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Antibiograms of canine urinary culture results in the light of current recommendation for use of antimicrobials

Kutyák vizelettenyésztésének rezisztenciavizsgálati eredményei a jelenlegi antimikróbás szerek használatára vonatkozó ajánlások tükrében

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Abstract

This thesis investigates the prevalence and antimicrobial resistance patterns of bacteria isolated from canine urinary tract infections (UTIs) at the University of Veterinary Medicine Budapest. As concerns about antimicrobial resistance (AMR) affecting animal and public health continue to grow, such studies provide essential data regarding UTI pathogens to inform guidelines for the prudent use of antimicrobial agents in veterinary medicine. Across 332 dogs, we collected 450 urine samples, between 01 November 2020 and 22 November 2023, and found a positive culture in 43.56% of cases. *Escherichia coli* was the most prevalent pathogen isolated (52.05% of positive cases), followed by *Staphylococcus spp.* (12.34%) and *Klebsiella spp.* (10.95%). High levels of resistance were shown, particularly for common antibiotics, including amoxicillin (28.95% resistance in E. coli) and high sensitivity for gentamicin (98.68%). The emergence of *Klebsiella spp*. as a notable pathogen, exhibiting a 93.75% resistance to amoxicillin, underscores the evolving landscape of UTI-associated bacteria. This study highlights the need for continued monitoring of antimicrobial resistance patterns as well as the importance of adhering to currently available veterinary guidelines to combat the threat of AMR. The findings help to gain a deeper understanding of UTI management in dogs and promote a One Health approach to address the interdependence of human and animal health.

Absztrakt

A dolgozat a budapesti Állatorvostudományi Egyetemen vizsgált, húgyúti fertőzésben szenvedő kutyákból izolált baktériumok előfordulását, valamint azok antimikrobiális rezisztenciáját vizsgálja. Az ember- és állatgyógyászatot is sújtó antibiotikum-rezisztencia egyre aggasztóbb méreteket ölt; az ehhez hasonló vizsgálatok értékes adatokat szolgáltatnak a húgyúti kórokozók kapcsán az ésszerű antibiotikum-használatra vonatkozó állatorvosi ajánlások megfogalmazásához. 332 kutyától 450 vizeletmintát gyűjtöttünk 2020. november 1. és 2023. november 22. között; a baktériumtenyésztés az esetek 43,56%-ában bizonyult pozitívnak. Az *Escherichia coli* volt a leggyakoribb izolált kórokozó (a pozitív esetek 52,05%-a), ezt követte a *Staphylococcus spp*. (12,34%) és a *Klebsiella spp*. (10,95%). A kórokozók rezisztenciája magas volt, különösen a gyakori antibiotikumokkal szemben, beleértve az amoxicillint (28,95%-os rezisztencia az *E. coli* esetében), míg a gentamicinnel szemben érzékenynek bizonyultak (98,68%). A *Klebsiella spp*. figyelemre méltó arányú megjelenése a minták között - 93,75%-os rezisztencia eredményével az amoxicillinnel szemben - rávilágít a húgyúti kórokozó baktériumok dinamikus fejlődésére. Ez a tanulmány kiemeli az antimikrobiális rezisztencia-minták folyamatos nyomon követésének szükségességét, valamint a jelenleg elérhető állatorvosi irányelvek betartásának fontosságát a növekvő antibiotikum-rezisztencia leküzdése érdekében. Eredményeink kutyák húgyszervi fertőzései esetében lehetővé teszik azok átgondoltabb kezelését, az Egy Egészség ("One Health") koncepciójának megfelelően, a humán és állategészségügy egymástól való kölcsönös függésére tekintettel.

Contents

1. Introduction

The one health concept is increasing in popularity and importance in today's society. It unifies the health of the ecosystem, humans and animals and presents it as an interlinking system. Regarding this the topic of antibiotic usage and resistance has been identified as one of the cornerstones of discussion due to its high threat level. It is projected that antimicrobial resistance (AMR) will result in an extra 1 trillion US$ in healthcare cost by 2050 [1] and furthermore has been the direct reason for 1.27 million death globally in 2019[1, 2]. The misuse of antimicrobials in each sector can contribute to the increase of AMR.

Ultimately, the realm of small animal veterinary medicine fosters an ever-deepening connection between human and animal health. In relation to this, urinary tract infections represent a prevalent affliction within canine practice, often addressed through the application of antimicrobial agents. Given the variability in practices and the universal use of antibiotics, the manifestations of antimicrobial resistance among urinary pathogens can differ significantly by region. For this reason, we have decided on the topic of Antibiograms of canine urinary culture results in the light of current recommendation for use of antimicrobials to contribute to the reduction of this arising multilevel threat and provide guideline for the antimicrobial use in case of urinary tract infection in dogs for the university of veterinary medicine in Budapest.

In our study 450 urine samples, 194 positive and 256 negative, of 332 dogs were collected at the clinic of the university of veterinary medicine in Budapest from the 25th of November 2020 to the 13th of November 2023. The treatment of these animals with empirically selected antibiotics can lead to an increase in resistance. The relevance of the spread of multi-resistant bacteria does not only concern the veterinary sector but as previously mentioned, also public health. For this reason, we analyzed and compared antibiograms in accordance with the gender, age and breed of the patients and reviewed them under the light of the current recommendation of antibiotic choice in pets.

2. Literature review

Urinary tract infections (UTIs) in dogs are familiar and serious clinical entities caused by the invasion of pathogenic microorganisms, mainly bacteria, into the urinary tract. Due to the broad scope of the urinary tract, differentiation between the upper-, consisting of the kidneys and ureters, and lower urinary tract, including bladder, urethra and, in male's prostate or in females, the vagina must be made. These anatomical parts prevent the contamination of urine by different physiological barriers and mechanism.

2.1. Pathomechanism

The bladder serves as a reservoir, with its muscle and mucosal layers acting as a protective layer against infection and coherently activating immune responses. However, there are multiple host factors that can put dogs at risk of an UTI. Anatomical defects, such as ectopic ureters, hormone-related diseases, especially in spayed females and concurrent illnesses that affect immune competence such as diabetes mellitus or Cushing's disease can produce failure of the hosts abilities to prevent infection. Moreover, the changes that are related to ageing, may increase the risk of UTIs, because during this period, both structure and function may vary [3]. Uropathogenic *Escherichia coli* (UPEC) are responsible for the majority of canine UTIs, but other bacteria like *Staphylococcus, Streptococcus* and *Proteus* species may also be involved. UPEC possesses specific virulence factors, including adhesins, enabling bacterial binding to the uroepithelium which promotes colonization and endurance within the urinary tract [4]. Once established these pathogens can enter the uroepithelium, leading to inflammation and damage to the mucosal barrier. The host's immune response, characterized by neutrophils and macrophages recruitment, aims for the elimination of invading microorganisms. Persistent infections, however, can cause tissue damage contributing to the clinical signs of UTIs such as dysuria, pollakiuria and hematuria [5].

The pathogenesis of UTIs is also an environmental matter. Dehydration, lack of hygiene and the presence of urinary calculi promote bacterial growth. Additionally, certain drugs, such as corticosteroids or immunosuppressive agents can impair the ability of the host to mount an effective immune response thus increasing their risk for infection.

2.2. Defence mechanisms

Several mechanisms act together and compose a line of defense against infection along the urinary tract of dogs, ensuring its function. Such mechanisms are critical to limit colonization and growth of pathogenic microorganisms in either intraluminal or myogenic sites (e.g. sphincters) and, as such, promote health of the urinary tract. This defense is largely anatomical related, such as the internal and external urethral sphincters, that prevent retrograding urine flow to reduce urinary tract infection (UTI) risk.

Another important line of defense is urinary flow, as regular urination facilitates flushing of bacteria and other pathogens from the urinary tract. Urine composition itself contributes to this protective effect; its high concentration of urea and salts, inhibits bacterial growth through its antimicrobial properties. In addition, local immune responses of the urinary tract are protected by an array of antibodies, such as immunoglobulin A [IgA] and antimicrobial peptides to neutralize invading pathogens [6]. The inflammatory response serves as a mechanism to combat an infection, through containing and eliminating the pathogens.

