The Molecular Characterization and Risk Factors of ST131 and Non-ST131 *Escherichia coli* in Healthy Fecal Carriers in Tehran, Iran

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Abstract

Background: Commensal extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* isolates in the gut can be the reservoir of virulence factors and resistance genes.

Objectives: We investigated the molecular feature, risk factors, and quinolone/fluoroquinolone (Q/FQ) resistance in sequence type 131 (ST131) and non-ST131: ESBL-producing *E. coli* (EPE) isolates in healthy fecal carriers.

Methods: A total of 540 fecal samples and its demographic data were collected from healthy adults in Tehran in 2018. ST131 isolates were identified by multilocus sequence typing (MLST) analysis, and the characteristics of the virulence factor, phylogenic assay, and Q/FQ resistance genes in ST131 and non-ST131 were determined by polymerase chain reaction (PCR).

Results: The EPE isolates mainly belonged to the commensal phylogenetic groups A (54.9%) and D (18.1%). The type 1 fimbriae (*fimH*; 89.6%) gene was the predominant virulence factor, and there was a significant correlation between ferric yersiniabactin uptake (*fyuA*; 52.9%), aerobactin receptor (*iutA*; 17.6%), and group II capsule synthesis (*kpsMII*; 35.3%) with ST131. In Q/FQ-resistant isolates, *qnrS* (19%) was the predominant gene, and mutations mostly occurred at codon S83 in *GyrA* The number of mutations in *gyrA* and *parC* genes was significantly higher in ST131 isolates than in non-ST131 isolates. There was a significant positive correlation between diabetes, male gender, and living in the south of the city with EPE carriage (P < 0.05).

Conclusions: Accumulation of multiple virulence factors and high- level resistance to Q/FQ in some phylogroups (B2 and D), particularly ST131 isolates, require to be considered in detecting resistant isolates in healthy carriers. According to the risk factor for spreading of EPE isolates (diabetes, living in low-income parts of the city, and male gender), the necessary strategies are required to be developed to control the dissemination of antimicrobial-resistant isolates in the community.

Keywords: Beta-Lactamase, Healthy People, Asymptomatic Carrier State, Phylogeny, Virulence Factors

1. Background

Escherichia coli, a member of *Enterobacteriaceae* and a prevalent gut normal microflora in the human body, causes intestinal and extraintestinal infections (ie, intestinal pathogenic *E. coli* [IPEC] and extraintestinal pathogenic *E. coli* [ExPEC]) (1). The isolates of *E. coli* are classified into distinct pathotypes mainly characterized by their specific virulence determinants, disease clinical symptoms, and phylogenetic background (2). Regarding phylogenetic typing, *E. coli* strains are categorized into 7 phylogenetic groups (phylogroups) named A, B1, B2, C, D, E, and F, which are interdependent with virulence patterns (3).

Pathogenic *E. coli* strains often differ from the commensal ones in terms of their accessory genes, allowing them to colonize the host mucosa and helping them induce pathogenicity and change their pathogenic potential (4). The gut population of *E. coli* contains one or more clones with dissimilar loads, though with antibiotic exposure, the number of resistant isolates will increase (5). Due to the broad-spectrum antimicrobial action, quinolone/fluoroquinolone (Q/FQ) and β-lactams are the most frequently prescribed antibiotics for treating infections (6), causing resistance to Q/FQ and β-lactams in intestinal gram-negative bacteria worldwide (6). Moreover, the global rate of extended-spectrum β-lactamase (ESBL)- and Q/FQ-resistant *E. coli* isolates, such as sequence type 131 (ST131) clone, has escalated in virtue of antibiotic resistance, rapid spread, and virulence factors (7). Researchers have focused on clinical *E. coli* isolates, but ExPEC strains,

such as ST131 isolates, commonly inhabit the gut of healthy subjects ([8]). By identifying the asymptomatic carriers of resistant bacteria, understanding the genetic characteristics of the isolates and risk factors for their carriage can contribute to the inhibition of multidrug-resistant bacteria spread in the community.

2. Objectives

We investigated the genetic characteristics, the risk factors, and Q/FQ resistance of ST131 and non-ST131 *E. coli* isolates in healthy individuals.

3. Methods

3.1. Study Design and Enrollment of Participants

In this cross-sectional study, a total of 540 fecal samples were collected from February to July 2018 from healthy adults. Exclusion criteria were any active intestinal infection and a history of antibiotic treatment or hospitalization in the 3 months before specimen collection. Informed consent was obtained from all participants to use their data in this study. A questionnaire was used to collect sociodemographic information (eg, age, sex, antibiotic consumption). Fecal samples were collected from various areas of Tehran City. Therefore, the city was divided into 6 locations according to the classification of Iran's Ministry of Health and Medical Education. Locations 1 to 5 are in the north, south, east, west, and center, respectively, and location 6 is a suburb of Tehran. Based on the referrals (the number of people) to various health centers, 350/540 (70/center) stool samples were obtained from healthy adults who referred to all the locations (except location 2), and 190/540 isolates were from health centers in location 2.

3.2. Antibiotic Susceptibility Testing

For the isolation of ESBL-producing *E. coli* (EPE), we inoculated the stool samples on CHROMagar ESBL (CHROMagar, Paris, France) and identified red colonies by standard microbiologic techniques ([9]). Susceptibility to cefotaxime (CTX; 30 μg), ceftazidime (CAZ; 30 μg), nalidixic acid (NA; 30 μg), and ciprofloxacin (CIP; 5 μg; Mast Group Ltd, Merseyside, United Kingdom) was tested using the disc diffusion method (10). The EPE isolates were confirmed by the combination disk test ([10]). *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as control strains. Minimum inhibitory concentration (MIC) of CIP

was determined by the E-test method (bioMérieux, Marcy-l'Étoile, France) ([10]). The MIC breakpoints applied for susceptibility and resistance to CIP were ≤ 1 μg/mL and ≥ 4 μg/mL, respectively. All the above-mentioned tests (ie, disc diffusion method, combination disk test, and E-test) were performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guideline ([10]).

