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### Occurrence of pathogens in urinary tract infection and their antibiogram in dogs in and around Bengaluru

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#### Abstract

**Aim:** This study aims to determine the etiology of urinary tract infection (UTI) in dogs, their occurrence and to develop an antibiogram of isolated organisms.

**Materials and Methods:** Urine samples were collected from 150 dogs suspected of having UTI who were brought to several hospitals in and around Bengaluru via catheterization or cystocentesis. Bacteria were identified in 86 samples based on cultural features, and all isolates were tested for antibiotic sensitivity *in vitro*.

**Results:** The study revealed that females are more susceptible (61.3%) to pathogenic infection than males (55.7%) also dogs more than 10 years old are more susceptible (80.49%) followed by 5 to 10 years old group (56.10%), less than 5 years old group (42.86%). *E. coli* (36.90%) is the most prevalent microorganism followed by *Enterococcus* (14.29%), *Klebsiella* spp. (10.71%), *Proteus* spp. (9.52%), *Coagulase negative Staphylococcus aureus* (8.33%), *Proteus mirabilis* (4.76%), *Staphylococcus aureus* (4.76%), *Proteus vulgaris* (3.57%), *Enterobacter* (2.38%), *MRSA* (2.38%), *Pseudomonas aeruginosa* (2.38%).

**Conclusion:** *E. coli* was the most predominant bacteria isolated from UTI affected in Bengaluru. Dogs of older age are more susceptible to UTI than younger dogs. Female dogs are more susceptible to UTI than male dogs. *In vitro* sensitivity revealed a significant proportion of bacteria to be resistant to multiple drugs which are commonly used in UTI in dogs.

Keywords: UTI, antibiotic susceptibility test, bacteria, E. coli

#### Introduction

A urinary tract infection (UTI) occurs when a breach (temporary or permanent) in host defensive mechanisms allows a virulent germ to attach, multiply, and survive in a part of the urinary tract. UTIs are typically caused by bacteria, although fungi and viruses can also infect the urinary tract. Infection can occur in a single location, such as the kidney (pyelonephritis), ureter (ureteritis), urinary bladder (cystitis), urethra (urethritis), prostate (prostatitis), or vagina (vaginitis), or in two or more of these locations (Bartges, 2004)<sup>[7]</sup>.

Bacterial urinary tract infections (UTIs) affect roughly 14% of dogs at some point in their lives (Ling, 1984)<sup>[9]</sup>. UTIs were found in 26.6% of females and 6.2% of males in a study of 237 euthanized dogs (Kivisto *et al.*, 1977)<sup>[2]</sup>. Urinary tract infections are classified as either simple uncomplicated (a sporadic bacterial infection in an otherwise healthy individual) or complicated (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). Wong *et al.* (2015)<sup>[5]</sup> define pyelonephritis as an infection of the renal parenchyma.

However, empirical antibiotic therapy is frequently initiated for a presumptive diagnosis of UTI based on clinical indicators of lower urinary tract disease (e.g., pollakiuria, stranguria, hematuria, or a combination of these signs), with or without urine culture results. Repeated treatment of animals with recurrent lower urinary tract indications in the absence of culture and susceptibility test data may result in inaccurate antibiotic selection, needless adverse medication effects, and the selection of resistant bacterial populations. Antimicrobial use in the past has been shown to promote the development of bacterial resistance in humans with UTIs, not only to the antimicrobial in use, but possibly to more than one class of antimicrobials, highlighting the importance of selecting the right antimicrobial to treat a UTI patient (Garraffo *et al.*, 2014; Hwang and Hooper, 2014)<sup>[1, 12]</sup>.

#### Materials and Methods Sample collection

A total of 150 dogs were collected using catheterization or cystocentesis after presenting to various hospitals in and around Bengaluru with clinical signs suggestive of UTI such as inappetence/anorexia, emaciation, vomiting, hematuria, polyuria, polydipsia, depression, weight loss, weakness, dehydration, nausea, anuria, stranguria, and oliguria.

#### Bacterial growth and identification

0.01 ml of the urine sample was inoculated on urochrome agar plate with a 4mm diameter nichrome loop using T streak method for semi quantitative analysis. The plate was then aerobically incubated at 37 °C for 24-48 hours. Depending on the significant growth of colonies, they were identified based on colony morphology, Gram's stain, and biochemical reactions. > 1,00,000 CFU/ml was considered as significant. 10,000- 1,00,000 CFU/ml was probable significant, to be correlated with clinical findings. < 10,000 CFU/ml was non-significant.

The Gram positive Cocci were subjected to catalase test to differentiate Staphylococcus species and Enterococcus species. Staphylococcus species are Catalase positive and Enterococcus species are catalase negative. The Gram negative Bacilli were identified based on the following reactions: Catalase test, oxidase test, indole production, urease production, citrate utilization, mannitol fermentation with motility and reaction on triple sugar iron agar.

#### Antimicrobial susceptibility testing

This was done by manual Kirby Bauer Disc diffusion method. The bacterial colony which needed to be processed was inoculated into peptone water and incubated at 37 °C for 3-5 hours to attain turbidity to match 0.5 Mc Farlands. With the help of a sterile cotton swab, the broth culture was spread evenly over Mueller Hinton agar plate by lawn culture method and antibiotic discs are placed. These plates were incubated at 37 °C for 24 hours and then zone of inhibition was measured in millimeters. Some commonly used antibiotics of different classes were selected for testing.

