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ORIGINAL ARTICLE

Molecular epidemiology of *Escherichia coli* sequence type 131 and its H30/H30-Rx subclones recovered from extra-intestinal infections: first report of OXA-48 producing ST131 clone from Iran

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Abstract Multidrug-resistant (MDR) O25b-ST131 clone of *Escherichia coli* is well established as a significant cause of extra-intestinal infections worldwide. However, there have been no studies about the prevalence of ST131 and its *H*30/*H*30Rx subclones from Iran. The prevalence of ST131 was 29.8% among phylogroups B2, D, and F of *E.coli* isolates recovered from extra-intestinal infections. Fifty-seven (90.4%) and six (9.6%) of isolates belonged to serogroups O25b and O16 respectively, and exhibited high rates of MDR (98.4% and 83.3%) and extended spectrum β-lactamase (ESBL) production (96.8% and 83.3%). The majority (56/57, 98.2%) of O25b isolates belonged to *H*30 lineage; of those, 24 isolates (42.8%) belonged to *H*30-Rx subclone. O16-ST131 isolates were *H*30-negative. The resistance rate values of O16-ST131subgroup were lower for fluoroquinolones/aminoglycosides and higher for carbapenems, cephalosporins, β-lactam/β-lactamase inhibitors and trimethoprim/sulfamethoxazole, as compared to O25b-ST131 isolates. Among *H*30 sub lineage and in comparison with non-Rx isolates, *H*30-Rx subclone showed higher resistance score and virulence genes (*papA* and *papC*), and was also associated with CTX-M group 1. $bla_{\text{OXA-48}}$ carbapenemase was detected in seven O25b and one O16 isolates; of those, one O25b-ST131 isolate was carbapenem-susceptible. The ST131 isolates comprised 15 'enterobacterial repetitive intergenic consensus' (ERIC) clusters, and O16 isolates remained distributed in five groups in cluster with O25b-ST131 isolates. In conclusion, this is the first report of the presence of MDR, $bla_{\text{OXA-48}}$/CTX-M-positive O25b/O16-ST131 isolates in Iran. Contrary to lower prevalence of O16-ST131 subgroup, higher resistance rates to β-lactam antibiotics may indicate the importance of this subgroup in the spread of MDR *E.coli* isolates.

Keywords ST131 · *H*30 subclone · ExPEC · OXA-48 · Carbapenem · Fluoroquinolone

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Introduction

Escherichia coli sequence type (ST) 131 is an emerging disseminated public health threat implicated in multidrug-resistant extra-intestinal infections worldwide [1–4]. Presently, the pandemic ST131 clonal group is dominant among the extra-intestinal pathogenic *E. coli* (ExPEC) isolates [5–7]. ST131 *E. coli* isolates typically exhibit multiple virulence factors, including adhesins, toxins, siderophores, and group 2 capsules [3, 8, 9]. Thus, this clonal group combines both resistance and virulence genes, which in the classical ExPEC isolates have been infrequently detected [10].

ST131 is frequently associated with fluoroquinolone resistance, CTX-M-15 enzyme, and *aac(6')-Ib-cr*, while bla_{TEM}, bla_{SHV} and other β-lactamases are less frequent. Extended spectrum β-lactamase (ESBL) and non-ESBL producing *E.coli* ST131 isolates often exhibit fluoroquinolone resistance, which may serve as a marker for ST131-positive *E.coli* [11]. The rise of fluoroquinolone resistance rate has caused concern in antimicrobial treatment of Enterobacteriaceae infections, whereby

carbapenems are considered the best therapeutic option [12]. Nevertheless, some Enterobacteriaceae can produce clinically important carbapenemases. In contrast to *K. pneumoniae*, much less is known about the carbapenemase-producing *E.coli*. The information that is available is in reference to the bla_{NDM} and bla_{OXA-48} classes. Of concern is the fact that the acquisition of carbapenemases by successful *E.coli* clones, such as ST131, has already occurred [13].

