



AKADÉMIAI KIADÓ

Acta Microbiologica et
Immunologica Hungarica


69 (2022) 4, 314–322

DOI:
[10.1556/030.2022.01826](https://doi.org/10.1556/030.2022.01826)
© 2022 Akadémiai Kiadó, Budapest

RESEARCH ARTICLE



Distribution and expression of virulence genes (*hlyA*, *sat*) and genotyping of *Escherichia coli* O25b/ST131 by multi-locus variable number tandem repeat analysis in Tehran, Iran

SAJJAD ASGHARZADEH¹, REZVAN GOLMORADI ZADEH¹,
MAJID TAATI MOGHADAM¹,
HAMED FARAHANI ERAGHIYE¹,
BEHROOZ SADEGHI KALANI²,
FARAMARZ MASJEDIAN JAZI¹ and
SHIVA MIRKALANTARI^{1*} 

¹ Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

² Department of Microbiology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran

Received: July 11, 2022 • Accepted: September 1, 2022

Published online: September 21, 2022

ABSTRACT

Escherichia coli ST131 is a pandemic clone with high antibiotic resistance, and it is a major causative agent of urinary tract infection (UTI) and bloodstream infections. This study evaluated the distribution and expression of virulence genes and genotyping of *E. coli* O25b/ST131 by Multi-locus variable number tandem repeat analysis (MLVA) method among UTI in patients at Tehran hospitals, Iran.

A total of 107 *E. coli* isolates were collected from UTI patients. Polymerase chain reaction (PCR) amplification of the *pabB* gene was used to identify *E. coli* O25b/ST131 and the prevalence of *sat* and *hlyA* virulence genes was also analyzed. The microtiter method quantified biofilm formation ability in *E. coli* O25b/ST131. The Real-Time PCR (qRT-PCR) was performed to evaluate the expression of *sat* and *hlyA* genes. Finally, MLVA was performed for *E. coli* O25b/ST131 genotyping by targeting seven tandem repeats. SPSS-16 software was used for statistical analysis. Molecular study showed that 71% of isolates carried the *pabB* gene and were considered *E. coli* O25b/ST131 strains. Also, 45.8% and 17.8% of isolates carried *sat* and *hlyA* genes, respectively. The 57.9% isolates had biofilm formation ability. Expression of the studied virulence genes showed an increase in strong biofilm producing *E. coli* O25b/ST131 strains. A total of 76 (100%) *E. coli* O25b/ST131 strains were typed by the MLVA method.

High prevalence of *E. coli* O25b/ST131 isolates in UTI patients can be a serious warning to the treatment due to the high antibiotic resistance rate, expression of virulence genes, and biofilm formation.

KEYWORDS

E. coli ST131, MLVA, biofilm production, urinary tract infection

INTRODUCTION

Escherichia coli is one of the most important commensal bacterium in the human body. This bacterium can cause a wide range of diseases, including diarrhea, urinary tract infections (UTI), meningitis, and opportunistic infections. The main cause of UTI is the dissemination of bacteria from the intestine to the urinary tract [1–3]. About half of women experience a symptomatic UTI at least once in their lifetime, and uropathogenic *E. coli* (UPEC) accounts for more than 85% of these infections. UTI can cause more serious complications such as pyelonephritis, bacteremia, and sepsis [4]. Most strains of UPEC are associated with B2 or D

*Corresponding author.
E-mail: Mirkalantari.sh@iums.ac.ir

phylogroups, and they belong to the ST131, ST69, ST10, ST405, ST38, ST95, ST648, ST73, ST410, ST393, ST354, ST12, ST127, ST167, ST58, ST617, ST88, ST23, ST117, and ST1193 sequences types [5, 6]. *E. coli* ST131 clone discovered in 2008, was the main sequence type isolated from UTI, bloodstream infections, and animal infections [7]. Over the past two decades, resistance to first-line antibiotics has increased significantly among UPEC strains, and *E. coli* ST131 became the dominant clone among multi-drug resistant (MDR) strains [8]. This clone can develop antibiotic resistance by various mechanisms; one of the most important of these mechanisms is the production of extended-spectrum beta-lactamases (ESBLs) such as CTX-M. ESBLs are capable to hydrolyze oxyimino-cephalosporins (cefotaxime, ceftriaxone, ceftazidime, and cefepime) and monobactams but are inhibited by beta-lactamase inhibitors such as clavulanic acid and tazobactam. Another note is that CTX-M-15 is usually associated with other enzymes that confer resistance to aminoglycosides and fluoroquinolones [9]. Besides, there have been reports of resistance to trimethoprim-sulfamethoxazole (TMP-SMX), colistin, and carbapenem among *E. coli* ST131 strains. Moreover, biofilm formation is another mechanism that causes antibiotic resistance. Mechanisms of resistance caused by biofilms include: i) reducing the penetration of antimicrobial agents (lysozyme, complement, antibiotics), ii) reducing the growth of bacteria due to nutrient restrictions, iii) stimulating the expression of stress response genes, iv) the emergence of a biofilm specific phenotype [9]. Biofilm also plays an essential role in increasing the expression of virulence genes, leading to the worsening of the disease [10]. Additionally, to antibiotic resistance and resistance genes, this clone carries many genes involved in the host's binding, colonization, invasion, and survival. For instance, *sat*, *fimH*, *fyuA*, *kpsM II*, *usp*, *malX*, *iha*, *iroN*, *ompT*, *iucD*, *iutA*, *hlyA*, and *trtA* are some genes involved in mentioned mechanisms [11–13]. Because *E. coli* ST131 has become a pandemic strain and has high antibiotic resistance, the use of bacterial typing is recommended to investigate the relationship between the strains and the source of infection. There are various techniques for typing in which each of these methods has many advantages or disadvantages. Multi-locus variable number tandem repeat analysis (MLVA) technique has been considered for bacterial typing in recent years. Because of the various advantages of this technique, it makes it ideal for epidemiological studies. This technique is based on the analysis of

variable number tandem repeats (VNTRs). The VNTRs are chromosomal regions in which a short DNA sequence motif is repeated in variables. The number of repeats per locus varies between strains of the same species [14, 15]. Here, we evaluated the distribution and expression of *hlyA* and *sat* genes, also genotyping of *E. coli* O25b/ST131 among UTI patients.

