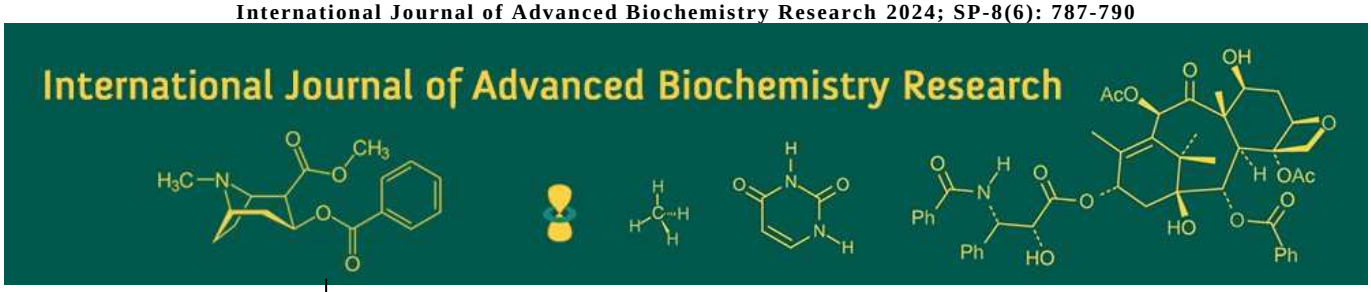

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Isolated pathogens and antibiogram in clinical cases of urinary tract infection in dogs

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Abstract
Aim: The objective of this study is to identify the causes of urinary tract infections (UTIs) in dogs and to establish an antibiogram of the isolated organisms.
Materials and Methods: Urine samples were collected via catheterization from 51 dogs suspected of having UTIs and admitted to VCC, LUVAS, Hisar. Bacteria were identified in 46 of these samples based on cultural characteristics and confirmed using the Vitek2 compact system. All isolates were subjected to vitro antimicrobial sensitivity testing.
Results: The urine samples positive for bacteria showed pure colony growth in 84.78% of cases and mixed growth in 15.21%. Among the 46 positive isolates, 19 (37.25%) were identified as *E. coli*, 9 (17.64%) as *Staphylococcus* spp., 4 (7.84%) as *Pseudomonas* spp., 3 (5.88%) as *Klebsiella* spp., 2 (3.92%) as *Proteus* spp., 1 (1.96%) as *Acinetobacter lwoffii*, and 1 (1.96%) as *Cronobacter dublinensis*. The isolates exhibited the highest sensitivity to Imipenem (65.32%) and Meropenem (65.04%), while showing complete resistance to Oxytetracycline (100%) and Tetracycline (100%).
Conclusion: Among dogs affected by UTIs, *E. coli* and *Staphylococcus* spp. were the most frequently isolated gram-negative and gram-positive bacteria, respectively. Antimicrobial sensitivity testing showed a notable portion of these bacteria to be resistant to multiple drugs.

Keywords: Canine urinary tract infections, multidrug resistance, *Escherichia coli*, antibiogram

