

Distribution of ciprofloxacin-resistance genes among ST131 and non-ST131 clones of *Escherichia coli* isolates with ESBL phenotypes isolated from women with urinary tract infection

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ABSTRACT

Background and Objectives: *Escherichia coli* (*E. coli*) sequence type 131 (ST131) is associated with extended-spectrum beta-lactamase (ESBL) production and fluoroquinolone resistance. This study aimed to investigate the prevalence of ST131, ESBL, and plasmid-mediated quinolone resistance (PMQR) genes in the ciprofloxacin-resistant (CIP^R) and ESBL producers from women with UTI.

Materials and Methods: The CIP-resistant ESBL producing (CIP^R/ESBL⁺) *E. coli* isolates were screened for ST131-by specific PCR of *mdh* and *gyrB*. The ESBL and PMQR genes were screened by single PCR. The ST131 and non-ST131 isolates were selected to determine the mutations of *gyrA* and *parC* using PCR and sequencing, and also their genetic background by the Pasteur-MLST scheme.

Results: Overall, 55% (33/60) CIP^R/ESBL⁺ isolates were identified as ST131 (94% O25b-ST131). Resistance rate to ampicillin-sulbactam (70%), aztreonam (97%) and gentamicin (61%), the prevalence of *aac(6)-Ib-cr* (66%), *bla*_{CTX-M-15} (82%), the profile of *qnrS+aac(6)-Ib-cr* (30%), and the double mutation in the *parC* was significantly higher in ST131 than non-ST131 isolates. The coexistence of PMQR and ESBL genes was found in more than 50% of ST131 and non-ST131 isolates. ST131 isolates differentiated into PST43 and PST506.

Conclusion: Management of women with UTI caused by the CIP^R/ESBL⁺ isolates (ST131) co-harbored PMQR, ESBL, and chromosomal mutations, is important for their effective therapy.

Keywords: Urinary tract infections; Uropathogenic *Escherichia coli*; Fluoroquinolones; Beta-lactamase CTX-M-15; Multilocus sequence typing

INTRODUCTION

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Urinary tract infection (UTI) is one of the most common bacterial infections encountered in clinical practice after respiratory and gastrointestinal infections (1). Women are at increased risk for developing

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UTI because of their anatomy, shorter urethra and pregnancy (2). Antibiotic therapy of complicated UTI has become problematic because of the inappropriate use of antibiotics, the emergence and dissemination of antibiotic-resistant clones e.g. sequence type 131 (ST131) and subsequently reduced therapeutic choices for UTI (2, 3).

Escherichia coli ST131 is one of the most important causes of community- and hospital-acquired UTI. According to the Achtman- multilocus sequence typing (MLST) system, ST131 designated as globally multidrug-resistant (MDR) clone with high virulence potential, has emerged dramatically over the past decade (4). The majority of ST131 strains belonged to phylogenetic group B2 and serotype O25b:H4. ST131 exhibits multi-resistance to different antibiotics classes such as trimethoprim/sulfamethoxazole, aminoglycosides, 3rd-generation cephalosporins and fluoroquinolones (FQs). ST131 lineage is associated with extended-spectrum beta-lactamas (ESBL) production and main cause of the spread of CTX-M-15 ESBL gene variant. Prevalence of *E. coli* ST131 accounted for over 50% of ESBL-producing or FQ-resistant (FQ^R) isolates in Asia and the US (5, 6).

The FQs e. g. ciprofloxacin (CIP), a class of bactericidal/broad-spectrum antibiotics, are being used frequently as a potent and first-line drug in the treatment of UTI caused by *E. coli*. The major mechanism of FQ-resistance is chromosomal mutations in regions so-called quinolone resistance-determining regions (QRDR). Besides, plasmid-mediated quinolone resistance (PMQR) has been increasingly reported over the past decade worldwide. The PMQR is often mediated by quinolone resistance (Qnr) proteins and aminoglycoside acetyltransferase [FQ-modifying enzyme; *aac(6')-Ib-cr* variant] (7, 8). PMQR genes often have a strong association with the ESBL genes and are typically located on the plasmids contributing to the spread of MDR isolates. Therefore, the emergence and rapid global spread of ST131, and infections caused by ST131 co-harboring ESBLs and PMQR genes that reduced therapeutic choices for UTI become a major therapeutic problem (9).

