ORIGINAL ARTICLE

Spectrum and Antibiotic Resistance Pattern of Uropathogens Causing Urinary Tract Infection Among Inpatients and Outpatients: An Experience of a Tertiary Care Hospital in Karachi, Pakistan

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Abstract

Background: UTI leads to irrational use of antibiotics and may cause higher antibiotic resistance which is a major public health issue. Therefore, it is crucial for clinicians to acquire knowledge of etiological agents and susceptibility patterns in their population for the optimistic use of antibiotic medicines.

Objective: To determine the spectrum and antibiotic resistance pattern of uropathogens causing urinary tract infection among inpatients and outpatients in a tertiary care hospital in Karachi.

Methods: This descriptive cross-sectional study was conducted in the Department of Microbiology, Sindh Institute of Urology and Transplant, Karachi, The study was conducted from March 2016 to March 2017 after taking approval from the Hospital Ethics Committee. Urine specimens were analyzed to establish a diagnosis of UTI and identify uropahtogens. The antibiotic susceptibility pattern of uropathogens was studied using disc diffusion method against the following antibiotics; fosfomycin, ampicillin, amoxicillin-clavulanate, nitrofurantoin, cefotaxime, ceftazidime, amikacin, cefoxitin, imipenem and vancomycin.

Results: A total of 480 samples of UTI were received during the study period. The average age of patients was 54.79±12.09 years. The majority of samples came out positive from the male gender (65%) and in-patient department (n=400, 83.3%). The highest prevalent microorganism was *E.coli* (82.1%) followed by *Klebsiella spp* (14%), *Pseudomonas aeruginosa* (1%), *Proteus mirabilis* (1%), *Morganella morgannii* (1%) and *Staphylococcus aureus* (0.8%). All microorganisms were highly resistant to augmentin, cefoxitin, cefotaxime, ceftazidime. Only *pseudomonas aeruginosa* was highly resistant to imipenem (60%). *Pseudomonas aeruginosa* (100%), *E.coli* (86.8%) and *Klebsiella spp* (71.6%) were highly sensitive for Amikacin (100%). *Morganella morgannii* (80%) and *Proteus Mirabilis* (40%) were mainly resistant to Fosfomycin. Only *E.coli* was sensitive to nitrofurantoin (74.1%).

Conclusion: The present study demonstrated that gram-negative bacteria was the most frequent cause of urinary tract infection. Microorganisms showed variable resistance to different antibiotics. The first line of antibiotics should be rationally selected by physicians to treat urinary tract infections.

Keywords: *Urinary tract infections, antimicrobial drugs, antibiotic resistance, gram-positive bacteria, gram-negative bacteria.*

INTRODUCTION

Urinary tract infection (UTI) is termed as a health condition that is associated with clinical signs and detection of pathogens in the kidney, urine, urethra, prostate and bladder [1]. Globally, UTI ranks as the second most frequently occurring bacterial infection that affects individuals of different age groups. Globally, UTI accounts for 150-250 million cases in a year and is the most frequent reason for a patient to seek medical advice [2, 3].

Nearly 40% to 50% of women and 5% of men develop UTI at least once in their life [4-6]. Both the gender are affected by UTI regardless of their age but the high prevalence among females may be due to anatomical structures due to anatomical structures or high bacterial load in urothelial mucosa or other factors such as obstructed urinary tract, sexual activity and pregnancy [7].

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Gram-positive and gram-negative bacteria are involved in the etiology of UTI but, uropathogenic *Escherichia coli* (*E.coli*) is a cause of the majority of cases UTI among the general population [8, 9]. Other most probably uropathogens involved in UTI are *Klebsiella, Enterobacter, Serratia, Proteus, Pseudomonas,* and *Enterococcus, Staphylococcus aureus* [10]. A study showed a predominance of *E.coli* in 31.5% of UTI patients whereas the prevalence of other pathogens among the general population was 3.5% [11].

There is evidence to conclude that UTI is associated with huge consumption of antibiotics in and out of the hospital and irrational use of antibiotics may cause higher antibiotic resistance which is a major public health issue around the world [12]. It is crucial for clinicians to acquire knowledge of etiological agents and susceptibility patterns in their population for the optimistic use of antibiotic medicines. The susceptibility pattern of uropathogens varies with time and geographical region, different settings thus, each

institute should monitor the drug sensitivity pattern in their settings to identify changes causing agents and their susceptibility pattern for appropriate management of the patient. Therefore, we planned to conduct the current study to determine the spectrum and antibiotic resistance pattern of uropathogens causing urinary tract infection among inpatients and outpatients in tertiary care hospitals in Karachi.

