Acta Microbiologica et Immunologica Hungarica 65 (1), pp. 15–25 (2018)
DOI: 10.1556/030.64.2017.030
First published online November 14, 2017

EMERGENCE OF FOSFOMYCIN RESISTANCE AMONG ISOLATES OF *ESCHERICHIA COLI* HARBORING EXTENDED-SPECTRUM AND AmpC β-LACTAMASES

AGHIL BAHRAMIAN[1], GITA ESLAMI[2]*, ALI HASHEMI[2], ALI TABIBI[1] and MOHSEN HEIDARY[3]

[1]Urology and Nephrology Research Center, Shahid Labbafinejad Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

(Received: 8 December 2016; accepted: 22 May 2017)

Urinary tract infection (UTI) is a common type of infectious disease globally. The aim of this study was to detect the frequency of *fosA3* and *fosC2* genes in extended-spectrum β-lactamases (ESBL) and bla_{DHA}, $bla_{\text{CMY-2}}$, and $bla_{\text{CMY-42}}$ genes in AmpC β-lactamases-producing isolates of *Escherichia coli*. In total, 120 isolates of *E. coli* were collected from three teaching hospitals between March 2014 and February 2015. Antibiotic susceptibility tests were carried out by disk diffusion method. The presence of $bla_{\text{CMY-2}}$, $bla_{\text{CMY-42}}$, bla_{DHA}, *fosA3*, and *fosC2* genes was detected by polymerase chain reaction (PCR) and sequencing. Of the 120 strains, 92 (76.6%) were identified as ESBL producers, 30 (25%) were determined as AmpC β-lactamase producers, and 24 (20%) had both ESBL and AmpC β-lactamase enzymes. Imipenem, fosfomycin, and nitrofurantoin had the best effect against isolates of *E. coli*. PCR assay demonstrated that the frequency of $bla_{\text{CMY-2}}$, $bla_{\text{CMY-42}}$, and bla_{DHA} genes among AmpC β-lactamases-producing strains were 39%, 1%, and 17.5%, respectively. This study reports the first detection of fosfomycin resistance in Iran. This study indicated the increasing prevalence of UTI isolates of *E. coli*-harboring ESBL and AmpC β-lactamases genes in Iran. Therefore, due to the high rate of bla_{DHA} and bla_{CMY} genes and emergence of fosfomycin-resistant *E. coli* isolates, we recommend continuous monitoring of antibiotic resistance as well as attention to guidelines of infection controls.

Keywords: *Escherichia coli*, urinary tract infection, Iran

*Corresponding author; E-mail: gita_eslami@yahoo.com

Introduction

Urinary tract infection (UTI) with approximately 200 million cases per year is one of the most common types of infectious disease globally. It is estimated that 5% of men and 45% of women will be infected with UTI at least once during their lifetime. UTIs are major contributors to global antibiotic use and resistance, due to their high incidence rate. Many urological methods would carry high risk, without effective drugs against common uropathogens [1–4]. *Escherichia coli* is the most common uropathogen associated with UTIs in the world. A main increase in the prevalence of extended-spectrum β-lactamase (ESBL)- and AmpC β-lactamase-producing clinical strains of *E. coli* has been reported in the last decades [5, 6]. ESBLs and AmpC β-lactamases are the most frequently detected groups of β-lactam ring hydrolyzing enzymes in UTI isolates of *E. coli* worldwide. Both are the main causes of treatment failure in patients when they are produced by pathogen [7–10]. Plasmid-mediated AmpC enzymes, such as CMY- and DHA-type β-lactamases, have been reported in clinical isolates of *E. coli*. Due to the plasmid-mediated characterization, which enables them to spread very rapidly, a rapid development of resistance to AmpC genes has been observed in UTI isolates of *E. coli* worldwide [11, 12]. Fosfomycin is one of the first-line drugs recommended for patients with UTIs, due to its activity against ESBL-producing and fluoroquinolone-resistant *E. coli* [13]. Fosfomycin resistance rates in UTI isolates of *E. coli* are often lower than 10% but are higher than 30% when ESBL producers are considered [14]. Recently, plasmid-mediated fosfomycin resistance genes *fosA3* and *fosC2* emerged in *E. coli* clinical and non-clinical strains [15]. The most prevalent gene is *fosA3* that has been mainly detected in clinical isolates of *E. coli* in Asian countries [13, 16–18] and *fosC2* is found in the fragment cloned from the conjugative plasmid of *E. coli* strain C316 [19]. The purpose of this study was to identify the extended-spectrum- and AmpC β-lactamases-producing clinical isolates of *E. coli* isolated from patients with UTIs and to detect the frequency of *fosA3* and *fosC2* genes in ESBLs-producing strains and bla_{DHA}, $bla_{\text{CMY-2}}$, and $bla_{\text{CMY-42}}$ genes in AmpC β-lactamases-producing strains.

