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Research Article

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BACTERIOLOGICAL ANALYSIS OF URINARY TRACT INFECTIONS AT HYDERABAD, SINDH

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ABSTRACT

Urinary Tract Infections (UTI) is a condition in which micro-organisms multiply within the tract to establish infection. The aim of this study was to assess bacteriological profiling of UTI in Hyderabad, Pakistan. Isolation and identification of bacterial uropathogens were carried out using standard microbiological methods such as microscopic analysis, cultural characteristics, and biochemical testing. A total of 320 urine samples were investigated for bacteriological analysis. Among them, 116 (36.26%) urine samples yielded bacterial growth, which included 74 (23.12%) female and 42 (13.13%) male patients. Bacterial distribution revealed that the most frequent isolate was *Escherichia. coli* which accounted 60.34% (n=70) of the isolates followed by *Klebsiella pneumoniae* 13.79% (n=16), *Pseudomonas aeruginosa* 7.76% (n=9), Enterococcus spp. 7.76% (n=9), Streptococcus spp. 4.31% (n=5), *Staphylococcus aureus* 3.4% (n=4), *Proteus mirabilis* 2.59% (n=3). It is concluded that the, bacteriological profiling of urine samples revealed seven different uropathogens circulating in Hyderabad with high prevalence of *E. coli*.

Keywords: *UTI, Bacteria, Uropathogens, E. coli, Pakistan,*

INTRODUCTION

In healthy individuals, the urine is considered sterile, free from actively growing bacteria except the distal region of urethra. UTI is the second most frequent infection in community practice and both outdoor and hospitalized patients can contract UTIs (Najmul & Tanveer, 2013). UTI in asymptomatic individual is the growth of bacteria more than 10^5 colony forming unit (CFU) for each milliliter of urine and colony forming unit of 10^3 per ml for persons with symptoms (Bano *et al.*, 2014). According to Kass, significant bacteriuria is considered if only one species of bacteria found in a clean catch midstream urine sample in concentration of 10^5 CFU (Nandy *et al.*, 2007). According to recent studies a significant infection is considered if any single bacterial counts as low as 10^2 per ml or as few coliform as 10^2 /ml, especially if white blood cells appear in the urine and thus traditional guideline of > 10^5 /ml for UTIs has been modified (Amaeze *et al.*, 2013). Adults are more frequently infected with UTI than children. Signs and symptoms are not clear in children. Boys are repeatedly infected with UTI at the age of less than one year and later on girls are more infected due to short urethra. During reproductive period male female ratio is 1:50. It becomes almost equal in males and females with a ratio 1:1.5 after the age of 50 years (Ali, 2001). Age, sex and nosocomial infection are major factor responsible to prevent natural resistance from UTI (Amaeze *et al.*, 2013). Most of the UTIs are not serious and are treatable. However, the kidney infection can lead to permanent tissue damage and bacteria may enter the blood cause bacteremia (Manikandan *et al.*, 2011). The most common infection after respiratory tract is urinary infection. In developing countries, 250 million people are found to be infected with UTIs yearly (Piranfar *et al.*, 2013). It is estimated that annually 10% of the population is affected by UTI. It is the most common infection in patients admitted to hospitals and according to National Nosocomial Infection Surveillance (NNIS) about 35% - 40% of all hospital acquired infections are UTIs (Najmul & Tanveer, 2013). Urinary tract infections are among the widespread extra-intestinal infections; that infect the people of all ages and at all times, including neonates, preschool girls, young and older men, and women. Bacterial cystitis is the most prevalent infection in women before the age of 24 with a risk of 60% than in men (13%). At the age of 11, girls develop UTIs more commonly (5%) than boys (1%). Urinary tract infections are diagnosed in 150 million people in the world (Javed *et al.*, 2015). It has been estimated that seven million outpatient's visits to clinics all around the world as a result of symptomatic UTIs while at the emergency department there are one million visits and one hundred thousand patients admitted to hospital in a year (Mansour *et al.*, 2009). A previous study conducted in Pakistan in 2014 showed a prevalence rate of UTI as 83.7% and *E. coli* was found to be a more frequent cause of UTI with a rate of 59.8% followed by 7.2% Pseudomonas, 14.7% Proteus, 6.4% Klebsiella, 8.0% Enterococcus and 4.0% *S. aureus* (Yasir, 2014). Urinary tract

