Prevalence and molecular characteristics of sequence type 131 clone among clinical uropathogenic *Escherichia coli* isolates in Riyadh, Saudi Arabia

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ABSTRACT

Background: The antimicrobial resistance of extraintestinal pathogenic *Escherichia coli* (ExPEC) has progressively been reported worldwide. This resistance has been ascribed to global dissemination of a single *E. coli* clone, namely *E. coli* sequence type 131 (*E. coli* ST131). The main goal of this study is to determine the prevalence and molecular traits of ST131 and its subclones among *E. coli* clinical urine isolates in Riyadh, Saudi Arabia.
Methods: Sixty *E. coli* urine isolates, of different extended spectrum β-lactamase (ESBL) carriage, were involved in this study. Molecular characterization was carried out to determine the ST131 status, phylogenetic groups and virulence carriage of these isolates. ST131 isolates were further tested to evaluate the prevalence of different phylogenetic groups, subclones and virulence carriage.
Results: Group B2 was the most common phylogroup from which *E. coli* isolates derived. Overall, 37 of 60 (61.7%) isolates belonged to ST131 clones. Of these, 19 (31.7%) isolates were from the *H30* subclone, including 10 (16.7%) *H30* non-Rx and 9 (15%) *H30*Rx. The remaining 18 (30%) ST131 isolates belonged to other non *H30* subclones. *H30* subclone was significantly higher in the virulence carriage in comparison to non *H30* ST131 subclones.
Conclusion: This study reported the prevalence and traits of clinical *E. coli* ST131 main subclones in Saudi Arabia. It also demonstrated the high prevalence of *E. coli* ST131 locally, and found different virulence genotypes and antimicrobial resistance phenotypes among ST131 subclones. In the future, preforming whole genome sequence-based studies on ST131 and its subclones is crucial to elucidate factors that drive the success of these organisms.

1. Introduction

Extraintestinal infections, usually urinary tract infections (UTIs), represent a main source of morbidity and mortality globally (Foxman, 2010). *Escherichia coli* (*E. coli*) is reported to be the key cause of UTIs (Farajnia et al., 2009), and the uropathogenic *E. coli* (UPEC), a subset of extraintestinal pathogenic *E. coli* (ExPEC) group, is associated with causing more than 80% of all UTIs (Ronald, 2002). Over the past twenty years, the antimicrobial resistance of ExPEC to first-line antibiotics, such as ciprofloxacin and amoxicillin, has increased markedly (Foxman, 2010). Additionally, an increased extended spectrum β-lactamase (ESBL) carriage has been reported among ExPEC strains (Pitout et al., 2005b).

As reported in 2008, *E. coli* sequence type 131 (*E. coli* ST131) was described among ESBL-producing *E. coli* in many continents throughout the world, and it has disseminated to become the predominant ExPEC clone that drives multidrug resistance (MDR) globally (Nicolas-Chanoine et al., 2014). *E. coli* ST131 is associated with fluoroquinolone (FQ)-resistance and CTX-M production, and is considered a main source of the spread of CTX-M-15 ESBL gene variant (Nicolas-Chanoine et al., 2008; Peirano and Pitout, 2010). *E. coli* ST131 belongs to the phylogroup B2 (Nicolas-Chanoine et al., 2008), and to the serotype O25b:H4 (Nicolas-Chanoine et al., 2014), although some ST131 members of serotype O16:H5

have been identified (Blanc et al., 2014). ST131 harbors the *fimH* gene that associated with a remarkable degree of allelic diversity, and *fimH30* is the dominant *fimH* type (Nicolas-Chanoine et al., 2014). ST131 *H30* subclone includes two important subsets (*H30*R and *H30*Rx) (Johnson et al., 2013; Mathers et al., 2015; Nicolas-Chanoine et al., 2014). The *H30R* isolates commonly display fluoroquinolone resistance (Sauget et al., 2016), while the *H30*Rx isolates frequently produce CTX-M ESBLs (Nicolas-Chanoine et al., 2014; Sauget et al., 2016).

