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ORIGINAL ARTICLE

Observational Study

Prevalence, serotyping and drug susceptibility patterns of *Escherichia coli* isolates from kidney transplanted patients with urinary tract infections

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Author contributions: Hakemi-Vala M proposed the subject of the project, supervised the proposal, practical steps, revised the draft, and submitted the article to this journal; Samavat S, as the urologist in Labafi Nejad Hospital, introduced the patients who were compatible with this subject; Najafi Khah A is an MSc student in medical microbiology and this paper is part of her thesis, she also carried out all practical processes such as sampling, cooperated with two private clinical laboratories and drafted the paper; Nasiri MJ, a consultant, contributed to statistical analysis, paper preparation, and revision, data collection and analysis.

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Abstract

BACKGROUND

Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) are among the main pathogens in urinary tract infections (UTIs) among kidney transplant patients (KTPs).

AIM

To estimate the prevalence of ESBL-producing *E. coli* in KTPs and to evaluate the most prevalent serotypes and antibacterial susceptibility patterns of isolated bacteria in Tehran, Iran.

METHODS

A total of 60 clinical isolates of uropathogenic *E. coli* were collected from 3 kidney transplant centers from April to May 2019. Antimicrobial susceptibility testing was performed by the disk diffusion method as recommended by the Clinical Laboratory and Standards Institute. The serotyping of *E. coli* isolates was performed by the slide agglutination method. The presence of bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes was evaluated by polymerase chain reaction.

RESULTS

The frequency of ESBL-producing *E. coli* in KTPs was found to be 33.4%. All of the 60 *E. coli* isolates were found to be susceptible to doripenem (100%) and ertapenem (100%). High resistance rates to ampicillin (86%), cefotaxime (80%), and cefazolin (77%) were also documented. The most frequent serotypes were serotype I (50%), serotype II (15%), serotype III (25%), and serotype VI (10%). The gene most frequently found was bla_{TEM} (55%), followed by bla_{CTX-M} (51%) and bla_{SHV}

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(41%).

CONCLUSION

Molecular analysis showed that bla_{TEM} was the most common ESBL-encoding gene. The high resistance to β-lactams antibiotics (*i.e.*, ampicillin, cefotaxime, and cefazolin) found in *E. coli* from KTPs with UTIs remains a serious clinical challenge. Further efforts to control ESBL-producing *E. coli* should include the careful use of all antibiotics as well as barrier precautions to reduce spread.

Key Words: Kidney transplantation; Urinary tract infection; Drug resistance; *Escherichia coli*; Serotyping; β-Lactamase

Core Tip: Extended-spectrum β-lactamases (ESBLs)-producing *Escherichia coli* (*E. coli*) are among the main pathogens in urinary tract infections among kidney transplant patients (KTPs). The aims of this study were: To estimate the prevalence of ESBL-producing *E. coli* in KTPs, and to evaluate the most prevalent serotypes and antibacterial susceptibility patterns of isolated bacteria in Tehran, Iran. The most important findings were: (1) The frequency of ESBL-producing *E. coli* in KTPs was 33.4%; (2) High resistance rates to ampicillin (86%) and cefotaxime (80%) were documented; (3) The most frequent serotype was serotype I (50%); (4) The most frequently found related gene was *blaTEM* (55%); and (5) Further efforts to control ESBL-producing *E. coli* should include the careful use of all antibiotics as well as barrier precautions to reduce spread.

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INTRODUCTION

Urinary tract infection (UTI) remains one of the most common bacterial infections in kidney transplant patients (KTPs)[1,2]. *Escherichia coli* (*E. coli*) is one of the main uropathogens isolated from KTPs with UTIs[3]. Recently, several studies have reported a high incidence of extended-spectrum β-lactamases (ESBLs)-producing *E. coli* among KTPs[4]. Infections caused by ESBL-producing bacteria are usually associated with increased morbidity and mortality[5-7]. Therefore, UTI caused by ESBL-producing *E. coli* in KTPs is an important challenge in healthcare settings.

The ESBL-producing strains are resistant to all penicillins, cephalosporins (including first-, second-, and third-generation) and aztreonam. This event occurs due to the production of CTX-M, TEM, and SHV β-lactamases which are encoded by bla_{CTX-M}, bla_{SHV}, and bla_{TEM} genes, respectively[5-7]. To date, several studies have reported the rates of ESBL-producing *E. coli* in Iran; however, very few studies have evaluated ESBL-producing bacteria in KTPs or their antimicrobial susceptibility profiles. Therefore, the aims of this study were to estimate the prevalence of ESBL-producing *E. coli* in KTPs, to serotype the ESBL-producing *E. coli*, and to identify the antibacterial susceptibility patterns of isolated bacteria in Tehran, Iran.

MATERIALS AND METHODS

Setting and samples

In this study, urine samples were collected using the mid-stream clean catch method. A total of 60 *E. coli* isolates from 60 KTPs referred to Labofinejad Hospital and two private laboratories, Yekta and Gholhak, were collected from April to May 2019. All

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isolates were confirmed as *E. coli* by standard bacteriologic methods and kept in 10% glycerol and TSB at -70°C for further evaluation.