The majority of ST131 isolates belong to the O25b:H4 serotype [3]. Furthermore, most isolates of this serotype are identified as subclone *H*30 and PST43 according to *fimH* allele and the "multi locus sequence type" (MLST) scheme of the Pasteur Institute respectively. Within the *H*30 subclone, the *H*30-Rx subset often carries $bla_{CTX-M-15}$ as a member of CTX-M group 1 cluster. It has been shown that *E. coli* O25b-ST131 exhibits a high virulence score compared to other lineages [14], and is capable of acquiring antibiotic resistance by different mechanisms [11, 15–17]. The fact that *E.coli* O25b-ST131 is able to exhibit antibiotic resistance means that the clinical environment within a hospital or community may actively select certain resistant strains [18], making the treatment of the infections caused by these strains increasingly difficult.

To our knowledge, there have been no studies of *E.coli* ST131 isolates in Iran. Thus, the present study was carried out to evaluate the prevalence of the O25b/O16 subgroups of ST131clone and the *H*30/*H*30-Rx lineages among *E.coli* isolates causing extra-intestinal infections, and to understand their contributions to the spread of antimicrobial resistance. Furthermore, the virulence attributes and genetic diversity of ST131 isolates were also determined using PCR-based approaches.

Material and methods

Hospital setting and samples investigated

A cross-sectional study was conducted at Kowsar University Hospital, which serves patients in Semnan (Iran) and provides medical and surgical care in all medical specialties. During the 1-year study period (March 2015–March 2016), 580 Enterobacterial isolates were cultured from hospitalized patients with extra-intestinal infections, of which 339 *E.coli* isolates were identified by routine laboratory methods. In this study, *E.coli* isolates cultured from patients with recurrent urinary tract infections (UTI) were also included (recurrent UTI refers to at least two episodes of repeatedly occurring UTI within an interval of more than 2 weeks [19]).

Phylogenetic analysis

DNA extraction was performed using CTAB method [20]. Each isolate was assigned to one of the four main phylogenetic groups by targeting three marker genes (*chuA*, *yjaA*, *arpA*) and a DNA fragment TspE4.C2. Based on the PCR products, the strains were classified into one of the four major *E.coli* phylogenetic lineages: A/C, B1, B2, D/E and F [21].

Screening of ST131clonal group

All phylogroups B2, D, and F isolates were screened by PCR for the ST131-associated single nucleotide polymorphism (SNP) in *mdh* and *gyrB* genes [22]. The thus-identified ST131 isolates were screened using a triplex PCR based in the detection of the new operon *afa* FM955459 and the targets *rfbO25b* and $bla_{CTX-M-15}$ gene 3′ end [23]. To confirm these clonal assignments, selected isolates underwent multilocus sequence typing according to the Achtman scheme using seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) (http://mlst.ucc.ie.mlst/dbs/Ecoli). The O25b and O16 subgroups of ST131 positive isolates were molecularly detected as described earlier [24]. The ST131 isolates were also tested by allele-specific primers for allele 30 of *fimH* corresponding with the main fluoroquinolone resistance associated subset within ST131, the *H*30 subclone [25]. The *H*30-Rx subclone was identified by PCR detection of a specific SNP (G723A) within the allantoin-encoding gene *ybbW* [6]. Furthermore, virotypes of all ST131 isolates were determined using two multiplex PCR, as described previously [26].

