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Original Article

Diversity and antibiotic resistance profile analysis of uropathogenic bacteria in human and canines

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ABSTRACT

In Pakistan, Urinary tract infections (UTI) are increasing day by day. The study therefore was designed for isolation identification and antibiotic resistance assessment of UTI causing bacteria. Bacterial pathogens causing UTI in dogs and human are becoming more resistant to antibiotic use. To evaluate the diversity and antibiotic resistance of uropathogenic bacteria, a total of 80 urine samples were collected in sterile containers. A total of 15 urine samples were taken from each gender of human and dogs with UTIs and 5 from each gender of healthy human and dogs. Samples were cultured for isolation and confirmed by biochemical tests and their antibiotic resistance pattern was checked by Kirby baur disk diffusion test. Samples that were taken from UTI males, *E.coli, Proteus spp., Klebsiella spp.* and *Staph aureus* was isolated from 93%, 6.67%,20% and 53% processed samples whereas from UTI females *E.coli, Proteus spp., klebsiella spp.*, and *Staph aureus* was isolated 100%,13%,33%and 40% of samples respectively. Similarly 60% *Staph aureus* was isolated from healthy males and females urine samples. While Samples that were taken from UTI dogs *E.coli., Proteus spp., Klebsiella spp.,* and *Staph aureus* was isolated from 66.7%, 6.67%, 33% and 26.6% of processed samples whereas Samples that were taken from bitches, *E.coli., Proteus spp., klebsiella spp.,* and *Staph aureus* was isolated from 73%,13%,26.6% and 26.6% of samples respectively. In case of healthy dogs and bitches urine smalpes 60% and 80% of *Staph aureus* was isolated. Antibiotic resistance pattern of isolates *E.coli, Proteus spp., klebsiella spp.* and *Staph aureus* from human (male and female) samples showed resistance to ceftriaxone, Levofloxecine, and mild type of resistance to ofloxacin while sensitive to ciprofloxacin and sulfamethazole.The antibiotic resistance pattern of isolates *E.coli, Proteus spp., Klebsiella spp.* and *Staph aureus* from dogs and bitches samples showed high resistance to Lincomycin and kanamycin, and lower resistance to norfloxacin and doxycyclin while sensitive to ciprofloxacin, amoxaclin.

1. Introduction

Urinary tract infections (UTIs) stand out as prevalent bacterial infections, impacting around 150 million individuals globally each year [1, 2]. In 2007, solely within the United States, there were an approximate 10.5 million instances of clinic visits due to UTI symptoms (constituting 0.9% of total

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ambulatory visits), coupled with 2–3 million visits to the emergency department [3, 4].

The economic burden of these infections, encompassing healthcare expenses and work absenteeism, presently accounts for approximately $3.5 billion annually in the United States alone. UTIs pose a significant health concern for infant boys, elderly men, and females across all age groups. Notable complications include frequent recurrences, pyelonephritis leading to sepsis, renal impairments in young children, pre-term births, and complications arising from excessive antimicrobial usage, such as heightened antibiotic resistance and *Clostridium difficile* colitis [5-8].

From a clinical perspective, UTIs are classified into two categories: uncomplicated and complicated. Uncomplicated UTIs generally affect individuals who enjoy good health otherwise and exhibit no structural or neurological anomalies within the urinary tract5,6. These infections are further differentiated into lower UTIs (cystitis) and upper UTIs (pyelonephritis) [9, 10].

Urinary tract infection (UTI) is considered as most prevalent and second most common type of infection in the body. The infection is caused by the invasion of pathogens in the urinary tract and produces the inflammatory response of urothelium [11-13]. Main cause of urinary tract infection is the proliferation of bacteria in urinary tract. Both male and female are susceptible for this type of infection irrespective of age but females are more susceptible for UTI infection [14] due to greater bacterial load or anatomical predisposition in the urothelial mucosa.

UTI infection is classified in to two groups; the uncomplicated UTI that occurs in those patients who are sexually active and with structurally and functionally normal urinary tract and complicated UTI patients are those who are associated with co -morbid conditions that increase the chances for therapeutic failure [15-17]. Mostly diverse group (Gram positive and gram negative) of uropathogens are considered to be responsible for UT infection but most common are the facultative anaerobes [18-20].

Various bacteria like *Staphylococcus spp. Streptococcus spp. and Enterococcus spp, Escherichia spp. Klebsiella spp. Enterobacter spp. Citrobacter spp. Proteus spp. Serratia spp. Salmonella spp. and Pseudomonas spp* are of major concern. UTI treatment is based on the bacterial sensitivity toward the antibiotics but prolong use of antibiotic may cause the antibiotic resistance in the patients. Like human, UT infections in dogs are also common and 14 percent dogs are affected by UTI, those presented for veterinary cares [21-23].

