Pathogens isolated from clinical cases of urinary tract infection in dogs and their antibiogram

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Received: 19-03-2018, Accepted: 14-06-2018, Published online: 02-08-2018

doi: 10.14202/vetworld.2018.1037-1042 How to cite this article: Punia M, Kumar A, Charaya G, Kumar T (2018) Pathogens isolated from clinical cases of urinary tract infection in dogs, Veterinary World, 11(8): 1037-1042.

Abstract

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

Materials and Methods: Urine samples were collected either through catheterization or cystocentesis from 35 dogs suspected of UTI admitted to VCC, LUVAS, Hisar. Bacteria were identified on the basis of cultural characteristics in 22 samples, and all the isolates were subjected to in vitro antimicrobial sensitivity testing.

Results: The urine samples found positive for bacteria yielded pure colony growth in 77.27% and mixed growth in 22.73% samples, respectively. Escherichia coli (29.62%) and Streptococcus spp. (29.62%) were the most prevalent microorganisms followed by Staphylococcus spp. (22.22%), Klebsiella spp. (11.11%), Pseudomonas spp. (3.7%), and Bacillus spp. (3.7%). Overall, maximum sensitivity of isolates was found toward ceftriaxone/tazobactam (88.88%) and least toward amoxicillin and cloxacillin (29.62%).

Conclusion: E. coli and Streptococcus spp. were the most predominant bacteria isolated from UTI affected dogs. In vitro sensitivity revealed a significant proportion of bacteria to be multidrug resistant.

Keywords: antibiogram, multidrug resistance, Escherichia coli, Staphylococci, Streptococci.

Introduction

Urinary tract infection (UTI) refers to the microbrial colonization of the urinary tract or of any urinary tract organ, except the distal urethra, which has a normal bacterial flora. Various microorganisms have been involved in the etiology of UTI in dogs. Approximately, 14% of all dogs encounter at least one episode of bacterial UTI during their lifetime [1]. Among bacterial causes, Escherichia coli has been the most frequently isolated bacteria causing UTI in dogs which can go up to 30% [2-4]. Other commonly isolated bacteria include Staphylococcus spp., Enterococcus spp., Proteus spp., and Klebsiella spp. [2-5].

Microbiological culture combined with susceptibility testing is the cornerstone of UTI diagnosis and the best instrument for guiding treatment decisions in individual dogs [6,7]. Periodic monitoring of pathogens isolated from UTI and their susceptibility patterns help in the choice of first-line empirical therapy and can also be used to monitor the presence of resistant bacteria.

Treatment with antibiotics is one of the most critical components to control UTI. Increasing antimicrobial resistance in canines is of concern as it complicates therapy in dogs, contributes to therapeutic failure, increased patient morbidity and mortality, and health-care costs in UTI infection. In addition, it is also a public health concern being pathogens/diseases zoonotic [8,9]. Therefore, definitive diagnosis of etiological agents and their antimicrobial sensitivity before treatment will help in selecting suitable and cost-effective antibiotic to treat the affected animal timely and adequately.

Therefore, the present study was aimed to determine the etiological agent(s) responsible for causation of UTI in dogs and to determine their antimicrobial sensitivity to institute proper line of treatment.

Materials and Methods

Ethical approval

The samples used in the current study were received from the clinical cases. As per University rule, approval of Institutional Animal Ethics Committee is not required for the clinical cases.

Sample collection

A total of 35 dogs admitted to the VCC, LUVAS, Hisar, showing clinical signs suggestive of UTI such as inappetence/anorexia, emaciation, vomition, hematuria, polyuria, polydipsia, depression, weight loss, weakness, dehydration, nausea, anuria, stranguria, and oliguria were considered in the present study. Urine
samples were collected using catheterization or cysto-
centesis. Of the 35 samples, 22 samples were collected
by catheterization and 13 samples by cystocentesis.

