AMLS Archives of Medical Laboratory Sciences

Original Article

Prevalence and Resistance Pattern of Extended-Spectrum β-Lactamase Producing *Escherichia coli* Isolated from Patients with Urinary Tract Infection

Mohammad Ahanjan[1], Maryam Salehian[2], Mehrdad Gholami[2]*

1. Antimicrobial Resistance Research Center, Mazandaran University of Medical Sciences, Sari, Iran.
2. Department of Microbiology and Virology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

Received: October 7, 2020, Accepted: December 1, 2020

Abstract

Background and Aim: Urinary tract infections (UTIs) are one of the most common infectious diseases. Although UTI is mostly associated with several members of the family of *Enterobacteriaceae*, *Escherichia coli* (*E. coli*) is the most common pathogen among them. This study aims to determine the prevalence and resistance pattern of ESBL producing *E. coli* isolated from patients with urinary tract infection in Sari, Iran.

Methods: From December-2016 to June-2017**,** a hospital-based cross-sectional work was accompanied, and a total of 200 urine samples were cultured on blood agar and MacConkey agar for the identification of etiologic agents. After detection and confirmation of *E. coli* isolates, susceptibility testing was assessed using the following antibiotics: cefotaxime, ceftazidime, imipenem, nalidixic acid, cefixime, amikacin, ofloxacin, ceftriaxone, cefepime, gentamicin, tobramycin, meropenem, piracetam, and ciprofloxacin with Kirby–Bauer disk-diffusion technique according to the CLSI guidelines. Double-disk synergy (DDS) methods were used for the detection of ESBL-producing strains.

Results: In the current study, 120 urinary isolates of *E. coli* were detected, which ESBL-producing phenotypes were detected in 55% (n=66) of the isolates. ESBL producing strains of *E. coli* showed the highest susceptibility to meropenem (100%) and ofloxacin (96%); and showed the highest rates of resistance to ceftazidime (91%), cefepime (87%), cefotaxime, and ceftriaxone (84%).

Conclusion: Markedly high resistance to third-generation cephalosporins among *E. coli* strains was found in the current study. Considering the high prevalence of resistance to third-generation cephalosporins in infections caused by organisms producing ESBL, performing comprehensive tests before prescribing antibiotics is essential for the management of infections caused by these strains in community/hospital-acquired UTIs. Furthermore accompanying molecular-based works on ESBL variants will assistance to achieve better results.

Keywords: *Escherichia coli*; Antimicrobial Resistance; Extended-Spectrum β-Lactamase; ESBL; UTI.

***Corresponding Author:** Mehrdad Gholami; **Email:** me.gholami@mazums.ac.ir

Please cite this article as: Ahanjan M, Salehian M, Gholami M. Prevalence and Resistance Pattern of Extended-Spectrum β-Lactamase Producing Escherichia coli Isolated from Patients with Urinary Tract Infection. Arch Med Lab Sci. 2020;6:1-7 (e13). https://doi.org/10.22037/amls.v6.33081

Introduction

Urinary tract infections (UTIs) are one of the most common infectious diseases after respiratory tract system infections; they infect a large number of people in developing countries each year (1). Due to anatomical structures and the hormonal settings of the urinary tract in females, women are remarkably more affected than men to develop a UTI. Approximately 50% of them will have a urinary tract infection during their lifetime (2, 3). UTI is common at all ages but aged people, children, and sexually active people may be at higher risk than others (4). UTIs can affect multiple organs including the urethra, bladder, ureter, and kidney (5). UTI is characterized by the existence of considerable numbers of pathogenic bacteria in the urinary system which needs convenient antibiotic intermediation to impede complications (6). Although UTI is mostly associated with several

