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Urinary tract infections among kidney transplant patients due to extended-spectrum beta-lactamase-producing bacteria

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1. Introduction

At present, in patients with end stage of renal failure, kidney transplantation is a selective therapeutic strategy due to better rates of life participation [1,2]. However, receiving of immunosuppressant medication encounter kidney transplant patients (KTPs) at increased risk of cancer, infection, diabetes and cardiovascular disease [3]. The presence of infections greatly influences the success of kidney transplantation since they cause higher morbidity and mortality and also increase the length and expenditure of hospitalization [4]. Of the infections, urinary tract infection (UTI) has been considered a common problem, occurring in 60% of KTPs during the first year post-transplant [5]. Some studies reported an increased incidence of bacterial infection with multiple antibiotic resistances among transplant patients [6]. Of the bacteria, extended spectrum beta-lactamases (ESBLs) producing gram negative bacteria most commonly *Escherichia coli* and *Klebsiella* spp have the ability to hydrolyze and cause resistance to oxyiminocephalosporins and monobactams [7,8]. Recently, the prevalence of CTX-M-type ESBLs (*bla* $_{CTX-M}$) has been increased and such ESBLs may cause resistance to some unrelated classes of antibiotics [9]. CTX-M enzymes are currently divided into five clusters on the basis of amino acid sequence, including: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25 [10]. Since the infections can affect renal transplantation outcomes and little information were available regarding the frequency of ESBLs among KTPs, this study was designed to investigate the susceptibility pattern, frequency of ESBLs and *bla* $_{CTX-M}$ among the isolated *E. coli* and *Klebsiella* spp from urine specimens of KTPs using phenotypic and molecular methods.

2. Methods

In this analytical, descriptive study, during the hospitalization (based on patients' condition, the length of hospitalization varied from two weeks up to one month), the urine specimen of KTPs through systematic urine sampling (twice a week) and also following the initial diagnosis of UTI have been collected and cultivated for isolation and identification of *E. coli* and *Klebsiella* spp in the bacteriology laboratory of Imam Khomeini university teaching hospital (a tertiary referral center), Urmia- Iran, between May 2013 to November 2014. For specimen collection from an existing, indwelling urinary catheter, the catheter collection port has been cleaned with an alcohol pad and punctured directly with a needle and syringe. The samples inoculated in both Blood agar and Mac Conkey agar plates and incubated at 37 °C for 24 h. In recent years, this center has performed about 100 kidney transplants annually, which most of them have been done with living donors. All KTPs received preoperative prophylaxis with Cefazolin and post operation prophylaxis, including; two days Cefazolin, for next six days with Cephalexin, after the eighth day the urinary catheter removal was performed and Trimethoprim-sulfamethoxazol was started (for nine to 12 months according to clinician's choice). Meanwhile the routine immunosuppressive regimen was Cyclosporine, Mycophenolate mofetil, and Prednisolone. For each isolate, antimicrobial susceptibility test was performed using the disk diffusion method based on the Clinical and Laboratory Standards Institute (CLSI 2014) recommendations [11]. The ESBL status was confirmed by double disc diffusion test (DDDT) with cefotaxime (30 μg) and ceftazidime (30 μg) alone and in combination with clavulanic acid (10 μg) (Mast Diagnostics, Merseyside-UK). Isolates with a ≥ 5-mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid and the zone diameter of the agent when tested alone were classified as probable ESBL-producers [11]. All ESBL positive isolates checked in respect of their susceptibility to Minocycline (30 μg) or Fosfomycin (200 μg)

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as alternative therapeutic options. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as quality control strains for performing antimicrobial tests. The DNA of isolates was extracted using a commercial kit (YTA Genomic DNA Extraction; Iran). Multiplex PCR was performed on isolates for the simultaneous detection of genes encoding five clusters of the CTX-M- beta-lactamases, as described previously [12] and characterized them by sequencing of PCR products. All the molecular analysis has been carried out in laboratory of Cellular and Molecular Research center of faculty of medicine, Urmia- Iran. Statistical analysis was performed with the SPSS (version 16) statistical program. $P < 0.05$ was regarded as statistically significant.

