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Etiology and Antimicrobial Susceptibility Pattern of Urinary Tract Infection in the First Year after Pediatric Renal Transplantation: A Preliminary Study

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Abstract

Background: Urinary tract infection (UTI) is common after pediatric renal transplantation, and the emergence of multidrug-resistant (MDR) bacteria causing UTI is a therapeutic challenge in this regard. The main purpose of this study was to determine the UTI frequency, its etiologic agents, and the antibiotic susceptibility pattern in the first year following renal transplantation in Iranian pediatric recipients.

Methods: In a retrospective cohort study, all of the 81 children who had undergone renal transplantation in Hazrat Rasoul Akram Hospital between 2012 and 2017 were enrolled. Confirmed episodes of UTI during the first year following renal transplantation were analyzed. The pattern of antibiotic resistance was determined for the causative agents of UTI. The data were analyzed using the IBM SPSS Statistics software (version 20). and the $P < 0.05$ was considered significant.

Results: Totally, from 81 enrolled cases, 37(44.7%) cases were in the age group of 11-15 years. Overall, 19, 10, and 3 UTI episodes had occurred in the first month, from the first to sixth month, and between the sixth month and one year after transplantation, respectively. The four most common isolated bacteria were *Escherichia coli* (*E. coli*; 31.2%), *Pseudomonas aeruginosa* (*P. aeruginosa*; 25%), *Enterococci* (21.9%) and *Klebsiella pneumoniae* (*K. pneumoniae*; 12.5%). The highest rate of resistance was reported to trimethoprim/sulfamethoxazole (TMP/SMX), cephalosporins, and fluoroquinolones among gram-negative bacteria. However, none of the *Enterococci* isolates were resistant to linezolid and nitrofurantoin.

Conclusion: Resistance to antibiotics is increasing among the pathogens causing UTI in pediatric renal transplanted cases. It is suggested to stop the administration of TMP/SMX and third-generation cephalosporins for empiric treatment of UTI in Iranian pediatric renal transplant recipients. Ciprofloxacin might be administered cautiously secondary to the increasing rate of antibiotic resistance in this group.

Keywords: Pediatrics, Renal transplantation, Urinary Tract Infection, Antimicrobial resistance

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Introduction

Urinary tract infection(UTI) is the most common bacterial infection in childhood following renal transplantation (1). At present, renal transplantation is recognized as the most effective treatment for patients with advanced chronic

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↑What is "already known" in this topic:
Urinary tract infection(UTI) is the most common bacterial infection in childhood following renal transplantation. The widespread use of antibiotics among renal transplant recipients has resulted in increased antibiotic resistance. Despite numerous studies about antibiotic resistance in Iran, the number of studies on UTI and antibiotic resistance in the field of pediatric renal transplantation is scarce.

→What this article adds:
It seems that TMP-SMX and third-generation cephalosporins may not be appropriate for empiric treatment of UTI in pediatric renal transplantation recipients, especially during the first months following transplantation. According to the results of this study, imipenem may be used successfully as an empiric treatment in this regard.

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kidney disease (CKD), which can increase life expectancy and lead to a better quality of life in these individuals (2). Despite significant developments in this field, the emergence of infections, especially UTIs mostly due to immunosuppressive drug consumption, may cause rejection in the first months after renal transplantation (2, 3). The major risk factors of UTI in pediatric renal transplant patients are a history of pre-transplant UTI, vesicoureteral reflux (VUR), immunosuppressive therapy, external devices (e.g., stents and urinary catheters), and suture materials (4, 5). UTI occurs in 25% of renal transplant recipients during the first year of renal transplantation and accounts for 45% of infections in this population (1, 6). Regarding the results of a study, asymptomatic bacteriuria, uncomplicated UTI, as well as complicated UTI represented %44.4, %32.3, and 23.3% of UTI cases, respectively (6, 7). This type of infection is similarly associated with increased risks of graft rejection, renal allograft dysfunction/loss, and mortality (8). In renal transplantation recipients, UTIs are mainly caused by *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K.pneumoniae*), *Pseudomonas aeruginosa(P. aeruginosa*) and *Enterococcus faecalis* (*E.faecalis*) (9, 10). The widespread use of antibiotic prophylaxis among renal transplant recipients has also resulted in increased resistance to some antibiotics such as trimethoprim-sulfamethoxazole (TMP/SMX) which is routinely used for UTI prophylaxis in these patients (8, 11). Despite numerous studies in the field of antibiotic resistance in Iran, studies on UTI and antibiotic resistance in the field of pediatric renal transplantation are scarce. (12). The results of such local studies can help to identify the common uropathogens and their antibiotic resistance causing UTI in the pediatric renal transplant population and determine the appropriate empiric treatment of UTI in this population. Also, it is essential for providing cost-effective management of UTIs.