3.3. Genomic DNA Extraction

Bacterial isolates were harvested in Luria-Bertani broth (Merck, Germany) for genomic DNA extraction by the boiling method.

3.4. Molecular Detection of ST131 Isolates

The molecular identification of the ST131 clone was carried out by polymerase chain reaction (PCR) to detect ST131-specific single nucleotide polymorphisms in *mdh* and *gyrB* genes, which was confirmed by multilocus sequence typing (MLST) analysis ([8]).

3.5. Phylogroup Determination

Phylogroups of EPE isolates were determined by PCR according to a described method by Clermont ([3]). Isolates not matching any of the profiles were reported as a nontypable (N) phylogroup.

3.6. Molecular Detection of Genes Encoding Virulence Determinants

Detection of genes encoding virulence determinants was associated with ExPEC and identified by PCR. The isolates were screened for 9 virulence genes, including type 1 fimbriae (*fimH*), P fimbriae (*pap*), S fimbriae (*sfa*), afimbrial adhesins (*afa*), aerobactin receptor (*iutA*), ferric yersiniabactin uptake (*fyuA*), hemolysin (*hlyA*), cytotoxic necrotizing factor 1 (*cnf1*), and group II capsule synthesis (*kpsMII*) ([11]). The isolates were designated as ExPEC if positive for $\geq$ 2 virulence genes ([12]). Gene scores were determined as the total number of virulence genes per strain.

3.7. Characterization of Plasmid-Mediated Quinolone Resistance Genes

The plasmid-mediated quinolone resistance (PMQR) in NA/CIP non-susceptible isolates was extracted using a plasmid kit (GeneAll Biotechnology, Seoul, Korea). All the NA/CIP non-susceptible isolates were checked for the presence of *qnrA*, *qnrB*, *qnrS*, *qepA*, and *aac(6')-Ib-cr* as PMQR genes by previously described PCR methods ([13]).

3.8. Detection of Mutation in Quinolone-Resistant Determining Region

Mutations in the quinolone-resistant determining region (QRDR) in *gyrA* and *parC* genes were detected by PCR in selected NA-resistant isolates. Purified PCR products were sequenced, and the nucleotide sequences were analyzed using the BLAST program (blast.ncbi.nlm.nih.gov). The mutations were determined by comparing with the sequences of QRDR regions in *E. colik12*; GenBank accession numbers were AF052256 for *gyrA* and D88981 for *parC* by MEGA 4 software (14).

3.9. Statistical Analysis

Categorical variables were compared and tested using the chi-square and Fisher exact tests to assess the correlation between the ExPEC status, phylogroups, and the presence of antimicrobial resistance genes. All tests were performed using R version 3.3.3. The Mann-Whitney U test was used to assess the correlation of the virulence score with phylogroups and the presence of antimicrobial resistance genes. P-values $\leq$ 0.05 were considered statistically significant.

4. Results

Of the 540 fecal samples included in this study, 43.1% (n = 233/540) of EPE isolates were detected. Additionally, 7.2% (n = 17/233) of EPE isolates were identified as the ST131 clone by MLST. The prevalence rates of CAZ- and CTX-resistant isolates were 90% (n = 210/233) and 98% (n = 228/233), respectively. In addition, 52% (n = 121/233) and 28% (n = 65/233) were found to be non-susceptible to NA and CIP, respectively, and all the CIP-resistant isolates were also resistant to NA. The MIC of CIP for *E. coli* isolates ranged from 0.01 to 32 μg/mL. Based on the antibiogram and MIC of the CIP results, the NA non-susceptible isolates were assigned to 4 groups: (1) NA-resistant isolates (MIC of CIP $\leq$ 1 μg/mL, n = 56/233, 24.1%), (2) NA/CIP intermediate susceptibility isolates (MIC of CIP = 2 μg/mL, n = 13/233, 5.5%), (3) NA/CIP-resistant isolates (MIC of CIP = 4-16 μg/mL, n = 29/233, 12.4%), and (4) NA/CIP-resistant isolates (MIC of CIP $\geq$ 32 μg /mL, n = 23/233, 9.8%).

4.1. Risk Factors of EPE Carriage

The demographic information about samples is shown in Table 1. According to the acquired information, 49.6% (n = 268/540) of the participants were male, and 50.4% (n = 272/540) were female. Additionally, 55.4% (n = 129/233) of EPE carriers were male, and 44.6% (n = 104/233) were female

(P = 0.02). EPE fecal carriers varied across municipalities, and their prevalence rates were 11.7% (n = 20) in location 1, 21.1% (n = 98) in location 2, 18.1% (n = 31) in location 3, 14.6% (n = 25) in location 4, 17.0% (n = 29) in location 5, and 17.5% (n = 30) in location 6, with a P-value of 0.02. In addition, intestinal EPE colonization was associated with underlying diseases (P = 0.03). Other putative risk factors, such as urinary tract infection (UTI) within 6 months (P = 0.08), daily animal contacts (P = 0.5), using boiling water (P = 0.8), food intake (P = 0.07), health care occupation (P = 0.1), and traveling abroad (P = 0.1), were not associated with intestinal EPE colonization.

4.2. Phylogroups Distribution of the EPE Isolates

The majority of the 233 EPE isolates belonged to phylogenetic groups A (54.9%, n = 128/233) and D (18%, n = 42/233), followed by groups B2 (14.2%, n = 33/233), B1 (3.9%, n = 9/233), F (3.4%, n = 8/233), C (3%, n = 7/233), and E (0.4%, n = 1/233). The phylogroup of 5 isolates (2.2% n = 5/233) could not be classified into distinct groups. As represented in Table 2, the prevalence of phylogroups in each location is significantly different (P = 0.02), indicating that phylogroup A is predominant, followed by phylogroups D and B2.