Healthy microbial flora in the urinary tract can help prevent pathogenic bacteria from entering and establishing bioburden, thereby lowering the possibility of UTIs. Uroepithelial cells lining the urinary tract contribute to mucosal defense by producing mucins and other substances which entrap pathogens and inhibit adherence of pathogens onto epithelial surfaces. The constant sloughing of epithelial cells helps carry away bacteria that adhere to them, further enhancing the defenses for the urinary tract.

2.3. Predisposing factors

2.3.1. Uroliths

Uroliths, commonly referred to as urinary stones, represent a significant predisposing factor in the pathogenesis of urinary tract infections (UTIs) in canines. These crystal structures can form in the urinary bladder, urethra, or kidneys from a variety of genetic, nutritional and metabolic factors. Uroliths can irritate the urothelium to the point where bacteria are able to colonize and/or create an obstruction resulting in stasis of urine (possibly leading to infection as well). Such stasis not only allows the pathogenic microorganisms to multiply, but also erodes host innate immune responses.

Finally, some uroliths including struvite stones can occur in conjunction with urine tract infections as well. This type of stones is favored by alkaline urine, and the frequent presence of

urease-producing bacteria in such environment play a role in promoting new stone formation and create a cycle of infection and inflammation [7].

Thus, treatment of urolithiasis is essential to relieve the discomfort and possible obstruction incident on the affected dog and for preventing recurrent UTI. The complexity of this relationship reinforces the need for a diagnostic strategy, which may involve urinalysis, imaging modalities, and dietary management when treating canine uroliths to also account for UTI susceptibility.

2.3.2. Diabetes mellitus

Canine diabetes mellitus is a growing recognized risk for urinary tract infections (UTI). Chronic hyperglycemia, due to inadequate insulin secretion or resistance from associated metabolic disorders, initiates a series of pathophysiological changes that threaten the structure and function of the urinary system.

Glucosuria (high glucose in urine), is one of the classical characteristics of diabetes mellitus. Not only does this excess amount of glucose provide a nutrient-dense substrate for diverse uropathogenic bacteria, but also change the normal urinary pH and osmolarity which helps to promote bacterial colonization and proliferation.

In addition, diabetes-related and other common causes of immunocompromised stage also increase susceptibility to UTIs. Neutrophils are an important part of the immune response, and hyperglycemia affects neutrophil function, reducing their capability and the canine's overall immune response to combatting a bacterial invasion. Moreover, there is chronic inflammation and tissue damage that sometimes may accompany diabetes which also disrupts the uroepithelium integrity subsequently allowing pathogens to access urinary tract [4].

Urinary tract infections are a common circumstance wherein diabetes mellitus and UTI relate; therefore, the interaction of diabetes with this condition is complex, requiring monitoring and preventive management. An adequate glycemic control together with an appropriate diagnosis and treatment of UTIs can reduce the complications due to this process.

2.3.3. Kidney failure

Chronic kidney failure, also referred to as chronic kidney disease (CKD), is prevalent among older dogs and is a considerable risk factor for urinary tract infections (UTIs). The kidneys are responsible for maintaining homeostasis, balancing electrolytes, and excreting waste. In CKD,

as the kidney's function deteriorates, the capacity to concentrate urine declines, leading to the formation of a diluted urinary stream. The compromised urine composition not only impairs the ability of the urinary tract to eliminate pathogens, but also allows for a niche where bacteria might be more readily able to colonize.

Also, the physiological alterations that happen in kidney failure can trigger a cascade of complications, eventually leading to an increased risk of UTIs. As an example, an excess of uremic toxins may affect the immune system and, in this way, reduce hosts ability to cope with infectious agents. Such immunosuppression, along with urinary stasis caused by impaired renal function and bladder atony allows a perfect environment for UTI development.

Furthermore, the concurrent diseases which frequently accompany CKD (e.g. diabetes mellitus or hyperadrenocorticism) increase UTI susceptibility. Combined, these comorbidities, as well as the physiological changes that occur with kidney failure, can really increase the risk of developing a new bacterial infection in affected dogs.

Thus, CKD requires overall management with ongoing surveillance for urinary tract infection. Early identification and treatment are key as patients who do not get treated for UTIs can go on to develop more serious issues like pyelonephritis and systemic infection.

2.3.4. Neurogenic bladder

The neurogenic bladder is defined as the inadvertent or insufficient emptying of the urinary bladder secondary to neurologic disease and is recognized as an important risk factor for UTIs in dogs. The causes of this dysfunction are diverse, ranging from spinal cord injuries to intervertebral disc disease to congenital defects compromising neural pathways controlling bladder function. The resulting disruption of normal bladder functions usually results in incomplete emptying, urinary retention and higher post void residual volume which creates a favorable environment for bacterial growth [8].

In normal dogs, the bladder contracts well during urination so that urine can be easily completely voided – a natural modality for flushing out pathogens. In contrast, in neurogenic bladder the disruption of normal signaling between the bladder and CNS leads to detrusor muscle atony or hyperreflexia with resultant urinary stasis that not only allows bacteria to colonize, but additionally, can alter urinary pH and solute concentration causing an increase in uropathogenic bacteria. Finally, neurogenic bladder can contribute to increased frequency of UTIs by means of chronic irritation and chronic inflammation of the bladder wall that disrupt the protective urothelial barrier.

Neurogenic bladder assessment in dogs is a complex operation including regular monitoring for urinary tract infection clinical signs, application of bladder expression procedures, and occasionally the administration of medications to improve bladder function.

2.3.5. Miscellaneous causes

Hyperadrenocorticism, commonly known as Cushing's disease, is a condition characterized by excessive production of cortisol from the adrenal glands, as well as an iatrogenic form, due to the long-term use of glucocorticoids or through high doses of corticosteroids. This endocrine disorder can lead to a variety of systemic effects, including immunosuppression and alterations in the normal flora of the urinary tract. Elevated cortisol levels associated with hyperadrenocorticism reduce the immune response, diminishing the ability of the dog’s body to combat infections effectively. Additionally, the resultant hormonal imbalances can cause changes in the urinary tract environment, such as polyuria causing decreased urine concentration and altered pH and therefore creating a more favorable setting for bacterial proliferation [9].

Immunosuppressive therapy is another factor that might predispose dogs to urinary tract infections. This treatment is often applied to manage various autoimmune diseases, neoplastic conditions, or following organ transplantation. The immune system’s ability to respond to pathogens can be severely inhibited through the administration of corticosteroids and other immunosuppressive agents. These therapies not only increases the risk of opportunistic infections but in addition to that alter the microbiome of the urinary tract, further predisposing dogs to UTIs [10].

As dogs age, there is a progressive decline in immune function, often referred to as immunosenescence. Additionally, age-related changes in the urinary tract, such as decreased bladder elasticity, reduced urine concentration ability, and alterations in hormone levels, can further facilitate the development of infections [11].

2.4. Clinical presentation, diagnosis and therapy

The clinical manifestation may appear in numerous forms, depending on the anatomical location of the inflammation and whether there are other coexisting diseases. Reason for their presentation usually include lower urinary tract signs such as dysuria, hematuria, polyuria or anuria [5].

Through the physical examination and history taking the presence or absences of given predisposing factors may in part be revealed. A physical examination of the urinary tract, for example, may reveal palpable masses or uroliths, evidence of pain in the bladder, and alterations in thickness of the bladder wall which are all indicative of disease.

Following the physical examination, urinalysis is recommended. By utilizing dipstick tests, assessing the specific gravity, conducting a cytological examination of sediment and the aerobic culturing of the urine sample, a diagnosis of UTIs can be established.

Urine samples should be collected via cystocentesis, except in cases where contraindications or potential complications are expected and cultured 24 hours after collection. Ultrasonography is a valuable tool to aid in the case of cystocentesis, while also providing insight into the status of the bladder. Voided samples should only be considered when cystocentesis is contraindicated but should be processed within a few hours.

Color and pH of urine can play a crucial role, as they provide rapid insights. Urine composition of a healthy dog typically appears transparent and ranges from light yellow, to amber, with pH varying between 6-7,5 [12].

