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ORIGINAL RESEARCH

Characterization of Extended-Spectrum β-Lactamase-Producing Uropathogenic *Escherichia coli* Among Iranian Kidney Transplant Patients

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Introduction: The aim of this study was to investigate the antimicrobial susceptibility pattern and the presence of ESBLs among the uropathogenic *Escherichia coli* (UPEC) isolated from kidney transplant patients (KTP) and community-acquired urinary tract infections (UTIs) using phenotypic and molecular methods.

Materials and Methods: A total of 111 pure cultures of UPEC isolates were collected from 65 and 46 of non-KTP and KTPs with UTIs. The pattern and ESBL production of the strains were evaluated. PCR reaction to detect the presence of bla_{SHV}, bla_{TEM}, and $bla_{CTX\text{-}M}$ genes was performed.

Results: The results revealed that most of UPEC isolates obtained from KTPs and control group were resistant to trimethoprim/sulfamethoxazole (84.8% vs 46.2%), while carbapenems (100% sensitivity) were the most effective against UPEC isolates. ESBL-producing strains were significantly more frequent in KTPs compared with control group (43.5% vs 23.1%, $P = 0.021$). The molecular results revealed that 53.2% (59/111), 45% (50/111), and 5.4% (6/111) of isolates harbored $bla_{CTX\text{-}M}$, bla_{TEM}, and bla_{SHV} genes, respectively. Of the genes investigated, $bla_{CTX\text{-}M}$ and bla_{TEM} genes were significantly higher among KTP than the control group.

Conclusion: Our results showed a high proportion of multidrug-resistant and ESBL-producing isolates, which most of them harbor blaCTX-M. A significant high co-resistance to different classes of antibiotics was reported from ESBL-producing UPEC from KTPs, which remains a serious clinical challenge.

Keywords: UTIs, ESBL, PCR, KTP

Introduction

Bacterial urinary tract infections (UTIs) are the most common infectious complications among kidney transplant patients (KTPs) with diseases ranging from asymptomatic bacteriuria (AB) to potentially life-threatening acute such as cystitis and pyelonephritis.[1] In most cases, the first year following transplantation is the period of the highest risk for UTI and recurrent infections occur in up to 72% of kidney transplant (KT) recipients.[2,3]

Female sex, longer duration of catheterization, immunosuppression, diabetes mellitus, and manipulation of the urinary tract are the most important risk factors for UTI after kidney transplantation (KTx).[2] Previous studies indicated that uropathogenic *Escherichia coli* (UPEC) is the major causative agent of UTIs after KTx.[4] Although UTIs should be controlled using antimicrobial therapy, the

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increased rate of antimicrobial resistance has become a major concern causing the emergence of multiple drug resistance in UPEC, especially among KTPs.[5,6]

Antimicrobial resistance leads to higher medical costs, prolonged hospital stay, and increased mortality. This outcome is excessive and inappropriate use of antibiotics, the default in treatment, poor infection prevention and control, and microbial characteristics.[7,8] UTIs caused by extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* account for one of the major therapeutic and epidemiologic challenges in KTPs.[9] The prevalence of infections caused by ESBL-producing *E. coli* has steadily been increased in recent years that significantly reduces therapeutic options and is followed by an increased mortality rate. ESBL-producing bacteria can hydrolyze and confer resistance to cephalosporins, penicillins, and monobactams and are inhibited by clavulanic acid. Genes encoding ESBL are often located on transferrable bacterial plasmid DNA. ESBL genes have also been established within integron-like structures.[10] Furthermore, β-lactamases may be chromosomally encoded and universally present in a species.[3,11] The plasmid-mediated β-lactamase in gram-negative bacteria contains TEM, SHV, and CTX-M enzymes.[3,12] Among different plasmid-mediated β-lactamases, several reports suggest that CTX-M-type ESBLs may now be the most predominant ESBL type worldwide.[13] Owing to the high rate of UTIs affecting renal transplantation and outcomes of ESBL phenotype, it is necessary to assess the weight and control the spread of ESBLs producing isolates among KTPs.[14] In this study, we aimed to investigate the antimicrobial susceptibility pattern and the presence of ESBLs among the UPESs isolated from KTPs and non-KTPs with UTIs using phenotypic and molecular methods.