4.3. Prevalence of Virulence Determinants of the EPE Isolates

Data on the prevalence of virulence determinants indicates that among EPE isolates, the most prevalent virulence genes are *fimH* (89.6%), *afa* (29.6%), *fyuA* (20.6%), and *kpsMII* (12.8%), whereas *sfa* (0%), *Cnf* (5.5%), *iutA/hlyA* (6.4%), and *papA* (9%) are the least prevalent genes.

4.4. Distribution of Virulence Genes in Different Phylogroups in EPE Isolates

The distribution of virulence genes in EPE isolates with different phylogenetic profiles is summarized in Table 3. The prevalence of ExPEC was 18.5% (n = 43/233) in EPE isolates, with a significant difference in each phylogroup (P = 0.007). Most of the isolates with virulence scores > 2, defined as ExPEC, belonged to phylogroups D (35.7%, n = 15/42) and B2 (30.3%, n = 10/33). The number of virulence factors detected in a single strain varied from 0 to 5, and their average number was 1.8. The virulence score differed significantly in each phylogroup (P = 0.03). Phylogroups D and B2 showed higher virulence scores than the isolates belonging to groups A, B1, C, and N. There was also a significant correlation between different phylogroups with *kpsMII* (P = 0.02) and *fyuA* (P = 0.02) virulence genes.

Table 1. Demographic and Epidemiological Characteristics of Healthy Individuals and Their Association with ESBL-Producing *Escherichia coli*[a]

Characteristics	ESBL (N = 233)	Non-ESBL (N = 307)	P-Value
Gender			0.02
Male	129 (55.4)	139 (45.4)	
Female	104 (44.6)	168 (54.7)	
Travel abroad [b]			0.1
Positive	79 (34.3)	85 (28.5)	
Negative	151 (65.7)	213 (71.5)	
Missing data	3 (1.2)	9 (2.9)	
Food intake [c]			0.07
Leafy greens	4 (1.7)	2 (0.6)	
Chicken	63 (27.1)	78 (25.4)	
Beef	70 (30.1)	96 (31.2)	
Chicken/beef	81 (34.7)	120 (39.1)	
Missing data	15 (6.4)	11 (3.5)	
Health care occupation			0.1
Positive	24 (10.3)	28 (9.1)	
Negative	203 (87.1)	272 (88.5)	
Missing data	6 (2.5)	7 (2.2)	
Boiled water use			0.8
Positive	67 (29.0)	89 (28.9)	
Negative	164 (71.0)	211 (68.7)	
Missing data	2 (0.8)	7 (2.2)	
Underlying diseases			
Positive	24 (10.4)	14 (5.2)	0.03
Diabetes	11 (45.8)	4 (28.5)	0.04
Cancer	4 (16.6)	3 (21.4)	0.1
Liver disease	3 (12.5)	1 (7.1)	0.6
Heart disease	1 (4.1)	2 (14.2)	0.5
Other	5 (20.8)	4 (28.5)	-
Negative	206 (89.6)	280 (94.8)	
Missing data	3 (1.2)	13 (4.2)	
UTI [b]			0.8
Positive	22 (9.4)	27 (8.7)	
Negative	198 (84.9)	265 (86.3)	
Missing data	13 (5.5)	15 (4.8)	
Daily animal contact [d]			0.5
Positive	80 (35.9)	99 (32.2)	
Negative	143 (64.1)	190 (61.8)	
Missing data	10 (4.2)	18 (5.8)	
Tehran municipality			0.02
1	20 (11.6)	49 (19.9)	
2	98 (21.1)	92 (13.6)	
3	31 (18.1)	39 (15.6)	
4	25 (14.6)	46 (18.2)	
5	29 (16.9)	41 (16.4)	
6	30 (17.5)	40 (16.1)	

[a] Values are expressed as No. (%). A P-value $\leq$ 0.05 was considered statistically significant.
[b] In the past 3 months
[c] $\geq$ 3 meals per week
[d] Daily contact is defined as contact with pets/domestic animals on a daily basis.

Table 2. Phylogenetic Groups of 233 ESBL-Producing *Escherichia coli* Isolates in 6 Different Locations in Tehran in Healthy Volunteers [a]

Tehran Municipality	Phylogenetic Groups, No. (%)						
	A	**B2**	**C**	**D**	**E, F**	**B1**	**N**
	128 (54.9)	**33 (14.1)**	**7 (3)**	**42 (18)**	**9 (3.8)**	**9 (3.8)**	**5 (2.1)**
1	15 (75.0)	0	0	2 (10.0)	0	2 (10.0)	1 (5.0)
2	59 (60.2)	22 (22.4)	0	13 (13.3)	4	0	0
3	14 (45.2)	3 (9.7)	2 (6.5)	7 (22.6)	1 (3.2)	3 (9.7)	1 (3.2)
4	13 (52.0)	1 (4.0)	1 (4.0)	6 (24.0)	1 (4.0)	2 (8.0)	1 (4.0)
5	9 (31.0)	5 (17.2)	1 (3.4)	8 (27.6)	2 (6.9)	2 (6.9)	2 (6.9)
6	18 (60.0)	2 (6.7)	3 (10.0)	6 (20.0)	1 (3.3)	0	0
P-value				0.02			

[a] A P-value ≤ 0.05 was considered statistically significant.

