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Aetiology and antimicrobial profile of canine urinary tract infections#

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Abstract

Bacterial isolation and identification were carried out from the urine samples collected from 50 dogs with clinical signs of urinary tract infection (UTI). After conducting biochemical tests and observing growth on selective media, the isolates were identified as Klebsiella (36%), E. coli (28%), Proteus (28%) and Staphylococcus (8%) after conducting biochemical tests and observation of colony growth on selective media. The identities of those isolates were confirmed using genus-specific PCR. Antibiogram of the isolates revealed that the most effective antibiotic was cotrimoxazole against E. coli and Klebsiella, ceftriaxone against Proteus and amikacin against Staphylococcus. The most widely used antibiotic enrofloxacin against UTI was found to be the least effective against all the four types of isolates. Fifty per cent of the isolates were found to be multidrug resistant within which, 44 per cent were Klebsiella, 28 per cent E. coli, 24 per cent Proteus and four per cent Staphylococcus. The high resistance levels observed in the four bacteria in this study underscore the critical need for prudent antibiotic use across all medical practices.

Keywords: *Urinary tract infection, antibiogram, multidrug resistance, PCR*

Canine urinary tract infection is one of the more frequently encountered conditions in clinical veterinary practice which may progress to kidney diseases and finally result in death of the animal. Animals having clinical signs like pollakiuria, unproductive straining at the end of urination, urgency to urinate, haematuria, pyrexia, incontinence between urination and offensive odour in urine could be considered as having UTI (Senior, 2007). Burton *et al*. (2017) found that the urinary

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bladder of a healthy dog was not a sterile environment but had its own unique, diverse and rich bacterial microbiota. Bacteria are the most common infectious causes of canine UTI, which can occur either due to aggressive multiplication of the resident bacteria or invasion from outside through various routes. Changes in antimicrobial susceptibility, combined with emergence of antimicrobial resistance among the plethora of the bacterial population within the urinary tract have become a serious problem in both veterinary and human medicine. Identification of the aetiology and the most effective antibiotic are thus of paramount importance in the treatment of UTI. Wong *et al*. (2015) reported that when UTI was suspected, routine urine culture and susceptibility test were recommended, especially in dogs that had recurrent UTI and had undergone previous antimicrobial therapy. Hence, this research work was undertaken to detect the causative species of bacteria for canine UTI, and their susceptibility to the antibiotics routinely used in the treatment of UTI. Emergence of antimicrobial resistance among them was also investigated.

Materials and methods

Study samples

The present study was carried out in the Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy, during the period from December 2022 to August2023. Fifty dogs with dysuria, pollakiuria, stranguria, haematuria or malodorous urine presented to the Teaching Veterinary Clinical Complex (TVCC), Mannuthy and University Veterinary Hospital (UVH), Kokkalai formed the subjects of the study.

Fifteen millilitres of urine sample were collected from each dog by bladder catheterization through the urethral route using infant feeding tube into 10- and 5-mL disposable syringes under aseptic precautions. Ten millilitres of urine were subjected to routine urine analysis. Five millilitres were transferred to a sterile urine collection vial and subjected to isolation of bacteria using standard microbial culture techniques.

Isolation and identification of bacteria

Isolation of bacteria from urine was carried out by direct streaking on to brain heart infusion agar (BHIA; M211) followed by incubation of the plates at 37 °C for 24 h. Plates were examined after 12 to 24 h for colony growth. Isolates for immediate use were streaked on to BHIA slants, plugged tightly with cotton, incubated at 37°C for 18-24 h. The tubes were examined after 24 h and followed by wrapping with parafilm and refrigerated at 4°C. They were kept viable by sub culturing every two weeks. The isolates were identified based on morphology, cultural characteristics and biochemical tests as per the protocol described by Barrow and Feltham (1993) and Quinn *et al*. (2013).

Genotypic characterisation

All the clinical isolates obtained and identified by biochemical test in the study were subjected to genotypic characterisation and molecular confirmation by amplification of

Table 1. Primers used and their references for identification of four bacterial organisms from the urine samples with UTI

