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ORIGINAL ARTICLE

Bacterial Profiles And Antibiotic Sensitivity Of Uro-Pathogens In Patients With Urinary Tract Infection: Insights From A Tertiary Care Hospital.

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Abstract:

Background: Urinary tract infection (UTI) is one of the most common illnesses, the mankind is suffering from and around 150 million people have been affected worldwide. UTI is defined as when a bacterium, mainly from the skin or rectum, enter the urethra and infect the urinary system. It affects kidney, ureter, bladder, and urethra, and other parts of the urinary tract

Objective: This study aimed to determine the common microorganisms that causes UTIs and evaluate the in vitro susceptibility and resistance pattern of these microorganisms to common antibiotics.

Materials and Methods: This cross-sectional observational and prospective study was conducted after ethical approval (IRB No. 1665) at the Pathology Department, Hayatabad Medical Complex, from December 5, 2023, to January 5, 2024. Patients with clinically diagnosed UTIs undergoing urine culture and sensitivity tests were included. The sample size (150) was calculated using Open Epi, assuming an 11% prevalence, 95% confidence interval, and 5% margin of error. Consecutive sampling was used, and demographic data were collected via a self-structured questionnaire. Data was entered and analyzed through SPSS version 2020.

Results: Among 150 patients (80 males, 70 females), *E. coli* was the most common uro-pathogen (69%), followed by *Candida*, *Enterobacter*, *Providencia*, *Klebsiella*, and *Serratia*. No significant gender-based difference in uro-pathogen prevalence was observed ($p = 0.339$). *E. coli* showed high sensitivity to Clindamycin (82.6%), while Nitrofurantoin was most effective against *Candida* (94.1%). Tigecycline exhibited strong activity against *Enterobacter* (83.3%) and *Providencia* (94.7%), while Colistin was 100% effective against *Klebsiella*. *E. coli* had the highest resistance to Imipenem (34.8%), while *Candida* showed high resistance to Colistin (64.7%).

Conclusion: This study concluded that *E. coli* was the most usually isolated bacteria and the most frequent cause of urinary tract infections among the studied population. The urinary tract infection was mostly reported in males and the infectious bacterial isolates showed multi-drug resistance against the tested antibiotics that are commonly used to treat urinary tract infection.

Keywords: UTI, Antibiotics, Culture & Sensitivity, Urine

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Introduction:

Urinary tract infection (UTI) is one of the most common illnesses, the mankind is suffering from and around 150 million people have been affected worldwide (1). UTI is defined as when a bacterium, mainly from the skin or rectum, enter the urethra and infect the urinary system (2). It affects kidney, ureter, bladder, and urethra, and other parts of the urinary tract. Among humans the disease is most common in females and that is because of the shorter urethra in females as compared to males which makes it easier for the microorganism to ascend and infect the urinary system (3). It may also be because prostatic secretion lacks bactericidal properties, making it easier for fecal flora to contaminate the urinary system (4). There are various microorganisms that can cause UTIs, the most common pathogens in the community responsible for simple UTIs, and account for about 75% of isolates are *Escherichia coli* and other *Enterobacteriaceae*. In patients who have been hospitalized the resistant Gram-negative rods, such as, *Enterococcus faecalis*, and *Pseudomonas spp* takes precedence in causing UTIs. The proportion of the pathogens varies with sex, age, catheterization and hospitalization, (5). In underdeveloped nations, such as Pakistan, the usual treatment of UTIs is typically empirical, which contribute to the emergence and spread of antibiotic resistance strains, and a major cause of treatment failure (6). Resultantly, rising in prevalence of antibiotic resistance in urinary pathogens globally. The chances of successfully completing another empirical attempt are significantly reduced by associated resistance, where a bacterium that is resistant to one antibiotic is much more likely to be resistant to other antibiotics as well (7). All over the world, there are profound regional variations in the rates of resistance to the most widely prescribed medications used to treat UTIs.

Estimating the local antibiogram profile may help determine the best empirical course of action (8). Therefore, it is important for healthcare providers and administrators to have a blue print of the local microorganism's prevalence, antibiotic resistance and susceptibility in order to make informed decision. The purpose of this study is to determine the common microorganisms that causes UTIs and evaluate the in vitro susceptibility and resistance pattern of these microorganisms to common antibiotics.

Material and Methods:***Study Design and Setting***

This cross-sectional observational and prospective study was conducted in the Department of Pathology, Hayatabad Medical Complex, Peshawar, from December 5, 2023, to January 5, 2024, after obtaining ethical approval from the Institutional Review Board (**IRB No. 1665**).