With regard to virulence potential of ST131, although previous reports showed ST131 with moderate virulence capacity compared to other major ExPEC STs such as ST73 and ST127 (Alghoribi et al., 2015; Gibreel et al., 2012), high virulence potential among ST131 isolates has been identified (Coelho et al., 2010). In Saudi Arabia, few studies on the molecular epidemiology of MDR ExPEC isolates have been performed (Abd El Ghany et al., 2018; Alghoribi et al., 2015; Alyamani et al., 2017; Yasir et al., 2018). Most reports have found that *E. coli* ST131 was the most dominant ST among the tested ExPEC isolates, however the overall distribution of ExPEC STs was greatly variable (Alghoribi et al., 2015; Alyamani et al., 2017; Yasir et al., 2018). Nonetheless, information on the local prevalence of *E. coli* ST131 subclones and their virulence capacity is very scarce.

Recent work conducted in our laboratory has investigated the antibiogram and ESBL carriage of 100 clinical UPEC isolates, and demonstrated that 67% of these isolates were MDR, that 33% of isolates were ESBL-producing and that the CTX-M-15 was the predominant CTX-M variant (Alqasim et al., 2018). Here we determined and compared various molecular traits of non-ESBL-producing and ESBL-producing UPEC isolates including their ST131 status, phylogenetic groups and virulence capacity. We further sought to characterize the *E. coli* ST131 isolates to identify subclones they belong to, and to compare between these subclones in terms of ESBL carriage, phylogenetic distribution and virulence potential.

2. Methods

2.1. Isolation of bacterial strains

In our research work, sixty *E. coli* urine isolates, thirty ESBL-producing and thirty non ESBL-producing, were involved and these isolates were obtained between January and March of 2018, from urine specimens of in-patients hospitalised at a tertiary hospital in Riyadh, Saudi Arabia. The antibiotic susceptibility patterns and ESBL gene carriage of these isolates have been previously described (Alqasim et al., 2018). Table 1 shows information on the ESBL-producing *E. coli* isolates.

2.2. Phylogenetic grouping

Phylogenetic groups were identified by multiplex polymerase chain reaction (PCR) as recommended by Clermont et al (Clermont et al., 2013).

2.3. Screening for E. coli ST131 and identification of its subclones

Screening for *E. coli* ST131 status was performed using a triplex PCR assay that combines the identification of O25b and O16 ST131 clades using the primer sets and PCR conditions previously published (Clermont et al., 2009; Johnson et al., 2014; Lacher et al., 2007). Afterwards, *E. coli* ST131 isolates belonging to *H30* subclone and the *H30*Rx subset were determined using allele-specific PCRs (Banerjee et al., 2013; Colpan et al., 2013).

Table 1
Details on ESBL-producing *E. coli* isolates used in this study.

Isolate ID	FQ phenotype[a]	MDR phenotype[b]	ESBL gene type(s)			Reference
			CTX-M-15	TEM	OXA	Alqasim et al. (2018)
U1	S	+	+	–	–	
U4	R	+	+	–	–	
U7	R	+	+	–	–	
U9	R	+	+	–	–	
U10	R	+	+	–	–	
U12	R	+	+	–	–	
U15	R	+	+	–	–	
U16	S	+	+	–	–	
U20	R	+	+	–	–	
U24	R	+	+	–	–	
U27	R	+	+	–	–	
U28	R	+	+	–	+	
U31	R	+	+	–	+	
U46	R	+	+	–	+	
U55	R	+	+	+	+	
U57	R	+	+	–	–	
U63	R	+	+	–	+	
U66	R	+	+	+	–	
U68	S	+	+	–	–	
U71	S	+	+	–	–	
U73	R	+	+	–	+	
U74	R	+	+	–	–	
U75	S	+	–	+	–	
U78	R	+	+	–	–	
U82	R	+	+	+	+	
U85	R	+	+	+	+	
U87	R	+	+	–	+	
U93	S	+	+	+	–	
U95	R	+	–	+	+	
U98	R	+	+	+	–	

[a] R: resistant, S: susceptible.
[b] MDR phenotype refers to displaying resistance to at least 1 antibiotic in ≥3 antibiotic groups (Magiorakos et al., 2012).