**Bacterial isolation**

Urine samples from the affected animals were
collected aseptically in a sterile container. After
receiving, the urine samples were inoculated in
0.01 ml volume on 5% sheep blood agar (BA) and
MacConkey lactose agar (MLA) plates with the help
of a 4 mm diameter platinum loop. The plates were
incubated aerobically at 37°C for 24-48 h. Subcultures
of the resulting growth were made on BA for purifica-
tion of isolates and identified on the basis of Gram’s
reaction, morphology, and colony characteristics. The
Gram-positive cocci were subjected to catalase test to
differentiate between staphylococci and streptococci.
To rule out the possibility of micrococci, all the cata-
lase-positive cocci were subjected to oxidase test and
oxidation fermentation test. Other organisms such as
E. coli and Klebsiella spp. were differentiated on the
basis of growth on MLA and eosin methylene blue
agar. Butyrous large colonies and no growth on eosin
methylen blue were taken as Klebsiella spp., more-
over, small shiny colonies with typical metallic sheen
growth were taken as E. coli.

**In vitro antibiotic sensitivity**

Different strains of various organisms isolated
from urine samples of the infected animals were sub-
jected to in vitro drug sensitivity testing, using 15 anti-
microbials by the disc-diffusion method. With the help
of a platinum loop, a small amount of test culture was
transferred into a tube of brain heart infusion broth
and incubated for 2-5 h at 37°C, to obtain turbidity.
With the help of a sterile cotton swab, the broth culture
was then evenly spread by smearing over the surface
of BA/Mueller-Hinton agar plates. The antimicrobial
discs were placed on the agar and gently pressed. These
were then incubated at 37°C for 24 h. The sensitivity
was observed on the basis of zone size interpretation
chart, provided by the manufacturer. Different anti-
microbials used were amikacin (10 mcg), amoxicillin
(10 mcg), amoxicillin-clavulanic acid (30 mcg), ampi-
cillin (25 mcg), cefoperazone (75 mcg), ceftriaxone
(10 mcg), ceftriaxone–tazobactam (30mcg), chloram-
phenicol (25 mcg), cloxacicillin (30 mcg), enrofloxacin
(10 mcg), gentamicin (30 mcg), neomycin (30 mcg),
oxytetracycline (30 mcg), penicillin-G (10 units), and
streptomycin (25 mcg) (Hi-Media). To remain conser-
vative in our estimates of resistance, isolates exhibit-
ing intermediate zones of inhibition were interpreted
as resistant.

**Determination of multidrug-resistant (MDR) bacteria**

On the basis of sensitivity pattern, isolates were
categorized into MDR, extreme drug resistant
(XDR), and pandrug-resistant. Isolates resistant to
three or more antibiotics belonging to different groups
were classified as MDR. Among MDR isolates, isol-
ates susceptible to only two antibiotics belonging to
two different groups were considered XDR, while
resistance to all the antibiotics was considered as
pandrug-resistant.

**Statistical analysis**

Results of overall antimicrobial sensitivity were
statistically analyzed for the determination of confi-
dence interval of percent number and Z test to reveal
any significant difference between the different groups
of antibiotics.

**Results**

**Bacteriological examination**

Of the 35 samples, 22 urine samples were found
positive for bacterial isolation, of which 22 sam-
ples, 17 (77.27%) samples yielded pure culture, whereas
mixed growth was observed in 5 (22.72%). Single isolation
of E. coli was isolated in 5 (22.7%), Streptococcus
spp. in 2 (9.09%), Klebsiella spp. in 2 (9.09%),
and Bacillus spp. in 1 (4.54%). Among mixed cul-
tures, one sample each was found positive for com-
bination of Streptococci + Klebsiella, Streptococci +
E. coli, Staphylococcus + E. coli, Staphylococcus +
Streptococci, and Pseudomonas + E. coli. A total of
27 isolates were recovered from urine samples. The
frequency of isolation of different bacterial species is
depicted in Table-1.

**Antimicrobial sensitivity testing**

Overall, maximum sensitivity of isolates was
found toward ceftriaxone/tazobactam combination
(88.88%) followed by ceftriaxone (77.77%), chlor-
amphenicol (74.07%), gentamicin and cefoper-
aze (70.30%), amoxicillin/clavulanic acid and
neomycin (55.55%), amikacin (48.14%), strepto-
mycin (44.44%), ampicillin (40.74%), enrofloxacin
(37.03%), oxytetracycline and penicillin-G (33.33%),
and amoxicillin and cloxacillin (29.62%) as shown
in Table-2. Statistically, Z test was applied to reveal
any significant difference among the antibiotic used
for in vitro sensitivity. Cephalosporin and macrolide
group of antibiotics were found to be significantly
sensitive as compared to penicillin group, whereas
non-significant difference existed in sensitivity of tetr-
cycline and penicillin groups of antibiotics. Among
in the present study, large numbers of bacteria were isolated. Among the various microorganisms isolated, E. coli (29.62%) and Staphylococcus spp. isolate was sensitive toward ceftriaxone-tazobactam, gentamicin, and amikacin (75%). \( E. coli \) was found most sensitive to neomycin (87.5%) followed by ceftriaxone-tazobactam, chloramphenicol, amikacin and gentamicin (75%), ceftriaxone, cefoperazone and ceftriaxone-tazobactam (62.5%), streptomycin (50%), amoxicillin-clavulanic acid (37.5%), and oxytetracycline and enrofloxacin (25%).