infections may be divided into uncomplicated and complicated UTIs. Infection with normal structure and function of the urinary tract in sexually active females is called uncomplicated urinary tract infection (Anusha *et al.*, 2014). Urinary tract infections in which abnormalities are found in the urinary tract like difficulty in urinating, indwelling urethral catheters and need prolonged treatment and there is a risk for failure treatment, are related to complicated urinary tract infection, men are considered to have complicated urinary tract infections (Anusha *et al.*, 2014). Urinary tract infection may be classified according to the location where it causes infection: infection in the bladder is called Cystitis, infection in the kidney is called Pyelonephritis and infection in the urethra is called Urethritis. The majority of urinary tract infections are caused by bacteria (95%). Fungi and viruses may also involve (Nandy *et al.*, 2007). Enterobacteriaceae are the major causative agents of UTIs. About 75 -90% of urinary tract infections are caused by *E. coli* (Shahzad *et al.*, 2013). *E. coli* is a normal flora of the colon and may stick to the urethra, causing infection most commonly catheter-associated UTIs (Komala & Kumar, 2013). Major causative bacteria in nosocomial infections are Klebsiella, Staphylococci, Enterobacter, Proteus, Pseudomonas, Enterococci species, whereas *E. coli* causes infection in outpatients more commonly and *Staphylococci saprophyticus* causes infection in young sexually active women (Mansour *et al.*, 2009). The present study was designed to assess the bacteriological profiling of urine samples at Hyderabad, Sindh.

MATERIALS AND METHODS

Sample Collection: A total of 320 urine samples were collected during seven months period from June 2015 to December 2015. Urine samples were collected randomly from UTI-suspected individuals aged between 6 months to 90 years old who were referred to diagnostic laboratories in Hyderabad. Midstream urine samples were collected by a clean catch method from all patients. Patients were advised to first cleanse the urethral area properly and asked to void the first few drops of urine. The urine of midstream was then collected into a sterile, dry, wide mouth leakproof container. The containers were labelled properly and brought to the laboratory as soon as possible.

Processing of urine specimens: All urine samples were transported to the Research laboratory at the Institute of Microbiology and processed immediately. Before inoculation, the urine samples were mixed by rotating the container and then a loopful was inoculated onto MacConkey Agar and Cystine lactose electrolyte deficient (CLED) agar. The plates were incubated at 37°C for 24h. On the next day, plates were observed for growth. Samples that demonstrated pure growth of isolate in a count of $\geq 10^5$ CFU/ml of urine were considered to indicate significant bacteriuria.

Identification of Uropathogens: The isolates were identified using conventional microbiological methods, on the basis of Gram staining reactions; morphology and cultural growth characteristics then isolated uropathogens were identified using different biochemical tests such as Triple Sugar Iron (TSI) test, Urease test, Simmons' citrate test, Sulfide Indole motility (SIM), Catalase test, Coagulase test and Oxidase test. All tests were performed as per standard methods (Cheesbrough, 2005).

RESULTS

Overall distribution of urine samples: The current study was focused on bacteriological analysis of UTIs in Hyderabad, Sindh. A total of 320 urine samples were investigated for bacteriological analysis. The data demonstrated that 116 samples (36.25%) were positive for bacterial growth. Amongst positive samples, the majority 23.12% samples were from female and 13.13% samples were from male patients (Fig 01). Bacteriological analysis showed higher frequency of Gram-negative bacteria accounting 84.48% (n=98) bacteria whereas the positive Gram-positive bacteria were 15.52% (n=18)

Age wise distribution of positive urine samples: Urne samples of six months age to 90 years old individuals were analysed and distributed in 10 years age groups. Age and Gender-wise distribution of all UTI positive samples demonstrated that the infections were more common in females than males in all age groups except the age group consisting of 61 years and above age, where male ratio was slightly higher than females. The data showed that urine samples of patients of age group under 10 years yielded the highest number of positive cultures of uropathogens. The age group of 31-40 years however showed lowest positivity for uropathogens (Figure. 02).

Distribution of uropathogens: The bacteriological analysis demonstrated that out of 116 cases of bacteriurea, *E. coli* was the most frequent uropathogen accounting 60.34% (*n*=70) followed by *K. pneumoniae* 13.79% (*n*=16) in all patients (Table 01). Whilst the remaining 25.87% isolates included six types of bacteria belonging to both Gram-negative and Gram-positive bacteria: i.e. Proteus spp., Pseudomonas spp., Klebsiella spp., Staphylococci spp., Streptococci spp., and Enterococci spp. (Table 01).