Detection of ESBLs

ESBL production was detected according to the Clinical Laboratory and Standards Institute (CLSI) confirmatory test using cefotaxime 30 mg and ceftazidime (CAZ) 30 mg disks alone and in combination with clavulanic acid (CA) 10 mg[8]. The test was considered positive when an increase in the growth-inhibitory zone around either the cefotaxime or the CAZ disk with CA was 5 mm or greater than the diameter around cefotaxime or CAZ alone[9]. *E. coli* ATCC 25922 and *Klebsiella. pneumonia*e ATCC 700603 were used as negative and positive controls, respectively.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was performed by the disk diffusion method on Mueller-Hinton agar as recommended by the CLSI[10]. The tested antibiotics were purchased from Mast (England) or Rosco (Denmark) companies and were used for AST: Ceftriaxone 30 mg, cefotaxime 30 mg, cefixime 30 mg, cefazolin 30 µg, cephalexin 30 mg from Rosco Company and ampicillin 10 µg, ampicillin-sulbactam 20/10 µg, piperacillin/tazobactam 100/10 µg, cefpodoxime 30 µg, doripenem 10 µg, imipenem 10 µg, ertapenem 10 µg, meropenem 10 µg, gentamicin 10 µg, tobramycin 10 µg, amikacin 30 µg, ciprofloxacin 5 µg, trimethoprim 5 µg, and nitrofurantoine 200 µg from Mast Company, respectively.

A bacterial suspension with turbidity equal to a homemade 0.5 MacFarland standard (1.5×10^8 CFU/mL) was prepared for each bacterial isolate, a bacterial lawn was performed on a Mueller Hinton agar plate using a sterile cotton swab and selected antibiotic disks were placed on the agar plate with sterile forceps. The plates were then incubated at 37°C for 24 h. The diameter of the zone of inhibition was measured and the results were reported as susceptible (S), resistant (R) or intermediate (I) based on the CLSI criteria[11]. *Escherichia coli* ATCC 25922 was used as a control.

Serotyping

Agglutination (Bahar Afshan_Iran) reactions were performed in triplicate following the manufacturer's protocol: 25 µL of test solution and 25 µL of bacterial suspension were added to a black slide. They were then thoroughly mixed, and the slide was incubated for 5 min at room temperature on a rotator set to 100 rpm[12].

DNA extraction and polymerase chain reaction method

A 1000 µL aliquot of cell suspension containing 10^7 cells/mL was transferred to microtubes and incubated at 100°C in a boiling water-bath for 5 min. The suspension containing DNA was vigorously homogenized by vortex for 10 s and the tube was frozen on ice. The DNA sample was stored at -18°C[13].

β-Lactamase genes were amplified by the polymerase chain reaction (PCR) using a panel of primers for the detection of bla_{TEM}, $bla_{SHV,}$ and bla_{CTX-M} genes[13]. PCR amplification of bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes was performed in 25 µL reaction mixtures containing 25 units/mL of Taq DNA polymerase, 200 µmol/L each of dATP, dGTP, dTTP, and dCTP, 0.2 µmol/L of each primer, 1.5 mmol/L $MgCl_2$, and 5 µL of DNA template[14]. The PCR products were analyzed by gel electrophoresis using 0.8% gel[15].

RESULTS

Based on the demographic data of the enrolled patients[15], 25% were male and 45 (75%) were female. The age of the patients ranged from 12 to 67 years. All of the 60 *E. coli* isolates were found to be susceptible to doripenem (100%) and ertapenem (100%). High resistance rates to ampicillin (86%), cefotaxime (80%), and cefazolin (77%) were also found in the collected isolates (Table 1). Based on the CLSI confirmatory test, the frequency of ESBL-producing *E. coli* in KTPs was found to be 33.4%. Using the slide agglutination method, the most frequent serotypes were found to be serotype I (including: O126, O55 and O111; 50%), serotype II (O86, O127; 15%), serotype III (O44, O125, O128; 25%), and serotype VI (O120, O114; 10%). The genes most frequently found were bla_{TEM} (55%), followed by bla_{CTX-M} (51%) and bla_{SHV} (41%).

Table 1 Antimicrobial susceptibility patterns of *Escherichia coli* isolates from kidney transplant patients

Antibiotic	Susceptible (%)	Intermediate (%)	Resistant (%)
Ampicillin	5 (8)	1 (2)	54 (90)
Amoxicillin-clavulanic acid	28 (46)	28 (46)	23 (38)
Ampicillin-sulbactam	26 (44)	8 (12)	26 (44)
Piperacillin-Tazobactam	40 (67)	6 (8)	14 (24)
Cefazolin	40 (67)	8 (12)	12 (20)
Cefepime	27 (45)	7 (12)	25 (43)
Cefotaxime	10 (17)	1 (2)	39 (65)
Doripenem	60 (100)	0 (0)	0 (0)
Ertapenem	60 (100)	0 (0)	0 (0)
Fosfomycin	57 (95)	2 (3)	1 (1)
Imipenem	57 (95)	3 (5)	0 (0)
Meropenem	36 (60)	10 (17)	0 (0)
Amikacin	40 (67)	14 (25)	14 (23)
Tobramycin	41 (68)	10 (17)	9 (15)
Trimethoprim	10 (17)	13 (22)	37 (61)
Nitrofurantoin	48 (82)	6 (8)	6 (8)
Ciprofloxacin	16 (27)	4 (6)	40 (67)
Gentamycin	43 (71)	6 (8)	6 (8)
Cefpodoxime	20 (34)	2 (2)	38 (64)

