

DISTRIBUTION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF UROPATHOGENS CAUSING URINARY TRACT INFECTIONS IN THE PATIENTS VISITING PIMS ISLAMABAD

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Abstract

Urinary tract infections (UTIs) pose a significant burden on healthcare systems worldwide, with a prevalence rate of 20% among patients attending Pakistan Institute of Medical Sciences (PIMS) Islamabad. This study aimed to investigate the distribution and antimicrobial susceptibility patterns of uropathogens causing UTIs among patients visiting PIMS Islamabad. A total of 180 urine samples, collected from patients presenting clinical evidence of UTIs, were analyzed over a two-month period. The majority of patients affected were females (65%) compared to males (35%), with a higher prevalence observed in the age group of 15-25 years. Escherichia coli emerged as the most prevalent uropathogen (33.88%), followed by Candida albicans (30%) and Klebsiella pneumoniae (23.33%). Gram-negative bacteria constituted 62.77% of isolates, with Gram-positive cocci accounting for 7.06%. Antimicrobial susceptibility testing revealed varying resistance patterns among uropathogens, with Penicillin exhibiting the highest resistance (100%) and Vancomycin demonstrating the highest sensitivity (100%). Notably, Amikacin and Nitrofurantoin displayed notable efficacy against E. coli. These findings underscore the importance of targeted antimicrobial therapy in UTI management. The study provides valuable insights into the epidemiology and antimicrobial resistance profile of UTIs in the region, aiding in the development of effective treatment strategies tailored to local conditions. This research contributes to the understanding of UTI dynamics, facilitating improved clinical management and patient outcomes in PIMS Islamabad and beyond.

INTRODUCTION

Urinary tract infections are highly prevalent, with approximately 250 million cases occurring worldwide each year. Diagnosis is based on the presence of over 10⁵ colony forming units (CFU) per ml of urine for

asymptomatic individuals, and around 10³ CFU/ml for symptomatic individuals (Gupta, Sahm, Mayfield, & Stamm, 2001). Urinary tract infection is second most frequent infection and has been analyzed

normally in outpatient and hospitalized (Gonzalez & Schaeffer, 1999). These are also the most broadly recognized infections experienced in therapeutic practices (Dimitrov, Udo, Emara, Awni, & Passadilla, 2004). Approximately 10% of the population is diagnosed positive for urinary tract infection annually (Fluit & Schmitz, 2001). UTIs account for 35 % of all nosocomial infections reported to the National Nosocomial Infection Surveillance (NNIS) system U.S.A (Gales, Jones, Gordon, Sader, Wilke, & Beach, 2000). UTIs are prevalent among people of all ages and genders, with females at higher risk. The prevalence varies based on age and sex. Literature suggests that at least 20% of males and 20–80% of females experience UTIs at least once in their lifetime (Griebing, 2013;Kunin, 1994). In Pakistan, the prevalence of urinary tract infections in febrile children is assessed to be 12% (Afzal & Naemullah, 2008).

Urinary tract infection is thought to be the most broadly recognized bacterial infection and is said to be accountable for with respect to 5% of all visits to crucial thought specialists (Michael, Wilson, & Gaido, 2004). UTIs affect around 40% of females and 12% of males at least once in their lifetime. Recurrence rates are challenging to determine due to underreporting. Certain groups, such as infants, pregnant women, spinal cord injury patients, and those with certain medical conditions, are at higher risk. Catheter-related UTIs are prevalent in healthcare settings, with over one million cases reported annually. In the elderly, UTIs rank as the second most common infection, comprising nearly 25% of all infections. UTIs carry significant medical and financial implications (Foxman, 2002).

Uncomplicated urinary tract infections (UTIs) occur in a normal genitourinary tract without prior instrumentation, while complicated infections involve abnormalities or instrumentation such as indwelling urethral catheters, often presenting with no symptoms (Gonzalez & Schaeffer, 1999). Frequency of UTI is higher in female than male, 40% to half of whom will suffer at least one clinical episode in their lifetime (Leigh, 1990). UTIs can affect both lower and upper urinary tracts. Cystitis refers to lower UTIs, characterized by symptoms like dysuria, frequency, urgency, and suprapubic tenderness. These symptoms differ from those of upper UTIs, which are often

present in most UTI cases (Mandell, Bennett, & Dolin, 2010). UTIs are common in clinical practice and the community, affecting about 10% of the population over their lifetime (j, T.T, A.R, K, A, & W, 2000). The common bacterium responsible for UTIs is *Escherichia coli*, both in the community and hospitals (Gorbach, Bartlett, & Balcklow, 2004).

