Observational Study

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

Atefeh Najafi Khah, Mojdeh Hakemi-Vala, Shiva Samavat, Mohammad Javad Nasiri

**ORCID number:** Atefeh Najafi Khah 0000-0002-4677-4920; Mojdeh Hakemi-Vala 0000-0002-8355-6885; Shiva Samavat 0000-0001-6707-7844; Mohammad Javad Nasiri 0000-0002-3279-0671.

**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.

**Supported by** Research Department of School of Medicine Shahid Beheshti University of Medical Sciences, No. 17920, and accepted by the ethic committee, Code. IR.SBMU. MSP.REC.1398.349.

**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*<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>.
Urinary tract infection (UTI) remains one of the most common bacterial infections in kidney transplant patients (KTPs)\(^\text{1,3}\). *Escherichia coli* (*E. coli*) is one of the main uropathogens isolated from KTPs with UTIs\(^\text{3,9}\). Recently, several studies have reported a high incidence of extended-spectrum β-lactamases (ESBLs)-producing *E. coli* among KTPs\(^\text{3,9}\). Infections caused by ESBL-producing bacteria are usually associated with increased morbidity and mortality\(^\text{20}\). 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 *blaCTX,M*, *blaTEM*, and *blaSHV* genes, respectively\(^\text{6\text{-}11}\). 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.

**INTRODUCTION**

**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 of isolates from kidney transplanted patients. The 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 ceftazidine (CAZ) 30 mg disks alone and in combination with clavulanic acid (CA) 10 mg\(^{[3]}\). 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\(^{[3]}\). *E. coli* ATCC 25922 and *Klebsiella pneumoniae* 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\(^{[3]}\). 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, cephalixin 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 nitrofurantoin 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\(^{[3]}\). *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\(^{[3]}\).

### 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\(^{[3]}\).

\(\beta\)-Lactamase genes were amplified by the polymerase chain reaction (PCR) using a panel of primers for the detection of *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>CTX-M</sub> genes\(^{[3]}\). PCR amplification of *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, and *bla*<sub>CTX-M</sub> 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\(^{[3]}\). The PCR products were analyzed by gel electrophoresis using 0.8% gel\(^{[3]}\).

### RESULTS

Based on the demographic data of the enrolled patients\(^{[3]}\), 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*<sub>TEM</sub> (55%), followed by *bla*<sub>CTX-M</sub> (51%) and *bla*<sub>SHV</sub> (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*<sub>TEM</sub> (55%), followed by *bla*<sub>CTX-M</sub> (51%) and *bla*<sub>SHV</sub> (41%). In Portugal, studies from individual hospitals have reflected a common spread of *bla*<sub>CTX-M</sub> and *bla*<sub>TEM</sub>. Studies reporting different ESBL-producing bacteria are increasing among European countries[21,22]. 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*<sub>CTX-M-15</sub> 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*<sub>TEM</sub> 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-beta-lactamases* should be determined.

**REFERENCES**

1. Halaji M, Shahidi S, Atapour A, Ataei B, Feizi A, Havaei SA. Characterization of Extended-Spectrum β-Lactamase-Producing Uropathogenic *Escherichia coli* Among Iranian Kidney Transplant Patients. *Infect Drug Resist* 2020; 13: 1429-1437 [PMID: 32523361 DOI: 10.2147/IDR.S248572]

2. Shams SF, Eidgahi ES, Lotfi Z, Khalodi A, Shakeri S, Sheikhi M, Bahrami A. Urinary tract infections in kidney transplant recipients 1st year after transplantation. *J Res Med Sci* 2017; 22: 20 [PMID: 28458711 DOI: 10.4103/1735-1995.200274]

3. Abo Basha J, Kiel M, Görlich D, Schütte-Nütgen K, Witten A, Pavenstädt H, Kahl BC, Dobrindt U, Reuter S. Phenotypic and Genotypic Characterization of *Escherichia coli* Causing Urinary Tract Infections in Kidney-Transplanted Patients. *J Clin Med* 2019; 8: 988 [PMID: 31284699 DOI: 10.3390/jcm8070988]

4. Gołąbewska JE, Krawczyk B, Wysocka M, Ewiak A, Komarnicka J, Bronk M, Rutkowski B, Dębeka-
Szlizei A. Host and pathogen factors in Klebsiella pneumoniae upper urinary tract infections in renal transplant patients. J Med Microbiol 2019; 68: 382-394 [PMID: 30747620 DOI: 10.1099/jmm.0.00942]

Govindaswamy A, Bajpai V, Khurana S, Aravinda A, Batra P, Malhotra R, Malhotra P. Prevalence and characterization of beta-lactamate-producing Escherichia coli isolates from a tertiary care hospital in India. J Lab Physicians 2019; 11: 123-127 [PMID: 31160850 DOI: 10.4103/jlp.JLP_122_18]