In the case of urinary tract infections, discoloration such as red or dark yellow may indicate the infections severity. Additionally, increase in the urinary pH towards alkalinity may suggest urease producing bacteria such as *Staphylococcus, Proteus spp. or Klebsiella spp.,* as well as be influenced by the diet (recent meals or low protein diets) [12].

Incorporating diagnostic imaging techniques such as radiography, contrast imaging, or cystoscopy can yield important information. Additionally deep-seated bladder infections may warrant a biopsy of the bladder [5].

According to the updated clinical guidelines, it is recommended to treat those patients of whom not only the culture/sediment exam is positive, but also clinical signs are present.

It is important to differentiate between sporadic bacterial cystitis and recurrent bacterial cystitis as their background, treatment and follow up may vary greatly.

When referring to sporadic cystitis patients, it is necessary that they have experienced fewer than three episodes of cystitis within the prior 12 months. Furthermore, animals in this category should have no known anatomical abnormalities and should be non-pregnant females or neutered males. In case of sporadic cystitis, a predictable pathogen pattern or little to no previous antibiotic exposure, empirical treatment is acceptable [5].

Recurrent bacterial cystitis is characterized by individuals experiencing three or more episodes within a 12-month period, or two or more occurrences within a span of six months.

If repeated antibiotic use fails to fully resolve the urinary tract infection it should not be continued without identifying the underlying disease, as it can lead to resistance and is unlikely to result in a long-term cure.

In instances of relapsing, refractory, or persistent infections, it is crucial to ensure that antibiotic levels can achieve adequate concentrations in the bladder. Reevaluation of antibiotic dosage and administration is essential, along with assessing the owner's adherence to home treatment protocols, to enhance treatment outcomes.

2.5. Pathogens

2.5.1. Bacteria

2.5.1.1.Escherichia coli

Escherichia coli (E. coli) is a gram-negative, enteric bacillus that is part of the normal intestinal flora. Non-diarrheagenic extraintestinal pathogenic *Escherichia coli (ExPEC)* include those which may infect the urogenital tract, hence are referred to as uropathogenic *E. coli (UPEC).* The most frequent outcome of an infection is cystitis, but the bacterium can also lead to pyelonephritis, prostatitis and urethritis [13] . The infection mostly starts in the intestine and then travels up through the urethra, first colonizing the periurethral area and later moving upward to adhere to bladder epithelium causing tissue damage. This is the result of toxins produced by a strong neutrophil response (IL-6 and IL-8) primarily induced by the TLR4 pathway, as well as toxin production intrinsic to *UPEC* [13].

The capsule of *Escherichia coli* (K1 and K5) and type 1 (unspecific) and P (highly associated with UPEC) fimbriae aid in evading host defense mechanisms, including phagocytosis and urinary flushing. Additionally, adhesin, cytotoxic protein and iron acquisition systems all contribute to the virulence and facilitate survival within the urinary system. This may enable ascension to the kidneys if not treated, leading to pyelonephritis (though this occurs only infrequently. Through multiplication inside cells, a biofilm-like structure is formed, shielding

them from the host's immune system and antibacterial treatments. This phenomenon frequently accounts for repeated infections.
Of note, several studies are theorizing canine *UPEC* as a potential source for human UTIs, highlighting its public health importance [14].

2.5.1.2.Staphylococcus spp. (pseuointermedius)

Staphylococcus is a gram-positive cocci-shaped facultative anaerobic bacterium, that can be categorized as an opportunistic bacterium.
Staphylococcus is commensally located on the skin and nose while also being part of the normal flora isolated from the oral cavity. Due to its localization, it is usually more related to pyoderma, yet it was among the more abundant resident bacteria in our study.
MRSP (methicillin-resistant *S. pseudointermedius)* strains increase in frequency, with many isolates showing multidrug resistance [15] and represent a potential public health threat.
The primary virulence factors associated with *Staphylococcus* strains encompass surface adhesins, coagulase, hemolysin, proteolytic and lipolytic enzymes, leukocidins that damage membranes and leukocytes, as well as their capacity to produce biofilms.

2.5.1.3.Klebsiella pneumoniae

Klebsiella pneumoniae is a gram-negative bacillus of Enterobacteriaceae family and ubiquitous in our environment, soil, water and intestinal flora of humans and animals. This bacterium is an opportunistic pathogen associated with many different infections in immunocompromised individuals and represents an emerging cause of urinary tract infections in dogs [16].
The reasons for this resistance are varied, including the production of extended-spectrum beta-lactamases (ESBLs) that hydrolyze penicillins and cephalosporins, as well as efflux pumps that pump drugs out of bacterial cells.
The rising occurrence of multidrug-resistant strains have inhibited the efficacy of conventional treatment regimens for UTIs in dogs [16].

2.5.1.4.Proteus mirabilis

Proteus mirabilis is a facultative anaerobic gram-negative rod that inhabits the intestines of animals and the environment, specifically soil and contaminated waters [17]. In addition to these traits, virulence factors of *Proteus spp.* are further increased by the production of toxins such as hemolysin, flagella, and fimbriate. The unique characteristic of this bacterium includes

the 'swarming' phenomenon, wherein vegetative rods are capable of producing approximately 50 times more flagella per unit cell surface area [17].
This bacterium is more prevalent in patients with urinary catheters and may contribute to the formation of uroliths, through the urease activity which raises the pH of the urine.

2.5.1.5.Pseudomonas aeruginosa

Facultative pathogen, gram-negative and rod-shaped. Adherence, colonization, growth and multiplication of this pathogen are regulated by different cell-surface components including flagella and lipopolysaccharides as well as a system involved in regulating virulence factors such as type-3 secretion system [13]. It commonly occurs after surgeries or through wound infections and through its high intrinsic antibiotic resistance, can be difficult to manage [13].

2.5.1.6.Enterococcus spp.

Commonly found in faeces, due to being natural habitants of the intestinal tract of humans and animals. They are gram-positive opportunistic pathogens and are responsible for various infections such as septicemia or urinary tract infection. They exhibit intrinsic resistance to several antimicrobial agents and can acquire resistance through plasmids and transposons.
It is possible for zoonotic transmission of antimicrobial resistant enterococci. Most common isolated types include *Enterococcus faecalis* and *E. faecium.*

2.5.1.7.Mycoplasma

Mycoplasma is a wall-less, obligate pathogen considered to have the smallest cell size and genome of all free-living organisms [13]. Most commonly, they are resident in the mucosal surfaces of their host. Without their cell wall, *Mycoplasma spp.* are susceptible to disinfectants and desiccation, preventing them from surviving in the environment for a long time. For treatment, tetracycline should be effective considering the structure of mycoplasma gives it natural resistance to many β-lactam antibiotics.

2.5.2. Fungi

Although fungal UTI is rare, it must be part of the differential diagnosis in cases of failure to respond to antibiotics. *Candida spp.* is the leading etiological agent of fungal UTI in dogs [18]. It is an opportunistic pathogen that colonizes mucosal surfaces without symptoms, but it can

produce diseases in hosts who are immuno-compromised. Predisposing factors such as antibiotic use, diabetes mellitus and urinary bladder catheterization, among others are believed to play a major role in the expression of fungal UTI [19].
Clinical signs are not different from those seen with the more common bacterial UTIs and include one or many of the following signs: dysuria, pyrexia, anorexia and dehydration.
Antifungal drugs such as fluconazole are the suggested treatment.

2.5.3. Parasites

Urinary tract infections due to parasitic infection are extremely rare and almost always self-limiting. But under a heavy infection, they can express clinical signs.
Capillaria plica is the nematode of the urinary tract in carnivores that is most frequently identified [20]. Fenbendazole and ivermectin can help to fully recover from such a disease.