Materials and Methods

Study Population and Bacterial Isolates and Identification

A case–control study was performed on a collection of 65 non-duplicate UPEC isolates obtained from non-KTP with UTIs, as control group and 46 non-repetitive UPEC isolates obtained from KTPs with UTIs as a case-group, from June 2019 to October 2019. Data were collected from non-KTP who referred to the three laboratory center and KTP were referred from two nephrology private clinic. After screening of the electronic records of the patients, repetitive samples of patients and isolates obtained from the non-KTP group with a history of antibiotic usage in the previous 2 weeks were excluded from the study. This study was evaluated and approved by the Ethics Committee of Isfahan University of Medical Sciences (IR.MUI.MED.REC.1398.202). Moreover, all participants provided written informed consent.

All UPEC isolates were identified using standard microbiological tests such as Gram-staining and biochemical testing (oxidase, sugar fermentation, IMViC, Kliger's iron agar, nitrate reduction, motility, etc.). To confirm the species, we carried out a polymerase chain reaction (PCR) detection based on the *uid* gene for β-glucuronidase of *E. coli*.[15] Confirmed *E. coli* isolates were preserved at −80ºC for further evaluation.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility pattern was performed on Muller–Hinton agar (HiMedia Co., India) using the disk diffusion method, as recommended by the Clinical and Laboratory Standards Institute (CLSI).[16]

For this assay, 16 antibiotics (BD BBL™ Sensi-Disc™) including amoxicillin/clavulanic acid, piperacillin/tazobactam, cefoxitin, ceftazidime, cefepime, cefixim, imipenem, meropenem, trimethoprim/sulfamethoxazole, ciprofloxacin, ofloxacin, nalidixic acid, norfloxacin, nitrofurantoin, gentamicin, amikacin were used. *E. coli* ATCC 25922 was utilized as the quality control strain for antibacterial susceptibility testing.[16] The isolates non-susceptible to ≥1 agent in ≥3 different antimicrobial categories were considered as MDR.[17]

ESBLs Screening Test

ESBL production was detected using the double-disk synergy test (cefotaxime (30 μg) and ceftazidime (30 μg)) alone and in combination with clavulanic acid (10 μg) based on CLSI recommendations. Briefly, the phenotypic confirmatory test was performed by comparing the inhibition zone of disks containing cefotaxime and ceftazidime with and without clavulanic acid. After overnight incubation at 37°C, an increase in the inhibition zone diameter of ≥5 mm between the single disk and the double disk was confirmed as ESBL production. *Klebsiella pneumoniae* ATCC 700603 (Positive control) and *E. coli* ATCC 25922 (Negative control) were used as control strains in accordance with CLSI guidelines.[16]

Molecular Characterization of β-Lactamases

Genomic DNA was extracted from fresh colonies as described previously. To detect antibiotic resistance genes including bla_{SHV}, $bla_{CTX\text{-}M}$, bla_{TEM} -F (AGTATTCAAC ATTTCCGTGTC), and bla_{TEM}-R (GCTTAATCAGTGA

GGCACCTATC), separate PCR reactions were performed as previously described.[18,19]

PCR was performed using commercially available PCR Master Mix (AMPLIQON, Denmark) based on the manufacturer's instructions. Briefly, 6 µL of Master Mix, 0.3 µL of each primer (10 pmoles/µL), and 4.4 µL DNase-free distilled water and finally, 1 µL template DNA was added in a final volume of 12.5 µL. PCR amplifications of genes were performed with an initial denaturation step at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing for 55–57 s at primer-specific temperatures, extension at 72°C for 30 min, and a final extension step at 72°C for 5 min. The PCR-products were separated by electrophoresis in 1% agarose gels with 1 X TAE (Tris/Acetate/EDTA) buffer, stained with safe stain load dye (CinnaGen Co., Iran) and visualized under ultraviolet illumination.

Statically Analysis

Statistical analysis was carried out through the SPSS™ software, version 16 (IBM Corp., USA). Categorical variables were expressed as counts and percentages. The Fisher's exact or Chi-square ($\chi 2$) tests were used to analyze significant differences. A P -value <0.05 was considered as statistically significant.