Table 3. Phylogenetic Distribution of Virulence Genes Among Fecal *Escherichia coli* Isolates in Healthy Subjects

Virulence Gene	Phylogroups							
	A	**B2**	**B1**	**C**	**D**	**E, F**	**N**	**P-Value**
fimH	111 (86.7)	32 (96.9)	8 (88.9)	5 (71.4)	39 (92.9)	9 (100)	5 (100)	0.3
papA	13 (10.1)	3 (9.1)	0	0	4 (9.5)	1 (12.5)	0	0.9
afa	33 (25.8)	9 (27.2)	5 (55.5)	3 (42.8)	15 (35.7)	3 (37.5)	1 (20.0)	0.5
fyuA	23 (17.9)	15 (45.4)	2 (22.2)	1 (14.3)	5 (11.9)	1 (12.5)	1 (20.0)	0.02
iutA	7 (5.4)	3 (9.1)	1 (11.1)	2 (28.5)	2 (4.7)	0	0	0.3
hlyA	10 (4.6)	2 (6.1)	0	0	2 (4.7)	0	1 (20.0)	0.8
cnf1	6 (4.7)	2 (6.1)	1 (11.1)	0	2 (4.7)	0	2 (40.0)	0.07
kpsMII	10 (7.8)	10 (30.3)	0	1 (14.3)	8 (19.1)	1 (12.5)	0	0.02
Average virulence score	213 (1.6)	76 (2.3)	18 (2.0)	12 (1.7)	77 (1.8)	15 (1.7)	10 (2.0)	0.03
ExPEC	17 (13.2)	10 (30.3)	0	1 (14.2)	15 (35.7)	0	0	0.007

Abbreviations: N, non-typable; ExPEC, extraintestinal pathogenic *Escherichia coli*, defined operationally as the presence of > 2 of the virulence genes investigated.
[a] Data are presented as No. (%); A P-value ≤ 0.05 was considered statistically significant.

4.5. Characterization of PMQR Genes in NA/CIP Non-susceptible to EPE Isolates

The PMQR determinants were found in 51.2% (n = 62/121) of NA/CIP-resistant isolates. The most prevalent PMQR genes were *qnrS* (19%, n = 23/121) and *aac(6)-Ib-cr* genes (13.2%, n = 16/121), followed by *qnrB* (6.6%, n = 8/121), *qnrB* + *qnrS* (4.1%, n = 5/121), *qnrB* + *aac(6)-1b-cr* (2.5%, n = 3/121), and *qnrB* + *qnrS* + *aac(6)-1b-cr* (5.8%, n = 7/121). Neither *qnrA* nor *qepA* was detected (Figure 1). PMQR genes were carried by isolates with high-level resistance phenotype (60.8%, n = 14/23) and resistant phenotype (58.6%, n = 17/29) to CIP, those with intermediate resistance phenotype to NA/CIP (53.8%, n = 7/13) and CIP-susceptible isolates that were resistant to NA only (42.8%, n = 24/56). Detection of the specific PMQR gene was not correlated with the susceptibility pattern to NA/CIP antibiotics, as these genes were found in all groups. In addition, there was no correlation between the existence of PMQR genes and the increase in the MIC of CIP isolates (P = 0.07).

4.6. Mutations Analysis in gyrA and parC Genes in NA/CIP Non-susceptible EPE Isolates

Forty isolates selected randomly from different groups in NA non-susceptible isolates were screened for mutation in *gyrA* and *parC* genes. Mutation at codon S83 (S83L substitution) of *GyrA* was detected in 100% (n = 40/40) of isolates, while 20% (n = 8/40) had double amino acid substitutions at codon D87 (D87N substitution) and were high-level CIP resistance. In the *ParC*, 5% (n = 2/40) of CIP sensitive isolates had amino acid substitution S80I, 5% (n = 2/40) of re-
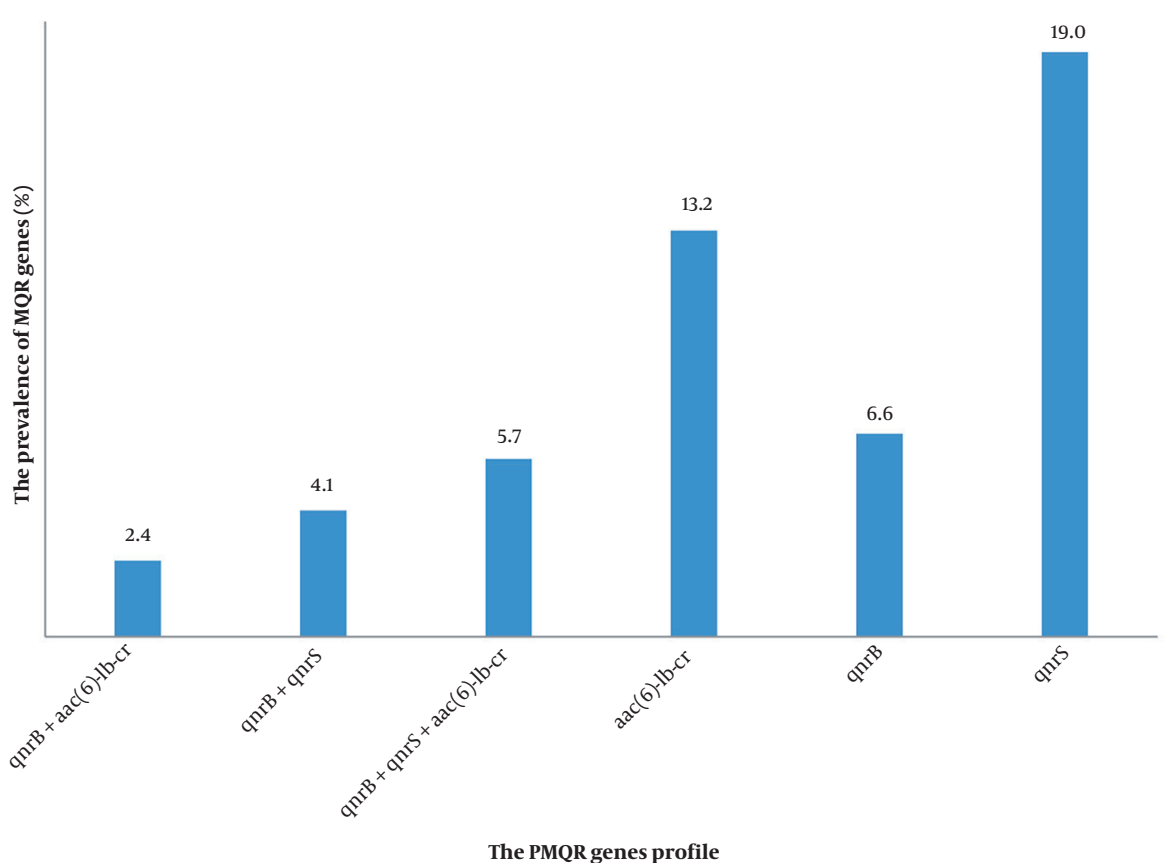
Figure 1. Prevalence of plasmid-mediated quinolone resistance gene profile in *Escherichia coli* isolates

sistant and intermediate isolates had amino acid substitution E84V, and 7.5% (n = 3/40) of CIP resistant isolates had amino acid substitution E84G. In NA/CIP intermediate isolates, 2.5% (n = 1/40) had double amino acid substitutions S80I + E84V, whereas 7.5% (n = 3/40) of high-level CIP resistance isolates had double amino acid substitutions S80I + E84G in *ParC*. There was a significant correlation between the number of mutations and an increase in MIC for CIP (P = 0.002).