Materials and Methods

Clinical isolates

This study was a descriptive investigation. A total of 120 unduplicated UTI isolates of *E. coli* were collected from Labbafinejad, Shohada Tajrish, and Taleghani Hospitals between March 2014 and February 2015. *E. coli* strains

were identified by classical bacteriological and biochemical tests, such as triple sugar iron, motility, methyl red, Voges–Proskauer, ornithine decarboxylation, and lysine decarboxylation [20].

Susceptibility testing

Susceptibility testing to 11 antibiotics (Mast Group, Merseyside, UK) was carried out by disk diffusion method according to recommendations of the Clinical and Laboratory Standards Institute (CLSI) [21]. The antibiotics tested were as follows: cefoxitin (30 μg), levofloxacin (10 μg), gentamicin (10 μg), amikacin (30 μg), cefotaxime (30 μg), tobramycin (10 μg), ampicillin (10 μg), fosfomycin (10 μg), imipenem (10 μg), cefpodoxime (30 μg), nitrofurantoin (300 μg), ciprofloxacin (10 μg), ceftriaxone (10 μg), ceftazidime (30 μg), and cotrimoxazole (25 μg). *E. coli* ATCC 25922 was used as a control strain.

ESBL confirmatory test

ESBL-producing UTI isolates of *E. coli* were detected by screening with 30 μg cefotaxime disk and then further testing with cefotaxime/clavulanic acid disks to detect clavulanic acid enhancement ≥5 mm. Similarly, for confirmation of detection of ESBL-producing isolates, screening was performed using 30 μg ceftazidime disk with and without clavulanic acid [11]. *Klebsiella pneumoniae* ATCC 700603 was used as a control strain.

AmpC β-lactamase confirmatory test

AmpC β-lactamase confirmatory test was performed to detect AmpC-producing UTI strains of *E. coli*. This test was performed by screening with 30 μg cefotaxime disk and then with cefotaxime/cloxacillin disks. Second screening stage was performed using 30 μg ceftazidime disk with and without cloxacillin [22].

Molecular detection of antibiotic resistance genes

The DNA was extracted by Roche Company and used as a template for polymerase chain reaction (PCR). The master mix including 3 mmol/ml $MgCl_2$ and 0.08 mmol/ml Taq polymerase was used (Sinclon Bioscience Company, Iran). The presence of $bla_{\text{CMY-2}}$, $bla_{\text{CMY-42}}$, *fosA3*, *fosC2*, and bla_{DHA} genes was

Table I. Primer sequence and product size

Primer	Sequence (5′→3′)	Gene	Product size (bp)	Reference
CMY-2-F	ACGAAGAGGCAATGACCAGA	$bla_{\text{CMY-2}}$	451	This study
CMY-2-R	CCAGTGGAGCCCGTTTTATG			
CMY-42-F	ACGAAGAGGCAATGACCAGA	$bla_{\text{CMY-2}}$	451	This study
CMY-42-R	CCAGTGGAGCCCGTTTTATG			
FOSA3-F	CCTGGCATTTTATCAGCAGT	*fosA3*	234	This study
FOSA3-R	CGGTTATCTTTCCATACCTCAG			
FOSC2-F	TGGAGGCTACTTGGATTTG	*fosC2*	217	This study
FOSC2-R	AGGCTACCGCTATGGATTT			
DHA-F	TGTATGCAAACAGCAGTATC	bla_{DHA}	312	This study
DHA-R	ACATTGCCATTTCCAGATCC			

determined for all UTI isolates of *E. coli*-harboring ESBLs and AmpC β-lactamases by PCR technique (Bio Intellectica PCR). The primer sets and thermal cycling conditions described in Tables I and II. One of the PCR products was purified and direct sequencing was performed. Two negative controls (molecular grade water) and positive control were included in each PCR run. KX342010, KX342011, and KP696465.1 strains of *E. coli* harboring the $bla_{\text{CMY-2}}$, $bla_{\text{CMY-42}}$, and bla_{DHA} genes were confirmed by sequencing method and were used as positive controls.