DISCUSSION

UTI is the main infectious complication in patients with kidney transplants. The high incidence of ESBL-producing *E. coli* among KTPs has been frequently reported[4]. In the current study, the frequency of ESBL-producing *E. coli* in KTPs was found to be 33.4%. A similar observation was noted by Linares *et al*[16], who reported that the incidence of ESBL-producing gram-negative bacteria in renal transplantation was 11.8%. Previous antibiotic therapy is an important risk factor for the development of ESBL-producing bacteria[17,18]. ESBL-producing *E. coli* infection is commonly associated with a significantly longer hospital stay and greater hospital charges[19].

According to the current study, high resistance rates to ampicillin (86%), cefotaxime (80%) and cefazolin (77%) were documented. Our results were comparable to a previous study that was conducted in Iran and reported a similar resistance rate to ampicillin[20].

In the current study, the most frequent ESBL genes were bla_{TEM} (55%), followed by bla_{CTX-M} (51%) and bla_{SHV} (41%). In Portugal, studies from individual hospitals have reflected a common spread of bla_{CTX-M} and bla_{TEM}[21]. Studies reporting different ESBL-producing bacteria are increasing among European countries[22]. A high prevalence of *E. coli* and *K. pneumoniae* isolates exhibiting two or three ESBL genes was also reported in a similar study from Iran[23]. The epidemiology of ESBL-producing bacteria is becoming more complex[24]. For example, *E. coli* harboring bla_{CTX-M}-15 and -14 have consistently been reported as the predominant ESBL types in clinical isolates from adult centers worldwide[25-27], yet a wide diversity of CTX-M enzymes was observed in children[28-30]. Moreover, it should be taken into consideration that bacterial isolates producing ESBLs are responsible for serious healthcare-related infections[31].

CONCLUSION

In conclusion, the frequency of ESBL-producing *E. coli* in KTPs was found to be 33.4% in the current study. Molecular analysis showed that bla_{TEM} was the most common ESBL encoding gene. The high resistance to β-lactams antibiotics (*i.e.*, ampicillin,

cefotaxime, and cefazolin) found in *E. coli* from KTPs with UTI remains a serious clinical challenge. Further efforts to control ESBL-producing *E. coli* should include the careful use of all antibiotics as well as barrier precautions to reduce spread.

ARTICLE HIGHLIGHTS

Research background

Escherichia coli (*E. coli*) isolates are the main pathogens in urinary tract infections (UTIs). Their effect is more important in kidney transplant patients (KTPs). Based on several studies and documents, the frequency of *E. coli* resistant to common drugs is increasing. Their resistance to antimicrobial drugs is mediated by different mechanisms such as producing extended-spectrum beta-lactamase (ESBLs). Therefore, UTIs caused by ESBL-producing *E. coli* in KTPs is an important challenge in healthcare settings.

Research motivation

However, different studies have reported the frequency of ESBLs *E. coli* isolates from different origins in Iran, but there are few studies on their frequency and role in KTPs and their antimicrobial susceptibility profile.

Research objectives

The aims of this study were: (1) To estimate the prevalence of ESBL-producing *E. coli* in KTPs; (2) To serotype the ESBL-producing *E. coli*; and (3) To identify the antibacterial susceptibility patterns of isolated bacteria in Tehran, Iran.

Research methods

Bacterial culture and isolation based on standard bacteriologic methods were carried out. Antimicrobial susceptibility testing based on the Clinical Laboratory and Standards Institute was performed. The minimum inhibitory concentration was determined using Epsilon strips during the E-test. The frequency of genes responsible for ESBLs coding was assessed after DNA extraction and polymerase chain reaction. Statistical analysis of the data was performed.

Research results

The most important findings were: (1) The frequency of ESBL-producing *E. coli* in KTPs was found to be 33.4%; (2) High resistance rates to ampicillin (86%) and cefotaxime (80%) were documented; (3) The most frequent serotype was serotype I (50%); (4) The most frequently found related gene was bla_{TEM} (55%); and (5) All of the *E. coli* isolates were susceptible to doripenem and ertapenem.

Research conclusions

Further efforts to control ESBL-producing *E. coli* isolates should include the careful use of all antibiotics as well as barrier precautions to reduce their spread.

Research perspectives

More *E. coli* isolates from different parts of Iran should be obtained and their antimicrobial profiles evaluated. Also, the frequency of ESBLs production and the existence of other ESBLs genes such as *KPC* and *metalo-betalactamases* should be determined.

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