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Isolation and Identification of Multidrug-Resistant Isolates of Uropathogenic *Klebsiella* spp in Dogs

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ABSTRACT

The most widespread ailments in dogs are urinary tract infections (UTIs) caused by bacterial species. It is necessary to recognize the prevailing bacterial pathogens and their susceptibility to antimicrobial agents to effectively treat UTIs. The present study aimed to classify the bacterial organism that causes UTIs in dogs and their patterns of antimicrobial resistance. A total of 141 urine samples were collected from diseased dogs in Veterinary Clinical Complex LUVAS in Hisar, India. Culture, biochemical and sensitivity testing were performed for each of the urine samples based on standard method. Of the total 141 urine samples from dogs, 21 (14.9%) isolates were identified as Klebsiella spp. The isolates were found to be highly resistant to ampicillin (100%), penicillin G (100%), oxytetracycline (100%), enrofloxacin (85.7%), chloramphenicol (80.6%), ceftriaxone (76.2%) and cloxacillin (71.4%), while susceptibility was observed against gentamicin (100%), amikacin (100%) and neomycin (90.5%). In the current study, 19 out of 21 identified isolates were found to be multidrug-resistant. This study indicates that dogs in the study area are found to harbor highly resistant Klebsiella spp. Therefore, when deciding on the antibiotic regimen for UTIs cases, Vets should consider resistance profile of chosen antibacterial agents before usage in order to discourage dissemination of resistant organisms in the study area.

Keywords: *Klebsiella* spp, Urinary tract infection, dog, Multidrug-resistant

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INTRODUCTION

Urinary tract infections (UTIs) are frequently observed in pets and humans and most often require antimicrobial therapy [1, 2]. UTIs in pets and humans might also be caused by *Klebsiella* spp, although mostly less common than *Escherichia coli* [2, 3]. Hence, infection with one single bacterial species has become more common than mixed infections [4]. *Klebsiella* spp is a leading pathogen of nosocomial infections, as well as UTIs which are often responsible for resistance [5].

Bacteriological culture, especially in combination with the antibiotic susceptibility test, has become an essential part of diagnosis of UTIs and the best tool for initiating therapeutic decisions for individual dogs [6, 7]. Periodic testing of susceptibility trends of organisms isolated from UTIs is the first-line of effective therapy of choice, and it can also be used to track the presence of resistant organisms. Rising antibiotic resistance in canines is of concern as it significantly affects dog treatment, which leads to medication failure, increase in high morbidity, mortality, and UTIs management cost. Moreover, it is indeed a public health issue for zoonotic diseases [6, 9]. The milestones of UTIs therapy are antimicrobials yet many patients with recurrent UTIs are poorly treated with multiple antimicrobial agents, thus potentiating the resistance of the microorganisms. If clinical symptoms are present or even not, lower urinary tract disorder, urolithiasis, prostatitis, pyelonephritis, or septicemia following the renal failure are the consequences of untreated UTIs [10].

Thorough pre-treatment diagnosis and antimicrobial sensitivity of bacterial pathogens can help to select relevant and cost-effective antibiotics to treat the infected animal in a timely and adequate manner. The aim of the present study, was to isolate and identify multidrug-resistant isolates of uropathogenic *Klebsiella* spp in dogs within the study area.

MATERIALS AND METHODS

Study Area

The current study was conducted in Hisar, Haryana State of India. The studied area is the administrative headquarters of Hisar district of Hisar division in the state of Haryana in northwestern India [Figure 1]. Hisar district lies between 28 53 45N and 29 34 50 N latitude and between 75 1944 E and 76 18 15 E longitude. It has a geographical area of 3,983.00 square kilometers comprising 3,835.53 square kilometers of rural area and 147.47 square kilometers of urban area [19].

Figure 1: *Map of Haryana State indicating Hisar (Red Arrow); Source: Google map*

Study design

The present study employed a cross sectional study design to assess dogs with UTIs in clinics of Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS) Hisar Haryana, India.

Sample collection

Urine samples were collected by cystocentesis from 141 dogs who were diagnosed with UTIs in clinics of Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS) Hisar Haryana, India.

Bacterial Isolation and Identification

The urine samples were inoculated and streaked onto MacConkey agar (MA) plates (HiMedia, Mumbai, India). The plates were incubated aerobically at 37℃ for 24-48 hours till adequate growth was observed. Suspected colonies were picked for further analysis of the pure culture of *Klebsiella* spp using standard microbiological techniques of colony identification which involved gram staining and biochemical tests (Indole, Methyl Red, Voges Proskauer, Citrates tests) using commercially available KB010 HiE.Coli™ Identification Kit (HiMedia Mumbai, India) following the manufacturer's instructions.