Results

In this study, a total of 111 confirmed UPEC were isolated, 69.6% (32/46) and 30.4% (14/46) isolates were collected from female and male KTP patients, while 69.2% (45/65) and 30.8 (20/65) isolates were obtained from female and male control group, respectively. Demographic and characterization of KTP and non-KTP are summarized in Table 1.

Table 1 Distribution of Characteristics of Kidney Transplant Recipients

Characterization	KTx Recipients* n (%)	Non-KTP n (%)
Sex (female/male)	32/14	45/20
Age (Mean, Median, Range)	50.8; 53; (15–82)	45:42; (1–80)
Hypertension	50% (19/38)	-
Diabetes mellitus	28.9% (11/38)	7.7% (5/65)
Immunosuppression	100 (46/46)	0 (0/65)
Time from last KTx to UTI (Range)	4 month-21 year	-
Ureteral stent or urinary catheter	0 (0/38)	0(0/65)
Hospitalization	0 (0/46)	0 (0/65)
Recurrent UTI	42.1% (16/38)	-
History of UTI	57.9% (22/38)	-
Antibiotic therapy	31.6% (12/38)	0(0/65)
Clinical data of a three-month period before the UTI diagnosis		
Hospitalization	7.9% (3/38)	(0/65)
Surgery	(0/38)	-
Ureteral stent or urinary catheter	(0/38)	-
Antibiotic therapy	39.5% (15/38)	0 (0/65)

Note: *Eight KTP information was missed.

Antibiotic Susceptibility Pattern of UPEC Isolates

The results of antibiotic susceptibility pattern revealed that more than 52% of UPEC strain isolated from KTP were resistant to trimethoprim/sulfamethoxazole (84.8%), nalidixic acid (78.3%), cefixim (58.7%), ciprofloxacin (54.3%), norfloxacin (56.5%), and ofloxacin (52.2%), while carbapenems (100%), amikacin (100%), and nitrofurantoin (71.7%) were most effective in vitro antibiotics against UPEC isolates.

Antibiotic susceptibility pattern on the control group revealed that the highest resistance rate was to nalidixic acid (58.3%) followed by ofloxacin (47.7%), while the most effective antibiotic was carbapenems (100%), amikacin (100%), and nitrofurantoin (89.2%). The frequencies of ESBL-positive strains were 31.5% (35/111), out of which 20 (43.5%) and 15 (23.1%) strain isolated from KTP and control group, respectively. Moreover, the MDR phenotype was found in 71.7% and 41.5% of UPEC strain obtained from KTP and control group, respectively. Statistical analysis showed that resistance rate against cefepime, cefixim, trimethoprim/sulfamethoxazole, norfloxacin, and nalidixic acid were significantly higher among KTP than CA-UTI. Moreover, resistance rates of norfloxacin, piperacillin-tazobactam, ceftazidim, ofloxacin, ciprofloxacin, cefixim, and cefepime in ESBL-positive isolates were found to be higher than ESBL-negative isolates in KTP, while, resistance to cefoxitin, ceftazidim, cefixim, ciprofloxacin, ofloxacin, norfloxacin, and gentamicin ESBL-producing isolates in the control group was significantly higher than in non-ESBL producers (P,0.001). The full results of antibiotic susceptibility pattern and comparison between ESBL-negative and ESBL-positive between two groups are shown in Tables 2 and 3.

Table 2 Antibiotic Susceptibility Pattern of UPEC Isolates Among KTP and Control Group