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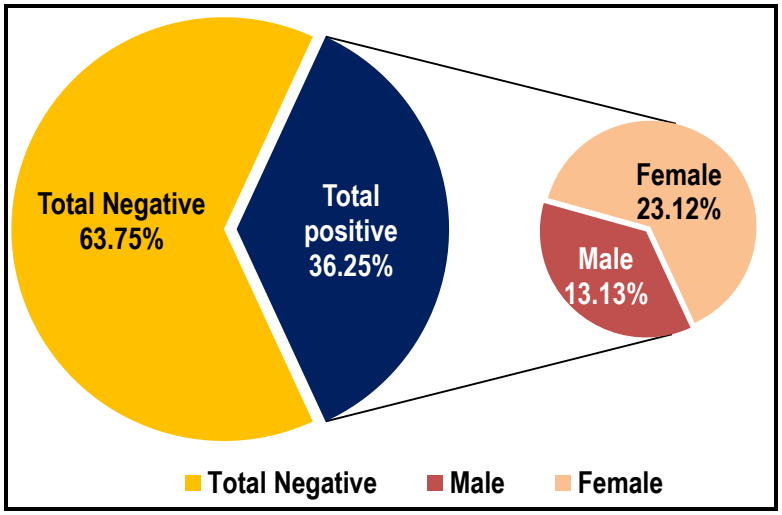

Figure 1. Pie chart showing the gender-wise prevalence of UTI.

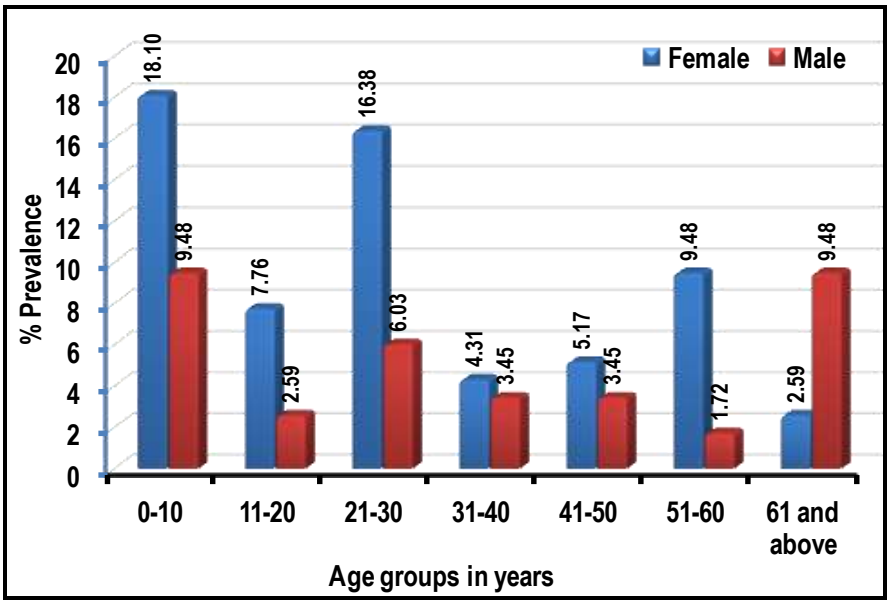

Figure 02. Graph showing the gender and age wise distribution of UTIs.

Table 1. Distribution of uropathogens

Organism (n=116)		**(=n)**	**(%)**
Gram-negative (n=98)	*E. coli*	70	60.34
	Klebsiella spp.	16	13.79
	Pseudomonas spp.	9	7.76
	Proteus spp.	3	2.59
Gram-positive (n=18)	*Enterococcus spp.*	9	7.76
	S. aureus	4	3.45
	Streptococcus spp.	5	4.31

Morphological, Cultural, and Biochemical characteristics of uropathogens: Color, size, and colony appearance of isolates were observed after growth on diagnostic media. On MacConkey agar, *E. coli* showed smooth pink colonies, *Klebsiella pneumoniae* showed pink mucoid colonies. While the Pseudomonas produced green colored colonies (Fig 03).