Sl. No.	Organism	Name of gene	Primer	Amplicon size	Reference
1	*E. coli*	16S rRNA	F: GAC CTC GGT TTA GTT CAC AGA	585 bp	Haque *et al*. (2014)
			R: CAC ACG CTG ACG CTG ACC A		
2	*Klebsiella*	gyr A	F: CGC GTA CTA TAC GCC ATG AAC GTA	441 bp	Brisse and Verhoef (2001)
			R: ACC GTT GAT CAC TTC GGT CAG G		
3	*Staphylococcus*	16S rRNA	F: AACTCTGTTATTAGGGAAGAACA	756 bp	Ciftici *et al*. (2009)
			R: CCACCTTCCTCCGGTTTGTCACC		
4	*Proteus*	ureR	F: GGTGAGATTTGTATTAATGG	225 bp	Zhang *et al*. (2013)
			R: ATAATCTGGAAGATGACGAG		

16SrRNA, gyrA, 16SrRNA and ureR genes of the bacteria *E. coli*, Klebsiella, Staphylococcus and Proteus, respectively. The DNA extraction from 50 bacterial isolates was done by the snap-chill method (Junior *et al*., 2016) and the extracted DNA were subjected to PCR. The primers used for PCR amplification and their references are given in Table 1.

Disc diffusion assay for antimicrobial profiling

Phenotypic antimicrobial susceptibility profiling was conducted for all 50 bacterial isolates which included 18 *Klebsiella* spp. isolates, 14 each of *E.coli* and *Proteus* spp. isolates and four *Staphylococcus* spp. This profiling was accomplished through in vitro disc diffusion assays, where the diameter of the zone of inhibition was measured. The recorded zone diameter values included the six-millimeter disc diameter too. Antibiotic discs containing predetermined concentrations of five antimicrobial agents *viz*., amoxicillin -clavulanate, ceftriaxone, cotrimoxazole, enrofloxacin and amikacin were employed for this assessment.

Results and discussion

Four bacteria species were identified by culture method which was confirmed by PCR from the urine samples of dogs with UTI in this study. They were Klebsiella, *E. coli*, Proteus and Staphylococcus. The result of biochemical tests for identification of bacterial isolates from 50 dogs with UTI is represented in Table 2. Out of 50 samples streaked on BHI agar, colonies of 14 isolates appeared as swarming growth with characteristic fishy odour and were identified as *Proteus spp*. (Fig. 1). All the Gram-negative isolates were streaked on MacConkey agar of which colonies of 18 isolates were pink and

Table 2. Results of biochemical tests for identification of bacterial isolates from 50 dogs with UTI

Sl. No.	Particulars of identification test	Observations	Number of positive samples	Presumptive bacterial isolate
1	Catalase test	Positive	50 (100%)	*E. coli* *Klebsiella* spp. *Proteus* spp. *Staphylococcus* spp.
		Negative	0	_
2	Oxidase test	Positive	0	_
		Negative	50 (100%)	*E. coli* *Klebsiella* spp. *Proteus* spp. *Staphylococcus* spp.
3	Indole test	Positive	14 (28%)	*E. coli*
		Negative	36 (72%)	*Klebsiella* spp. *Proteus* spp. *Staphylococcus* spp.
4	MR test	Positive	18 (36%)	*E. coli* *Staphylococcus* spp.
		Negative	32 (64%)	*Klebsiella* spp. *Proteus* spp.
5	VP test	Positive	22 (44%)	*Klebsiella* spp. *Staphylococcus* spp.
		Negative	28 (56%)	*E. coli* *Proteus* spp.
6	Citrate utilisation test	Positive	36 (72%)	*Klebsiella* spp. *Proteus* spp. *Staphylococcus* spp.
		Negative	14 (28%)	*E. coli*

Fig. 1. Growth of Proteus on BHI agar

Fig. 2. Mucoid colonies of Klebsiella and Colourless colonies of Proteus on Macconkey agar

Fig. 3. Metallic sheen of *E. coli* on EMB agar

Fig. 4. Yellow colonies of Staphylococcus on MSA

mucoid which were presumed as *Klebsiella spp*. (Fig. 2). The other 14 bright pink colonies observed on MacConkey agar were streaked onto EMB agar (Eosin-Methylene Blue Agar), where they exhibited a metallic sheen and were identified as *E. coli*. (Fig. 3). The Proteus suspected isolates appeared as colourless in MacConkey agar (Fig. 2). Gram positive cocci isolates streaked on MSA (Mannitol Salt Agar) appeared as yellow growths and were presumed as *Staphylococcus aureus* (Fig. 4).

According to Scarborough *et al*. (2020), the analysis of 6196 urinary isolates from dogs and cats in Australia revealed *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus pseudintermedius* and *Proteus* spp. as the most prevalent organisms. According to Chew and Westropp (2012), *E. coli* was the primary cause of uncomplicated UTIs in healthy dogs, comprising 45 percent of all pathogens, while others such as *Staphylococcus* spp., *Proteus* spp., *Klebsiella* spp. and *Enterococcus* spp. collectively accounted for an additional 40 percent. Variations in aetiology based on geography and locally prevalent management methods can be expected for nonspecific infections.