Population and Sample Size

The study included patients aged 18 years and above of either gender with clinically diagnosed urinary tract infections (UTIs) who presented to outpatient departments for routine medical checkups and were advised to undergo urine culture and sensitivity testing. The clinical diagnosis was based on urinary symptoms (dysuria, suprapubic pain, fever) and urinalysis findings (presence of pus cells and positive nitrites). Patients who were catheterized, or having recent history of urinary tract instrumentation, or those who were pregnant were excluded from the study. The sample size was calculated using OpenEpi, assuming a uro-pathogen prevalence of 11%, 95% confidence interval, and 5% margin of error, yielding a total of 150 patients. A consecutive sampling technique was employed. Demographic and clinical

data were collected using a self-structured questionnaire. Data were entered and analyzed using SPSS version 20.

Specimen Collection and Processing:

After obtaining informed consent, patients provided 5–10 mL of midstream clean-catch urine (MSU) under sterile conditions. Specimens were transported to the microbiology laboratory and processed within 2–3 hours; if delayed, samples were stored under optimum refrigeration conditions until analysis. Urine samples were cultured on 5% Nutrient Agar and Mueller-Hinton Agar using the streak plate method, following standard microbiological protocols. Plates were incubated aerobically at 37°C for 24 hours and examined by experienced microbiologists (≥ 3 years of practical experience) for bacterial growth. The colony count was determined, and any culture with $\geq 10^5$ CFU/mL of pure bacterial growth was considered significant bacteriuria, while negative cultures showed no growth or mixed urogenital flora (>2 different isolates). Urine cultures with significant bacteriuria were further analyzed based on their physical characteristics such as colony morphology, swarming, odor, spread on the culture medium, Gram-reaction, and biochemical reaction pattern using the standard procedures for identification

significant bacteriuria were obtained, a bacterial suspension was prepared by emulsifying colonies in 5 mL of sterile saline (0.85% NaCl) to achieve 0.5 McFarland turbidity standards. The inoculated plates were allowed to dry for 3–5 minutes at room temperature before antibiotic discs were applied. For susceptibility testing the following antimicrobial discs were used with a specific concentration: Gentamicin (CN, 10 μ g), tetracycline (TTC, 30 μ g), colistin (CT, 10 μ g), nitrofurantoin (F, 30 μ g), imipenem (IPM, 10 μ g), meropenem (MEM, 30 μ g), ciprofloxacin (CIP, 5 μ g), clindamycin (DA, 10 μ g), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 μ g), chloramphenicol (C, 30 μ g), nalidixic acid (NA, 30 μ g), ceftazidime (CAZ, 30 μ g), norfloxacin (NOR, 10 μ g), amoxicillin-clavulanic acid (AMC, 20/10 μ g), erythromycin (E, 15 μ g), and cefotaxime (CTX, 30 μ g). Notably, meropenem (MEM, 30 μ g), nalidixic acid (NA), cefotaxime (CTX, 30 μ g), tetracycline (TTC, 30 μ g), ceftazidime (CAZ, 30 μ g) can be used only for gram-negative bacteria, while clindamycin (DA, 10 μ g), and erythromycin (E, 15 μ g) can be used only for gram-positive bacteria. The zone of inhibition around each antibiotic disc was measured in millimeters, and susceptibility was classified as sensitive, intermediate, or resistant based on CLSI 2020 criteria. All antimicrobial discs were sourced from Oxoid Ltd.

Antibiotic Susceptibility Testing:

Antibiotic susceptibility testing was performed using the Kirby–Bauer disc diffusion method, following Clinical Laboratory Standards Institute (CLSI) 2020 guidelines. Once pure cultures with

Results:

150 patients were included in the study, consisting of 80 males and 70 females. The results of culture and sensitivity testing, as shown in Table 1, indicate the distribution of bacterial growth among the study participants. *Escherichia coli* (*E. coli*) was

the most frequently isolated uro-pathogen, accounting for 69% of cases. Other identified uro-pathogens included Candida, Enterobacter, Providencia, Klebsiella, and Serratia, though these were detected at lower frequencies. The prevalence of each uro-pathogen is detailed in Table 2. Delving deeper into the distribution of uro-pathogens by gender, the study revealed a p-value of 0.339. This statistic indicated no significant gender-based difference in the prevalence of these uro-pathogens. In essence, the distribution of uro-pathogens appeared similar among both males and females. Table 3 displays the gender-wise distribution of uro-pathogens. In a separate aspect of the study, the sensitivity and resistance of uro-pathogens to various antibiotics were explored. *E. Coli* exhibited notable sensitivity to Clindamycin, with an impressive sensitivity rate of 82.6%. Against Candida, Nitrofurantoin emerged as highly effective, achieving a remarkable sensitivity rate of 94.1%. Tigecycline