2.4. Detection of virulence-associated gene (VAG) carriage

The presence of 20 VAGs was tested for all isolates using established PCR-based assays described previously (Bauer et al., 2002; Johnson et al., 2000; Johnson and Stell, 2000; Johnson et al., 2006; Restieri et al., 2007). The virulence scores of isolates were calculated as previously shown (Oteo et al., 2014). ExPEC status of all isolates was also identified using procedure from the previous reference study Pitout et al. (2005a).

2.5. Statistical analysis

IBM SPSS software (version 21.0) was employed for data analysis. Comparison study in different groups was conducted using Fisher's exact test (FET) and virulence scores were estimated by Mann-Whitney *U* test. *P* value of ≤0.05 used as threshold for statistical significance.

3. Results

3.1. Characteristics of ESBL-producing and non ESBL-producing E. coli isolates

Of all the 60 *E. coli* isolates, 6 (10%) were from phylogroup A, 5 (8.3%) from group B1, 37 (61.7%) from group B2, 0 (0%) from group C, 10 (16.7%) from group D, 0 (3.3%) from group E and 2 (3.3%) from group F (Table 2). The association between phylogroup B2 and ESBL-producing isolates (*P* = 0.007) was statistically significant, however groups A and B1 were significantly found among non ESBL-producing *E. coli* isolates (Table 2).

Table 2
Characteristics of *E. coli* isolates in relation to ESBL carriage.

Category	Specific trait	Number of isolates (%)			*P* value[a]
		ESBL-producing (*n* = 30)	Non-ESBL-producing (*n* = 30)	Total (*n* = 60)	
Phylogroups	A	0 (0%)	6 (20%)	6 (10%)	**0.02**
	B1	0 (0%)	5 (16.7%)	5 (8.3%)	**0.05**
	B2	24 (80%)	13 (43.3%)	37 (61.7%)	**0.007**
	C	0 (0%)	0 (0%)	0 (0%)	1.000
	D	6 (20%)	4 (13.3%)	10 (16.7%)	0.73
	E	0 (0%)	0 (0%)	0 (0%)	1.000
	F	0 (0%)	2 (6.7%)	2 (3.3%)	0.49
ST131 isolates	ST131	24 (80%)	13 (43.3%)	37 (61.7%)	**0.007**
	ST131 O25b	24 (80%)	13 (43.3%)	37 (61.7%)	**0.007**
	ST131 O16	0 (0%)	0 (0%)	0 (0%)	1.000
ST131 subclones	ST131 *H30*	14 (46.7%)	5 (16.7%)	19 (31.7%)	**0.03**
	*H30*Rx	7 (23.3%)	2 (6.7%)	9 (15%)	0.15
	Non *H30*Rx	7 (23.3%)	3 (10%)	10 (16.7%)	0.3
	ST131 non- *H30*	10 (33.3%)	8 (26.7%)	18 (30%)	0.8
FQ phenotype	FQ R[b]	24 (80%)	12 (40%)	36 (60%)	**0.003**
MDR phenotype	MDR	30 (100%)	15 (50%)	45 (75%)	**<0.001**
Adhesions	*papA*	8 (26.7%)	6 (20%)	14 (23.3%)	0.76
	papC	8 (26.7%)	6 (20%)	14 (23.3%)	0.76
	papG allele I	0 (0%)	0 (0%)	0 (0%)	1.000
	papG allele II	7 (23.3%)	5 (16.7%)	12 (20%)	0.75
	sfa/focDE	2 (6.7%)	2 (6.7%)	4 (6.7%)	1.000
	afa/draBC	9 (30%)	3 (10%)	12 (20%)	0.10
	fimH	29 (96.7%)	24 (80%)	53 (88.3%)	0.10
	iha	19 (63.3%)	14 (46.7%)	33 (55%)	0.30
Siderophores	*iutA*	23 (76.7%)	20 (66.7%)	43 (71.6%)	0.57
	iroN$_{E.\ coli}$	2 (6.7%)	9 (30%)	11 (18.3%)	**0.04**
Toxins	*hlyA*	6 (20%)	4 (13.3%)	10 (16.7%)	0.73
	cnf1	3 (10%)	3 (10%)	6 (10%)	1.000
Polysaccharide coatings	*kpsMT* II	17 (56.7%)	14 (46.7%)	31 (51.7%)	0.60
	kpsMT III	0 (0%)	2 (6.7%)	2 (3.3%)	0.49
Miscellaneous	*PAI*	19 (63.3%)	9 (30%)	28 (46.7%)	**0.02**
	cvaC	1 (3.3%)	4 (13.3%)	5 (8.3%)	0.35
	traT	20 (66.7%)	18 (60%)	38 (63.3%)	0.79
	ompT	13 (43.3%)	11 (36.7%)	24 (40%)	0.79
	usp	17 (56.7%)	11 (36.7%)	28 (46.7%)	0.19
	sat	17 (56.7%)	12 (40%)	29 (48.3%)	0.30
	ExPEC[c]	23 (76.7%)	15 (50%)	38 (63.3%)	0.06
	Virulence scores[d]	6.8 (1–11)	5.5 (1–13)	6.2 (1–13)	0.09