4.7. Distribution of Risk Factors, Virulence Factors, and Molecular Characterization of Q/FQ Resistance in the ST131 Clone

The comparison of risk factors in ST131 carriers, as shown in Table 4, suggested no significant difference between ST131 and non-ST131 clone isolates. Concerning virulence factors, the distribution of *fyuA*, *iutA*, and *kpsMII* was significantly higher in ST131 compared to that of non-ST131 isolates (P = 0.001, P = 0.05, and P = 0.004, respectively). The virulence score in ST131 was 2.4, which was significantly

higher than non-ST131 isolates (P = 0.03). The rates of resistance to NA and CIP among ST131 isolates were 88.2% (n = 15/17) and 47.05% (n = 8/17), respectively. All ST131 isolates were screened for mutations in *gyrA* and *parC* genes. Mutations S83L + D87N (80%, n = 12/15) in *GyrA* and S80I + E84V (20%, n = 3/15) in *ParC* of the ST131 isolate were predominant (Table 5). The PMQR gene associated with ST131 isolates was *aac(6)-Ib-cr* (P = 0.001), and the prevalence rate of double subsituation in the *ParC* was significantly higher among ST131 clones than non-ST131 isolates (P = 0.002).

5. Discussion

Fecal carriage of EPE represents an important reservoir for person-to-person transmission, and the gut content with this bacterium has been recognized as a major source in the dissemination of antimicrobial-resistant isolates in the community (15). Most commensal *E. coli* isolates belonged to phylogroups A and B1, while isolates causing extraintestinal infections belonged to B2 and D phylogroups

Table 4. Risk Factor of the ST131 Fecal Carriage and Distribution of Virulence Factors [a]

Characteristics	ST131 (N = 17)	Non-ST131 (N = 216)	P-Value
Gender			0.2
Male	8 (47.05)	121 (56.01)	
Female	9 (52.9)	95 (43.9)	
Underlying diseases	2 (11.7)	22 (10.1)	0.6
Health care occupation	3 (17.6)	21 (9.7)	0.09
Boiled water use	6 (35.2)	61 (28.2)	0.8
Daily animal contact [b]	5 (29.4)	75 (34.7)	0.8
UTI [c]	2 (11.7)	20 (9.2)	0.2
Travel abroad [c]	7 (41.1)	72 (33.3)	
Food intake [d]			0.6
leafy greens	0	4 (1.8)	
Chicken	5 (29.4)	48 (22.2)	
Beef	2 (11.8)	52 (24.4)	
Chicken/beef	6 (35.3)	83 (38.4)	
Tehran municipality			0.6
1	1 (5.9)	19 (8.7)	
2	8 (47.05)	90 (41.6)	
3	1 (5.9)	30 (13.8)	
4	1 (5.9)	24 (11.6)	
5	2 (11.7)	27 (12.5)	
6	3 (17.6)	27 (12.5)	
Virulence factors			
fimH	16 (94.1)	193 (89.4)	0.5
papA	2 (11.8)	19 (8.8)	0.6
fyuA	9 (52.9)	18.1 (39)	0.001
afa	29.4 (5)	64 (29.6)	0.9
iutA	3 (17.6)	12 (5.6)	0.05
hlyA	1 (5.9)	14 (6.5)	0.9
cnf1	2 (11.7)	11 (5.1)	0.2
kpsMII	6 (35.3)	24 (11.1)	0.004
Average virulence score	41 (2.4)	379 (1.7)	0.03
ExPEC [e]	7 (41.2)	36 (16.7)	0.01

[a] Values are expressed as No. (%).
[b] Daily contact is defined as contact with pets/domestic animals on a daily basis.
[c] In the past 6 months
[d] $\geq$ 3 meals per week
[e] Extraintestinal pathogenic *Escherichia coli*, defined operationally as the presence of > 2 of the virulence genes investigated.

Table 5. The Prevalence Rate of Plasmid-Mediated Quinolone Resistance and Mutation in Quinolone-Resistant Determining Region in ST131 *Escherichia coli* and Non-ST131 Isolates [a]

Resistant Genes	ST131 (N = 15)	Non-ST131 (N = 106)	P-Value
qnrS	4 (26.6)	19 (17.9)	0.4
qnrB	1 (6.6)	7 (6.6)	0.8
aac(6)-Ib-cr	6 (40.0)	10 (9.4)	0.01
qnrS + *qnrB*	2 (13.3)	3 (2.8)	0.09
qnrB + *aac(6)-Ib-cr*	1 (6.6)	2 (1.8)	0.3
qnrS + *qnrB* + *aac(6)-Ib-cr*	1 (6.6)	6 (5.6)	0.6
Mutation in QRDR	n = 15	n = 40	
gyrA			0.1
S83L	3 (20.0)	32 (80.0)	
S83L + D87N	12 (80.0)	8 (20.0)	
parC			0.002
S80I	2 (13.3)	5 (2.0)	
S80I + E84V	3 (20.0)	1 (2.5)	
E84G	-	3 (7.5)	
S80I + E84G	1 (6.6)	3 (7.5)	
E84V	2 (13.3)	2 (5)	

[a] Values are expressed as No. (%). *qnrA* and *qepA* genes were not found in any of the isolates studied. A P-value ≤ 0.05 was considered statistically significant. The mutation was screened in all ST131 isolates (n = 15) and 40 randomly selected isolates based on MIC in 106 non-ST131 isolates.