demonstrated noteworthy effectiveness against Enterobacter (83.3%) and Providencia (94.7%). Colistin notably showed perfect sensitivity against Klebsiella (100%). Furthermore, Gentamicin, Cefoperazone/sulbactam, and nitrofurantoin were identified as the most effective antibiotics against Serratia, each boasting a remarkable 100% sensitivity rate. Table 4 illustrates the frequency distribution of uro-pathogens' sensitivity to different antibiotics. In terms of resistance, *E. Coli* generally displayed low resistance to most antibiotics, with the highest resistance observed for Imipenem at 34.8%. Candida, conversely, exhibited a high resistance rate to Colistin, reaching 64.7%. Enterobacter was found to be highly resistant to Ceftazidime (46.7%). Providencia was notably resistant to Meropenem, with a significant resistance rate of 63.2%. Table 5 shows the frequency distribution of uro-pathogens' resistance to different antibiotics.

Table 1. Gender-wise distribution for growth seen on culture and sensitivity.

Gender	Yes	No
Male	53	27
Female	50	20

Table 2. Frequency distribution of prevalence of uro-pathogens

Uro-pathogen	Number (%)
E. Coli	69 (46%)
Candida	17 (11.3%)
Enterobacter	30 (20%)
Providencia	19 (12.7%)
Klebsiella	11 (7.3%)
Serratia	4 (2.7%)

Table 3. Gender-wise distribution of prevalence of uro-pathogens.

Gender	E-coli	Candida	Enterobacter	Providencia	Klebsiella	Serratia
Male	34	13	14	12	5	2
Female	35	4	16	7	6	2

Table 4. Frequency distribution of uro-pathogens sensitivity to different antibiotics. N=(%)

Antibiotics	E-coli	Candida	Enterobacter	Providencia	Klebsiella	Serratia
Gentamicin	51(73.9%)	13 (76.5%)	17 (56.7%)	15(78.9%)	10(90.9%)	4(100%)
Tetracycline	50(72.5%)	12(70.6)	25(83.3%)	18(94.7%)	8(72.7%)	0(0.0%)
Polymyxin-B	48(69.6%)	13(76.5%)	21(70%)	15(78.9%)	9(81.8%)	3(75%)
Colistin	54(78.3%)	6(35.3%)	22(73.3%)	14(73.7%)	11(100%)	
Cefoperazone/sulbactam	50(72.5%)	9(52.9%)	18(60%)	14(73.7%)	5(45.5%)	4(100%)
Fosfomycin	49(71%)	14(82.4%)	24(80%)	15(78.9%)	8(72.7%)	2(50%)
Nitrofurantoin	55(79.7%)	16(94.1%)	23(76.7%)	13(68.4%)	7(63.6%)	4(100%)
Piperacillin Tazobactam	55(79.7%)	12(70.6%)	24(80%)	14(73.7%)	7(63.6%)	2(50%)
Amikacin	54(78.3%)	13(76.5%)	23(76.7%)	13(68.4%)	7(63.6%)	2(50%)
Imipenem	45(65.2%)	11(64.7%)	18(60%)	13(68.4%)	6(54.5%)	3(75%)
Meropenem	49(71%)	12(70.6%)	19(63.3%)	7(36.8%)	7(63.6%)	2(50%)
Ciprofloxacin	49(71%)	13(76.5%)	20(66.7%)	10(52.6%)	8(72.7%)	3(75%)
Clindamycin	57(82.6%)	14(82.4%)	22(73.3%)	15(78.9%)	9(81.8%)	3(75%)
Cefepime	56(81.2%)	15(88.2%)	21(70%)	10(52.6%)	9(81.8%)	3(75%)
Ceftriaxone	49(71%)	12(70.6%)	23(76.7%)	12(63.2%)	7(63.6%)	
Co-amoxiclav	53(76.8%)	11(64.7%)	24(80%)	15(78.9%)	9(81.8%)	
Ceftazidime	43(62.3%)	7(41.2%)	16(53.3%)	12(63.2%)	9(81.8%)	0(0%)

Table 5. Frequency distribution of uro-pathogens resistance to different antibiotics. N=(%)