Antimicrobial Susceptibility Testing

The Antimicrobial susceptibility testing was determined according to the method of Bauer-Kirby [11], by using a commercially prepared disc (HiMedia Mumbai, India) with the known concentration of antibiotics. A small amount of test culture was transferred into a tube of brain heart infusion (BHI) broth using a platinum loop and incubated for 2-5 hours at 37°C to obtain turbidity. The broth culture was then uniformly distributed over the surface of Mueller-Hinton agar plates with the aid of a sterile cotton swab. The antimicrobial discs were gently pressed onto the agar with a sterile forceps to ensure uniform close contact with the medium. This were then incubated for 24 hours at 37°C. A zone of inhibition was measured and interpreted as sensitive (S), intermediate (I), and resistant (R) according to the interpretation chart provided by the manufacturer.

The antibiotics tested were ampicillin (AMP) 10mcg, Enrofloxacin (EN) 10mcg, amikacin (AK) 30mcg, ceftriaxone (CRT) 10cmg, gentamycin (GEN), 30mcg, Neomycin (N) 30mcg, Cloxacillin (COX) 1mcg, Chloramphenicol (C) 25mcg Penicillin G (P) 10 units and Oxytetracycline (O) 30mcg (HiMedia, Mumbai, India).

Data analysis

The data was analyzed using descriptive statistics with JMP Version 11(SAS, Inc. NC, USA)

RESULTS

Bacterial Isolates Confirmation

Bacterial isolates were identified using morphological and biochemical tests, which confirmed the presence of gram-negative bacteria. Out of a total of 141 samples of urine collected from dogs, 21 (14.9%) of the samples yielded positive growth of the species *Klebsiella*.

Biochemical Test

Biochemical (IMViC) test for *Klebsiella* spp indicated Indole negative, Methyl-Red negative, Voges-Proskauer test positive and Citrate test positive as presented in table 1.

Antibiotic Susceptibility Test

The isolated *Klebsiella* were more resistant to ampicillin (100%), penicillin (100%), oxytetracycline (100%), enrofloxacin (85.7%), chloramphenicol (80.6%), ceftriaxone (76.2%), and cloxacillin (71.4%), while susceptibility was observed against gentamicin (100%), amikacin (100%) and neomycin (90.5%) as illustrated in Table 2.

In the current study, 19 out of 21 identified isolates were found to be multidrug-resistant as indicated in Table 3.

Table 1: Biochemical (IMViC) test for *Klebsiella* spp

Characteristics	Properties
Indole	Negative
Methyl-Red	Negative
Voges -Proskauer test	Positive
Citrate test	Positive

Table 2: Antimicrobial susceptibility of K*lebsiella* spp isolates from the urine of dogs

Antibiotics	Number of Susceptible Klebsiella spp (%)	Number of Intermediate Klebsiella spp (%)	Number of Resistant *Klebsiella* spp (%)
Tetracycline			
Oxytetracycline	0 (0.0)	0 (0.0)	21 (100)
Penicillins			
Ampicillin	0 (0.0)	0 (0.0)	21 (100)
Cloxacillin	1(4.8)	5 (23.8)	15 (71.4)
Penicillin G	0 (0.0)	0 (0.0)	21 (100)
Fluoroquinolones			
Enrofloxacin	0 (0.0)	3 (14.3)	18 (85.7)
Aminoglycosides			
Gentamicin	21 (100)	0 (0.0)	0 (0.0)
Amikacin	21 (100)	0 (0.0)	0 (0.0)
Neomycin	19 (90.5)	0 (0.0)	2 (9.5)
Amphenicols			
Chloramphenicol	2 (9.5)	2 (9.5)	17 (80.6)
Cephalosporin			
Ceftriaxone	4(19.0)	1(4.8)	16 (76.2)

Table 3: Distribution of Multidrug-resistant among the *Klebsiella* isolates

Number of Isolates	Resistance Pattern
2	O, AMP, C, CRT
3	O, AMP, P, EN, N, CRT
5	EN, P, AMP, N, COX
9	EN, O, AMP, C, P, COX, N

AMP, Ampicillin; C, Chloramphenicol; EN, Enrofloxacin; O, Oxytetracycline; CRT, Ceftriaxone; N, Neomycin; COX, Cloxacillin; P, Penicillin G

DISCUSSION

In the present study, the most common infectious disease in dogs was urinary tract infections (UTIs) cause by *Klebsiella* organisms. Although, it is more often seen in older dogs in the present study. These findings was in accord with the study carried out by [2, 3].