Antimicrobial category	Antibiotics	KTP					*p*-value	Control-group					*p*-value	*p*-value KTP vs control-group
		Total resistance	ESBL-positive isolates (20)		ESBL-negative isolates (26)			Total resistance	ESBL-positive isolates (15)		ESBL-negative isolates (50)			
			R	S	R	S			R	S	R	S		
Beta-lactams	Amoxicillin/clavulanic	15 (32.6)	7 (35)	10 (50)	8 (30.8)	18 (69.2)	0.098	15 (23.1)	7 (46.7)	7 (46.7)	8 (16)	40 (80)	0.079	0.53
	Piperacillin-tazobactam	6 (13)	6 (30)	14 (70)	0	26 (100)	0.004	7 (10.8)	3 (20)	12 (80)	4 (8)	45 (90)	0.37	0.6
	Cefoxitin	10 (21.7)	7 (35)	13 (65)	3 (11.5)	23 (88.5)	0.06	15 (23.1)	6 (40)	9 (60)	9 (18)	41 (82)	0.08	0.86
	Ceftazidim	23 (50)	20 (100)	0	3 (11.5)	23 (88.5)	≤ 0.001	22 (33.8)	15 (100)	0	7 (14)	43 (86)	≤ 0.001	0.08
	Cefepime	10 (21.7)	9 (45)	6 (30)	1 (3.8)	23 (88.5)	≤ 0.001	7 (10.8)	2 (13.3)	12 (80)	5 (10)	45 (90)	0.16	0.004
	Cefixim	27 (58.7)	15 (75)	5 (25)	12 (46.2)	14 (53.8)	0.04	22 (33.8)	13 (86.7)	2 (13.3)	9 (18)	49 (80)	≤ 0.001	0.02
Carbapenem	Imipenem	0	0	26 (100)	0	20 (100)	-	0	0	15 (100)	0	50 (100)	-	0.2
	Meropenem	0	0	26 (100)	0	20 (100)	-	0	0	15 (100)	0	50 (100)	-	0.2
Sulfonamides	Trimethoprim/ sulfamethoxazole	39 (84.8)	17 (85)	3 (15)	22 (84.6)	4 (15.4)	0.9	30 (46.2)	9 (60)	6 (40)	21 (42)	28 (56)	0.43	≤ 0.001
Quinolones	Ciprofloxacin	25 (54.3)	16 (80)	4 (20)	9 (34.6)	16 (61.5)	0.008	24 (36.9)	10 (66.7)	5 (33.3)	14 (28)	35 (70)	0.024	0.17
	Ofloxacin	24 (52.2)	16 (80)	4 (20)	8 (30.8)	18 (69.2)	≤ 0.001	31 (47.7)	12 (80)	3 (20)	19 (38)	30 (60)	0.017	0.6
	Nalidixic acid acid	36 (78.3)	18 (90)	2 (10)	18 (69.2)	8 (30.8)	0.15	38 (58.5)	12 (80)	2 (13.3)	26 (52)	21 (42)	0.12	0.047
	Norfloxacin	26 (56.5)	16 (80)	4 (20)	10 (38.5)	16 (61.5)	0.005	23 (35.4)	10 (66.7)	5 (33.3)	13 (26)	37 (74)	0.004	0.027
Nitrofurans	Nitrofurantoin	11 (23.9)	3 (15)	15 (75)	8 (30.8)	18 (69.2)	0.14	6 (9.2)	0	14 (93.3)	6 (12)	44 (88)	0.075	0.06
Aminoglycosides	Gentamicin	15(32.6)	10 (50)	10 (50)	5 (19.2)	21 (80.8)	0.27	11 (16.9)	6 (40)	9 (60)	5 (10)	45 (90)	0.014	0.055
	Amikacin	0	0	26 (100)	0	20 (100)	-	0	0	15 (100)	0	48 (100)	0.43	0.2

Table 3 Antibiotic Susceptibility Pattern Among ESBLs and Non-ESBL Isolates Among KTP and Control Group