members of the family of *Enterobacteriaceae* (7), *Escherichia coli* (*E. coli*) is the most common pathogen among them. Some pathotypes of *E. coli* are systematically associated with uropathogenicity and are designated as uropathogenic *E. coli* (UPEC) (6, 8, 9). The UPEC strains are responsible for most UTIs among ambulatory patients and also up to half of all hospital-acquired UTIs (10). As time goes on, *E. coli* is becoming resistant to the diversity of antibiotics that are vastly prescribed. This can cause a variety of complications in the management and therapeutic strategies of UTI. Various strategies are applied by bacteria to avoid the harmful effects of antibiotics (11,12). Extended-spectrum ß-lactamases (ESBLs) are enzymes generated by some gram-negative bacteria such as *E. coli* and can cause resistance to ß-lactam antibiotics. ESBLs catalyze the hydrolysis of the ß-lactam ring and subsequently make the antibiotics inactive (13). ESBL-producing bacteria are also ordinarily resistant to trimethoprim-sulfamethoxazole, quinolones, and aminoglycosides (14). Recent researches described that the mortality ratio amongst patients infected with ESBL-positive *E. coli* was higher than those infected with non-ESBL producing strains (15-17). Introducing the new generation of antibiotics such as broad-spectrum cephalosporins and aztreonam, as well as increasing their extended use in the treatment of bacterial infectious diseases, leads to the emergence of a new class of extended-spectrum beta-lactamase (18). The rise in ESBL-producer gram-negative strains ordinarily displays a multidrug resistance phenotype (MDR). Clinically, treatment of these multidrug-resistant bacteria still is a major concern (19). So, in this study, we aimed to investigate the frequency of ESBL production and the resistance pattern of ESBL-producer *E. coli* strains recovered from patients with urinary tract infections at Sari city hospitals.

Methods

Bacterial culture and identification

In this descriptive, cross-sectional study, 200 urine samples were collected by midstream clean-catch during December-2016 and June-2017 from the educational hospitals of Sari. Specimens representing the UTIs among inpatients and outpatients attending teaching hospitals Mazandaran University of Medical Sciences were included in the work. The same samples recovered from one patient were excluded. A single type of bacteria with a colony count ≥of 105 CFU/mL for midstream urine was taken as a positive urine culture.

To identify the gram-negative bacilli, the samples were cultured on blood and MacConkey agar plates (Merck, Darmstadt, Germany). The plates were incubated in aerobic conditions for 24 hours at 37°C. Following the incubation, some bacteria were collected from colonies, and subsequently, gram staining and oxidase tests were performed. Oxidase-negative samples were evaluated by deferential biochemical tests such as citrate, urea, indole, and movement test and they were identified precisely (20).

ESBL screening

The E. coli strains were analyzed for ESBL-production by the combined disc method (CDT) in line with the clinical laboratory standard institute (CLSI) recommendation. For the CDT test, the standard concentration of bacterium was grass-cultured on Muller- Hinton agar plate.

As ceftazidime disk placed against ceftazidime clavulanic acid combined disk (10 µg, 30 µg) and cefotaxime disk placed against cefotaxime clavulanic acid combined disk (10 µg, 30 µg) (Mast Co., United Kingdom) with 15-millimeter spacing. Plates were incubated for 18-24 hours at 37°C. Zone of inhibition diameter around the clavulanic acid disk, as the ESBL enzyme inhibitor, was interpreted in the respect of that disk alone. Therefore, if the diameter of the zone of inhibition around clavulanic acid disks is larger than or equal to 5mm compared to the same disk alone, the strain can be taken as ESBL (20).

Antimicrobial susceptibility testing

Disc diffusion antibiotic sensitivity testing (Kirby-Bauer antibiotic testing) was done for ESBL-producer strains based on the CLSI guideline 2016. Antibiogram was performed using the Kirby-Bauer method on Muller–Hinton agar plate.

In this respect, one sterile swab immersed in 0.5 McFarland suspension, and the strains were swabbed uniformly across a Muller–Hinton culture plate (Merck, Darmstadt, Germany).

Antibiotic filter-paper disks were then placed on the surface of the agar by sterile forceps and plates have been incubated at 37° C for 18 hours. In this interval, the antibiotic diffused from the filter paper into the agar.

The tested antibiotic disks were ceftazidime (30µg), ceftriaxone (30µg), cefotaxime (30µg), cefepime (30µg), nalidixic acid (30µg), ciprofloxacin (5µg), ofloxacin (5µg), imipenem (10µg), meropenem (10µg), and cotrimoxazole (10µg) (Mast Co., United Kingdom).

