Original Article

Inconsistent and multiple bacterial species from different sample types of dogs with urolithiasis and bacterial cystitis

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Abstract

Urinary tract infection is the most common complication found in dogs with urolithiasis. Bacterial cystitis can play an important role in dogs with urolithiasis; it can either be the cause of cystic formation or be a complication following urolithiasis. Moreover, urolithiasis cases that come with bacterial cystitis can lead to a condition of complicated cystitis which will require more complicated treatment and management. Proper bacterial identification should be performed in order to follow an appropriate treatment. Specific treatments require bacterial identification in order to select the most appropriate antibiotics. There are three main possible sites for bacterial sample collection in bacterial cystitis cases (urine, uroliths and urinary bladder mucosa), while the most representative sample is still questionable. In this study, urine, urinary bladder mucosa from the body and the neck and urolith were collected from ten dogs with bacterial cystitis and urolithiasis. All dogs were firstly diagnosed for all types of urolithiasis which needed to undergo surgical treatment at the Surgery Unit, Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University. Urinalysis and urine sediment cytology were then performed to confirm the condition of bacterial cystitis in all samples. In the surgery field, all samples were cultured for bacterial species identification by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF). Uroliths retrieved from the surgical procedure were submitted for stone analysis at the Minnesota Urolith Center, College of Veterinary Medicine, University of Minnesota, USA. Positive bacterial culture results from urolith, urine (>10^3 CFU/mL), mucosa from the body of the bladder and mucosa from the neck of the bladder were 90%, 87.5% (no data available in 2 patients), 50%, and 40% respectively. The most common bacteria cultured from UTI patients in this study were *Escherlichia coli* and *Pseudomonas aeruginosa*. In conclusion, urine culture should be done in every patient either prior to or in the surgical field. Moreover, due to the result of inconsistent bacterial culture and bacterial species from some types of samples, a urolith culture is recommended as the appropriate culture in case of a negative result from a urine culture.

Keywords: Canine, Urolithiasis, Bacterial culture, Urinary tract infection

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Introduction

Urolithiasis is the general term referring to aggregates of crystalline and non-crystalline solid substances that form in one or more locations within the urinary tract (Koehler *et al*., 2009). Regardless of the underlying mechanism(s), uroliths will not be formed unless sufficiently high urine concentrations of urolith-forming constituents exist and the transit time of crystals within the urinary tract is prolonged, and for certain stones (eg, struvite, cystine, urate) other favorable conditions such as proper pH for crystallization must also exist. These criteria can be affected by urinary tract infection, diet, intestinal absorption, urine volume, frequency of urination, therapeutic agents and genetics (Tion *et al*., 2015). Many types of urolithiasis in dogs that can be classified depend on the causative agents such as struvite, oxalate, cysteine and urate.

On average the most common urolith in canines is struvite and the second most common is calcium oxalate. From 2005 to 2009, there were recordings of 8,560 canine urolith from Thailand sent to be analyzed at the Minnesota Urolith Center. Most of the samples (97.2%) were collected from the lower urinary tract. (Hunprasit *et al*., 2017).

Urinary tract infection is one of the most common complications found in dogs with urolithiasis. The struvite uroliths especially predispose the urinary tract infection by urease-producing bacteria, mainly coagulase-positive Staphylococcus spp. Other most prevalent microbes found to cause UTIs include Eschelichia coli. Proteus mirabilis, Pseudomonas aeruginosa and Klebsiella pneumoniae (Gatoria *et al*., 2006). It has been described that UTIs which relate to an abnormality (such as lower urinary tract urolithiasis) can cause persistent infection or recurrent infection due to failure of UTI treatment. This condition can also be described as complicated UTI which requires specific diagnostic and treatment protocols. Identification of the underlying case microbial is strongly recommended in complicated UTIs (Teh, 2022).

As in humans (Krieger, 2002) and cats (Litster *et al*., 2007), the most common pathogen isolated from the canine urinary tract is E. coli, which accounts for 33–55% of isolates obtained from UTI cases (Forrester *et al*., 1999; Norris *et al*., 2000; Ling *et al*., 2001; Cohn *et al*., 2003; Seguin *et al*., 2003).

