatogeni mogu koristiti kliničarima prilikom izbora antimikrobnih lijekova, a mogu biti i osnova za praćenje antimikrobne rezistencije u nadolazećim godinama.

**Ključne riječi:** IMS, pas, uropatogen, antimikrobna rezistencija