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Dr. Nidhi Patel

Veterinary Officer, Department
of Animal Husbandry,
Mahisagar, Gujarat, India

Dr. SK Raval

Ex Professor, College of
Veterinary Science and Animal
Husbandry, Kamdhenu
University, Anand, Gujarat,
India

Dr. Nilesh Gohil

Veterinary Officer, Department
of Animal Husbandry, Narmada,
Gujarat, Gujarat, India

Dr. Neha Rao

Veterinary Clinical Complex,
College of Veterinary Science and
Animal Husbandry, Kamdhenu
University, Anand, Gujarat,
India

Dr. BB Bhandari

Department of Veterinary
Microbiology, College of
Veterinary Science and Animal
Husbandry, Kamdhenu
University, Himmatnagar,
Gujarat, India

Corresponding Author:

Dr. Nidhi Patel

Veterinary Officer, Department
of Animal Husbandry,
Mahisagar, Gujarat, India

Isolation of bacterial pathogens and their antibiotic sensitivity in the cases of urinary tract infection in dogs

Nidhi Patel, SK Raval, Nilesh Gohil, Neha Rao and BB Bhandari

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Abstract

One of the most prevalent diseases in companion animals is urinary tract infection. Bacterial urinary tract disease is a common cause of morbidity in dogs and cats, and among the leading reason for antimicrobial use. Improper therapy can lead to variety of health concerns for the dog or cat (e.g., failure to resolve infection, development of antimicrobial resistance). Present study is conducted to isolate bacterial pathogens and to determine their antibiotic sensitivity from the dogs affected with urinary tract infection. A total of 28 urine samples from the dogs suspected of UTI were collected by catheterization and subjected to the cultural isolation and antimicrobial susceptibility testing. Out of 28 samples subjected to cultural isolation total 17(60.71%) samples were found positive for bacterial growth. Out of total 17 positive samples 4 (23.52%) samples were positive for *E. coli*, 3(17.64%) for *Proteus* spp., 3(17.64%) for *Staphylococcus* spp., 2(11.67%) for *Klebsiella* spp., 2(11.67%) for *Pseudomonas* spp., 2 (11.67%) samples were found to be positive for mix infection, with one case showing *E. coli* + *Staphylococcus* spp. and the other showing *E. coli* + *Proteus* spp. Whereas 1(5.88%) sample were found positive for *Streptococcus* spp. Ceftriaxone + Tazobactam was shown to have the highest overall sensitivity (88.23%), followed by Amoxicillin + Sulbactam (76.47%), gentamicin (76.47%), ciprofloxacin (70.58%), ampicillin (47.05%), and penicillin (11.76%).

Keywords: *E. coli*, Ceftriaxone+Tazobactam, Amoxicillin+Sulbactam, urinary tract infection, antimicrobial sensitivity

1. Introduction

The use of unconventional feedstuffs especially in monogastric animal feeding continue to attract positive interest among researchers, livestock farmers, animal feed manufactures and other stakeholders in the agricultural and agro-allied industries. According to Iyayi *et al* (2005) agro-industrial by-products (AIBs) have in recent years become important feed components in poultry diets in Nigeria mainly due to the increased competition for the conventional ingredients by humans and the food industries. Those of high fibre contents are being used either as fillers or as energy diluents.

One of the most prevalent diseases in companion animals is urinary tract infection (Ling 1984). Positive urine cultures are seen in 15-65% of dogs and up to 12% of cats that had urinary tract illness clinically manifested (Hall *et al.*, 2013; Windahl *et al.*, 2014; Teichmann-Knorrn *et al.*, 2018) ^[4, 21, 16]. Bacterial urinary tract disease is a common cause of morbidity in dogs and cats, and among the leading reason for antimicrobial use. Improper therapy can lead to variety of health concerns for the dog or cat (e.g., failure to resolve infection, development of antimicrobial resistance), economic (e.g., need for repeated or prolonged treatment), public health (e.g., antimicrobial resistance) and regulatory (e.g., antimicrobial use) concerns (Wees *et al.*, 2021) ^[20]. Most UTIs are the result of ascending bacteria from rectal or fecal contamination or from the distal urogenital tract. Large, retrospective studies have documented the most common species of uropathogens in dogs and cats, with *Escherichia coli* being the single most common pathogen in both acute and recurrent UTIs. The other pathogens include, *Staphylococcus*, *Proteus*, *Pseudomonas*, *Streptococcus* and *Klebsiella* spp. (Roopali *et al.*, 2018) ^[15].