The bold numbers refer to presence of significant difference between groups for some traits.
[a] *P* values for 2-group comparison: ESBL and non ESBL.
[b] FQ resistant.
[c] Number of isolates showing ExPEC status.
[d] Median number of virulence factors (range).

Our data found that 37 of 60 (61.7%) isolates belonged to the ST131 clone (Table 2). Of these, 24 (80%) were ESBL-producing while only 13 (43.3%) were non ESBL-producing, and this difference was significant (*P* = 0.007). Additionally, screening the O16 and O25b ST131 clades revealed that all the 37 *E. coli* ST131 isolates (100%) were from O25b clade. Subclone typing of ST131 isolates showed that the *H30* was the most prevalent subclone, with 19 of 60 (31.7%) isolates were *H30*, while 18 (30%) belonged to subclones other than non *H30*. Among *H30* isolates, 10 (16.7%) were from *H30* non-Rx whereas 9 (15%) belonged to *H30*Rx subset.

We also found that ESBL-producing isolates were more capable of displaying FQ resistance and MDR phenotypes compared to non ESBL-producing isolates, and there was a statistically significant difference between ESBL-producing and non producing isolates in terms of FQ resistance (*P* = 0.003) and MDR phenotype (*P* ≤ 0.001) (Table 2).

Considering the virulence capacity of all *E. coli* isolates, the most commonly expressed VAGs were *fimH*, *iutA*, *iha*, *traT* and *kpsMTII*, **and these were found** in ≥50% of the isolates. However, all *E. coli* isolates were negative for *papG* allele I. There was a slight difference in the virulence carriage between ESBL-producing and non ESBL-producing isolates, and the median virulence scores (ranges) were 6.8 (1–11) for ESBL-producing isolates and 5.5 (1–13) for non ESBL-producing isolates (Table 2).

3.2. Characteristics of E. coli ST131 and non ST131 isolates

All 37 *E. coli* ST131 isolates (100%) were from B2 group. Most of the non ST131 isolates derived from group D followed by group A, while none of these isolates were derived from group B2. ST131 isolates were significantly associated with group B2 (*P* ≤ 0.001) (Table 3).

With regard to ESBL carriage, ST131 isolates were higher in their ability to carry ESBL genes than non-ST131 isolates. Our data found that 24 of 37 (64.9%) ST131 isolates were ESBL producers while ESBL production was only detected in 6 of 23 (26.1%) of non ST131 isolates, and the overall difference in ESBL carriage between ST131 and non ST131 isolates was significant (*P* = 0.007). ST131 isolates were significantly higher in their ability to exhibit FQ resistance than non ST131 isolates (*P* ≤ 0.001), while the difference between these groups in showing MDR phenotype was insignificant (*P* = 0.07) (Table 3).