2.6. Antimicrobials

Antibiotics can be split into 4 major categories by the European Medicine's Agency.
Category A encompasses antibiotics that are not permitted for usage within the European union but can rarely be administered in exceptional circumstances for individual non- food producing animals. Examples include vancomycin, rifampicin and carbapenems [21].
Category B includes quinolones, 3rd- and 4th generation cephalosporins, as well as polymyxins. Given their significance in human healthcare, their application in the veterinary sector should be restricted to mitigate potential risk to public health [21].
Category C consists of antibiotics that have alternatives in human medicine but are seldom found in veterinary medicine and should only be used if category D antibiotics prove ineffective. This category includes aminopenicillins in combination with beta-lactamase inhibitors (e.g. amoxicillin-clavulanic acid), 1st and 2nd generation cephalosporins (e.g. cefuroxime), aminoglycosides, amphenicols and lincosamides [21].
Category D, regarded as the first line treatment option, should be used in a prudent manner. This category comprises penicillins, sulfonamides and certain tetracyclines [21].
In our research, we conducted thorough sensitivity testing for amoxicillin, amoxicillin/clavulanic acid, ciprofloxacin, cefuroxime, enrofloxacin, gentamicin, and sumetrolim. These agents will be emphasized in the subsequent chapters.

2.6.1. Beta lactam

Beta-lactam antibiotics promote cell wall synthesis inhibition through the binding to transpeptidases and penicillin-binding proteins (PBPs). As a result, such drugs have the ability to display an apparent time-dependent bactericidal mechanism of action in cells that are actively synthesizing their cell wall. Among the factors affecting their efficacy are the ability to permeate through the outer cell membrane of gram-negative bacteria, beta lactamase production, amount of peptidoglycan and expression of receptor sites (PBP). For beta-lactamase-producing bacteria, penicillins should be given in combination with a beta-lactamase inhibitor. These drugs are assumed be very safe with little to no toxic effects. Still, negative effects include anaphylaxis or less severe hypersensitivity symptoms.

2.6.1.1.Amoxicillin/Amoxicillin and clavulanic acid

Amoxicillin is a member of the broad-spectrum penicillin family but is still susceptible to beta-lactamase. To combat this susceptibility to beta-lactamase, a combination with the beta-lactamase inhibitor clavulanic acid can be used.

The broad-spectrum activity of amoxicillin against gram-negative bacteria (*e.g. Salmonella, E. coli and Proteus mirabilis*) exceeds other narrow-spectrum penicillins. The systemic availability of the drug is between about 60% and 70%, approximately twice that of ampicillin (20–40%), which belongs to the same group of broad-spectrum penicillin. Due to its pharmacokinetic properties of almost completely excretion via the kidneys, it is a perfect agent for empirical treatment for urinary tract infections (UTIs), though acquired resistance has significantly reduced the effectiveness through involve plasmid or integron - mediated factors [22].

2.6.1.2.Cephalosporins

Another member of the beta lactam antibiotic family and same mode- and mechanism of action. They generally show the well-known features of beta-lactams and are considered as one of the safest groups of antimicrobials. The reason that makes these antibiotics very different from other groups is their extra resistance to *Staphylococcal* beta-lactamase. Additionally, they show great activity against hemolytic *Streptococci*.

Currently four generations of cephalosporins have been developed (two generations belong to AMEG classification C and the other two to B): The first has mainly activity against gram positive microbes. Next, those second-generation cephalosporins are active against gram-

positive organisms and some gram-negative bacteria. The third generation has reduced activity against gram-positive bacteria but improved activity against gram-negative bacteria. Fourth and last generation improve the activity for both gram-positive and negative bacteria.
Modification of penicillin-binding-proteins (PBP), along with diminished efflux and decreased permeability, particularly the production of beta-lactamase, can contribute to the development of resistance.
Cefuroxim, a second-generation cephalosporin, has a broad spectrum and is therefore considered a great choice for the treatment of UTIs, as cefalexin (first generation) shows ineffectiveness against *pseudomon*as and *proteus*.
Recent concept considers a reduction of third- and fourth-generation cephalosporin treatment as one of the main pillars of prudent antimicrobial use.

2.6.2. Lincosamide

Lincosamides are able to inhibit protein synthesis at the 50S ribosomal subunit by blocking the peptidyl transferase. Their antimicrobial spectrum is moderate, because many gram-negative bacteria develop resistance to these agents by failing to penetrate the ribosomal binding site. Clindamycin is equally effective against some bacteria as lincomycin but has superior activity against anaerobes compared with lincomycin. Both are universally considered effective for gram-positive bacteria and some mycoplasma. Resistance can arise as cross-resistance to macrolides, either constitutive (high-level resistance to all macrolide, lincosamide and streptogramin B antibiotics) or dissociated (thought to be primarily due to mutations in 23S rRNA that confer initially macrolide resistance but after exposure leading to the emergence of resistance against lincosamides).
Compared with herbivorous animals, the toxicity of lincosamide has been found to be mild in canines, which puts it firmly into group C for AMEG.

2.6.3. Macrolides

These agents reversibly bind to the 50S subunits of the ribosome and inhibit protein synthesis. This leads to the inhibition of transpeptidation resulting in the early detachment of polypeptide chains. They have a bacteriostatic effect. Bacterial resistance involves ribosomal RNA (rRNA) modification, enzymatic inactivation and active efflux. Their classification falls under AMEG Group C.

2.6.4. Aminoglycosides

Aminoglycosides are antibiotics with enhanced activity against aerobic gram-negative bacteria and *Staphylococci*. However, their application has been overshadowed by the introduction of fluoroquinolones, which offer superior safety and enhanced distribution kinetics.

Their effectiveness relies on their ability to penetrate bacterial cells. This may occur in at least two distinct ways: Through the co-administration of drugs that disrupt cell wall synthesis, such as beta lactam antibiotics, and the second way is that aerobic gram-negative bacteria actively take them into the cell based on an oxygen-dependent pump. Upon entering the bacterial cell, aminoglycosides bind to the 30S ribosomal subunit, resulting in misreading of the genetic code and interfering with the proper protein synthesis. This is due to their bactericidal activity which is concentration-dependent [22].Furthermore there is a significant post-antibiotic effect (PAE) a period where antimicrobial levels fall below MIC yet render bacteria more vulnerable to host defenses. Gentamicin belongs to this group.

2.6.5. Fluoroquinolones

These substances show general properties such as high distribution throughout the body through penetration of most types of tissue. Bactericidal effects dependent on concentration. Most fluoroquinolones are recognized as second-generation agents with an increased spectrum covering aerobic gram-negative bacteria (eg. *Enterobacteriaceae, Pasteurella spp*). Second-generation fluoroquinolones have decreased activity against gram-positive bacteria, and even less for anaerobic bacteria. To correct this lack of efficiency, newer generations have been developed. As members of the broadest class of antibiotics they should not be used as a first line choice for bacterial infections. Instead, administration should be limited to cases where the infection poses a major danger to the patient's life. In addition to that, they are considered moderately safe, which also provides a reason for them being used so often in such critical scenarios. Its mechanism of action is facilitated through the inhibition of DNA synthesis.

2.6.6. Potentiated sulfonamides

Sulfonamides inhibit the biosynthesis of folic acid in bacterial cells by competing with para-aminobenzoic acid (PABA) for incorporation into the folate (pteroylglutamic acid) molecule. This selective bacteriostatic effect can be differentiated by the mechanism in which bacteria and mammalian cells obtain folic acid. Susceptible microorganisms must synthesize folic acid,

while mammalian cells can utilize the preformed folic acid. If an excess of PABA is available, it will counteract this mechanism, so any tissue exudate or necrotic material shall be removed prior to the administration of these agents [22].

These agents are recognized as broad spectrum antimicrobials, but due to extensive resistance they have been significantly hampered. Resistance can develop through chromosomal mutations, such as plasmid and integron mediated patterns linked to special genes. Especially enteric bacteria are able to develop high resistance through the diminishing of drug penetration [22].

Sumetrolim is a potentiated sulfonamide, developed through a synergistical mix of sulfonamide and diaminopyrimidine. In the case of sumetrolim, sulfamethoxazole and trimethoprim are mixed in a 5:1 ratio, increasing the spectrum and lowering the risk of resistance development.

3. Materials and methods

3.1. Animals

The veterinary university of Budapest serves as a referral clinic, frequently treating patients with multiple comorbidities, as well as cases directed to it after diagnosis and treatment by field veterinarians.