MATERIALS AND METHODS

Bacterial isolates

A total of 107 *E. coli* isolates were collected from April 2019 to January 2021 from UTI patients who were referred to Fir-oozgar and Firooz Abadi hospitals in Tehran, Iran. According to conventional bacteriological tests, the isolates were identified as *E. coli* [16, 17]. Isolates were stored in a trypticase soy broth (TSB) medium containing 10% glycerol at -70°C .

Identification of *E. coli* ST131 clones and *sat* and *hlyA* virulence genes

Genomic DNA was extracted by boiling method [18]. PCR amplification of the *pabB* gene was used to identify *E. coli* O25b/ST131 clones, furthermore, to evaluate the prevalence of *sat* and *hlyA* virulence genes PCR was used (Table 1). PCR was performed in the following conditions: 2 μL of DNA template, 10 μL master mix 2x (Ampliqon, Denmark), 1 μL of each $10\ \mu\text{mol}^{-1}$ primer (forward and reverse), and 11 μL distilled sterile water, for a total reaction volume of 25 μL . The Thermocycler program was as follows; an initial denaturation step at 95°C for 5 min followed by 34 cycles of denaturation at 95°C for 45s, annealing at 61°C for 30 s and extension at 72°C for the 40s. A final extension step was performed at 72°C for 5 min finally, amplicons were run on 1.0% (w/v) agarose gels [19].

Biofilm formation assay

The microtiter method quantified the biofilm formation ability in *E. coli* O25b/ST131 clones [20]. In summary, *E. coli* O25b/ST131 clones were cultured in TSB medium with 1% glucose and incubated overnight at 37°C . The cultures were diluted in the same medium to reach an optical density (OD) of 0.08 at a 600 nm wavelength. Inoculate 200 μL of the obtained bacterial suspension into each well of the

Table 1. Study primers and their characterization for identification of *E. coli* O25b/ST131 isolates and their virulence genes

Gene		Primer sequence (5' → 3')	Product size	Tm ($^{\circ}\text{C}$)	Origin of the primer
<i>sat</i>	F	AGAAATATGGCATCTGTCACC	97bp	60	[43]
	R	CAGACGATATAGTCGGTGTTC			
<i>hlyA</i>	F	TTCGTGAAAGGAGGCAGTCC	88bp	61.5	this study
	R	ACCGACTGATGCATGCTGAA			
<i>pabB</i>	F	TCCAGCAGGTGCTGGATCGT	347 bp	60	[44]
	R	GCGAAATTTTCGCCGTACTGT			
16s rRNA	F	AGGCCCGAAACTGACGATTT	166 bp	60	this study
	R	CATGTCCGCAATGGCATCAG			



96-well polystyrene plate and incubate for 24 h at 37 °C. Next, plates were washed twice with phosphate-buffered saline (PBS), and then 100 µL of 1% crystal violet dye were added. After 20 min and twice washed with PBS, 100 µL of 95% ethanol was added to the wells and incubated at room temperature for 15 min. Finally, cell density was measured at 570 nm by Microplate reader Varioskan Flash (Thermo Scientific, Billerica, MA, USA). Each assay was performed in triplicate. Based on the adherence capabilities, all isolates were classified into four categories: non-adherent bacteria ($OD \leq OD_c$); weakly adherent bacteria ($OD_c < OD \leq 2 \times OD_c$); moderately adherent bacteria ($2 \times OD_c < OD \leq 4 \times OD_c$); strongly adherent bacteria ($4 \times OD_c < OD$) [21].

Gene expression analysis by qRT-PCR

The expression of *sat* and *hlyA* genes in strong and weak biofilm formation strains was evaluated by qRT-PCR. Next, the biofilm cells were isolated using sonication and twice washed with PBS. RNA extraction was performed according to manufacturer's protocols by Kit (Favorgen, Taiwan). The quantity and quality of extracted RNA were evaluated by a nanodrop device (Thermo Fisher Scientific, USA). The aliquots were treated by the DNase kit (Roche, Germany) according to the manufacturer's protocols to remove the remaining DNA. The total RNA was transcribed into the cDNA by Kit (Betabairn, Germany). The qRT-PCR was recruited in the Rotor-Gene thermal cycler system (Corbett 6000; Australia) based on the SYBR green method (Taiwan, ExceITaq™ 2X Q-PCR Master Mix). Total 20 µL reaction volume contained 1 µL of each primer, 10 µL SYBR Green master mix (SMOBIO, Taiwan), 1 µL cDNA template, and 7 µL of RNase-free water. The three-step cycling reaction protocol was set as follows: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 59 °C for 30 s and 72 °C for 30 s. The 16S rRNA gene was used to calculate mRNA expression fold changes based on the $2^{-\Delta\Delta Ct}$ method [22].

MLVA

MLVA was recruited as described previously by François Camélina (2019) by targeting seven tandem repeats [(RDB1 (*rhaD*), *ms11* (*rsxC*), *ms21* (*tRNA-Arg*), CNV001 (*ftsK*), CNV004 (*tolA*), CNV014 (*hemY*) and O157-33 (*ytfL*)] using singleplex PCR technique [23]. The primer details used to MLVA are shown in Table 2. The following equation calculated the number of tandem repeats:

$$\text{Number of repeats} = \frac{(\text{amplicon size} - \text{flanking region})}{\text{repeat size}}.$$

The number of repeats was entered into BIONUMERICS (Applied Maths) software, and the dendrogram was drawn based on categorical coefficients and the Ward algorithm.

