

The antibiotic susceptibilities and microorganisms isolated from urinary tract infections of dogs

Enes Seyhan¹, Dilek Öztürk¹

¹Department of Microbiology, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Türkiye

Key Words: antibiotic susceptibility dog urinary tract infection

 Received
 : 02.01.2023

 Accepted
 : 16.06.2023

 Published Online
 : 31.12.2023

 Article Code
 : 1228243

Correspondence: D. ÖZTÜRK (dozturk@mehmetakif.edu.tr)

ORCID E. SEYHAN : 0000-0002-7142-2054 D. ÖZTÜRK : 0000-0002-9643-8570

This study was presented in 2. International Veterinary Medicine Students Scientific Research Congress (IYRSC 2020), 17-19 December, 2020, Burdur, TÜRKİYE and 22nd International Veterinary Medicine Student Scientific Research Congress, 8-9 May, 2021, Online, İstanbul, TÜRKİYE.

INTRODUCTION

Urinary tract infections (UTI) are commonly seen in dogs. Some drugs, gravels, bladder neoplasms and problems concerning neural system are among the predisposed factors. UTI caused by microorganisms are commonly seen in dogs and almost 14% of dogs have these infections all their lives. Escherichia coli is the most frequently isolated agent and might exist by itself or together with other bacteria (Thompson et al., 2011). During the studies carried out in Canada, Turkey and Italy, it was stated that E. coli had been the most frequently isolated microorganisms from urinary tract infections of dogs and other Gram-negative and Gram-positive bacteria might also be isolated (Kalınbacak et al., 2004; Papini et al., 2006; Ball et al., 2008). The other bacteria isolated from UTI of dogs are Proteus spp., Staphylococcus spp., Streptococcus spp., Klebsiella spp., Proteus spp., Pseudomonas spp., Enterococcus spp. and Enterobacter spp. (Ball et al., 2008). Notwithstanding quite rarely, Acinetobacter types can also be isolated (Siğirci et al., 2012). Although fungi and yeast are rarely isolated from urinary tract infections of cats and dogs, Candida spp. is the most frequently isolated yeast. Rhodotorula mucilaginosa and Cryptococcus neoformans are the opportunist pathogens and might cause urinary tract infec-

ABSTRACT

In this study, we aimed to isolate microorganisms from urine samples taken from dogs with urinary system infection and to determine an antibiotic susceptibility. For this purpose, urine samples taken from 30 dogs by cystocentesis were inoculated on Blood Agar, MacConkey Agar and Sabourraud's Dextrose Agar. The isolated microorganisms were identified by conventional microbiological methods. In this study, while 19 bacteria were isolated from 15 dogs (63.33%), no isolation was detected within the mycological culture. The most isolated bacteria were Escherichia coli (26.32%) and coagulase negative Staphylococci (26.32%). The isolates were susceptible 84.2% to ceftriaxone, 78.9% to enrofloxacine, 73.7% to ciprofloxacin, gentamicin and trimethoprim + sulfamethoxazole, 73.7% to amoxicillin-clavulanic acid and cefixime, 57.9% to cephalexin, 52.6% to oxytetracycline and 47.4% to ceftiofur. We concluded that Escherichia coli and coagulase negative Staphylococci were the most common reason for urinary tract infections in dogs and due to the differences to antibiotic of the bacterial isolates that antibiotic susceptibility tests is necessary for treatment.

tions (Pressler et al., 2003; Jin et al., 2005).

The most common symptoms of UTI in dogs are thamuria, dysuria, stanguria and haematuria (Byron, 2019). UTI is usually noticed by chance in cats and dogs. Clinical symptoms appear depending on the amount and virulence of the pathogen, presence of predisposed factors, response of the body to the infection and the area and duration of the infection (Bartges, 2004). UTI is seen more in female dogs than male ones. *E. coli* isolation was able to be carried out from 58% of UTI in dogs while this rate was detected 55% in male dogs (Norris et al., 2000). Treatment of UTI is generally carried out with antibiotics.