The main purpose of the current study was to assess the coexistence of PMQR with ESBL genes, and chromosomal mutations in the CIP^R ESBL producing ST131 (CIP^R/ESBL⁺) isolates, and compared with non-ST131 isolates from women with UTIs. Also, the allele profiles of Pasteur-MLST (P-MLST) of selective isolates were determined to a comprehensive

understanding of the population structure of CIP^R/ESBL⁺ isolates.

MATERIALS AND METHODS

Place and duration of the study. The current study was carried out in the Microbiology Department at Pasteur Institute of Iran (Tehran) during 2017-2019. This study was approved by the Ethical Committee of the Pasteur Institute of Iran (Ethical code: IR.PII.REC.1396.20). Written informed consent was provided by all the patients.

***E. coli* isolates.** A total of 215 *E. coli* isolates were obtained from the urine of women with symptomatic UTIs at referral university hospitals in Tehran. The patients included in the study were in different age groups. The *E. coli* isolates were identified by using conventional bacteriology tests (10). All isolates was screened by ST131-specific PCR for *mdh* and *gyrB* genes (11) and an O25b-ST131-specific PCR for the *pabB* gene (12) using sets of specific primers (Table 1).

Phenotypic identification of CIP-resistant ESBL producers. The primary ESBL screening of the *E. coli* isolates was accomplished by the Clinical and Laboratory Standards Institute (CLSI) guidelines (20) through the Kirby-Bauer disc diffusion method using cefotaxime and ceftazidime (30 µg each). The ESBL confirmatory test was carried out using the combination disc test (CDT) by including cefotaxime/cefotaxime+clavulanic acid (CT/CTL) and ceftazidime/ceftazidime+clavulanic acid (TZ/TZL). *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as the ESBL- negative and -positive control strains, respectively. Also, antimicrobial susceptibility to other antimicrobial agents was tested for all the ESBL producers. The antimicrobial agents (BD BBL Sensi-Disc susceptibility discs, BD Biosciences, US) used were as follows: amoxicillin-clavulanic acid (20/10 µg), ampicillin-sulbactam (10/10 µg), piperacillin-tazobactam (100/10 µg), aztreonam (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), gentamicin (10 µg), amikacin (30 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), and nalidixic acid (30 µg), imipenem (10 µg), meropenem (10 µg), and erapenem (10 µg).

The ESBL producers associated with resistance to

Table 1. Characteristics of primers used in this study

Primer	Sequence (5' - 3')	Target gene	Product length (bp)	Annealing temp (°C)	Reference
Mdh36	F: GTTTAACGTTAACGCCGGT R: GGTAACACCAGAGTGACCA	<i>mdh</i>	270	65	(11)
GyrB	F:CGCGATAAGCGCGAC R: ACCGTCTTTTTTCGGTGGAA	<i>gyrB</i>	132	65	(11)
O25pabBspe	F: TCCAGCAGGTGCTGGATCGT R: GCGAAATTTTTTCGCCGTACTGT	<i>PabB</i>	347	63	(12)
CTX-M-15	F: CACACGTGGAATTTAGGGACT R: GCCGTCTAAGGCGATAAACA	<i>bla</i> _{CTX-M-15}	995	55	(13)
TEM	F: ATGAGTATTCAACATTTCCGTG R: TTACCAATGCTTAATCAGTGAG	<i>bla</i> _{TEM}	861	56	(14)
SHV	F: TTATCTCCCTGTTAGCCACC R: GATTTGCTGATTTTCGCTCGG	<i>bla</i> _{SHV}	795	57	(15)
QnrB	F: GGMATHGAAATTCGCCACTG R: TTTGCGYGYCGCCAGTCGAA	<i>qnrB</i>	264	57	(16)
QnrS	F: GCAAGTTCATTGAACAGGCT R: TCTAAACCGTCGAGTTCGGCG	<i>qnrS</i>	428	57	(16)
QnrA	F: AGAGGATTTCTCACGCCAGG R: GCAGCACTATKACTCCCAAGG	<i>qnrA</i>	619	57	(17)
AAC	F: ATGACTGAGCATGACCTTGC R: TTAGGCATCACTGCGTGTTG	<i>aac(6)-Ib-cr</i>	519	55	(18)
ParC	F: TCGTTGCCGTTTATTGG R: GCAGGTTATGCGGTGGAAT	<i>parC</i>	470	56	(19)
GyrA	F: GCGATGTCGGTCATTGTT R: ACTTCCGTCAGGTTGTGC	<i>gyrA</i>	490	56	(19)