After 18 hours, growth halo diameter was measured by ruler and the results were interpreted using CLSI instruction. In the condition that the antibiotic was effective against bacteria; no colonies grow around the disk which calls a zone of inhibition. The diameter of the zone of inhibition around each disk estimates the bacteria's sensitivity to that particular antibiotic disk. *E. coli* ATCC 25922 was used as a control strain.

Data analysis

The data were analyzed using SPSS version 16.0. The p-value <0.05 was regarded as statistically significant.

Results

Of total 200 urine samples were handled. Among which, 120 (60%) E. coli was isolated from samples. Among the E. coli positive samples, eighty-six (of 120, 71.66%) isolates were obtained from the inpatients, and thirty-four (of 120, 28.33%) isolates were obtained from the outpatients. Out of all E. coli positive samples, 74 patients (62%) were women and 46 of them (38%) were men. The age range of the patients infected with ESBL producing E. coli is shown in Figure 1. Their age ranged from 5 to 76 years old. The highest occurrence of infection with ESBL producer E. coli strains was observed in the first decade of life (32 patients; 27%); and the lowest incidence was detected in the 7th decade of life (6 patients; 5%).Based on the results of CDT, among 120 tested isolates, 55% (n=66) of *E. coli* strains were confirmed as ESBL producers. According to the results of antibiogram tests, meropenem (n= 66/66, 100%) and ofloxacin (n=63/66, 96%) were found to be the most effective antibiotics against ESBL producing strains of *E. coli*. While, ceftazidime (n=60/66, 91%), cefepime (n=57/66, 87%), cefotaxime and ceftriaxone (n=56/66, 84%) showed the highest rates of resistance. Detailed results of antibiogram tests are presented in Figure 2.

