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Isolation and antimicrobial susceptibility profiling of bacteria from canine cystitis

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Abstract
The present study was envisaged to record the prevalence of different bacteria in canine cystitis cases and their antibiogram to select suitable antibiotic for the effective treatment. Early and specific use of antimicrobials significantly limits the severity of the in present study the bacterial isolates from canine cystitis cases were identified up to species level disease and in many cases prevent the appearance of any visible sign of infection. However, the indiscriminate use of antimicrobial is leading to development of resistance in microbes. Thus the regular screening for the selection of suitable and effective antimicrobial is need of the day. With this objective based on cultural and biochemical characteristics. Among 30 samples, 23 samples yielded single isolates and the percentage of isolates were *Staphylococcus* 12 (60%), *Escherchia coli spp.* 08 (32%), *Proteus* spp. 01 (4%), *Klebsiella* spp. 01 (4%) and *Enterobacter* spp. 01 (4%).The development of antimicrobial resistance is the important drawback in the treatment of urinary tract infections. The present study had maximum sensitivity to Enrofloxacin, Amikacin and Trimethoprim/sulfamethoxazole and least sensitivity to Cefpodoxime.

Keywords: Cystitis, isolation, antibiogram, drug resistance and sensitivity

Introduction
Urinary tract infections (UTIs) are associated with a temporary or permanent breach in host defense mechanisms, and often (Bacterial UTIs) they occur as a consequence of ascending migration of pathogens through the genital tract and urethra to the bladder [2]. Improper diagnosis and treatment can lead to over use or misuse of antimicrobials, failure to resolve the problem, need for repeated and prolonged treatment, public health and regulatory concerns
Bacterial urinary tract infection (UTI) results from normal skin and gastrointestinal tract flora ascending the urinary tract and overcoming the normal urinary tract defense that prevents colonization [1]. The bacteria most frequently involved in dog urinary tract infection include the gram negative rods *Escherichia* spp., *Proteus* spp., *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas* spp., and the gram positive cocci *Staphylococcus* spp. and *Streptococcus* spp. [5]. The most important drawback in the treatment of urinary tract infections is the development of antimicrobial resistance. Therefore, the action of currently available antimicrobial substances and their structure-property relationships have to be understood for their prudent use [8]. Understanding drug pharmacokinetics (PK) and pharmacodynamics (PD) is essential when determining the most effective antibiotic therapy. In addition, successful antimicrobial therapy requires appropriate choice of antibiotic, including dose, frequency, and duration. The Present study describes the bacteria involved in canine cystitis cases and their antibiogram to select suitable antibiotic for the effective treatment.

Materials and Methods
The Present study was carried out on 30 dogs, which were presented to Veterinary College Hospital, Hebbal, Bangalore with different clinical manifestation such as chronic recurrent vomiting, pollakiuria, urination in inappropriate places, hematuria, stranguria/dysuria, decreased volume of urine voided and urinary incontinence. These cases were subjected for through clinical examination and the urine was collected by catheterization [4]. Care was taken during catheterization to prevent contamination from external structures by clipping surrounding hair and cleaned the external genitalia prior to the procedure [10]. About 5 ml of urine were collected from affected dogs and subjected to bacterial isolation.
About 0.1ml of urine sample was initially enriched in Brain heart infusion broth and incubated at 37 °C for 12 hours. The cultures were streaked on different selective media namely, Mannitol salt agar, McConkey's agar, EMB agar and incubated at 37 °C for 24 hours.

Each of the different isolated colonies was streaked on to BHI agar plates to get pure cultures for further identification.

Bacterial isolates were identified up to genus level based on colony characteristics of individual primary isolates, hemolytic patterns on blood agar, growth on Mannitol salt agar, McConkey's agar, EMB agar and by Grams staining. Further, these isolates were subcultured on plain BHI agar plates and then subjected to primary tests such as catalase, using pure cultures and were preserved at 4^0C on nutrient agar slants. All the isolates were subjected to cultural and biochemical tests such as indole, methyl red, citrate, urease, catalase, oxidase as per standard procedures to identify and group them up to genus level.

A loop full of pure cultures were inoculated into nutrient broth and incubated for 12-24 hours. Then broth was centrifuged at 3000 rpm for 5 min to obtain the pellet of bacterial growth. The supernatant was discarded and pellet was suspended in Phosphate buffer saline (PBS, pH 7.2) and adjusted to 0.5 scale McFarland turbidity standard to carry out primary antibiotic susceptibility test by agar disc diffusion method on Mueller-Hinton agar plates as per the guidelines of Clinical and Laboratory Standards Institute (CLSI) [9].