bp, base pair; F, forward; R, reverse

CIP were defined as CIP^R/ESBL⁺ isolates. The minimum inhibitory concentration (MIC) of CIP was determined using the Epsilon test (E-test) (20). In the end, CIP^R/ESBL⁺ ST131 and CIP^R/ESBL⁺ non-ST131 isolates were further investigated in molecular studies.

Molecular detection of ESBLs. Genomic DNA of CIP^R/ESBL⁺ *E. coli* isolates was extracted by the commercially available DNA extraction kit (Bioneer Company, Korea) following the manufacturer's protocol and stored at -20°C until use. Isolates were screened for ESBL genes (*bla*_{CTX-M-15}, *bla*_{TEM} and *bla*_{SHV}) by single PCR assays using primers specific as described previously (21). Data of the primers used in this study are shown in Table 1.

Detection of PMQR genes. Detection of PMQR genes (*qnrA*, *qnrB*, *qnrS*, and *aac(6)-Ib-cr*) was performed by single PCR using primers described previ-

ously (16-18). PCR was performed in 25 µl reaction volume containing 12.5 µl of Taq DNA polymerase 2× Master Mix RED at 1.5 mM of MgCl₂ final concentration (Co. Amplicon, Denmark), 10 ng of extracted DNA, 0.6 µl of each primer (10 pmol), and distilled water to obtain a 25-µl total volume. PCR reactions were carried out at the optimized annealing temperatures for each primer pair, as shown in Table 1.

Detection of QRDR mutations in *gyrA* and *parC* genes. Among the CIP^R/ESBL⁺ *E. coli* isolates identified, 15 ST131 and 15 non-ST131 isolates were randomly selected to determine chromosomal mutations. For this purpose, the *gyrA* gene (encoding subunit A of DNA gyrase; primary FQ target), and the *parC* gene (encoding subunit A of topoisomerase; secondary FQ target) were amplified by PCR (19). Amplicons were sequenced using an ABI 3730XL DNA analyzer apparatus (Macrogen Inc., Korea). Nucleotide sequences in the *gyrA* and *parC* genes were com-

pared with *E. coli* K-12 MG1655 genome sequences (reference sequences) using BLAST tools of the National Center for Biotechnology Information; (NCBI; <http://www.ncbi.nlm.nih.gov/blast>).

P-MLST. Initially, 25 (12 ST131 and 13 non-ST131) CIP^R/ESBL⁺ *E. coli* isolates exhibiting chromosomal mutation were selected for MLST typing to determine their Pasteur sequence type (PST). Typing was performed using eight housekeeping genes, including *dinB*, *icdA*, *pabB*, *polB*, *putP*, *trpA*, *trpB*, and *uidA*. Then their allelic profiles were characterized by comparing with the corresponding allele available in P-MLST database (https://bigsdatabase.pasteur.fr/cgi-bin/bigsdatabase/bigsdatabase.pl?db=pubmlst_ecoli_seqdef) to recognize the PST of each isolate.