In case of \( \text{Staphylococcus} \) spp., the maximum sensitivity 100% was seen toward ceftriaxone, cefoperazone, chloramphenicol, and ceftriaxone-tazobactam followed by amoxicillin-clavulanic acid (87.5%), penicillin-G and ampicillin (75%), oxytetracycline (62.5%), gentamicin, amikacin and cloxacinil (50%), enrofloxacin (37.5%), and streptomycin (25%). Amikacin and neomycin (12.5%) were found to be least sensitive (Table-3).

Maximum sensitivity of \( \text{Staphylococcus} \) spp. was observed for ceftriaxone-tazobactam (83.33%), followed by gentamicin and neomycin (66.67%), chloramphenicol, ceftriaxone, cefoperazone, streptomycin, oxytetracycline, amoxicillin-clavulanic acid, enrofloxacin, and amikacin (50%) and least toward amoxicillin, ampicillin, claxacinil, and penicillin-G (33.33%) (Table-3). \( \text{Klebsiella} \) spp. showed maximum sensitivity toward ceftriaxone-tazobactam, gentamicin, and ceftriaxone (100%) followed by chloramphenicol and amoxicillin-clavulanic acid (66.67%), neomycin, amikacin, cefoperazone, streptomycin, oxytetracycline, enrofloxacin, and amoxicillin (33.33%) (Table-3).

\( \text{Pseudomonas} \) spp. obtained in the present study was found to be sensitive toward enrofloxacin, gentamicin, amikacin, neomycin, streptomycin, ceftriaxone, cefoperazone, and ceftriaxone-tazobactam combination and resistant toward chloramphenicol, oxytetracycline, ampicillin, amoxicillin, cloxacinil, penicillin, and amoxicillin-clavulanic acid (Table-3). \( \text{Bacillus} \) spp. isolate was sensitive toward ceftriaxone, cefoperazone, ceftriaxone-tazobactam, chloramphenicol, gentamicin, amikacin, oxytetracycline, and claxacinil and resistant toward ampicillin, amoxicillin, penicillin-G, amoxicillin-clavulanic acid, enrofloxacin, neomycin, and streptomycin (Table-3).
Table 3: *In vitro* antibiotic sensitivity pattern of different bacterial isolates.