3. Results

During the period of study, in total, 127 sequential and non-duplicate isolates, including 57 *E. coli,* 39 *K. pnumoniae*, 13 *Pseudomonas aeruginosa*, eight *staphylococcus aureus*, three Enterococci, two *Acinetobacter baumannii* and five other bacteria were collected from 630 urine specimens of KTPs. Of the mentioned isolates, only 96 isolates, namely; *E. coli* (57 [59.4%]) and *K. pnumoniae* (39 [40.6%]) were included in this study. Of KTPs, 42 (43.75%) were males and 54 (56.25%) were females ranging in age from 8 to 76 years with a mean of 47.07 years.

Antibiotic susceptibility test using disc diffusion revealed high resistance to Trimethoprim/Sulfamethoxazole (78.1%), and the least levels of resistance was observed to Imipenem (10.4%). The overall frequencies of ESBL positive strains were 56 (58.3%), including 39 (40.6%) *E. coli* and 17 (17.7%) *K. pneumoniae* isolates using DDDT. All the ESBL-producing isolates had higher resistance to tested antibiotics except Imipenem, Ertapenem and Nitrofurantoin in compare with ESBL-negative ones (Table 1). The MICs of 10 imipenem resistant isolates (detected by disc diffusion method) were determined using Etest (Liofilchem, Italy) method. Based on the Etest results, the MIC was ≥32 mg/ml for four isolates and >1–2 mg/ml (intermediate) for five isolates; a MIC of 6 mg/ml was seen for only in one isolate.

Among 56 ESBL isolates; 41 (73.2%) vs. 13 (23.2%) were sensitive and 8 (14.3%) vs. 36 (64.3%) showed resistance for Fosfomycin and Minocycline, respectively. In addition, 7 (12.5%) isolates revealed intermediate resistant to both Fosfomycin and Minocycline.

The results of Multiplex- PCR on 96 isolates showed that 53 (55.2%) isolates were producing CTX-M type ESBLs, which 53.1% (51/96) and 2.1% (2/96) harbored CTX-M genogroup-1 and genogroup-9, respectively (Fig. 1). Among the CTX-M genogroup-1; 31 and 20 isolates were *E. coli* and *K. pnumoniae*, respectively. Both strains with CTX-M genogroup- 9 were *E. coli*. Three of 56 phenotypically detected ESBLs were not carrying any of CTX- M genogroups.

The isolates producing CTX-M type ESBLs were subjected for sequence analyses (Bioneer- Korea). On performing the BLAST search on isolates belonging to CTX-M genogroup-1 and genogroup-9, 100% similarity was found with CTX-M-15 and CTX-M-65 types ESBL, respectively.

4. Discussion

Urinary tract infection is the most common type of bacterial infection contracted by KTPs after transplantation. Immunosuppression and inappropriate antibiotic therapy of UTIs can affect the outcome of transplantation [13] and need to more attention to the prevention of such infections and its consequences. For this purpose, in our transplantation center the same as others, prophylaxis with different antibiotics are used for the avoidance of the infections in KTPs, but it seems to be a link between perioperative prophylaxis along with the higher hospitalization period and the development of antibiotic resistance over time. Some researchers suggested an association between UTI and perioperative cephalosporin prophylaxis in KTPs [14]. Consistent with other studies [15,16], in our research, *E. coli* was the most commonly isolated bacteria, followed by *K. pneumoniae*. Such bacteria can create many problems with gaining of resistance against a large number of beta-

Table 1
Results of antibiotic susceptibility test between ESBLs and non- ESBLs using disc diffusion method.