With respect to virulence capacity, compared to non ST131 isolates, *E. coli* ST131 isolates were linked to higher VAG carriage. ST131 isolates were significantly associated with carrying 8 VAGs, *papA*, *papC*, *papG* allele II, *fimH*, *iha*, *hlyA*, *PAI*, *ompT*, *usp* and *sat*, compared to non ST131 isolates. The median virulence scores (ranges) were 7.5 (1–13) for ST131 isolates and 4 (1–7) for non ST131 isolates, and this difference was found to be significant

Table 3
Characteristics of *E. coli* isolates in relation to clonal group.

| Category | Specific trait | Number of isolates (%) | | | P value[a] |
		ST131 (*n* = 37)	Non-ST131 (*n* = 23)	Total (*n* = 60)	
Phylogroups	A	0 (0%)	6 (26.1%)	6 (10%)	**0.002**
	B1	0 (0%)	5 (21.7%)	5 (8.3%)	**0.006**
	B2	37 (100%)	0 (0%)	37 (61.7%)	**<0.001**
	C	0 (0%)	0 (0%)	0 (0%)	1.000
	D	0 (0%)	10 (43.5%)	10 (16.7%)	**<0.001**
	E	0 (0%)	0 (0%)	0 (0%)	1.000
	F	0 (0%)	2 (8.7%)	2 (3.3%)	0.15
ESBL type(s)	ESBL producing	24 (64.9%)	6 (26.1%)	30 (50%)	**0.007**
	CTX-M-15	12 (32.4%)	3 (13%)	15 (25%)	0.13
	CTX-M-15 + TEM	1 (2.7%)	2 (8.7%)	3 (5%)	0.55
	CTX-M-15 + OXA	7 (18.8%)	0 (0%)	7 (11.7%)	**0.04**
	CTX-M-15 + TEM + OXA	3 (8.1%)	0 (0%)	3 (5%)	0.28
	TEM	0 (0%)	1 (4.3%)	1 (1.7%)	0.38
	TEM + OXA	1 (2.7%)	0 (0%)	1 (1.7%)	0.38
	Non-ESBL	13 (35.1%)	17 (73.9%)	30 (50%)	**0.007**
FQ phenotype	FQ R	35 (94.6%)	2 (8.7%)	37 (61.7%)	**<0.001**
MDR phenotype	MDR	31 (83.7%)	14 (60.9%)	45 (75%)	0.07
Adhesions	*papA*	13 (35.1%)	1 (4.3%)	14 (23.3%)	**0.005**
	papC	13 (35.1%)	1 (4.3%)	14 (23.3%)	**0.005**
	papG allele I	0 (0%)	0 (0%)	0 (0%)	1.000
	papG allele II	12 (32.4%)	0 (0%)	12 (20%)	**0.002**
	sfa/focDE	3 (8.1%)	1 (4.3%)	4 (6.7%)	1.000
	afa/draBC	9 (24.3%)	3 (13%)	12 (20%)	0.34
	fimH	36 (97.3%)	17 (73.9%)	53 (88.3%)	**0.01**
	iha	25 (67.6%)	8 (34.8%)	33 (55%)	**0.02**
Siderophores	*iutA*	29 (78.4%)	14 (60.9%)	43 (71.6%)	0.24
	iroN$_{E.\ coli}$	6 (16.2%)	5 (21.7%)	11 (18.3%)	0.73
Toxins	*hlyA*	10 (27%)	0 (0%)	10 (16.7%)	**0.009**
	cnf1	6 (16.2%)	0 (0%)	6 (10%)	0.07
Polysaccharide coatings	*kpsMT* II	23 (62.2%)	8 (34.7%)	31 (51.7%)	0.06
	kpsMT III	0 (0%)	2 (8.7%)	2 (3.3%)	0.14
Miscellaneous	*PAI*	28 (75.7%)	0 (0%)	28 (46.7%)	**<0.001**
	cvaC	1 (2.7%)	4 (17.4%)	5 (8.3%)	0.07
	traT	23 (62.2%)	15 (65.2%)	38 (63.3%)	1.000
	ompT	20 (74.1%)	4 (17.4%)	24 (40%)	**0.007**
	usp	26 (70.3%)	2 (8.7%)	28 (46.7%)	**<0.001**
	sat	22 (59.5%)	7 (30.4%)	29 (48.3%)	**0.04**
	ExPEC[b]	29 (78.4%)	9 (39.1%)	38 (63.3%)	**0.005**
	Virulence scores[c]	7.5 (1–13)	4 (1–7)	6.2 (1–13)	**<0.001**