Genotypic identification of the isolates

Among the tested isolates, 14 yielded amplicons of 585 bp in size of the 16S rRNA gene for *E. coli* (Fig. 5), 18 isolates produced 441 bp amplicons of the *gyrA* gene for Klebsiella (Fig. 6), 14 isolates generated 225 bp amplicons

Table 3. Results of the antimicrobial susceptibility test of the 50 isolates from dogs with UTI

Sl. No.	Antibiotics	*E. coli*		*Klebsiella* spp.		*Proteus* spp.		*Staphylococcus* spp.	
		S – sensitive, R - resistant							
		S	R	S	R	S	R	S	R
1	Amoxicillin– clavulanate	5	9	7	11	9	5	3	1
2	Amikacin	8	6	7	11	9	5	4	0
3	Ceftriaxone	9	5	7	11	11	3	3	1
4	Cotrimoxazole	11	3	13	5	8	6	2	2
5	Enrofloxacin	2	12	1	17	1	13	2	2

Table 4. Results of the antimicrobial resistance analysis of 50 isolates from dogs with UTI

Multi drug resistant isolates	No. of MDR isolates	Per cent of isolates
E. coli	7	28
Klebsiella	11	44
Proteus	6	24
Staphylococcus	1	4

of the *ureR* gene for Proteus (Fig. 7) and four isolates resulted in 756 bp amplicons of the 16S rRNA gene for Staphylococcus (Fig. 8). The result of the biochemical characterisation of the isolates was in complete agreement with the PCR method confirmation for all 50 organisms of this study, attesting to the validity of biochemical methods of identification.

Antimicrobial susceptibility testing

Results of the antimicrobial susceptibility test of the 50 isolates are given in Table 3. Cotrimoxazole was found to be the most effective antibiotic against both *Klebsiella* and *E. coli*, whereas it was ceftriaxone for Proteus and amikacin for Staphylococcus. The increased susceptibility of cotrimoxazole can be explained by the relatively little use of this antibiotic in general veterinary practice, and especially in urinary tract and renal infections because of chances of crystalluria development. This is contrary to the finding of Chang *et al*. (2015) who reported that enrofloxacin was more effective than cotrimoxazole in case of *E. coli* isolates from the urine of dogs with UTI. All the organisms, Klebsiella, *E. coli*, Proteus, and Staphylococci exhibited maximum resistance against enrofloxacin. Results are given in Table 4. Enrofloxacin is widely regarded as the drug for treating UTIs in dogs. The extensive veterinary use of this antibiotic, especially for the treatment of UTIs during the last decade,

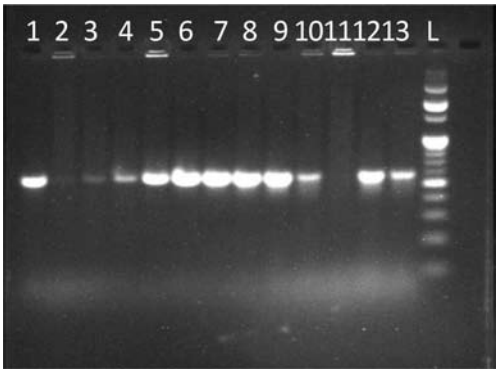

Fig. 5. Agarose gel electrophoresis of 16SrRNA specific PCR of *E. coli*

Lane L : DNA marker 100 bp
Lanes 1,2, 3, 4, 5,6,7 8, 9,10,13 : Positive samples (585 bp)
Lane 11 : Negative control
Lane 12 : Positive control

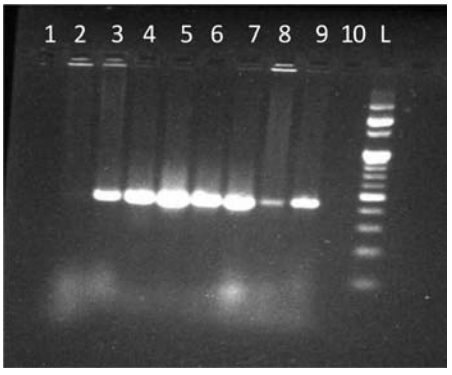

Fig. 6. Agarose gel electrophoresis of *gyrA* specific PCR of Klebsiella

Lane L : DNA marker 100 bp
Lanes 4, 5, 6, 7, 8, 9 : Positive samples **(441 bp)**
Lane 2 : Negative control
Lane 3 : Positive control