Statistics. All data analyses were performed using SPSS software (version 19.0). The prevalence of antibiotic resistance (resistance genes) were compared between the ST131 and non-ST131 isolates by Pearson chi-square test and Fisher exact test. In all experiments, the threshold for statistical significance was $p\text{-value} \leq 0.05$.

RESULTS

Characteristic of patients, ESBL producers, and the prevalence of ST131 clone. Of the 215 *E. coli* isolates, 94 (44%) were confirmed as ESBL producer. The confirmed ESBL⁺ isolates showed high rates of resistance to aztreonam ($n = 78$, 82.9%) and trimethoprim-sulfamethoxazole ($n = 74$, 78.7%), followed by amoxicillin-clavulanic acid ($n = 58$, 61.7%), ampicillin-sulbactam ($n = 44$, 46.8%), gentamicin ($n = 32$, 34%), piperacillin-tazobactam ($n = 24$, 25.5%), nitrofurantoin ($n = 13$, 13.8%), and amikacin ($n = 11$, 11.7%). All the ESBL⁺ isolates were susceptible to carbapenems, and resistant to both ciprofloxacin and nalidixic acid. All CIP^R/ESBL⁺ isolates exhibited high-level CIP resistance ($\text{MIC} \geq 32 \mu\text{g/mL}$). Among 94 ESBL⁺ isolates screened, 52 (55%) isolates were detected as the ST131 and the remaining 42 (45%) isolates as non-ST131. Meanwhile, 50 out of the 52 ST131 *E. coli* isolates were identified as the O25b-ST131 clone and two isolates as the non-O25b-ST131 clone.

All patients ranged in age from 1 to 85 years (Table 2), and were allocated to group 1–15 years ($n =$

39), and group 16–45 years ($n = 14$), and group 46–85 years ($n = 26$). Both the ST131 and the non-ST131 isolates were mostly detected in patients under 1–15 years old (40% vs. 43%) and 46–85 years old (46% vs. 40%). Among inpatients (hospital patients), the ST131 isolates were significantly associated with the age range 46–85 years compared to the non-ST131 (79% vs. 48%; $p = 0.027$). No significant difference in resistance patterns was observed among different age groups between ST131 and non-ST131.

Antimicrobial resistance in *E. coli* ST131 and non-ST131 isolates. CIP resistance among ESBL producers was exhibited in 63.4% ($n = 33/52$) ST131 isolates and 64.2% ($n = 27/42$) non-ST131 isolates (CIP^R/ESBL⁺). A significant difference was detected in the prevalence of resistance to aztreonam ($p = 0.021$), ampicillin-sulbactam ($p = 0.048$), and gentamicin ($p = 0.017$) in the CIP^R/ESBL⁺ ST131 comparing the CIP^R/ESBL⁺ non-ST131 isolates (Table 3).

There was a significant correlation between CIP^R/ESBL⁺ ST131 isolates and resistance to sulbactam, piperacillin-tazobactam and gentamicin in compare with CIP^S/ESBL⁺ isolates (Table 3). Similar finding was detected only in gentamicin resistance among CIP^R/ESBL⁺ non-ST131 isolates.

Data analysis of the QRDR sequence in the *gyrA* and *parC* genes. Thirty CIP^R/ESBL⁺ isolates (15 ST131 and 15 non-ST131) were randomly analyzed by PCR and direct sequencing of QRDR of *gyrA* and *parC*. Substitutions of both serine 83 to leucine (S83L) and aspartic acid 87 to asparagine (D87N) in *gyrA*, as well as serine 80 to isoleucine (S80I) and glutamic acid 84 to valine (E84V) in *parC* were the mutations found in the isolates. The present study also detected that 23.3% ($n = 7$) of the isolates as carrying S83L or D87N alone and 76.7% ($n = 23$) of the isolates co-harboring S83L and D87N substitutions. Besides, 26.7% ($n = 8$) isolates carried S80I or E84V alone and 63.3% ($n = 19$) isolates co-harbored S80I and E84V substitutions. There was a significant difference in the double mutations (S80I+ E84V) between ST131 (80%) and non-ST131 (47%) isolates.