Antibiotic susceptibility testing and phenotypic detection of ESBL producers

Antibiotic susceptibility profiles were obtained for all *E.coli* isolates by using the standard disc diffusion method on Mueller–Hinton agar. The Clinical Laboratory Standard Institute (CLSI) recommendations for antimicrobial susceptibility testing were followed [27]. The antibiotic panel used was as follows: imipenem, meropenem, ertapenem, ceftazidime, cefepime, cefotaxime, aztreonam, piperacillin/tazobactam, ampicillin/sulbactam, amoxicillin/clavulanic acid, trimethoprim/sulfamethoxazole, gentamicin, amikacin, tobramycin, ciprofloxacin, and levofloxacin. Intermediate susceptibility was interpreted as resistant. Isolates were considered as carbapenem-resistant when they were identified as non-susceptible to any of imipenem, meropenem, or ertapenem (inhibition zone <23 mm) [27]. The number of antibiotics to which an isolate was resistant was considered as the resistance score. Multidrug-resistant (MDR) isolates were those resistant to at least one representative of ≥3 antimicrobial classes, including "β-lactam/β-lactamase inhibitors", extended-spectrum cephalosporins, aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole [28]. Screening for the presence of ESBLs was performed with combined disc test using cefpodoxime, ceftazidime, and

cefotaxime with or without clavulanic acid, according to the recommendations of the CLSI [27].

Virulence genotyping

All *E.coli*-collected isolates were investigated to the following six ExPEC-related virulence genes by single multiplex PCR. P fimbriea *papC* and *papA*, central region of *sfaS* and *focG* operons (*sfa/focDE*), aerobactin siderophore receptor (*iutA*), group 2 capsule (*KpsM II*), and secreted autotransporter toxin (*sat*). Isolates were classified as ExPEC if positive for ≥2 of these genes. The virulence score was the number of virulence genes that were detected in an isolate [29].

Detection of resistance genes

The presence of carbapenemase (bla_{NDM}, bla_{OXA-48}, bla_{IMP-}, bla_{VIM-} and bla_{KPC}) [30], Extended Spectrum β-lactamase (ESBL) (bla_{TEM-}, bla_{SHV-} and bla_{CTX-M} groups 1, 2 8, 9, 25) [31, 32] and plasmid mediated quinolone resistance (PMQR) (*qnrA*, *qnrB*, *qnrS* and *aac(6')-Ib-cr*) [33] genes were assessed by multiplex PCR according to previously published methods. As positive controls, previously characterized isolates carrying resistance gene sequences understudy were used.

ERIC-PCR analysis

Enterobacterial repetitive intergenic consensus sequence polymerase chain reaction (ERIC-PCR) was performed on all isolates. BioNumerics software, version 6.1, (Applied Maths, Sint-Martens-Laten, Belgium) was used for analyzing the ERIC-PCR fingerprints. The similarities in amplicon profiles were compared using a Dice coefficient at 1% tolerance and 0.5% optimization, and a dendrogram was constructed using the unweighted-pair group method with arithmetic mean clustering (UPGMA) method, with a cut-off of 80% similarity [34].

Statistical analysis

Comparisons of proportions and scores were tested using Fisher's exact test and the Mann–Whitney *U* test respectively. $P < 0.05$ was considered to denote significant differences.

Results

Prevalence of ST131 clonal group and *H*30 and *H*30-Rx subclones:

Of the 339 collected *E.coli* isolates, 136, 33, and 42 isolates belonged to phylogroups B2, D, and F respectively, and were finally selected for screening of ST131 clonal group.

According to PCR-based detection, *E.coli* ST131 clonal group accounted for 63 (29.8%) of the 211 selected isolates, and MLST confirmed the PCR typing of ST131 isolates. These non-duplicate ST131 isolates were cultured from 52 urine (82.5%), eight sputum (12.6%), and three wound samples (4.7%). The age range of the individuals (35 females and 28 males) recruited into the study was 13–92 years. Sixty-two of these isolates belonged to phylogroup B2, and the one remaining strain belonged to group F. O25b/O16 subgrouping PCR identified 57 (90.4%) and six (9.6%) ST131 isolates as O25b and O16 subclonal lineages respectively. With regard to the type of sample, O16-ST131 isolates were predominantly obtained from UTI (five cases) and sputum (one case). The majority (56/57; 98.2%) of O25b-ST131 belonged to *fimH*30 subclone, and 24 of 56 (42.8%) *fimH*30 isolates belonged to the *H*30-Rx subclone. All of the O16-ST131 isolates belonged to phylogroup B2 and also were *fimH*30 negative.