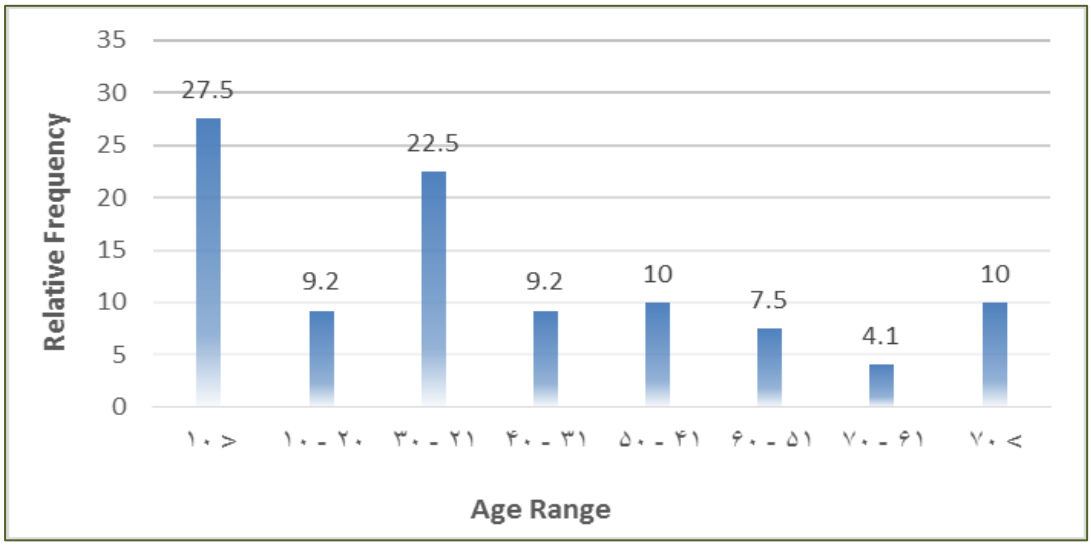

Figure 1. The age range of the patients infected with ESBL producing *E. coli*

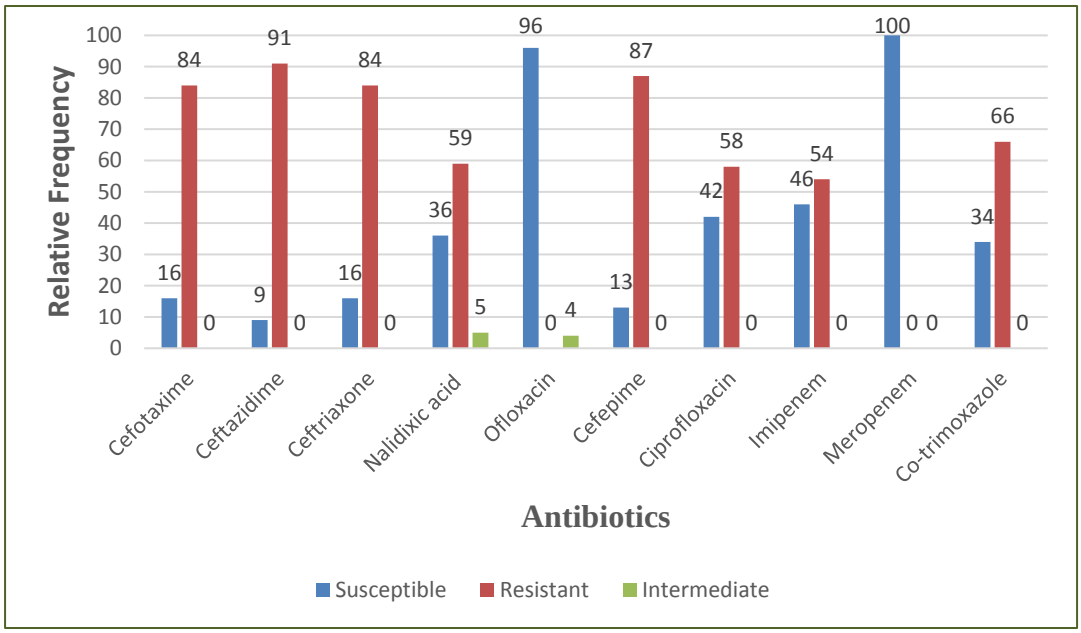

Figure 2. Percentage of antimicrobial susceptibility in ESBL-producing *E. coli* strains.

Discussion

Although bacterial urinary tract infections are very common, it is not always possible to perform urine culture analysis and antibiotic sensitivity tests for the treatment, particularly in small therapy centers (21, 22). E. coli as one of the most important bacteria causing urinary tract infection is widely involved in several clinical infections (23, 24). In recent years, resistance towards antimicrobials has considerably increased and antibiotic-resistant infections are becoming an important clinical problem. Antibiotic resistance rates vary in different geographic places (25, 26). The incidence of ESBL-producing strains of E. coli isolated from urinary tract infections is diverse in different countries and from a hospital to another hospital; it depends on the infection control systems and therapeutic strategies (27). During the past few years, we have observed a significant increase in the resistant strains of E. coli which are resistant to the most first-line antibiotics, including third-generation cephalosporin, aminoglycosides, and even fluoroquinolones (25, 26). This research displayed that 60% of samples were E. coli positive and our data was in coordination with Hamid-Farahani et al (28), Madani et al (29), and Jha et al (30) studies; in which the E. coli infection was reported 54.1%, 60.3%, 45.4%, and 50%, respectively. In this study, 66% of isolated *E. coli* were ESBL-producing strain which was in accordance with Yazdi et al (27), Babypadmini et al (31), and Ejaz et al (32) findings that were 44.3%, 41%, and 57.4%, respectively. Our results indicated that the most effective antibiotic against ESBL-producing E. coli strains isolated from urinary tract infections was meropenem and ofloxacin. According to outcomes in the present study, we should use ceftazidime, cefepime, ceftriaxone, and cefotaxime less in initial treatment, due to high levels of resistance of ESBL-producing E. coli to these antibiotics. Rajabnia et al (33) demonstrated the resistance of ESBL-producing E. coli to ceftazidime 87.9% and cefepime 86.24% which were in accordance with our findings, 91%, and 87%, respectively. In a study was done by Akya et al (34) the antibiotic resistance rate to cefotaxime and cotrimoxazole was 95.5% and 58.8%, respectively; which was almost in accordance with our results of 84% and 66%, respectively. Soltan Dalal et al (35) and Molaabaszadeh et al (36) among clinical isolates and urinary strains of E. coli displayed an antibiotic resistance to cotrimoxazole