Bacteriological culture and antibiotics susceptibility tests are not always performed during these infections, yet the treatment is immediately started with antibiotics. The most crucial problem in treatment is the resistance against antibiotics depending.

The aim in this study was to determine the microorganisms causing UTI in dogs and to detect the susceptibilities of the isolated bacteria to antibiotics.

MATERIALS and METHODS

Samples

From October 2020 to September 2021, the urine samples were collected from 30 dogs with suscepted UTI by cystocentesis. The animals used in the study were not made previously antimicrobial treatment. The urine samples were sent to the Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Microbiology for bacteriological and mycological examination.

This study was conducted within the scope of the Board decision of Burdur Mehmet Akif Ersoy University Rectorate Animal Experiments Local Ethics Committee dated August 26, 2020 and numbered 668.

ceftriaxone (5µg, Oxoid, Hamshire, UK), cefixime (30µg, Oxoid, Hamshire, UK) disks was used in antibiotic susceptibility test.

RESULTS

In this study, while 19 (63.33%) bacteria were isolated from 15 dogs, no growth could be detected in mycological culture. One bacterial agent was isolated from urine samples of 12 dogs while more than one bacteria was obtained from 3 dogs. Gram-negative bacteria were constituted 57.89% of the isolated bacteria and Gram-positive bacteria was 42.1%. *E. coli* (26.32%) and CNS (26.32%) were the most frequently isolated bacteria. Other bacteria isolated from urine samples were 15.79% *Streptococcus* spp., 10.53% *Enterobacter* spp., 5.26% *Aeromonas* spp., 5.26% *Citrobacter* spp., 5.26% *Proteus* spp. and 5.26% *P. aeruginosa* (Table 1).

Bacteria	n	⁰∕₀		
E. coli	5	26.32		
CNS	5	26.32		
Streptococcus spp.	3	15.79		
Enterobacter spp.	2	10.53		
Aeromonas spp.	1	5.26		
Citrobacter spp.	1	5.26		
Proteus spp.	1	5.26		
P. aeruginosa	1	5.26		
Total	19	100		

Table 1. Bacterial isolates identified in urinary tract infections of dogs.

Bacteriological and Mycological Examinations

The urine samples were centrifuged at 3000 rpm for 20 minutes and the supernatant was taken out from the urine, and the remaining sediment was used for bacteriological culture. Samples were inoculated onto Blood Agar (Oxoid, Hamshire, UK) with 5% sheep blood, MacConkey Agar (Oxoid, Hamshire, UK) and Sabouraud's Dextrose Agar (SDA, Oxoid, Hamshire, UK) plates. The petris were incubated for 24 hours at 37°C in an aerobic environment, except for SDA. The samples were inoculated on two SDA petris. One petri was incubated at 25 °C and the other at 37 °C for 7-10 days. The colonies were identified by conventional microbiological methods.

Antibiotic Sensitivity Tests

Disk diffusion method was performed according to Clinical and Laboratory Standards Institute (CLSI) standards (CLSI 2013). For this purpose, 0,1 mL of the bacteria suspensions adjusted according to the turbidity of the McFarland 0.5 standard suspension was taken and inoculated on Muller-Hinton Agar (Oxoid, Hamshire, UK). In this study, amoxicillin- clavulanic acid (30µg, Oxoid, Hamshire, UK), cephalexin (30µg, Oxoid, Hamshire, UK), ciprofloxacin (5µg, Oxoid, Hamshire, UK), enrofloxacin (5µg, Oxoid, Hamshire, UK), gentamicin (10µg, Oxoid, Hamshire, UK), trimethoprim/sulfamethoxazole (25µg, Oxoid, Hamshire, UK), oxytetracycline (30 µg, Oxoid, Hamshire, UK), ceftiofur (30µg, Oxoid, Hamshire, UK), The isolates were found susceptible to ceftriaxon 84.2 %, to enrofloxacin 78.9 %, to ciprofloxacin, gentamicin and trimethoprim + sulfamethoxazole 73.7 %, to amoxicillin-clavulanic acid and cefixime 73.7 %, to cephalexin 57.9 %, to oxytetracycline 52.6 % and to ceftiofur 47.4 % (Table 2). All *E. coli* isolates were found susceptible to amoxycillin clavulonic acid, cefixime and ceftriaxon. Four (80%) of the *E. coli* isolates were susceptible to amoxicillin clavulonic acid, ceftriaxon, enrofloxacin and trimethoprim sulfamethoxazole. In addition, CNS isolates were detected susceptible to ciprofloxacin, cefalexin, ceftiofur and oxytetracycline. All *Streptococcus* spp. isolates were susceptible to amoxicillin clavulonic acid, cefixim, ceftriaxon, sefalexin and ceftiofur. In this study, *Citrobacter* spp. isolate was found susceptible against to all tested antibiotics (Table 2).