The main purpose of this study was to determine the UTI frequency, type of microorganisms causing UTI, and antibiotic susceptibility pattern in the first year after renal transplantation in Iranian pediatric recipients.

Methods

Study design and patients

All of the 81 children who had undergone renal transplantation in Hazrat Rasul Akram Hospital between 2012 and 2017 were enrolled in this retrospective cohort study. The patients who had UTIs during the first year following renal transplantation were included. Positive cultures with common contaminants such as nonpathogenic Neisseria were excluded. Then, their demographic and clinical data including age, gender, underlying cause of the end-stage renal disease (ESRD), first or recurrent episode of the infection, as well as history and modality of dialysis before transplantation, were collected by reviewing hospital records of the cases.

Specimen collection

After renal transplantation and during hospitalization, a urine sample was taken every three days through the Foley catheter and every five to seven days when the Foley catheter was removed using the mid-stream clean catch method. Then, the urine was examined weekly during the first two months for analysis and cultures and every two weeks in the third and fourth months after transplantation. In the fifth- and sixth- months following transplantation the urine examination and culture were performed every three weeks and once a month after six months.

Laboratory diagnosis of UTI

Urine samples were firstly examined by means of diagnostic strips (Behring, Germany), and then 10-15 ml of urine was centrifuged (2000 rpm) for 5 min. After that, the precipitate was placed on a glass slide, undergoing light microscopy. All of the samples were cultured on MacConkey agar and blood agar (Merck Co, Germany), then incubated at 37°C for 24 h. Based on guidelines, UTI was defined as: A bacterial growth $\geq 10^5$ CFU/mL in clean catch midstream urine culture or $\geq 10^4$ CFU/mL from a urine sample obtained by catheterization; test (13, 14). All bacteria were identified by using conventional biochemical tests such as TSI Agar, SIM medium, Urea agar, Simons citrate, MR/VP and lysine decarboxylase.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was also performed according to the guideline Clinical and Laboratory Standards Institute (CLSI) using the Kirby-Bauer Disk Diffusion method (15).

Statistical analysis

Qualitative variables were represented by means of numbers and percentages, and descriptive statistics were expressed as frequency and mean± standard deviations (SD). The association of two categorical variables was assessed using the Chi-square or Fisher exact tests. Also, the student's t-test or Mann-Whitney test was used to compare a quantitative variable among the subgroups of binomial variables. The data were analyzed using the IBM SPSS Statistics software (version 20). and the p-value<0.05 was considered significant.

Results

In this study, 81 cases undergone renal transplantation, including 51 (63%) male and 49 (27%) female cases (P =0.030), were enrolled. Totally, 37 cases (44.7%) were in the age group of 11-15 years. The frequency of distribution of cases by age group is shown in Table 1. The most common causes of renal failure resulting in renal transplantation were reflux nephropathy with and without neurogenic bladder followed by focal segmental glomerulosclerosis (FSGS). The frequency of etiologies of renal failure is presented in Table 2. Before transplantation, 43 (53.1%) and 15 (18.5%) cases were on hemodialysis and peritoneal dialysis, respectively. In addition, 4 cases (4.9%) had a history of using a combination of both methods, and 19 cases (23.5%) were preemptive (i.e., without prior dialysis). Regarding the source of renal allograft donation, 60 (74.1%) and 21 (25.9%) patients had received the organ from living (namely, 2 related and 19 unrelated) and cadaveric donors, respectively. A prophylactic antibiotic regimen of trimethoprim-sulfamethoxazole (TMP-SMX) was further used