As urinary tract infections (UTIs) are most common in females, most studies on the treatment of common UTIs in outpatients have been performed on females, normally premenopausal females (lipskyba, 1989). Although the rate of UTI is lower at young age, it rises with age; 33% of each of the 80-year-old males will have had an episode of bacteriuria (TL, 2005).

UTIs are generally caused by *Escherichia coli* representing more than 70% of uncomplicated cases both in outpatients and inpatients (Gupta *et al.*, 2001). Other Gram-negative microorganisms include *Klebsiella* spp., *Enterobacter* spp., *Pseudomonas aeruginosa.*, *Proteus* spp. Gram positive bacteria represent 5 to 15% of UTIs and include *Enterococcus* spp, *Staphylococci* and *Streptococci* (Hryniewicz *et al.*, 2001; Akram *et al.*, 2007).

Materials and methods:

The study employed a variety of materials including urine containers, bacteriuritest strips, Petri dishes, CLED agar, autoclaves, incubators, nutrient agar, cotton swabs, and antibiotic disks. CLED agar, a selective medium containing enzymatic digests and lactose, was utilized for the isolation and characterization of urinary pathogens (MacFaddin & D, 1985). Nutrient agar, augmented with peptic and beef extracts, served as a versatile substrate for culturing microorganisms (Lapage, Shelton, & Mitchell, 1970). Antibiotic susceptibility testing was conducted using discs from Oxoid Limited, USA, employing in vitro diffusion techniques.

Study area:

The study was carried out in Microbiology Laboratory of Pakistan Institute of Medical Sciences (PIMS), Islamabad. The urine samples were collected from OPDs (out patients departments). The duration of study was two months from August 2023 to September 2023.

Study population:

The urine samples of 180 patients comprised of 67 males and 113 females who attended the OPDs (out patients department) of PIMS and had clinical evidence of urinary tract infection, determined by physicians, were included in this study. The age range of patients included in this study was from 5 years to 100 years.

Sample collection:

Clean catch midstream urine was collected from each patient into 20ml calibrated sterile screw -capped universal container which were provided to patients. The specimens were labeled, transported to laboratory and analyzed within 6 hours. All patients were well instructed on how to collect sample aseptically prior to sample collection to avoid contamination from urethra. The study was conducted after due ethical approval granted by hospital administration.

Sample processing:

A bacteruritest strip method was used for isolation of bacterial pathogen for urine samples. The sterile bacteruritest strip was used which delivered 1ml of urine. The bacteruritest strip was placed on cysteine-lactose -electrolyte deficient agar (CLED). The inoculated plate was incubated at 37°C for 24 hours and for 48 hours in negative cases. The number of isolated colonies was multiplied by 1000 for estimation of bacterial load /ml of urine sample. The specimen was considered positive for UTI if an organism was culture at a concentration of $\geq 10^5$ cfu/ml or when an organism was cultured at a concentration of $\geq 10^4$ cfu/ml and 5 pus cell per high power field were observe on microscopic examination of the urine.

Identification and maintenance of pure bacterial isolates:

The Identification of bacterial isolates was done on the basis of their culture characteristics and biochemical characteristics under the supervision of a qualified lab supervisor having 5 years of experience. On the basis of colony morphology; Klebsiella spp was read as pink with mucoid colonies, greenish to bluish colonies for Proteus, creamy and raised colonies for *Staphylococcus aureus*, yellow and slightly raised colonies for Pseudomonas, white small colonies for

E.coli and colorless small colonies for streptococcus. Biochemical tests; catalase, coagulase and DNase were also performed.