Despite the fact that urinary tract infections (UTIs) are a prevalent problem in dogs, their treatment has grown more difficult as the frequency of antibiotic-resistant strains of different pathogens has increased (Jessen *et al.*, 2015) [6]. Hence it is important to try to prevent or reduce the incidence of antimicrobial resistance in clinical treatment by performing drug susceptibility testing and resistance monitoring. Based on antibiotic sensitivity test, the most appropriate antibiotic regimen can be selected. Keeping this in view present study was conducted to carry out cultural isolation and antimicrobial susceptibility testing in dogs affected with urinary tract infection. Microbiological culture combined with susceptibility testing is the cornerstone of UTI diagnosis and the best instrument for guiding treatment decisions in individual dog (Punia *et al.*, 2018) [14].

2. Materials and Methods

2.1 Collection of Urine Sample

Total 28 dogs with clinical signs of urinary tract infection presented to Veterinary Clinical Complex and Department of Veterinary Medicine, Department of Veterinary Surgery of Veterinary College, Anand, and Government polyclinics and some of the private clinics of nearby Anand were taken into consideration during the study after obtaining consent from the dog owner. Urine samples were collected aseptically by catheterization in sterile containers on day '0'. Collected urine samples were subjected to bacteriological examination.

2.2 Isolation and Identification of Bacteria

Urine samples from the affected animals was collected aseptically in a sterile container. Samples were centrifuged at 6000 rpm for 5 minutes. Supernatant was removed and sediment portion of the samples were used for inoculation. Samples were inoculated on 5% sheep blood agar plates with the help of 4 mm diameter platinum loop and incubated at 37 °C for 24 hrs. The identification of bacterial organisms was done based on colony characteristics on agar, microscopic examination, and biochemical tests. To distinguish between *Staphylococci* spp. and *Streptococci* spp., the Gram-positive cocci were subjected to a catalase test. Based on growth on eosin methylene blue agar (EMB) and MLA, other species such as *Escherichia coli* and *Klebsiella* spp. were distinguished. The identification of bacterial isolates was done as per method described by Cowan and Steel (2004).

2.3 Antibiotic Susceptibility Testing

The *in-vitro* antimicrobial susceptibility testing of the isolated bacteria was carried out by disc diffusion method (Bauer *et al.*, 1966) as follows. A loopful of colony from BHI agar plate transferred to test tube containing 4-5 ml BHI broth. Then broth was incubated at 37 °C for 8-10 hrs or until the visible turbidity is observed. The turbidity of inoculated broth was adjusted by comparing in with 0.5 McFarland standard using sterile BHI broth. The turbidity of test suspension and standard was adjusted against the white background with black contrasting lines. A sterile swab was dipped into adjusted broth culture of bacteria. Excess fluid was removed by rotating and pressing swab inside the wall of tube above the fluid level. The lawn culture over the entire surface of Muller-Hinton agar (MHA) was carried with the help of swab in 3-4 direction after rotating the plate at 60° angle. Antibiotic discs were placed on the agar surface with the sterile forceps. The disc was gently pressed against the agar surface. The plates were inverted and then incubated at 37 °C for 24 hrs. After the incubation diameter of zone of inhibition were

measured using ruler and interpretation made according to the guidelines provided by manufacturer. Antimicrobial used in this study were Ceftriaxone + Tazobactam (30 mcg), Amoxicillin + Sulbactam (30 mcg), Gentamicin (10 mcg), Ampicillin (2 mcg), Ciprofloxacin (5 mcg), Penicillin (10 mcg)

3. Result and Discussion

3.1 Bacteriological isolation

Out of 28 samples subjected to cultural isolation total 17(60.71%) samples were found positive for bacterial growth and 11(39.28%) samples were found negative for bacterial growth on culture. Out of total 17 positive samples 4 (23.52%) samples were positive for *E. coli*, 3(17.64%) for *Proteus* spp., 3(17.64%) for *Staphylococcus* spp., 2(11.67%) for *Klebsiella* spp., 2(11.67%) for *Pseudomonas* spp., 2 (11.67%) samples were found to be positive for mix infection, with one case showing *E. coli* + *Stephylococcus* spp. and the other showing *E. coli* + *Proteus* spp. Whereas 1(5.88%) sample were found positive for *Streptococcus* spp (Table 1). The present findings are well concurred with findings of Hernando *et al.* (2021) [5], found that *Escherichia* spp. (45.3%), *Proteus* spp. (13.2%), *Staphylococcus* spp. (11%), *Streptococcus* spp. (6.5%), *Klebsiella* spp. (5.1%), *Pseudomonas* spp. (4.1%) were the bacterial species that were most often identified from the urine samples in dogs with urinary tract infection and Dupont *et al.* (2020) observed mix infection in 10% dogs affected with urinary tract infection. Similar observations were reported by Chang *et al.* (2014) [1], Thungrat *et al.* (2015) [18], McMeekin *et al.* (2016) [11], Merkel *et al.* (2017) [12], Yogeshpriya *et al.* (2018a) [22], Mohamed *et al.* (2019) [13] and Madhvi (2021) [10] who identified *E. coli* spp. as a major pathogen in urine of dogs affected with urinary tract infection.