Bacterial species such as Pseudomonas aeruginosa and Enterococcus spp. have a higher prevalence in persistent or recurrent canine UTIs compared to uncomplicated UTIs (Seguin *et al*., 2003). In two large retrospective studies of dogs with persistent or recurrent UTIs, the six most prevalent bacteria were *E. coli*, *Klebsiella spp*., *Staphylococcus spp*., *Enterococcus spp*., *Proteus spp.* and *Pseudomonas spp.* (Norris *et al*., 2000; Seguin *et al*., 2003). In these cases, multiple bacterial species were often isolated from the urine, which sometimes complicated treatment options (Norris *et al*., 2000; Seguin *et al*., 2003).

These bacterial UTIs frequently occur in association with canine urolithiasis and the bacteria isolated are similar to those found in patients with UTIs without urolithiasis.

Large, retrospective studies have documented the most common species of uropathogens in dogs and cats, Escherichia coli is one of the most common pathogens in both acute and recurrent UTIs. Other common pathogens are *Staphylococcus*, *Proteus*, *Streptococcus*, *Klebsiella* and *Pseudomonas spp.* (Dowling, 2015).

Treatment of cystic calculi is recommended in the 2016 ACVIM Small Animal Consensus Recommendation (Lulich *et al*., 2016). Some uroliths are dissolvable by nutritional management such as struvite urolithiasis. Some are related to other systemic diseases such as urate urolithiasis which is related to liver disease in dogs such as a portosystemic shunt. However, the actual cause of some urolithiasis is still not completely understood. Surgery may be the choice of treatment in many cases of urolithiasis. As mentioned earlier, urinary tract infection can be one of the most common complications in cases with urolithiasis or in some cases can be the predisposing cause of urolithiasis. Bacterial identification and treatment of urinary tract infection is essential in cases with all types of urolithiasis with urinary tract infection. In cases which are treated without the procedure of surgery, cystocentesis of the urine in the urinary bladder is the ideal method to identify the pathogen in cases with urinary tract infection (Smee *et al*., 2013). However, in cases which need surgical treatment for urolithiasis removal, there are many specimens that can be sampled for bacterial identification such as uroliths, urinary bladder mucosa or the urine. A study in 2006 showed that the best area to do sampling for bacterial identification is the urinary bladder mucosa (Gatoria *et al*., 2006). However, the major population of urolithiasis in that previous study was mainly calcium oxalate urolithiasis and may not be able to represent every type of urolithiasis which led to our study.

We aim to ascertain appropriate sampling techniques for bacterial culture to identify the pathogen that causes urinary tract infections in dogs with urolithiasis.

Materials and Methods

All animal and bacterial uses in this study were approved by the Faculty of Veterinary Science-Animal Care and Use Committee (FVS-ACUC) protocol No.1931975 and the Faculty of Veterinary Science Institutional Biosafety Committee (CUVET-IBC) Protocol No. IBC 1931050 respectively. Ten dogs (n = 10) were of all breeds, ages and gender diagnosed with cystic calculi (final diagnosis by urinalysis and either plain film x-ray or ultrasonography) with urinary tract infection (by urine sediment cytology and urinalysis) and requiring surgical treatment at the Surgery Unit, Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University. Fully informed consent from owners was submitted before surgery.

On the day of surgery, four samples including urine, urinary bladder mucosa from the body and the neck of the urinary bladder (UB) and the calculi were collected from each animal. At least 5 mL of urine was collected by direct cystocentesis after laparotomy with a sterile needle (23 G) and a sterile 5 ml syringe then

placed into a sterile screw-capped tube for urinalysis and urine quantitative culture. Every sample sent for urine culture was evaluated before sample submission by urinalysis for a screening test of bacterial urinary tract infection. Cystic calculi were collected in a sterile centrifuge tube. Uroliths retrieved from the surgical procedure were submitted for chemical composition analysis of uroliths at the Minnesota Urolith Center, College of Veterinary Medicine, University of Minnesota, USA and were also submitted for bacterial culture. Mucosa at UB neck and body, of the size of five cm^3 per sample, were collected before closure then placed into a sterile microcentrifuge tube with 1 mL of Brain Heart Infusion Broth (BHIB).