Antimicrobial Category	Antibiotics	ESBL-Positive Isolates				*p*-value	ESBL-Negative Isolates				*p*-value
		KTP		Control Group			KTP		Control-Group		
		R	S	R	S		R	S	R	S	
Beta-lactams	Amoxicillin/clavulanic	7 (35)	10 (50)	7 (46.7)	7 (46.7)	0.6	8 (30.8)	18 (69.2)	8 (16)	40 (80)	0.32
	Piperacillin-tazobactam	6 (30)	14 (70)	3 (20)	12 (80)	0.39	0	26 (100)	4 (8)	45 (90)	0.25
	Cefoxitin	7 (35)	13 (65)	6 (40)	9 (60)	0.76	3 (11.5)	23 (88.5)	9 (18)	41 (82)	0.35
	Ceftazidim	20 (100)	0	15 (100)	0	-	3 (11.5)	23 (885)	7 (14)	43 (86)	0.53
	Cefixim	15 (75)	5 (25)	13 (86.7)	2 (13.3)	0.39	12 (46.2)	14 (53.8)	9 (18)	40 (80)	0.02
	Cefepime	9 (45)	6 (30)	2 (13.3)	12 (80)	0.14	1 (3.8)	23 (88.5)	5 (10)	45 (90)	0.09
Carbapenem	Imipenem	0	20 (100)	0	15 (100)	-	0	26 (100)	0	26 (100)	0.2
	Meropenem	0	20 (100)	0	15 (100)	-	0	26 (100)	0	26 (100)	0.2
Sulfonamides	Trimethoprim/sulfamethoxazole	17 (85)	3 (15)	9 (60)	6 (40)	0.1	22 (84.6)	4 (15.4)	21 (42)	28 (56)	0.002
Quinolones	Ciprofloxacin	16 (80)	4 (20)	10 (66.7)	5 (33.3)	0.3	9 (34.6)	16 (61.5)	14 (28)	35 (70)	0.7
	Ofloxacin	16 (80)	4 (20)	12 (80)	3 (20)	1.00	8 (30.8)	18 (69.2)	19 (38)	30 (60)	0.6
	Nalidixic acid	18 (90)	2 (10)	12 (80)	2 (13.3)	0.46	18 (69.2)	8 (30.8)	26 (52)	21 (42)	0.22
	Norfloxacin	16 (80)	4(40)	10 (66.7)	5 (33.3)	0.3	10 (38.5)	16 (61.5)	13 (26)	37 (74)	0.26
Nitrofurans	Nitrofurantoin	3 (15)	15 (75)	0	14 (93.3)	0.25	8 (30.8)	18 (69.2)	6 (12)	44 (88)	0.063
Aminoglycosides	Gentamicin	10 (50)	10 (50)	6 (40)	9 (60)	0.5	5 (19.2)	21 (80.8)	5 (10)	45 (90)	0.29
	Amikacin	0	20 (100)	0	15 (100)	-	0	26 (100)	2 (4)	48 (96)	0.3

The molecular results revealed that 53.2% (59/111), 45% (50/111), and 5.4% (6/111) of isolates harbored bla_{CTX-M}, bla_{TEM} and bla_{SHV} genes, respectively (Table 4). Also, 3 (2.7%) of the isolates were found positive for all ESBL genes. Out of 35 ESBL-positive isolates (20 from KTP and 15 from control group), 29 isolates (82.9%) harbored bla_{CTX-M} followed by 14 (40%) bla_{TEM}, and 1 (2.9%) bla_{SHV}. Of the studied resistance genes, bla_{CTX-M} and bla_{TEM} genes were significantly higher among KTP than the control group, while bla_{CTX-M} were found significantly higher in ESBL-positive isolates in comparison with ESBL-negative isolates (Table 4). Moreover, 29 (26.1%) isolates had both bla_{CTX-M} and bla_{TEM}, simultaneously.

Discussion

UTI is the common complication in KTPs. It is considered a particular risk factor for graft loss and patient death in kidney recipients, and it has seen in the first few weeks following transplantation.[20] Gram-negative bacteria are the leading cause of 70% of UTIs, leading to renal failure of which, UPEC is the most common etiology. Furthermore, the extensive use of antibiotics in human medicine as treatment, prevention, and prophylactic is associated with the increasing emergence of MDR and ESBL-producing strains.[21] Thus, it needs more attention to the prevention of UTI and its consequences in KTPs.[22] Here, we investigated the antibiotic resistance pattern and ESBL production in a collection of UPEC obtained from KTPs and compared them to the control group. Physicians can use these regional antibiotic resistance rates to improve antimicrobial stewardship programs and alter antibiotic administration in these patients.

In the present study, 31.5% of the isolates were ESBL producers. According to our findings, ESBL-producing strains were significantly more frequent in KTPs compared to the control group. (43.5% vs 23.1%, $P = 0.021$).