Table 1: Relative frequency of organisms isolated from urine samples of dogs (N=17) suffering from urinary tract infection

Sr. No.	Bacterial isolates	No of samples positive (N=17)	Percent positivity
1	<i>E. coli</i>	4	23.52
2	<i>Proteus</i> spp.	3	17.64
3	<i>Staphylococcus</i> spp.	3	17.64
4	<i>Klebsiella</i> spp.	2	11.67
5	<i>Pseudomonas</i> spp.	2	11.67
6	Mixed infection	2	11.67
7	<i>Streptococcus</i> spp.	1	5.88

Mixed infection: *E. coli* + *Proteus* spp and *E. coli* + *Stephylococcai* spp

3.2 Antibiotic Susceptibility Testing

The sensitivity of the isolated organism is the primary factor in the selection of an appropriate antibiotic for therapy. In our study there are various isolates shows resistance towards different antibiotic it might be because to treat UTI symptoms while awaiting the results of antimicrobial susceptibility testing, antimicrobials are routinely prescribed empirically based on the existence of compatible clinical signs, urine cytological findings, and in the absence of urine culture. Also, antimicrobials are used indiscriminately in treating bacterial infection which leads to antimicrobial resistance. Ceftriaxone+Tazobactam was shown to have the highest overall sensitivity (88.23%), followed by Amoxicillin+Sulbactam (76.47%), gentamicin (76.47%), ciprofloxacin (70.58%), ampicillin (47.05%), and penicillin (11.76%) as per Table 2. In the present study, Ceftriaxone+Tazobactam was shown to have the highest

overall sensitivity (88.23%) which was in accordance with Thirunavukkarasu *et al.* (2010) [17] found that all ten isolates isolated from canine UTI were 100% sensitive to Ceftriaxone+Tazobactam. Punia *et al.* (2018a) [14] reported overall, isolates were most sensitive to Ceftriaxone+Tazobactam (88.88 percent) and Madhvi (2021) [10] observed 90.91% sensitivity of Ceftriaxone+Tazobactam and amoxicillin+ sulbactam was sensitive in 76.47% of isolates. Which agreed with Madhvi (2021) [10] reported 81.82% of tested isolates were susceptible to amoxicillin sodium + sulbactam as per Table 3.

Gentamicin was found to be sensitive in 76.47% of isolates which was in consistence with the findings of Cetin *et al.* (2003) reported 78.43% of the strains isolated from dogs with urinary tract infection were sensitive towards gentamicine. Similar observations were found by Villarroel *et al.* (2002) [19], Chang *et al.* (2014) [1], Thungrat *et al.* (2015) [18] while Madhvi (2021) [10] reported higher (93.94%) sensitivity of gentamicin. (Table 3).

In present study, 70.58% of the isolates were found to be sensitive towards ciprofloxacin. Similar findings were reported by Madhvi (2021) [10] observed 75.76% sensitivity of ciprofloxacin. However, Fernandes *et al.* (2019) [3] observed high (86.67%) sensitivity in contrast Macotpet *et al.* (2009) [9] reported 100% sensitivity of ciprofloxacin and ampicillin was found to be sensitive in 47.05% of isolates which agreed with Feria *et al.* (2000) [2] reported 52.8% sensitivity, Chang *et al.* (2014) [1] observed 50.00% sensitivity. However, Fernandes *et al.* (2019) [3] reported higher sensitivity (60%) compared to our present study. Penicillin (11.76%) shown to have least sensitivity in all the antibiotics which is in agreement with the findings of Punia *et al.*, (2018) [14]. It might be due to indiscriminate and repeated use of the same antibiotics for the treatment in veterinary field.