Virotyping of studied ST131 isolates showed four discrete virotypes based on the presence or absence of four distinctive virulence genes. The 63 isolates were distributed among four virotypes as follows: virotype A; six (five O25b and one O16) isolates, virotype B; one O25b isolate, C; 55 (51: O25b and four: O16) isolates, and D; one O16 isolate. As in recent Spanish and British studies [26, 35], virotype C (afa^-, $iroN^-$, $ibeA^-$, sat^+) was frequently detected.

Based on the molecular definition of ExPEC, all ST131 isolates except one O16 strain were attributed with the status of ExPEC. The most prevalent virulence factor was *sat* (61, 96.8%), followed by *iutA* (59, 93.7%), *kpsMII* (30, 47.6%), *papC* (27, 42.9%) and *papA* (24, 38.1%) respectively. In comparison between O25b and O16 isolates, O25b-ST131 subgroup exhibited significantly higher prevalence of two virulence genes, *sat*, and *iutA* (*P*: 0.05, for both). Furthermore, virulence scores of O25b isolates were significantly higher in comparison with the O16-ST131 subgroup [median, 3 (2–5) vs 2 (1–4); *P*: 0.01]. Among *H*30 isolates, *papA* and *papC* were more prevalent among Rx isolates (*P*: 0.006 and *P*: 0.03 respectively), while *kpsMII* was detected more frequently among non-Rx subclone (*P*: 0.01) (Table 1).

Antibiotic susceptibility and resistance determinants:

According to the results of antibiotic susceptibility testing, the highest resistance rate was obtained against cefotaxime (59, 93.6%), followed by aztreonam (58, 92%), ciprofloxacin/levofloxacin (55, 87.3%) and ceftazidime (50, 79.3%) respectively. The O16-ST131 isolates exhibited different resistance rate values compared with O25b-ST131 isolates, being significantly lower for ciprofloxacin/levofloxacin (0 vs 100%) ($P < 0.001$) and significantly higher for imipenem (16.7% vs 1.8%) and ampicillin/sulbactam (83.3% vs 42.1%) (*P*: 0.05, for both). There was no significant difference in resistance score between O25b-ST131 and O16-ST131 (*H*30-negative)

Table 1 Antibiotic resistance, virulence, and resistance genes among studied ST131 isolates