80.5% and 63.92%, respectively that were in agreement with our results of 66%. Mohajeri et al (37) reported the antibiotic resistance rate among ESBL positive E. coli strains, to cotrimoxazole, ofloxacin were 85.2%, 55.6%, and imipenem 0%; their results were different to our findings 66%, 0%, and 54% respectively; but their results about antibiotic resistance rate to ceftriaxone 90.7% and ciprofloxacin 55.5% were in agreement with our data (84% and 58% respectively). The results of this study and comparison with other studies indicated the antibiotic resistance in E. coli isolated from urinary tract infections. Therefore, applying new drugs such as meropenem that have shown very positive performance in different articles is recommended (38). Meropenem is a member of the carbapenems that is resistant to beta-lactamase enzymes (36). ESBL strains are a major threat to the cephalosporins administration. Therefore, estimating the prevalence of ESBL-producing E. coli, rapid identification of strains in the microbiology laboratories, and evaluating the pattern of antibiotic sensitivity is significantly and crucially important (27).

Conclusion

The occurrence of infections caused by ESBL-producing organisms has increased noticeably in recent years. Our work showed an increase in the frequency of ESBL producing *E. coli* from 2007 to 2017 for UTIs in Sari therapeutic centers. The resistance rates of antimicrobial agents, mainly third-generation broad-spectrum cephalosporins, showed a noteworthy increase in ESBL producing *E. coli*. Also, meropenem demonstrates good activity against ESBL-producing *E. coli*, in vitro. In conclusion, it is suggested, routine identification of ESBLs production of Enterobacteriaceae in hospitals' laboratory units along with antibiogram test and strong infection prevention strategies would be suitable to primary management of community/hospital-acquired UTIs.

Conflict of Interest

The authors declared that they have no conflict of interest.

Acknowledgment

Thanks to the Department Microbiology and Virology of Mazandaran University of Medical Sciences and Antimicrobial Resistance Research Center of Mazandaran University of Medical Sciences for their contribution to this research.

Funding/Support

This study was supported by the grant of Mazandaran University of medical sciences and molecular and cell biology research center

Ethics

The study was approved by the ethical committee of Mazandaran University of Medical Sciences, Sari, Iran (IR.MAZUMS.REC.1397.628).

Authors Contributions

MA and MG: Design of the study and supervision. MS: collected the data, cultured the samples, and performed experiments. MG drafting of the manuscript in collaboration with MA. All authors read and approved the final manuscript.

References

1. Oyaert M, Van Meensel B, Cartuyvels R, Frans J, Laffut W, Vandecandelaere P, et al. Laboratory diagnosis of urinary tract infections: Towards a BILULU consensus guideline. J Microbiol Methods. 2018;146:92-9.

2. Singh N, Gandhi S, McArthur E, Moist L, Jain AK, Liu AR, et al. Kidney function and the use of nitrofurantoin to treat urinary tract infections in older women. Cmaj. 2015;187(9):648-56.

3. Mohsin R, Siddiqui KM. Recurrent urinary tract infections in females. J Pak Med Assoc. 2010;60(1):55–59.

4. SS Thakre, SN Dhakne, SB Thakre, SN Ughade. Hygiene Practices and Sexual Activity Associated with Urinary Tract Infection in Rural Pregnant Women of Nagpur, India. Indian J Med Microbiol. 2015;33(1):177-8.

٥. Olin SJ, Bartges JW. Urinary tract infections: treatment/comparative therapeutics. Vet Clin North Am Small Anim Pract. 2015;45(4):721-46.

6. Goneau LW, Hannan TJ, MacPhee RA, Schwartz DJ, Macklaim JM, Gloor GB, et al. Subinhibitory antibiotic therapy alters recurrent urinary tract infection pathogenesis through modulation of bacterial virulence and host immunity. MBio. 2015;6(2):e00356-15.

7. Lyonga EE, Toukam M, Nkenfou C, Gonsu HK, Assoumou MO, Mesembe MT, et al. Resistance pattern of enterobacteriaceae isolates from urinary tract infections to selected quinolones in Yaoundé. Pan Afr Med J. 2015;21(1.(

8. Daoud Z, Salem Sokhn E, Masri K, Cheaito K, Haidar-Ahmad N, Matar GM, et al. Escherichia coli isolated from urinary tract infections of Lebanese patients between 2005 and 2012: epidemiology and profiles of resistance. Front Med. 2015;1:1–11.