Statistical analysis

Current survey was a descriptive study. Analysis of results was carried out by MINITAB 16 software. The p value and confidence intervals were <0.05% and 95%, respectively.

Results

Overall, 60 (50%) strains were isolated from Labbafinejad Hospital, of which 10 were related to dialysis patients, 25 (20.8%) strains were isolated from Taleghani Hospital, of which 15 were related to dialysis patients and 35 (29.2%) strains were isolated from Shohada Tajrish Hospital, of which 12 were related to dialysis patients. Eighty-seven strains were isolated from female patients (72.5%) and 33 from males (27.5%). The age range of the patients with UTIs was 2–70 years. The isolates were obtained from patients in different age groups: 2–5 years ($N = 3$), 6–18 years ($N = 20$), 19–40 years ($N = 48$), and 41–65 years

Table II. Temperature and time of PCR assay

Step	Temperature (°C)					Time				
	$bla_{\text{CMY-2}}$	$bla_{\text{CMY-42}}$	*fosA3*	*fosC2*	bla_{DHA}	$bla_{\text{CMY-2}}$	$bla_{\text{CMY-42}}$	*fosA3*	*fosC2*	bla_{DHA}
Initial denaturation	94	94	94	94	94	5 min	5 min	5 min	5 min	3 min
Denaturation	94	94	94	94	94	45 s	45 s	45 s	45 s	30 s
Annealing	51	51	50	50	51	45 s	45 s	45 s	45 s	45 s
Extension	72	72	72	72	72	45 s	45 s	45 s	45 s	1 min
Final extension	72	72	72	72	72	5 min	5 min	5 min	5 min	10 min
Cycle	36	36	36	36	36			–		

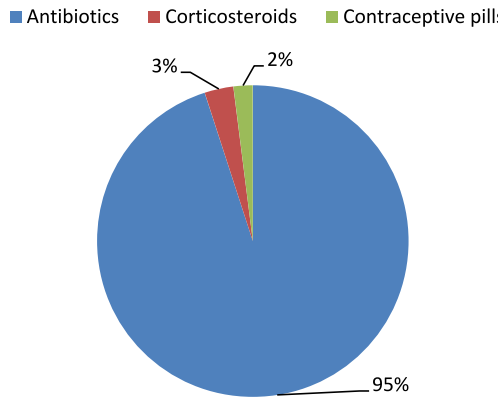

Figure 1. Previous use of antibiotics, corticosteroids, or contraceptive pills

($N = 42$), and seven isolates were isolated from patients of more than 65 years of age. The previous use of antibiotics, corticosteroids, or contraceptive pills is shown in Figure 1. Of the 120 strains, 92 (76.6%) were identified as ESBL producers, 30 (25%) were determined as AmpC β-lactamase producers, and 24 (20%) had both ESBL and AmpC β-lactamase enzymes (Table III). In this study, imipenem, fosfomycin, and nitrofurantoin against clinical isolates of *E. coli* had the best effect in antimicrobial susceptibility tests. The patterns of the antibiotic susceptibility tests in *E. coli* isolates have been shown in Table IV. Screening of antibiotic resistance genes, *fosA3*, *fosC2*, bla_{DHA}, $bla_{\text{CMY-2}}$, and $bla_{\text{CMY-42}}$, by PCR assay demonstrated that the frequency of $bla_{\text{CMY-2}}$, $bla_{\text{CMY-42}}$, and bla_{DHA} genes among AmpC β-lactamases-producing strains were 39%, 1%, and 17.5% (21) isolates, respectively. To the best of our knowledge, this is the first report of emergence of $bla_{\text{CMY-42}}$ genes in *E. coli* isolates in Iran. Among the bla_{CMY}-positive isolates, 12 strains and among the bla_{DHA}-positive isolates, 6 strains were isolated from dialysis patients. The nucleotide sequence data that reported in this

Table III. Sources of *E. coli* isolates harboring ESBL and AmpC β-lactamases

	Hospitals			
β-lactamase enzymes	Labbafinejad	Shohada Tajrish	Taleghani	Total no. of isolates
ESBL	48	24	20	92
AmpC	9	9	12	30
ESBL + AmpC	5	7	12	24