The samples were therefore collected from dogs exhibiting clinical symptoms of cystitis, or potentially from patients with multiple health issues who showed indications or laboratory findings of inflammatory diseases, septic conditions, or disorders that predispose them to urinary tract infections (such as diabetes, chronic kidney disease, hyperadrenocorticism, or urolithiasis).

3.2. Sample taking

The standard procedure for obtaining a urine sample intended for microbiological culture and resistance testing at the clinic involves ultrasound guided cystocentesis. Prior to the submission of samples for microbiological analysis, routine urinalysis and sediment examination are conducted. The identification of an active sediment, characterized by a minimum of five leukocytes per visual field, is classified as cystitis. Such samples are subsequently forwarded to the microbiology laboratory for detailed evaluation.

Since the microbiology laboratory is located off-site, any samples that cannot be transported immediately are stored in a refrigerator until they can be sent, typically within a 24-hour period.

3.3. Laboratory Examination

During the microbiological examination, the samples were cultured on Columbia agar containing 5% sheep blood (COS, bioMérieux, Marcy l'Etoile, France) and MacConkey agar (MAC, bioMérieux, Marcy l'Etoile, France).

The media were incubated for 4 x 24 hours at 35 °C in normal atmospheric conditions enriched with 5% carbon dioxide, and bacterial growth was checked every 24 hours. The identification of bacteria was based on colony type and morphology, Gram staining, and standard biochemical tests. Negative results were recorded and released after 24-48 hours. Further incubation was

conducted to detect slower-growing potential uropathogens (*Ureaplasma, Corynebacterium urealiticum*). The results of this were recorded and communicated only in positive cases.

The antibiotic sensitivity testing was performed using the Kirby-Bauer disk diffusion method (test disk: Oxoid, Basingstoke, UK) on Mueller-Hinton 2 agar (MH2, bioMérieux, Marcy l'Etoile, France). The range of antibiotics that can be tested for a given group of bacteria and the breakpoint values (mm) were determined based on the guidelines of EUCAST (European Committee on Antimicrobial Susceptibility Testing, 2023) and CLSI Vet (2022).

Laboratory testing was conducted at the Doubakt Veterinary Microbiological Laboratory (Veresegyház, Hungary)

4. Results

From the total of 450 samples of the 332 dogs, 194 samples (43,56%) were positive while 256 (56,44%) samples were negative. Out of the 194 positive samples, 48 (24,74%) were recurrent cases.

Reasons for negative samples include sterile cystitis, urolith or tumor related inflammations, or previous antibiotic administration close to the time of sampling, done by the referring vet or the clinic itself, that might influence the result. Additionally, negative samples which were falsely interpreted or might have been positive due to contamination of a glass slide, along with borderline leucocytes in the sediment and immune compromised patients add to this list.

4.1. Age, breed and gender distribution

Among the tested dogs, 76 (52,1%) were females and 70(47,9%) were male. The average age of the dogs was 8,7 years, with the youngest being a 5-month-old male Golden retriever and the oldest a 16-year-old female Englisch bullterrier (Figure 1).

The most frequently treated breed was the Beagle and Yorkshire terrier, each represented by 8 cases (5,48%), followed by the French bulldog and Border collie with 6 specimens (4,11%) (Figure 2).

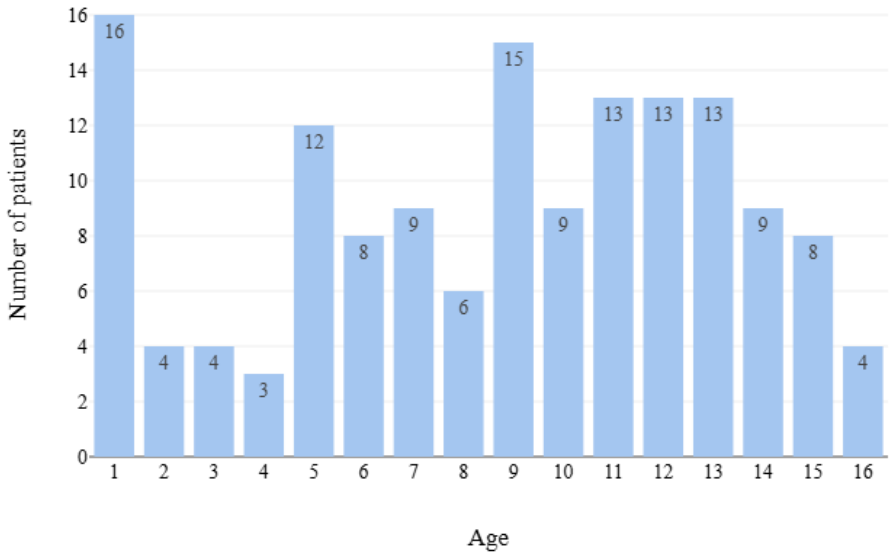

Figure 1. Age distribution of dogs tested

Breeds distribution in this research tended to be directed into smaller to medium sized dogs instead of larger dogs. Several factors may contribute to this trend, including frequent anatomical issues in smaller breeds like Pug or Yorkshire terrier, as well as breed predisposition to urinary abnormalities like the Bulldog or Bichon frisé, the latter being associated with higher risk of urinary stones [23].

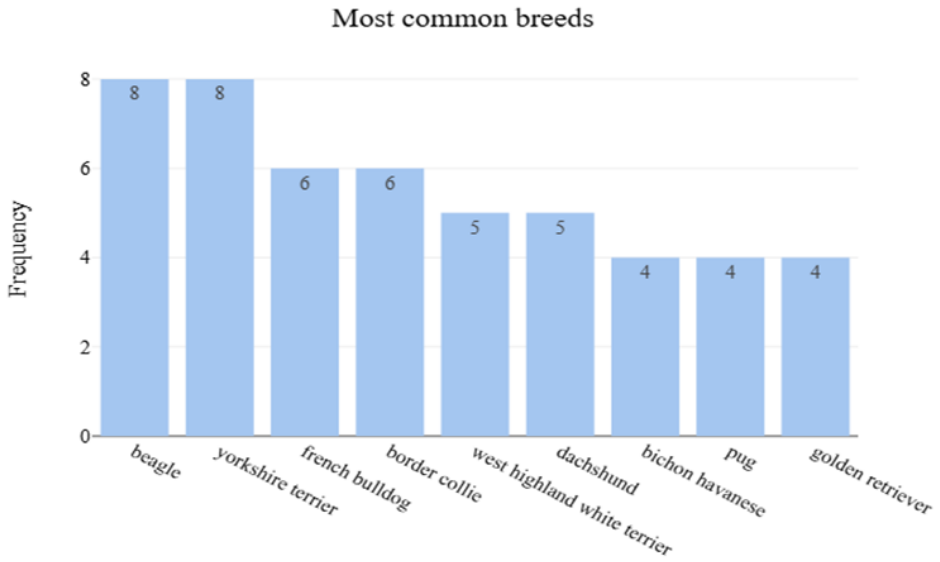

Figure 2. Distribution among the most common breeds

4.2. Bacterial prevalence

The predominant bacterial species identified was *Escherichia coli*, which made up over 52% of the total. This was followed by *Staphylococcus spp.,* accounting for 12,34%, with *Staphylococcus pseudointermedius* being the most frequent among them, followed by *Staph. aureus* and *coagulase negative Staphylococcus*. The third most widespread bacteria were found to be *Klebsiella spp.* at 10,95%, in which the *K. pneumoni*ae strain was most abundant compared to *K. oxytoca* and *K. aerogenes*. Additionally, *Proteus mirabilis* emerged as a significant bacterium, comprising 8,92%. *Enterobacter spp, Enterococcus, Pseudomonas, Streptococcus* were present but in lower frequency accounting for around 10% of total bacterial infections. Finally, we detected the presence of *Mycoplasma, Serratia* and C*orynebacterium spp.*, but these accounted for only about 6% of the infection, indicating them as less clinically relevant.

