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Antimicrobial Susceptibility Patterns in Urinary Tract Infections in Dogs (2010–2013)

C. Wong, S.E. Epstein, and J.L. Westropp

Background: Urinary tract infections (UTIs) are common in dogs. The responsible bacterial populations have evolved with increasing resistance to many antimicrobials.

Objective: To characterize the antimicrobial susceptibility patterns of canine urinary tract isolates over a 51-month period.

Animals: One thousand six hundred and thirty-six bacterial isolates from 1,028 dogs.

Methods: Aerobic bacterial isolate growth and susceptibility data from urine cultures of dogs were identified, retrospectively. Medical records were reviewed to obtain signalment, comorbidities, and antimicrobial use in the previous 30 days. The UTIs were further categorized as uncomplicated, complicated, or pyelonephritis.

Results: Common bacterial isolates identified were *Escherichia coli* (52.5%), *Staphylococcus* spp. (13.6%), and *Enterococcus* spp. (13.3%). In vitro susceptibility among all isolates varied for commonly prescribed antimicrobials (amoxicillin [59%], amoxicillin/clavulanic acid [76%], cephalexin [66%], enrofloxacin [74%] and trimethoprim-sulfamethoxazole [86%]). For all antimicrobials tested (except aminoglycosides), in vitro susceptibility was higher in uncomplicated versus complicated infections ($P < .05$). Uncomplicated infection isolate susceptibility rates remained ≤90% for PO administered antimicrobials. Administration of amoxicillin, doxycycline, and enrofloxacin, but not amoxicillin/clavulanic acid in the previous 30 days was associated with resistance to that antimicrobial. Multidrug resistant isolates of *E. coli* and *Staphylococcus* spp. were more common in dogs with complicated than uncomplicated UTIs (36% versus 21%, $P < .0001$).

Conclusions and Clinical Importance: In vitro susceptibility was highly variable and no PO administered antimicrobial had >90% efficacy among isolates tested. Multidrug resistance was frequent among isolates tested suggesting that routine culture and susceptibility testing is indicated. Previously prescribed antimicrobials may affect empirical choices made pending susceptibility testing.

Key words: Antimicrobial resistance; Bacterial resistance; Culture and susceptibility; Pyelonephritis; Uropathogens.

Bacterial urinary tract infections (UTIs) occur in approximately 14% of dogs in their lifetime with variable age of onset.[1] Furthermore, in 1 study of 237 euthanized dogs, the incidence of UTIs was 26.6% in females and 6.2% in males.[2] Urinary tract infections can be classified as simple uncomplicated, which is a sporadic bacterial infection in an otherwise healthy individual, or complicated, which is defined as a UTI that occurs in the presence of an anatomic or functional abnormality or a comorbidity that may predispose the patient to persistent infection, recurrent infection or treatment failure. Pyelonephritis is defined as an infection of the renal parenchyma.

Abbreviations:

UTI(s)	urinary tract infection(s)
MDR	multidrug resistant
CFU	colony forming unit
CKD	chronic kidney disease
TMS	trimethoprim-sulfamethoxazole

Collection of urine by cystocentesis followed by complete urinalysis and quantitative aerobic bacterial culture are recommended to confirm the presence of bacterial UTI in dogs with signs of lower urinary tract disease.[3] However, empirical antimicrobial treatment often is started for a presumptive diagnosis of UTI based on clinical signs of lower urinary tract disease (eg pollakiuria, stranguria, hematuria, or a combination of these signs) with or without results of urine culture. Repeated treatment of animals with recurrent lower urinary tract signs without culture and susceptibility test results may lead to incorrect antimicrobial choices, unnecessary adverse effects of drug treatment, and possible selection of resistant bacterial populations.

Widespread antimicrobial resistance is an emerging problem in small animal medicine[4] and in human beings.[5] Empirical antimicrobial administration is not without concerns because this practice may select for multidrug resistant (MDR) organisms, disturb normal flora and encourage colonization or infection.[6] Historical use of antimicrobials has been shown to encourage development of bacterial resistance in human beings

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with UTIs, not only to the antimicrobial in use, but possibly to more than 1 class of antimicrobials, highlighting the importance of the correct selection of an antimicrobial to treat a patient with a UTI.[7–10]

Although guidelines for treating UTIs have been published for dogs and human beings,[3,11,12] regional variability exists among isolates as well as susceptibility to various antimicrobials.[10,13] The population of affected individuals can vary, with some hospitals treating more patients with uncomplicated UTIs, whereas others may see primarily patients with complicated UTIs. In addition, treatment for uncomplicated versus complicated UTI may be different. For example, pyelonephritis, which is categorized as a complicated UTI, initially may be treated with parenterally rather than PO administered antimicrobials in some cases.[14] In addition to considering published guidelines for treating UTIs, current local data and recent antimicrobial surveillance play important roles in antimicrobial use as bacterial populations evolve.