To select the effective antimicrobial agent for successive treatment, all the cultures were tested for their sensitivity against eight different antimicrobials commonly used in veterinary practice. The antimicrobial discs obtained from Himedia (Mumbai) were used in the study included, Amoxycillin with Clavulanic acid**,** Doxycycline**,** Trimethoprim/sulfamethoxazole, Amikacin, Ampicillin**,** Ciprofloxacin**,** Cephalexin**,** Cefpodoxime and Enrofloxacin. The zone of inhibitions were measured and interpreted as either susceptible or resistant to the exposed agent according to the zone diameter interpretative standards provided by the manufacturer. All the results were compiled and compared to ascertain the pattern of drug resistance in common bacterial pathogens of canine cystitis.

Results and Discussion

Microscopic examination of the urine sediment revealed RBCs, WBCs, pus cells and epithelial cells. Presence of blood cells and epithelial cells in the present study might be due to inflammation of urinary tract and the presence of more number of these cells may suggest bacterial urinary tract infection [6]. Among 30 samples, 23 samples yielded single isolates and no growth was observed in other seven samples. The percentage of isolates were *Staphylococcus* 12 (60%), *Escherchia coli* 8 (32%), *Proteus* spp. 01 (4 %), *Klebsiella* spp. 01 (4 %) and *Enterobacter* spp. 01 (4 %). The findings of the present study are in agreement with [11] who reported that *E. coli, Proteus, Staphylococci, Streptococci, Enterococus, Pseudomonas* were the common pathogens associated with urinary tract infections in dogs.

Table 1: Result of Biochemical tests of isolates

Etiological agents	No. of isolates	Catalase	Oxidase	Citrate utilization	Methyl red	Indole	Urease	H_2S Production
Staphylococcus aureus	12	+ve	-ve	-ve	+ve	-ve	-ve	-ve
Escherichia coli	0 8	+ve	-ve	-ve	+ve	+ve	-ve	-ve
Proteus spp.	01	-ve	-ve	-ve	+ve	+ve	+ve	+ve
Klebsiella spp.	01	-ve	-ve	+ve	-ve	-ve	+ve	-ve
Enterobacter *spp*	01	+ve	-ve	-ve	-ve	+ve	-ve	-ve

Study on antibiotic sensitivity of bacterial species isolated from the urine sample

The antibiotic susceptibility tests of the cultures from cystitis revealed resistance to multiple antibiotics tested in the pathogens involved. The number of samples showing sensitivity or resistance was expressed in percentage. The present study describes the sensitivity pattern, *E. coli* are moderately resistant, *Klebsiella* spp. and *Enterobacter* spp. are completely resistant to most of the antibiotics. In the present study, Enrofloxacin was reported to be highly effective towards urinary infections in dogs. This might be due to concentrations of the drug will be much higher in these sites than the serum levels can be achieved [7, 11]. The isolates of *Staphylococcus* spp. are sensitive to most of the antibiotics and *Proteus* spp. are 100% sensitive to all the antibiotics except for Cefodoxime. This sensitivity pattern of enteric gram negative bacilli causing UTI such as *E. coli, Klebsiella* spp. and *Enterobacter* spp., emphasizes judicious use of antibiotics and the choice of antibiotics requires a different strategy.