Table IV. Antibiotic susceptibility testing results

Antibiotic	Resistant no. (%)	Sensitive no. (%)	Intermediate no. (%)
Cefoxitin	80 (66.6)	40 (33.4)	0 (0)
Levofloxacin	40 (33.4)	75 (62.5)	5 (4.1)
Gentamicin	62 (51.6)	46 (38.3)	12 (10)
Amikacin	15 (12.5)	75 (62.5)	30 (25)
Imipenem	7 (5.8)	113 (94.2)	0 (0)
Cefotaxime	87 (72.5)	33 (27.5)	0 (0)
Tobramycin	88 (73.3)	32 (26.6)	0 (0)
Ampicillin	120 (100)	0 (0)	0 (0)
Fosfomycin	8 (6.6)	112 (93.4)	0 (0)
Cefpodoxime	112 (93.4)	8 (6.6)	0 (0)
Nitrofurantoin	13 (10.8)	107 (89.2)	0 (0)
Ciprofloxacin	90 (75)	25 (20.8)	5 (4.2)
Ceftriaxone	86 (71.6)	34 (28.4)	0 (0)
Ceftazidime	65 (54.2)	55 (45.8)	0 (0)
Cotrimoxazole	95 (79.2)	25 (20.8)	0 (0)

study have been submitted to the GenBank sequence database and assigned accession number KX342010 for $bla_{\text{CMY-2}}$ gene, KX342011 for $bla_{\text{CMY-42}}$ gene, and KP696465.1 for bla_{DHA} gene.

Discussion

UTIs, due to their high incidence rate, are one of the most important contributors to global antibiotic resistance. This study was conducted to identify the clinical isolates of *E. coli* isolated from patients with UTIs and detect drug resistance genes in the extended-spectrum- and AmpC β-lactamases-harboring strains. A main increase in the prevalence of ESBL- and AmpC β-lactamase-producing strains of *E. coli* has been reported in the last decades. In current survey, the prevalence of ESBL- and AmpC β-lactamase producers were identified 76.6% and 25%, respectively. This high prevalence of resistance to broad-spectrum β-lactams is a global threat. Uncontrolled use of broad-spectrum drugs, less relation between physicians and laboratories, and lack of attention to laboratory screening of ESBL and AmpC β-lactamase production by clinical isolates *E. coli* are the most important risk factors for this high rate of drug resistance. The patterns of the antibiotic susceptibility tests demonstrated that imipenem, fosfomycin, and nitrofurantoin had the best effect against *E. coli* isolates in our investigation. Although fosfomycin was one of the best drugs, 8 (6.6%) isolates were resistant to this oldest and most effective drug for treatment of UTIs. Due to the few

therapeutic choices for UTIs treatment, increasing rate of resistance to fosfomycin can become a great concern around the world. Lob et al. [22] performed a study in Canada and the United States on 3498 *E. coli* UTI isolates and confirmed that imipenem had the most susceptibility, more than 95%, against clinical strains of *E. coli*. Plasmid-mediated CMY- and DHA-type β-lactamases have been reported in *E. coli* isolates. This study showed that prevalence of $bla_{\text{CMY-2}}$, $bla_{\text{CMY-42}}$, and bla_{DHA} genes among AmpC β-lactamases producers were 39%, 1%, and 17.5%, respectively. In a study carried out by Shayan et al. [23] in Iran, the prevalence of bla_{CMY} gene among 392 isolates of *E. coli* was investigated. They reported that 13 (3.3%) isolates were identified as AmpC producers, which 11 of the 13 isolates contained the bla_{CMY} gene. In another survey performed by Saffar et al. [24] in Iran (Tehran), bla_{DHA} gene was detected in 14.8% isolates of *E. coli*. Their results confirmed this hypothesis that geographical location plays the most important role in the distribution of bla_{DHA} gene, due to the closer regions have a relatively similar distribution of bla_{DHA} gene [24]. Recently, plasmid-mediated quinolone resistance determinants have been also reported among *E. coli* isolates, furthermore, plasmid-mediated fosfomycin-modifying enzymes, *fosA3* and *fosC2*, were identified in *E. coli* clinical isolates. Acquisition of these fosfomycin resistance determinants, which inactivate fosfomycin by exerting glutathione-S-transferase activity, has also been seem to confer resistance [19, 25]. Although phenotypic test reported eight isolates of *E. coli* as fosfomycin-resistant strains, molecular test showed that none of them had the *fosA3* or *fosC2* genes. Our research was the first investigation in Iran about detection of fosfomycin resistance genes, *fosA3* and *fosC2*. Therefore, additional studies using PCR or a probe-based assay can simplify the actual dissemination of these resistance genes. In conclusion, this study demonstrated that UTI isolates of *E. coli*-harboring ESBL and AmpC β-lactamases are increasing, which may lead to more cost and mortality rate. Therefore, efforts must be undertaken to diagnose ESBL- and AmpC β-lactamases-producing isolates to allow for targeted treatment. Furthermore, due to the high rate of bla_{DHA} and bla_{CMY} genes and emergence of fosfomycin-resistant *E. coli* isolates, we recommend continuous monitoring of antibiotic resistance, attention to guidelines of infection controls, use of sensitive methods for laboratory diagnosis, and close relation between physician and laboratories.