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during the first year after renal transplantation. The rate of UTI was 19, 10, and 3 episodes in the first month, from the first to sixth months, and between the sixth month and one year following renal transplantation, respectively. The highest incidence rate of UTI occurred in the first month after transplantation. In addition, 10 cases out of 19 patients with UTI showed recurrences (one episode of recurrence: n = 7; two episodes of recurrences: n = 3). The four most common isolated bacteria were *E. coli* (31.2%), *P. aeruginosa* (25%)**,** Enterococci (21.9%) and K. *pneumoniae*. (12.5%). More details about isolated uropathogens are represented in Table 3. The rate of resistance to TMP/SMX and cephalosporins was 100% to *E. coli*. In addition, 75% of *K. pneumoniae* isolates were resistant to gentamicin, ciprofloxacin, TMP/SMX and cephalosporins. In contrast, Klebsiella isolates had lower levels of resistance to amikacin, imipenem and nitrofurantoin (25%, 50% and 50%, respectively).

Also, imipenem and ciprofloxacin were more effective against *P. aeruginosa*. However, none of the Enterococci isolates were resistant to linezolid and nitrofurantoin. The antimicrobial susceptibility details of the uropathogens are shown in Table 4.

Discussion

In renal transplant recipients, UTI is the most frequent infection following transplantation (16, 17). A number of studies have shown the association between acute rejection and UTI (18). In addition, UTIs lead to increased hospitalization and treatment costs. In recent years, the emergence of multidrug-resistant (MDR) organisms increased in both immunocompetent and immunocompromised hosts (19).

Table 1. Frequency of renal transplantation according to age group

Age group	1-5 years	6-10 years	11-15 years	16-20 years
Numbers(%)	10 (12.4%)	33 (40.7%)	37 (45.7%)	1 (1.2%)

Table 2. Causes of renal transplantation in the studied cases

Underlying disorder	Number	%	95% confidence interval
Reflux Nephropathy ±neurogenic bladder	18	22.3	(13.1-31.2)
FSGS	16	19.8	(11.1-21.4)
Renal Hypoplasia+ Agenesia	15	18.5	(10.0-26.9)
CKD with unknown etiology	12	14.8	(7.0-22.5)
Cystinosis	8	9.9	(3.3-16.3)
Congenital Nephrotic Syndrome	3	3.6	(0.004-7.8)
ARPKD	2	2.5	(0.003-5.8)
Nephronophthisis	2	2.5	(0.003-5.8)
Alport Syndrome	2	2.5	(0.003-5.8)
CGN	1	1.2	(0.001-3.6)
Bartter syndrome	1	1.2	(0.001-3.6)
HUS	1	1.2	(0.001-3.6)

FSGS Focal segmental glomerulosclerosis; PUV: Posterior urethral valve; CGN: Chronic glomerulonephritis; HUS: Hemolytic uremic syndrome; ARPKD: Autosomal recessive polycystic kidney disease; ADPKD: Autosomal dominant polycystic kidney disease

Table 3. Distribution of uropathogens in patients following kidney transplantation.

Organisms	0-1 month	2-6 month	7-12 month	Total Numbers [P(95% Confidence Interval)]
E. coli	5	3	2	10 [31.25(15.1-47.3)]
Pseudomonas aeruginosa	7	1	-	8 [25(10.0-40.0)]
Klebsiella pneumoniae	2	2	-	4 [12.5(1.0-23.9)]
Acinetobacter baumannii	1	1	-	2 [6.25(0.02-14.6)]
Enterobacter cloacae	-	1	-	1 [3.1(0.01-9.1)]
Enterococcus spp	4	2	1	7 [21.9(7.0-36.1)]