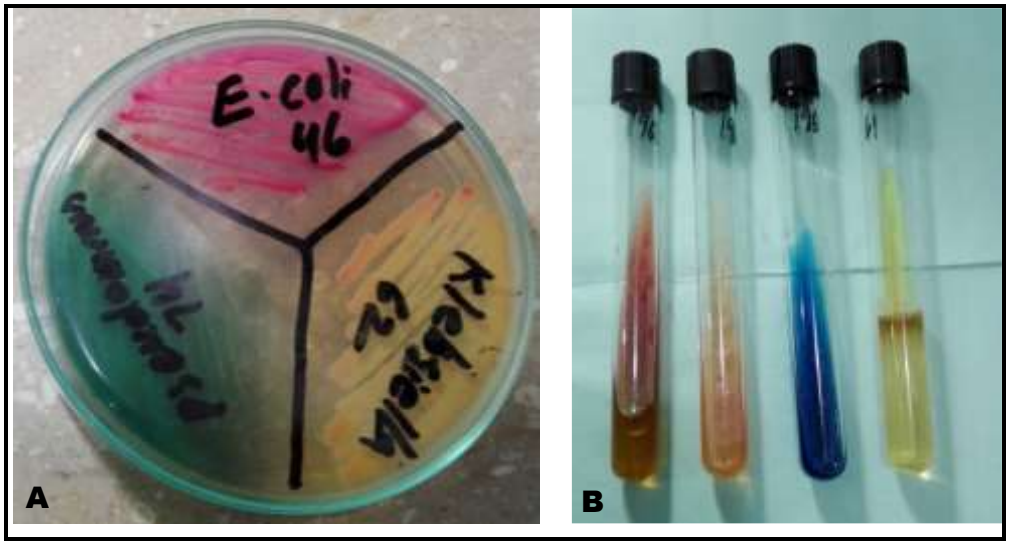

Figure 03. Representative pictures demonstrating (A) Pure cultures of uropathogen on MacConkey's Agar (B) Biochemical profiling of Pseudomonas spp. isolated from urine samples.

DISCUSSION

The present study reports the bacteriological profiling of UTI at Hyderabad. UTIs are the common cause of illness and major public health problems and are among the most common infections worldwide. The causative agents of UTI are developing resistance to frequently used antibiotics (Yasir, 2014). The current study pointed out the relationship between gender, isolated uropathogens and antibiotic sensibility. One of the most common bacterial infections that clinicians treat frequently in developing countries is UTI. Monitoring studies focused on a particular area to obtain more knowledge about the bacteria causing UTIs and how they respond to the treatment might help medical professionals to choose the best treatment strategy for preventing the infection based on the published data (Mustafai *et al.*, 2023).

This study indicated that children are more commonly infected with urinary infections. Approximately 1 in 3 women will require antimicrobial treatment for a UTI before age 24, and 40% to 50% of women will have a UTI during their lifetime. In this study, the greater part of the positive samples was female, a similar trend has been reported in other studies (Mansour *et al.*, 2009). The increased frequency of UTI in females may be due to various factors including the shortness of the urethra and its closeness to the anus (Khan *et al.*, 2013).

One of the most prevalent infectious disorders in humans is urinary tract infection. Gram-negative bacteria such as *E coli*, Acinetobacter spp., *P. aeruginosa*, Proteus spp., and Klebsiella spp. are usually responsible for causing UTIs in humans (Ahmed *et al.*, 2020). These findings agree with the published data from other regions indicating that *E. coli*, Gram-negative bacteria was the most common isolated bacteria from urine samples. The bacteriological profiling revealed that *E. coli* was a highly prevalent bacterial uropathogen followed by Klebsiella spp. and Pseudomonas spp. and Enterococcus spp. The increasing resistance to antibiotics is making it more difficult to treat urinary tract infections. Urinary tract infections cannot be diagnosed based only on clinical criteria, rather, the confirmation of a UTI requires an analysis of bacteria in the urine of patients bladder (Mehboob *et al.*, 2021).

CONCLUSION

In conclusion, seven different types of bacteria belonging to both Gram-negative and Gram-positive groups were recovered from UTIs. The most frequent isolate was *E. coli*, followed by Klebsiella, Pseudomonas, Enterococcus spp. The majority of samples belonged to female patients and age group under 10 years were found to be favorable to UTI at Hyderabad.

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