The main objectives of this study were (1) to identify common urinary isolates involved in uncomplicated UTI, complicated UTI and pyelonephritis, as well as their associated susceptibility patterns at our tertiary care facility, (2) to evaluate potential changes in susceptibility patterns over time, and (3) to determine whether historical antimicrobial use was associated with future antimicrobial resistance in dogs with UTIs.

Materials and Methods

Data Collection

All urine samples obtained from dogs by cystocentesis that had positive quantitative aerobic urine culture results and subsequent antimicrobial susceptibility testing at the William R. Pritchard Veterinary Medical Teaching Hospital between January 1, 2010 and September 10, 2013 were identified from an electronic database. The William R. Pritchard Veterinary Medical Teaching Hospital is a tertiary facility receiving cases from a large area of northern California. We included antimicrobial susceptibility testing on samples with bacterial counts ≥100 colony forming units (CFU)/mL, because this is the minimal detection limit by the technique used for urine cultures in our hospital's laboratory. To provide a description of the population being studied, a colony count was performed on 10% of isolates that had positive growth. The corresponding medical records from these patients were reviewed and the signalment including age (calculated in years based on urine submission time), breed, and sex were recorded. An enteric designation was given if the isolate was from the family Enterobacteriaceae.

Urinary tract infections were further categorized from analysis of the available medical record as uncomplicated, complicated or pyelonephritis as previously described.[3] For dogs with complicated UTI, comorbidities were identified by the clinician's assessment at time of diagnosis combined with the client or referral history. Comorbidities included diabetes mellitus, kidney disease (both chronic kidney disease [CKD], and acute kidney injury), hyperadrenocorticism, pyoderma, immune suppression (corticosteroid or other immunosuppressive agent, chemotherapy administration, or some combination of these), urolithiasis (upper or lower urinary tract), anatomical abnormalities (eg recessed vulva, ectopic ureters, urethral stricture) and urinary incontinence (including dogs with thoracolumbar myelopathy and urethral sphincter mechanism incompetency). Pyelonephritis was presumptively diagnosed based on the final clinical diagnosis of the attending clinician which was made based on clinical signs, clinicopathologic data and ultrasound examination results as available. Recurrent infections were identified as ≥3 episodes of UTI documented by urine culture within a 1-year time period, which categorizes the infection as a complicated UTI. Historical antimicrobial use was identified by previous prescription dispensation from pharmacy records or by client or referral veterinarian history. If the antimicrobial was administered to the dog within 30 days of the urine culture, the antimicrobial used was recorded.

Isolates were considered susceptible if a susceptible classification was assigned and resistant if an intermediate or resistant classification was assigned according to the Clinical and Laboratory Standards Institutes recommendations for serum breakpoints.[15] If not available for dogs, human breakpoints were utilized. MDR organisms were identified based on definitions proposed by a collaborative effort between the Centers for Disease Control and Prevention and the European Centre for Disease Prevention and Control for enteric organisms and *Staphylococcus* spp.[16] Bacteria were considered MDR if found to be resistant to 1 agent in ≥3 separate antimicrobial categories in which the wild type bacteria would normally be susceptible.

Statistical Analysis

Categorical data between groups were evaluated for a difference in proportion with chi square or Fisher's exact testing where appropriate with commercially available software.[a] Susceptibility patterns over years were compared using chi-squared test for trends. For all comparisons, a value of $P < .05$ was considered significant.

Results

From January 1, 2010 through September 30, 2013 1,636 aerobic bacterial isolates were identified from 1,028 dogs. A total of 54 (5.2%) of the dogs were <1 year of age, 313 (30.4%) were between 1 and 7 years, and 661 (64.3%) were >7 years old. In 1 patient, the age was not documented. Seven-hundred and sixty (74%) were females, of which 684/1028 (66.5%) were spayed females and 76/1028 (7.4%) were intact females. Two-hundred and sixty-eight (26%) of the isolates obtained were from males with 216/1028 (21.0%) being castrated males and 52/1028 (5.1%) intact males. The breeds most commonly identified were Labrador Retrievers 148 (14.4%), German Shepherds 44 (4.3%), Golden Retrievers 44 (4.3%), and Dachshunds 41 (4.0%).

Of the 164 randomly selected isolates for CFU analysis all were >100 CFU/mL and 143 were >1000 CFU/mL (87.2%). Of the isolates with <1000 CFU/mL: 3 (1.8%) were from dogs on antimicrobials for treatment of complicated UTIs, 3 (1.8%) were from dogs with concurrent lower urinary tract signs and pyuria on urinalysis, 2 (1.2%) were from dogs with uncontrolled diabetes mellitus, and 1 each (0.6%) were from a dog receiving immunosuppressive drugs, a dog with chronic perivulvar dermatitis being treated with antimicrobials, and a dog with no clinical signs of lower urinary tract disease.