DISCUSSION

Urinary tract infection is one of the most frequent infections seen in cats and dogs. Though many factors cause these infections, microorganisms are the most crucial reason. In urinary tract infections of dogs, Gram-negative bacteria are reported to have been isolated more than Gram-positive ones (Kogika et al., 1995; Çetin et al., 2003; Yu et al., 2020). Although *E. coli* was reported to have been the most frequently isolated agent from UTI of dogs, Gram-negative bacteria such as *Klebsiella* spp, *Proteus* spp., *Pseudomonas* spp. and Gram-positive ones such as *Staphylococcus* spp., *Streptococcus* spp., *Corynebac*-

	Antimicrobial agents																			
Bacteria	A	MC	C	FM	(CIP		CL	(CN	C	RO	E	NR	F	UR		Т	S	XT
Isolated	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
E. coli (n=5)	5	100	5	100	4	80	4	80	4	80	5	100	4	80	1	20	3	60	4	80
CNS (n=5)	4	80	2	40	3	60	3	60	5	100	4	80	4	80	3	60	3	60	4	80
Streptococcus spp. (n=3)	3	100	3	100	2	66.7	3	100	1	33.3	3	100	2	66.7	3	100	2	66.7	2	66.7
Enterobacter spp. (n=2)	0	0	1	50	1	50	0	0	1	50	1	50	1	50	0	0	0	0	1	50
Aeromonas spp. (n=1)	0	0	0	0	1	100	0	0	1	100	1	100	1	100	0	0	1	100	1	100
<i>Citrobacter</i> spp. (n=1)	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100	1	100
Proteus spp. (n=1)	0	0	1	100	1	100	0	0	0	0	1	100	1	100	1	100	0	0	1	100
P. aeruginosa (n=1)	0	0	0	0	1	100	0	0	1	100	0	0	1	100	0	0	0	0	0	0
Total (n=19)	13	68.4	13	68.4	14	73.7	11	57.9	14	73.7	16	84.2	15	78.9	9	47.4	10	52.6	14	73.7

Table 2. Antibiotic susceptibility o	bacteria isolated from urinar	y tract infection of dogs.

AMC: Amoxicillin- clavulanic acid, CFM: Cefixime, CIP: Ciprofloxacin, CL: Cephalexin, CN: Gentamicin, CRO: Ceftriaxone, ENR: Enrofloxacin, FUR: Ceftiofur, T: Oxytetracycline, SXT: Trimethoprim/Sulphamethoxanole.

terium spp. could also be isolated (Cohn et al., 2003; Çetin et al., 2003; Papini et al., 2006; Sığırcı et al., 2012; Wong et al., 2015; Harihan et al., 2016; Marques et al., 2016; Bağcıgil et al., 2018; Yu et al., 2020; Vercelli et al., 2021). Kogika et al. (1995) stated that Gram-negative bacteria were the most common ones isolated from UTI of dogs, the primary isolated agent was E. coli (35.3%) and Staphylococcus spp. (23.5%) and other bacteria were isolated at various rates. Cetin et al. (2003) reported that 51 bacteria were isolated from 100 dogs with UTI, 52.94% of these bacteria were Gram-negative and the most frequently isolated bacteria was E. coli (23.52%). Adsanychan et al. (2019) stated that Gram-negative bacteria were the most frequently isolated ones from UTI of dogs. In this study, researchers found Staphylococcus intermedius as the most commonly isolated agent and Proteus mirabilis and E. coli followed respectively. Yu et al. (2020) stated that they isolated 129 bacteria from 103 out of 336 urine samples they collected from dogs in China and 33.3% of Gram-negative bacteria were E. coli, 12.4% Klebsiella spp. and 6.2% Pseudomonas spp. In the presented study, 7 different bacteria were isolated from UTI of dogs, E. coli and KNS were the most commonly isolated bacteria and E. coli was the most frequently isolated Gram-negative bacteria (57.89%). The obtained results in the presented study support the studies (Papini et al., 2006; Wong et al., 2015; Harihan et al., 2016;