Diversity of bacteria causing UTI is still need to be explored. Limited information are available regarding UTI causing bacteria in human and dogs. Therefore, this study was designed to examine the diversity of uropathogenic bacteria in both human and dogs along with their antibiotic resistance profile.

2. Materials and methods

2.1. Sample collection

A total of 80 urine samples were collected in sterile containers. A total of 15 urine samples were taken from each gender of human and dogs with UTIs and 5 from each gender of healthy human and dogs. After collection samples were transported on ice packs to University diagnostic lab (UDL) of The University of Agriculture, Department Veterinary and Animal Sciences Peshawar for isolation, identification of diversity of uropathogens and their antibiotic resistance profile.

2.2. Bacterial Isolation and identification Procedure

Samples were centrifuged and sediments were cultured on the blood macconkey and nutrient agar by spread plate method followed by 24 hour incubation at 37 °C and bacteria colonies which have different morphology were selected, purified and identified by their biochemical profiles accronding to OIE manual 2008.

2.3. Antimicrobial susceptibility testing

In the present study antimicrobial susceptibility testing was done on Mueller-Hinton agar (Merck, Germany) using disk diffusion (Kirby Bauer's) technique. This method was done according to Clinical and Laboratory Standards Institute CLSI [24] guidelines to determine susceptibility of UTI agents. Sensitivity pattern of human isolates were checked out against 7 antibiotics including Fosfomycin (30µg), Urixin (30µg), Ofloxacin (15µg), Ciprofloxacin (5µg), Levofloxacin (30µg), Ceftriaxone; (30µg), and Sulfamethoxazole (10µg).

The confirmed dog's bacterial isolates were subjected to antibiotic susceptibility testing by disk diffusion method. Isolates were screened for resistance against 7 antibiotics including Amoxicillin (30µg), Doxycilne (30µg), Norfloxacin (15µg), Ciprofloxacin (5µg), Kanamycin (30µg), tetracycline (30µg), and Lincomycin (10µg). Zones of inhibition were measured of each bacteria against antibiotics and then compared with standards zones in CLSI and marked as sensitive, intermediate and resistance

2.4. Statistical Analysis

Data was analyzed by chi square using Statistical Package for Social Science (SPSS 18.0 software).

3. Results

In a study involving urine samples, a total of 40 samples were collected, comprising 20 from males and 20 from females. These samples were processed to identify the presence of specific bacteria, including *E. coli, Proteus spp., Klebsiella spp*., and *Staph aureus*. The isolation rates varied among different categories. Among the processed male samples, *E. coli* was isolated from 93% of the samples, while *Proteus spp., Klebsiella spp.*, and *Staph aureus* were isolated from 6.67%, 20%, and 53% of the samples, respectively.In the case of UTI females, all four bacteria were found in higher isolation rates: *E. coli* (100%), *Proteus spp*. (13%), *Klebsiella spp*. (33%), and *Staph aureus* (40%).

Among healthy males and females, *Staph aureus* was isolated at rates of 60% and 80%, respectively. Moving on to canine samples, out of the 40 collected samples (20 from male dogs and 20 from female dogs), *Proteus spp., Klebsiella spp*., and *Staph aureus* were found in 66.7%, 6.67%, and 33% of processed dog samples, respectively. For samples from female dogs, *E. coli, Proteus spp., Klebsiella spp.*, and *Staph aureus* were isolated at rates of 73%, 13%, 26.6%, and 26.6%, respectively. In healthy canine samples, *Staph aureus* was present in 60% of the male samples and 80% of the female samples.

The antibiotic resistance patterns, isolates of *E. coli, Proteus spp., Klebsiella spp.*, and *Staph aureus* from human samples showed resistance to ceftriaxone and levofloxacin, with a mild level of resistance to ofloxacin. However, they exhibited sensitivity to ciprofloxacin and sulfamethazole. On the other hand, isolates from dogs and bitches displayed high resistance to lincomycin and kanamycin, and relatively lower resistance to norfloxacin and doxycycline. They were found to be sensitive to ciprofloxacin and amoxicillin.

The variations in the prevalence of specific bacteria in different sample groups, along with varying antibiotic resistance profiles for each bacterial species across human and canine samples shown in table 1 and figure 1.