All samples were sent for bacterial identification within 3 hours. The UB mucosa sample in BHIB was shaken for 5 minutes using a vortex mixer then fifteen µL of BHIB was inoculated on to 5% sheep blood agar and MacConkey agar for bacterial identification of the UB mucosa samples. Calculi were washed 3 - 4 times with sterile normal saline before crushing, 15 µL of the calculi suspension was inoculated onto 5% sheep blood agar and MacConkey agar for bacterial identification. Urine quantitative culture was conducted. Positive bacterial culture results were obtained from urolith, urine (>10^3 CFU/mL). After incubation at 37 C for 24 h, colonies were subcultured for species identification by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI TOF MS) (Microflex, Bruker, Germany). Bacterial colonies spotted on the target plate were lysed with 1 µL 10% formic acid and overlaid with 1 µL alpha-hydroxyl-4-cinnamic acid (HCCA) matrix. The protein spectral fingerprint was analyzed by Biotyper 3.0 software.

Results

Demographic information, clinical presentations, imaging diagnosis of the dogs included in this study are shown in Table 1. Urinalysis results are shown in Table 2.

Chemical composition of uroliths and bacterial findings in all samples are shown in Table 3. In our study, we found that at least one of the samples from all dogs showed positive bacterial culture. The uroliths identified from the urolithiasis with bacterial infection cases were struvite uroliths (n=6) and calcium oxalate uroliths (n=4). Bacterial culture was positive in 9 of 10 of the urolith samples. Urine was collected in 8 dogs (2 dogs had insufficient urine in the bladder at surgery) and 7 of those samples were positive for bacterial culture. For the samples of the UB mucosa at the neck and at the body, 4 and 5 samples, respectively, out of 10 were positive for bacterial culture.

In the struvite urolithiasis cases, *Escherichia coli* was cultured from 3 cases, from the urine samples in all 3 cases and from uroliths and mucosal biopsy from 2 cases each. *Klebsiella pneumoniae* was cultured from all three samples in one case (with *Pseudomonas aeruginosa* as a complex infection in the urine culture). In one case (16.67 percent), *Staphylococcus pseudintermedius* was cultured only from the urolith. In two cases, *Pseudomonas aeruginosa* was coinfected with *Klebsiella aeruginosa* and was cultured in urine in one case and in the urolith in another case. In one case, *Enterobacter cloacae* was cultured from both urolith and urine with coinfection of *Enterococcus faecalis* which was cultured from both mucosal sites.

Table 1 Demographic information, clinical signs and imaging diagnosis of the dogs.

Sample	Age (years)	Breed	Weight (Kg)	Sex	Clinical signs presented	Imaging diagnosis results
1	10	Mixed	7.4	Female (Spayed)	Incidental finding (Chief complaint of bite wound)	2 radiopaque material in the UB (X-ray)
2	9	Shih Tzu	9	Female (Intact)	Incidental finding (Chief complaint of caesarian section)	Multiple cystic calculi (X-ray)
3	3	American Pit Bull	28	Female (Intact)	Chronic urinary tract infection with stranguria	Uretheral cacilus and cystitis (X-ray and Ultrasonography)
4	5	Pomeranian	5.5	Female (Intact)	Hematuria and Stranguria	Multiple cystic calculi (X-ray and Ultrasonography)
5	10	Mixed	8.5	Female (Spayed)	Stranguria	Multiple cystic calculi (X-ray)
6	5	Pomeranian	4.5	Female (Intact)	Hematuria and Stranguria	Multiple cystic calculi (X-ray)
7	8	Pomeranian	2.5	Male (Castrated)	Cystitis and Hematuria	Cystic calculus (X-ray and Ultrasonography)
8	10	Pomeranian	3.8	Male (Intact)	Recurrence of cystic calculi from following up	Urethral and Cystic calculi (X-ray and Ultrasonography)
9	5	Shih Tzu	9.9	Male (Intact)	Stranguria	Multiple cystic calculi (X-ray)
10	10	Pomeranian	9.6	Male (Castrated)	Stranguria	Sand calculi (X-ray)

Table 2 Urinalysis results of all samples.