Introduction
Urinary tract infections in dogs are frequent and arise due to various factors, typically happening when the immune system is weakened and either pathogens or normal bacteria enter the urinary tract (Ettinger and Feldman, 2010) [5]. Urinary tract infections (UTIs) can originate from either internal or external factors, with external factors being more prevalent. Furthermore, medical procedures like cystocentesis and catheterization can cause iatrogenic infections by introducing pathogens into the urinary system through the urethra (Thompson *et al*., 2011) [22]. Around 14% of dogs experience at least one episode of bacterial UTI in their lifetime (Ling *et al*., 1984) [10]. Pathogens such as bacteria, mycoplasma, fungi, and viruses are the main culprits of canine UTIs (Nelson and Couto, 2009) [14]. Among bacterial infections, *Escherichia coli* is the most commonly isolated, accounting for up to 30% of cases (Hall *et al*., 2013) [9]. Other bacteria, including *Klebsiella* spp., *Proteus* spp., *Enterococcus* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Citrobacter* spp., *Actinomycetes*, *Haemophilus* spp., and *Brucella* spp., have also been identified as causes of UTIs in dogs (Norris *et al*., 2000; Seguin *et al*., 2003) [16, 20]. UTIs in canines can be caused by a single pathogen, resulting in a simple infection, or by multiple pathogens, leading to a mixed infection (Thompson *et al*., 2011) [22]. Optimal practices for diagnosing and managing UTIs in companion animals include performing bacterial culture and sensitivity testing on urine-isolated pathogens before initiating treatment (Bartges *et al*., 2004) [2]. Despite this, antimicrobial treatment is frequently started empirically to alleviate clinical symptoms, bypassing these diagnostic steps (Guardabassi *et al*., 2004) [7]. Skipping urine culture and antimicrobial susceptibility tests can result in inappropriate antimicrobial selection and the emergence of multidrug-resistant (MDR) bacteria in recurrent UTI cases (Wong *et al*., 2015) [23]. The rising antimicrobial resistance in dogs is troubling as it complicates treatment, leading to therapeutic failures, increased morbidity and mortality, and higher healthcare costs associated with UTIs. This issue is also a public health concern due to the zoonotic potential of these pathogens (Ewers *et al*., 2011) [6]. Retrospective analysis of the most frequently isolated bacteria from urine samples of dogs with suspected UTIs and their antimicrobial

resistance patterns can help clinicians make informed decisions about first-line treatments (McMeekin *et al*., 2016) [12]. This approach facilitates the selection of appropriate, cost-effective antibiotics for timely and effective treatment. Consequently, the present study aims to identify the pathogens causing UTIs in dogs and to determine their antimicrobial sensitivity, enabling the establishment of an effective treatment protocol.

Materials and Methods

Sample collection

In this study, we collected 51 urinary samples from dogs which were preliminary diagnosed as UTIs or other relevant urinary system diseases by catheterization presented to small animal section of Veterinary Clinical Complex (VCC), Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar. Urine samples from the affected animals were collected aseptically in a sterile container. The samples were transported on ice to department of veterinary public health and epidemiology laboratory and processed on the same day. The history was collected by face-to-face interview with the dog owners using a self-designed questionnaire.

Bacterial Isolation and Identification

After receiving, the urine samples were streaked with the help of a 4 mm diameter platinum loop on 5% sheep blood agar (BA) (Hi-Media, Mumbai, India), MacConkey's lactose agar (MLA) (Hi-Media, Mumbai, India) and Brain Heart Infusion (BHI) (Hi-Media, Mumbai, India) plates separately. The plates were then incubated aerobically at 37 °C for 24-48 hours. Colonies that grew on MLA were further sub-cultured on EMB agar after Gram staining. Colonies displaying purple-bluish precipitation on EMB, with or without a metallic sheen, were tentatively identified as *E. coli* and subjected to Polymerase Chain Reaction (PCR) amplification for confirmation using the *E. coli*-specific universal stress protein (uspA) gene (Osek, 2001) [17]. Isolates that tested negative for *E. coli* by PCR were identified using the Vitek2 compact system. Additionally, samples that did not show growth on MLA but grew on either BA or BHI were also identified using the Vitek2 compact system after Gram staining. Gram-negative (GN) and Gram-positive (GP) reagent cards were used for the identification of isolates other than *E. coli*, following the manufacturer's recommendations and the method described by Mittal *et al*. (2014) [13].

