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Research Article

Bacteriological Examination of Urine Samples from Dogs with Symptoms of Urinary Tract Infection

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Abstract: In the present study, a total of 100 urine samples obtained by antepubic cystocentesis from dogs with symptoms of urinary tract infection were examined bacteriologically. Bacteria were isolated from 38 of 100 urine samples. No significant difference was determined when the frequency of bacteriuria was compared between female and male dogs and in 2 different age groups (<2 and ≥2 years). The organisms were isolated in pure culture from 29 (76.31%) samples and were isolated in mixed culture from 9 (23.68%) samples. A total of 51 strains were isolated from the urine samples. Of the strains isolated, 12 (23.52%) were identified as *Escherichia coli*, 8 (15.68%) as *Streptococcus* spp., 6 (11.76%) as *Micrococcus* spp., 5 (9.80%) as *Staphylococcus* spp., 5 (9.80%) as *Corynebacterium* spp., 4 (7.84%) as *Klebsiella pneumoniae*, 3 (5.88%) as *Proteus vulgaris*, 2 (3.92%) as *Pseudomonas aeruginosa*, 2 (3.92%) as *Citrobacter freundii*, 2 (3.92%) as *Acinetobacter calcoaceticus* and 2 (3.92%) as *Enterobacter agglomerans*. Of the 51 strains examined, 46 (90.19%) were sensitive to amoxycillin/clavulonic acid, 40 (78.43%) to gentamicin, 39 (76.47%) to ampicillin/sulbactam, 31 (60.78%) to enrofloxacin, 30 (58.82%) to nitrofurantoin, 29 (56.86%) to kanamycin, 28 (54.90%) to oxytetracycline, 26 (50.98%) to cephalothin and danofloxacin, and 23 (45.09%) to sulphamethoxazole/trimethoprim.

Key Words: Urine, bacterium, dog

Üriner Sistem İnfeksiyonu Semptomlu Köpeklerin İdrar Örneklerinin Bakteriyolojik İncelemesi

Özet: Bu çalışmada üriner sistem infeksiyonu semptomlu 100 köpekten antepubik sistosentez ile alınan idrar örnekleri bakteriyolojik yönden incelendi. Yüz idrar örneğinin 38'inden bakteri izolasyonu yapıldı. Bakteriuri'nin görülme sıklığı erkek ve dişi köpeklerde ve iki farklı yaş (<2 ve ≥ 2 yaş) grubunda karşılaştırıldığında önemli bir fark saptanmadı. Bakteriler 29 (% 76,31) örnekten saf kültür, 9 (% 23,68) örnekten ise karışık kültür şeklinde izole edildi. İdrar örneklerinden toplam 51 suş izole edildi. İzole edilen suşların 12'si (% 23,52) *Escherichia coli*, 8'i (% 15,68) *Streptococcus* spp., 6'sı (% 11,76) *Micrococcus* spp., 5'i (% 9,80) *Staphylococcus* spp., 5'i (% 9,80) *Corynebacterium* spp., 4'ü (% 7,84) *Klebsiella pneumoniae*, 3'ü (% 5,88) *Proteus vulgaris*, 2'si (% 3,92) *Pseudomonas aeruginosa*, 2'si (% 3,92) *Citrobacter freundii*, 2'si (% 3,92) *Acinetobacter calcoaceticus* ve 2'si (% 3,92) *Enterobacter agglomerans* olarak identifiye edildi. İncelenen suşların, 46'sı (% 90,19) amoxycillin/clavulonic acid'e, 40'ı (% 78,43) gentamicin'e, 39'u (% 76,47) ampicillin/sulbactam'a, 31'i (% 60,78) enrofloxacin'e, 30'u (% 58,82) nitrofurantoin'e, 29'u (% 56,86) kanamycin'e, 28'i (% 54,90) oxytetracycline'e, 26'sı (% 50,98) cephalothin ve danofloxacin'e ve 23'ü (% 45,09) sulphamethoxazole/trimethoprim'e duyarlı bulundu.