Table 1. Different antibiotic resistance profiles for each bacterial species across human and canine samples

Antibiotic	*E. coli*	*Proteus spp.*	*Klebsiella spp.*	*Staph aureus*
Ceftriaxone	Resistant	Resistant	Resistant	Resistant
Levofloxacin	Resistant	Resistant	Resistant	Resistant
Ofloxacin	Mild Resistant	Mild Resistant	Mild Resistant	Mild Resistant
Ciprofloxacin	Sensitive	Sensitive	Sensitive	Sensitive
Sulfamethazole	Sensitive	Sensitive	Sensitive	Sensitive
Lincomycin	-	High Resistant	High Resistant	High Resistant
Kanamycin	-	High Resistant	High Resistant	High Resistant
Norfloxacin	-	Lower Resistant	Lower Resistant	Lower Resistant
Doxycycline	-	Lower Resistant	Lower Resistant	Lower Resistant
Amoxicillin	-	-	-	Sensitive

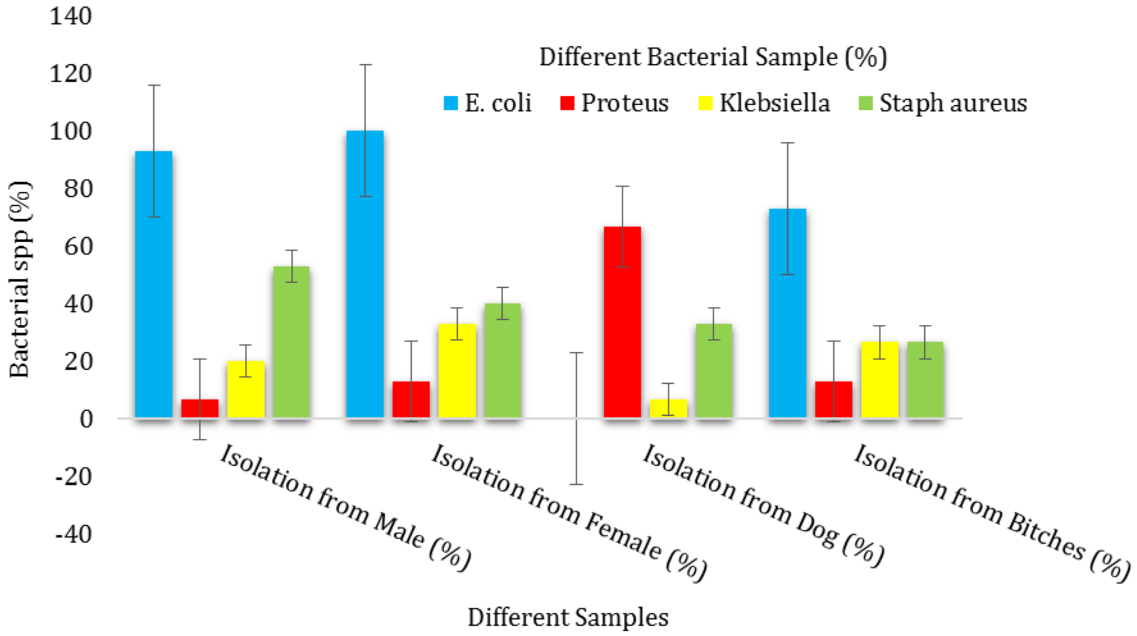

Fig. 1. Variations in the prevalence of specific bacteria in different sample groups (Human and canine)

4. Discussion

Urinary tract infections are common conditions worldwide and the pattern of antimicrobial resistance varies in different regions [25-28]. In this study the diversity of uropathogenic bacteria in both human and dogs along with their antibiotic resistance profile was designed to examine. Male samples investigation showed involvement of *E.coli most* frequently (93%) as compared to *Klebsiella spp.* (20%), *Staph aureus* (33%) and *Proteus spp*. (6.67%). Our findings regarding occurrence of *E.coli* more frequently as compared to other isolates are in agreement with the findings of previous research [29] where *E.coli* was isolated most frequently (80.9%) as compared to *Staph aureus*, *Klebsiella spp and Proteus spp.* Similarly females' samples investigation showed the involvement of *E. coli*, most frequently (100%) as compared to *Klebsiella species* (33%), *Staph aureus* (40%) and *Proteus spp.* (13%) in our hands. These results are concordance with the findings of previous research [30] that In their study *E.coli* was isolated most abundantly as compared to other bacteria. *Escherichia coli* (70%), followed by *Klebsiella pneumonia* (14%), *Streptococcus faecalis* (5.7%), *Acinetobacter* (4.2%), *Staphylococcus aureus* (2.8%), *Candida* (1.4%), *Pseudomonas* (1.4%) and *Proteus* (1.4%).