Sample	pH	Urine Specific Gravity	Protein	Glucose	Ketone	Blood	Leukocytes	Cells and casts
1	6	1.036	Negative	Negative	Negative	Negative	Negative	Squamous epithelial cells, Bacteria (Rods)
2	7	1.028	Negative	Negative	Negative	Negative	Negative	Bacteria (Rods)
3	7	1.014	Negative	Negative	Negative	Negative	Negative	W.B.C. (10 – 20 Cells/HPF), Bacterial (Cocci)
4	8	1.026	3+	Negative	Negative	4+	2+	W.B.C. (10 – 20 Cells/HPF), R.B.C. (20 – 30 Cells/HPF), Calcium oxalate cast, Transitional epithelial cells (10 – 15 Cells/HPF), Bacteria (Rods)
5	9	1.028	1+	Negative	Negative	3+	1+	W.B.C. (10 – 30 Cells/HPF), R.B.C. (10 – 40 Cells/HPF), Struvite cast, Transitional epithelial cells (5 – 10 Cells/HPF), Bacteria (Rods)
6	7	1.026	2+	Negative	Negative	4+	2+	W.B.C. (0 – 5 Cells/HPF), R.B.C. (TNTC), Hyaline cast (0 – 2 Cells/HPF) , Bacteria (Rods)
7	5	1.018	1+	Negative	Negative	Negative	Negative	R.B.C. (5 – 10 Cells/HPF), Squamous and Transitional epithelial cells (0 – 5 Cells/HPF), Bacterial (Cocci)
8	6	1.034	Negative	Negative	Negative	Negative	Negative	Calcium oxalate cast, Squamous and Transitional epithelial cells (0 – 3 Cells/HPF), Bacterial (Cocci)
9	8	1.028	1+	Negative	Negative	Negative	Negative	W.B.C. (0 – 2 Cells/HPF), Bacterial (Cocci)
10	7	1.048	2+	Negative	Negative	Negative	Negative	Bacterial (Cocci)

Table 3 The composition of uroliths and bacterial findings in all samples

Sample	Bacterial findings				
	Chemical composition of Urolith	Urolith	Urine	Mucosa	
				Neck	Body
1	Struvite	*Escherichia coli*	*Escherichia coli*	*Escherichia coli*	*Escherichia coli*
2	Struvite	*Klebsiella aeruginosa*	*Klebsiella aeruginosa, Pseudomonas aeruginosa*	Negative	*Klebsiella aeruginosa*
3	Struvite	*Enterobacter cloacae*	*Enterobacter cloacae*	*Enterococcus faecalis*	*Enterococcus faecalis*
4	Struvite	*Escherichia coli*	N/A	*Escherichia coli*	*Escherichia coli*
5	Struvite	*Escherichia coli*	*Escherichia coli*	Negative	Negative
6	Struvite	*Staphylococcus pseudintermedius*	Negative	Negative	Negative
7	CaOX	*Staphylococcus pseudintermedius , Pseudomonas aeruginosa*	*Staphylococcus pseudintermedius, Staphylococcus schleiferi, Pseudomonas aeruginosa*	*Staphylococcus haemolyticus, Staphylococcus warneri*	Negative
8	CaOX	*Escherichia coli, Enterococcus fecium*	N/A	Negative	*Escherichia coli*
9	CaOX	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	Negative	Negative
10	CaOX	Negative	*Pseudomonas aeruginosa*	Negative	Negative

In dogs with CaOX uroliths, *Pseudomonas aeruginosa* was cultured from both urolith and urine in 2 cases and from only the urine in one case. In one case, *Escherichia coli* was cultured from the urolith and body of mucosa. In one case, *Staphylococcus pseudintermedius* and *Pseudomonas aeruginosa* were cultured from urolith and urine. *Staphylococcus schleiferi* was cultured from the urine alone. *Staphylococcus haemolyticus* and *Staphylococcus warneri* were cultured from the neck of mucosa.