#### Results

Bacteriological examination of the 150 samples, 86 urine samples were found positive for bacterial isolation. Single isolation of *E. coli* was isolated in 31 (36.90%), Enterococcus in 12 (14.29%), *Klebsiella* spp. in 9 (10.71%), *Proteus* spp. in 8 (9.52%), *Coagulase negative Staphylococcus aureus* in 7 (8.33%), *Proteus mirabilis* in 4 (4.76%), Staphylococcus aureus in 4 (4.76%), *Proteus vulgaris* in 3 (3.57%), *Enterobacter* in 2 (2.38%), MRSA in 2 (2.38%), *Pseudomonas aeruginosa* in 2 (2.38%), and some organisms with unknown identity in 2 (2.38%) samples. The frequency of isolation of different bacterial species is shown in Table-1.

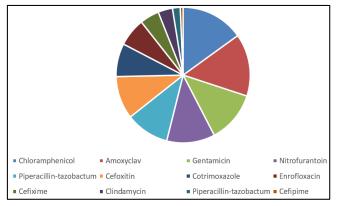
Table 1: Relative frequency of organism isolated from urine samples of dogs suffering from urinary tract infections.

Bacterial isolates	Number of positive samples	Percentage positivity
E. coli	31	36.90
Enterococcus	12	14.29
Klebsiella spp.	9	10.71
Proteus spp.	8	9.52
Coagulase negative Staphylococcus aureus	7	8.33
Proteus mirabilis	4	4.76
Staphylococcus aureus	4	4.76
Proteus vulgaris	3	3.57
Enterobacter	2	2.38
MRSA	2	2.38
Pseudomonas aeruginosa	2	2.38
Others	2	2.38

#### Antibiogram of the isolates

Antibiogram was performed on all the bacterial isolates isolated from the urine sample by using antibiotics such as; cefixime, cefoxitin, chloramphenicol, Amoxyclay, nitrofurantoin, clindamycin, cotrimoxazole, gentamicin, Piperacillin-Tazobactum, enrofloxacin, cefepime, piperacillin. Among used antibiotics against the 86 isolates

chloramphenicol was found to be most sensitive antibiotic (65 isolates) followed by Amoxyclav (64 isolates), gentamicin (53 isolates), nitrofurantoin (50 isolates), Piperacillin-Tazobactum (45 isolates), cefoxitin (44 isolates), cotrimoxazole (34 isolates), enrofloxacin (29 isolates), cefixime (20 isolates), clindamycin (15 isolates), piperacillin (8 isolates). Least sensitivity was observed with Cefipime (3 isolates).



Antibiotic sensitivity of the isolates  $\sim$   $_{1587}\sim$ 

#### Discussion

In the present study among 150 samples collected with different signs of urinary tract infection, 86 samples (57.3%) were found positive for different bacterial infection. (Wierup, 1978) <sup>[13]</sup> reported bacterial growth in 55.7% of 199 urinary specimens from dogs with symptoms of UTIs. In a study conducted by Lefmann., 1977 <sup>[8]</sup> it is reported that 44 (48.35%) of the urine samples from 91 dogs were bacteriologically positive. (Al kocabiyik., 2003) <sup>[3]</sup> reported 38 (38%) samples among 100 samples collected as positive. 427 (80.6%) of 530 urine samples from dogs with UTIs or suspected infections were reported positive for bacterial infections by (Zschock *et al.*, 1988) <sup>[14]</sup>. By these reports even though urinary tract infections of UTIs should not be made only on the basis of clinical symptoms.

The current study revealed that among 84 samples collected from male dogs 46 (54.8%) were positive for bacterial infection and among 66 samples collected from female dogs 40 samples (60.6%) were found positive. In the study conducted by (CR Norris, 2000) <sup>[4]</sup> among 383 dogs 54% of infections were found in females and rest (45.7%) were males. In another study conducted by (C. Wong, 2015) <sup>[5]</sup> among 1028 dogs, 760 (74%) were females and 268 (26%) of the isolates obtained were from males. Supported by all these works UTIs are more common among female dogs than male dogs. This may be due to shorter urethral length and close proximity to external urethra orifice, vagina and anus.

Among the isolates most of the isolates were sensitive to Amoxyclav (74.4%) this is in agreement with (Moyaert *et al.*, 2016) <sup>[16]</sup> who reported that 98.0% of isolates were sensitive to Amoxyclav. The same author also reports that the 94.6% of the isolates were sensitive to enrofloxacin, this statement is in contrast with the present study as only 33.7% of the isolates were sensitive for enrofloxacin. This variations in the sensitivity may be due to over usage or under usage of particular antibiotic in that particular area.

#### Conclusion

*E. coli* was the most predominant bacteria isolated from UTI affected in Bengaluru. Dogs of older age are more susceptible to UTI than younger dogs. Female dogs are more susceptible to UTI than male dogs. *In vitro* sensitivity revealed a significant proportion of bacteria to be resistant to multiple drugs which are commonly used in UTI in dogs.

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