Characterization of ESBL and PMQR genes in the CIP^R/ESBL⁺ producers. Among 60 CIP^R/ESBL⁺ isolates, 31 (51.6%) and eight (13.4%) contained only the *bla*_{CTX-M-15} or *bla*_{TEM} respectively, and only 10 (16.6%) of isolates co-harbored the *bla*_{CTX-M-15}

Table 2. Characteristics of patients infected with *E. coli* ST131 and non-ST131 isolates

Isolates	Service type	Range of age groups (Years)		
		No. of isolates (%)		
		1-15	16-45	46-85
ST131	Inpatient, n=24	3 (12)	2 (8)	19 (79)
	Outpatient, n=28	18 (64)	5 (18)	5 (18)
Non-ST131	Inpatient, n=21	6 (28)	5 (24)	10 (48)
	Outpatient, n=21	12 (57)	2 (9)	7 (33)
<i>P</i> -v of In/out-patient	(ST131 vs. non- ST131)			
Inpatient		0.179	0.153	0.027
Outpatient		0.612	0.409	0.213

Table 3. Resistance to antimicrobial agents in the ESBL producers

Antimicrobial agents	No. (%) of isolates				p- value of			
	CIP ^R		CIP ^S		CIP ^R (ST131 vs. non-ST131)	CIP ^S (ST131 vs. non-ST131)	AS (CIP ^R non-ST131 vs. CIP ^S non-ST131)	AS (CIP ^R ST131 vs. CIP ^S ST131)
	ST131 (n= 33)	non- ST131 (n= 27)	ST131 (n= 15)	non-ST131 (n= 19)				
AUG	24 (73)	14 (52)	8 (53)	12 (63)	0.095	0.728	0.446	0.186
SAM	23 (70)	12 (44)	2 (13)	7 (37)	0.048	0.123	0.606	< 0.0001
PTZ	11 (33)	8 (29)	1 (7)	4 (21)	0.759	0.240	0.514	0.048
ATM	32 (97)	21 (77)	13 (87)	13 (68)	0.047	0.312	0.477	0.172
SXT	26 (79)	22 (81)	11 (73)	15 (79)	0.795	0.732	0.831	0.677
GM	20 (61)	8 (30)	3 (20)	1 (5)	0.017	0.185	0.040	0.009
AN	5 (15)	3 (11)	1 (7)	2 (10)	0.647	0.694	0.950	0.410
IN	3 (9)	5 (18)	3 (20)	2 (10)	0.265	0.439	0.457	0.289

AS, Antimicrobial susceptibility; AUG, amoxicillin-clavulanic acid; SAM, ampicillin-sulbactam; PTZ, piperacillin-tazobactam; ATM, aztreonam; SXT, trimethoprim- sulfamethoxazole; GM, gentamicin; AN, amikacin; IN, nitrofurantoin; CIP^R, fluoroquinolone resistant; CIP^S, fluoroquinolone sensitive. The CIP^R isolates studied were defined as resistant isolates to ciprofloxacin. The ESBL positive isolates studied were confirmed by the combined disk test (CDT) as ESBL producers. All the isolates were susceptible to carbapenems and resistant to cefotaxime and nalidixic acid.

A p-value ≤ 0.05 was considered statistically significant.

and *bla*_{TEM} genes. The ESBL genes were detected in 93.3% (n=31/33) and 66.6% (n=18/27) of ST131 and non-ST131 isolates, respectively. The prevalence of the *bla*_{CTX-M-15} was significantly higher in ST131 isolates than non-ST131 isolates (n= 27/33; 82% vs. n= 14/27; 52%, p= 0.013).