STATISTICAL ANALYSIS

Data were analyzed using SPSS software version 16 by chi-squared (X²). In addition, the *P*-value less than 0.05 was considered significant.

Table 2. Primers used in this study and their properties to perform MLVA by PCR technique

PCR	Gene name	primers	sequence 5'-3'	Repeat unit size (bp)	Product size range (bp)	flanking region size (bp)	Annealing temperature	Origin of the VNTR
ECMLV1	RDB1	ECMLV1 - F	TCCCTGGACAAACCCAGGACTG	92	162–1,597	135	61 °C	[23]
	<i>rhaD</i>	ECMLV1 - R	CGTGGGACTTATGAGAAAG					
ECMLV2	<i>ms11</i>	ECMLV2 - F	GAAACAGGCCCGGCTACAC	96	575–869	259	61.5 °C	[23]
	<i>rsxC</i>	ECMLV2 - R	CTGGCGCTGTTATGGGTAT					
ECMLV3	<i>ms21</i>	ECMLV3 - F	TTCAGGAAATGGATAAAGTAG T	139	616–1,157	311	59.7 °C	[23]
	<i>tRNA-Arg</i>	ECMLV3 - R	GGGAGTATGGGTCAAAAGC					
ECMLV4	CNV001	ECMLV4 - F	ACAAACGGCTGGGGGGAATCC	39	413–539	216	65.5 °C	[23]
	<i>ftsK</i>	ECMLV4 - R	GTCAGCAATCCAGAGAAAGGCA					
ECMLV5	CNV004	ECMLV5 - F	GCGGCGCTGAAGAAGAAAGC	48	375–438	172	69.2 °C	[23]
	<i>tolA</i>	ECMLV5 - R	CTCCCGGCGGCGAAGCATTTGT					
ECMLV6	CNV014	ECMLV6 - F	CAAAGAGCAATAACACTTTTAGCA	6	102–149	80	57 °C	[23]
	<i>hemY</i>	ECMLV6 - R	GCAGCAGGACAAACGGGAAGCTAA					
ECMLV7	O157-33	ECMLV7 - F	GTGAAGGATAAGCTGCATTGTCA	17	176–211	159	59 °C	[23]
	<i>ytfL</i>	ECMLV7 - R	GCCTGACGCTAAAGATAAAGAAGA					



RESULT

In the present study, 107 *E. coli* isolates were collected from UTI patients referred to Firoozgar and Firooz Abadi hospitals, Tehran, Iran. Among the isolates, 71 (66.3%) and 36 (33.6%) samples were isolated from patients referred to Firoozgar and Firooz Abadi hospitals, respectively. Altogether 39 (36.4%) samples were obtained from men, and 68 (63.6%) samples were obtained from women. Molecular studies showed that 76 (71%) isolates carried the *pabB* gene and were considered *E. coli* O25b/ST131 strains. Thirty (77%) and forty-six (67.7%) *E. coli* O25b/ST131 were identified in men and women, respectively. It was found that the frequency of *E. coli* O25b/ST131 strains in Firoozgar hospital was higher than in Firooz Abadi hospital. Out of 71 samples isolated from Firoozgar hospital, 59 (83.1%) were identified as *E. coli* O25b/ST131 strains, but in Firooz Abadi hospital, out of 36 samples, only 17 (47.3%) were identified as *E. coli* O25b/ST131. Statistical analysis showed a significant relationship between the frequency of *E. coli* O25b/ST131 strains and different hospitals ($P < 0.05$). Out of 107 samples, 49 (45.8%) isolates carried *sat* gene as one of the virulence factors. Statistical analysis showed that the frequency of *sat* gene in *E. coli* O25b/ST131 strains was higher than non-*E. coli* O25b/ST131 isolates. Of the 76 strains of *E. coli* O25b/ST131, 45 (59.3%) had the *sat* virulence factor, but out of 31 non-*E. coli* O25b/ST131 isolates, this virulence factor was detected in only 4 (13%) isolates. A significant correlation was observed between the high frequency of *sat* virulence factor and *E. coli* O25b/ST131 ($P < 0.05$). Examination of the frequency of the *hlyA* virulence factor in 107 samples showed that 19 (17.8%) isolates harbored the *hlyA* gene. All 19 *hlyA* genes were found in *E. coli* O25b/ST131 strains and non-*E. coli* O25b/ST131 isolates had this virulence factor. Statistical analysis showed a significant relationship between the high frequency of *hlyA* and *E. coli* O25b/ST131 strains

compared to isolates of non-*E. coli* O25b/ST131 strains ($P < 0.05$) (Fig. 1). Out of 107 isolates, 62 (57.9%) isolates were able to form biofilms, of which 21 (19.6%), 14 (13.1%), and 27 (25.2%) isolate formed weak, medium, and strong biofilms, respectively. On the other hand, 45 (42.1%) isolates could not form biofilms. Our analysis showed that most biofilm-producing isolates were *E. coli* O25b/ST131 strains, which had a significant relationship between this strain and biofilm formation ($P < 0.05$). Among the weak, medium, and strong biofilm-producing isolates were *E. coli* O25b/ST131 strains 19 (90.4%), 14 (100%), and 27 (100%), respectively.

The results of qRT-PCR and CT examination of *sat* and *hlyA* virulence genes showed an expression increase in strong biofilms producing *E. coli* O25b/ST131 compared to weak ones.