Of the 1,028 incidents of infection, we classified 363 (35.3%) as uncomplicated and 665/1028 (64.7%) as complicated. Of the complicated UTIs, 51/665 (7.7%)

of dogs had pyelonephritis. Five-hundred and thirty-two of 665 dogs with complicated UTI had a defined comorbidity (Table 1). The most common comorbidities were immune suppression (185 [34.7%]), kidney disease (162 [30.4%]), and anatomic abnormality (135 [25.3%]). Of those with anatomic abnormalities, the majority (71/135 [52.5%]) had recurrent UTIs. The predominant anatomical abnormality observed in recurrent UTIs was recessed vulva 46/71 (64.7%), followed by ectopic ureter 6/71 (8.4%) with 1 patient having both. In 70/665 (10.5%) complicated UTI cases, an underlying comorbidity was not documented for the patient but the dogs were classified as having complicated UTI based on recurrent infections (Table 1). Two hundred and seventy-nine (27.1%) dogs had recurrent or a persistent infections.

Table 1. Distribution of comorbidities among dogs with complicated urinary tract infections.

	% (n) of Comorbidities
Comorbidities	(532)
Immune suppression	34.7 (185)
Renal disease	30.4 (162)
Hyperadrenocorticism	8.2 (44)
Diabetes mellitus	8.0 (43)
Urolithiasis	8.0 (43)
Pyoderma	4.8 (26)
Neoplasia	4.7 (25)
Incontinence	1.8 (10)
Anatomic abnormalities	25.3 (135)
Recessed vulva	17.4 (93)
Other[a]	3.3 (18)
Ectopic ureter(s)	2.2 (12)
Urethrostomy	1.1 (6)
Stricture (ureteral, urethral, or vaginal)	1.1 (6)

Total number of comorbidities exceeds number of dogs as some dogs had multiple comorbidities listed.

[a]Hymenal remnant 0.37 (2), abnormal bladder wall 0.75 (4), vestibulovaginal stenosis 0.18 (1) urethral duplication/diverticulum 0.56 (3), urethral prolapse 0.37 (2), renal dysplasia 0.18 (1), perivulvar hyperplasia/mass 0.75 (4), paraphimosis 0.18 (1).

Most infections, (793/1028 [77.1%]), were monomicrobial whereas (235/1028 [22.9%]) were polymicrobial. The bacterial species isolated are reported in Table 2. Most isolates were gram negative 1128 (68.9%), and of these (1065/1128 [94.4%]) were enterics. In pyelonephritis, the most common bacterial isolates were *Escherichia coli* (50 [58.1%]), *Enterococcus* spp. (8 [9.3%]), *Staphylococcus* spp. (6 [7.0%]), *Proteus mirabilis* (6 [7.0%]), and *Enterobacter* spp. (6 [7.0%]). Of the subgroup of complicated UTIs that had recurrent infections, regardless of underlying comorbidity, the most common bacterial isolates were *E. coli* (273 [51.7%]), *Enterococcus* spp. (90 [17.0%]), *Staphylococcus* spp. (65 [12.3%]), *Klebsiella* spp. (27 [5.1%]), and *Pseudomonas aeruginosa* (16 [3.0%]).

Isolates from dogs with uncomplicated UTI were more likely to be gram negative ($P = .04$) and an enteric organism ($P = .006$) compared with isolates obtained from dogs with complicated UTI. Among the enteric organisms, the proportion of *E. coli* was higher in uncomplicated infections when compared with isolates from dogs with complicated UTI ($P = .03$; Table 2). One-hundred and fifty-three (50.8%) of *E. coli* UTIs in uncomplicated cases were nonhemolytic compared with 312 (61.5%) in complicated infections ($P = .003$). The proportion of *Staphylococcus* spp., *Proteus* spp., *Klebsiella* spp., and *Enterobacter* spp. were not significantly different

Table 2. Bacterial isolates identified in canine urinary tract infections (UTI) classified as uncomplicated UTI, complicated UTI and pyelonephritis. *P* value based on Fisher's exact test for difference in proportion of isolates in uncomplicated and complicated UTIs.