Our study revealed the less number of bacterial species as compared to previous one So , Similarities and differences in the type and distribution of uropathogens may result from different environmental conditions and host factors, and practices such as healthcare and education programmers, socioeconomic standards, hygiene practices and sample size variation [31-34]. Similarly *E.coli* bacteria were isolated more frequently in the female than males similar to previous study [31].

In the present study 20 urine samples were taken from UTI dogs and bitches each and processed for bacterial diversity. Dogs' samples study revealed the involvement of *E.coli* most common (66.7%), as compared to other isolates *Klebsiella spp.*, (33%) *Staph aureus* (26.6%) and *Proteus spp.* (6.67%). These finding are in agreement with other studies of a previous research [35] where *E.coli* was isolated most frequently among other UTI pathogens *including Staph aureus* (11.6%), *Proteus spp.* (9.3%) and *Klebsiella spp*. (9.1%). In case of bitches, *E.coli* was isolated most frequently as compared to other uropathogens like *Klebsiella spp., (*26.6%), *Staph aureus (*26.6%) *and Proteus spp*. (13%).

These results are in line with the previously reported study results of various researchers [35]. Like in human, in UT infected dogs *E.coli* was isolated most frequently followed by *Staphylococcus spp.*, *Proteus spp, Klebsiella spp.* and *Enterococcus spp.,* So, Case nature and geographical factors may contribute to the differences in the prevalence of bacterial uropathogens. In our study more positivity of uropathogens was observed in bitches as compared to dogs that is in line with the findings of a previous research [36].

The antibiotic resistance profile of human (males and females) isolates was also determined in this study. Among the most prevalent Gram negative bacilli, *E. coli* showed more resistance against ceftriaxone (35.7%) and Levofloxacine (28.5 %) and low rank of resistance to Ofloxacin and (33.3%) *Klebsiella Spp. depicted* resistance to Fosfomycin, Ofloxacin and Levofloxacin and *Proteus spp*. showed 50% resistance against Ceftriaxone. The kind of resistance pattern observed in this study are matching with previous studies of a previous research [37].

In general, Gram negative bacilli were susceptible to the Ciprofloxacin, Sulfamethazole and Urixin. Based on these results, it could be inferred that antibiotics such as ciprofloxacin sulfamethazole and urixin might be useful for the management of Gram negative uropathogens isolated in the study area. In case of Gram positive cocci, it was evidenced that *Staph. aureus* displayed resistance to Cefatriaxone which is comparable to another study conducted by a previous research [37] where tetracycline, ampicillin, amoxicillin and Penicillin resistance were observed and *Staph aureus* showed sensitivity against ciprofloxacin. Hence, overall results of present study could be useful to improve therapeutic tactics of uropathogens in the study area.

Antibiotic resistance profile of isolates originated from dogs and bitches (*Proteus spp. E. coli, Staph aureus, and Klebsiella spp)* exhibited high level of drug resistance to Kanamycin and Lincomycin and 10% of total *E.coli* isolates, showed lowest resistance to tetracyclin while 30% of *Klebsiella spp isolates, depicted* resistances to norfloxacin and 20% to doxycycline and tetracyclin. Similarly 25-28 % resistance was observed in case of total isolated *Staph aureus* to Doxycyclin and tetracyclin our findings regarding occurrence of resistance pattern compared to other study are in disagreement with a previous research [38] where *Proteus spp. E. coli, Staph aureus α-haemolytic Streptococci, and Klebsiella spp.* exhibited high level of drug resistance to all the antibiotics. Although it is inaccurate to compare prevalence data of studies which used different antibacterial sensitivity tests, the results we obtained are different to those described in other publications for the same pathogens in other areas of the world.

Overall total 60-80% isolates originated from dogs and bitches showed the sensitivity against antibiotics used in this study with in the agreement of a previous research [38, 39] where susceptibility of the different isolates varied from 80 to 100% to some of the tested bactericidal agents slight variation regarding to the susceptibility of present and past study was observed due to the different geographical differences ,bioburden and irrational use of antibiotics.

5. Conclusion

Gram-negative bacilli (Enterobacteracea) were mainly responsible for urinary tract infections and most common isolated bacteria from urinary tract infections was *E. coli* both in human and dogs followed by Gram positive cocci and ciprofloxacine best drug of choice in both human and dogs UT infection.

Conflict of Interest

The authors hereby declare that they have no conflict of interest.

Author's contributions

All authors equally participated in designing experiment analysis and interpretation of data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors have read and approved the final manuscript for publication.

Availability of data and material

The authors have embedded all data in the manuscript.

Informed Consent

The authors declare not used any patients in this research.

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