Marques et al., 2016) also stating *E. coli* as the most commonly isolated bacteria. Likewise in this study, *Klebsiella* spp., *P. aeru-ginosa* and *Citrobacter* spp. were isolated from urine samples and these results supported many studies stating that many bacteria might cause UTI (Cohn et al., 2003; Papini et al., 2006; Sığırcı et al., 2012; Wong et al., 2015; Harihan et al., 2016; Marques et al., 2016). Besides, some researchers (Punia et al., 2018; Adsanychan et al., 2019) reported that Gram-positive bacteria, especially *Streptococcus* and *Staphylococcus*, could be isolated more than *E. coli* in UTI of dogs. In the presented and reported studies, differences in isolation rates of bacteria might originated from geographical and climatic conditions and resistance to antibiotics due to antibiotics use (Wong et al., 2015).

In this study, *in vitro* susceptibility of the bacteria isolated from UTI of dogs was searched to ten different antibiotics. The isolates showed different susceptibilities to antibiotics in this study. The researchers (Çetin et al., 2003; Windahl et al., 2014; Bağcıgil et al., 2018; Ukah et al., 2018; Adsanychan et al., 2019; Yu et al., 2020) reported that the microorganisms were found susceptible to antibiotics at different rates. Çetin et al. (2003) stated that the most effective antibiotics in UTI of dogs were amoxycillin clavulonic acid, gentamicin, ampiciline sulbactam and enrofloxacin. Adsanychan et al. (2019) reported that the isolates obtained from urine samples were between 66%-86% and were most susceptible to amoxycillin clavulonic acid. In this presented study, the obtained isolates were susceptible to ceftriaxone, enrofloxacin, gentamicin and ciprofloxacin but resistant to ceftiofur, oxytetracycline and cephalexin. It is thought that the differences in the susceptibilities of isolates to antibiotics might be related with the resistance developing against antibiotics used for various infections of animals.

CONCLUSION

As a result, with this study, it was revealed that Gram-negative bacteria usually caused UTI of dogs and *E. coli* and CNS were the most commonly isolated agents. Due to the differences in antibiotic susceptibilities of isolates, in UTI of dogs, antibiotics susceptibility tests were absolutely advised to be applied before treatment.

DECLARATIONS

Ethics Approval

This study was approved by Burdur Mehmet Akif Ersoy University Rectorate, Animal Experiments Local Ethics Committee (Decision No: 668/2020)

Conflict of Interest

Authors declare that there are no conflicts of interest for this study.

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: DÖ, ES

Data and analysis: ES, DÖ

Drafting of the manuscript: DÖ, ES

Critical review: DÖ, ES

Acknowledgments

This study was supported by the "TÜBİTAK 2209-A University Students Research Projects Support Program

REFERENCES

Adsanychan, N., Hoisang, S., Seesupa, S., Kampa, N., Kunkitti, P., Jitpean, S. (2019). Bacterial isolates and antimicrobial susceptibility in dogs with urinary tract infection in Thailand: A retrospective study between 2013–2017. Veterinary Integrative Sciences, 17(1), 21-31.