Antibiotics	E-coli	Candida	Enterobacter	Providencia	Klebsiella	Serratia
Gentamicin	18(26.1%)	4(23.5%)	13(43.3%)	4(21.1%)	1(9.1%)	0(0%)
Tetracycline	19(27.5%)	5(29.4%)	5(16.7%)	1(5.3%)	3(27.3%)	4(100%)
Polymyxin-B	21(30.4%)	4(23.5%)	9(30%)	4(21.1%)	2(18.2%)	1(25%)
Colistin	15(21.7%)	11(64.7%)	8(26.7%)	5(26.3%)	0(0%)	2(50%)
Cefoperazone/sulbactam	19(27.5%)	8(47.1%)	12(40%)	5(26.3%)	6(54.5%)	0(0.0%)
Fosfomycin	20(29%)	3(17.6%)	6(20%)	4(21.1%)	3(27.3%)	2(50%)
Nitrofurantoin	14(20.3%)	1(5.9%)	7(23.3%)	6(31.6%)	4(36.4%)	0(0%)
Piperacillin Tazobactam	14(20.3%)	5(29.4%)	6(20%)	6(26.3%)	4(36.4%)	2(50%)
Amikacin	15(21.7%)	4(23.5%)	7(23.3%)	6(31.6%)	4(36.4%)	2(50%)
Imipenem	24(34.8%)	6(35.3%)	12(40%)	6(31.6%)	5(45.5%)	1(25%)
Meropenem	20(29%)	5(29.4%)	11(36.7%)	12(63.2%)	4(36.4%)	2(50%)
Ciprofloxacin	20(29%)	4(23.5%)	10(33.3%)	9(47.4%)	3(27.3%)	1(25%)
Clindamycin	12(17.4%)	3(17.6%)	8(26.7%)	4(21.1%)	2(18.2%)	1(25%)
Cefepime	13(18.8%)	2(11.8%)	9(30%)	9(47.4%)	2(18.2%)	1(25%)
Ceftriaxone	20(29%)	5(29.4%)	7(23.3%)	7(36.8%)	4(36.4%)	1(25%)
Co-amoxiclav	16(23.2%)	6(35.3%)	6(20%)	4(21.1%)	2(18.2%)	3(75%)
Ceftazidime	26(37.7%)	10(58.8%)	14(46.7%)	7(36.8%)	2(18.2%)	4(100%)

Discussion:

Urinary tract infections (UTIs) remain a prevalent concern in healthcare, causing substantial morbidity and mortality globally (9). Among the most commonly diagnosed infections, UTIs affect approximately 150 million people each year, representing a significant burden on society and clinical practice (10). The spectrum of pathogens contributing to UTIs is diverse, with a multitude of identified enteropathogenic organisms being accountable for these infections (11). Primarily bacterial in nature, UTIs can inflict damage on the kidneys, bladder, and urethra, typically attributed to various bacterial genera. Mostly, confined to the lower urinary tract, extreme cases can extend to the kidneys, resulting in acute pyelonephritis, potentially leading to bacteremia and sepsis, and posing life-threatening complications (12). Effective management and prevention strategies are critical in addressing UTIs, given their widespread prevalence and potential to cause severe systemic complications. Understanding the evolving landscape of microorganisms and the associated antimicrobial resistance patterns is pivotal in optimizing treatment strategies and

mitigating the burden imposed by these infections on global healthcare systems (13). In our study we found that the E-coli is leading the list of the total identified uropathogens constituting 46%, which is consistent with studies on urinary infections (14). Enterobacter and Providencia were the second and third most frequently isolated bacteria with 20% and 12.7% respectively in addition to a wide range of other pathogens that constitute low percentage. E-coli was noted to be more resistant to Ceftazidime and least resistant to Clindamycin which is contrast to the previous literature where studies showed imipenem as least resistant antibiotic to E-coli (14,15). Enterobacter was found to be highly resistant to Ceftazidime while least resistant to tigecycline. Providencia was more resistant to meropenem and least resistant to tigecycline which are consistent with literature (15). In our study, the second most common bacterial isolates after E. coli were Enterobacter (30%), Providencia (12.7%), Candida (11.3%), Klebsiella (7.3%) and Serratia (2.7%) respectively. However, a study by Asaduzzaman et al, reported that the second most frequent organism after E. coli was Klebsiella followed

by *Staphylococcus aureus*, *Pseudomonas*, and *Enterococcus* spp., *Proteus*, and *Enterobacter*(16). The current study concludes that *E. coli* was the most usually isolated bacteria and the most frequent cause of urinary tract infections among the studied population. The urinary tract infection was mostly reported in males and the infectious bacterial isolates showed multi-drug resistance against the tested antibiotics that are commonly used to treat urinary tract infection(17-19). There are some limitations of our study. One is the very short duration of one month, which can not give information about seasonal variations. Second, our study is single center so the results could not be generalized. Further research carrying a multi centric design with large sample size and longer duration may better provide impactful data.

Conflict of Interest Statement:

The authors declare no conflicts of interest regarding the publication of this study.

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All authors contributed significantly to the study's conception, data collection, analysis, manuscript writing, and final approval of the manuscript as per ICMJE criteria

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