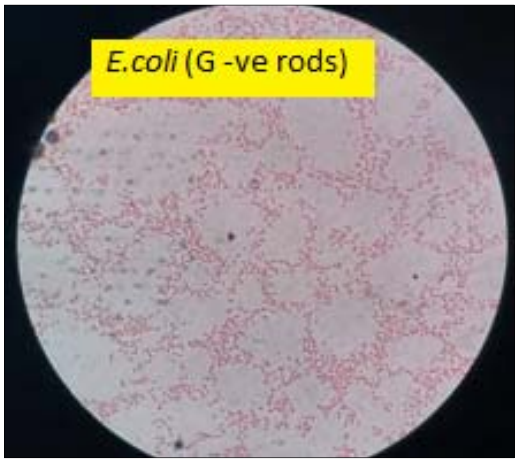


Fig 1: Microscopc view of E.coli

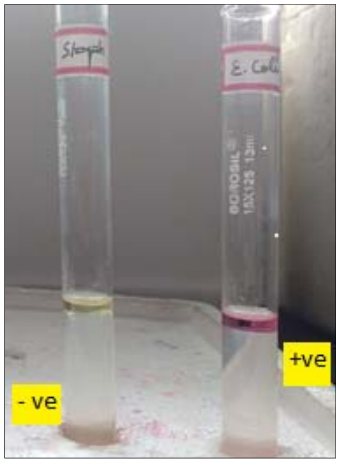


Fig 2: Indole test

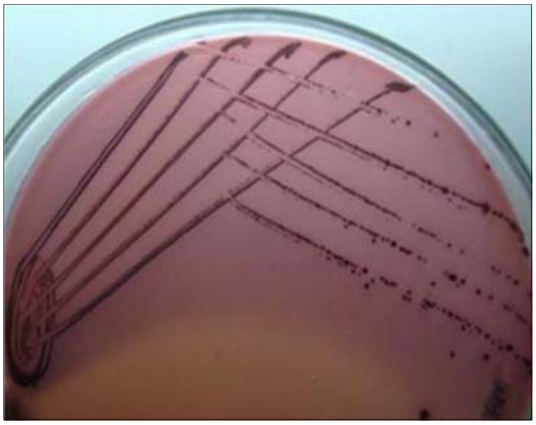


Fig 4: Pink colonies on Mac conkey agar

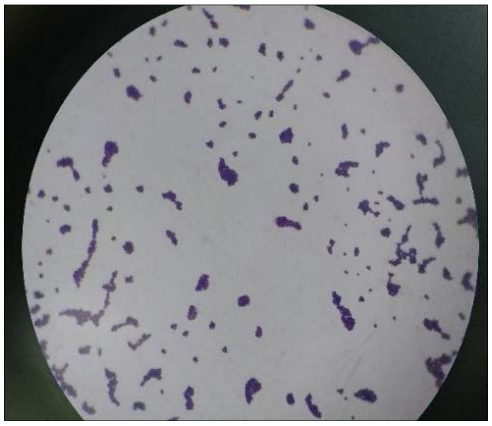


Fig 3: Microscopic view of Staphaureus

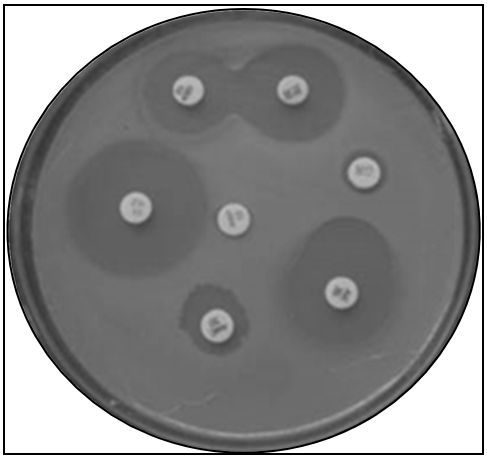
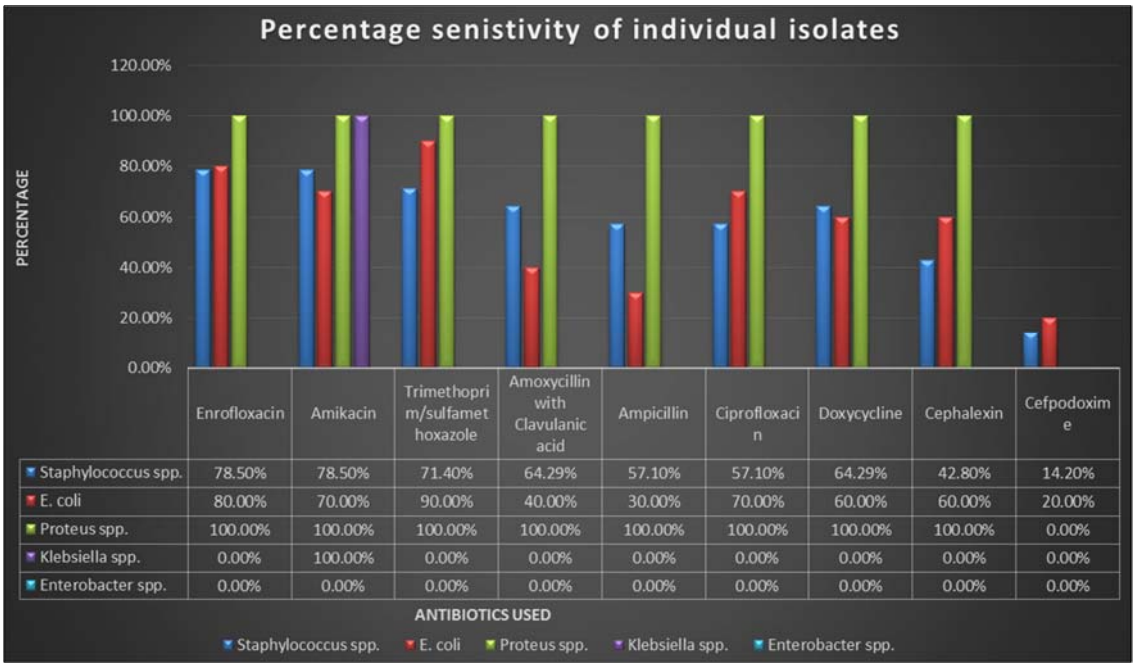