In the present study, the prevalence of *Klebsiella* spp isolated and identified as the organism responsible for the UTIs infections was high. This was probably due to the higher number of urine samples collected from the study area. However, Punia *et al*. [12] reported a lower prevalence of *Klebsiella* organism from 35 dogs in urine samples. This could be due to the lower number of samples used in her study. The current finding concurs with the study of Liu *et al*. [13] who detected higher number of *Klebsiella* spp in 285 urine samples from dogs with symptoms of UTIs. Additionally, Cetin *et al*. [14] reported that *Klebsiella* spp were cultured from 51 urine samples of dogs with UTIs. Whereas, Robert *et al*. [15] also isolated *Klebsiella* spp from urine specimens of dogs suffering from UTIs. This could be due to the frequency of contacts amongst dogs in the respective study areas.

In the current study, and other research works showed that the vast majority of canine UTIs are caused by a single bacterial species. However, different microorganisms could be involved in the etiology of UTIs in dogs. Punia *et al*. [12] reported that infection with a single organism was found in 2 dogs with bacteriuria and with more than one organism in 5 dogs. In another study, Cetin *et al*. [14] studied urinary tract infections in 100 dogs and mixed infections were detected in 9 dogs. Furthermore, Ling *et al*. [4] reported that infection with a single microbial species was responsible for 72% of UTIs in both sexes. Diagnosis and treatment of bacterial diseases are an integral part of the practice of small animal medicine, where in-vitro antibiotic sensitivity testing of bacterial strains is a necessary tool for the treatment of bacterial infections.

The antibiotic resistance pattern of *Klebsiella* spp observed in the current study showed high resistance to ampicillin, penicillin, oxytetracycline, enrofloxacin, chloramphenicol, ceftriaxone, and cloxacillin which is in accordance with the finding of Punia *et al*. [12] and Windahl *et al*. [16]. The isolate's resistance to penicillin can be due to the development of an antibiotic-destroying β-lactamase enzyme. On the other hand, however, overuse and misuse of this antibiotic to treat various disease conditions in dogs can induce a mutation and transformation of microorganisms. The findings of the present study, indicate an increase in the amount of induced resistance and the potential spread of multidrug-resistant bacteria strains in the study area.

In the current study, *Klebsiella* spp demonstrated strong susceptibility to gentamicin, amikacin, and neomycin. This finding correlates with those other previous studies [12, 14, 17]. Similarly, Kogita *et al*. [18] reported high susceptibility of *Klebsiella* spp to these antibiotics which is consistent with the findings of the present study. Furthermore, the results of the present study vary with that of Liu *et al*. [13] who reported high resistance to aminoglycoside. This is due to the nephrotoxic nature, and the susceptibility of *Klebsiella* organisms to aminoglycoside and this could be attributed to the lower use of these antibiotics.

In the present study, 19 out of 21 *Klebsiella* spp were seen to be multi-drug resistant. This was buttressed by the work of Punia *et al*. [12] who reported that all the 22 isolates were multidrug-resistant. These variable susceptibilities of the multiple antimicrobial agents against *Klebsiella* isolates showed that the antimicrobial agent should indeed be chosen on the basis of bacterial culture and antimicrobial susceptibility test results and the clinical reaction to the antibiotic.

Conclusion

The current study revealed that *Klebsiella* spp

isolated from the urine of dogs with UTIs were highly resistant to tetracyclines, penicillins, fluoroquinolones, cephalosporins, and macrolides. However, they were highly susceptible to aminoglycosides. Therefore, when deciding on the antibiotic regimen for UTIs cases, vets should consider the choice of the most potent antibacterial agent to control the phenomenon of resistance.

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Competing interests
There are no potential conflicts of interest declared by the authors.

REFERENCES

1. Weese, J. S., Blondeau, J. M., Boothe, D., Breitschwerdt, E. B., Guardabassi, L., Hillier, A., Lloyd, D.H., Papich, M.G., Rankin, S.C., Turnidge, J.D. and Sykes, J. E. (2011). 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. *Veterinary Medicine. International* 263-768.

2. Flores-Mireles, A. L., Walker, J. N. Caparon, M., and Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature reviews microbiology,* 13(5), 269-284.