Bağcıgil, A.F., Dokuzeylül, B., Göçmen, H., Yalçın, E., İkiz, S., Özgür, N.Y., Seyyal, A.K. (2018). *Escherichia coli* strains isolated from cats and dogs with urinary tract infections. Journal of Anatolian Environmental and Animal Sciences, 3(3), 131-136. https://doi.org/10.35229/jaes.431221

Ball, K.R., Rubin, J.E., Chirino-Trejo, M., Dowling, P.M. (2008). Antimicrobial resistance and prevalence of canine uropathogens at the Western College of Veterinary Medicine Veterinary Teaching Hospital, 2002–2007. Canadian Veterinary Journal, 49:985–990. Bartges, J.W. (2004). Diagnosis of urinary tract infections. *Veterinary* Clinical North American Small Animal Practise, 34(4), 923-933. https://doi.org/10.1016/j.cvsm.2004.03.001

Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M. (1966). Antibiotic susceptability testing by a standardized single disk method. American Journal of Clinical Pathology, 45: 493- 496.

Byron, J.K. (2019). Urinary tract infection. Veterinary Clinics of North America: Small Animal Practice, 49(2), 211-221. https://doi.org/10.1016/j.cvsm.2018.11.005

CLSI. (2013). Performance Standards for antimicrobial susceptibility testing: 23rd international supplement CLSI 2013, Wayne, Pennsylvania, M100-S23.

Cohn, L.A., Gary, A.T., Fales, W.H., Madsen, R.W. (2003). Trends in fluoroquinolone resistance of bacteria isolated from canine urinary tracts. Journal of Veterinary Diagnostic Investigation, 15(4), 338-343. https://dx.doi. org/10.1177/104063870301500406

Çetin, C., Şentürk S, Kocabıyık, A.L., Temize, I M., Özel, E. (2003). Bacteriological examination of urine samples from dogs with symptoms of urinary tract infection. Turkish Journal of Veterinary Animal Sciences, 27: 1225-1229.

Diren Sığırcı, B., Koenhemsi, L., Gönül, R., Uysal, A., Özgür, N.Y. (2012). Bir köpeğin idrar yolu înfeksiyonunda saptanan *Acinetobacter baumannii* ve *Streptococcus canis*. İstanbul Üniversitesi Veteriner Fakültesi Dergisi, 38 (1), 73-78.

Dowling, P.M. (1996). Antimicrobial therapy of urinary tract infections. Canadian Veterinary Journal, 37(7), 438.

Hall, J.L., Holmes, M.A., Baines, S.J. (2013). Prevalence and antimicrobial resistance of canine urinary tract pathogens. Veterinary Record, 173(22), 549-549. https://doi.org/10.1136/vr.101482

Hariharan, H., Brathwaite-Sylvester, E., Belmar, V.M., Sharma, R. (2016). Bacterial isolates from urinary tract infection in dogs in Grenada, and their antibiotic susceptibility. Open Journal of Veterinary Medicine, 6(6), 85-88. https://dx.doi. org/10.4236/ojvm.2016.66010

Jin, Y., Lin, D. (2005). Fungal Urinary Tract infections in The dog and cat: A retrospective study (2001–2004). Journal of the American Animal Hospital Association, 41(6), 373-381. https://doi.org/10.5326/0410373

Kalınbacak, A., Atalay, Ö., Kırmızıgül, A.H., Noyan, D., Karakurum, M.Ç. (2004). Kedi ve köpeklerde sistitis'in tanısında çift kontrast sistografi tekniğinin kunanımı ve tedavide enrofloksasin'in etkinliğinin araştırılması. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 51, 111-115. https://doi. org/10.1501/Vetfak_0000002280

Kogika, M.M., Fortunato, A.B.V., Mamizuka, E.M., Hagiwara, K.M., de Pavan, F.B.M., Grosso, SNA. (1995). Etiologic study of urinary tract infection in dogs. Brazilian Journal of Veterinary Research and Animal Science, 32(1), 31-36.https:// doi.org/10.11606/issn.1678-4456.bjvras.1994.52087 Marques, C., Belas, A., Franco, A., Aboim, C., Gama, L.T., Pomba, C. (2018). Increase in antimicrobial resistance and emergence of major international high-risk clonal lineages in dogs and cats with urinary tract infection: 16 year retrospective study. Journal of Antimicrobial Chemistry, 73(2), 377-384. https://doi.org/10.1093/jac/dkx401