METHODOLOGY

The current descriptive cross-sectional study was conducted at the Department of Microbiology at Sindh Institute of Urology and Transplantation from March 2016 to March 2017. Patients of both the gender of age range 18 to 60 years diagnosed with UTI for at least 2 months ago and showing the presence of single organism with pure growth more than 104 organisms on CLED plate were included in the study. Patients of a mixed growth of multiple organisms or single organism but with growth less than 104 organisms on CLED plate and taking antibiotics in the past 14 days were excluded from the study. The prevalence of pathogens other than *E.coli* in the general population was 3.5% [11]. Therefore, the sample size of 450 was calculated taking a prevalence of 3.5% at a 95% confidence interval and a margin of error of 1.7% using World Health Organization (WHO) calculator version 2.1.

The urine specimen was taken from the patient and sent to the Microbiology department for analysis. The final diagnosis of UTI was established with detection of positive culture in urine specimen which was defined as $>10^4$ colony-forming units per milliliter from voided specimens or 50,000 colony-forming units per milliliter from a catheterized specimen. The volume of 0.1ml of urine was inoculated on a cystine lactose electrolyte deficient medium plate (CLED) (Oxoid, UK) to isolate uropathogens which was incubated at 37°C for 24 hours. Inoculation of urine was done with the help of a sterilized calibrated wire loop and the number of colonies was counted on each plate. Significant bacterial growth was considered for colony count >105 organisms/mL of urine [13]. International guidelines were used to biochemically characterize the colonies [14]. MacConkey agar (Oxoid, UK) was used to subculture the isolated which was kept for incubation at 37°C for 24 hours to achieve pure growth.

The antimicrobial susceptibility pattern of uropathogens was evaluated using Kirby-Bauer disk diffusion. The inhibition zone of bacterial growth was interpreted according to Clinical and Laboratory Standard guidelines [15]. Isolates showing intermediate susceptibility were defined as resistant. *Escherichia coli* American Type Culture Collection (ATCC) 25922, *Pseudomonas aeruginosa* 27853 and *Staphylococcus aureus* 29213 were used as quality control strains. All gram-positive and gram-negative isolates were tested for the following antibiotics; fosfomycin, ampicillin, amoxicillin-clavulanate, nitrofurantoin, cefotaxime, ceftazidime, amikacin, cefoxitin, imipenem and vancomycin. Statistical package SPSS version 21 was used for data entry and analysis. Variables were summarized as frequencies with percentages and mean ± standard deviation for categorical and numerical variables respectively.

RESULTS

During the study period, a total of 480 samples were found UTI positive with a mean age of 54.79±12.09 years. Most of specimen came out to be positives from males (n=312, 65%) and in-patient department (n=400, 83.3%). The majority of the detected isolates were gram-negative bacteria (n=476, 99.2%). Out of 480 samples, the most frequently detected organism was *E.coli* (n=394, 82.1%) followed by *Klebsiella spp* (n=67, 14%), *Pseudomonas aeruginosa* (n=5, 1%), *Proteus mirabilis* (n=5, 1%), *Morganella morgannii* (n=5, 1%) and *Staphylococcus aureus* (n=4, 0.8%). Gender-wise frequency of isolates is presented in Table **1**.

The resistance pattern of isolates against the tested antibiotics is depicted in Table **2**. All microorganisms were highly resistant to augmentin, cefoxitin, cefotaxime, ceftazidime. Only *pseudomonas aeruginosa* was highly resistant to imipenem (60%). *Pseudomonas aeruginosa* (100%), *E.coli* (86.8%) and *Klebsiella spp* (71.6) were highly sensitive for Amikacin (100%). *Morganella morgannii* (80%) and *Proteus mirabilis* (40%) were mainly resistant to Fosfomycin. Only *E.coli* was sensitive to nitrofurantoin (74.1%).

Table 1: Frequency of isolated microorganisms among males and females.

Microorganism	Males n(%)	Females n(%)
Gram-negative organisms		
Escherichia coli	255 (64.7)	139 (35.3)
klebsiella spp	40 (59.7)	27 (40.3)
Pseudomonas aeruginosa	4 (80)	1 (20)
Proteus mirabilis	5 (100)	0 (0)
Morganella morganii	5 (100)	0 (0)
Gram-positive organisms		
Staphylococcus aureus	3 (75)	1 (25)

Table 2: Resistance pattern of isolates against the tested antibiotics.