Joshi P, Khan ZA, Tandan R, Harshe A, Bhutada A, Gogavale S. Antibiotic Susceptibility Profile and Prevalence Pattern of Gram Negative Pathogens in Tertiary Care Hospital. AJMIPS 2019; 7: 1-9 [DOI: 10.9734/ajmips/2019/v7i430125]

Rameshkumar G, Ramakrishnan R, Shivkumar C, Meenakshi R, Anitha V, Venugopal Reddy YC, Maneksha V. Prevalence and antibacterial resistance patterns of extended-spectrum beta-lactamate producing Gram-negative bacteria isolated from ocular infections. Indian J Ophthalmol 2016; 64: 303-311 [PMID: 27221683 DOI: 10.1016/j.ijopth.2015.07.023]

Kuch A, Zieniuk B, Zabicka D, Van de Velde S, Literacka E, Skoczyszyka A, Hyniewicz W. Activity of temocillin against ESBL-, AmpC-, and/or KPC-producing Enterobacteriaceae isolated in Poland. Eur J Clin Microbiol Infect Dis 2020; 39: 1185-1191 [PMID: 32096107 DOI: 10.1007/s10095-020-03844-5]

Dirar M, Bilal N, Ibrahim ME, Hamid M. Resistance Patterns and Phenotypic Detection of β-lactamate Enzymes among Enterobacteriaceae Isolates from Referral Hospitals in Khartoum State, Sudan. Cureus 2020; 12: e7260 [PMID: 32195070 DOI: 10.7759/cureus.7260]

Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing, 29th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute. 2019. Available from: https://shop.clsi.org/media/3226/m100-s29_definitions_correction_notice_2.pdf

Sanchez DG, de Melo FM, Savazzi EA, Stehling EG. Detection of different β-lactamases encoding genes, including bla NDM, and plasmid-mediated quinolone resistance genes in different water sources from Brazil. Environ Monit Assess 2018; 190: 407 [PMID: 29909525 DOI: 10.1007/s10661-018-6801-5]

Ori ELF, Takagi EH, Andrade TS, Miguel BT, Cergole-Novella MC, Guth BEC, Hernandez RT, Dias RCB, Pinheiro SRS, Camargo CH, Romero EC, Dos Santos LF. Diarrhoeagenic Escherichia coli and Escherichia albertii in Brazil: pathotypes and serotypes over a 6-year period of surveillance. Epidemiol Infect 2018; 147: 1-9 [PMID: 30229714 DOI: 10.1017/S0950268818002593]

Dilhari A, Sampath A, Gunasekara C, Fernando N, Weerasekara D, Sissoms C, McBain A, Weerasekara E. Evaluation of the impact of six different DNA extraction methods for the representation of the microbial community associated with human chronic wound infections using a gel-based DNA profiling method. J Am Acad Dermatol 2020; 82: 2008-2019 [PMID: 32923838 DOI: 10.1186/s13630-017-0477-z]

Haghhasilipanah M, Mozaffari Nejad AS, Mojtahedi A, Amirmozaffari N, Zeighami H. Detection of extended-spectrum beta-lactamate (ESBL) and plasmid-borne bla CTX-M and bla TEM genes among clinical strains of Escherichia coli isolated from patients in the north of Iran. J Glob Antimicrob Resist 2016; 7: 110-113 [PMID: 27721192 DOI: 10.1016/j.jgar.2016.08.005]

Singh V, Wilks C, Reddy J, Granger J. Outpatient Urinary-Tract-Infection-Like Symptoms: Causative Microbial Survey Utilizing Multiplex Quantitative Polymerase Chain Reaction Methodology. Adv Infect Dis 2020; 10: 26-36 [DOI: 10.4236/aid.2020.101003]

Linares L, Cervera C, Cofán F, Lizaso D, Marco F, Ricart MJ, Esforzado F, Oppenheimer P, Campistol JM, Moreno A. Risk factors for infection with extended-spectrum and AmpC beta-lactamate-producing gram-negative rods in renal transplantation. Am J Transplant 2008; 8: 1000-1005 [PMID: 18727176 DOI: 10.1111/j.1600-6143.2008.02197.x]

Zerr DM, Miles-Jay A, Kronman MP, Zhou C, Adler AL, Haaland W, Weissman SJ, Elward A, Newland JG, Zaatout T, Qin X. Previous Antibiotic Exposure Increases Risk of Infection with Extended-Spectrum-β-Lactamate- and AmpC-Producing Escherichia coli from etc. [PMID: 27721192 DOI: 10.1016/j.jgar.2016.08.005]

Antimicrob Agents Chemother 2016; 60: 4237-4243 [PMID: 27139486 DOI: 10.1128/AAC.01917-16]