It was found that *E. coli* O25b/ST131 strains were typable by the MLVA method. Dendrogram results showed that among 76 isolates of *E. coli* O25b/ST131, we have 73 different allelic profiles (Fig. 2). *E. coli* O25b/ST131 strains formed two main clusters (A and B) and four sub-clusters. Most isolates belonged to cluster A (74), and cluster B consisted of 2 isolates separated by differences in 4 VNTR markers. Cluster A was divided into A1 and A2 subclusters. The A1 subcluster hosted five types, but most were in the A2 sub-cluster. Using MST analysis, we observed that our isolates consisted of 46 singletons and 8 clonal complexes (CC) (Fig. 3). CC 4 hosted the most strains. These CC belonged to Firoozgar hospital and subcluster A2. No CC was observed in strains isolated from Firooz Abadi hospital. Although 6 strains had the same genotype, they showed different phenotypes. The Hunter-Gaston diversity index calculated the variation degree of all seven loci. We recognized that the highest variation was related to the VNTR4 (0.45) in our collection, and thus it was the most polymorphic locus in the present study. Also, VNTR6 loci had the least (0.16) variation.

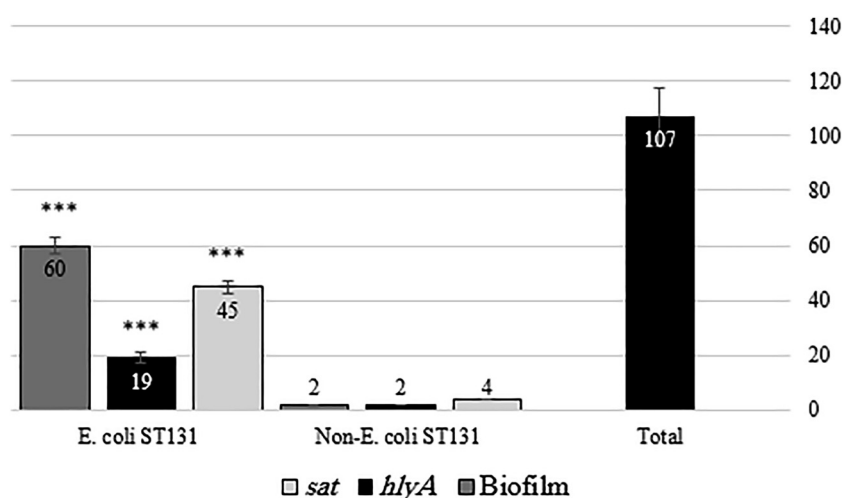


Fig. 1. Comparison of the frequency of virulence genes and biofilm production in *E. coli* O25b/ST131 and non-*E. coli* O25b/ST131 strains that the significance correlation of the frequency of the virulence factors is marked with "*" in which P -value less than 0.05 = *, less than 0.005 = **, and less than 0.0005 = ***

