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*WJBC* World Journal of Biological Chemistry
ABOUT COVER

Editorial board member of *World Journal of Biological Chemistry*, Dr. Jian-Xun Ding is a Professor at Changchun Institute of Applied Chemistry (CIAC), Chinese Academy of Sciences (CAS). Dr. Ding received his Bachelor of Science degree from the University of Science and Technology of China in 2007 and obtained his PhD degree from CIAC, CAS in 2013. His research focuses on the synthesis of functional biodegradable polymers, the development of smart polymer platforms for controlled drug delivery, and the exploitation of polymer-based adjuvants for immunotherapy. Heretofore, he has published more than 100 articles, which have amassed over 7000 citations. Moreover, he has applied for over 70 patents in China and has won more than 10 awards for his career accomplishments. He was selected for the Young Talents Promotion Project of Jilin Province and joined the CAS Young Innovation Promotion Association in 2019. (L-Editor: Filipodia)

AIMS AND SCOPE

The primary aim of the *World Journal of Biological Chemistry* (*WJBC, World J Biol Chem*) is to provide scholars and readers from various fields of biological chemistry a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

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INDEXING/ABSTRACTING

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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 were performed by the slide agglutination method. The presence of *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>CTX-M</sub> 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*<sub>TEM</sub> (55%), followed by *bla*<sub>CTX-M</sub> (51%) and *bla*<sub>SHV</sub>
INTRODUCTION
Urinary tract infection (UTI) remains one of the most common bacterial infections in kidney transplant patients (KTPs)\[3\]. 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\[5\]. 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\textsubscript{CTX-M}, bla\textsubscript{TEM}, and bla\textsubscript{SHV} genes, respectively\[6\]. 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 isolates from kidney transplanted patients with urinary tract infections, and to evaluate the most prevalent serotypes and antibacterial susceptibility patterns of isolated bacteria in Tehran, Iran.

Therefore, UTI caused by ESBL-producing E. coli in KTPs is an important challenge in healthcare settings.

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
isolation and polymerase chain reaction method

A 1000 μL aliquot of cell suspension containing 10⁷ 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.

β-Lactamase genes were amplified by the polymerase chain reaction (PCR) using a panel of primers for the detection of blaTEM, blaSHV, and blaCTX-M genes. PCR amplification of blaTEM, blaSHV, and blaCTX-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 of each dNTP, 0.2 mg/mL of bovine serum albumin, and 5 μL of DNA template. The PCR products were analyzed by gel electrophoresis using 0.8% agarose gel.

RESULTS

Based on the demographic data of the enrolled patients, 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 blaTEM (55%), followed by blaCTX-M (51%) and blaSHV (41%).
Table 1 Antimicrobial susceptibility patterns of *Escherichia coli* isolates from kidney transplant patients

<table>
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<tr>
<th>Antibiotic</th>
<th>Susceptible (%)</th>
<th>Intermediate (%)</th>
<th>Resistant (%)</th>
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<tr>
<td>Ampicillin</td>
<td>5 (8)</td>
<td>1 (2)</td>
<td>54 (90)</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>28 (46)</td>
<td>28 (46)</td>
<td>23 (38)</td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>26 (44)</td>
<td>8 (12)</td>
<td>26 (44)</td>
</tr>
<tr>
<td>Piperacillin-Tazobactam</td>
<td>40 (67)</td>
<td>6 (8)</td>
<td>14 (24)</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>40 (67)</td>
<td>8 (12)</td>
<td>12 (20)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>27 (45)</td>
<td>7 (12)</td>
<td>25 (43)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>10 (17)</td>
<td>1 (2)</td>
<td>39 (65)</td>
</tr>
<tr>
<td>Doripenem</td>
<td>60 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>60 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>57 (95)</td>
<td>2 (3)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>57 (95)</td>
<td>3 (5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>36 (60)</td>
<td>10 (17)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>40 (67)</td>
<td>14 (25)</td>
<td>14 (23)</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>41 (68)</td>
<td>10 (17)</td>
<td>9 (15)</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>10 (17)</td>
<td>13 (22)</td>
<td>37 (61)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>48 (82)</td>
<td>6 (8)</td>
<td>6 (8)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>16 (27)</td>
<td>4 (6)</td>
<td>40 (67)</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>43 (71)</td>
<td>6 (8)</td>
<td>6 (8)</td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td>20 (34)</td>
<td>2 (2)</td>
<td>38 (64)</td>
</tr>
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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. 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. 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_. Studies reporting different ESBL-producing bacteria are increasing among European countries.[21] 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,
Najafi Khah A et al. E. coli isolates from kidney transplanted patients

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.

**REFERENCES**