Three genes, i.e. *qnrS*, *qnrB*, and *aac(6')-Ib-cr*, detected in CIP^R/ESBL⁺ producers. The *aac(6')-Ib-cr* (n=33, 55%) and *qnrS* (n= 32, 53.3%) were the most predominant genes in all the isolates, followed by *qnrB* (n= 17, 28.3%). In comparative analysis, 78.7% (26/33) ST131 and 88.8% (24/27) non-ST131 isolates possessed at least one PMQR gene (p= 0.971). The

prevalence of PMQR genes in the ST131 vs. non-ST131 were as follows: *qnrS* (n= 16, 48.4% vs. n= 16, 59.2%; p= 0.895), *qnrB* (n= 7, 21.2% vs. n= 11, 40.7%; p= 0.101), and *aac(6')-Ib-cr* (n= 22, 66.6% vs. n= 11, 40.7%; p= 0.045). Our results indicated a significant association of *qnrS+aac(6')-Ib-cr* combination in ST131. The dominant PMQR genes in ST131 and non-ST131 isolates were *aac(6')-Ib-cr* (66.6%) and *qnrS* (59.2%), respectively. Profiles of resistance genes are illustrated in (Table 4). The *qnrA* and *bla*^{SHV} were not found in ST131 and non-ST131 isolates.

Coexistence of ESBL and PMQR genes among

Table 4. Profiles of resistance genes in the CIP^R/ESBL⁺ isolates

Resistance genes	No. (%) of CIP ^R / ESBL ⁺ isolates		p-value (ST131 vs. non-ST131)	
	ST131 (n= 33)	non- ST131 (n= 27)		
ESBL genes				
<i>bla</i> _{CTX-M-15}	20 (61)	11 (44)	0.126	
<i>bla</i> _{TEM}	4 (11)	4 (17)	0.760	
<i>bla</i> _{CTX-M-15} + <i>bla</i> _{TEM}	7 (19)	3 (13)	0.296	
PMQR genes				
<i>qnrS</i>	2 (6)	7 (26)	0.032	
<i>qnrB</i>	-	2 (7)	0.460	
<i>aac(6')-Ib-cr</i>	8 (21)	4 (11)	0.364	
<i>qnrS</i> + <i>qnrB</i>	2 (6)	3 (11)	0.481	
<i>qnrS</i> + <i>aac(6')-Ib-cr</i>	9 (30)	2 (7)	0.048	
<i>qnrB</i> + <i>aac(6')-Ib-cr</i>	2 (3)	2 (7)	0.835	
<i>qnrS</i> + <i>qnrB</i> + <i>aac(6')-Ib-cr</i>	3 (12)	4 (15)	0.492	
Co-existence of ESBL & PMQR genes				
<i>bla</i> _{CTX-M-15} + <i>qnrS</i>	2 (5)	3 (11)	0.201	
<i>bla</i> _{CTX-M-15} + <i>qnrS</i> + <i>qnrB</i>	-	1 (5)	0.270	
<i>bla</i> _{CTX-M-15} + <i>aac(6')-Ib-cr</i>	5 (11)	1 (9)	0.141	
<i>bla</i> _{CTX-M-15} + <i>qnrS</i> + <i>aac(6')-Ib-cr</i>	6 (24)	3 (5)	0.445	
<i>bla</i> _{CTX-M-15} + <i>qnrB</i> + <i>aac(6')-Ib-cr</i>	1 (3)	-	-	
<i>bla</i> _{CTX-M-15} + <i>qnrS</i> + <i>qnrB</i> + <i>aac(6')-Ib-cr</i>	2 (3)	3 (11)	0.481	
<i>bla</i> _{TEM} + <i>qnrS</i>	-	2 (7)	0.427	
<i>bla</i> _{CTX-M-15} + <i>bla</i> _{TEM} + <i>qnrS</i> + <i>qnrB</i> + <i>aac(6')-Ib-cr</i>	2 (6)	-	0.193	
<i>bla</i> _{TEM} + <i>qnrS</i> + <i>qnrB</i>	-	1 (4)	0.270	
<i>bla</i> _{CTX-M-15} + <i>bla</i> _{TEM} + <i>aac(6')-Ib-cr</i>	3 (11)	2 (9)	0.257	
QRDR mutations*				
<i>gyrA</i>	S83L	2 (13)	3 (20)	0.624
	D87N	-	2 (13)	0.560
	S83L+ D87N	13 (87)	10 (66)	0.099
<i>parC</i>	S80I	1 (6.5)	1 (6.5)	1.000
	E84V	2 (13)	4 (27)	0.361
	S80I+ E84V	12 (80)	7 (40)	0.058