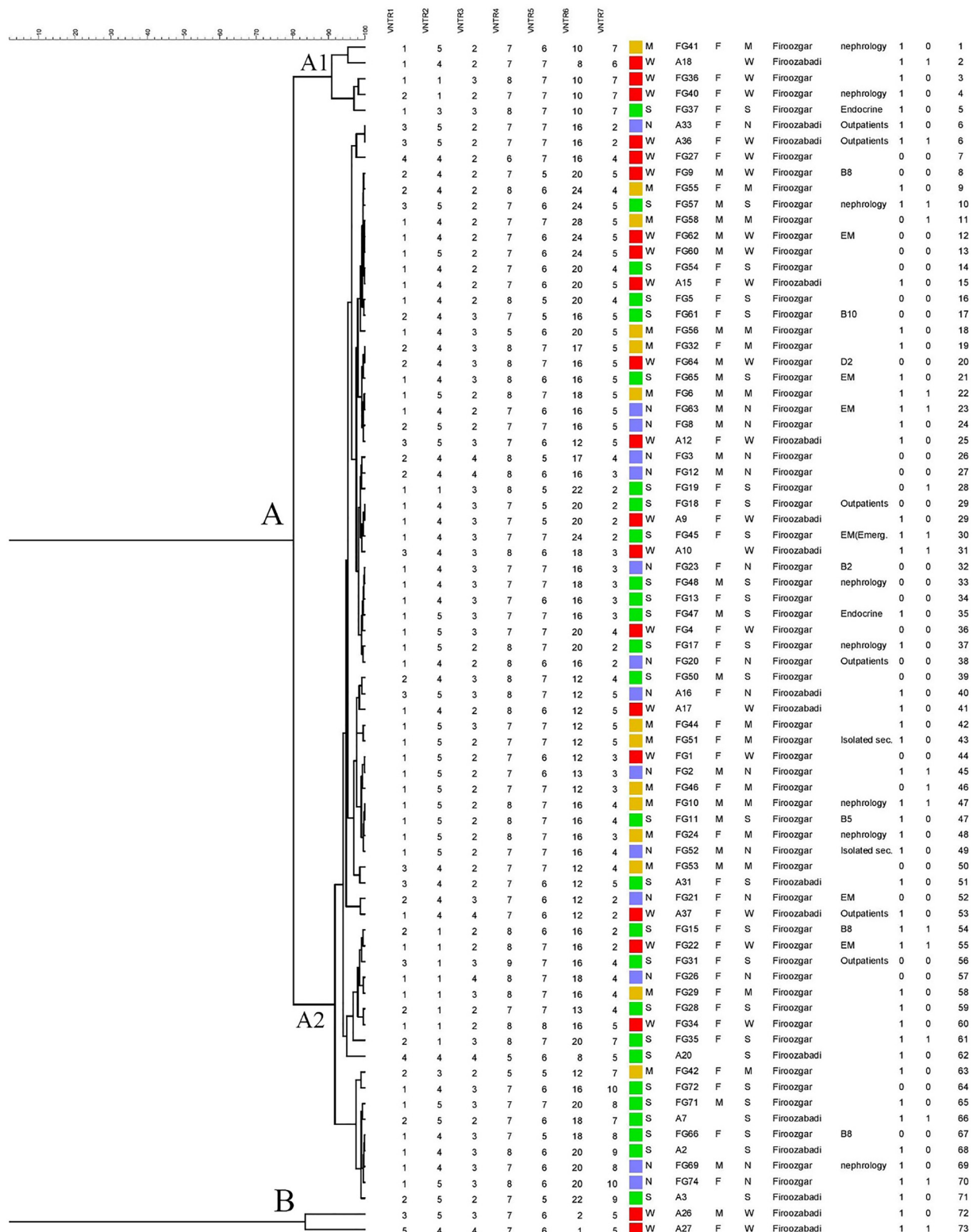


Fig. 2. MLVA based clustering of *E. coli* O25b/ST131 isolates investigated in this study. Colored squares indicate the ability to produce biofilm in the strains. And the numbers 0 and 1 indicate the presence or absence of virulence genes

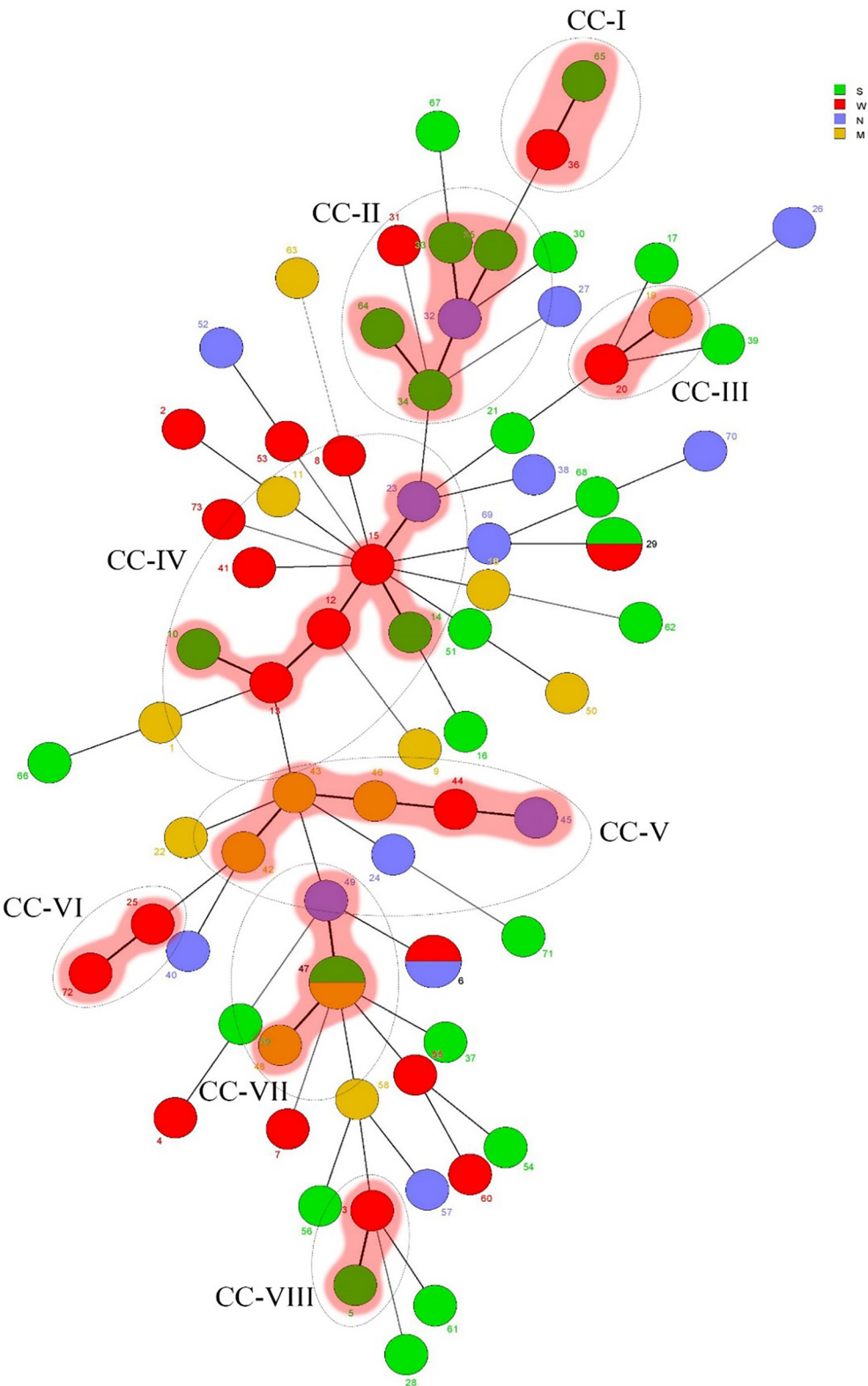


Fig. 3. Data analysis by using the Minimum Spanning Tree (MST) algorithm from 76 *E. coli* O25b/ST131 isolates. Each circle shows an isolate with a unique profile. The colors inside the circle are related to the biofilm production power