Antibiotics	Within ST131			Within H30 subclone		
	No. (%) of resistant isolates		P^{+} value	No. (%) of resistant isolates		
	O25b-ST131 (n = 57)	O16-ST131 (n = 6)		Rx (n = 24)	Non-Rx (n = 33)	*P value*
Imipenem	1 (1.8)[a]	1 (16.7%)[a]	0.05	0	1 (3)	-
Meropenem	1 (1.8)	0 (0)	-	1 (4.2)	0	-
Ertapenem	7 (12.5)	1 (16.7)	-	4 (16.7)	3 (9.1)	-
Ceftazidime	45 (78.9)	5 (83.3)	-	20 (83.3)	25 (75.8)	-
Cefepime	35 (61.4)	4 (66.7)	-	15 (62.5)	20 (60.6)	-
Cefotaxime	54 (94.7)	5 (83.3)	-	23 (95.8)	31 (93.9)	-
Aztreonam	53 (93)	5 (83.3)	-	24 (100)	29 (87.9)	-
Piperacillin/tazobactam	10 (17.5)	2 (33.3)	-	7 (29.2)	3 (9.1)	-
Ampicillin/sulbactam	24 (42.1)[a]	5 (83.3)[a]	0.05	14 (58.3)[a]	10 (30.3)[a]	0.05
Amoxicillin/clavulanate	33 (57.9)	5 (83.3)	-	20 (83.3)[a]	13 (39.4)[a]	0.001
Ciprofloxacin/levofloxacin	57 (100%)[a]	0[a]	< 0.001	24 (100)	33 (100)	-
Trimethoprim/sulfamethoxazole	39 (68.4)	5 (83.3)	-	18 (75)	21 (63.6)	-
Amikacin	7 (12.3)	0	-	7 (29.2)[a]	0[a]	0.001
Tobramycin	28 (49.1)	1 (16.7)	-	18 (75)[a]	10 (30.3)[a]	0.001
Gentamicin	25 (43.9)	2 (33.3)	-	15 (62.5)[a]	10 (30.3)[a]	0.03
MDR[¶]	55 (96.5)	5 (83.3)	-	23 (95.8)	32 (97)	-
Resistance score (median)	(8)	(8)	-	(8.50)[a]	(6)[a]	0.001
ESBL phenotype [†]	55 (96.5)	5 (83.3)	-	24 (100)	31 (93.2)	-
Resistance genes	No.(%) of positive isolates					
CTX-M-G-1	49 (86%)	4 (66.7)	-	24 (100)[a]	25 (27.8)[a]	0.01
CTX-M-G-8	**0 (0)**	3 (50)[a]	0.001	0	0	-
CTX-M-G-9	18 (31.6)	0 (0)	-	2 (8.3)[a]	16 (48.5)[a]	0.001
CTX-M-G-25	18 (31.6)	2 (33.3)	-	4 (16.7)[a]	14 (42.4)[a]	0.04
$bla_{\text{TEM-}}$	40 (70.2)	4 (66.7)	-	16 (66.7)	24 (72.7)	-
$bla_{\text{SHV-}}$	27 (47.4)	2 (33.3)	-	12 (50)	15 (45.5)	-
$bla_{\text{OXA-48}}$	7 (12.3)	1 (16.7)	-	4 (16.7)	3 (9.1)	-
aac6Ib-cr	38 (66.7)	2 (33.3)	-	19 (79.2)	19 (57.6)	-
qnrB	7 (12.3)	0 (0)	-	2 (8.3)	5 (15.2)	-
qnrS	4 (7)	0 (0)	-	0	4 (12.1)	-
Virulence genes	No. (%) of positive isolates					
papC	26 (45.6)	1 (16.7)	-	15 (62.5)[a]	11 (33.3)[a]	0.03
kpsMII	28 (49.1)	2 (33.3)	-	7 (29.2)[a]	21 (63.6)[a]	0.01
iutA	55 (96.5)[a]	4 (66.7)[a]	0.05	24 (100)	31 (93.8)	-
sfa	1 (1.8)	0	-	0	1 (3)	-
papA	23 (40.4)	1 (16.7)	-	15 (62.5)[a]	8 (24.2)[a]	0.006
sat	56 (98.2)[a]	5 (83.3)[a]	0.05	23 (95.8)	33 (100)	-
Virulence score (median)	(3)[a]	(2)[a]	0.01	(4)[a]	(3)[a]	0.04

[+] Determined by Fisher's exact test. Values are shown where P is ≤0.05; for all other comparisons, P was >0.1. [a] indicates statistically significant values

[¶] Multidrug-resistant

[†] ESBL-producing isolates confirmed by phenotypic combined disc test

subgroups. In contrast, among *H*30 isolates, antimicrobial resistances differed between Rx and non-Rx subclones, showing *H*30-Rx isolates a higher prevalence of tobramycin, amikacin (P: 0.001, for both), gentamicin (P: 0.03), amoxicillin/clavulanate (P: 0.001), and ampicillin/sulbactam resistance (P: 0.05) ,and also higher resistance scores

([median, 8.50 vs 6], *P*: 0.01). Three isolates, including two O25b (isolates; 556 and 656) and one O16 (isolate; 587) were ESBL-negative using phenotypic test; of those, isolates 656 and 587 were also detected as non-MDR (Table 1).