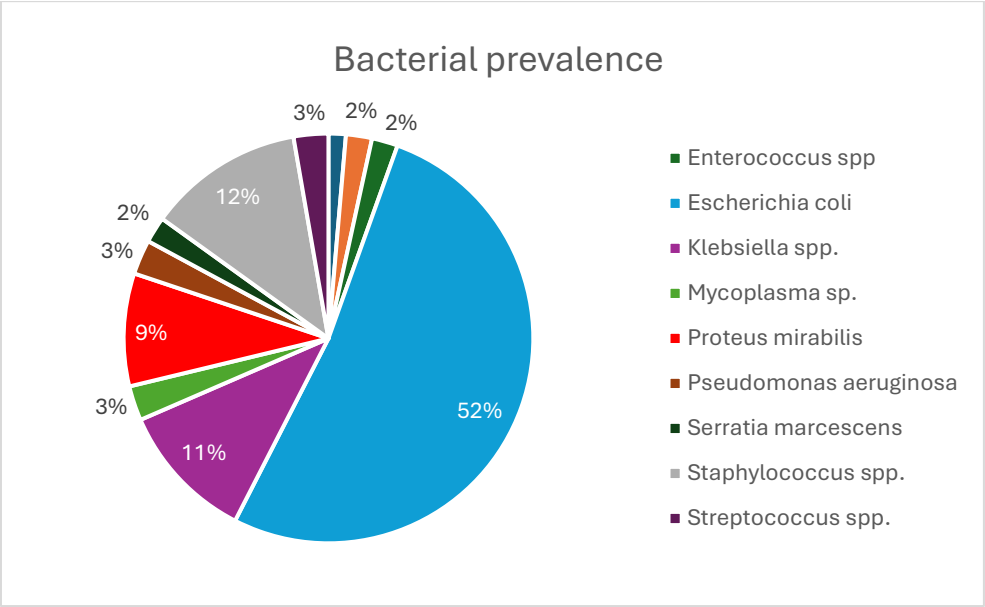

Figure 3. Bacterial prevalence in relation to the most common isolates

In relation to the recurrent instances, of the 47 cases analyzed more than 61% were attributed to *E.Coli*, with Klebsiella accounting for 17,02% followed by the lower distribution of *Staphylococcus, Enterococcus, Proteus* and *Mycoplasma* (Table 1).

Table 1. Positive recurrent cases

BACTERIA	**Recurrent cases**	
	n	%
Enterococcus spp.	3	6,38%
E. coli	29	61,70%
Klebsiella spp.	8	17,02%
Proteus mirabilis	2	4,26%
Staphylococcus spp.	4	8,51%
Mycoplasma spp.	1	2,13%
Total	**47**	100%

E.coli exhibited significant resistance to amoxicillin, with a resistance rate of 28,95%. Meanwhile amoxicillin/clavulanic acid showed a resistance level of 15,79%, while ciprofloxacin and enrofloxacin demonstrated lower resistance rates at 11,84%. Gentamicin proved to be the most effective treatment with an efficacy rate of 98,68%, closely followed by cefuroxime. Among the 76 *E.coli* strains that were tested, 8 (10,53%) displayed resistance towards a single antimicrobial agent, 3 (3,95%) against 2 different groups, while 12 (15,79%) were identified as multidrug resistant, exhibiting resistance to three or more agents (Table 2).

Table 2. E.coli's sensitivity and resistance

ESCHERICHIA COLI						
Antibiotic name	Sensitive		Resistance		Intermediate	
	n	%	n	%	n	%
Amoxicillin	54	71,05%	22	28,95%		
Amoxicillin & Clavulanic acid	64	84,21%	12	15,79%		
Ciprofloxacin	66	86,84%	9	11,84%	1	1,32%
Cefuroxim	73	96,05%	3	3,95%		
Enrofloxacin	66	86,84%	9	11,84%	1	1,32%
Gentamicin	75	98,68%	1	1,32%		
Sumetrolim	69	90,79%	7	9,21%		

Klebsiella spp. demonstrated a remarkable resistance of 93,75% to amoxicillin. There was significant resistance observed against the antimicrobials tested, however this assessment is difficult to judge due to the lower number of positive *Klebsiella spp.* samples. Among the 16 cases analyzed, 4 possessed multidrug resistance, while 10 showed resistance solely towards amoxicillin (Table 3)

Table 3. Klebsiella spp. sensitvity and resistance

KLEBSIELLA SPP.						
Antibiotic name	Sensitive		Resistance		Intermediate	
	n	%	n	%	n	%
Amoxicillin	1	6,25%	15	93,75%		
Amoxicillin & Clavulanic acid	12	75%	4	25%		
Ciprofloxacin	13	81,25%	2	12,5%	1	6,25%
Cefuroxim	13	86,66%	1	6,67%	1	6,67%
Enrofloxacin	13	81,25%	2	12,5%	1	6,25%
Gentamicin	14	87,5%	1	6,25%	1	6,25%
Sumetrolim	14	87,5%	2	12,5%		

Proteus mirabilis demonstrated complete sensitivity towards amoxicillin/clavulanic acid, cefuroxime and gentamicin. Nevertheless, it revealed varying degrees of resistance to amoxicillin and sumetrolim. From the 13 strains, 3 out of the 4 cases were resistant to two antimicrobials (Table 4).

Table 4. Proteus mirabilis sensitivity and resistance

PROTEUS MIRABILIS						
Antibiotic name	Sensitive		Resistance		Intermediate	
	n	%	n	%	n	%
Amoxicillin	10	76,92%	3	23,08%		
Amoxicillin & Clavulanic acid	13	100%	0	0%		
Ciprofloxacin	11	84,62%	1	7,69%		
Cefuroxim	13	100%	0	0%		
Enrofloxacin	11	84,62%	1	7,69%	1	7,69%
Gentamicin	13	100%	0	0%		
Sumetrolim	11	84,62%	2	15,38%		

Staphylococcus spp. illustrated the highest sensitivity to gentamicin, with amoxicillin/clavulanic acid, ciprofloxacin, enrofloxacin and sumetrolim following in efficacy. Amoxicillin presented a significant resistance rate at 75%, albeit based on only four infections assessed. 3 out of the 18 strains that were tested proved to be multidrug resistant, while an additional two were exclusively resistant to amoxicillin (Table 5).

Table 5. Staphylococcus spp. sensitivity and resistance

STAPHYLOCOCCUS SPP.						
Antibiotic name	Sensitive		Resistance		Intermediate	
	n	%	n	%	n	%
Amoxicillin	1	25%	3	75%		
Amoxicillin & Clavulanic acid	15	83,33%	3	16,67%		
Ciprofloxacin	15	83,33%	3	16,67%		
Cefuroxim	2	66,67%	1	33,33%		
Enrofloxacin	15	83,33%	3	16,67%		
Gentamicin	15	83,33%	2	11,11%	1	5,56%
Sumetrolim	15	83,33%	3	16,67%		

4.3. Antimicrobial resistance patterns

Amoxicillin shows a significant higher level of resistance compared to other commonly used antimicrobials, including amoxicillin in combination with clavulanic acid. When analyzing the data collected for *E. coli*, identified as the most prevalent bacterium, it presents a resistance of 28,95%. Contrarily, amoxicillin/clavulanic acid demonstrates a lower resistance rate of 15,79%. Cumulatively, the overall resistance to amoxicillin reached 41,46% across 123 cases, which is equal to approximately 1 in every 3 instances (Table 6).

Table 6. Amoxicillin resistance and effectiveness compared to the different Microbial

AMOXICILLIN					
Microbial Name	**Sensitive**		**Resistance**		**Total**
	n	%	n	%	n
Escherichia coli	54	71,05%	22	28,95%	76
Klebsiella spp.	1	6,25%	15	93,75%	16
Proteus mirabilis	10	76,92%	3	23,08%	13
Staphylococcus spp.	1	25%	3	75%	4
Streptococcus spp	4	100%	0	0%	4
Enterococcus spp.	2	66,7%	1	33,3%	3
Serratia marcescens	0	0%	3	100%	3
Enterobacter cloacae	0	0%	3	100%	3
Corynebacterium urealyticum	0	0%	1	100%	1
Total	72	**58,54%**	51	**41,46%**	**123**

In the case of amoxicillin/clavulanic acid, strains of *Serratia marcescens* and *Enterobacter cloacae* demonstrated a complete resistance of 100% in six instances. An overall resistance rate of 19,7% was analyzed across a total of 132 cases, over 20% lower compared to amoxicillin. (Table 7).