(3). In our study, the phylogenetic pattern of isolates varied in different parts of the city, and the majority of the isolates belonged to the commensal groups (phylogroup A); however, the prevalence rate of virulent extraintestinal phylogenetic groups D and B2 was noticeable. These results are in line with a study that showed the location had an important role in the phylogenetic pattern of *E. coli* in the gut (16). These differences in the phylogroup pattern of commensal *E. coli* isolates can be ascribed to the geographic, climatic conditions, use of antibiotics, host diet, health status, and genetic factors.

Extraintestinal *E. coli* infections caused mainly by *E. coli* with various virulence factors related to some phylogroups when entering the normally sterile body site such as the bladder (17). The most commonly detected virulence genes among our EPE included isolates were *fimH* (89.6%) in agrees with studies on hospitalised patients and healthy individuals (18, 19). In line with other studies, the phylogenetic distribution of virulence genes was concentrated in isolates clustered in groups B2 and D (20). On the other hand, some virulence factors, such as *fyuA* (52.9%), *iutA* (17.6%), and *kpsMII* (35.3%), were more common in the ST131 clone (which is in line with the results of Hojabri et al and can explain the persistence of this clone in the gut)

(21).

Virulence factors plus quinolone resistance can cause fitness advantage in *E. coli*, particularly the ST131 clone (22). The prevalence rate of the NA/CIP resistance in *E. coli* varies geographically (23). In healthy subjects of our study, the *E. coli* isolates showed relatively high-level resistance to NA/CIP (51.9%/27.8%), indicating the widespread use of NA/CIP for treating and preventing disease in humans and animals. In addition, our findings showed a close relationship with data from clinical samples in Iran (24). Based on our results, the presence of PMQR genes probably is not sufficient to increase resistance to CIP, as the isolates with different resistance phenotypes harbored one or more PMQR genes. The *qnrS* gene (19%) was dominant, followed by *aac(6)-Ib-cr* (13.2%), which is in line with the dominance prevalence of the *qnrS* (53.3%) gene among clinical *E. coli* strains (25). In a number of studies on the city population, despite the relative agreement on the higher prevalence of *qnrS* and *qnrB* genes among *E. coli*, the distribution of predominate genes varied over different regions (26, 27).

The difference between the results may be explained by the variation in geographical distribution, type of sample, or the methods used. Our results indicated that cer-

tain single mutations in *gyrA* were sufficient to generate resistance to NA, and additional mutations in *gyrA* or *parC* genes were necessary for high-level resistance to CIP. An earlier study similarly reported that multiple substitutions, such as S83L and D87N in *GyrA* with S80I in *ParC* mediate high-level resistance to CIP (28). In agreement with a previous study, we found that the number of mutations in QRDR in ST131 isolates was significantly higher than that of non-ST131 isolates (2). Our findings also demonstrate that healthy people carry ST131 *E. coli* isolates in their gut with co-harboring PMQR, such as *aac(6)-Ib-cr*, which can increase double mutations in *gyrA* and *parC* genes (29). Thus, these isolates can be considered because they may be a source of infection and can spread resistance to Q/FQ in the community (30).

There is limited information on risk factors related to the fecal carriage of EPE (31). Different surveys have indicated adverse data on the association between sex and carriage of EPE; meanwhile, our results, in line with some studies, demonstrate an association between the males with EPE carriage (9, 32, 33). Therefore, these discrepancies may reflect the regional habit, social activity, and employment status related to gender. In addition, the present study found that people with diabetes had an increased risk of fecal EPE carriage, which was in accordance with other studies (34). Furthermore, in a different part of the city, location 2 in Tehran with lower economic income and many immigrants had significantly high EPE carriers. Therefore, this area of the city has to be taken into account because the accommodation of high population density, a high proportion of immigrant residents, and the people of a lower socioeconomic class can affect the incidence of EPE carriage (35).

The true risk factor for the transmission of the ST131 clone in the community remained unknown in this study. Our subjects did not use antibiotics 3 months prior to the start of the study; thus, our results can be influenced by the long-term impact of antibiotic consumption. The present research had some limitations. First, the impossibility of using MLST for all isolates in EPE and non-EPE groups; thus, ST131 isolates were identified only in EPE isolates; thus, the results should be interpreted with caution. Second, the relatively small number of ST131 isolates restricted statistical power.

5.1. Conclusions

In this study, we revealed that the asymptomatic healthy people colonized by *E. coli* isolates with concentrated virulence factors belong to phylogroups B2 and D in their guts, which can increase the risk of infection and dissemination of pathogenic isolates in the community. We also observed that ST131 isolates were significantly resistant to Q/FQ antibiotics that can make treatment more difficult in infectious diseases; thus, healthy carriers of high virulent isolates with high antibiotic-resistant should be considered.

Footnotes

References

1. Sora VM, Meroni G, Martino PA, Soggiu A, Bonizzi L, Zecconi A. Extraintestinal pathogenic *Escherichia coli*: Virulence factors and antibiotic resistance. *Pathogens.* 2021;**10**(11). doi: 10.3390/pathogens10111355. [PubMed: 34832511]. [PubMed Central: PMC8618662].

2. Yang HS, Kim Y, Cho SY, Nam YS, Oh TS, Park KS, et al. Fluoroquinolone resistance mechanisms by molecular epidemiologic study of ciprofloxacin-nonsusceptible Escherichia coli sequence types isolated from clinical specimens in a tertiary care university hospital in Korea: emergence of clone ST131, 2006-2008. *Ann Clin Lab Sci*. 2017;**47**(4):511–5. [PubMed: 28801381].

3. Clermont O, Christenson JK, Denamur E, Gordon DM. The Clermont Escherichia coli phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. *Environ Microbiol Rep*. 2013;**5**(1):58–65. doi: 10.1111/1758-2229.12019. [PubMed: 23757131].