Table 4. Antimicrobial resistance rates in the most common uropathogens

	Escherichia coli	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Enterococcus spp*
Antibiotics	N (R%)	N (R%)	N (R%)	N (R%)
Amikacin	10 (50)	4 (25)	8 (75)	ND
Gentamicin	10 (80)	4 (75)	6 (83.3)	ND
Ampicillin	ND	ND	ND	7 (57.2)
Cefazolin	3 (100)	4 (75)	ND	ND
Ceftazidime	ND	ND	6 (100)	ND
Cefotaxime	10 (100)	4 (75)	ND	ND
Cefepime	6 (100)	4 (75)	7 (100)	ND
Imipenem	9 (22.2)	4 (50)	7 (42.9)	ND
Pipracillin-Tazobactam	ND	ND	4 (100)	ND
Ampicillin-sulbactam	ND	4 (75)	ND	ND
Ciprofloxacin	8 (87.5)	4 (75)	8 (37.5)	5 (80)
Trimethoprim/sulfamethoxazole	9 (100)	4 (75)	ND	ND
Nitrofurantoin	10 (10)	4 (50)	ND	6 (0)
Vancomycin	ND	ND	ND	7 (57.2)
Linezolid	ND	ND	ND	7 (0)

N, numbers of tested. R%, Percentage of resistance. ND, not determined

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However, MDR organisms have become a major public health problem worldwide (20, 21). Overall, it seems that UTI frequency in pediatric renal transplant recipients is significantly more than the normal population of the same age group. In the current study, 23.5% of the cases (19 children and 32 episodes of UTI) had suffered from UTI during the first year of the transplantation, while the incidence of UTI in Iranian children is reported to be 4.92 % (22). A study by John et al. demonstrated that 40 children (36.3%) out of 110 cases had developed UTI within the first year after renal transplantation (23). In another study, 24 cases (17.4%) out of 138 children had UTI over a 4.5-year period (24). As mentioned above, the highest rate of UTI was detected in the first month after transplantation which may be due to prolonged hospitalization, interventions leading to renal or ureteral tissue injury during surgery and immunosuppressive therapy. A study from Poland also revealed that 38% of UTI episodes were diagnosed during the first month after transplantation in adults (25). In our study, similar to previous studies, *E. coli* was the most common etiology of UTI (9, 26). It should be noted that UTI frequency decreases over time after renal transplantation and the highest rate is reported in the first year following transplantation (27).

In the present study, 63% of the enrolled cases were male (P =0.030), and no significant difference was observed in the incidence rate of UTI in the male and female in the UTI cases. Nevertheless, most of the studies on pediatric UTI indicate that the incidence of UTI in females is more than in males, other than the neonatal group (28).

In this study, 19 out of 81 children had 32 episodes of UTI, of whom 7 cases (36.8%) had an underlying cause of VUR-inducing ESRD emergence and renal transplantation. In a retrospective study published in 2011, 24 cases out of 138 patients had UTI, of whom 14 cases (58%) had VUR and urinary tract obstruction (24). It seems that the most prevalent underlying disorder in pediatric cases that leads to chronic kidney disease is congenital anomalies of the kidney and urinary tract (29). However, a study on an adult population of kidney transplant recipients showed primary glomerulonephritis (33.5%) as the most prevalent underlying disorder (25).