Antibiotic susceptibility test:

Isolates were tested for antimicrobial susceptibility by Kirby-Bauer disc diffusion methods. Standard inoculums adjusted to sterile cotton swab sticks were streaked on whole Petri dish having nutrient agar for susceptibility. Nutrient agar plates were incubated at 37°C for 24 hours. After 24 hours, zones of inhibition were measured and interpreted by suggestion of clinical and research facility standard. The following standard antibiotic discs were used for isolates, Amikacin (AK), Amoxiclavate (AMC), Azithromycin (AZM), Chloramphenicol (C), Ceftazidime (CAZ), Cefixime (CFM) Ciprofloxacin (CIP), Ceftraxone (CRO), Clindamycin (DA), Erthromycin (E), Nitrofeurotin (F), Fosfomycin (FOS), Gentamycin (CN), Imipenem (IPM), Levofloxacin (LEV), Minocycline (MH), Nalidixic acid (NA), Norfloxacin (NOR), oxacillin (OX), Penicillin (P), Polymxin B(PB), Piperacillin (PRL), Cefaperazone (SCF), Cotrimizole (SXT), Tetracyclin (TE), Tigacycline (TGC), Tobramycin (TOB), Tazobactam (TZP), Vancomycin (VA).

Results:

The overall prevalence of UTI's in females and males was found to be 20 %. Total 180 urine samples showed the significant bacterial growth which comprised of 63 (35%) samples from males and 117 (65%) from females. These results indicated that the prevalence of UTI was higher in female patients than in males. The distribution value showed the significant variation between male and female patients.

Among 180 positive culture 113 (62.77%) were to be found Gram negative while 13 (7.06%) were Gram positive. *Escherichia coli* was found to be the dominant bacterium among all isolated uropathogens with the distribution rate of 33.88%. The second most prevalent isolated organisms was *Candida albicans* (30%), followed by *Klebsiella pneumonia* (23.33%), *Pseudomonas aeruginosa* (5.55%), *Enterobacter* spp. (2.77%), *Staphylococcus aureus* (1.66%) and *Enterococcus* species (1.66%) (Figure 1).

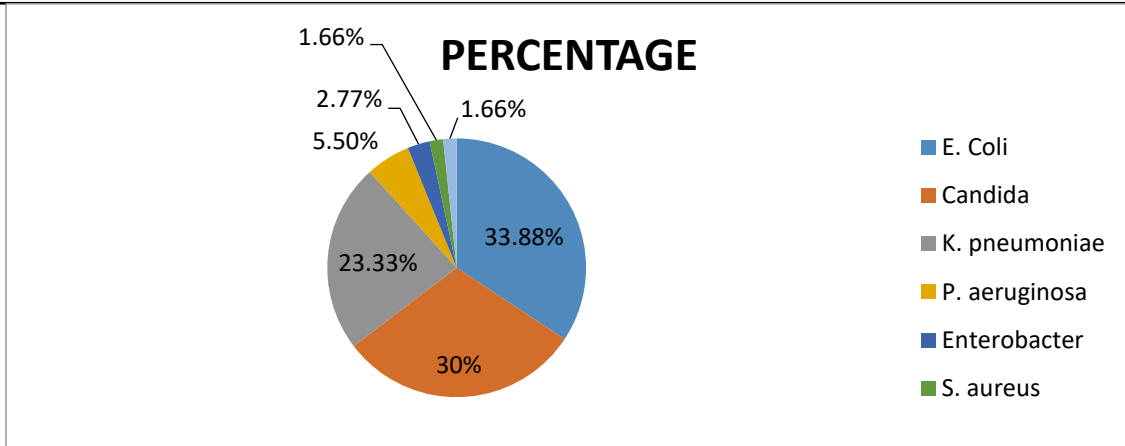


Figure 1: Distribution of isolated uropathogens according to percentage

Distribution of uropathogens according to sex showed that streptococci (95%) are most abundant uropathogens found in females followed by Staphylococcus aureus (90%), Candida albicans (73.68%), E.coli (68.80%), Klebsiella pneumoniae (52.38%) and Pseudomonas aeruginosa (20%) respectively. In male the distribution of uropathogens showed

that Pseudomonas aeruginosa (80%) are most abundant uropathogen followed by Klebsiella pneumoniae (47.60%), E. coli (31.40%), Candida albicans (22.22%), Staphylococcus aureus (10%) and streptococci (5%) respectively (Figure 2).