DISCUSSION

Although *E. coli* is a commensal bacterium in the humans and animals intestines, it is one of the most common microorganisms that cause extra-intestinal infections in animals. UTI is one of the most prominent infections caused by *E. coli*. Reports have shown that UTI is the most common bacterial infection in humans, and UPEC is responsible for more than 80% of these cases [9, 24, 25]. In the present study, the prevalence of UTI in women (63.6%) was much higher than in men (36.4%), which was consistent with the study of Dadi, B.R et al. [26]. The study results by Al-Jamei et al. were very similar to our research, which confirms the higher prevalence of UTI in women [27], which is predictable due to the structure of the female urinary system being inefficient in preventing the entry of bacteria. Anatomical differences in women, such as shortness of the urethra, proximity of the urethra to the anus and vagina, and lack of antimicrobial compounds similar to those found in male prostate fluid, make them more susceptible to UTIs [28, 29]. We found that among the UPEC isolated from UTI patients, the overall rate of *E. coli* O25b/ST131 clones was significantly high (71%) ($P < 0.05$). The prevalence ratio of these clones was slightly higher in men, too. Recently, due to the particular importance of this strain, several studies have been underway. In the study by Demirci-Duarte et al., only 30% of the strains were ST131 [30]. Another study also reported a 24% prevalence of this sequence type [31]. However, in Hojabri et al., *E. coli* ST131 had a higher prevalence in women [32]. In general, reports on the frequency of *E. coli* ST131 are diverse, and this diversity is due to geographical, economic, and health conditions; as well, diagnostic methods are critical. A high prevalence of *sat* and *hlyA* virulence genes was observed among *E. coli* O25b/ST131 isolates, which may be necessary for the pathogenesis of this strain in various infections. The study by Coelho et al. also confirmed the obtained results of our study [33]. Hojabri et al. found that the *sat* and *hlyA* virulence genes were twice as common among the *E. coli* ST131 isolates [34]. Due to the incomparable role of biofilm in the development of antibiotic resistance, much research has been done on the ability of *E. coli* ST131 biofilm formation. We examined the biofilm formation ability among all strains and found that *E. coli* O25b/ST131 isolates were much superior in this capability, according to Kudinha et al.'s study [35]. In contrast, Zhang et al. reported most *E. coli* ST131 isolates could produce weak biofilms [36]. Sarkar et al. revealed that type I fimbriae play a vital role in biofilm formation and inhibition of FimH adhesion, which significantly reduces biofilm production [37].

In general, there was a high correlation between biofilm production capacity and expression of *sat* and *hlyA* virulence genes. Studies have shown that the strains of potent biofilm producers expressed *sat* and *hlyA* genes several times more often. Rim Al Safadi et al. indicated virulence genes of *E. coli* have the highest expression during biofilm formation [38]. This study successfully used the MLVA method for typing *E. coli* O25b/ST131 isolates from clinical specimens.

The MLVA is easy and cheap and provides acceptable results, as mentioned in the previous sections. Therefore, it can be widely used for typing in developing countries in the future. Numerous studies have confirmed the effectiveness of this method for typing *E. coli* strains [39, 40]. This method is not limited to *E. coli* and can be used for typing different types of bacteria. To perform MLVA, we used seven VNTR-related loci, and these loci had special features such as easy separation in agarose gel and high resolution. The use of seven loci for *E. coli* typing with the MLVA technique was first described by Lindstedt et al. (ref). However, the drawback of his method was the need for sequencing, which increased costs [41]. More loci can improve the differentiating power, so eight to ten loci have been used [39, 42]. All strains had the above loci, while in the study of Caméléna et al., 23% of strains did not have one or two VNTR loci [23]. These corresponding loci may be missing in some strains due to the long VNTR or mutation at the primer binding site of the DNA. Dendrogram results showed extensive heterogeneity in the strains so that only 6 strains had a similar allelic profile. The high prevalence of *E. coli* O25b/ST131 in Firoozgar hospital and the presence of multiple CC in this hospital was probably due to the transfer of similar strains between patients.

CONCLUSION

Due to the high antibiotic resistance of *E. coli* O25b/ST131, our observations about the prevalence in patients with UTIs and further virulence factors of this strain can be a warning to the medical system. The results of strain typing with the new MLVA technique can be cheaper, faster, and more accurate in identifying the origin of various strains.

Ethical approval and consent to participate: The ethics committee of Iran University of Medical Sciences has approved the study protocol (code: IR.IUMS.FMD.REC.1399.713). All methods were performed in accordance with the relevant guidelines and regulations/declaration of Helsinki. The patient signed a written informed consent form.

Consent for publication: Not applicable

Availability of data and material: All the datasets supporting the conclusions of this article are available. Additional data of this paper can be obtained upon request. The corresponding author (Shiva Mirkalantari: shiva.mirkalantari@yahoo.com) should be contacted if someone wants to request the data from this study.

Competing interests: The authors declare that they have no competing interests.

Funding: This study was financially supported by a research grant (No.18525) for an M.Sc thesis at Iran University of Medical Sciences (Tehran, Iran), for which we are very grateful



Author's contributions: This article was part of a study that contributed to an MSC thesis by Sajjad Asgharzadeh. and Shiva Mirkalantari designed the study. Sajjad Asgharzadeh and Rezvan Golmoradi zadeh performed experiments of the research and drafted the manuscript and Majid Taati Moghadam performed the statistical analysis. All authors read and approved the final manuscript.

Conflicts of interest: We have no conflicts of interest to disclose.

ACKNOWLEDGEMENTS

We would like to thank the members of the Microbiology Department for their valuable contributions for this research project. Sajjad Asgharzadeh¹, Rezvan Golmoradi Zadeh¹, Majid Taati Moghadam¹, Hamed Eraghiye Farahani¹, Behrooz Sadeghi Kalani², Faramarz Masjedian Jazi¹, Shiva Mirkalantari¹