Anahtar Sözcükler: İdrar, bakteri, köpek

Introduction

Urinary tract infection (UTI) refers to a condition in the urinary bladder (sometimes also involving the kidneys) caused by infection, usually with bacteria. UTI is thought to be the most common infectious disease in dogs. It has been estimated that as many as 10% of all canine patients seen by veterinarians for any reason have UTI in addition to the problems for which they are presented (1). In most studies UTIs have been found in female dogs more often than in males, and older animals have been thought by some investigators to be at increased risk of UTI (1,2). The bladder stones, tumors of the bladder, nervous system problems, some diseases (diabetes mellitus, Cushing's disease) and medications (cortisone-like drugs, anti-cancer drugs) may predispose dogs to bacterial UTI (3). A presumptive diagnosis of UTI is often based on clinical and urinalysis findings, but urine culture is required to conclusively diagnose UTI (4). Various microorganisms may be involved in the etiology of UTI in dogs. *Escherichia coli*, *Proteus* spp., *Psedomonas* spp., *Staphylococcus* spp. and *Streptococcus* spp. are the most common infecting bacteria (2,5). Infections with a single bacterial species are more prevalent than mixed infections (1,2,6,7). The great majority of UTIs are benign and readily respond to antimicrobial therapy. However, some patients diagnosed as having bacterial UTIs fail to respond to antimicrobial therapy. The bacterial isolates from the urine of dogs may have acquired resistance to one or more commonly used antimicrobials because animals are more frequently exposed to antimicrobials and/or antimicrobial residues (1,4).

The aim of this study was to determine the frequency and antibiotic sensitivity patterns of bacterial pathogens from dogs with UTI.

Materials and Methods

Dogs

A total of 100 dogs with symptoms of UTI were used as material in this study. The age and sex distributions of the dogs are shown in Table 1.

Blood samples

After clinical examination, blood samples from each dog were placed in tubes with and without anticoagulant (EDTA) for a hemogram.

Table 1. Age and sex distributions of dogs with symptoms of urinary tract infection.

Age (years)	Sex		Total
	Female	Male	
<2	7	8	15
≥2	37	48	85
Total	44	56	100

Urine samples

Urine samples were obtained by antepubic cystocentesis from dogs for urinalysis and bacteriological examination.

Hemogram

White blood cell (WBC), hematocrit (HCT), neutrophil, lymphocyte, eosinophil and erythrocyte values were evaluated by Hemocell counter. Serum urea and creatinine concentrations were measured by Reflotron®.

Urinalysis

The physical properties of the urine were examined. Urine pH, proteinuria, glucosuria, ketonuria, bilirubinuria, urobilinogen, nitrite and urine specific gravity levels were evaluated by lipstick test (Lapstrip®). Proteinuria was in addition checked by sulfosalicylic test. Urine samples were centrifuged at 3000 rpm for 3 min. Urinary sediment examination was made to evaluate RBC, WBC, epithelial cell casts and crystals in centrifuged urine

Bacteriological examination

Urine samples were streaked onto sheep blood agar and MacConkey agar plates. Plates were incubated at 37 °C in aerobic conditions and examined daily for 5 days for the presence of bacterial growth. The isolated bacteria were identified on the basis of cultural, morphological and biochemical characteristics (8).

Antibiotic sensitivity testing was performed using the disk diffusion method (9) with the following antibiotics: amoxycillin/clavulanic acid (30 µg), ampicillin/sulbactam (20 µg), cephalothin (30 µg), danofloxacin (5 µg), enrofloxacin (5 µg), gentamicin (10 µg), kanamycin (30 µg), nitrofurantoin (300 µg), oxytetracycline (30 µg) and sulphamethoxazole/trimethoprim (25 µg).

Statistical analysis

The chi square test was used to detect significant differences between the groups (GrandphPad InStat TM, ©1990-1993 GraphPad Software V2.02).

Results

Clinical examination

Depression, lethargy, anorexia, abdominal pain and fever (39.5-40.5 °C) were determined in the dogs.