The bold numbers refer to presence of significant difference between groups for some traits.
[a] *P* values (by Fisher's exact test) are for 2-group comparison: ST131 and non ST131.
[b] Number of isolates showing ExPEC status.
[c] Median number of virulence factors (range).

($P \leq 0.001$). The number of isolates showing ExPEC status was also significantly higher among ST131 isolates than that of non ST131 isolates ($P = 0.005$) (Table 3).

3.3. Characteristics of E. coli ST131 subclones

ESBL carriage was detected in 14 of 19 (73.7%) *H30* isolates [7 (77.8%) *H30*Rx and 7 (70%) *H30* non-Rx], however ESBL production was found in 10 of 18 (55.6%) non *H30* isolates. Our data found that 19 *H30* isolates (100%) were FQ resistant while 16 (88.9%) of non *H30* isolates were FQ insusceptible. In contrast, MDR phenotype was more predominant in non *H30* compared to *H30* isolates. The difference between various ST131 subclones in their ESBL carriage, FQ phenotype and MDR phenotype remained insignificant (Table 4).

Considering the virulence capacity of ST131 subclones, each of *H30* subsets (*H30*Rx and non-Rx) was significantly associated with 5 VAGs, *iha*, *iutA*, *PAI*, *traT* and *usp*, compared to non *H30* (Table 4). Additionally, *H30* non-Rx subset was significantly associated with carrying the *sat* gene in comparison to non *H30*. Generally, *H30* non-Rx subset was the highest in terms of the virulence capacity compared to *H30*Rx and to non *H30*. The median virulence scores (ranges) were 9.3 (7–12) for non-Rx isolates, 8.9 (7–12) for

ST131 *H30*Rx isolates and 5.9 (1–13) for non *H30* isolates. Our data also found a significant difference in the median virulence score between *H30* non-Rx and non *H30* isolates ($P = 0.009$). The number of isolates showing ExPEC status was also significantly higher among *H30* non-Rx compared to non *H30* ($P = 0.03$) (Table 4).

4. Discussion

E. coli ST131 has currently emerged as a major rapidly disseminated pandemic *E. coli* clone worldwide, with a high capability of causing extraintestinal infections, particularly UTIs (Nicolas-Chanoine et al., 2014). Additionally, ST131 *H30* subclone known to be a major factor for MDR ExPEC illnesses (Johnson et al., 2017). This study explored the genotypic traits of *E. coli* urine isolates from Riyadh, Saudi Arabia, and provided comparisons between ESBL-producing and non-producing *E. coli* isolates, between *E. coli* ST131 and non ST131 isolates, and between different *E. coli* ST131 subclones.

Here we showed that most of our *E. coli* were derived from phylogenetic B2 group, this concurs with earlier reports showing the dominance of this phylogroup among ExPEC isolates that cause UTIs (Johnson and Stell, 2000). However, we found some isolates that derived from other groups such as D, A and B1. A previous

Table 4
Characteristics of isolates belonging to various *E. coli* ST131 subclones.