4. Stromberg ZR, Van Goor A, Redweik GAJ, Wymore Brand MJ, Wannemuehler MJ, Mellata M. Pathogenic and non-pathogenic Escherichia coli colonization and host inflammatory response in a defined microbiota mouse model. *Dis Model Mech*. 2018;**11**(11). doi: 10.1242/dmm.035063. [PubMed: 30275104]. [PubMed Central: PMC6262807].

5. Ruppe E, Lixandru B, Cojocaru R, Buke C, Paramythiotou E, Angebault C, et al. Relative fecal abundance of extended-spectrum-beta-lactamase-producing Escherichia coli strains and their occurrence in urinary tract infections in women. *Antimicrob Agents Chemother*. 2013;**57**(9):4512–7. doi: 10.1128/AAC.00238-13. [PubMed: 23836184]. [PubMed Central: PMC3754361].

6. Hamed SM, Elkhatib WF, El-Mahallawy HA, Helmy MM, Ashour MS, Aboshanab KMA. Multiple mechanisms contributing to ciprofloxacin resistance among Gram negative bacteria causing infections to cancer patients. *Sci Rep*. 2018;**8**(1):12268. doi: 10.1038/s41598-018-30756-4. [PubMed: 30115947]. [PubMed Central: PMC6095922].

7. Fasciana T, Giordano G, Di Carlo P, Colomba C, Mascarella C, Tricoli MR, et al. Virulence factors and antimicrobial resistance of Escherichia coli ST131 in community-onset healthcare-associated infections in Sicily, Italy. *Pharmacol Online*. 2017;**1**:12–21.

8. Johnson JR, Clermont O, Johnston B, Clabots C, Tchesnokova V, Sokurenko E, et al. Rapid and specific detection, molecular epidemiology, and experimental virulence of the O16 subgroup within Escherichia coli sequence type 131. *J Clin Microbiol*. 2014;**52**(5):1358–65. doi: 10.1128/JCM.03502-13. [PubMed: 24501035]. [PubMed Central: PMC3993632].

9. Onduru OG, Mkakosya RS, Rumisha SF, Aboud S. Carriage prevalence of extended-spectrum β-lactamase producing enterobacterales in outpatients attending community health centers in Blantyre, Malawi. *Trop Med Infect Dis*. 2021;**6**(4). doi: 10.3390/tropicalmed6040179. [PubMed: 34698289]. [PubMed Central: PMC8544730].

10. Wayne PA. Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing. *Inform Suppl*. 2011;**31**(1):100–21.

11. Johnson JR, Stell AL. Extended virulence genotypes of Escherichia coli strains from patients with urosepsis in relation to phylogeny and host compromise. *J Infect Dis*. 2000;**181**(1):261–72. doi: 10.1086/315217. [PubMed: 10608775].

12. Johnson JR, Murray AC, Gajewski A, Sullivan M, Snippes P, Kuskowski MA, et al. Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic Escherichia coli from retail chicken products. *Antimicrob Agents Chemother*. 2003;**47**(7):2161–8. doi: 10.1128/AAC.47.7.2161-2168.2003. [PubMed: 12821463]. [PubMed Central: PMC161843].

13. Park CH, Robicsek A, Jacoby GA, Sahm D, Hooper DC. Prevalence in the United States of aac(6')-Ib-cr encoding a ciprofloxacin-modifying enzyme. *Antimicrob Agents Chemother*. 2006;**50**(11):3953–5. doi: 10.1128/AAC.00915-06. [PubMed: 16954321]. [PubMed Central: PMC1635235].

14. Kotb DN, Mahdy WK, Mahmoud MS, Khairy RMM. Impact of co-existence of PMQR genes and QRDR mutations on fluoroquinolones resistance in Enterobacteriaceae strains isolated from community and hospital acquired UTIs. *BMC Infect Dis*. 2019;**19**(1):979. doi: 10.1186/s12879-019-4606-y. [PubMed: 31752702]. [PubMed Central: PMC6868749].

15. Aghamohammad S, Nikbin VS, Badmasti F, Shahcheraghi F. High heterogeneity of fecal carriage extended-spectrum beta-lactamase-producing E. coli isolated from iranian community and clinical settings. *BMC Infect Dis*. 2022;**22**(1):1–9. doi: 10.1186/s12879-022-07304-7. [PubMed: 35361158]. [PubMed Central: PMC8973555].

16. Stoppe NC, Silva JS, Carlos C, Sato MIZ, Saraiva AM, Ottoboni LMM, et al. Worldwide phylogenetic group patterns of Escherichia coli from commensal human and wastewater treatment plant isolates. *Front Microbiol*. 2017;**8**:2512. doi: 10.3389/fmicb.2017.02512. [PubMed: 29312213]. [PubMed Central: PMC5742620].

17. Aghamohammad S, Badmasti F, Shirazi AS, Dabiri H, Solgi H, Sabeti S, et al. Considerable rate of putative virulent phylo-groups in fecal carriage of extended-spectrum beta-lactamase producing Escherichia coli. *Infect Genet Evol*. 2019;**73**:184–9. doi: 10.1016/j.meegid.2019.04.035. [PubMed: 31054921].

18. Halaji M, Shahidi S, Ataei B, Atapour A, Feizi A, Havaei SA. Molecular epidemiology of blaCTX-M gene-producing uropathogenic Escherichia coli among Iranian kidney transplant patients: clonal dissemination of CC131 and CC10. *Ann Clin Microbiol Antimicrob*. 2021;**20**(1):1–11. doi: 10.1186/s12941-021-00470-7. [PubMed: 34496873]. [PubMed Central: PMC8424993].

19. Raimondi S, Righini L, Candeliere F, Musmeci E, Bonvicini F, Gentilomi G, et al. Antibiotic resistance, virulence factors, phenotyping, and genotyping of *E. coli* isolated from the feces of healthy subjects. *Microorganisms*. 2019;**7**(8). doi: 10.3390/microorganisms7080251. [PubMed: 31405113]. [PubMed Central: PMC6722543].