9. Tewary K, Narchi H. Recurrent urinary tract infections in children: Preventive interventions other than prophylactic antibiotics. World J Methodol. 2015;5(2):13-9.

10. Rattanaumpawan P, Nachamkin I, Bilker WB, Roy JA, Metlay JP, Zaoutis TE, et al. Risk factors for ambulatory urinary tract infections caused by high-MIC fluoroquinolone-susceptible Escherichia coli in women: results from a large case–control study. J Antimicrob Chemother. 2015;70(5):1547-51.

11. Fagan M, Lindbæk M, Grude N, Reiso H, Romøren M, Skaare D, et al. Antibiotic resistance patterns of bacteria causing urinary tract infections in the elderly living in nursing homes versus the elderly living at home: an observational study. BMC Geriatr. 2015;15(1):98–104.

12. Nor NS, Abu NA, Rashid MA, Ismail MM, Razak RK. Bacterial pathogens and antibiotic resistance patterns in children with urinary tract infection in a Malaysian tertiary hospital. Med J Malays. 2015;70(3):153-7.

13. Cho YH, Jung SI, Chung HS, Yu HS, Hwang EC, Kim SO, et al. Antimicrobial susceptibilities of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in health care-associated urinary tract infection: focus on susceptibility to fosfomycin. Int Urol Nephrol. 2015;47(7):1059-66.

14. Rodríguez-Bano J, Navarro MD, Romero L, Martínez-Martínez L, Muniain MA, Perea EJ, et al. Epidemiology and clinical features of infections caused by extended-spectrum beta-lactamase-producing Escherichia coli in nonhospitalized patients. J Clin Microbiol. 2004;42(3):1089-94.

15. Sianipar O, Asmara W, Dwiprahasto I, Mulyono B. Mortality risk of bloodstream infection caused by either Escherichia coli or Klebsiella pneumoniae producing extended-spectrum β-lactamase: a prospective cohort study. BMC Res Notes. 2019;12(1):719.

16. Paterson DL, Ko W, Von Gottberg A, Mohapatra S, Casellas JM. International prospective study of Klebsiella pneumoniae bacteremia: implications of extended-spectrum β-lactamase production in. Ann Intern Med. 2004;140:26–32.

17. Melzer M, Petersen I. Mortality following bacteraemic infection caused by extended spectrum beta-lactamase (ESBL) producing E. coli compared to non-ESBL producing E. coli. J Infect. 2007;55:254–9.

18. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clin Microbiol Rev. 2005;18(4):657-86.

19. Teklu DS, Negeri AA, Legese MH, Bedada TL, Woldemariam HK, Tullu KD. Extended-spectrum beta-lactamase production and multi-drug resistance among Enterobacteriaceae isolated in Addis Ababa, Ethiopia. Antimicrob Resist Infect Control. 2019;8(1):39-50.

20. Amiri P, Pournajaf A, Shavalipour A, Tayebi Z, Goudarzi H, Eslami G, et al . Evaluation of Antimicrobial Resistance in the Beta-lactamase Producing Escherichia Coli Isolated from Urinary Tract Infection in the Patients Referring to Taleghani Hospital of Tehran. Tabari Biomed Stu Res J. 2015;1 (2):11-19

21. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 2015;13(5):269-84.

22. Kumar MS, Lakshmi V, Rajagopalan R. Occurrence of extended spectrum beta-lactamases among Enterobacteriaceae spp. isolated at a tertiary care institute. Indian J Med Microbiol. 2006;24(3):208-11.

23. Esteve-Palau E, Solande G, Sánchez F, Sorlí L, Montero M, Güerri R, et al. Clinical and economic impact of urinary tract infections caused by ESBL-producing Escherichia coli requiring hospitalization: a matched cohort study. J Infect. 2015;71(6):667-74.

24. Dan M, Yair Y, Samosav A, Gottesman T, Yossepowitch O, Harari-Schwartz O, et al. Escherichia coli isolates from patients with bacteremic urinary tract infection are genetically distinct from those derived from sepsis following prostate transrectal biopsy. Int J Med Microbiol. 2015;305(4-5):464–468.