E. coli was shown to have highest antibiotic sensitivity towards ceftriaxone + tazobactam (100%) followed by amoxicillin sodium + sulbactam (75.00%), gentamicin (75.00%), ampicillin (25.00%). Penicillin resistance was found in all the isolates. In case of *Staphylococcus spp.* all the isolates were found sensitive towards ceftriaxone + tazobactam, ciprofloxacin and ampicillin followed by amoxicillin sodium + sulbactam (66.67%), gentamicin (66.67%) and penicillin (33.33%). *Proteus spp.* revealed highest antibiotic sensitivity towards ceftriaxone + tazobactam and ciprofloxacin followed by amoxicillin sodium + Sulbactam (66.67%), gentamicin (66.67%), ampicillin (66.67%) and penicillin (33.33%). as per showed in Table 3

In case of *Klebsiella spp.* all the isolates were found sensitive towards ceftriaxone + tazobactam, ciprofloxacin, amoxicillin sodium + sulbactam, gentamicin followed by ampicillin (50.00%) and penicillin (50.00%). However, in *Pseudomonas spp.* all the isolates were found sensitive towards Ceftriaxone+Tazobactam, ciprofloxacin, amoxicillin sodium + sulbactam, gentamicin, followed by ampicillin (50.00%) and penicillin (50.00%) as per showed in Table 3.

In case of Mix infection all the isolates were found sensitive towards ceftriaxone + tazobactam (50.00%) followed by amoxicillin sodium + sulbactam (50.00%), gentamicin (50.00%), nitrofurantoin (50.00%). whereas in *Streptococcus spp.* All the isolates were found sensitive towards Ceftriaxone+Tazobactam, amoxicillin sodium + sulbactam, gentamicin, nitrofurantoin, ciprofloxacin, gentamicin, and all isolates were resistance towards penicillin and in case of *Streptococcus spp.* All the isolates were found sensitive towards Ceftriaxone+Tazobactam, amoxicillin sodium + sulbactam, gentamicin, nitrofurantoin, ciprofloxacin, gentamicin, and all isolates were resistance towards penicillin as per showed in Table 3.