	All Organisms % (n)	Pyelonephritis % (n)	Uncomplicated % (n)	Complicated % (n)	*P* Value
Total	(1636)	(86)	(522)	(1028)	
Gram positive	31.0 (508)	18.6 (16)	28.3 (148)	33.5 (344)	.04
Staphylococcus spp.	13.7 (224)	7.0 (6)	12.3 (64)	15.0 (154)	.75
Enterococcus spp.	13.4 (220)	9.3 (8)	12.3 (64)	14.4 (148)	.96
Other Gram positive[a]	3.9 (64)	2.3 (2)	3.8 (20)	4.1 (42)	.76
Gram negative	68.9 (1128)	81.4 (70)	71.6 (374)	66.5 (684)	.04
Enterics	65.1 (1065)	76.7 (66)	69.5 (363)	61.9 (636)	.006
Escherichia coli	52.4 (858)	58.1 (50)	57.7 (301)	49.3 (507)	.03
Non-hemolytic	29.9 (489)	27.9 (24)	29.3 (153)	30.3 (312)	.14
Hemolytic	22.7 (371)	30.2 (26)	28.4 (148)	19.0 (195)	.003
Proteus mirabilis	5.4 (89)	7.0 (6)	5.9 (31)	5.1 (52)	.20
Enterobacter spp.	1.8 (29)	7.0 (6)	1.3 (7)	1.6 (16)	.66
Klebsiella spp.	3.7 (61)	3.5 (3)	2.9 (15)	4.2 (43)	.09
Other gram negative enterics[b]	1.7 (28)	1.1 (1)	1.7 (9)	1.8 (18)	1.0
Non-enterics	3.9 (63)	4.7 (4)	2.1 (11)	4.7 (48)	.005
Pseudomonas spp.	2.0 (33)	2.3 (2)	1.5 (8)	3.6 (37)	.01

Nonenterics: *Acinetobacter baumannii*, *Alcaligenes faecalis*, *Pasteurella* spp., *Providencia rettgeri*, *Stenotrophomonas maltophilia*.

[a]*Aerococcus viridans*, *Corynebacterium* spp., *Lactobacillus* spp., *Lactococcus* spp., *Micrococcus* spp., *Streptococcus* spp.

[b]*Citrobacter* spp., *Salmonella enteritidis*, *Serratia marcescens*, *Yersinia enterocolitica*.

in dogs with uncomplicated and complicated infections. Isolates from dogs diagnosed with pyelonephritis were more likely to be gram negative ($P = .003$) and be an enteric species ($P = .007$) than isolates from dogs with other types of complicated UTI. The proportions of all other bacteria were not significantly different between these 2 groups.

Isolates obtained from dogs with complicated UTIs were significantly more resistant to amoxicillin/clavulanic acid, ampicillin, chloramphenicol, doxycycline, enrofloxacin, ticarcillin/clavulanic acid and trimethoprim-sulfamethoxazole (TMS) when compared with isolates from dogs with uncomplicated UTIs (Table 3). However, isolates obtained from dogs with documented pyelonephritis only were more resistant to amoxicillin/clavulanic acid and TMS when compared with isolates from dogs with uncomplicated UTI (Table 3). On a longitudinal basis, an increase in resistance rates over time among all isolates was observed with amikacin ($P = .027$), gentamicin ($P = .021$) and ticarcillin/clavulanic acid ($P = .014$), but not for any other antimicrobial tested.

Isolates identified from dogs that had received amoxicillin, doxycycline, or enrofloxacin 30 days before culture results were obtained showed significantly higher resistance rates to those antimicrobials ($P = .003$, $P < .0001$, $P < .0001$, respectively) when compared with dogs that did not receive those antimicrobials before urine cultures were obtained. There was no difference in resistance rates of isolates identified from dogs that were treated with cephalexin or amoxicillin/clavulanic acid in the previous 30 days than from isolates from dogs not on those specific antimicrobials, respectively (Table 4).

Among *Staphylococcus* spp. and enteric spp. isolated, 401/1285 (31.2%) were MDR with 79/224 (79%) Staphylococcus spp. and 322/1061 (30.3%) enteric species being categorized MDR. The proportion of MDR isolates from dogs with complicated UTI was significantly higher 294/790 (36%) compared with isolates from dogs with uncomplicated infections 91/427 (21%; $P < .0001$).

Discussion

In this study, we documented a stable level of resistance against commonly prescribed antimicrobials over the study period, but a significantly higher frequency of bacterial resistance from isolates obtained from dogs with complicated UTI compared with those with uncomplicated UTI. Furthermore, many of these dogs had received antimicrobials previously that may have contributed to the overall increase in bacterial resistance in dogs with complicated UTI. We also documented multidrug resistance in many dogs with UTIs. Recurrent infections often were associated with various comorbidities.

Dogs included in this study more commonly had complicated UTI. The higher frequency of complicated infections likely is attributable to our hospital being a tertiary care facility, receiving referrals for recurrent UTI, as well as dogs undergoing treatment for many of

Table 3. Percentage of isolates susceptible for various antimicrobial agents among dogs with uncomplicated urinary tract infections (UTIs), complicated UTIs and those diagnosed with pyelonephritis (%). Chi-square test for difference in percent susceptibility for uncomplicated UTIs versus complicated UTIs.

