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Antimicrobial Susceptibility of Bacterial Isolates from Domestic Dogs with Urinary Tract Infection

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Abstract : Bacterial pathogens were isolated from dogs with urinary tract infection (UTI) in local animal hospitals between August 2003 and December 2009. Bacteria were isolated from urine of 47 dogs. The isolated pathogens were *Escherichia coli* (n = 27), *Streptococcus* spp. (n = 7), *Staphylococcus* spp. (n = 5), *Enterobacter* spp. (n = 3), *Proteus* spp. (n = 2), other species were 3 strains, respectively. *E. coli* were susceptible to imimpenem, polymyxin B, amikacin, cephalosporins, aztreonam, amoxicillin clavulate, cephalosporins, tricarcillin, and amoxicillin clavulate, while were resistant bacitracin, erythromycin, lincomycin, oxacillin, penicillin, and novobiocin. *Streptococcus* spp. were susceptible to bacitracin, imimpenem, and trimethoprime-sulfa, while were highly resistant amikacin, cefotaxim, cefoxitin, cloxacillin, gentamicin, lincomycin, oxacillin, penicillin, streptomycin, and tobramycin. *Staphylococcus* spp. were susceptible to cefoxitin, doxycycline, enrofloxacin, imimpenem, and tobramycin, but were resistant aztreonam and tetracycline.

Key words **:** Dogs, urinary tract infection (UTI), antibiotics susceptibility, bacteria.

Introduction

Bacterial urinary tract infection (UTI) is one of the most commonly diagnosed infectious diseases in canine practice and affects approximately 14% of dogs presented for veterinary care (21,32). Uropathogenic strains of *Escherichia coli* are the most common cause of UTIs in both humans and dogs, and strains of this species are often abundant in the gastrointestinal tract at the time of infection (13,22). In contrast to most intestinal strains of *E. coli*, uropathogenic strains possess virulence factors which facilitate survival and persistence in the urinary tract (22). The risk of UTI recurrence is increased when highly pathogenic bacteria or underlying problems (such as, anatomic abnormalities, neoplasia, diabetes mellitus) are present (7,31). Microbiological culture and susceptibility testing is the cornerstone of UTI diagnosis and the results are used by veterinarians to select antimicrobial therapy (3). Susceptibility results from specific populations are used to select empirical therapy and to monitor trends in antimicrobial resistance (5,31). Antimicrobial resistance in uropathogens complicates therapy in dogs and is also a public health concern because these pathogens may be zoonotic (12,17,18,22).

The objectives of this study were to estimate the prevalence of uropathogens in dogs with urinary tract infections, to apply the formula to help select rational antimicrobial therapy, and to identify changes in antimicrobial resistance among canine uropathogens.

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Materials and Methods

Experimental specimens

Samples of dogs with urinary tract infection were collected at 2 veterinary clinics in Seoul, Korea between 2003 and 2009. Urine specimens were obtained aseptically by cystocentesis. Samples were directly transported to microbiological laboratory, where the bacteriological analysis was carried out immediately after sample arrival.

Isolation and identification of bacteria

For the isolation of staphylococci and streptococci, samples were streaked onto 5% bovine blood agar, Staphylococcus medium 110 agar (Difco) and mannitol salt agar (Difco) and incubated 37°C for 24-48 hours. Identification of bacteria were performed by conventional methods. For the isolation of *E. coli*, samples were cultured on McConkey agar (Difco) and incubated 37°C for 24-48 hours. *E. coli* was identified by colony morphology, oxidase and indol test. The identity was further confirmed with API20E (bioMeieux).

Antimicrobial susceptibility test

Antimicrobial susceptibility was assessed by the Kirby-Bauer disk diffusion method on Muellet-Hinton agar with commercial antibiotic discs (BBL), according to the standards of the Clinical Laboratory Standards Institute (CLSI). The disks of

antibiotics used were listed in Table 1. Zone of growth inhibition were interpreted according to instructions of the manufacturers of the antibiotic discs. And then the strains were classified as resistant, susceptible and intermediate (1).

Results

Bacteria isolated from dogs with urinary tact infection

Forty-seven organisms were isolated from urine of dogs. Total numbers of isolates from urine that were, in order of frequency: *E. coli* (n = 27), *Streptococcus* spp. (n = 7), *Staphylococcus* spp. (n = 5), *Enterobacter* spp. (n = 3), *Proteus* spp. (n = 2), other species were 3, respectively.

Antimicrobial susceptibility of *E. coli*, *Streptococcus* spp. and *Staphylococcus* spp.