25. Hanna-Wakim RH, Ghanem ST, El Helou MW, Khafaja SA, Shaker RA, Hassan SA, et al. Epidemiology and characteristics of urinary tract infections in children and adolescents. Front Cell Infect Microbiol. 2015;5:45-9.

26. Abujnah AA, Zorgani A, Sabri MA, El-Mohammady H, Khalek RA, Ghenghesh KS. Multidrug resistance and extended-spectrum β-lactamases genes among Escherichia coli from patients with urinary tract infections in Northwestern Libya. Libyan J Med. 2015;10(1).

27. Yazdi M, Nazemi A, Mir nargasi MS, Khataminejad M, Sharifi S, Babai kochkaksaraei M. Prevalence of SHV/CTX-M/TEM (ESBL) Beta-lactamase Resistance Genes in Escherichia Coli Isolated from Urinary Tract Infections in Tehran, Iran. Med Lab J. 2010;4(1).

28. Hamid FR, Tajik AR, Noorifard M, Keshavarz A, Taghipour N, hossieni SJ. Antibiotic resistance pattern of E. coli isolated from urine culture in 660 Army clinical laboratory center in Tehran 2008. Ann Mil Health Sci Res, 2012;10(1):45-9.

29. Madani SH, Khazaei S, Kanani M, shahi M. Antibiotic resistance pattern of E. coli isolated from urine culture in Imam Kermanshah-2006. J Kermanshah Univ Med Sci. 2008;12:287–95.

30. Jha N, Bapat SK. A study of sensitivity and resistance of pathogenic micro organisms causing UTI in Kathmandu valley. Kathmandu Univ Med J. 2005;3(2):123-9.

31. Babypadmini S, Appalaraju B. Extended spectrum-lactamases in urinary isolates of Escherichia coli and Klebsiella pneumoniae-prevalence and susceptibility pattern

in a tertiary care hospital. Indian J Med Microbiol. 2004;22(3):172-4.

32. Ejaz H, Zafa A, Mahmood S, Javed MM. Urinary tract infections caused by extended spectrum β-lactamase (ESBL) producing Escherichia coli and Klebsiella pneumoniae. Afr J Biotechnol. 2011;10(73):16661-6.

33. Rajabnia M, Forghani MS, Hasani S, Bahadoram M, Mohammadi M, Barahman M. Prevalence and antibiotic resistance pattern of extended spectrum beta lactamase producing Escherichia coli isolated from urinary tract infection. J Renal Inj Prev. 2019;8(2):78-81.

34. Akya A, Gheisari H, Mohammadi G, Khodadoost M. Study of the pattern of plasmid and antibiotic resistance of Escherichia coli isolated from outpatients with urinary tract infection. Sci J Kurdistan Univ Medical Sci. 2015;20:89–96.

35. Soltan Dallal MM, Sabbaghi A, Fallah Mehrabadi J, Aghamirzaei H, Rastegar Lari A, Eshraghian MR, et al. Evaluation of presence of the bla-SHV and bla-AmpC (CITM, FOX) β-lactamase genes in clinical isolates of Escherichia coli. J Med Counc I.R. Iran. 2010;28(3):269-76

36. Molaabaszadeh H, Hajisheikhzadeh B, Mollazadeh M, Eslami K, MohammadzadehGheshlaghi N. Study of sensibility and antimicrobial resistance in Escherichia coli isolated from urinary tract infection in Tabriz city. J Fasa Univ Med Sci. 2013;3(2):149-54.

37. Mohajeri P, Izadi B, Rezai M, Falahi B, Khademi H, Ebrahimi R. Assessment of the frequency of extended spectrum beta lactamases producing Escherichia coli isolated from Urinary tract infections and its antibiotic resistance pattern in kermanshah. J Ardabil Univ Med Sci. 2011;11(1):86-94.

38. Horcajada JP, Soto S, Gajewski A, Smithson A, De Anta MT, Mensa J, et al. Quinolone-resistant uropathogenic Escherichia coli strains from phylogenetic group B2 have fewer virulence factors than their susceptible counterparts. J Clin Microbiol. 2005;43(6):2962-4.