Biset S, Moges F, Endalamaw D, Shewa S. Multi-drug resistant and extended-spectrum β-lactamases producing bacterial uropathogens among pregnant women in Northwest Ethiopia. Environ Monit Assess 2020; 192: 1-12 [PMID: 32493343 DOI: 10.1007/s12649-020-03635-z]

Ho PL, Yip KS, Chow KH, Lo JY, Que TL, Yuen KY. Antimicrobial resistance among uropathogens that cause acute uncomplicated cystitis in women in Hong Kong: a prospective multicenter study in 2006 to 2008. Diagn Microbiol Infect Dis 2010; 66: 87-93 [PMID: 19446980 DOI: 10.1016/j.diagmicrobio.2009.03.027]

Molina AS, Soltani B, Taghavi Ardakani A, Moravejii A, Erami M, Haji Rezaei M, Namaazi M. Multidrug-Resistant Escherichia coli and Klebsiella pneumoniae Isolated From Patients in Kashan, Iran. Jundishapur J Microbiol 2015; 8: e27517 [PMID: 26587220 DOI: 10.5812/jjm.27517]

Cantón R, Novaes A, Valverde A, Machado E, Peix L, Baqueiro F, Coque TM. Prevalence and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Europe. Clin Microbiol Infect 2008; 14 Suppl 1: 144-153 [PMID: 18154538 DOI: 10.1111/j.1469-0691.2007.01850.x]

Coque TM, Baqueiro F, Cantón R. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Euro Surveill 2008; 13: 19044 [PMID: 19021958]

Mehrgan H, Rahbar M, Arab-Halavi Z. High prevalence of extended-spectrum beta-lactamate-producing Klebsiella pneumoniae in a tertiary care hospital in Tehran, Iran. J Infect Dev Ctries 2010; 4: 132-138 [PMID: 20351452 DOI: 10.3855/jidc.488]

Martinez P, Garzón D, Mattar C. CTX-M-producing Escherichia coli and Klebsiella pneumoniae isolated from community-acquired urinary tract infections in Valledupar, Colombia. Braz J Infect Dis 2012; 16: 420-425 [PMID: 22964287 DOI: 10.1016/j.bjid.2012.05.001]

Pitout JD, Nordmann P, Laupland KB, Poirel L. Emergence of Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs) in the community. J Antimicrob Chemother 2005; 56: 52-59 [PMID: 15971288 DOI: 10.1093/jac/dki166]

Hawkey PM. Prevalence and clonality of extended-spectrum beta-lactamases in Asia. Clin Microbiol Infect 2008; 14 Suppl 1: 159-165 [PMID: 18154540 DOI: 10.1111/j.1469-0691.2007.01855.x]
Najafi Khah A et al. *E. coli* isolates from kidney transplanted patients

27 Shu JC, Chia JH, Kuo AJ, Su LH, Wu TL. A 7-year surveillance for ESBL-producing Escherichia coli and Klebsiella pneumoniae at a university hospital in Taiwan: the increase of CTX-M-15 in the ICU. *Epidemiol Infect* 2010; 138: 253-263 [PMID: 19619387 DOI: 10.1017/S0950268809999049]

28 Yu F, Chen Q, Yu X, Li Q, Ding B, Yang L, Chen C, Qin Z, Parsons C, Zhang X, Huang J, Luo Y, Wang L, Pan J. High prevalence of extended-spectrum beta lactamases among Salmonella enterica Typhimurium isolates from pediatric patients with diarrhea in China. *PLoS One* 2011; 6: e16801 [PMID: 21390297 DOI: 10.1371/journal.pone.0016801]

29 Poirel L, Ortiz de la Rosa JM, Richard A, Aires-de-Sousa M, Nordmann P. CTX-M-33, a CTX-M-15 derivative conferring reduced susceptibility to carbapenems. *Antimicrob Agents Chemother* 2019; 63: e01515-19 [PMID: 31527021 DOI: 10.1128/AAC.01515-19]

30 Díaz-Agero Pérez C, López-Fresneña N, Rincón Carlavilla AL, Hernandez Garcia M, Ruiz-Garbajosa P, Aranzaz-Andrés JM, Muechler F, Gastmeier P, Bonten MJM, Canton R. Local prevalence of extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* intestinal carriers at admission and co-expression of ESBL and OXA-48 carbapenemase in *Klebsiella pneumoniae*: a prevalence survey in a Spanish University Hospital. *BMJ Open* 2019; 9: e024879 [PMID: 30826764 DOI: 10.1136/bmjopen-2018-024879]

31 Latour K, Huang TD, Jans B, Berhin C, Bogaerts P, Noel A, Nonhoff C, Dodémont M, Denis O, leven M, Loens K, Schoevaerdts D, Catry B, Glupczynski Y. Prevalence of multidrug-resistant organisms in nursing homes in Belgium in 2015. *PLoS One* 2019; 14: e0214327 [PMID: 30921364 DOI: 10.1371/journal.pone.0214327]