Fig.7. Agarose gel electrophoresis of *ureR* specific PCR of Proteus

Lane L : DNA marker 100 bp
Lanes 2, 3 : Positive samples **(225 bp)**
Lane 4 : Positive control
Lane 5 : Negative control

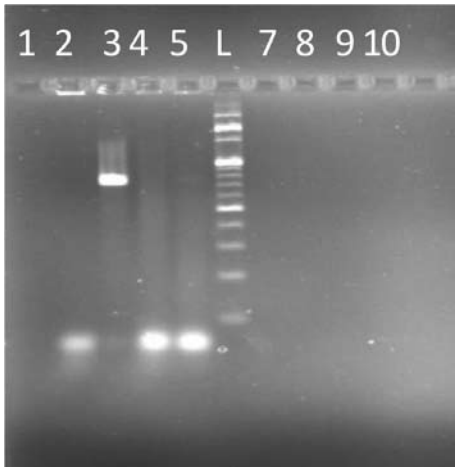

Fig. 8. Agarose gel electrophoresis of 16*S*rRNA specific PCR of Staphylococci

Lane L : DNA marker 100 bp
Lane 2 : Negative control
Lane 3 : Positive sample **(756 bp)**

might have contributed to the emergence of resistance to this drug. According to Punia *et al*. (2018), *Klebsiella* spp. identified from urine of dogs with UTI showed the highest susceptibility to ceftriaxone, (100 percent) followed by amoxicillin-clavulanic acid (66.67 percent) and lowest sensitivity to amikacin and enrofloxacin. As per the findings of Kaustubh *et al*. (2021), amikacin was the most effective antibiotic, while amoxicillin-clavulanic acid was found to be the least effective against Klebsiella in urine samples. Unlike the two-Gram negative bacteria obtained from urine culture in this study, the Proteus isolates were most susceptible to ceftriaxone, and enrofloxacin was the least effective. This is contrary to the finding of Decome *et al*. (2020) who reported that Proteus isolated from urine of dogs with UTI had 98 per cent susceptibility to enrofloxacin. They have found cotrimoxazole to be the most effective antibiotic (100 percent). Even though their study identified five multi-drug resistant isolates, the overall sensitivity for all tested antibiotics remained very high. All of the four isolates of Staphylococcus in this study were susceptible to amikacin. Due to the limited number of isolates, a comprehensive description of the resistance patterns for this organism could not be provided. Yogeshpriya *et al*. (2013) reported that Staphylococcus isolated from urine of dogs with UTI had only 25 per cent susceptibility to amikacin which was contrary to our findings. The wide variation in the antimicrobial sensitivity expressed by the same pathogens in different locations lays emphasis on the observations by Wong *et al*. (2015) who suggested routine culture and susceptibility test before treating UTI, especially in dogs that had recurrent UTI and had undergone previous antimicrobial therapy.

Multi-drug resistance of the isolates

Out of the 50 selected isolates, 25 (50 per cent) were found to be multi drug resistant (MDR) organisms. This included seven out of the 14 isolates of *E. coli*, 11 out of the 18 isolates of Klebsiella, six out of 14 isolates of Proteus and one out of the four isolates of the Staphylococcus. The result of the antimicrobial resistance analysis is given in Table 4.

According to Yudhanto *et al*. (2022) multidrug resistance, defined as resistance to at least one agent in a minimum of three antimicrobial classes, was identified in 85 out of 366 (23.22 per cent) *E. coli* isolates, 18 per cent of Proteus isolates and 20 per cent of Klebsiella isolates. The rise of antimicrobial resistance poses a significant challenge for clinicians worldwide, and the elevated levels of resistance exhibited by the four bacteria in this

study underscore the crucial need for prudent antibiotic use in all types of medical practices.

Conclusion

Klebsiella, *E. coli*, Proteus and Staphylococcus were identified as the predominant causes of bacterial UTI in dogs. The most effective antibiotic against those organisms were cotrimoxazole (Klebsiella and *E. coli*), ceftriaxone (Proteus) and amikacin (Staphylococcus). The least effective antibiotic against the organisms identified in this study was enrofloxacin. Fifty per cent of the selected isolates were found to be multi-drug resistant, stressing on the importance of the prudent use of antibiotics for managing the emergence of antibiotic resistance.

Conflict of interest

The authors declare that they have no conflict of interest.

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