	Amikacin	Amoxicillin/ Clavulanic acid	Ampicillin	Cefazolin	Chloramphenicol	Doxycycline	Enrofloxacin	Gentamicin	Ticarcillin/ Clavulanic acid	Trimethoprim/ Sulfamethoxazole
All organisms	83.7	76.7	59.4	66.0	85.1	71.6	74.6	80.6	74.4	85.7
Pyelonephritis	88.2	71.8	57.6	63.5	85.9	73.2	80.0	78.9	80.2	80.5
Uncomplicated UTI	84.8	83.0	68.0	73.7	86.6	77.4	83.0	83.1	77.9	90.3
Complicated UTI	82.7	73.8	55.2	82.6	82.6	68.5	69.6	79.4	71.9	83.9
P value	.30	<.0001	<.0001	<.0001	.04	.003	<.0001	.09	.01	.001

Table 4. Percent isolates susceptible to designated antimicrobial cultured from dogs that had received (column A) or not received (column B) the antimicrobial within 30 days before obtaining the urine culture.

Antimicrobial	A % (n) of Isolates that were Susceptible to the Designated Antimicrobial for Dogs being Administered that Antimicrobial within 30 days Before Culture	B % (n) of Isolates that were Susceptible to the Designated Antimicrobial from Dogs that had not Received any Antimicrobials within 30 days Before Culture	*P* Value
Amoxicillin/ clavulanic acid	73.8 (107)	76.9 (1118)	.86
Cephalexin	72.1 (31)	66.0 (1034)	.38
Amoxicillin	41.3 (26)	60.2 (903)	.003
Doxycycline	40.0 (17)	72.6 (1125)	<.0001
Enrofloxacin	28.4 (23)	77.2 (1103)	<.0001

the comorbidities reported in this study. Different results might have been obtained from a primary care facility or in other geographical locations.

In this large group of dogs with UTIs, large variability was observed in the susceptibility rates of bacterial isolates, with none achieving >90% susceptibility to any PO administered antimicrobial option. This patient population is similar to that of other studies, with the average age being >7 years old, as well as the documented higher frequency in female dogs.[2,17–19] Similar to other studies, no breed predispositions were found for UTI and the breeds represented likely reflected breed popularity during the study period.

Evaluating patients for comorbidities is particularly important for cases of recurrent UTI.[20] In our study, the most common concurrent disease in dogs with complicated UTI was immune suppression. This finding is similar to a previous study that identified impaired immunity as a disorder that may contribute to reinfections or persistent infections.[20] These are not unexpected findings because it is estimated that 18–39% of dogs on long-term corticosteroids treatment will develop UTIs.[21,22] In addition to immune suppression, kidney disease was the second most common comorbidity identified. These included cases of acute and CKDs, as reported by the attending clinician, but pyelonephritis was deemed unlikely based on the other clinical features of the case. However, subclinical pyelonephritis cases could have been inadvertently included in this population because of the retrospective nature of the study and difficulty in documenting pyelonephritis. In human beings, patients with CKD are reported to have impaired host immunity at all stages of CKD,[23] thus it is not surprising that dogs with CKD also may be susceptible to recurrent infections. Other comorbidities are similar between dogs and humans presented with recurrent UTI.[24]

In a study of 100 dogs with recurrent UTI, anatomic defects were the second most common disorder associated with infection,[20] and it was the third most common problem noted at our institution. The larger population we evaluated or the referral nature of the institution might have contributed to this slight difference. Evaluating patients for anatomic abnormalities is essential, because their correction can help prevent future UTIs. In 1 study, episioplasty for correction of a recessed vulva improved outcome in 13/16 dogs with respect to UTI.[25]

A total of 8% of our dogs had diabetes mellitus documented as a comorbidity. Although diabetes mellitus has been associated with UTI in the dog,[26] it was not a common comorbidity documented in this study. Patients with diabetes mellitus are predisposed to infection because urinary and tissue glucose can impair neutrophil function and affect a wide variety of other factors that might alter the immune system and micturition.[27,28] Therefore, controlling diabetes may improve the health of the urinary tract.

Escherichia coli comprised 80% of the gram-negative enterics in our study, representing the most common isolate in all categories of UTIs, which is consistent with the findings of other studies.[2,17–19] This observation likely is associated with the many mechanisms of virulence that these bacteria possess as discussed in other studies.[13,18,29] Additional studies are indicated to differentiate the origins of these isolates as gastrointestinal commensals, environmental organisms or extraintestinal uropathogenic *E. coli*. The second most common organism identified was *Staphylococcus* spp. These results are consistent with previous studies in which *Staphylococcus* spp. were the second or third most common isolate reported in dogs.[17,18]

Approximately 30% of dogs in this study had recurrent UTI, which is higher than in previous studies, in which recurrent or persistent UTI were reported to be present in up to 4.5% of dogs with UTI.[29] In a previous study performed at this hospital, such infections were identified in 0.3% of all hospital patients.[29,30] This finding likely reflects our hospital now being primarily a tertiary care facility, to which patients with recurrent infections are referred, and the nature of the disease for which the dogs were presented required multiple urine cultures. However, the frequency of recurrent UTI in the general population is likely to be lower than that observed in this study. Identifying the infections as recurrent, relapsing or persistent was beyond the scope of our study because many dogs were lost to follow-up and subsequent re-evaluations were performed by the primary care veterinarians. Therefore, the incidence of recurrent UTI actually may have been higher in this

population. The most common isolates obtained from dogs with complicated UTIs in other studies (*E. coli, Enterococcus* spp., *Staphylococcus* spp., and *Klebsiella* spp.) are in agreement with these findings.[20,30] There was a higher prevalence of *Pseudomonas* spp. (3.0%) in recurrent cases, which is similar to what was observed in other studies,[20,30] likely reflecting the intrinsic pathogenicity of these bacteria.