Of the studied resistance genes, CTX-M gene cluster showed the highest prevalence among ST131 isolates. ESBL subtype varied significantly by subclone, with CTX-M group 1 being associated with *H*30-Rx, and CTX-M groups 9 and 25 with non-Rx isolates. Thus,all 24 *H*30-Rx isolates were CTX-M group 1 positive. Among the PMQR determinants, *aac(6')-Ib-cr* was the highest prevalent element, and detected in 38 ciprofloxacin/levofloxacin non-susceptible and also two O16-ST131-susceptible isolates respectively. Of the studied carbapenemase genes, $bla_{\text{OXA-48}}$ was detected in seven (six O25b and one O16) carbapenem non-susceptible isolates and also in one carbapenem-susceptible strain (O25b). Four out of six O25b-ST131 carbapenem non-susceptible isolates belonged to *H*30-Rx subclone (Table 1).

ERIC-PCR profiles of ST131 isolates

Figure 1 shows a dendogram with ERIC-PCR profiles of 61 ST131 isolates. The remaining two strains were non-typeable, as their ERIC-PCR failed to yield distinct bands despite multiple efforts to repeat the experiment. The ERIC-PCR analysis showed that 59 O25b/O16 ST131 isolates belonged to 15 different clusters, including between two and 12 isolates each, whereas the remaining two O25b isolates (isolates 12 and 372 [phylogroup F]) were singleton. In particular, the six O16-ST131 isolates remained distributed in five groups in cluster with other O25b-ST131 isolates (Fig. 1).

Discussion

Our study is the first survey in Iran to investigate and compare the prevalence and genotypes of O25b/O16-ST131 isolates, including the *H*30 and *H*30-Rx subclones. During our 1-year surveillance study, the O16-ST131 isolates accounted for only 1.76% compared with 16.8% of the O25b-ST131 subgroup. We confirmed the well-established association between ST131 and ESBL production [6], and found that *H*30 ST131 subclone has expanded in the study region more than other ST131 subclones.

Our results, and the observation of a high prevalence of phylogroup B2 isolates among the ST131 clone, suggest the clonal expansion of ST131 from a common phylogenetic ancestor; this observation reinforces the explanations for ST131's remarkable epidemiological success, as phylogroup B2 is thought to be epidemiologically associated with extra-intestinal virulence by means other than through the defined extra-intestinal virulence traits [36].

An important sub-lineage within *H*30 subclone, called *H*30-Rx (Rx, extensively resistant) subclone because of its more extensive antimicrobial resistance profile, was identified using whole genome phylogenetic analysis [37]. A similar study conducted in 2014 in Spain showed that 99 (78%) of ST131 isolates belonged to *H*30 subclone, and 79% of these *H*30 isolates belonged to *H*30-Rx subclone [10]. In the present study, *H*30 subclone was the most prevalent (88.8%) among the studied ST131 isolates, and accounted for nearly all (56 out of 57 isolates) fluoroquinolone-resistant isolates, while 46.8% of *H*30 isolates belonged to *H*30-Rx subclone. The *H*30-Rx subclone was highly resistant to antimicrobials, and its CTX-M-group 1 production differed from the *H*30-non-Rx isolates. Peirano and Pitout have reported that a rapid influx of the *H*30-Rx clone is responsible for the rise in quinolone resistance [38]. Our study was conducted in a region where quinolone resistance rates were high. Despite an increase in resistance to quinolones, a drop in the proportion of *H*30-Rx suggests that there might be other resistant subclones of ST131 responsible for high quinolone resistance.

The O16-ST131 isolates exhibited different resistance rate values as compared with O25b-ST131 isolates, being lower for fluoroquinolones/aminoglycosides and higher for imipenem, ceftazidime, cefepime, trimethoprim/sulfamethoxazole, piperacillin/tazobactam, ampicillin/sulbactam, and amoxicillin/clavulanic acid. In Japan, USA, and Spain, O16-ST131 isolates were associated with resistance to gentamicin and trimethoprim/sulfamethoxazole. However, O16-ST131 isolates in our study exhibited higher trimethoprim/sulfamethoxazole resistance value (71.4%) compared with O16-ST131 isolates from Spain (52%), Japan (50%) and USA (66%) [10, 24, 39]. Our findings also uniquely document a continuum of increasing antimicrobial resistance within ST131, from the non-*H*30 lineage (O16-ST131, susceptible to fluoroquinolones and aminoglycosides) to the *H*30-Rx subclone (most resistant).