In-vitro Antimicrobial Susceptibility Testing

A total of 46 isolates were tested for AST was determined according to the method of Bauer-Kirby (Bauer *et al*., 1966) [3] by using commercially prepared disc (Hi-media, India) with known concentration of antibiotics. The following 24 antibiotics belonging to nine different classes were employed for the susceptibility testing; Amikacin (AK, 30 µg), Amoxycillin (AMX, 30 µg), Amoxyclav (AMC, 50/10 µg), Ampicillin (AMP, 10 µg), Cephalothin(CF, 22 µg), Ceftizoxime (CZX, 30 µg), Ceftriaxone/sulbactum (CIS, 30/15 µg), Cephalexin (CN, 30 µg), Chloramphenicol (C, 30 µg), Ciprofloxacin (CIP, 10 µg), Co-trimoxazole (COT, 25 µg), Doxycycline (DO, 30 µg), Enrofloxacin (EX, 10 µg), Gentamicin (GEN, 10 µg), Imipenem (IPM, 10 µg), Meropenem (MRP, 10 µg), Moxifloxacin (MO, 5 µg), Metronidazole (MT, 5 µg), Norfloxacin (NX, 10 µg), Ofloxacin (OF, 5 µg), Oxytetracycline (O, 30 µg), Penicillin G (P, 10 µg), Streptomycin (S, 10 µg), Tetracycline (TE, 30 µg). To ensure a conservative estimation of resistance, isolates with intermediate zones of inhibition were classified as resistant.

MDR bacteria

Based on their sensitivity and resistance patterns, isolates were categorized into three groups: Multi-Drug Resistant (MDR), Extreme Drug Resistant (XDR), and Pan-Drug Resistant. Isolates were classified as MDR if they were resistant to three or more antibiotics from different classes. Those resistant to all antibiotics were labeled as Pan-Drug Resistant. Isolates showing sensitivity to only two antibiotics from two different classes were categorized as XDR, a specific subset of MDR.

Results and Discussion

Urinary tract infection (UTI) is a significant health issue in canines. Recently, UTIs in domestic animals have been found to impact both production and reproductive status (Yerhuam *et al*., 2006) [24]. Among the 46 samples analyzed, 15.21% exhibited mixed bacterial growth, while 84.77% showed pure growth of a single colony. Contrary to our findings, reported a higher incidence of mixed growth, whereas similar results were observed by Nikvand *et al*. (2014) [15], Hajikolaei *et al*. (2015) [8], Al-Iraqi *et al*. (2016) [1], and Solomon *et al*. (2020) [21]. Out of 46 samples, 90.19% tested positive for bacterial growth, while 9.80% showed no growth upon culturing. Among the bacterial isolates, 65.21% were Gram-negative, and 19.56% were Gram-positive. The Gram-negative bacteria included *E. coli* (41.30% of total positive isolates), *Pseudomonas* spp. (8.69%), *Klebsiella* spp. (6.52%), *Proteus* spp. (4.34%), *Acinetobacter lwoffii* (2.17%), and *Cronobacter dublinensis* (2.17%). The Gram-positive bacteria identified were *Staphylococcus* spp. (19.56% of total positive isolates) (Table 1). Consistent with the present study, Liu *et al*. (2017) [11] and Roopali *et al*. (2018) [19] also identified *E. coli* as the major cause of UTIs in canines. Conversely, Nikvand *et al*. (2014) [15] and Hajikolaei *et al*. (2015) [8] reported *Staphylococcus* spp. as the most prevalent. Unlike the present study, which found no *Corneybacterium* sp. isolates, Al-Iraqi *et al*. (2016) [1] reported it as a primary cause of UTI.

Table 1: Groupwise distribution of isolated bacteria from canine UTIs

Bacterial groups	Bacterial isolates	No. of isolates (n=46)	Isolates (%)
Gram negative	*E. coli*	19	41.30%
	Pseudomonas spp.	4	8.69%
	Klebsiella spp.	3	6.52%
	Proteus spp.	2	4.34%
	Acinetobacter lwoffii	1	2.17%
	Cronobacter dublinensis	1	2.17%
	Total	30	65.21%
Gram positive	*Staphylococcus* spp.	9	19.56%
	Mixed infections	7	15.21%

The overall antimicrobial sensitivity analysis revealed a high degree of resistance, with sensitivity ranging from 2.17% to 65.21%, as illustrated in Table 2. This high resistance rate can likely be attributed to the indiscriminate

use of antibiotics, irregular dosing, or underdosing. Complete resistance (100%) was observed against Moxifloxacin, Ofloxacin, Penicillin G, Oxytetracycline, and Tetracycline. Conversely, the highest sensitivity was recorded for Imipenem and Meropenem.