20. 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):1–12. doi: 10.1186/s12879-020-4844-z. [PubMed: 32033541]. [PubMed Central: PMC7006406].

21. 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**:893–903. doi: 10.2147/IDR.S199759. [PubMed: 31118697]. [PubMed Central: PMC6475099].

22. Matsumura Y, Yamamoto M, Nagao M, Ito Y, Takakura S, Ichiyama S, et al. Association of fluoroquinolone resistance, virulence genes, and IncF plasmids with extended-spectrum-beta-lactamase-producing Escherichia coli sequence type 131 (ST131) and ST405 clonal groups. *Antimicrob Agents Chemother*. 2013;**57**(10):4736–42. doi: 10.1128/AAC.00641-13. [PubMed: 23856781]. [PubMed Central: PMC3811414].

23. Boyd LB, Atmar RL, Randall GL, Hamill RJ, Steffen D, Zechiedrich L. Increased fluoroquinolone resistance with time in Escherichia coli from >17,000 patients at a large county hospital as a function of culture site, age, sex, and location. *BMC Infect Dis*. 2008;**8**:4. doi: 10.1186/1471-2334-8-4. [PubMed: 18197977]. [PubMed Central: PMC2258293].

24. Halaji M, Shahidi S, Atapour A, Ataei B, Feizi A, Havaei SA. Characterization of extended-spectrum β-lactamase-producing uropathogenic Escherichia coli among Iranian kidney transplant patients. *Infect Drug Resist*. 2020;**13**:1429–37. doi: 10.2147/IDR.S248572. [PubMed: 32523361]. [PubMed Central: PMC7237106].

25. Rasoulinasab M, Shahcheraghi F, Feizabadi MM, Nikmanesh B, Hajihasani A, Aslani MM. 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. *Iran J Microbiol*. 2021;**13**(3):294–302. doi: 10.18502/ijm.v13i3.6389. [PubMed: 34540167]. [PubMed Central: PMC8416580].

26. Malekzadegan Y, Rastegar E, Moradi M, Heidari H, Sedigh Ebrahim-Saraie H. Prevalence of quinolone-resistant uropathogenic Escherichia coli in a tertiary care hospital in south Iran. *Infect Drug Resist*. 2019;**12**:1683–9. doi: 10.2147/IDR.S206966. [PubMed: 31354317]. [PubMed Central: PMC6590898].

27. Salah FD, Soubeiga ST, Ouattara AK, Sadji AY, Metuor-Dabire A, Obiri-Yeboah D, et al. Distribution of quinolone resistance gene (qnr) in ESBL-producing Escherichia coli and Klebsiella spp. in Lome, Togo. *Antimicrob Resist Infect Control*. 2019;**8**:1–8. doi: 10.1186/s13756-019-0552-0. [PubMed: 31244995]. [PubMed Central: PMC6582466].

28. Johnning A, Kristiansson E, Fick J, Weijdegard B, Larsson DG. Resistance mutations in gyrA and parC are common in Escherichia communities of both fluoroquinolone-polluted and uncontaminated aquatic environments. *Front Microbiol*. 2015;**6**:1355. doi: 10.3389/fmicb.2015.01355. [PubMed: 26696975]. [PubMed Central: PMC4673309].

29. Volcao LM, Lacava JP, Gewehr MF, Leal VL, Ramis IB, Ramos DF, et al. High frequency of aac(6')-Ib-cr gene associated with double mutations in gyrA and parC in Escherichia coli isolates from patients with urinary tract infections. *J Glob Antimicrob Resist*. 2018;**13**:180–3. doi: 10.1016/j.jgar.2017.12.013. [PubMed: 29307860].

30. Mathers AJ, Peirano G, Pitout JD. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. *Clin Microbiol Rev*. 2015;**28**(3):565–91.

doi: 10.1128/CMR.00116-14. [PubMed: 25926236]. [PubMed Central: PMC4405625].

31. Hu Y, Matsui Y, W. Riley L. Risk factors for fecal carriage of drug-resistant Escherichia coli: a systematic review and meta-analysis. *Antimicrob Resist Infect Control*. 2020;**9**(1):1–12. doi: 10.1186/s13756-020-0691-3. [PubMed: 32046786]. [PubMed Central: PMC7014593].

32. Shashwati N, Kiran T, Dhanvijay AG. Study of extended spectrum beta-lactamase producing Enterobacteriaceae and antibiotic coresistance in a tertiary care teaching hospital. *J Nat Sci Biol Med*. 2014;**5**(1):30–5. doi: 10.4103/0976-9668.127280. [PubMed: 24678193]. [PubMed Central: PMC3961948].

33. Kang CI, Wi YM, Lee MY, Ko KS, Chung DR, Peck KR, et al. Epidemiology and risk factors of community onset infections caused by extended-spectrum beta-lactamase-producing Escherichia coli strains. *J Clin Microbiol*. 2012;**50**(2):312–7. doi: 10.1128/JCM.06002-11. [PubMed: 22162561]. [PubMed Central: PMC3264158].

34. Al Yousef SA, Younis S, Farrag E, Moussa H, Bayoumi FS, Ali AM. Clinical and laboratory profile of urinary tract infections associated with extended spectrum β-lactamase producing Escherichia coli and Klebsiella pneumoniae. *Ann Clin Lab Sci*. 2016;**46**(4):393–400. [PubMed: 27466299].

35. Letara N, Ngocho JS, Karami N, Msuya SE, Nyombi B, Kassam NA, et al. Prevalence and patient related factors associated with Extended-Spectrum Beta-Lactamase producing Escherichia coli and Klebsiella pneumoniae carriage and infection among pediatric patients in Tanzania. *Sci Rep*. 2021;**11**(1):1–9. doi: 10.1038/s41598-021-02186-2. [PubMed: 34815472]. [PubMed Central: PMC8611091].