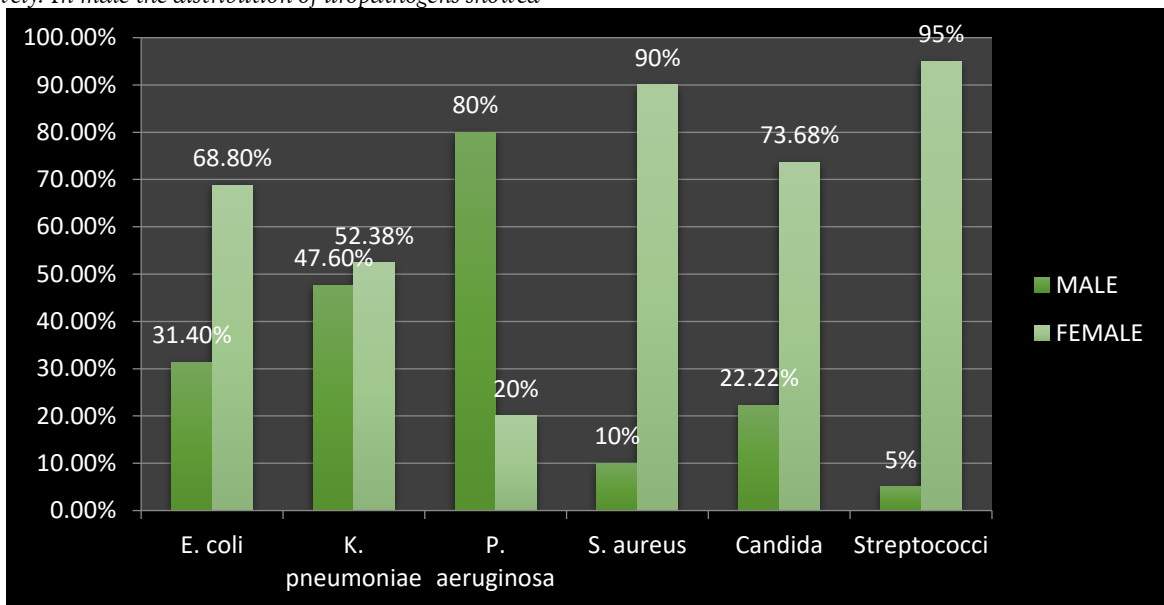


Figure 2: Distribution of uropathogens according to sex

The most susceptible age group of patients to UTI was between 15-25 years (23%) followed by 40-55 years (20%), 25-40 years (19.4%), 55-70 years (15%), 70-85 years (8.8%), 5-15 years (3.3%) and 85-100 years (1.1%). Comparatively, however, more cases of UTI were observed in females than in males in all age

groups. The highest prevalence of UTI in females was found in the age group of 25-40 years (17.22%); however, in males the highest susceptible age group to UTI was 15-25 years (9.4%) (Figure 3).