| Antimicrobials used | Sensitivity (%) |
|---------------------|-----------------|
|                     | *E. coli* n=8 (%) | *Streptococcus* spp. n=8 (%) | *Staphylococcus* spp. n=6 (%) | *Klebsiella* spp. n=3 (%) | *Pseudomonas* spp. n=1 (%) | *Bacillus* spp. n=1 (%) |
| Tetracyclines       |                |                             |                             |                          |                          |                          |
| Oxytetracycline     | 2 (25)         | 5 (62.5)                    | 3 (50)                      | 1 (33.33)                | 0                         | 1 (100)                   |
| Penicillins         |                |                             |                             |                          |                          |                          |
| Ampicillin          | 1 (12.5)       | 6 (75)                      | 2 (33.33)                   | 0                        | 0                         | 0%                        |
| Amoxicillin         | 1 (12.5)       | 4 (50)                      | 2 (33.33)                   | 1 (33.33)                | 0                         | 0%                        |
| Cloxacillin         | 1 (12.5)       | 4 (50)                      | 2 (33.33)                   | 0                        | 0                         | 0%                        |
| Penicillin          | 1 (12.5)       | 6 (75)                      | 2 (33.33)                   | 0                        | 0                         | 0%                        |
| Amoxicillin/clavulanic acid | 3 (37.5)     | 7 (87.5)                    | 3 (50)                      | 2 (66.67)                | 0                         | 0%                        |
| Fluoroquinolones    |                |                             |                             |                          |                          |                          |
| Enrofloxacin        | 2 (25)         | 3 (37.5)                    | 3 (50)                      | 1 (33.33)                | 1 (100)                   | 0                         |
| Aminoglycosides     |                |                             |                             |                          |                          |                          |
| Gentamicin          | 6 (75)         | 4 (50)                      | 4 (66.67)                   | 3 (100)                  | 1 (100)                   | 1 (100)                   |
| Amikacin            | 6 (75)         | 1 (12.5)                    | 3 (50)                      | 1 (33.33)                | 1 (100)                   | 1 (100)                   |
| Neomycin            | 7 (87.5)       | 1 (12.5)                    | 4 (66.67)                   | 1 (33.33)                | 1 (100)                   | 0                         |
| Streptomycin        | 4 (50)         | 2 (25)                      | 3 (50)                      | 1 (33.33)                | 1 (100)                   | 0                         |
| Cephalosporins      |                |                             |                             |                          |                          |                          |
| Ceftriazone         | 5 (62.5)       | 8 (100)                     | 3 (50)                      | 3 (100)                  | 1 (100)                   | 1 (100)                   |
| Cefoperazone        | 5 (62.5)       | 8 (100)                     | 3 (50)                      | 1 (33.33)                | 1 (100)                   | 1 (100)                   |
| Ceftriazone/tazobactam | 6 (75)        | 8 (100)                     | 5 (83.33)                   | 3 (100)                  | 1 (100)                   | 1 (100)                   |
| Macrolides          |                |                             |                             |                          |                          |                          |
| Chloramphenicol     | 6 (75)         | 8 (100)                     | 3 (50)                      | 2 (66.67)                | 0                         | 1 (100)                   |

*E. coli*=Escherichia coli
isolates were found to be resistant to a different group of antibiotics. This resistance can be attributed to the indiscriminate use of antibiotics against bacteria resulting in the emergence of drug-resistant mutants which often leads to treatment failure in dogs. Overall, in vitro antibiotic sensitivity revealed cephalosporins and macrolides as the most sensitive and penicillins showed the least sensitivity toward all the isolates.

Of all the antibiotics tested for the determination of in vitro sensitivity of E. coli isolates, aminoglycosides outstood with the range of 50-87.5%. Among aminoglycosides, neomycin was found to be most effective with 87.5% sensitivity. This can be attributed to lesser use of these antibiotics due to nephrotoxic nature of these drugs. Least sensitivity of E. coli was seen toward penicillin group, especially ampicillin, amoxicillin, cloxacillin, and penicillin-G while higher sensitivity of E. coli toward penicillin drugs has been found most sensitive toward cephalaxin/tazobactam combination and least toward gentamicin and ampicillin. The resistance of S. aureus to penicillin group of antibiotics in our study which is due to the production of beta-lactamase, an enzyme that inactivates penicillin and closely related antibiotics.

Klebsiella isolates have been found 100% sensitive toward gentamicin and ampicillin which was in accordance with the findings of Kogika et al. [11]. 100% resistance of Klebsiella isolates toward ampicillin, cloxacillin, and penicillin in the present study is in accordance with the findings of Windahl et al. [14] showing complete resistance toward ampicillin. This resistant behavior of Klebsiella isolates toward ampicillin and other penicillins can be attributed to the production of β-lactamase enzyme destroying the antibiotics.

These findings of variable sensitivity of bacteria isolated toward different antimicrobial suggest that the antimicrobial agent to be used should be selected on the basis of bacterial culture and results of antibiotic sensitivity tests and clinical response to the antibiotic.

**Conclusion**

The present study indicated considerable resistance in pathogens associated with UTI in dogs. E. coli and Streptococcus spp. were the most prevalent bacteria isolated from UTI affected dogs. In vitro antimicrobial sensitivity testing revealed cephalosporins and macrolide group to be the most effective group of antibiotics.

**Authors’ Contributions**

AK supervised the whole study. MP and AK designed the study. MP and TK performed the cultural
examination and determination of sensitivity. GC and MP analyzed the data and wrote the manuscript. The final manuscript has been read and approved by all the authors.

Acknowledgment
The authors acknowledge Head of the Department, Veterinary Medicine, LUVAS, and In charge, College Central Laboratory, LUVAS, for providing the facilities. Funds from the department were used for the present study.

Competing Interests
The authors declare that they have no competing interests.

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