Antibiotics	ESBL positive isolates 56 (58.3%)						Resistance among all *ESBLs* No. (%)	ESBL negative isolates 40 (41.7%)						Resistance among all *Non-ESBLs* No. (%)	Resistance among all isolates No. (%)	***Pv***[e]
	E. coli (n = 39) No. (%)			*K. pneumoniae* (n = 17) No. (%)				*E. coli* (n = 18) No. (%)			*K. pneumoniae* (n = 22) No. (%)					
	S[b]	**I**[c]	**R**[d]	S	I	R		S	I	R	S	I	R			
Ampicillin	0 (0)	0 (0)	39 (100)	nc[f]	nc	nc	**39 (100)**	3 (16.7)	0 (0)	15 (83.3)	nc	nc	nc	**15 (83.3)**	**54 (94.7)**	0.028
Cotrimoxazole[a]	4 (10.3)	0 (0)	35 (89.7)	1 (5.9)	0 (0)	16 (94.1)	**51 (91.1)**	4 (22.2)	0 (0)	14 (77.8)	11 (50.0)	1 (4.5)	10 (45.5)	**24 (60.0)**	**75 (78.1)**	0.001
Aztreonam	0 (0)	2 (5.2)	37 (94.8)	0 (0)	1 (5.9)	16 (94.1)	**53 (94.6)**	15 (83.3)	3 (16.7)	0 (0)	17 (77.3)	5 (22.7)	0 (0)	**0 (0)**	**53 (55.2)**	0.000
Ciprofloxacin	9 (23.1)	0 (0)	30 (76.9)	0 (0)	0 (0)	17 (100)	**47 (83.9)**	7 (38.9)	0 (0)	11 (61.1)	13 (59.1)	0 (0)	9 (40.9)	**20 (50.0)**	**67 (69.8)**	0.001
Gentamicin	12 (30.8)	0 (0)	27 (69.2)	1 (5.9)	0 (0)	16 (94.1)	**43 (76.8)**	11 (61.1)	0 (0)	7 (38.9)	15 (68.2)	0 (0)	7 (31.8)	**14 (35.0)**	**57 (59.4)**	0.000
Nitrofurantoin	26 (66.7)	8 (20.5)	5 (12.8)	3 (17.6)	1 (5.9)	13 (76.5)	**18 (32.1)**	15 (83.3)	0 (0)	3 (16.7)	1 (4.5)	1 (4.5)	20 (91.0)	**23 (57.5)**	**41 (42.7)**	0.25
Ertapenem	36 (92.3)	0 (0)	3 (7.7)	9 (52.9)	6 (35.3)	2 (11.8)	**5 (8.9)**	14 (77.8)	0 (0)	4 (22.2)	13 (59.1)	2 (9.1)	7 (31.8)	**11 (27.5)**	**16 (16.7)**	0.151
Imipenem	38 (97.4)	0 (0)	1 (2.6)	15 (88.2)	0 (0)	2 (11.8)	**3 (5.4)**	16 (88.9)	0 (0)	2 (11.1)	17 (77.3)	0 (0)	5 (22.7)	**7 (17.5)**	**10 (10.4)**	0.058

Bold columns indicated the percent of resistance among ESBLs, non-ESBLs and total isolates, respectively.
[a] Trimethoprim/Sulfamethoxazole.
[b] Sensitive.
[c] Intermediate.
[d] Resistant.
[e] *Pv* for resistance rate between ESBLs and non- ESBLs.
[f] Not cheked; *Klebsiella* spp. are intrinsically resistant to penicillins.