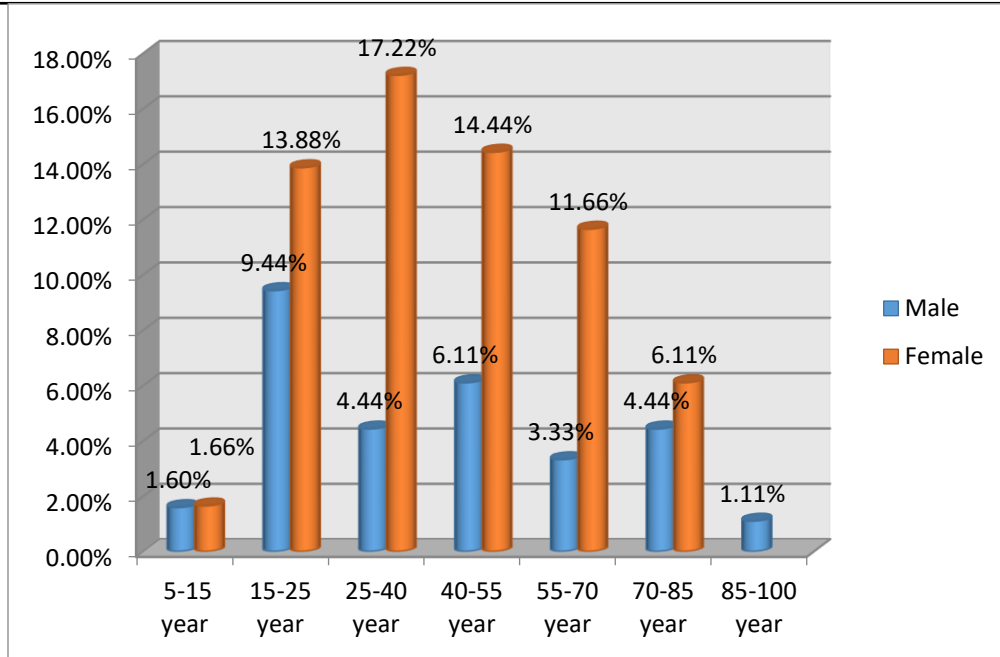


Figure 3: Distribution of UTIs according to age and gender

Out of 118 Gram negative bacteria 70 (59%) were isolated from females and 48 (40%) were from male patients. Out of 8-gram positive bacteria Only 2 (25%) gram positive bacteria were isolated from male and 6 (75. %) were isolated from female patients. The highest number of gram positive

and negative uropathogens 23 was found in the female patients of the age group 25–40 years followed by 8 uropathogens which were isolated from the male patients with the age group 15-25 years (Figure 4).

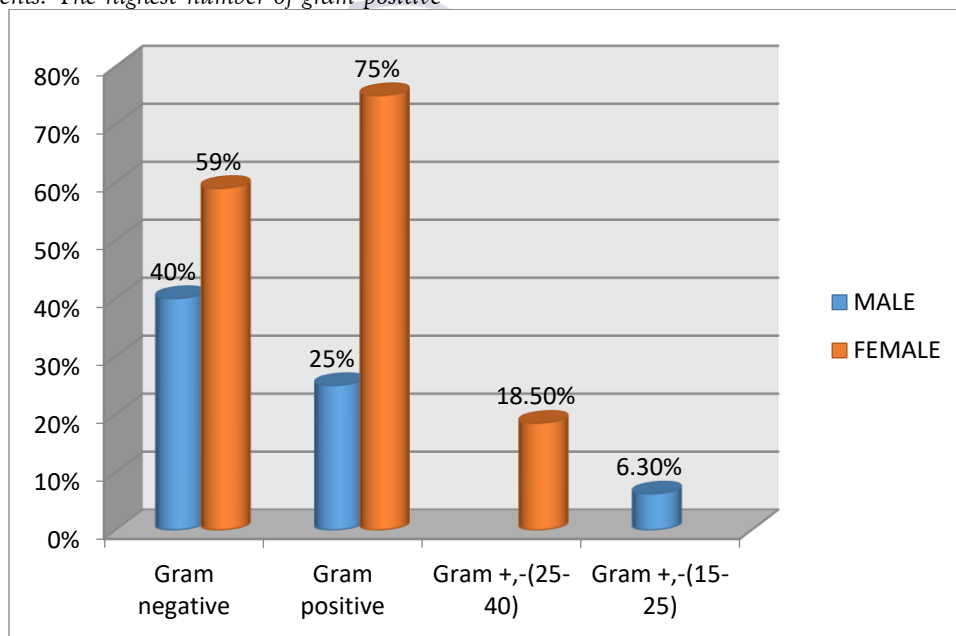


Figure 3: Distribution of gram positive and gram-negative bacteria according to age groups and gender

Antibiotic susceptibility results showed the resistant and susceptible antibiotics for the tested

uropathogens. Overall uropathogens were found the most resistant against Penicillin 8 (100%) followed by

Gentamycin 2 (90%) Norofloxacin 2 (90%) respectively. However, uropathogens were found most sensitive to Vancomycin 8 (100%) followed by Amikacin 93 (80%), Imipenim 97 (78.86%), Nitroferotin 74 (63.15%), Tozobactem 73 (60.33%),

Tobramycin 6 (60%), Tecoplanine 7 (50%), Levofloxacin 8 (47%), Choloromphenicol 3 (40%), and Monocycline 7 (40%) respectively (Figure 5).