Acknowledgements

The authors would like to thank the Urology and Nephrology Research Center, Shahid Labbafinejad Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Funding Sources

This work was supported by a research grant from Urology and Nephrology Research Center, Shahid Labbafinejad Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran (Grant No. unrc-650).

Conflict of Interest

None.

References

1. Zowawi, H. M., Harris, P. N., Roberts, M. J., Tambyah, P. A., Schembri, M. A., Pezzani, M. D., Williamson, D. A., Paterson, D. L.: The emerging threat of multidrug-resistant Gram-negative bacteria in urology. Nat Rev Urol **12**, 570–584 (2015).
2. Heidary, M., Bahramian, A., Hashemi, A., Goudarzi, M., Omrani, V. F., Eslami, G., Goudarzi, H.: Detection of acrA, acrB, aac (6′)-Ib-cr, and qepA genes among clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*. Acta Microbiol Immunol Hung **64**, 63–69 (2017).
3. Barber, A. E., Norton, J. P., Spivak, A. M., Mulvey, M. A.: Urinary tract infections: Current and emerging management strategies. Clin Infect Dis **57**, 719–724 (2013).
4. Heidary, M., Bahramian, A., Goudarzi, H., Eslami, G., Hashemi, A., Khoshnood, S.: To study the association between AcrAB and Qep A efflux pumps and ciprofloxacin resistance among *Escherichia coli* and *Klebsiella pneumoniae* clinical strains. Arak Med Univ J **19**, 1–10 (2016).
5. Sallem, R. B., Slama, K. B., Estepa, V., Jouini, A., Gharsa, H., Klibi, N., Saenz, Y., Ruiz-Larrea, F., Boudabous, A., Torres, C.: Prevalence and characterisation of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* isolates in healthy volunteers in Tunisia. Eur J Clin Microbiol Infect Dis **31**, 1511–1516 (2012).
6. Jones, G. L., Warren, R., Skidmore, S., Davies, V., Gibreel, T., Upton, M.: Prevalence and distribution of plasmid-mediated quinolone resistance genes in clinical isolates of *Escherichia coli* lacking extended-spectrum β-lactamases. J Antimicrob Chemother **62**, 1245–1251 (2008).
7. Roshani, M., Heidary, M., Goudarzi, H., Hashemi, A., Eslami, G., Yousefi, N.: Investigating the antibacterial effect of methanol and acetone extracts of *Urtica dioica* and *Zataria* multiflora against metallo beta-lactamase producing *Pseudomonas aeruginosa*. J Ilam Uni Med Sci **24**, 70–78 (2016).
8. Karisik, E., Ellington, M., Pike, R., Warren, R., Livermore, D., Woodford, N.: Molecular characterization of plasmids encoding CTX-M-15 β-lactamases from *Escherichia coli* strains in the United Kingdom. J Antimicrob Chemother **58**, 665–668 (2006).
9. Goudarzi, H., Hashemi, A., Fatemeh, F., Noori, M., Erfanimanesh, S., Yosefi, N., Heidary, M., Koshnood, S., Houri, H. R.: Detection of bla_{DIM}, bla_{AIM}, bla_{GIM}, bla_{NDM} and bla_{VIM} genes among *Acinetobacter baumannii* strains isolated from hospitalized patients in Tehran hospitals, Iran. Iran J Med Microbiol **9**, 32–39 (2016).