Table 2: Overall antimicrobial sensitivity pattern of different bacterial isolates recovered from clinical cases of urinary tract in canines

S.no.	Class of Antimicrobials	Antimicrobials used	Sensitivity (%)
1.	Aminoglycosides	Amikacin (AK 30)	(34.78)
2.		Gentamicin (GEN 10)	(39.13)
3.		Streptomycin (S 10)	(23.91)
4.	Carbapenem	Imipenem (IPM 10)	(65.21)
5.		Meropenem (MRP 10)	(63.04)
6.	Cephalosporins	Cephalothin (CF 22)	(2.17)
7.		Cephalexin (CN 30)	(2.17)
8.		Ceftizoxime (CZX 30)	(26.08)
9.		Ceftriaxone/sulbactum (CIS 30/15)	(32.60)
10.	Fluoroquinolones	Ciprofloxacin (CIP 10)	(8.69)
11.		Enrofloxacin (EX 10)	(4.34)
12.		Moxifloxacin (MO 5)	(0.00)
13.		Norfloxacin (NX10)	(2.17)
14.		Ofloxacin (OF 5)	(0.00)
15.	Macrolides	Chloramphenicol (C30)	(41.30)
16.	Nitroimidazole	Metronidazole (MT 5)	(2.17)
17.	Penicillins	Amoxycillin (AMX 30)	(6.52)
18.		Amoxyclav (AMC 50/10)	(39.13)
19.		Ampicillin (AMP 10)	(4.34)
20.		Penicillin G (P10)	(0.0)
21.	Sulfonamides	Co-trimoxazole (COT 25)	(56.52)
22.	Tetracyclines	Doxycycline (DO 30)	(10.86)
23.		Oxytetracycline (O 30)	(0.00)
24.		Tetracycline (TE 30)	(0.00)

The antibacterial sensitivity pattern of *E. coli* isolates showed the highest sensitivity to Chloramphenicol, followed by Co-trimoxazole, Imipenem, and Meropenem. Contrary to these findings, Chang *et al.* (2015) [4] reported greater sensitivity of *E. coli* to amoxicillin and ampicillin. This discrepancy could be attributed to the indiscriminate use of these antibiotics, leading to decreased sensitivity and increased resistance among bacterial strains. *Staphylococcus* spp. isolates exhibited the highest sensitivity to Imipenem and Meropenem, followed by Amikacin, Ceftizoxime, and Co-trimoxazole. These findings differ from those of Penna *et al.* (2010) [18], and McMeekin *et al.* (2016) [12], who reported higher sensitivity of *Staphylococcus* spp. to enrofloxacin and amoxicillin with clavulanic acid. In this study, both *Pseudomonas* spp. and *Klebsiella* spp. showed the greatest antibacterial sensitivity to Imipenem and Meropenem, followed by Ceftriaxone/sulbactam. In contrast, *Proteus* spp. were found to be resistant to Ceftriaxone/sulbactam.

Table 3: Overall antibiotic sensitivity pattern of bacterial isolates from dog urine sample (sensitivity**%)**