The results of antimicrobial susceptibility of *E. coli* (n = 27) from urine showed that the highest rates were IPM (100.0%), followed by PB (70.4%), and AN (63.0%). Other drugs were susceptible less than 60.0%. *E. coli* behaves as highly resistant to BC, EM, LM, OX, PIP, and NB (100.0%, respectively), CX, SM, and RA (96.3%, respectively), DC and TE (92.6%, respectively), AM and CB (85.2%, respectively), CP, GM, and TIC (81.5%, respectively).

All *Streptococcus* spp. isolates (n = 7) from urine had antimicrobial susceptibility less than 60.0%, except for BC (71.4%). The highest rates of resistance were shown to AN, ATM, CTX, FOX, CX, GM, LM, OX, PIP, SM, and NN (100.0%, respectively), CL, PB, and TE (85.7%, respectively). *Staphylococcus* spp. (n=5) from urine were higher susceptible to antibiotics than two major pathogens of UTI, described above. Antibiotics had the highest susceptibility were FOX, DC, ENR, IPM, and NN (100.0%, respectively), followed by AN, BC, CTX, CIP, CX, OX, PB, and RA (80.0%, respectively). The highest resistance was shown to ATM and TE (100.0%), followed by CP and PIP (80.0%, respectively).

Discussion

Urinary tract infection(UTI) is a condition that occurs when bacteria from outside the body get into the urinary tract and cause infection and inflammation. UTIs impose a tremendous health and economic burden in humans (11,17,33).

UTI develops when a breach occurs in host defense mechanisms and a virulent microbe in sufficient numbers is allowed adhere, multiply, and persist in a portion of the urinary tract (4). Cystic calculi, tumors of the bladder, neurologic problems, some diseases (diabetes mellitus, Cushing's disease) and medications (cortisone-like drugs, anticancer drugs) may predispose dogs to bacterial UTI (4,17).

E. coli is the most common uropathogen in humans, dogs and cats (26). This pathogen accounts for one third to one half of all organisms isolated from urine (4). Gram-positive cocci are the second major group of pathogens. Staphylococci and streptococci account for one fourth to one third of isolates recovered (4). Other uncommon bacterial species include *Proteus* spp., *Klebsiella* spp., *Enterobacter* spp., *Pasteurella* spp., *Pseudomonas* spp., *Corynebacterium* spp. and *Mycoplasma*

Table 1. List of antimicrobial drugs used in disc diffusion test and disc contents

Class/Antimicrobial agents	Disc content (μg)
Aminoglycoside	
Amikacin (AN)	30
Gentamicin (GM)	10
Streptomycin (SM)	10
Tobramycin (NN)	10
Carbapenem	
Imipenem (IPM)	10
Cephalosporin	
Cefazolin (CZ)	30
Cefotaxim (CTX)	30
Cefoxitin (FOX)	30
Macrolide	
Erythromycin (EM)	15
Lincomycin (LM)	2
Monobactam	
Aztreonam (ATM)	30
Penicillin	
Ampicillin (AM)	10
Carbenicillin (CB)	100
Cloxacillin (CX)	1
Oxacillin (OX)	1
Penicillin G (PIP)	10*
Tricarcillin (TIC)	75
Penicillin & beta-lactamase inhibitor	
Amoxicillin clavulanic acid (AmC)	20
Quinolone	
Ciprofloxacin (CIP)	5
Enrofloxacin (ENR)	5
Norfloxacin (NOR)	10
Sulfonamide	
Trimethoprim/sulfamethoxazole (SXT)	1.25 /23.75
Tetracycline	
Doxycycline (DC)	30
Tetracycline (TE)	30
Others	
Bacitracin (BC)	
Chloramphenicol (CP)	
Colistin (CL)	10
Novobiocin (NB)	30
Polymyxin B (PB)	300*
Rifampin (RA)	5

* U : International units

Table 2. Bacteria isolated from dogs with urinary tact infection

Species	Number of isolates
E. coli	27
Streptococcus spp.	7
Staphylococcus spp.	5
Enterobacter spp.	3
Proteus spp.	2
Other species	3
Total	47

spp. (4,19,30). Prescott *et al* (30) reported that total numbers of isolates from UTI that were identified in Canada from 1984 to 1998 were, in order of frequency: *E. coli* (657 isolates, 46%), *S. aureus* or *S. intermedius* (262, 18%), *Enterococcus* spp. (113, 7.9%), *Pseudomonas aeruginosa* (98, 6.8%), *Streptococcus* spp. (94, 6.6%), *Klebsiella* spp. (87, 6.1%), *Proteus* spp. (85, 5.9%), and *Enterobacter* spp. (36, 2.5%). In this study, similar to previous studies (20,26,30), *E. coli* was the most common pathogen (57.4%) in urine. Gram-positive cocci (staphylococci and streptococci) accounted for 25.6% of isolates. Isolation percentage of other 5 pathogens was up to 17.0%.