3. Marques, C., Belas, A., Franco, A., Aboim, C., Gama, L. T., and Pomba, C. (2018). Increase in antimicrobial resistance and emergence of major international high-risk clonal lineages in dogs and cats with urinary tract infection: 16 year retrospective study. *Journal of Antimicrobial Chemotherapy.* 73(2), 377-384.

4. Ling, G. V., Norris, C. R., Franti, C. E., Eisele, P. H., Johnson, D. L., Ruby, A. L. and Jang, S. S. (2001). Interrelations of organism prevalence, specimen collection method, and host age, sex, and breed among 8,354 canine urinary tract infections (1969–1995). *Journal of Veterinary Internal Medicine* 15(4), 341-347.

5. ECDC. (2017). Antimicrobial Resistance Surveillance in Europe 2015. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net).

6. Bartges, J. W. (2004). Diagnosis of urinary tract infections. Veterinary Clinics: *Small Animal Practice* 34(4), 923-933.

7. Ball, K. R., Rubin, J. E., Chirino-Trejo, M. and Dowling, P. M. (2008). Antimicrobial resistance and prevalence of canine uropathogens at the Western College of Veterinary Medicine Veterinary Teaching Hospital, 2002–2007. *Canadian veterinary journal* 49 (10), 985-990,

8. Guardabassi, L., Schwarz, S. and Lloyd, D. H. (2004). Pet animals as reservoirs of antimicrobial-resistant bacteria. *Journal of Antimicrobial Chemotherapy*. 54(2), 321-332.

9. Ewers, C., Grobbel, M., Bethe, A., Wieler, L. H. and Guenther, S. (2011). Extended-spectrum beta-lactamases-producing gram-negative bacteria in companion animals: action is clearly warranted. Berliner und Münchener Tierärztliche Wochenschrift, 124(3/4), 4-101.

10. Warren, J. W., Abrutyn, E., Hebel, J. R., Johnson, J. R., Schaeffer, A. J. and Stamm, W. E. (1999). Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. *Clinical Infectious Diseases* 29(4), 745-759.

11. Bauer, A.W., Kirby ,W.M.M., Sherris, J.C. and Turck, M. (1966). Antibiotic susceptibility testing by tandardized single disk method. *American journal of clinical pathology.* 45,493-496.

12. Punia, M., Kumar, A., Charaya, G., and Kumar, T. (2018). Pathogens isolated from

clinical cases of urinary tract infection in dogs and their antibiogram, *Veterinary World*., 11 (8): 1037-1042.

13. Liu, Y., Yang, Y., Chen, Y., and Xia, Z. (2017). Antimicrobial resistance profiles and genotypes of extended-spectrum β-lactamase- and AmpC β-lactamase-producing Klebsiella pneumoniae isolated from dogs in Beijing, China. *Journal Global Antimicrobial Resistance*.., 10, 219-222.

14. Çetin, C., Şentürk, S., Kocabiyik, A. L., Temizel, M. and Özel, E. (2003). Bacteriological examination of urine samples from dogs with symptoms of urinary tract infection. *Turkish Journal of Veterinary Animal Sciences*. 27(5), 1225-1229.

15. Roberts, M., White, J., and Lam, A. (2019). Prevalence of bacteria and changes in trends in antimicrobial resistance of *Escherichia coli* isolated from positive canine urinary samples from an Australian referral hospital over a 5-year period (2013–2017). *Veterinary Record Open*., 6(1), e000345.

16. Windahl, U., Holst, B. S., Nyman, A., Grönlund, U. and Bengtsson, B. (2014). Characterisation of bacterial growth and antimicrobial susceptibility patterns in canine urinary tract infections. *BMC Veterinary Research* 10(1), 1-10.

17. Yu, Z., Wang, Y., Chen, Y., Huang, M., Wang, Y., Shen, Z., Xia, Z., and Li, G. (2020). Antimicrobial resistance of bacterial pathogens isolated from canine urinary tract infections. *Veterinary Microbiology*. 241, 108540.

18. Kogika, M.M., Assunta Batistini Fortunato, V., Masae Mamizuka, E., Kuribayashi Hagiwara, M., de Fatima Borges Pavan, M. and Nonogaki Actis Grosso, S. (1995). Etiologic study of urinary tract infection in dogs. *Brazilian Journal of Veterinary Research and Animal Science*. 31-36.

19. Meena, R.S. (2016). District Census Handbook, Mansa, Part-XII A & B, Series-4, Punjab.