In vitro resistance to commonly tested antimicrobials was higher for isolates obtained from dogs with complicated UTI, which is not unexpected because a number of complicated infections may be recurrent and therefore more resistant because of previously used antimicrobial. Historic single or multiple antimicrobial usage can lead to the development of resistance over time as well as select for MDR bacteria.[6,8,11] In addition, resolution may be compromised by a comorbidity that promotes colonization. However bacteria with a higher level of intrinsic resistance were more likely to be found in complicated UTIs, and a higher proportion of nonhemolytic *E. coli* was found in dogs with complicated UTI.

Even in uncomplicated UTIs, no PO administered antimicrobial had a susceptibility rate >90%. This finding is in contrast to recent findings in a study of 27 dogs with uncomplicated UTI and positive cultures that found 100% susceptibility to both TMS and cephalexin,[31] but this study had fewer cases and in our study, we documented bacteria intrinsically resistant to TMS and cephalexin that were not present in the other study. Some of these dogs actually may have had complicated UTI, and underlying comorbidities may not have been identified because they are not always investigated on initial presentation. Our findings highlight the need for quantitative aerobic bacterial urine culture and susceptibility testing when UTI is suspected, even when it is the first UTI for a patient. Antimicrobial treatment should be based on these results whenever possible.

Over the study period, isolates were documented to be significantly more resistant to amikacin, ticarcillin/clavulanic acid, and gentamicin, but this was not observed with more commonly used antibiotics. One possible explanation could be that bacteria with intrinsic resistance to those antimicrobials were isolated rather than an increase in acquired resistance during the study period. If increasing antimicrobial resistance was caused by an increase in acquired resistance by bacterial pathogens, then cross resistance over variable classes of antimicrobials was likely implicated, because these 3 antimicrobials were not commonly prescribed for dogs with urinary tract problems. Development of resistance to other classes of antimicrobials, other than those prescribed, can occur with use of multiple antimicrobials suggesting plasmid-mediated mechanisms of resistance.[8,13] For example, in *E. coli* (the largest represented isolate in this study) aminoglycoside resistance often is plasmid mediated. Plasmids containing genes for fluoroquinolone resistance commonly contain genes for aminoglycoside resistance[32,33]. Fluoroquinolones frequently are prescribed in this setting and may have caused a change in resistance to aminoglycosides over the study period. These findings warrant further investigation.

The high incidence of MDR may be unique to our patients in this tertiary facility, because they often are presented for complicated or recurrent UTIs, or both and may not reflect the general population, or the organisms may represent an expanding population of MDR bacteria in veterinary medicine. Severe underlying illness, hospitalization for ≥3 days, and surgical intervention have been reported to be common in dogs with extraintestinal infections of MDR *E. coli* and *Enterobacter*.[34] Prior antimicrobial treatment with beta-lactams, fluoroquinolones and first generation cephalosporins also may be a risk factor.[34,35] In a study of MDR *E. coli* UTI in cats, the number of antibiotic groups used within a 3-month period was associated with increased risk for infection.[35] With the high incidence of recurrent infections in this study, antimicrobial use within the previous 30 days (as seen with amoxicillin, doxycycline, and enrofloxacin) could have led to the MDR isolates found in this study, further emphasizing the need for culture and susceptibility testing before initiating treatment. This is especially important when prior antimicrobial treatment has been administered.

One must be careful when comparing breakpoint results among populations. Our results were based on serum breakpoints, not urine breakpoints. Many antimicrobials attain higher concentrations in the urine compared with serum as long as renal function is normal because the majority of these drugs are eliminated through the kidneys. Therefore, a higher rate of susceptibility among isolates may be noted if traditional urinary breakpoints are used (amoxicillin and amoxicillin-clavulanic acid, which are higher than serum), whereas not with other antimicrobials such as the fluoroquinolones in which urine and serum breakpoints are the same.[15,36] This difference in established urinary breakpoints provided for dogs have been limited to ampicillin, enrofloxacin, difloxacin, marbofloxacin, and orbifloxicin during the study period.[15] After the end of the study period, an additional canine specific urinary tract breakpoint was developed for amoxicillin-clavulanic acid.[36] In uncomplicated UTIs, some investigators consider it reasonable to use urinary breakpoints to determine susceptibility, but for complicated UTIs and pyelonephritis serum breakpoints are preferred.[3] The disadvantage of urinary breakpoints for uncomplicated UTI cases is that if the uropathogen has invaded the bladder wall, these results could lead to ineffective treatment and the potential for persistent or recurrent UTIs. The Antimicrobial Guidelines Working Group of the International Society for Companion Animal Infectious Diseases recommends using serum breakpoint interpretive criteria for UTIs.[3] These recommendations have been applied at our institution's microbiology laboratory throughout the study period.