In addition, in KTPs, the risk of UTIs provided by ESBL-producing isolates was 2.56 times (95% CI 1.12–5.82) more than in the control group patients. These results highlight the importance of the presence and emergence of ESBL-producing isolates in these infections. According to the literature review, the high frequency of ESBL-positive as well as prophylactic antimicrobial therapy, and diabetes mellitus accounts for the potential risk factors for UTIs in KTPs.[14,23] Furthermore, the high level of antibiotic resistance and high ESBL-producing isolates may show that KTPs are more exposed to high antibiotic selective

Table 4 Distribution of ESBL Genes Among ESBLs and Non-ESBL Isolates

ESBL Genes	Total 111	ESBL-Positive Isolates			*p*-value	ESBL- Negative Isolates			*p*-value	*p*-value
		Total 35	KTP 20	Control Group 15		Total 76	KTP 26	Control-Group 50		
CTX-M	59 (53.2)	29 (82.9)	19 (95)	10 (66.7)	0.02	30 (39.5)	14 (53.8)	16 (32)	0.06	≤0.001
TEM	50 (45)	14 (40)	11 (55)	3 (20)	0.03	36 (47.4)	15 (57.7)	21 (42)	0.19	0.46
SHV	6 (5.4)	1 (2.9)	1 (5)	0	0.3	5 (6.6)	2 (7.7)	3 (6)	0.7	0.42
CTX-M+ TEM	29 (26.1)	13 (37.1)	11 (55)	2 (13.3)	0.01	16 (21.05)	9 (34.6)	7 (14)	0.03	-
CTX-M+ SHV	4 (3.6)	1 (2.9)	1 (5)	0	-	3 (3.9)	1 (3.8)	2 (4)	-	0.7
TEM+ SHV	3 (2.7)	1 (2.9)	1 (5)	0	-	2 (2.6)	1 (3.8)	1 (2)	-	0.9
CTX-M+TEM+SHV	3 (2.7)	1 (2.9)	1 (5)	0	-	5 (6.6)	2 (7.7)	3 (6)	-	0.4

pressure and excessive use of antibiotics like third-generation cephalosporins before and after transplantation. Various reports of ESBL-producing UPEC have been described from all parts of the world in KTPs. A lower frequency of ESBL-positive isolates has been reported in France (10.9%),[24] Spain (25.7%),[1] Canada (10.7%), and the United States (3.4%).[25] A recent meta-analysis of the literature has reported that the incidence of the UTI caused by ESBL-producing Enterobacteriaceae in KTPs is 10%.[9] In accordance with our results, one study in northwestern of Iran reported that 40.6% of the UPEC isolates were ESBL producers.[22]

The incidence of ESBL-producing isolates among community-acquired UPEC isolates detected in this study (23.1%) was higher than that reported among strains isolated in Brazil (7.1%),[26] Algeria (9%),[27] the United States (7% to 15%),[28] and Turkey (11%),[5] and it was lower than those reported in Iran (32%),[29] (29.6%)[30] and (41%).[31]

In addition to increasing antibiotic resistance in KTPs, several factors such as intolerance of patients to some antibiotics and drug toxicity, and standard and appropriate antibiotic therapy for UPEC in KTPs are regarded as one of the complicated challenges.[32] According to antibiotic susceptibility pattern, high resistance to trimethoprim/sulfamethoxazole and quinolones, as well as high effectiveness of carbapenems, amikacin, and nitrofurantoin were reported in KTPs. Moreover, relatively similar results were seen in the control group.

According to previous reports, third-generation cephalosporins and trimethoprim-sulfamethoxazole can be used as prophylaxis to reduce the incidence of UTI.[3] However, based on our results showing high-level resistance against trimethoprim-sulfamethoxazole, the use of this antibiotic may not be as effective as prophylaxis.

Although another common treatment option is the use of quinolones for UTIs in KTPs, there have been increasing reports of decrease in susceptibility to quinolones. This increase is commonly associated with ESBL production.[2,4,33] Compared to ESBL-negative isolates, ESBL-producing isolates were associated with high-level resistance to norfloxacin, piperacillin-tazobactam, ceftazidime, ofloxacin, ciprofloxacin, cefixime, and cefepime (P-value <0.05). This finding is one of the most worrisome aspects of ESBL-positive bacteria.