10. Heidary, M., Hashemi, A., Goudarzi, H., Khoshnood, S., Roshani, M., Azimi, H., Goudarzi, M.: The antibacterial activity of Iranian plants extracts against metallo beta-lactamase producing *Pseudomonas aeruginosa* strains. J Paramed Sci **7**, 13–19 (2016).
11. Mendonça, N., Leitão, J., Manageiro, V., Ferreira, E., Caniça, M.: Spread of extended-spectrum β-lactamase CTX-M-producing *Escherichia coli* clinical isolates in community and nosocomial environments in Portugal. Antimicrob Agents Chemother **51**, 1946–1955 (2007).
12. Yan, J. J., Ko, W. C., Jung, Y. C., Chuang, C. L., Wu, J. J.: Emergence of *Klebsiella pneumoniae* isolates producing inducible DHA-1 β-lactamase in a university hospital in Taiwan. J Clin Microbiol **40**, 3121–3126 (2002).
13. Ho, P. L., Chan, J., Lo, W. U., Lai, E. L., Cheung, Y. Y., Lau, T. C., Chow, K. H.: Prevalence and molecular epidemiology of plasmid-mediated fosfomycin resistance genes among blood and urinary *Escherichia coli* isolates. J Med Microbiol **62**, 1707–1713 (2013).
14. Mendes, A. C., Rodrigues, C., Pires, J., Amorim, J., Ramos, M. H., Novais, Â., Peixe, L.: Importation of fosfomycin resistance fosA3 gene to Europe. Emerg Infect Dis **22**, 346–348 (2016).
15. Sato, N., Kawamura, K., Nakane, K., Wachino, J. I., Arakawa, Y.: First detection of fosfomycin resistance gene fosA3 in CTX-M-producing *Escherichia coli* isolates from healthy individuals in Japan. Microb Drug Resist **19**, 477–482 (2013).
16. Lee, S. Y., Park, Y. J., Yu, J. K., Jung, S., Kim, Y., Jeong, S. H., Arakawa, Y.: Prevalence of acquired fosfomycin resistance among extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates in Korea and IS26-composite transposon surrounding fosA3. J Antimicrob Chemother **67**, 2843–2847 (2012).
17. Hou, J., Huang, X., Deng, Y., He, L., Yang, T., Zeng, Z., Chen, Z., Liu, J. H.: Dissemination of fosfomycin resistance gene fosA3 with CTX-M β-lactamase genes and rmtB carried on IncFII plasmids among *Escherichia coli* isolates from pets in China. Antimicrob Agents Chemother **56**, 2135–2138 (2012).
18. Ho, P., Chan, J., Lo, W., Law, P., Li, Z., Lai, E., Chow, K. H.: Dissemination of plasmid-mediated fosfomycin resistance fosA3 among multidrug-resistant *Escherichia coli* from livestock and other animals. J Appl Microbiol **114**, 695–702 (2013).
19. Wachino, J., Yamane, K., Suzuki, S., Kimura, K., Arakawa, Y.: Prevalence of fosfomycin resistance among CTX-M-producing *Escherichia coli* clinical isolates in Japan and identification of novel plasmid-mediated fosfomycin-modifying enzymes. Antimicrob Agents Chemother **54**, 3061–3064 (2010).
20. Heidary, M., Goudarzi, H., Hashemi, A., Eslami, G., Goudarzi, M., Chirani, A. S., Amraei, S.: Prevalence of quinolone resistance genes in *Klebsiella pneumoniae* strains isolated from hospitalized patients during 2013–2014. Arch Pediatr Infect Dis, e38343 (2016). doi:10.5812/pedinfect.38343 [Epub Ahead of Print].
21. Clinical and Laboratory Standards Institute (CLSI): Performance Standard for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement. CLSI document M100_s17. CLSI, Wayne, PA, USA, 2013.
22. Lob, S. H., Nicolle, L. E., Hoban, D. J., Kazmierczak, K. M., Badal, R. E., Sahm, D. F.: Susceptibility patterns and ESBL rates of *Escherichia coli* from urinary tract infections in Canada and the United States, SMART 2010–2014. Diagn Microbiol Infect Dis **85**, 459–465 (2016).

23. Shayan, S., Bokaeian, M., Shahraki, S.: Prevalence and molecular characterization of AmpC-producing clinical isolates of *Escherichia coli* from Southeastern Iran. Microb Drug Resist **20**, 104–107 (2014).
24. Saffar, H., Niaraki, N. A., Tali, A. G., Baseri, Z., Abdollahi, A., Yalfani, R.: Prevalence of AmpC β-lactamase in clinical isolates of *Escherichia coli*, *Klebsiella* spp., and *Proteus mirabilis* in a Tertiary Hospital in Tehran, Iran. Jundishapur J Microbiol **9**, e39121 (2016).
25. Domokos, J., Kristóf, K., Szabó, D.: Plasmid-mediated quinolone resistance among extended-spectrum beta-lactamase producing *Enterobacteriaceae* from bloodstream infections. Acta Microbiol Immunol Hung **63**, 313–323 (2016).