CIP^R/ESBL⁺, ciprofloxacin-resistant/ESBL producer. The *bla*_{SHV} and *qnrA* genes were not found in any of the isolates studied. A p-value ≤ 0.05 was considered statistically significant.

* Frequency of QRDR mutations was calculated for 15 isolates in each ST131 and non-ST131.

the CIP^R/ESBL⁺ isolates. The coexistence of ESBL and PMQR genes was detected in 37 (61.6%) out of 60 isolates. No significant difference was observed in the coexistences of ESBL and PMQR genes between ST131 and non-ST131 isolates (n= 21, 62% vs. n= 16, 48%; p= 0.729; Table 4).

PSTs among the CIP^R/ESBL⁺ isolates. PST of 12 CIP^R/ESBL⁺ ST131 and 13 CIP^R/ESBL⁺ non-ST131 isolates were obtained by the MLST technique. The O25b-ST131 and non-O25b-ST131 isolates showed

PST43 (n=10) and PST506 (n=2), respectively. Thirteen non-ST131 isolates were classified into 10 different PST, that the most frequent PST was PST53 (n=4, 30.7%), followed by PST355, PST477, PST945, PST446, PST8, PST731, PST487, PST86, and PST490 (n=1, 7.6% for each).

DISCUSSION

The FQ resistance in *E. coli* ST131 has spread

worldwide, particularly in developing countries and has become an increasing public health concern in clinical management (22). This study aimed to assess the prevalence of resistance genes in CIP^R/ESBL⁺ *E. coli* isolates (ST131 and non-ST131) in women with UTIs. In this study, nearly half of the ESBL producers belonged to the ST131. The majority of these ST131 isolates belonged to the O25b-ST131, which is in agreement with other investigations (23). In current study, the ST131 were significantly detected in the hospitalized patients in the age range 46-85 years (Table 2). Elderly patients who are hospitalized are more prone to infection with ST131, because of diminished immune response, recurrent and long-duration hospitalizations, urinary catheter use, exposure to stronger drug and underlying diseases (4, 24). Therefore, screening of ST131 particularly in older hospitalized patients is essential.

The CIP^R ST131 isolates accounted 68.7% of ESBL producers. In a study from Canada, 44% of all CIP^R *E. coli* isolates were ST131 (11). In the current study, the *E. coli* CIP^R/ESBL⁺ isolates, including ST131 and non-ST131 were shown the highest sensitivity to amikacin 87% (n= 52/60). In a study in Iran, the high sensitivity rates to fosfomycin followed by amikacin and nitrofurantoin were detected in the ESBL producers (25). It is worth noting that the use of older antibiotics such as fosfomycin can be a suitable treatment option for infections caused by MDR and ESBL producers.

With the analysis of PMQR genes, the most frequent genes were *aac(6')-Ib-cr* and *qnrS* in ST131 and non-ST131 isolates, respectively. Several studies in Iran and other Asian countries showed varied frequencies for PMQR genes (26, 27). This difference could be the lack of focus on ST131 in their studies. Another explanation can be the difference in their geographical area or sample source. The relatively high prevalence of PMQR genes in our CIP^R/ESBL⁺ isolates (with MIC₅₀ ≥ 32 µg/mL), especially in ST131, is alarming, as these genes may facilitate the selection of chromosomal mutations in QRDR and subsequently lead to high-level Qnr. Accordingly, the significant association of the *aac(6')-Ib-cr* with the ST131 isolates in our research is likely due to the higher rate of ST131 resistance to gentamicin. The *aac(6')-Ib-cr* encodes a bifunctional enzyme catalyzing the FQs and aminoglycosides (28).