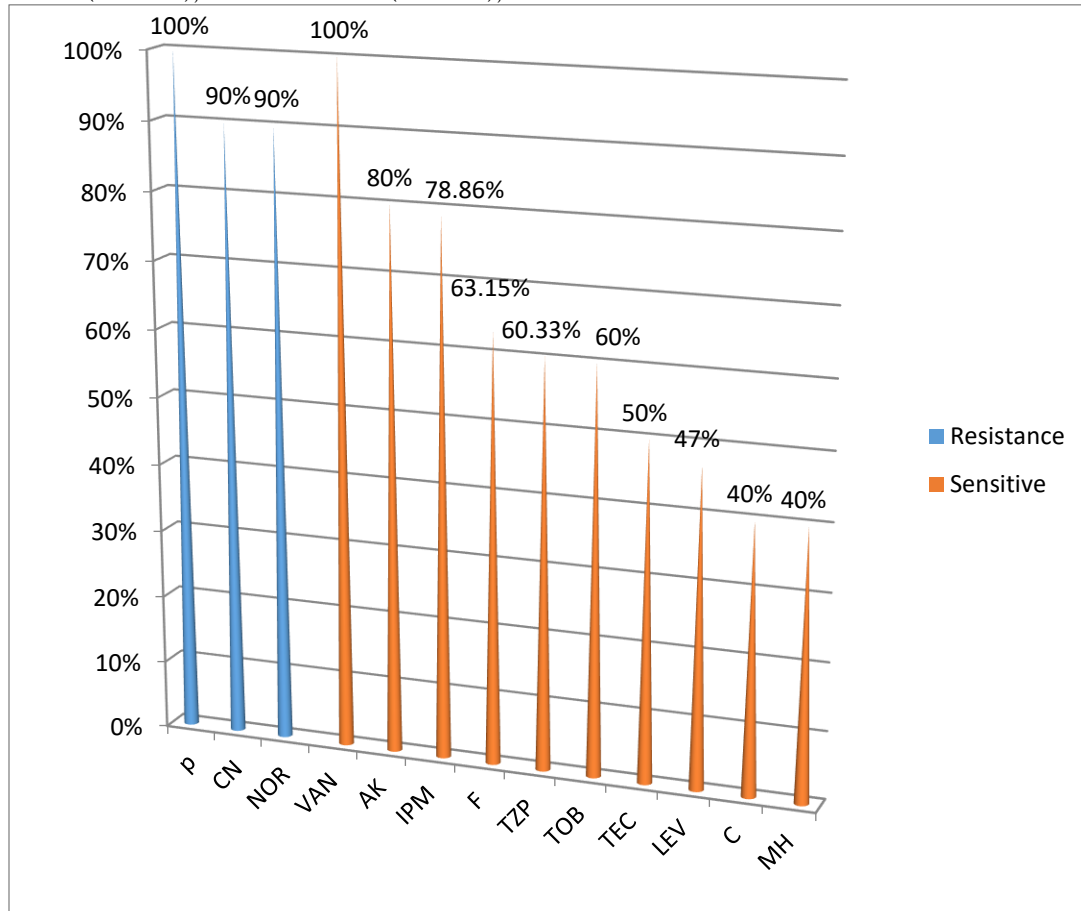


Figure 5: Resistance and sensitivity of antibiotics to uropathogens

Nalidixic acid was found the highest resistant drug against (83.61%) *E.coli* followed by Ceftriaxone (77%) and Ciprofloxacin (75%); however Imipenem (85.2%) followed by Tozobactem (78.6%). Amikacin and Nitroferotin were (68.1%) showed the highest sensitivity against *E. coli*. 90% of *K. pneumonia* were resistant to Cotrimoxazole and Amikacin was found to be the most susceptible drug against *K. pneumonia* with the rate of 80.9%. In case of *P. aeruginosa* the highest resistant and susceptible antibiotics were

Piperacillin (90%), and Amikacin (70%) respectively. 100% of tested Enterobacter were resistant against Cotrimoxazole and 100% sensitive against Amikacin. Enterococcus spp. showed (100) % resistance against Penicillin and Ciprofloxacin however, and (90%) were sensitive to Vancomycin, Amoxiclavate and Tecoplanin. All *S.aureus* (100%) showed resistance against Fosfomycin, Erythromycin and Tozobactem; however, Vancomycin was found 100% sensitive (Figure 6).