In the current study, all ST131 isolates except one O16 strain were found to associate with ExPEC status by harboring two to five of the ExPEC virulence factors. The O25b subclone is known to be highly virulent, as judged by virulent genes and animal models [39]. Like Matsumura et al. in Japan [39] and Dahbi et al. in Spain [10], O16-ST131 subgroup appeared to have low virulence and to differ from the O25b-ST131 isolates. Furthermore, concurring with Banerjee et al. and Dahbi et al. [6, 10], we document that *H*30-Rx isolates have higher virulence scores than non-Rx subclone, implying greater virulence potential. The mechanisms whereby specific accessory traits may facilitate the epidemiological success of ST131 and its principal subclones deserve further study.

The *E.coli* ST131 clonal group has achieved notoriety for its role in the rapid global dissemination of ESBLs, especially $bla_{\text{CTX-M-15}}$, including in community settings. This is the first report of isolates belonging to the *H*30, *H*30-Rx, and non-*H*30

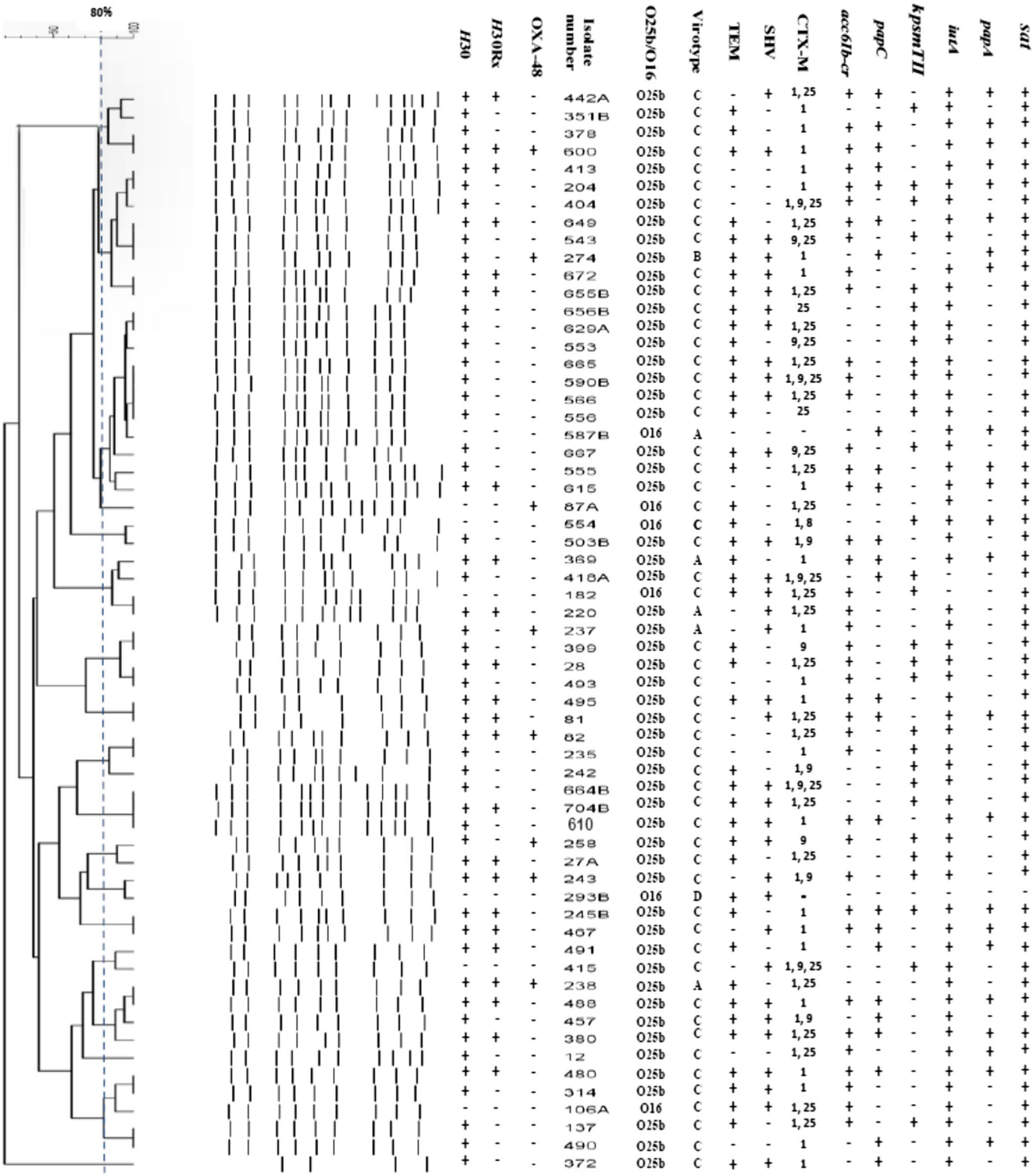

Fig. 1 ERIC-based dendogram of 61 ST131 isolates produced by use of the UPGMA algorithm based on Dice similarity coefficients

subclones of pandemic *E.coli* ST131 clone that produces an OXA-48 carbapenemase from Iran. Of the eight isolates carrying $bla_{\text{OXA-48}}$ carbapenemase, seven strains were non-susceptible to either of imipenem, meropenem, or ertapenem, while the remaining one isolate was susceptible. Laboratory detection of $bla_{\text{OXA-48}}$ producing *E.coli* may be challenging, because $bla_{\text{OXA-48}}$ does not confer frank resistance to the carbapenems as defined by current interpretive standards, and this enzyme must act in conjunction with reduced carbapenem permeability across the outer membrane and/or efflux to

confer frank resistance [40]. The present finding adds to a growing number of reports that this clone can host carbapenemases. The potential of this high-risk clone to serve as a vehicle for hospital-community spread of carbapenemases is profoundly alarming.