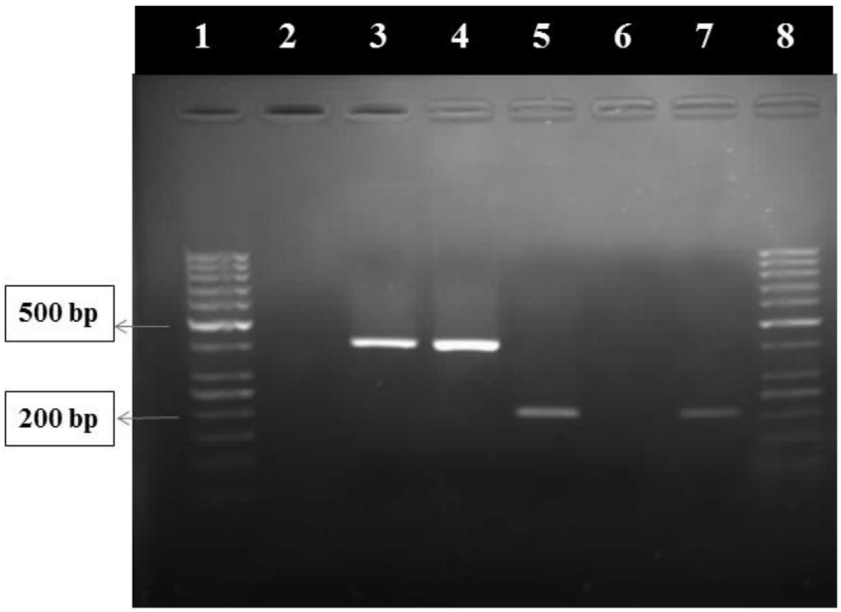

Fig. 1. Agarose gel electrophoresis of amplified to CTX-M genogroup by multiplex PCR. Lanes: 1and 8, 50 bp DNA ladder (Bioflux); 2, negative control; 3 and 4, *E. coli* positive for CTX-M genogroup-1(415 bp); 5 and 7, *E. coli* positive for CTX-M genogroup-9 (205 bp); 6, *E. coli* negative for CTX-M genogroup.

lactam antibiotics due to producing ESBLs [17]. The different prevalence of ESBLs producing bacteria has been reported from all parts of the world from KTPs [7,18]. In this study, ESBLs producing strains were more obvious in *E. coli* strains than *K. pneumonia* ($Pv = 0.021$). By contrast, in some studies, ESBLs positive *K. pneumoniae* was predominant isolates among organ transplant recipients [19]. In our setting, the frequency of ESBLs among *E. coli* and *Klebsiella* spp obtained from hospitalized patients with other clinical features (Non- KTPs) during a year, was 29.53% (176 out of 596) and 14.8% (37 out of 250), respectively (Unpublished data). The analysis of distribution of ESBLs in the two groups of patients reveals a higher frequency of ESBL isolates from KTPs (58.3%) than Non- KTPs (25.17%). Since, after kidney transplantation, the average length of hospitalization was fifteen days and in some cases may become a month or more, it seems that the period is enough for colonization of ESBLs in KTPs.