S.No.	Antimicrobials used	*E. coli* (n=19)	*Staphylococcus* spp. (n=9)	*Pseudomonas* spp. (n=4)	*Klebsiella* spp. (n=3)	*Proteus* spp.(n=2)
1.	Amikacin (AK 30)	10 (52.63)	5 (55.55)	1 (25.00)	0(0.00)	0(0.00)
2.	Gentamicin (GEN 10)	11 (57.89)	4 (44.44)	2 (50.00)	0(0.00)	1 (50.00)
3.	Streptomycin (S 10)	6 (31.57)	3 (33.33)	2(50.00)	0(0.00)	0(0.00)
4.	Imipenem (IPM 10)	13 (68.42)	9 (100)	4 (100)	2 (66.66)	2(100)
5.	Meropenem (MRP 10)	12 (63.15)	9 (100)	4 (100)	2(66.66)	2(100)
6.	Cephalothin (CF 22)	0 (0.00)	1 (11.11)	0(0.00)	0(0.00)	0(0.00)
7.	Cephalexin (CN 30)	0 (0.00)	1(11.11)	0(0.00)	0(0.00)	0(0.00)
8.	Ceftizoxime (CZX 30)	4 (21.00)	5 (55.55)	2(50.00)	1 (33.33)	0(0.00)
9.	Ceftriaxone/sulbactum (CIS 30/15)	5 (26.31)	6 (66.66)	3 (75)	1(33.33)	0(0.00)
10.	Ciprofloxacin (CIP 10)	0 (0.00)	2 (22.22)	1(25.00)	0(0.00)	1(50.00)
11.	Enrofloxacin (EX 10)	0 (0.00)	2 (22.22)	0(0.00)	0(0.00)	0(0.00)
12.	Moxifloxacin (MO 5)	0 (0.00)	0 (0.00)	0(0.00)	0(0.00)	0(0.00)
13.	Norfloxacin (NX10)	0 (0.00)	1 (11.11)	0(0.00)	0(0.00)	0(0.00)
14.	Ofloxacin (OF 5)	0 (0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
15.	Chloramphenicol (C30)	15 (78.94)	2 (22.22)	2(50.00)	0(0.00)	0(0.00)
16.	Metronidazole (MT 5)	0 (0.00)	1(11.11)	0(0.00)	0(0.00)	0(0.00)
17.	Amoxycillin (AMX 30)	0 (0.00)	1(11.11)	0(0.00)	1(33.33)	1(50.00)
18.	Amoxyclav (AMC 50/10)	8 (42.10)	3 (33.33)	3(75.00)	2(66.66)	2(100)
19.	Ampicillin (AMP 10)	0 (0.00)	1(11.11)	0(0.00)	1(33.33)	0(0.00)
20.	Penicillin G (P10)	0 (0.0)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
21.	Co-trimoxazole (COT 25)	13 (68.42)	5(55.55)	4 (100)	2(66.66)	2(100)

22.	Doxycycline (DO 30)	0 (0.00)	1(11.11)	2(50.00)	1(33.33)	1(50.00)
23.	Oxytetracycline (O 30)	0 (0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
24.	Tetracycline (TE 30)	0 (0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)

The observed variation in the prevalence of different isolates and differences in sensitivity among researchers can be attributed to the differences in empirical treatments used in various regions. The findings of this study underscore the importance of determining the antibiogram of bacterial urinary tract infections before initiating therapy, as empirical treatment may lead to treatment failure.

Conclusion

In conclusion, this study provides a detailed analysis of urinary tract infections (UTIs) in canines, highlighting significant findings regarding bacterial prevalence and antimicrobial sensitivity. The predominance of Gram-negative bacteria, particularly E. coli, underscores its role as a primary causative agent of UTIs in dogs, consistent with similar studies. High resistance rates observed against several antibiotics emphasize the critical need for prudent antibiotic use and tailored treatment strategies based on local antibiograms. Disparities in bacterial profiles and sensitivity patterns across studies underscore the importance of regional variability in empirical treatment approaches. These findings underscore the necessity for veterinarians to adopt evidence-based approaches in managing UTIs to mitigate resistance and improve treatment outcomes in canine patients.