Category	Specific trait	ST131 subclones, number of isolates (%)				P value[a]		
		*H30*Rx (9)	*H30* non- Rx (10)	Non *H30* (18)	Total (37)	*H30*Rx vs *H30* non-Rx	*H30*Rx vs Non *H30*	*H30* non-Rx vs Non *H30*
ESBL type(s)	ESBL producing	7 (77.8%)	7 (70%)	10 (55.6%)	24 (64.9%)	1.000	0.41	0.69
	CTX-M-15	3 (33.3%)	5 (50%)	4 (22.2%)	12 (32.4%)	0.64	0.38	0.08
	CTX-M-15 + TEM	0 (0%)	0 (0%)	1 (5.55%)	1 (2.7%)	1.000	1.000	1.000
	CTX-M-15 + OXA	2 (22.2%)	2 (20%)	3 (16.7%)	7 (18.9%)	1.000	1.000	1.000
	CTX-M-15 + TEM + OXA	2 (22.2%)	0 (0%)	1 (5.55%)	3 (8.1%)	0.47	0.28	1.000
	TEM	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.000	1.000	1.000
	TEM + OXA	0 (0%)	0 (0%)	1 (5.55%)	1 (2.7%)	1.000	1.000	1.000
	Non-ESBL	2 (22.2%)	3 (30%)	8 (44.4%)	13 (35.1%)	1.000	0.41	0.69
FQ phenotype	FQ R	9 (100%)	10 (100%)	16 (88.9%)	35 (94.6%)	1.000	0.54	0.52
MDR phenotype	MDR	7 (77.8%)	8 (80%)	16 (88.9%)	31 (83.7%)	1.000	0.58	0.60
Adhesions	*papA*	3 (33.3%)	3 (30%)	7 (38.9%)	13 (35.1%)	1.000	1.000	0.70
	papC	3 (33.3%)	3 (30%)	7 (38.9%)	13 (35.1%)	1.000	1.000	0.70
	papG allele I	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.000	1.000	1.000
	papG allele II	3 (33.3%)	3 (30%)	6 (33.3%)	12 (32.4%)	1.000	1.000	1.000
	sfa/focDE	0 (0%)	1 (10%)	2 (11.1%)	3 (8.1%)	1.000	0.54	1.000
	afa/draBC	2 (22.2%)	4 (40%)	3 (16.7%)	9 (24.3%)	0.63	1.000	0.21
	fimH	9 (100%)	10 (100%)	17 (94.4%)	36 (97.3%)	1.000	1.000	1.000
	iha	9 (100%)	10 (100%)	6 (33.3%)	25 (67.6%)	1.000	**0.001**	**0.0009**
Siderophores	*iutA*	9 (100%)	10 (100%)	10 (55.6%)	29 (78.4%)	1.000	**0.03**	**0.03**
	iroN$_{E.\ coli}$	0 (0%)	1 (10%)	5 (27.8%)	6 (16.2%)	1.000	0.14	0.37
Toxins	*hlyA*	1 (11.1%)	3 (30%)	6 (33.3%)	10 (27%)	0.58	0.36	1.000
	cnf1	1 (11.1%)	3 (30%)	2 (11.1%)	6 (16.2%)	0.58	1.000	0.32
Polysaccharide coatings	*kpsMT* II	5 (55.6%)	8 (80%)	10 (55.6%)	23 (62.2%)	0.35	1.000	0.25
	kpsMT III	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.000	1.000	1.000
Miscellaneous	*PAI*	9 (100%)	10 (100%)	9 (50%)	28 (75.7%)	1.000	**0.01**	**0.001**
	cvaC	0 (0%)	0 (0%)	1 (5.6%)	1 (2.7%)	1.000	1.000	1.000
	traT	9 (100%)	8 (80%)	6 (33.3%)	23 (62.2%)	0.47	**0.001**	**0.05**
	ompT	7 (77.8%)	4 (40%)	9 (50%)	20 (74.1%)	0.17	0.23	0.71
	usp	9 (100%)	9 (90%)	8 (44.4%)	26 (70.3%)	1.000	**0.009**	**0.04**
	sat	6 (66.7%)	10 (100%)	6 (33.3%)	22 (59.5%)	0.09	0.12	**0.0009**
	ExPEC[b]	8 (88.9%)	10 (100%)	11 (61.1%)	29 (78.4%)	0.47	0.2	**0.03**
	Virulence scores[c]	8.9 (7–12)	9.3 (7–12)	5.9 (1–13)	7.5 (1–13)	0.65	**0.03**	**0.009**

The bold numbers refer to presence of significant difference between groups for some traits.
[a] *P* values for 3-group comparisons: *H30*Rx, *H30* non-Rx and non *H30*.
[b] Number of isolates showing ExPEC status.
[c] Median number of virulence factors (range).

report described the occurrence of significant shift toward other phylogenetic groups, particularly A and D, among FQ resistant and/or ESBL producing *E. coli* isolates (Johnson et al., 2003), and this might provide an explanation of the phylogenetic diversity detected among our *E. coli* isolates.