REFERENCES

- Mohebi S, Hossieni Nave H, Norouzi A, Kandehkar Gharaman M, Taati Moghadam M. Detection of extended spectrum beta lactamases on class I integron in *Escherichia coli* isolated from clinical samples. *J Mazandaran Univ Med Sci* 2016; 26(138): 66–76.
- Jamali S, Tavakoly T, Mojtahedi A, Shenagari M. The phylogenetic relatedness of *bla*_{NDM-1} harboring extended-spectrum β -lactamase producing uropathogenic *Escherichia coli* and *Klebsiella pneumoniae* in the North of Iran. *Infect Drug Resist* 2020; 13: 651–7.
- Haghighatpanah M, Mojtahedi A. Characterization of antibiotic resistance and virulence factors of *Escherichia coli* strains isolated from Iranian inpatients with urinary tract infections. *Infect Drug Resist* 2019; 12: 2747–54.
- Bergeron CR, Prussing C, Boerlin P, Daignault D, Dutil L, Reid-Smith RJ, et al. Chicken as reservoir for extraintestinal pathogenic *Escherichia coli* in humans, Canada. *Emerg Infect Dis* 2012; 18(3): 415–21.
- Manges AR, Geum HM, Guo A, Edens TJ, Fibke CD, Pitout JDD. Global extraintestinal pathogenic *Escherichia coli* (ExPEC) lineages. *Clin Microbiol Rev* 2019; 32(3): e00135–18.
- Sadeghi M, Ebrahim-Saraie HS, Mojtahedi A, Nikokar I, Roushan ZA. Genetic diversity and prevalence of aminoglycoside modifying enzymes among *Escherichia coli* strains isolated from inpatients with urinary tract infections. *Gene Rep* 2020; 21: 100957.
- Platell JL, Johnson JR, Cobbold RN, Trott DJ. Multidrug-resistant extraintestinal pathogenic *Escherichia coli* of sequence type ST131 in animals and foods. *Vet Microbiol* 2011; 153(1–2): 99–108.
- Alqasim A, Abu Jaffal A, Alyousef AA. Prevalence and molecular characteristics of sequence type 131 clone among clinical uropathogenic *Escherichia coli* isolates in Riyadh, Saudi Arabia. *Saudi J Biol Sci* 2020; 27(1): 296–302.
- Moghadam MT, Mirzaei M, Moghaddam MFT, Babakhani S, Yeganeh O, Asgharzadeh S, et al. The challenge of global emergence of novel colistin-resistant *Escherichia coli* ST131. *Microb Drug Resist* 2021; 27: 1513–24.
- Roy PK, Ha AJ-W, Mizan MFR, Hossain MI, Ashrafudoulla M, Tushik SH, et al. Effects of environmental conditions (temperature, pH, and glucose) on biofilm formation of *Salmonella enterica* serotype Kentucky and virulence gene expression. *Poult Sci* 2021; 100(7): 101209.
- Nicolas-Chanoine MH, Bertrand X, Madec JY. *Escherichia coli* ST131, an intriguing clonal group. *Clin Microbiol Rev* 2014; 27(3): 543–74.
- Mandomando I, Vubil D, Boisen N, Quintó L, Ruiz J, Sigauque B, et al. *Escherichia coli* ST131 clones harbouring AggR and AAF/V fimbriae causing bacteremia in Mozambican children: emergence of new variant of fimH27 subclone. *Plos Negl Trop Dis* 2020; 14(5): e0008274.
- Cha MK, Kang C-I, Kim SH, Cho SY, Ha YE, Wi YM, et al. Comparison of the microbiological characteristics and virulence factors of ST131 and non-ST131 clones among extended-spectrum β -lactamase-producing *Escherichia coli* causing bacteremia. *Diagn Microbiol Infect Dis* 2016; 84(2): 102–4.
- Li W, Raoult D, Fournier P-E. Bacterial strain typing in the genomic era. *FEMS Microbiol Rev* 2009; 33(5): 892–916.
- Moghadam M, Shariati A, Mirkalantari S, Karmostaji A. The complex genetic region conferring transferable antibiotic resistance in multidrug-resistant and extremely drug-resistant *Klebsiella pneumoniae* clinical isolates. *New Microbes New Infections* 2020; 36: 100693.
- Hadizadeh M, Norouzi A, Taghadosi R, Mohebi S, Mohammadi M, Hasanazade A, et al. Prevalence of *qnr*, *intI*, and *intII* genes in extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* isolated from clinical samples in Iran. *Trop J Pharm Res* 2017; 16(1): 141–7.
- Yousefi S, Mojtahedi A, Shenagari M. A survey of *gyrA* target-site mutation and *qnr* genes among clinical isolates of *Escherichia coli* in the north of Iran. *Jundishapur J Microbiol* 2018; 11(9).
- Moghadam MT, Nave HH, Mohebi S, Norouzi A. The evaluation of connection between integrons class I and II and ESBL-producing and Non-ESBL *Klebsiella pneumoniae* isolated from clinical samples, Kerman. *Iran J Med Microbiol* 2016; 10: 1–9.
- Kangachar SA, Mojtahedi A. Evaluation of integrons classes 1–3 in extended spectrum β -lactamases and multi drug resistant *Escherichia coli* isolates in the North of Iran. *Gene Rep* 2018; 12: 299–303.
- Shahbazi S, Asadi Karam MR, Habibi M, Talebi A, Bouzari S. Distribution of extended-spectrum β -lactam, quinolone and carbapenem resistance genes, and genetic diversity among uropathogenic *Escherichia coli* isolates in Tehran, Iran. *J Glob Antimicrob Resist* 2018; 14: 118–25.
- Al-Kafaween M, Khan R, Bakar M, Tengku M. Characterization of biofilm formation by *Escherichia coli*: an in vitro study. *J Appl Biol Biotechnol* 2019; 7.
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 2001; 25(4): 402–8.
- Caméléna F, Birgy A, Smail Y, Courroux C, Mariani-Kurkdjian P, Le Hello S, et al. Rapid and simple universal *Escherichia coli* genotyping method based on multiple-locus variable-number tandem-repeat analysis using single-tube multiplex PCR and standard gel electrophoresis. *Appl Environ Microbiol* 2019; 85(6): e02812–18.