References

1. Al-Iraqi OM, Shareef AY, Dhahir SH. Urinary tract bacterial infection of local Iraqi buffaloes (Bubalus bubalis) in Mosul city. Iraqi J Vet Med. 2016;40(2):124-130.
2. Bartges JW. Diagnosis of urinary tract infections. Vet Clin N Am Small Anim Pract. 2004;34:923-933.
3. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by standardized single disk method. Am J Clin Pathol. 1966;45:493-496.
4. Chang SK, Lo DY, Wei HW, Kuo HC. Antimicrobial resistance of *Escherichia coli* isolates from canine urinary tract infections. J Vet Med Sci. 2015;77(1):59-65.
5. Ettinger SJ, Feldman EC. Textbook of Veterinary Internal Medicine: Diseases of the Dog and the Cat. 7th ed. St. Louis: Elsevier Saunders; c2010.
6. Ewers C, Grobbel M, Bethe A, Wieler LH, Guenther S. Extended-spectrum beta-lactamases producing gram-negative bacteria in companion animals: Action is clearly warranted! Berl Munch Tierarztl Wochenschr. 2011;124(3-4):94-101.
7. Guardabassi L, Schwarz S, Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. J Antimicrob Chemother. 2004;54(2):321-332.
8. Hajikolaei MRH, Jamshidian M, Mohammadian B. Bacteriological study of urine and its relationship with histopathological findings of bladder and kidney. Comp Clin Path. 2015;24:251-253.
9. Hall JL, Holmes MA, Baine SJ. Prevalence and antimicrobial resistance of canine urinary tract pathogens. Vet Rec. 2013;173:549.
10. Ling GV. Therapeutic strategies involving antimicrobial treatment of the canine urinary tract. J Am Vet Med Assoc. 1984;185(10):1162-1164.
11. Liu X, Liu H, Li Y, Hao C. Association between virulence profile and fluoroquinolone resistance in *Escherichia coli* isolated from dogs and cats in China. Infect Dev Ctries. 2017;11(4):306-313.
12. McMeekin CH, Hill KE, Gibson IR, Bridges JP, Benschop J. Antimicrobial resistance patterns of bacteria isolated from canine urinary samples submitted to a New Zealand veterinary diagnostic laboratory between 2005–2012. N Z Vet J. 2016;65(2):99-104.
13. Mittal S, Sharma M, Chaudhary U. Study of virulence factors of uropathogenic *Escherichia coli* and its antibiotics susceptibility pattern. Indian J Pathol Microbiol. 2014;57:61-64.
14. Nelson RW, Couto CG. Small animal internal medicine. Textbook of Veterinary Internal Medicine. 4th ed. St. Louis: Mosby/Elsevier; c2009.
15. Nikvand AA, Haji Hajikolaei MR, Ghadrdanmashhadi AR, Ghorbanpour M, Mohammadian B. Bacteriological study of urine and its relationship with histopathological findings of bladder and kidney in river buffalo (*Bubalus bubalis*). Iraqi J Vet Med. 2014;8(3):157-161.
16. Norris CR, Williams BJ, Ling GV, Franti CE, Johnson RA. Recurrent and persistent urinary tract infections in dogs: 383 cases (1969–1995). J Am Anim Hosp Assoc. 2000;36:484-492.
17. Osek J. Multiplex polymerase chain reaction assay for identification of enterotoxigenic *Escherichia coli* strains. J Vet Diagn Invest. 2001;13:308-311.
18. Penna B, Varges R, Mortins R, Lilenbaum W. *In vitro* antimicrobial resistance of Staphylococci isolated from canine urinary tract infection. Can Vet J. 2010;51:738-42.
19. Roopali B, Roy M, Roy S. Haemato-biochemical changes and therapeutic management of urinary tract infection in canines. Pharm Innov J. 2018;7(6):89-92.
20. Seguin MA, Vaden SL, Altier C, Stone E, Levine JF. Persistent urinary tract infections and reinfections in 100 dogs (1989–1999). J Vet Intern Med. 2003;17:622-31.
21. Solomon D, Shpigel NY, Salamon H, Goshen T. Epidemiology and risk factors of pyelonephritis in Israeli dairy cattle. Isr J Vet Med. 2020;75(1):6-11.
22. Thompson MF, Litster AL, Platell JL, Trott DJ. Canine bacterial urinary tract infections: new developments in old pathogens. Vet J. 2011;190:22-27.
23. Wong C, Epstein SE, Westropp JL. Antimicrobial susceptibility patterns in urinary tract infections in dogs. J Vet Intern Med. 2015 Jul;29(4):1045-1052.
24. Yeruham I, Elad D, Avidar Y, Goshen T. A herd level analysis of urinary tract infection in dairy cattle. Vet J. 2006;171(1):172-176.