Table 7. Amoxicillin and clavulanic acid resistance and effectiveness compared to the different microbials

AMOXCICILIN & CLAVULANIC ACID					
Microbial Name	**Sensitive**		**Resistance**		**Total**
	n	%	n	%	n
Escherichia coli	64	84,21%	12	15,79%	76
Klebsiella spp.	12	75%	4	25%	16
Proteus mirabilis	13	100%	0	0%	13
Staphylococcus spp.	15	83,33%	3	16,67%	18
Streptococcus spp	1	100%	0	0%	1
Enterococcus spp.	1	50%	1	50%	2
Serratia marcescens	0	0%	3	100%	3
Enterobacter cloacae	0	0%	3	100%	3
Total	106	**80,3%**	26	**19,7%**	**132**

Cefuroxim resistance was evaluated in 116 cases, revealing that 6.9% demonstrated resistance, while another 3,45% (equating 4 cases) displayed intermediate sensitive. Out of 76 *E. coli* cases, 3 were resistant and all 13 *Proteus mirabilis* cases had no resistance. *Klebsiella pneumoniae* only provided 1 resistance out of 12 cases (Table 8).

Table 8. Cefuroxim resistance and effectiveness compared to the different microbials

CEFUROXIM							
Microbial Name	**Sensitive**		**Resistance**		**Intermediate**		**Total**
	n	%	n	%	n	%	n
Escherichia coli	73	96,05%	3	3,95%	0	0%	76
Klebsiella spp.	13	86,66%	1	6,67%	1	6,67%	15
Proteus mirabilis	13	100%	0	0%	0	0%	13
Staphylococcus spp.	2	66,67%	1	33,33%	0	0%	3
Streptococcus beta-haemolytica	1	100%	0	0%	0	0%	1
Enterococcus faecalis	1	50%	0	0%	1	50%	2
Serratia marcescens	0	0%	3	100%	0	0%	3
Enterobacter cloacae	1	33,33%	0	0%	2	66,67%	3
Total	104	**89,66%**	8	**6,9%**	4	**3,45%**	**116**

Ciprofloxacin resistance was 12,5% in a total of 136 cases. Resistance of *Staphylococcus pseudointermedius* was the highest with 21,43% of the 14 cases presented. *E. coli* resistance showed 11.84%. It would be an important fluoroquinolone due to its antimicrobial effects

against *Pseudomonas,* but as there is no veterinary product available for dogs, enrofloxacin or pradofloxacin will be used if needed (Table 9).

Table 9. Ciprofloxacin resistance and effectiveness compared to the different microbials

CIPROFLOXACIN							
Microbial Name	**Sensitive**		**Resistance**		**Intermediate**		**Total**
	n	%	n	%	n	%	n
Escherichia coli	66	86,84%	9	11,84%	1	1,32%	76
Klebsiella spp.	13	81,25%	2	12,5%	1	6,25%	16
Proteus mirabilis	11	84,62%	1	7,69%	1	7,69%	13
Staphylococcus spp.	15	83,33%	3	16,67%	0	0%	18
Streptococcus beta-haemolytica	0	0%	0	0%	1	100%	1
Enterococcus faecalis	0	0%	2	100%	0	0%	2
Serratia marcescens	2	66,67%	0	0%	1	33,33%	3
Enterobacter cloacae	3	100%	0	0%	0	0%	3
Pseudomonas aeruginosa	4	100%	0	0%	0	0%	4
Total	114	**83,82%**	17	**12,5%**	5	**3,68%**	**136**

Enrofloxacin resistance was tested in 132 cases with an overall resistance of 12,88% and 3,79% intermediate susceptibility (Table 10).

Table 10. Enrofloxacin resistance and effectiveness compared to the different microbials

ENROFLOXACIN							
Microbial Name	**Sensitive**		**Resistance**		**Intermediate**		**Total**
	n	%	n	%	n	%	n
Escherichia coli	66	86,84%	9	11,84%	1	1,32%	76
Klebsiella spp.	13	81,25%	2	12,5%	1	6,25%	16
Proteus mirabilis	11	84,62%	1	7,69%	1	7,69%	13
Staphylococcus spp.	15	83,33%	3	16,67%	0	0%	18
Streptococcus beta-haemolytica	0	0%	0	0%	1	100%	1
Enterococcus faecalis	0	0%	2	100%	0	0%	2
Serratia marcescens	2	66,67%	0	0%	1	33,33%	3
Enterobacter cloacae	3	100%	0	0%	0	0%	3
Total	110	**83,33%**	17	**12,88%**	5	**3,79%**	**132**

Sumetrolim was tested in 132 cases and 10,61% of the microbes were proven to be resistant. Though it has a 100% efficacy against all the low number microbes present, such as *Serratia* or *Enterobacter* (Table 11).

Table 11. Sumetrolim resistance and effectiveness compared to the different microbials

SUMETROLIM					
Microbial Name	**Sensitive**		**Resistance**		**Total**
	n	%	n	%	n
Escherichia coli	69	90,79%	7	9,21%	76
Klebsiella spp.	14	87,5%	2	12,5%	16
Proteus mirabilis	11	84,62%	2	15,38%	13
Staphylococcus spp.	15	83,33%	3	16,67%	18
Streptococcus beta-haemolytica	1	100%	0	0%	1
Enterococcus faecalis	2	100%	0	0%	2
Serratia marcescens	3	100%	0	0%	3
Enterobacter cloacae	3	100%	0	0%	3
Total	118	**89,39%**	14	**10,61%**	**132**

Gentamicin was tested in 136 cases with a resistance of 5,15% and intermediate susceptibility of 2.21%. Its efficacy towards the low representative microbes, such as *Serratia marcescens, Enterobacter cloacae or Pseudomonas aeruginosa,* is high but falls short compared to Sumetrolim in the cases of *Streptococcus spp.* or *Enterococcus faecalis* (Table 12).

Table 12. Gentamicin resistance and effectiveness compared to the different microbials

GENTAMICIN							
Microbial Name	**Sensitive**		**Resistance**		**Intermediate**		**Total**
	n	%	n	%	n	%	n
Escherichia coli	75	98,68%	1	1,32%	0	0%	76
Klebsiella spp.	14	87,5%	1	6,25%	1	6,25%	16
Proteus mirabilis	13	100%	0	0%	0	0%	13
Staphylococcus spp.	15	83,33%	2	11,11%	1	5,56%	18
Streptococcus beta-haemolytica	0	0%	1	100%	0	0%	1
Enterococcus faecalis	0	0%	1	50%	1	50%	2
Serratia marcescens	3	100%	0	0%	0	0%	3
Enterobacter cloacae	3	100%	0	0%	0	0%	3
Pseudomonas aeruginosa	3	75%	1	25%	0	0%	4
Total	126	**92,64%**	7	**5,15%**	3	**2,21%**	**136**

5. Discussion

A Hungarian thesis from 2017 written at the university of veterinary medicine in Budapest, titled ”Analysis of antimicrobial resistance of bacteria isolated from dogs with bacterial cystitis” [24], will serve as a leading reference point for comparing antimicrobial resistance trends with those observed seven years prior. This comparison is crucial for understanding how antibiotic resistance has evolved over time and can help identify potential areas for intervention in veterinary medicine.

In our research female dogs were more frequently affected by urinary tract infections, than the male population, with rates of 52,1% in females versus 47,9% in males. This finding is validated by other studies that indicate an even more pronounced prevalence among females [3, 25]. The increased susceptibility is likely attributable to their shorter urethras which facilitates bacterial ascension.

Urinary tract infection typically occurs in middle aged dogs. Our study revealed a median age of 8,7 years. With a range spanning from 1-16 years, this data aligns with findings of other studies, such as the previous Hungarian study with an average age of 9,3 years [24]. These older patients might possess compromised immune systems or existing health conditions that render them more vulnerable to infections.

Determining breed predispositions present considerable challenges as there are multiple factors involved. Varying origins of studies for example will almost certainly yield different outcomes. Despite that, we noted a trend indicating that smaller and medium sized dogs were more prevalent. Breeds such as the French bulldog, Dachshund and Beagle are particularly common nowadays, especially in urban areas.