Drugs	*Escherichia coli* n(%)	*klebsiella spp* n(%)	*Pseudomonas aeruginosa* n(%)	*Proteus mirabilis* n(%)	*Morganella morganii* n(%)	*Staphylococcus aureus* n(%)
Augmentin	249(63.2)	37(55.2%)	3(60%)	5(100%)	5(100%)	4(100%)
Cefoxitin	276(70.1%)	49(73.1%)	5(100%)	3(60%)	3(60%)	4(100%)
Imipenem	29(7.4%)	9(13.4%)	3(60%)	0(0%)	1(20%)	1(25%)
Amikacin	52(13.2%)	19(28.4%)	0(0%)	2(40%)	2(40%)	2(50%)
Fostomycin	35(8.9%)	12(17.9%)	1(20%)	2(40%)	4(80%)	0(0%)
Nitrofurantoin	102(25.9%)	38(56.7%)	5(100%)	4(80%)	4(80%)	2(50%)
Cefotaxime	295(74.9%)	43(64.2%)	2(40%)	2(40%)	4(80%)	4(100%)
Ceftazidime	294(74.6%)	43(64.2%)	2(40%)	2(40%)	4(80%)	4(100%)

DISCUSSION

The present study focused on distributions of uropathogens in confirmed UTI cases from in-patients and out-patients. In our study, most of the samples were received from males than females which is contrary to locally and internationally available literature [2, 16-18]. The current study was conducted in SIUT which is a kidney transplant center. Most of the admitted patients comprise those requiring a kidney transplant or those who present with a major complaint of other kidney-related issues in which UTI is common. Secondly, the male gender was predominant among admitted patients during the study period. Moreover, the majority of the specimen included in the study were those collected from the in-patient department, which could be the most likely explanation for higher UTI samples from the male population in our study.

The bacterial spectrum in UTI of hospitalized patients is extensive, including Enterobacteria, non-fermenters and Gram-positive bacteria. However, the bacterial spectrum of pathogens from the urinary tract causing septicaemia is markedly shifted toward Gram-negative organisms [19, 20]. In our study, Gram-negative bacteria were isolated from 99.2% of UTI cases. A similar study was conducted in Punjab, Pakistan to ascertain the spectrum of isolates from UTI cases and their resistance pattern against commonly used antibiotics. The study reported that 70.2% of UTI cases were caused by Gram-negative bacteria [16]. The finding of a higher frequency of gram-negative bacteria is also in line with other study conducted in Pakistan [18]. The findings regarding the higher frequency of Gram-negative bacteria are also consistent with the studies conducted in Iran and Ethiopia [21, 22].

In the present study, the overall most frequent isolated microorganism was *E-coli* (82.1%) followed by *Klebsiella spp* (14%), *Pesudomonas Aer* (1%), *Proteus Mir* (1%), *Morganella* (1%) and *Staph Aureus* (0.8%). The findings of the current study were similar to another Pakistani study in terms of the most frequently detected organism as *E.coli* was also the most detected organism in that study. However, findings contrasted regarding Gram-positive bacteria as in our study the only detected Gram-positive bacteria was *Staph aureus* whereas in that study *Enterococcus faecalis* (14.8%) and *Candida* (14.3%) were also detected in 14.8% and 14.3% of the UTI cases which stood as second and third frequently detected organism and other detected organisms were *Pseudomonas* (5.9%), *Klebsiella spp*. (1.3%), *Staphylococcus aureus* (0.8%), *Proteus* (0.8%) [16]. Hrabcek *et al.* conducted a study in Central Europe and observed that the most frequently isolated bacteria was *E.coli* (26.0%), followed by Enterococcus spp. (22.4%), *Klebsiella spp*. (11.3%), P. aeruginosa (7.3%), and *Proteus spp.* (6.2%). Hrabcek *et al.* in their study also reported that the relative prevalence of microorganisms was varying with the time with an increase in the prevalence of *E. coli* and *Proteus spp* [17].

In the present study, we also determined the antibiotic susceptible pattern of detected uropathogens for tailoring the empirical antibiotic treatment according to the local epidemiological condition. A high resistance of *E.coli* was observed to cefotaxime (74.9%), ceftazidime (74.6%), cefoxitin (70.1%) and augmentin 63.2%). Lesser resistance rate of *E.coli* was observed for imipenem (7.4%), fostomycin (8.9%), amikacin (13.2%) and nitrofurantoin (25.9%). Sohail *et al*. in their study reported approximately the same resistance rate of *E.coli* to cefotaxime (72%), Ceftazidime (71%) however

resistance to Amoxicillin (84%) was quite higher than observed in our study. Similarly, Sohail *et al.* reported lower resistance of *E.coli* to imipenem (3%), fosfomycin (10%). In contrast to our study, high resistance of *E.coli* was observed to amikacin (91%). However, he did not test *E.coli* for nitrofurantoin and cefoxitin [18]. A study conducted in 2008 in Pakistan reported resistance pattern of *E.coli* for the following antibiotics; ampicillin (92%), co-trimoxazole (80%), ciprofloxacin (62%), gentamicin (47%), nitrofurantoin (20%), and amikacin (4%) [23].