As revealed in Table 1, most (94.8%) of our isolates were resistant to at least two of the antibiotics tested, and antibiotic resistance was more common among *E. coli* isolates than *K. pneumoniae*. Using both Cefotaxime and Ceftazidime disks improves the sensitivity of screening ESBL producing isolates as mentioned in CLSI (2014). However, in some isolates (11isolates) when zone diameter was zero for Cefotaxime and Ceftazidime disks, the DDD test as confirmatory for ESBL producing did not also show any increasing zone diameter in combination with clavulante to considering them as ESBLs but we considered them as ESBL producers because they carried *ctx*-M genes using Multiplex PCR. We couldn't find any comment related to such situation in CLSI, but the authors suggest for isolates without any zone of inhibition either in cefotaxime (30 μg) and ceftazidime (30 μg) alone or in combination with clavulanic acid be considered as ESBL-producers. In this study, three strains of ESBLs (by DDDT) that did not show any *ctx*-M genes may harbor other genes related to ESBLs such as TEM or SHV. By the way, the relatively high frequency of ESBL producing isolates is an alarming situation which could be due to use of various Cephalosporins before and after transplantation and antibiotic selection pressure. The ESBL producers were as well resistant to the other classes of antibiotic besides beta-lactams, including Ciprofloxacin, Gentamicin and Trimethoprim/Sulfamethoxazole, as indicated in the majority of studies on ESBL producer bacteria [20]. This condition can contribute to the selection and persistence of multidrug-resistant ESBL strains and plasmids in various settings [21]. Easy movement of extra chromosomal genetic material such as insertion sequences, integrons and transposons among bacteria played a crucial role in global spreading of the most common ESBL genes, namely CTX-M [22]. The prevalence of CTX-M and other types of ESBLs differs among patient groups, clinical and geographic settings. CTX-M-15 (group 1) is predominant in most of Europe, North America, the Middle East, and India, whereas CTX-M-14 (group 9) is most common in China, Southeast Asia, and Spain, and CTX-M-2 (group 2) is isolated predominantly in Argentina and Japan [23–25]. During this investigation, molecular analysis revealed that CTX-M- 15 subtype (53.1%) of group-1 was the most common among the isolates. In addition, two isolates showed CTX-65 subtype (group 9). To the best of our knowledge, this is the first report of sequencing outcomes of CTX-M-type ESBLs from north-west Iran. Other studies from Iran, also demonstrated that the CTX-M-15 is common in different settings and other groups have not an outbreak or have a small percentage into account [26].

In our study, ESBL-producing organisms showed less resistant to Imipenem (10.4%) and Ertapenem (16.7%) using disc diffusion method. Such carbapenems are generally resistant to ESBL-mediated hydrolysis. Using these antibiotics for empiric antibiotic therapy of KTPs with UTI is sensible, because any delay in treatment can cause severe adverse consequences for instance septicemia (two out of 96 cases). This is in agreement with previous studies in which Carbapenems are the drug of choice for ESBL producing pathogens [27]. On the other hand, Carbapenems therapy should be performed with caution, since the emergence of carbapenem-resistance tend to increase among Gram negative organisms with ESBLs [28]. In this research, two out of seven Imipenem resistant *K. pneumoniae* confirmed as carbapenemase producers using modified Hodge test (MHT) (CLSI, 2014) and were also ESBLs positive. As noted in CLSI (2014), MHT with the Imipenem disk may give poor result as a screen for carbapenemases, so for isolates positive with the disk screen and MHT, carrying out the MIC test before reporting any carbapenem results, has been recommended. For this reason, the MIC of the detected Imipenem resistant isolates

(by disk diffusion method) determined using Etest method and all were non susceptible. However, it seems to confirm that an isolate is a carbapenemase producer; the molecular methods should also be applied along with phenotypic methods. In addition, Carbapenems resistance was also found at a higher frequency among ESBL negative isolates than among ESBL producing isolates (17.5% vs. 5.4% for Imipenem). This may be due to inappropriate prescribing and overuse of these antibiotics in our setting. Among the antibiotics tested, in addition to Imipenem, Fosfomycine also exhibited better *in vitro* activity against ESBL isolates. Even so, continuous monitoring of the effectiveness of the antibiotics will be required to detect any changes in its current status.

In conclusion, our study highlighted the presence of a high frequency of multidrug resistance among the isolates that most of them were ESBLs producer with CTX-M-15 subtype. This is an alarming situation for KTPs and medical teams by limited the choice of antibiotics. Therefore, collaboration between clinicians and the medical microbiologist is essential to rapid diagnosis and use of appropriate antibiotics against the resistant isolates. In addition, permanent monitoring of the treatment outcome of infections among KTPs can reduce the spread of bacterial resistance, the length of hospitalization, expenditures and side effects of drugs. This requires compulsory testing of all isolates for ESBLs and other type of resistance, by phenotypic and molecular methods alongside each other.

Conflict of interest

All authors report no conflicts of interest relevant to this article.

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