Hemogram

The dogs had leukocytosis (17,000-22,000) and left shift (80-90%). Serum urea and creatinine concentrations were 3.2-4.5 mg/dl and 82-156 mg/dl, respectively.

Urinalysis

The urine samples were concentrated and cloudy. Urine specific gravity in most samples was low (1010-1015). Urine pH was between 6 and 9. Marked proteinuria was observed in all samples. Nitrite and bilirubin were determined in some samples. During microscopic examination of the urine sediment, leukocytes, erythrocytes, epithelial cells, squamous epithelial cells, epithelial casts and leukocytic casts were observed.

Bacteriological examination

Bacteria were isolated from 38 of 100 urine samples. The age and sex distributions of dogs with bacteriuria are shown in Table 2.

The organisms were isolated in pure culture from 29 (76.31%) samples and were isolated in mixed culture from 9 (23.68%) samples.

A total of 51 strains were isolated from urine samples. Of the strains isolated, 12 (23.52%) were identified as *E. coli*, 8 (15.68%) as *Streptococcus* spp., 6 (11.76%) as *Micrococcus* spp., 5 (9.80%) as *Staphylococcus* spp., 5 (9.80%) as *Corynebacterium* spp., 4 (7.84%) as *Klebsiella pneumoniae*, 3 (5.88%) as *Proteus vulgaris*, 2 (3.92%) as *Pseudomonas aeruginosa*, 2 (3.92%) as *Citrobacter freundii*, 2 (3.92%) as *Acinetobacter calcoaceticus* and 2 (3.92%) as *Enterobacter agglomerans*. Bacteria isolated from dogs with bacteriuria are presented in Table 3.

Of the 51 strains examined, 46 (90.19%) were sensitive to amoxycillin/clavulonic acid, 40 (78.43%) to gentamicin, 39 (76.47%) to ampicillin/sulbactam, 31 (60.78%) to enrofloxacin, 30 (58.82%) to nitrofurantoin, 29 (56.86%) to kanamycin, 28 (54.90%) to oxytetracycline, 26 (50.98%) to cephalothin and danofloxacin and 23 (45.09%) to sulphamethoxazole/trimethoprim. The antibiotic sensitivities of the bacteria isolated from dogs with bacteriuria are presented in Table 3.

Statistical analysis

The difference in the frequency of bacteriuria between female and male dogs was not statistically significant. Similarly, there was no statistically significant difference when the frequency of bacteriuria was compared in the 2 age groups (<2 and ≥2 years).

Discussion

UTI is thought to be the most common infectious disease of dogs. Establishing a diagnosis of bacterial UTI in dogs is dependent upon routine laboratory evaluation consisting of urinalysis and urine culture (4). The urine culture as the definitive diagnostic test for UTI is considered to be the gold standard (10). In this study, a total of 100 urine samples from dogs with symptoms of UTI were examined bacteriologically.

In the present study, bacteria were isolated from 38 of 100 urine samples from dogs with UTI symptoms. Lefmann (6) reported that 44 (48.35%) of the urine samples from 91 dogs were bacteriologically positive and the remainder were sterile. Wierup (11) detected bacterial growth in 55.7% of 199 urinary specimens

Table 2. Age and sex distributions of dogs with bacteriuria.

Age (years)	Sex				Total	
	Female		Male			
	No. of dogs examined	No. of dogs with bacteriuria	No. of dogs examined	No. of dogs with bacteriuria	No. of dogs examined	No. of dogs with bacteriuria
<2	7	1	8	4	15	5 (33.33%)
≥2	37	12	48	21	85	33 (38.82%)
Total	44	13 (29.54 %)	56	25 (44.64%)	100	38 (38.00%)

Table 3. Bacteria isolated from dogs with bacteriuria and their antibiotic sensitivities.