This pattern of antibiotic resistance was very close to the finding obtained by Mohammadzadeh et al,[32] Kashef Nejad et al,[22] and Azap et al[4] reporting a high level of resistance to trimethoprim/sulfamethoxazole and quinolones and also carbapenems had the least therapeutic effect. Commonly, in KTP, co-trimoxazole with 480 mg daily is used for 6 months after transplantation. In a meta-analysis study conducted by Moghaddam et al, antibiotic susceptibility pattern of UPEC isolates among Iranian KTP patients was investigated. Similar to our results, the results indicated that cotrimoxazole, nalidixic acid, cefixime, and ceftazidime had a high level of antibiotic resistance.[34] Moreover, a similar resistant pattern was also reported by Shokouhi Mostafavi et al[35] and Farajzadah Sheikh et al[8] in Iran.

In our region, based on the literature review, the resistance rate of the isolates was high to fluoroquinolones, sulfonamides, and third-generation cephalosporins. This high resistance rate may be due to the extensive and routine use of these agents in treatment patients with UTI.[8,22,30,32,35]

Similar to our finding, a report from China established that among KTPs, all gram-negative bacteria like UPEC were susceptible to carbapenems (imipenem or meropenem) that might be related to its low usage in routine treatments for UTIs. In this regard, carbapenems such as imipenem and meropenem represent a good choice for serious UTIs.[22] Nevertheless, therapy with carbapenems should be implemented with caution, since the emergence of carbapenem-resistance tends to increase among Gram-negative organisms with ESBLs.[36] Accordingly, our results suggest that nitrofurantoin and aminoglycoside can be used as drugs available for outpatient setting.

Molecular analysis of the three most prevalent ESBL genes ($bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV}) showed that $bla_{\text{CTX-M}}$ was the most common ESBL gene and following that bla_{TEM} (45%) was higher among all UPEC isolates. Furthermore, a significant association was detected between the presence of $bla_{\text{CTX-M}}$ and ESBL-positive strain ($P \leq 0.001$); however, bla_{TEM} was detected with a slightly higher frequency in ESBL-negative strain ($P=0.46$).

The considerable predominance of the $bla_{\text{CTX-M-15}}$ allele may be owing to the outstanding ability of its gene products to hydrolyze aztreonam, cefotaxime, and ceftazidime. According to some previous studies, the $bla_{\text{CTX-M}}$ is the most frequent beta-lactamase in UPEC isolates.[27,31,37] This finding confirms the importance of the spread of $bla_{\text{CTX-M}}$ types in ESBL production.

Additionally, this report demonstrates that the presence of a $bla_{\text{CTX-M}}$ gene in a bacterium may be an appropriate biomarker for high resistance to beta-lactam antibiotics, and it can be applied in protocols associated with testing routine antibiotic susceptibility.

Furthermore, in this study, the presence of bla_{CTX-M} and bla_{TEM} was significantly higher in KTP isolates than in the control group. In Portugal, Espinar et al investigated the prevalence of ESBL among KTPs. In this study, 25%, 18.8%, and 9.4% of isolates carried bla_{CTX-M}, bla_{TEM}, and bla_{SHV}, respectively.[38] This finding was inconsistent with our results revealing 82.9%, 40%, and 2.9% of isolates harbored bla_{CTX-M}, bla_{TEM}, and bla_{SHV}, respectively. Based on our findings, the occurrence of bla_{SHV} gene was lower than that reported in Iran[31,37,39] and Portugal,[38] but it was similar to that reported in Turkey.[5] Despite the relative agreement on the higher incidence of ESBLs genes among UPECs, a discrepancy in the prevalence of ESBL producers was observed over different regions, which is probably due to differences in geographical regions, infection control policy, and sample source. The present study has some limitations that we should discuss. The first limitation of this study is the lack of complete background details of patient history. The second is the incomplete data on documentation of symptoms and physical exam.

Conclusion

In conclusion, this study demonstrated a high frequency of MDR and ESBLs isolates that most of them harbor bla_{CTX-M} in KTPs. Significant high co-resistance to different classes of antibiotics was reported from ESBL-producing UPEC in UTIs from KTPs, which remains a serious clinical challenge. Thus, this high level of resistance and high frequency of MDR and ESBLs isolates may alert us to adjust our strategy regarding empirical antibiotic therapy and screening susceptibility pattern for UTI cases among KTPs.

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Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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