For treatment of UTI, generally considered antibiotics were any of the penicillins and cephalosporins, teteracycline, fluoroquinolones, aminoglycosides, and trimethoprim-sulfonamides (4). In this study, the most effective antibiotics to *E. coli* isolated from urine were IPM followed by PB, and AN. Cepha-

Table 3. Percentage of antimicrobial susceptibility of bacterial isolates from urine in dogs

Antibiotics	*E. coli* (n=27)			*Staphylococcus* spp. (n=5)			*Streptococcus* spp. (n=7)		
	R*	I**	S***	R	I	S	R	I	S
Amikacin	33.3	3.7	63	20	0	80	100	0	0
Gentamicin	81.5	0	18.5	40	0	60	100	0	0
Streptomycin	96.3	0	3.7	40	0	60	100	0	0
Tobramycin	63	7.4	29.6	0	0	100	100	0	0
Imipenem	0	0	100	0	0	100	42.9	0	57.1
Cefazolin	66.7	3.7	29.6	40	0	60	57.1	0	42.9
Cefotaxim	33.3	22.3	44.4	20	0	80	100	0	0
Cefoxitin	59.3	3.7	37	0	0	100	100	0	0
Erythromycin	100	0	0	40	20	40	71.4	14.3	14.3
Lincomycin	100	0	0	60	0	40	100	0	0
Aztreonam	40.7	26	33.3	100	0	0	100	0	0
Ampicillin	85.2	0	14.8	60	0	40	71.4	0	28.6
Carbenicillin	85.2	0	14.8	40	0	60	42.9	14.2	42.9
Cloxacillin	96.3	0	3.7	20	0	80	100	0	0
Oxacillin	100	0	0	20	0	80	100	0	0
Penicillin G	100	0	0	80	0	20	100	0	0
Tricarcillin	81.5	0	18.5	40	0	60	57.1	0	42.9
Amoxicillin/ clavulate	55.6	11.1	33.3	40	0	60	42.9	0	57.1
Ciprofloxacin	74.1	0	25.9	20	0	80	57.1	28.6	14.3
Enrofloxacin	74.1	0	25.9	0	0	100	57.1	28.6	14.3
Norfloxacin	74.1	0	25.9	40	0	60	57.1	14.3	28.6
Trimethoprim-sulfa	74.1	3.7	22.2	60	0	40	42.9	0	57.1
Doxycycline	92.6	3.7	3.7	20	0	80	71.4	14.3	14.3
Tetracycline	92.6	3.7	3.7	100	0	0	85.7	0	14.3
Bacitracin	100	0	0	20	0	80	28.6	0	71.4
Chloramphenicol	81.5	0	18.5	80	0	20	71.4	0	28.6
Colistin	44.4	0	55.6	20	40	40	85.7	0	14.3
Novobiocin	100	0	0	40	0	60	57.1	0	42.9
Polymyxin B	29.6	0	70.4	20	0	80	85.7	0	14.3
Rifampin	96.3	0	3.7	20	0	80	71.4	14.3	14.3

*:Resistant, **:Intermediate, ***:Susceptible

losporins, ATM, and AmC were more effective than aminoglycosides, macrolides, penicillin, quinolones, and sulfonamides. Penicillin and tetracycline were almost ineffective. IPM is a carbapenem antibiotic and may be useful in equine or small animal medicine to treat serious infections when other less expensive antibiotics are ineffective or have unacceptable adverse effect profiles (28). It can be used only when justified by culture and susceptibility results and in consultation with named infectious disease specialists (29, 30). Thus, it is not commonly used in clinics. PB is restricted to topical skin and ophthalmic applications. AN is a semisynthetic derivative of kanamycin. Gram-negative enteric bacteria which have acquired resistance to GM and NN by producing enzymes that conjugate these antibiotics, may remain susceptible to AN (2).