Furthermore, it should be noted that certain breeds might be more susceptible to UTI due to their genetic predisposition increasing their likelihood of facilitating urinary tract abnormalities or augmenting their risk of urolithiasis. As previously mentioned, breeds like Pugs, Yorkshire terrier and Bichon frisé fall under this category.

The occurrence of isolated bacteria in the urine samples analyzed in our research yielded varied outcomes when compared with findings from Europe, particularly those from Hungary.

In our study *Escherichia coli* emerged as the predominant bacterium in the urinary tract of dogs, accounting for 52,05% of all cases which, when compared to the other study, is slightly lower than the reported 57,4% [24]. This occurrence can be ascribed to the ubiquitous presence of

Escherichia coli in the environment, which is coupled with various virulence factors, increasing resistance to a wider array of antibacterial agents and the capability to endure within host cells. *Staphylococcus spp.* represented 12,34% of our samples, reflecting a modest increase compared to the Hungarian study's 9,8%[24], with a difference of approximately 2,5%. Despite that other studies in the last two decades suggest our findings to be comparable [26, 27].

The presence of *Klebsiella spp.* was recorded at 10,95%, making it the third most frequently identified bacterium, with *Klebsiella pneumoniae* being the most common strain detected. In contrast to the Hungarian thesis where this pathogen was not noted, our data indicates a significant shift. Other studies have suggested a more typical prevalence around 2% [26, 27]. This finding warrants caution since resistance to amoxicillin, one of the most used empirical antibiotics, was observed in 93,75% of cases.

The occurrence rate for *Proteus mirabilis* was found to be 8,92%, aligning with figures from other studies [26, 27]. Comparatively, Hungarian data indicated an occurrence rate of 18% [24], which despite their study having fewer samples, represents a substantial decline.

The rates for *Streptococcus spp., Pseudomonas aeruginosa and Enterobacter,* which were observed at rates of 2,74%, 2,74% and 2,05%, respectively, closely resemble the data found in both the Hungarian thesis and additional literature. However, our *Enterococcus spp.(2,05%)* findings were significantly lower compared to earlier studies (approximately 10%) [24, 26, 27].

Resistance patterns observed in vitro showed that amoxicillin had the highest resistance rate at 41,46%, followed by amoxicillin/clavulanic acid at 19,7%, enrofloxacin at 12,88%, ciprofloxacin at 12,5%, sumetrolim at 10,61%, cefuroxime at 6,9% and gentamicin at just over five percent (5,15%). Notably, amoxicillin resistance nearly doubled since previous assessments, while amoxicillin/clavulanic acids resistance rose by three percentage points compared to 2017 data. Meanwhile fluroquinolone resistance decreased by about six percent (previously being at 18,2%) and sulphonamide resistance fell by more than nine percentage (previously 19,7%) [24]. Gentamicin exhibited the most significant reduction over the last seven years with a decrease of exceeding 10%. This decrease may indicate that strategies to reduce antibiotic use or improved prescribing practices are having a positive impact.

Foundation for the amoxicillin resistance is the increased production of β-lactamase, particularly with the rise of extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase. These enzymes are commonly produced by *Klebsiella* strains and possess the ability to

hydrolyze a broad range of β-lactams [28]. Furthermore, horizontal gen transfer via plasmids or integrons consolidate resistance.

Amongst *E.coli* strains examined there was a recorded resistance level of 28,95% towards amoxicillin compared to amoxicillin/clavulanic acid with 15,79%, and proving the highest effectiveness in cefuroxime (96,05%), a 2nd generation cephalosporin and gentamicin (98,68%). When comparing these results with the previous study an overall decline in antimicrobial resistance by around 10% can be observed (amoxicillin resistance was at 37,1%, while gentamicin sensitivity was only at 88,6%) [24]. This result can be explained by the increased introduction of prudent use into the veterinary field.

The rise in amoxicillin resistance can likely be linked to the emergence of *Klebsiella spp*., which demonstrated a substantial resistance level reaching 93,75%. Cefuroxim (86,66%), Gentamicin (87,5%) and sumetrolim (87,5%) displayed the highest efficacy towards *Klebsiella spp.*

Meanwhile *Proteus mirabilis* exhibited complete sensitivity towards amoxicillin/clavulanic acid, cefuroxime and gentamicin, but showed resistances of 23,08% against amoxicillin and 15,38% towards sumetrolim. Compared to the previous findings, there has been an increase of five percentage points concerning amoxicillin (previously 18,2%), while showing a decrease from over 21% regarding sumetrolim (previously 36,4%).

Our study recorded 48 recurrent cases, with more than half suffering for more than two episodes.

Notably, a quarter of all recurrent bacterial strains exhibited multidrug resistance to three or more classes of antimicrobials. When considering bacteria resistant to two groups as well, the cumulative resistance rises to 37.5%, in contrast to the 22.6% observed in uncomplicated bacterial cystitis cases.

For instance, strains from the recurrent *E. coli* infections displayed a multidrug resistance rate of 27.59%.

The variation in resistance rates highlights the need for continued surveillance and research to ensure that treatment protocols remain effective in the face of evolving resistance patterns.

The limitations in our study were the missing medical records of patients. Recurrent cases may therefore have had underlying reasons, such as endocrine disorders or anatomical alterations.

6. Summary

Bacterial urinary tract infections are one of the most common infectious diseases affecting dogs. Typically, these infections arise from bacteria that are part of the normal skin or gastrointestinal microbiota and often lead to ascending infections. Cystitis can be classified into either sporadic cases or recurrent bacterial cystitis. Utilizing ultrasound-guided cystocentesis at the University of Veterinary Medicine in Budapest, we were able to collect 450 urine samples from 332 dogs for analysis. Conducted between November 2020 and November 2023 a positive culture rate of 43.56% was revealed.

The research identifies *Escherichia coli* as the most prevalent pathogen, responsible for 52.05% of positive cases, followed by *Staphylococcus spp*. (12.34%) and *Klebsiella spp*. (10.95%). Resistance testing proved that *E. coli* demonstrated a significant resistance of 28.95% towards amoxicillin, while gentamicin indicated high sensitivity at 98.68%. Notably, *Klebsiella spp.* displayed 93,75% resistance to amoxicillin.

The objective of this study was to investigate the prevalence and resistance patterns of microbial agents and compare them with other studies. The analysis was stratified by gender, age, and breed, following the current veterinary recommendations.

These data highlight the need for continued awareness of UTI pathogen epidemiology, along with examining the consequences of antibiotic resistance in veterinary practice. We therefore recommend continuous surveillance of resistance patterns and adherence to established guidelines for antimicrobial use, emphasizing a One Health approach that recognizes the connection between human and animal health. In conclusion, this study provides valuable insights into the management of UTIs in dogs, aiming to enhance treatment outcomes and decrease the risk of AMR.

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Thesis progress report for veterinary students

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Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2024.	~~FEBR~~	22.	TOPIC DEFINITION (TIME FRAME, SUBJECT), LITERATURE REVIEW.	d. Aradi
2.	2024.	MARC.	4.	LITERATURE SCANNING, STUDY PLANS	d. Aradi
3.	2024.	MARC.	18.	DATABASE, PATIENT POOL, STATISTICAL PLANNING.	d. Aradi
4.	2024.	APR.	19.	OVERSEEING TASKS (MAT. & METH., LAB. METHODOLOGY)	d. Aradi
5.	2024.	MAJ.	7.	FINALIZING STUDY PLAN, TASK LIST; LITERATURE DISCUSSION.	d. Aradi

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2.	2024.	OKT.	26.	STATISTICAL ANALYSIS, RESULTS DISC.	d. Aradi
3.	2024.	NOV.	9.	(RESULTS TO BE INCLUDED), DRAFT SCAN	d. Aradi
4.	2024.	NOV.	21.	SUGGESTED STRUCTURAL MODIFICATION (MINOR)	d. Aradi

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5.	2024.	NOV.	25.	(FORMAT CONTROL)	

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The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

.....
signature of the supervisor

Signature of the student: .....

Signature of the secretary of the department: .....

Date of handing the thesis in 28.11.2024