The results of antimicrobial susceptibility testing revealed high rates of resistance to cephalosporins, gentamicin, TMP/SMX, and ciprofloxacin in *E. coli* and *K. pneumoniae* isolates. This finding represents the high prevalence of MDR uropathogens among renal transplantation recipients. In Kiros et al. study, multidrug resistance was reported in 82% of the isolates among renal transplant recipients (30). In the study of Khosravi et al. (31) , the most common resistance was reported against cefixime, cephalothin, and TMP/SMX, which was consistent with our results. In the Shapouri Moghaddam et al. study (26), gram-negative bacteria isolated from urine samples of renal transplant recipients who suffered from UTI showed high resistance to ampicillin (91.2%) followed by ceftazidime (89.5%) which is in concordance with our results. The results of the present study showed that imipenem and ciprofloxacin were more effective against *P. aeruginosa* isolates, while *P. aeruginosa* isolates were resistant to cefepime, ceftazidime, gentamicin and pipracillin-tazobactam. This finding may be mediated by the production of extended-spectrum beta-lactamases. Furthermore, this organism as a prevalent organism in hospital settings, can cause healthcare-associated infections in immunocompromised patients. In the present study, all Enterococcus isolates were susceptible to linezolid, while 57.2% of them were resistant to ampicillin and vancomycin. In the current study, similar to the previous studies, Enterococcus spp was a frequent uropathogens in renal transplantation recipients. Unfortunately, the distribution of vancomycin-resistant enterococci strains has increased among both inpatient and outpatient cases(2, 32-34). Therefore, clinicians should consider the addition of an antibiotic active against Enterococcus spp especially in the early post-transplant period. In this study, no significant relationship was reported between gender and UTI frequency after transplantation (P = 0.323) as well as gender and frequency of recurrent UTI (P = 0.799); however, predominance in female patients was reported in the studies by Fallahzadeh et al. and John et al. (23, 24). It should be taken into account that the mean age of the cases in their study was relatively high, which might explain this point. In the present study, the highest rate of UTI belonged to the age group of 11-15 years but there was no significant relationship between age and UTI frequency (P = 0.93). No significant relationship was also found between age and UTI recurrence (P = 0.353). Nevertheless, in the study by Esezobor et al., an age below 5 years was considered a UTI risk factor (35). With respect to some reports revealing that cadaveric donation may increase the incidence rate of UTI probably due to increased cold ischemia and delayed graft loss, no significant relationship was found between the type of donation and UTI incidence rate. These findings were accordingly consistent with the findings reported by Fallahzadeh et al. (24). Our study had some limitations. The minimum inhibitory concentration (MICs) of the antimicrobial agents for each bacterium and molecular detection of antibiotic resistance was not performed. In addition, automation systems were not used for timely bacterial identification and determination of antimicrobial susceptibilities**.** Since the study was retrospective, the speciation for Enterococcus isolates had not been determined and the sample size was small. It is accordingly recommended to design a multi-center prospective study to obtain more extensive results. It is also suggested to provide continuous surveillance to update the antimicrobial resistance.

Since renal transplant recipients are prone to be infected with unusual opportunistic organisms, it is very important to provide the facilities for this population to be tested in the reference laboratories.

Conclusion

Although some guidelines recommend TMP/SMX for prophylaxis of UTI, the results of this study showed that resistance to commonly used antibiotics, including trimethoprim-sulfamethoxazole and third-generation cephalosporins are now more prevalent for many gram-negative bacterial isolates which are mostly the causes of UTI. Therefore, it seems that TMP-SMX and third-generation

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cephalosporins may not be appropriate for empiric treatment of UTI in pediatric renal transplant recipients especially during the first months following transplantation. The successful empirical treatment protocols should be based on local antimicrobial resistance rates. Therefore, according to the results of this study imipenem may be used as a successful empiric treatment in this regard. The use of pathogen-specific antibiotic therapy guided by culture, MIC and sensitivity data is recommended after the results are available. However, more studies are suggested for finding the optimal empiric treatment of UTI in pediatric renal transplantation recipients.

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Ethical issue

The present study was approved by the Ethics Committee of Iran University of Medical Sciences (IR.IUMS.FMD.REC1396.931165026).

Authors' contribution

Shirin Sayyahfar designed the project and wrote the first draft, Zahra Mohammadnezhad contributed to the data collection and analysis and interpretation of the results, Khosrow Zamani contributed to the data analysis, interpretation of the results and writing the manuscript, Rozita Hoseini and Hasan Otukesh contributed to the data collection and revision of the article. All authors discussed the results and approved the final manuscript. Nahid Rahimzadeh directed the project and is the guarantor.

Conflict of Interests

The authors declare that they have no competing interests.

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