Bacteria	No.of isolates	No. of isolates sensitive to antibiotics									
		AMC	CN	SAM	ENR	F	K	OT	KF	DFX	SXT
Escherichia coli	12 (23.52)	12 (100.0)	10 (83.33)	8 (66.66)	11 (91.66)	9 (75.00)	8 (66.66)	9 (75.00)	3 (25.00)	10 (83.33)	6 (50.00)
Streptococcus spp.	8 (15.68)	7 (87.50)	4 (50.00)	7 (87.50)	2 (25.00)	4 (50.00)	2 (25.00)	3 (37.50)	6 (75.00)	1 (12.50)	1 (12.50)
Micrococcus spp	6 (11.76)	6 (100.0)	4 (66.66)	5 (83.33)	3 (50.00)	4 (66.66)	3 (50.00)	4 (66.66)	5 (83.33)	2 (33.33)	4 (66.66)
Staphylococcus spp.	5 (9.80)	4 (80.00)	4 (80.00)	4 (80.00)	3 (60.00)	4 (80.00)	3 (60.00)	3 (60.00)	4 (80.00)	3 (60.00)	2 (40.00)
Corynebacterium spp.	5 (9.80)	4 (80.00)	3 (60.00)	2 (40.00)	3 (60.00)	0 (0.00)	2 (40.00)	1 (20.00)	4 (80.00)	1 (20.00)	2 (40.00)
Klebsiella pneumoniae	4 (7.84)	3 (75..00)	4 (100.0)	4 (100.0)	2 (50.00)	3 (75.00)	4 (100.0)	3 (75.00)	0 (0.00)	2 (50.00)	3 (75.00)
Proteus vulgaris	3 (5.88)	3 (100.0)	3 (100.0)	3 (100.0)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	3 (100.0)	0 (0.00)	0 (0.00)
Pseudomonas aeruginosa	2 (3.92)	1 (50.00)	2 (100.0)	1 (50.00)	2 (100.0)	0 (0.00)	1 (50.00)	1 (50.00)	0 (0.00)	2 (100.0)	1 (50.00)
Citrobacter freundii	2 (3.92)	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	0 (0.00)	2 (100.0)	2 (100.0)
Acinetobacter calcoaceticus	2 (3.92)	2 (100.0)	2 (100.0)	2 (100.0)	1 (50.00)	2 (100.0)	2 (100.0)	2 (100.0)	1 (50.00)	1 (50.00)	1 (50.00)
Enterobacter agglomerans	2 (3.92)	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	2 (100.0)	1 (50.00)	0 (0.00)	2 (100.0)	1 (50.00)
Total	51 (100.0)	46 (90.19)	40 (78.43)	39 (76.47)	31 (60.78)	30 (58.82)	29 (56.86)	28 (54.90)	26 (50.98)	26 (50.98)	23 (45.09)

Note: a) AMC: amoxycillin/clavulonic acid, CN: gentamicin, SAM: ampicillin/sulbactam, ENR: enrofloxacin, F: nitrofurantoin, K: kanamycin, OT: oxytetracycline, KF: cephalothin, DFX: danofloxacin, SXT: sulphamethoxazole/trimethoprim
b) Figures in parentheses are percentages

from dogs with symptoms of UTIs. Zschock et al. (12) reported that bacteria were cultured from 427 (80.6%) of 530 urine samples from dogs with UTIs or suspected infections. These results indicate that although clinical and urinalysis findings are consistent with bacterial infection, a diagnosis of bacterial UTI should not be based on these findings alone.

In most reports, UTIs are found in female dogs more often than in males (1,2). Kogika et al. (7) reported that UTIs were more common in males. Middle-aged and older dogs were more commonly affected. In another study (13), it was reported that older dogs were most commonly affected and no sex predilection was seen in the dogs. In this study, sex and age were not found to be significantly associated with the frequency of bacteriuria. These differences among the findings on the frequency of bacteriuria in dogs of different sexes and ages may be related to a number of predisposing factors.

One or more organisms can cause UTI in dogs. Lefmann (6) reported that infection with a single organism was found in 37 (84.09%) of 44 dogs with bacteriuria and with more than one organism in 7 dogs. In another study (7), UTIs were studied in 51 dogs and mixed infections were detected in 4 dogs. Ling et al. (2) reported that infection with a single microbial species was responsible for 72% of UTIs in both genders. In parallel with previous studies, the organisms were isolated in pure culture from 29 (76.31%) samples and were isolated in mixed culture from 9 (23.68%) samples in this study. The present study and other works show that the vast majority of canine UTIs are caused by a single bacterial species.