24. Shenagari M, Bakhtiari M, Mojtahedi A, Roushan ZA. High frequency of mutations in *gyrA* gene associated with quinolones resistance in uropathogenic *Escherichia coli* isolates from the north of Iran. *Iran J Basic Med Sci* 2018; 21(12): 1226.
25. Mojtahedi A. The presence of extended-spectrum β -lactamase as a risk factor for MDR in clinical isolation of *Escherichia coli*. *Trop Biomed* 2017; 34(1): 98–109.
26. Dadi BR, Abebe T, Zhang L, Mihret A, Abebe W, Amogne W. Distribution of virulence genes and phylogenetics of uropathogenic *Escherichia coli* among urinary tract infection patients in Addis Ababa, Ethiopia. *BMC Infect Dis* 2020; 20(1): 108.
27. Al-Jamei SA, Albsoul AY, Bakri FG, Al-Bakri AG. Extended-spectrum β -lactamase producing *E. coli* in urinary tract infections: a two-center, cross-sectional study of prevalence, genotypes and risk factors in Amman, Jordan. *J Infect Public Health* 2019; 12(1): 21–5.
28. Minardi D, d'Anzeo G, Cantoro D, Conti A, Muzzonigro G. Urinary tract infections in women: etiology and treatment options. *Int J Gen Med* 2011; 4: 333–43.
29. Moore KN, Day RA, Albers M. Pathogenesis of urinary tract infections: a review. *J Clin Nurs* 2002; 11(5): 568–74.
30. Demirci-Duarte S, Unalan-Altintop T, Eser OK, Cakar A, Altun B, Sancak B, et al. Prevalence of O25b-ST131 clone and fosfomycin resistance in urinary *Escherichia coli* isolates and their relation to CTX-M determinant. *Diagn Microbiol Infect Dis* 2020; 98(1): 115098.
31. Can F, Kurt-Azap Ö, İspir P, Nurtop E, Seref C, Loçlar İ, et al. The clinical impact of ST131 H30-Rx subclone in urinary tract infections due to multidrug-resistant *Escherichia coli*. *J Glob Antimicrob Resist* 2016; 4: 49–52.
32. Hojabri Z, Mirmohammadkhani M, Darabi N, Arab M, Pajand O. Characterization of antibiotic-susceptibility patterns and virulence genes of five major sequence types of *Escherichia coli* isolates cultured from extraintestinal specimens: a 1-year surveillance study from Iran. *Infect Drug Resist* 2019; 12: 93–903.
33. Coelho A, Mora A, Mamani R, López C, González-López JJ, Larrosa MN, et al. Spread of *Escherichia coli* O25b:H4-B2-ST131 producing CTX-M-15 and SHV-12 with high virulence gene content in Barcelona (Spain). *J Antimicrob Chemother* 2010; 66(3): 517–26.
34. Hojabri Z, Darabi N, Arab M, Saffari F, Pajand O. Clonal diversity, virulence genes content and subclone status of *Escherichia coli* sequence type 131: comparative analysis of *E. coli* ST131 and non-ST131 isolates from Iran. *BMC Microbiol* 2019; 19(1): 117.
35. Kudinha T, Johnson JR, Andrew SD, Kong F, Anderson P, Gilbert GL. *Escherichia coli* sequence type 131 as a prominent cause of antibiotic resistance among urinary *Escherichia coli* isolates from reproductive-age women. *J Clin Microbiol* 2013; 51(10): 3270–6.
36. Zhang S, Zhang Q, Huang J, Cao Y, Zhao Z, Li B. Epidemic potential of *Escherichia coli* O16:H41-ST131: compared with pandemic O25b:H30-ST131 lineage. *Infect Drug Resist* 2021; 14: 2625–32.
37. Sarkar S, Vagenas D, Schembri MA, Totsika M. Biofilm formation by multidrug resistant *Escherichia coli* ST131 is dependent on type 1 fimbriae and assay conditions. *Pathog Dis* 2016; 74(3).
38. Safadi RA, Abu-Ali GS, Sloup RE, Rudrik JT, Waters CM, Eaton KA, et al. Correlation between in Vivo biofilm formation and virulence gene expression in *Escherichia coli* O104:H4. *Plos One* 2012; 7(7): e41628.
39. Nielsen JB, Albayati A, Jørgensen RL, Hansen KH, Lundgren B, Schønning K. An abbreviated MLVA identifies *Escherichia coli* ST131 as the major extended-spectrum β -lactamase-producing lineage in the Copenhagen area. *Eur J Clin Microbiol Infect Dis* 2013; 32(3): 431–6.
40. Naseer U, Olsson-Liljequist BE, Woodford N, Dhanji H, Cantón R, Sundsfjord A, et al. Multi-locus variable number of tandem repeat analysis for rapid and accurate typing of virulent multidrug resistant *Escherichia coli* clones. *Plos One* 2012; 7(7): e41232.
41. Lindstedt B-A, Brandal LT, Aas L, Vardund T, Kapperud G. Study of polymorphic variable-number of tandem repeats loci in the ECOR collection and in a set of pathogenic *Escherichia coli* and *Shigella* isolates for use in a genotyping assay. *J Microbiol Methods* 2007; 69(1): 197–205.
42. Dolatyar Dehkharghani A, Haghighat S, Rahnamaye Farzami M, Rahbar M, Douraghi M. Clonal relationship and resistance profiles among ESBL-producing *Escherichia coli* 2021; 11(499).
43. Samarasinghe S, Reid R, Al-Bayati M. The anti-virulence effect of cranberry active compound proanthocyanins (PACs) on expression of genes in the third-generation cephalosporin-resistant *Escherichia coli* CTX-M-15 associated with urinary tract infection. *Antimicrob Resist Infect Control* 2019; 8(1): 181.
44. Clermont O, Dhanji H, Upton M, Gibreel T, Fox A, Boyd D, et al. Rapid detection of the O25b-ST131 clone of *Escherichia coli* encompassing the CTX-M-15-producing strains. *J Antimicrob Chemother* 2009; 64(2): 274–7.