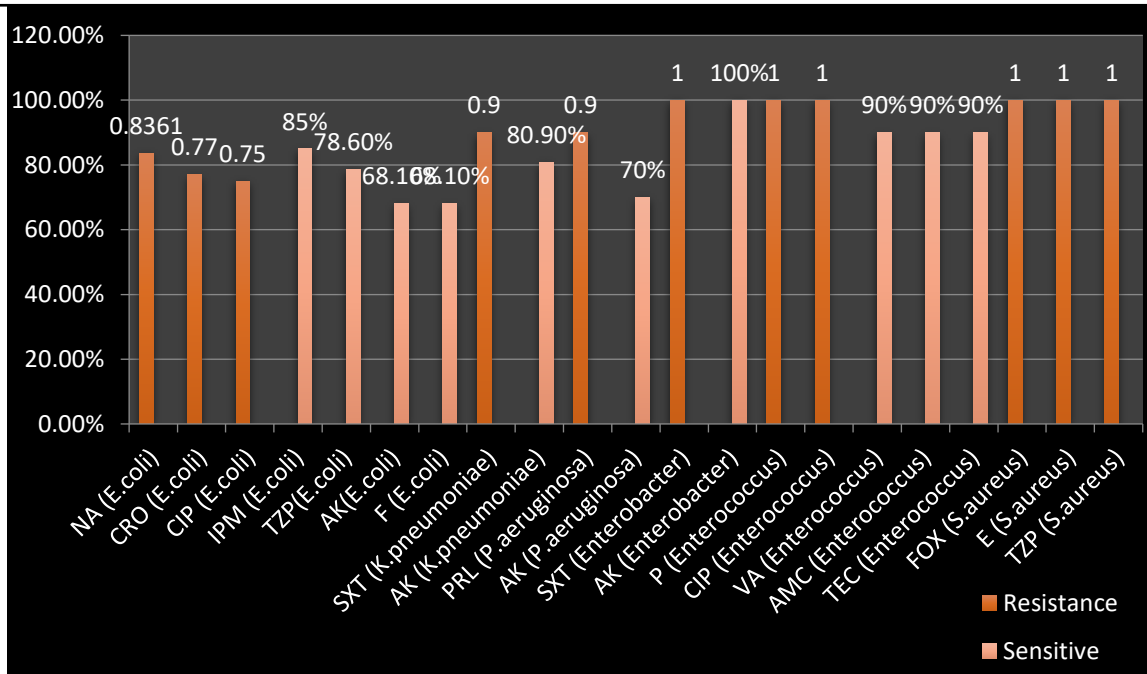


Figure 6: Resistance and sensitivity of antibiotics among different uropathogens

Discussion:

This study provides valuable data to compare and monitor the status of antimicrobial resistance among uropathogens to improve efficient empirical treatment. Increasing antimicrobial resistance has been documented globally (Nedolisa, 1998). The prevalence of UTI was found to be 20% in this study and this rate of prevalence is more than in the other studies which accounts for 25.5% in Argentina (Hugo E Villar, Mónica B Jugo, & Alejandro, 2014), 23.2% in KSA (Al-Jiffri, Zahira, El-Sayed, & Fadwa, 2011), 30.5% in Nepal (Lamichhane, Thapa, Banjara, & Acharya, 2015), 48% Nigeria (Agbagwa & Ifeanacho, 2015), 4.2% and 6.3% in Iran (Kashif, Djavid, & Sahba, 2010) however, the prevalence rate of UTI 35.5% in our study correlates with other studies done in Hazara division (Sajed, et al., 2014). In the Lahore Pakistan population, which showed such more highly significant uropathogens and 52.1% (Anis-ur-Rehman, Jahanzeb & Siddiqui, 2008).