However, resistance rates of *E. coli* from urine examined were significantly higher than other countries. Feria *et al* (8) demonstrated that canine uropathogenic *E. coli* isolates have resistance to TIC (36%) (81.5% in this study), cefalothin (25%), (CZ, 66.7%, in this study), and AmC (19%) (55.6% in this study) in Portugal. Other study(16) also reported resistance to AmC in European (4%) and American clinics (19%). Franklin and Moerner(9) found that Gram(-) bacilli causing UTIs in dogs were resistant to AM (16.3%) (85.2% in this study) and cefalothin (18.4%). Cetin *et al*(6) reported, in Turkey, resistance of *E. coli* to AmC (0.0%), GM (16.7%), AM (33.3%), ENR (8.3%) (74.1% in this study), SXT(50%) (74.1% in this study). These differences indicate that a number of antibiotics were randomly used several times in local animal hospitals without the susceptibility test. Emerging antimicrobial resistance among *E. coli* strains that cause UTIs makes treatment more difficult and increases complications(11, 14, 32).

Similar to susceptibility pattern of uropathogens, resistance of staphylococci isolated from our hospital was higher than other countries. Ganiere *et al*(10) demonstrated, in France, resistance of *S. intermedius* to sulphonamides (26%) (76% in this study), CP(30%) (52% in this study), SM (28%) (80% in this study), EM (28%) (84% in this study), DC (6%) (44% in this study), GM (2%) (68% in this study), and ENR (2%) (48% in this study). Kania *et al*(15) reported that, in USA, 26% of staphylococcal isolates was resistant of *S. intermedius* to methicillin (44% in this study). Petersen *et al*(27) founded resistance to PIP (72%) (92% in this study), AM (72%) (88% in this study), OX (1%), cephalothin (1%) (CZ, 32% in this study), EM (21%), trimethoprim sulfa (45%) (76% in this study), TE (45%) (88% in this study), GM (3%), and CIP (1%) (52% in this study).

This study shows a number of organisms isolated from clinical specimens had multiple drug resistance (MDR; 97% of all isolates). One study reported, in UK, percentage MDR in various bacterial pathogens isolated from clinical cases at small animal referral hospital (24). It indicated that 31% of all isolates, 39% of *E. coli*, 16% of *Staphylococcus* spp., and 11% of *Streptococcus* spp. showed MDR in 1997. For same period, in veterinary community practice of UK, 36% of all isolates, 46% of *E. coli*, 18% of *Staphylococus* spp., and 15% of *Streptococcus* spp. showed MDR(23). In this study, 100.0% of *E coli*, 93% of *Staphylococcus* spp, and 100% of *Streptococcus* spp. had MDR. These results indicate that clinician have come to a serious difficulty in selecting antibitocis and treating bacterial infections, due to high MDR and antibiotic resistance of bacterial pathogens.

This study emphasizes the need for bacterial culture, species identification, and susceptibility testing in order to choose appropriate antimicrobial agents and to improve the clinical therapeutic approach for canine UTIs.

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세균성 요로 감염증 애완견의 세균 분포 및 항생제 감수성

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요 약 : 2003년부터 2009년까지 서울 지역 동물병원에 의뢰된 세균성 요로 감염증 개의 병소에서 세균의 분리빈도와 항생제 감수성을 조사한 결과는 다음과 같다. 세균성 요로 감염증 개의 뇨에서 *Escherchia coli* 27주, *Streptoococcus* spp. 7주, *Staphylococcus* spp. 5주, *Enterobacter* spp. 3주, *Proteus* spp. 2주, 그리고 기타 세균 3주, 총 47주의 세균이 분리되었다. 이 중 분리 빈도가 높은 *E. coli*, *Streptoococcus* spp. 및 *Staphylococcus* spp.를 대상으로 항생제 감수성을 조사하였다. *E. coli*의 항생제 감수성은 imimpenem, polymyxin B, amikacin, cephalosporins, aztreonam, amoxicillin clavulate, cephalosporins, tricarcillin, amoxicillin clavulate 순으로 나타난 반면 bacitracin, erythromycin, lincomycin, oxacillin, penicillin, novobiocin 등에 대해서는 높은 내성을 나타내었다. *Streptoococcus* spp.의 항생제 감수성은 bacitracin, imimpenem, trimethoprime-sulfa 순이었고 amikacin, cefotaxim, cefoxitin, cloxacillin, gentamicin, lincomycin, oxacillin, penicillin, streptomycin, tobramycin에는 매우 높은 내성을 나타내었다. *Staphylococcus* spp.의 항생제 감수성은 cefoxitin, doxycycline, enrofloxacin, imimpenem, tobramycin에 매우 높았고 aztreonam, tetracycline에 내성을 나타내었다.

주요어 : 애완견, 요로 감염증, 항생제 감수성, 세균.