According to the study conducted by Nicolas-Chanoine et al. (2014), *aac(6')-Ib-cr* gene is car-

ried along with other resistance genes (*bla*_{CTX-M-15}, *bla*_{OXA-1}, *bla*_{TEM-1}, *mphA*, and *tetA*) on the plasmids, conferring resistance to aminoglycosides, ciprofloxacin, macrolides, chloramphenicol, and tetracycline. Similarly, we found *aac(6')-Ib-cr* -carrying isolates were associated with *bla*_{CTX-M-15} gene (4). The coexistence of PMQR and ESBL genes was displayed in all CIP^R/ESBL⁺ isolate, which is in line with previous studies (4, 28, 29). The association of these genes may be potential horizontal transfer of antibiotic resistance agents among isolates (9). Therefore, a complete understanding of resistance mechanisms can be helpful for treatment and infection control.

The sequence analysis of QRDR indicates that mutations occur in 2 positions at S83 in *gyrA* and, and E84 in *parC* which are consistent with other reports (30, 31). The mutations in positions S83 and E84 are likely suggestive of the higher levels of CIP-resistance. A higher rate of double mutations (S80I+E84V) was significantly observed in ST131 than non-ST131 isolates. Studies have revealed that the point mutations led to decreased susceptibility of *E. coli* to FQs, whereas the double mutations elevate high-level resistance to FQ (30, 32). A study in the Netherlands showed the replacement of isoleucine 529 to leucine (I529L) in *parE* in ST131 isolates (32). Therefore, future investigation of other chromosomal-mediated resistance agents will complete the understanding of the MDR clones, especially ST131.

In the present study, PST506 was accounted for a small subset (3.8%) among CIP^R/ESBL⁺ ST131 isolates by P-MLST analysis. PST506 belongs to the O16-ST131 clade that this clade accounts for one to five percent of *E. coli* isolates. According to studies, most O16-ST131 isolates are susceptible to FQs and extended-spectrum cephalosporins (33). However, our PST506-ST131 isolates (n= 2) were CIP^R/ESBL⁺ and contained the *aac(6')-Ib-cr* and *bla*_{CTX-M-15} genes but lacking *bla*_{TEM} and *qnr* genes.

Our finding revealed that PST53 was the most common PST (26.6%) in the non-ST131 isolates. In a recent study by Kim et al. (2017), PST53, PST8, PST2, PST594, PST13, PST39, PST44, PST53, PST253, PST478, and PST666 were found in community-associated bacteremia isolates (34). All PST53 isolates were associated with the variants of *aac(6')-Ib-cr* and *bla*_{CTX-M-15} genes and showed at least three chromosomal mutations. Furthermore, PST53 could play an important role in the distribution of antibiotic resistance in women with UTI.

Distribution of CIP^R/ESBL⁺ isolates co-harboring various antimicrobial resistance determinants can lead to enhanced MDR bacteria and subsequently limit the choice of empirical antimicrobial therapy. Due to the fact that women are more likely to suffer from UTIs and recurrences thus epidemiological studies and molecular screening of women with UTI for the presence of ESBL-producing and MDR *E. coli* clones are highly recommended. It is also of paramount importance to understand the resistance gene profiles of isolates to select the appropriate treatment for reducing resistance to therapeutic agents such as CIP and spreading such clones.

CONCLUSION

This study showed that the ST131 clone were accounted for more than half of the ESBL-producing isolates. The ESBL and CIP resistance genes were significantly higher in ST131 isolates compared to non-ST131 isolates. The majority of the CIP^R/ESBL⁺ isolates were exhibited the coexistence of PMQR genes, ESBL genes, and multiple mutations in QRDR that is a serious public health concern in older hospitalized patients. The combination of the multiple resistance genes in the ST131 may contribute to its successful spread and rapid worldwide dissemination. Therefore, continuous monitoring and surveillance requires to prevent the dissemination of resistant clones for effective treatment options.

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