This study has the limitations inherent to a retrospective study. We relied upon the attending clinician to properly categorize the infections as pyelonephritis and some infections may have been miscategorized. This could have resulted in cases of pyelonephritis not being

included in this subset of complicated UTI or cases included inappropriately. We chose to provide data from this group of complicated UTIs separately because the diagnosis of other comorbidities associated with complicated UTI (eg urolithiasis, neoplasia, recessed vulva) are more easily confirmed using routine diagnostic tests. We required that all infections be documented at our institution and therefore may have underestimated the true number of recurrent infections if subsequent urine cultures were performed by the primary care veterinarian. In addition, quantitation of bacterial counts was not evaluated, but our primary focus was to evaluate potential changes in trends related to antimicrobial susceptibility over time. Our results may not apply to the majority of simple UTIs, because our hospital is a tertiary care facility that receives a larger number of dogs with complicated infections.

Finally, although clinically relevant bacteriuria is considered >1000 CFUs/mL for dogs that have not received antimicrobials, we included urine samples obtained by cystocentesis that had bacterial counts >100 CFU/mL because of the varied histories and our interest in identifying cases that possibly had received antimicrobial treatment. The primary focus of our study was to evaluate for the development of bacterial resistance. Furthermore, random sampling of a subgroup of isolates from this study confirmed that most dogs (87%) fit the classical definition of a UTI (ie >1000 CFU/mL). Of the isolates from dogs that did not meet this criterion, all but 1 were either receiving antimicrobial treatment or had a concurrent disease that resulted in isosthenuria, likely lowering the colony count. Interpreting both colony counts and clinical signs are important considerations for the clinician in deciding whether or not to prescribe antimicrobial treatment. Recommendations for intention to treat however were beyond the scope of our paper.

Conclusion

Many recurrent UTIs were considered complicated and a concurrent comorbidity could be documented. In vitro susceptibility was highly variable and no PO administered antimicrobial had >90% efficacy among all isolates tested. A stable level of resistance for commonly prescribed antimicrobials was observed over the study period. Multidrug resistance was frequent in this population. Previously, prescribed antimicrobials may affect empirical choices pending susceptibility testing. Routine culture and susceptibility testing is recommended when UTI is suspected, especially when recurrent UTI is suspected and in those dogs that have received previous antimicrobial treatment.

Footnote

[a] GraphPad Prism 6.0, Graph Pad Software, La Jolla, CA

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Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

References

1. Ling GV. Therapeutic strategies involving antimicrobial treatment of the canine urinary tract. J Am Vet Med Assoc 1984;185:1162–1164.

2. Kivisto AK, Vasenius H, Sandholm M. Canine bacteruria. J Small Anim Pract 1977;18:707–712.

3. Weese JS, Blondeau JM, Boothe D, et al. Antimicrobial use guidelines for treatment of urinary tract disease in dogs and cats: antimicrobial guidelines working group of the international society for companion animal infectious diseases. Vet Med Int 2011;2011:1–9.

4. Cooke CL, Singer RS, Jang SS, et al. Enrofloxacin resistance in *Escherichia coli* isolated from dogs with urinary tract infections. J Am Vet Med Assoc 2002;220:190–192.

5. Rijal A, Ghimire G, Gautam K, et al. Antibiotic susceptibility of organisms causing urinary tract infection in patients presenting to a teaching hospital. J Nepal Health Res Counc 2012;10:24–27.

6. Wilcox MH. The tide of antimicrobial resistance and selection. Int J Antimicrob Agents 2009;34(Suppl 3):S6–S10.

7. Garraffo A, Marguet C, Checoury A, et al. Urinary tract infections in hospital pediatrics: many previous antibiotherapy and antibiotics resistance, including fluoroquinolones. Med Mal Infect 2014;44:63–68.

8. Friedrich LV, White RL, Bosso JA. Impact of use of multiple antimicrobials on changes in susceptibility of gram-negative aerobes. Clin Infect Dis 1999;28:1017–1024.

9. Metlay JP, Strom BL, Asch DA. Prior antimicrobial drug exposure: a risk factor for trimethoprim-sulfamethoxazole-resistant urinary tract infections. J Antimicrob Chemother 2003;51:963–970.

10. Hwang TJ, Hooper DC. Association between fluoroquinolone resistance and resistance to other antimicrobial agents among *Escherichia coli* urinary isolates in the outpatient setting: a national cross-sectional study. J Antimicrob Chemother 2014;69:1720–1722.

11. Prescott JF, Hanna WJ, Reid-Smith R, et al. Antimicrobial drug use and resistance in dogs. Can Vet J 2002;43:107–116.