Our study showed a high prevalence of UTI in females (65%) than in males (35%) which correlates with other findings which revealed that the frequency of UTI is greater in females as compared to males. The highest susceptible age group of patients to UTI was between 15-25 years (30%) followed by 40-55 years (20%), 25-40 years (19.4%), 55-70 years (15%), 70-85

years (8.8%), 5-15 years (3.3%) and 85-100 years (1.1%) this study differ from other studies done in Nigeria and KSA in which the highest group of incidences recorded of UTI among the age group 5-40 year (48.1%) and the lowest among the age group 50-100 year (47.2%). However, our results agree with the study done in Japan with a 20-year period in which a trend of increasing complicated UTI was reported in elderly patients (Prakash & Sahai, 2013). Females of age group (25-40) years were found more susceptible to UTI (17.22%) followed by females of age group (40-55) years for which rate of UTIs was found to be 14.44% while rate of UTIs was 13.88% for females of age group (15-25) years. The findings correlate with report which shows that females are more prone to UTI than male during adolescence and adulthood (Prakash & Sahai, 2013). The increased incidence of UTIs in young female are associated with high sexual activity of recent use of diaphragm with spermicide and history of recurrent UTI. In this study, the Gram-negative bacilli constituted 62.7% of the total bacteria isolates while Gram positive cocci constituted 7.06%. *Escherichia coli* (33.88%) was found the most prevalent gram-negative bacteria in the positive urine samples of UTI. The second most prevalent isolated was *Candida albicans* (30%), followed by *Klebsiella pneumoniae* (23.33%), *Pseudomonas aeruginosa* (5.55%),

Enterobacter spp. (2.77%), *Staphylococcus aureus* (1.66%), Enterococcus spp. (1.66%). However, *P. aeruginosa* was reported as the second most common bacterial isolate in UTI studies in India (D. H. Tambekar, 2006) and Lafia, Nigeria (A. S. Kolawole, 2009). Higher incidence of gram-negative bacteria, related to Enterobacteriaceae, in causing UTI has many factors which are responsible for their attachment to the uroepithelium. In addition, they are able to colonize in the urogenital mucosa with adhesins, pili, fimbriae, and P-1 blood group phenotype receptor (Warren, Abrutyn, Hebel, Johnson, Schaeffer, & Stamm, 1999).

The most sensitive drug against all uropathogens was Vancomycin 8 (100%) followed by Amikacin 93 (80%), Imipenem 97 (78.86%), nitrofurantoin 74 (63.15%), Tozobactam 73 (60.33%), Tobramycin 6 (60%), Tecoplanin 7 (50%), Levofloxacin 8 (47%), Chloramphenicol 3 (40%), and Minocycline 7 (40%). Amikacin and Nitrofurantoin were (68.1%) showed the highest sensitivity against *E. coli*. 90% of *K. pneumonia* was resistant against Cotrimaxazoles and Amikacin was found the most susceptible drug with the rate of 80.9%.

Penicillin was found the most resistant drug as 8 (100%) uropathogens were found resistant against penicillin. The second most resistant drug was gentamycin 2 (90%) followed by norfloxacin 2 (90%); however, the most sensitive drug against all uropathogens was vancomycin 8 (100%) followed by amikacin 93 (80%), imipenem 97 (78.86%), nitrofurantoin 74 (63.15%), tozobactam 73 (60.33%), tobramycin 6 (60%), tecoplanine 7 (50%), levfloxacin 8 (47%), Chloramphenicol 3 (40%), and Minocycline 7 (40%). Nalidixic acid was found the highest resistant drug against (83.61%) *E. coli* followed by ceftraxone (77%) and ciprofloxacin (75%); however imipenem (85.2%) followed by tozobactam (78.6%). In case of *P. aeruginosa* the highest sensitive and resistant antibiotics were Piperacillin (90%) and Amikacin (70%) respectively.

Conclusion:

Due to scarcity of reports of UTI in Rawalpindi and Islamabad, this study aimed to uncover the commonness of UTI, the impact of gender and age on its prevalence and susceptibility profile of uropathogens in the cities of Rawalpindi and

Islamabad. This study provides useful information regarding the status of antimicrobial resistance among uropathogens. Furthermore, the outcome of this research can be used to improve treatment suggestions specifically in studied geographic area and generally at national scale. Additionally, the results permit overview of the clinical picture of UTIs in PIMS Islamabad with different districts inside and outside the capital city.

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