The results of the comparative study of bacterial cultures of the urine, urinary bladder biopsy and urolith are shown in Table 3. *Escherichia coli* was the most common bacterial species that could be consistently isolated from more than 2 samples in 4 dogs, however there were 2 mucosa samples from two of these dogs that were negative. *Enterococcus faecalis*

was concurrently isolated with *Escherichia coli* in one urolith sample. Two dogs in this study (sample 3 and 7) had 2 different bacterial species between the culture from mucosa with the culture from the urolith and urine. More than one bacterial species was identified in urolith samples in two dogs and urine in two dogs. Mucosa sample from one dog (sample 7) had two different staphylococcal species that were different from those found in urolith and urine. The second most common bacterial species found was *Pseudomonas aeruginosa* which was isolated from urine samples, while mucosa samples in these dogs (sample 2, 7, 9 and 10) were negative for *Pseudomonas aeruginosa.* All bacteria found in this previous study were urease-producing except *Enterococcus faecalis* and *Enterococcus faecium.* There was no trend of association of bacterial species and urolith types in this study.

Discussion

From 10 cases of UTIs with calculi in dogs, 6 cases were struvite and 4 cases were calcium oxalate. The most common bacteria cultured from UTI patients in this study were *Escherlichia coli* and *Pseudomonas aeruginosa* which were similar to the results found many previous publications. In this study positive bacterial culture was also found in cases with calcium oxalate uroliths. Even though it is quite clear that the pathogenesis of calcium oxalate urolith are not associated with urinary tract infection, the finding of positive bacterial culture in this study could be the complication in individual dogs following the formation of calculi in the urinary tract. In the current study, 4 out of 10 cases of canine urinary tract infection resulted from multiple bacteria. Norris *et al*. (2000), and Seguin *et al*. (2003), suggests that multiple bacterial species were often isolated from urine. However, in this study, there were two cases where bacterial culture results from urine samples indicating a single infection and the results from the urolith sample in the same patient suggested otherwise.

As the number of positive results of CaOx urolith cases was fewer than those of struvite urolith cases, it might be assumed that UTI was one of the predisposing causes and the bacteria can also act as a nidus that leads to struvite urolithiasis. Uroliths can be an interesting sample for bacterial culture to uncover the underlying uropathogen of bacterial UTIs. The study of Perry *et al*., (2013) has shown that urolith culture can be clinically interrelated, even if urine bacterial culture is negative. Although the patients have been treated with antimicrobial medications, urolith culture can provide positive bacterial growth. The results between urine and urolith culture are not always correlated. Isolated pathogens may be different. This study shows that urolith culture provides bacterial culture results covering more than one bacterial species than those from mucosa. As uroliths can also be the source of bacterial infection and may be associated with recurrent UTI cases (Seguin *et al*., 2003), urolith culture should be considered when uroliths are presented.

Urine samples in this study were positive for one or more bacterial species, which is consistent with those from uroliths. Urine collection by cystocentesis is less complicated and less invasive compared to the collection from the UB mucosa and it can also be performed via abdominal cystocentesis before antimicrobial and surgical treatment. Therefore, the authors suggest that urine is a good sample for bacterial culture in UTI. According to the prior study, urine collected by cystocentesis is the most recommended sample for bacterial identification (Forrester *et al*., 1999) while mucosal biopsy is suggested if the urine culture is negative (Gatoria *et al*., 2006). On the contrary, this study finds mucosal culture is not the most dependable sample for bacterial identification. Mucosal cultures from the body of the urinary bladder appeared negative in 5 of the 10 samples and mucosa from the neck appeared negative in 6 of the 10 samples. This information differs from recommendations of previous studies suggesting that collections from the mucosa are good representative cultures in UTI for cases of dogs undergoing cystotomy (Gatoria *et al*., 2006). However, it might be difficult to localize the infected part of the mucosa in the surgical field.

In conclusions, according to the results of our study, urine culture techniques obtained by cystocentesis should be performed in every UTI patient. The results of this current study show the negative results from urolith culture were less than from mucosal culture. Consequently, the culture of uroliths was suggested as a second choice if the result of urine culture was negative in order to appreciate underlying uropathogens and provide effective treatments. Bladder mucosa culture techniques are not recommended because bladder mucosa biopsy requires the surgeon's experience for sampling infectious mucosal areas. Otherwise, the results can result in a false negative.

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