The second most isolated organism in our study was *Klebsiella spp* which had the highest resistance for cefoxitin (73.1%), cefotaxime (64.2%), ceftazidime (64.2%), augmentin (55.2%), nitrofurantoin (56.7%) and lower resistance was observed for imipenem (13.4%), fosfomycin (17.9%), and amikacin (28.4%). The lowest resistance of *Klebsiella spp* was observed against amikacin (37%) imipenem (37%) in other Pakistani investigation while ceftazidime (85.2%), cefotaxime (100%), nitrofurantoin (85.2%) were highly resistant antimicrobials against *Klebsiella spp*. In this study, a much higher resistance was observed for fosfomycin than our findings (48%) [18].

In the present study, *Pseudomonas spp* was found to be 100% resistant to cefoxitin and nitrofurantoin. High resistance was also observed for augmentin (60%), imipenem (60%), cefotaxime (40%) and ceftazidime (40%) whereas it was 100% and 80% sensitive for amikacin and fosfomycin respectively. A lower resistance for amikacin was also observed in a study conducted by Sohail *et al.* and resistance to all other antibiotics was not tested in his study [16]. A study conducted in Khyber Pakhtun Khuwa reported 8.3% resistance to amkacin, 25% resistance to augmentin, 66.7% resistance to cefotaxime, 75% resistance to ceftazidime, 58.3% resistance to fosfomycin, 41.7% resistance to imipenem and 100% resistance to nitrofurantoin. Resistance to cefoxitin was also not tested in this study [18].

We found *Proteus mir.* was highly resistant against augmentin (100%) followed by nitrofurantoin (80%), cefoxitin (60%), amikacin (40%), fosfomycin (40%), cefotaxime (40%), ceftazidime (40%) whereas highly sensitive to imipenem (100%). These findings are consistent with the study conducted by Muhmmad *et al.* who reported similar resistance of *Proteus mir.* against nitrofurantoin (83.3%), cefoxitin (66.7%), fosfomycin (33%). However, contradictory to our study, higher resistance was observed for amikacin (66.7%), ceftazidime (50%) and imipenem (16.7%) whereas augmentin was not tested in his study for *Proteus mir.* [18]. A resistance of 67% was observed for amoxicillin in a study by Sohail *et al.* [16].

Studies conducted in Pakistan, Kuwait and Central Europe did not observe M. *morganii* isolate in their study [2, 16-18]. However, a case report was published from Iran in which they reported that for a first time they observed M. *morganii* isolate harboring blaVIM, blaCTX-M, and blaSHV genes after kidney transplantation with persistent urinary infections. The current study was conducted in SIUT which is a Kidney transplant center more probably, unlike other studies, we also found the M. moraganii as a rare cause of UTI due to this fact. However, future research should be conducted with a larger sample size to further have the evidence. M. *morganii* is naturally resistant to tetracyclines, tigecycline, polymyxins, and nitrofurantoin [24]. Consistent with the literature, in our study, we also found 80% resistance of M. *morganii* against nitrofurantoin.

In our study, although microorganism Staph auerus was found in 0.83% cases of UTI however, it was 100% resistant to augmentin, cefoxitin, cefotaxime, ceftazidime. Resistance of 50% was also observed for amikacin and nitrofurantoin with high sensitivity for fosfomycin (100%) and imipenem (25%). In contrast to our study, a resistance of 84.6%, 85.7%, 42.9%, 50%, 14.3% was observed against augmentin, cefotaxime, ceftazidime, amikacin, fosfomycin and imipenem respectively. Resistance against cefoxitin was not determined in other studies [18].

CONCLUSION

The present study demonstrated that gram-negative bacteria was the most frequent cause of urinary tract infection. Microorganisms showed variable resistance to different antibiotics. The first line of antibiotics should be rationally selected by physicians to treat urinary tract infections.

ETHICS APPROVAL

The study was conducted after obtaining approval from Hospital Ethics Committee.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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