Genotyping by ERIC-PCR showed a relative relatedness among studied isolates, since 59 O25b/O16-ST131 isolates clustered into 15 different ERIC profiles. This suggests clonal expansion from a common ancestor. Even though it has been years since the emergence and dissemination of ST131 *E. coli* strains, they still maintain their clonal nature. This clonal nature, on the one hand, holds the danger of potentially causing disease outbreaks but, on the other hand, may facilitate effective control strategies involving vaccine development and transmission prevention [36].

This study has several limitations. The isolates were collected regionally, not nationwide, which limits the generalizability of our data. Furthermore, we have included the ST131 clonal group of *E.coli* isolates based on the *gyrB/mdh* SNP PCR screening rather than do MLST, so we did not study the other high-risk clones, such as ST405, ST127, and ST38, which are contributing to the spread of MDR *E.coli*.

In summary, the present study confirms that the *H*30 subclone is expanded in our locale and suggests that this subclone is the one which is mainly responsible for the successful spread of clonal group ST131. Following the recent reports of carbapenem-resistant NDM-1 and OXA-48 *E.coli* strains in the Middle East, we now describe the population structure of ST131 clonal group from a major medical center in Semnan, revealing the presence of MDR strains carrying $bla_{\text{OXA-48}}$ carbapenemase. Importantly, although O16-ST131 clonal subgroup showed a lower frequency than O25b-ST131, higher resistance rates to most of antibiotics except fluoroquinolones and aminoglycosides indicate the importance of this subgroup in the spread of MDR *E. coli* isolates.

Compliance with ethical standards

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Conflict of interest None to declare.

Ethical approval Not requierd.

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