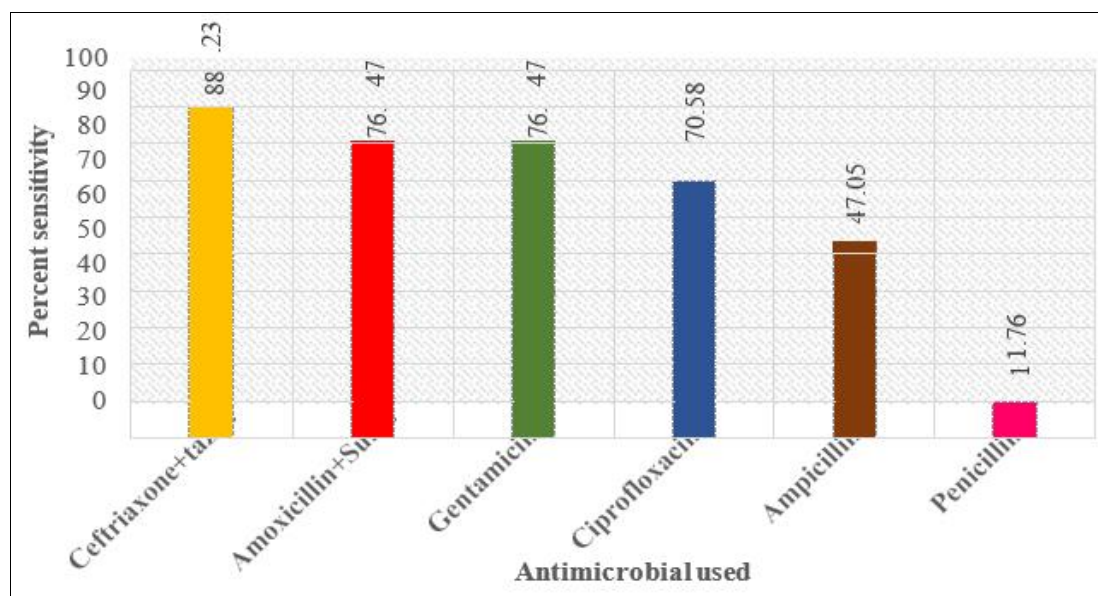


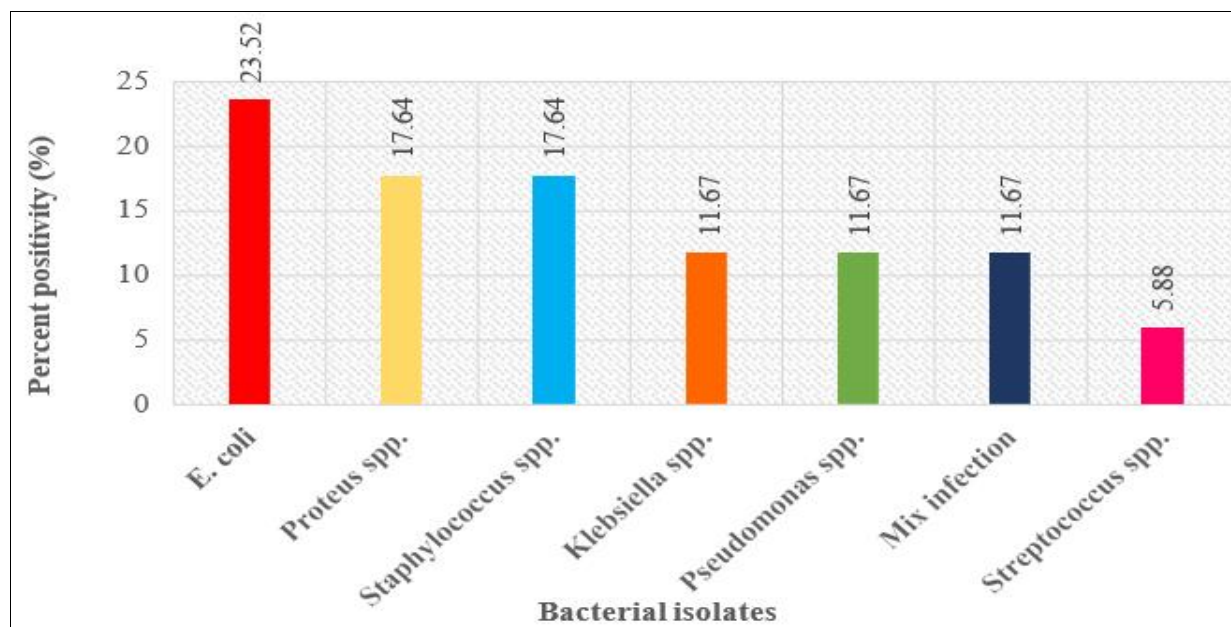
Fig 1: Relative frequency of organisms isolated from urine samples of dogs suffering from urinary tract infection (N=17)

Table 2: Antimicrobial sensitivity pattern of different bacterial isolates from urine of dogs affected with UTI (N=17)

Sr. No.	Antimicrobial used	Percent sensitivity
1	Ceftriaxone+ tazobactam	88.23
2	Amoxicillin+ Sulbactam	76.47
3	Gentamicin	76.47
4	Ciprofloxacin	70.58
5	Ampicillin	47.05
6	Penicillin	11.76

Table 3: *In vitro* antibiotic sensitivity pattern of different bacterial isolates (N=17)

Name of antibiotic	<i>E. coli</i> (N=4)	<i>Staphylococcus</i> spp. (N=3)	<i>Klebsiella</i> spp. (N=2)	<i>Proteus</i> spp. (N=3)	<i>Pseudomonas</i> spp. (N=2)	Mix infection (N=2)	<i>Streptococcus</i> spp. (N=1)
Ceftriaxone + Tazobactam	4 (100%)	3 (100%)	2 (100%)	3 (100%)	1 (50.00%)	1 (50.00%)	1 (100%)
Penicillin	0	1 (33.33%)	1 (50.00%)	1 (33.34%)	0	0	0
Amoxicillin+ Sulbactam	3 (75.00%)	2 (66.67%)	2 (100%)	2 (66.67%)	2 (100%)	1 (50.00%)	1 (100%)
Ciprofloxacin	2 (50.00%)	3 (100%)	2 (100%)	3 (100%)	0	1 (50.00%)	1 (100%)
Gentamicin	3 (75.00%)	2 (66.67%)	2 (100%)	2 (66.67%)	2 (100%)	1 (50.00%)	1 (100%)
Ampicillin	1 (25.00%)	3 (100%)	1 (50.00%)	2 (66.67%)	0	0	1 (100%)

**Fig 2:** Antimicrobial sensitivity pattern of different bacterial isolates from urine of dogs affected with UTI (N=17)

4. Conclusion

From the study it is concluded that *E. coli* is one of the most common organisms responsible for urinary tract infection in dogs and cephalosporins, potentiated penicillin and aminoglycosides are most effective in the treatment of urinary tract infection in dogs.

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Conflict of Interest

Not available

Financial Support

Not available

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