Various microorganisms may be involved in the etiology of UTI in dogs. In the present study, a total of 51 strains were isolated from urine samples. Of the strains isolated, 12 (23.52%) were identified as *E. coli,* 8 (15.68%) as *Streptococcus* spp., 6 (11.76%) as *Micrococcus* spp., 5 (9.80%) as *Staphylococcus* spp., 5 (9.80%) as *Corynebacterium* spp., 4 (7.84%) as *K. pnemoniae*, 3 (5.88%) as *P. vulgaris*, 2 (3.92%) as *P. aeroginosa*, 2 (3.92%) as *C. freundii*, 2 (3.92%) as *A. calcoaceticus*, and 2 (3.92%) as *Enterobacter agglomerans*. Sinha and Mishra (14) reported that the pathogens chiefly involved were *E. coli* (36%), *P. vulgaris* (16.6%), *Staphylococcus aureus* (16.6%), *P. aeruginosa* (19.4%), *Klebsiella* spp. (5.5%), *Streptococcus faecalis* (5.5%). In another study (15), the most important isolates were *E. coli* (34), *S. aureus* (25), *Streptococcus* spp. (15), *Enterobacter* spp. (14), *K. pneumoniae* (12) and *P. aeruginosa* (12). Mitra et al. (16) reported that *E. coli, S. aureus, Pseudomonas* spp. and *P. vulgaris* were isolated from 13 (54.17%), 6 (25%), 3 (12.50%) and 2 (8.33%) cases, respectively. Kogika et al. (7) isolated *E. coli* (35.3%), *Staphylococcus* spp. (23.5%), *Proteus mirabilis* (15.7%), *Streptococcus* spp. (13.7%), *Klebsiella* spp. (9.8%), *P. aeruginosa* (3.9%), *Enterobacter cloacae* (2.0%), *C. freundii* (2.0%) and *Providencia rettgeri* (2.0%). Ling et al. (2) reported that

10 bacterial genera accounted for 96.3% of the urinary isolates, including *E. coli* (44.1%), *Staphylococcus* spp. (11.6%), *Proteus* spp. (9.3%), *Klebsiella* spp. (9.1%), *Enterococcus* spp. (8.0%) and *Streptococcus* spp. (5.4%), and these were the most common isolates in both genders of dogs. It appears from these findings that although the prevalence of various species varies considerably from study to study, *E. coli* is the most common causative agent of UTIs in dogs.

Infectious diseases and their treatment are an important part of veterinary practice. Antibiotic sensitivity testing in veterinary practice is an essential tool for the treatment of infectious disease. In the present study, the in vitro antibiotic sensitivity tests of strains examined revealed amoxycillin/clavulonic acid to be the most effective antibiotic followed by gentamicin, ampicillin/sulbactam, enrofloxacin, nitrofurantoin, kanamycin, oxytetracycline, cephalothin and danofloxacin in decreasing order, while sulphamethoxazole/trimethoprim was found to be the least effective. In a previous study (11), it was reported that ampicillin was the drug to which resistance was least common (16.2%) while 79.1% of the strains examined were resistant to sulfonamide. Sharma et al. (15) reported that most bacterial strains from UTIs in dogs were sensitive to cephalexin, ciprofloxacin, nalidixic acid and gentamicin. In another study (16), it was reported that isolated organisms were most sensitive to norfloxacin, gentamicin and nitrofurantoin, followed by ampicillin, mendelamine and tetracycline. Yuri et al. (13) reported that most isolates were resistant to oxytetracycline, cephalexin, thiamphenicol and kanamycin, but sensitive to orbifloxacin. These findings indicate that the antimicrobial sensitivities of bacteria isolated from dogs with UTI are variable, and therefore antimicrobial agents should be selected on the basis of bacterial culture and sensitivity tests.

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