Fig 5: ABST of urine culture sensitivity for Enrofloxacin



	Enrofloxacin	Amikacin	Trimethoprim/sulfamethoxazole	Amoxycillin with Clavulanic acid	Ampicillin	Ciprofloxacin	Doxycycline	Cephalexin	Cefpodoxime
Staphylococcus spp.	78.50%	78.50%	71.40%	64.29%	57.10%	57.10%	64.29%	42.80%	14.20%
E. coli	80.00%	70.00%	90.00%	40.00%	30.00%	70.00%	60.00%	60.00%	20.00%
Proteus spp.	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	100.00%	0.00%
Klebsiella spp.	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Enterobacter spp.	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Fig 6: Percentage sensitivity of individual isolates.

Multidrug-resistant pathogens, *Enterobacter* Spp. *Klebsiella* Spp. and *E. coli* are becoming increasingly problematic [10, 12]. The prevalence of positive urine culture results in dogs with chronic kidney disease was lower than that reported for dogs with some systemic diseases that may predispose to infection [3]. High antibiotic concentrations achieved in renal tubules and the urine after routine therapy with the modest doses of antibiotics is often sufficient to cure lower urinary tract

infections, even those that are caused by organisms identified on a susceptibility tests as "intermediate" in sensitivity. Multidrug-resistant pathogens may be harder to treat because of limited drug choices. There are public health concerns with regard to the potential for zoonotic transmission of resistant pathogens. Additional concerns result from the increasing pressure to use antimicrobials in animals that are critically important in human medicine.

Conclusion

The present article concluded that selection of antibiotics through Antimicrobial susceptibility which helped in treating of cystitis cases.

Ethical Matters

In the present study, the urine samples were collected from the clinical cases presented to the veterinary hospital, Bangalore, as a part of Post graduate Programme indicating no ethical issue related in this study.

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Conflict of interest

All the authors declares that they have no conflict of interest.

References

1. Dowling PM. Antimicrobial therapy of urinary tract infections. Can. Vet. J. 1999; 37:43 441.
2. Elena B, Mariateresa C, Tiziana C, Mauro B, Natascia B, Elisa M. A pilot study to evaluate alternative approaches for treatment of urinary tract infections in dogs. Asian J Biomed Pharmaceut Sci. 2019; 9(67):7-9.
3. Jonathan DF, Harathi K, Stephen C. Characterization of subclinical bacteriuria, bacterial cystitis, and pyelonephritis in dogs with chronic kidney disease. J American Vet. Med. Assoc. 2018; 252:1257-1262.
4. Kurien BT, Nancy E, Everds E, Scofield RH. Experimental animal urine collection: a review. Laboratory Animals, 2004, 38.
5. Penna B, Varges R, Mortins R, Lilenbaum W. *In vitro* antimicrobial resistance of *Staphylococci* isolated from canine urinary tract infection. Can. Vet. J. 2010; 51:738-742.
6. Rajan R, Chand N, Dhaliwal. Diagnosis and therapeutic management of urinary tract infections in dogs. Indian Vet. J. 2007; 84:507-508.
7. Srikanth K, Satish Kumar K, Nagaraj P. Cultural studies of urine from cystitis dogs. The Pharma Innovation Journal. 2017; 6(8):247-250.
8. Wagenlehner, Weidner W. Anti-Infective Treatment of Bacterial Urinary Tract Infections. Curr. Med. Chem. 2008; 15:1412-1427.
9. Wayne P. A. Performance standards for antimicrobial susceptibility testing: twenty first information supplement. Clinical and Laboratory Standards Institute Document. Edn, U.S.A. 2016; 6:33.
10. 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, 263768.
11. Westropp JL, Sykes JE, Irom S, Daniels JB, Smith A, Keil D *et al.* Evaluation of the Efficacy and Safety of High Dose Short Duration Enrofloxacin Treatment Regimen for Uncomplicated Urinary Tract Infections in Dogs. J Vet Intern Med. 2012; 26:506-512.
12. Wong C, Epstein SE, Westropp JL. Antimicrobial Susceptibility Patterns in Urinary Tract Infections in Dogs. J Vet. Intern. Med. 2015; 29:1045-1052.