Norris, C.R., Williams, B.J., Ling, G.V., Franti, C.E., Ruby, A.L. (2000). Recurrent and persistent urinary tract infections in dogs: 383 cases (1969-1995). Journal of the American Animal Hospital Association, 36(6), 484-492. https://doi. org/10.5326/15473317-36-6-484

Olby, N.J., Mackillop, E., Cerda-Gonzalez, S., Moore, S., Muñana, K.R., Grafinger, M., Osborne, J.A., Vaden, S.L. (2010). Prevalence of urinary tract infection in dogs after surgery for thoracolumbar intervertebral disc extrusion. Journal of Veterinary Internal Medicine, 24(5), 1106-1111. https:// doi.org/10.1111/j.1939-1676.2010.0567.x

Papini, R., Ebani, V.V., Cerri, D., Guidi, G. (2006). Survey on bacterial isolates from dogs with urinary tract infections and their in vitro sensitivity. Revue Médecine Véterinaria, 157, 1, 35-41.

Pressler, B.M., Vaden, S.L., Lane, I.F, Cowgill, L.D., Dye, J.A. (2003). *Candida* spp. urinary tract infections in 13 dogs and seven cats: predisposing factors, treatment, and outcome. Journal of the American Animal Hospital Association, 39(3), 263-270. https://doi.org/10.5326/0390263

Punia, M., Kumar, A., Charaya, G., Kumar, T. (2018). Pathogens isolated from clinical cases of urinary tract infection in dogs and their antibiogram. Veterinary World, 11(8), 1037. https://doi.org/10.14202/vetworld.2018.1037-1042

Thompson, M..F, Litster, A.L., Platell, J.L., Trott, D.J. (2011). Canine bacterial urinary tract infections: new developments in old pathogens. Veterinary Journal, 190(1), 22-27. https://doi.org/10.1016/j.tvjl.2010.11.013

Ukah, U.V., Glass, M., Avery, B., Daignault, D., Mulvey, M.R., Reid-Smith, R.J., Parmley, E.J., Portt, A., Boerlin, P., Manges, A.R. (2018). Risk factors for acquisition of multidrug-resistant *Escherichia coli* and development of community-acquired urinary tract infections. Epidemiology & Infection, 146(1), 46-57. https://doi.org/10.1017/S0950268817002680

Vercelli, C., Della Ricca, M., Re, M., Gambino, G., Re, G. (2021). Antibiotic stewardship for canine and feline acute urinary tract infection: An observational study in a small animal hospital in Northwest Italy. Antibiotics, 10(5), 562. https:// doi.org/10.3390/antibiotics10050562

Weese, J.S., Blondeau, J., Boothe, D., Guardabassi, L..G, Gumley, N., Papich, M., Jessen, L.R., Lappin, M., Rankink, S., Westropp, J.L., Sykes, J. (2019). International society for companion animal infectious diseases (ISCAID) guidelines for the diagnosis and management of bacterial urinary tract infections in dogs and cats. Veterinary Journal, 247, 8-25. https://doi. org/10.1016/j.tvjl.2019.02.008

Windahl, U., Holst, B.S., Nyman, A., Grönlund, U., Bengts-

son, B. (2014). Characterisation of bacterial growth and antimicrobial susceptibility patterns in canine urinary tract infections. BMC Veterinary Research. 10(1), 1-10.

Wong, C., Epstein, S.E., Westropp, J.L. (2015). Antimicrobial susceptibility patterns in urinary tract infections in dogs (2010–2013). Journal of Veterinary Internal Medicine, 29(4), 1045-1052. https://doi.org/10.1111/jvim.13571

Yu, Z., Wang, Y., Chen, Y., Huang, M., Wang, Y, Shen, Z., Xia, Z., Li, G. (2020). Antimicrobial resistance of bacterial pathogens isolated from canine urinary tract infections. Veterinary Microbiology, 241, 108540. https://doi.org/10.1016/j. vetmic.2019.108540