12. Warren JW, Abrutyn E, Hebel JR, et al. Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. Infectious Diseases Society of America (IDSA). Clin Infect Dis 1999;29:745–758.

13. European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2012. Available at: http://ecdc.europa.eu/en/publications_layouts/forms/Publication_DispForm.aspx?List=4f55ad51-4aed-4d32-b960-af70113dbb90&ID=963 (2013). (last accessed November 2014)

14. Pressler B, Bartges JW. Urinary tract infections. In: Ettinger SJ, Feldman EC, eds. Textbook of Veterinary Internal Medicine, 7th ed. St. Louis: Saunders; 2010:2036–2047.

15. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. 3rd ed. M31-A3 Wayne, Pennsylvania: Clinical Laboratory and Standards Institute; 2008.

16. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268–281.

17. Ling GV, Norris CR, Franti CE, et al. Interrelations of organism prevalence, specimen collection method, and host age,

sex, and breed among 8,354 canine urinary tract infections (1969–1995). J Vet Intern Med 2001;15:341–347.

18. Ball KR, Rubin JE, Chirino-Trejo M, et al. Antimicrobial resistance and prevalence of canine uropathogens at the Western College of Veterinary Medicine Veterinary Teaching Hospital, 2002–2007. Can Vet J 2008;49:985–990.

19. Hall JL, Holmes MA, Baines SJ. Prevalence and antimicrobial resistance of canine urinary tract pathogens. Vet Rec 2013;173:549.

20. Seguin MA, Vaden SL, Altier C, et al. Persistent urinary tract infections and reinfections in 100 dogs (1989–1999). J Vet Intern Med 2003;17:622–631.

21. Peterson AL, Torres SM, Rendahl A, et al. Frequency of urinary tract infection in dogs with inflammatory skin disorders treated with ciclosporin alone or in combination with glucocorticoid therapy: a retrospective study. Vet Dermatol 2012;23:201–e243.

22. Torres SM, Diaz SF, Nogueira SA, et al. Frequency of urinary tract infection among dogs with pruritic disorders receiving long-term glucocorticoid treatment. J Am Vet Med Assoc 2005;227:239–243.

23. Kausz AT, Gilbertson DT. Overview of vaccination in chronic kidney disease. Adv Chronic Kidney Dis 2006;13:209–214.

24. Neal DE Jr. Complicated urinary tract infections. Urol Clin North Am 2008;35:13–22.

25. Lightner BA, McLoughlin MA, Chew DJ, et al. Episioplasty for the treatment of perivulvar dermatitis or recurrent urinary tract infections in dogs with excessive perivulvar skin folds: 31 cases (1983–2000). J Am Vet Med Assoc 2001;219:1577–1581.

26. Forrester SD, Troy GC, Dalton MN, et al. Retrospective evaluation of urinary tract infection in 42 dogs with hyperadrenocorticism or diabetes mellitus or both. J Vet Intern Med 1999;13:557–560.

27. Balachandar MS, Pavkovic P, Metelko Ž. Kidney infections in diabetes mellitus. Diabetol Croat 2002;31:85–104.

28. Balasoiu D, van Kessel KC, van Kats-Renaud HJ, et al. Granulocyte function in women with diabetes and asymptomatic bacteriuria. Diabetes Care 1997;20:392–395.

29. Thompson MF, Litster AL, Platell JL, et al. Canine bacterial urinary tract infections: new developments in old pathogens. Vet J 2011;190:22–27.

30. Norris CR, Williams BJ, Ling GV, et al. Recurrent and persistent urinary tract infections in dogs: 383 cases (1969–1995). J Am Anim Hosp Assoc 2000;36:484–492.

31. Clare S, Hartmann FA, Jooss M, et al. Short- and long-term cure rates of short-duration trimethoprim-sulfamethoxazole treatment in female dogs with uncomplicated bacterial cystitis. J Vet Intern Med 2014;28:818–826.

32. Strahilevitz J, Jacoby GA, Hooper DC, et al. Plasmid-mediated quinolone resistance: a multifaceted threat. Clin Microbiol Rev 2009;22:664–689.

33. Cohn LA, Gary AT, Fales WH, et al. Trends in fluoroquinolone resistance of bacteria isolated from canine urinary tracts. J Vet Diagn Invest 2003;15:338–343.

34. Gibson JS, Morton JM, Cobbold RN, et al. Multidrug-resistant *E. coli* and enterobacter extraintestinal infection in 37 dogs. J Vet Intern Med 2008;22:844–850.

35. Hernandez J, Bota D, Farbos M, et al. Risk factors for urinary tract infection with multiple drug-resistant *Escherichia coli* in cats. J Feline Med Surg 2014;16:75–81.

36. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. 2nd ed. VET 01-S2 Wayne, Pennsylvania: Clinical Laboratory and Standards Institute; 2013.