It was also found that ST131 clone accounted for 37 of 60 (61.7%) isolates, and this was higher compared to earlier local reports showing that the prevalence of ST131 isolates ranged between 17.3 and 37.5% (Alghoribi et al., 2015; Alyamani et al., 2017; Yasir et al., 2018). Additionally, we demonstrated a significant association between ST131 isolates and ESBL-production, which is similar to a previous local finding (Alghoribi et al., 2015). Phylogenetically, all ST131 isolates identified in this study were derived from group B2, which agrees with many reports globally (Johnson et al., 2017; Nicolas-Chanoine et al., 2014). The high local dissemination of this clone reported here merits detailed future investigation to understand the reasons behind its success over other major STs.

Subclone typing of *E. coli* ST131 isolates was identified, with *H30* being the most common ST131 subclone. This was concordant with a previous local study (Alghoribi et al., 2015), and with other reports from China (Li et al., 2017) and United States (Johnson et al., 2017). Of the *H30* isolates, 10 (16.7%) were members of *H30* non-Rx subset while 9 (15%) belonged to *H30*Rx subset. However, there is no previous report on the prevalence of different *H30* subsets in Saudi Arabia. Our ST131 subclone typing results concur with recent findings from China (Li et al., 2017) and United States

(Johnson et al., 2017) showing that the prevalence of *H30* non-Rx subset was higher that of *H30*Rx. As given the vast number of our *E. coli* ST131 isolates are FQ resistant (94.6%), the dominance of *H30* non-Rx subset among all *H30* members is expected since the *H30* subclone, particularly non-Rx subset, comprises most of the FQ-resistant ST131 isolates (Sauget et al., 2016). Additionally, we also found a strong association between ST131 *H30*Rx subset and CTX-M-15 production, which is consistent with many previous reports showing the frequent association between the *H30*Rx subset and CTX-M-15 carriage (Nicolas-Chanoine et al., 2014; Sauget et al., 2016).

Our data showing high virulence carriage of ESBL producers is in contrary to the previous finding showing that antimicrobial resistance was linked to a great reduction in virulence (Johnson et al., 2003). Additionally, given that 80% of ESBL producers in this study were FQ-resistant, this high virulence among ESBL-producing isolates seems to disagree with previous reports demonstrating higher virulence potential among FQ-susceptible ExPEC isolates compared to FQ-resistant isolates and ESBL-producing isolates (Johnson et al., 2017).

E. coli ST131 isolates tested here were significantly higher in terms of virulence score compared to non ST131 isolates, and this is in contrary to a previous local study showing ST131 isolates with a moderate virulence capacity compared to other ExPEC STs (Alghoribi et al., 2015). However, it concurs with previous reports showing high virulence potential of ST131 isolates (Coelho et al., 2010).

Among ST131 subclones, we found that the *H30* isolates were generally higher than non *H30* isolates in terms of the overall virulence carriage. Within *H30* subclone, *H30* non-Rx subset showed a slightly higher virulence score than *H30*Rx subset, and this disagrees with previous reports showing that the virulence carriage of *H30R*x isolates was higher than *H30* non-Rx isolates (Johnson et al., 2017; Li et al., 2017). The finding of our study showing distinctive virulence genotypes and antimicrobial resistance phenotypes among different ST131 subclones highlights the need of testing the traits of these subclones individually instead of considering them as one ST131 group only.

Our study has limitations; firstly the number of isolates used here was low compared to some molecular epidemiological studies on *E. coli*, and this might be a caveat to our findings. Additionally, it was performed on a population from Riyadh and this might not reflect the prevalence of *E. coli* ST131 and its subclones in other regions within Saudi Arabia.

In conclusion, we provided the first local description of prevalence and traits of clinical *E. coli* ST131 main subclones. Our study showed high prevalence of ST131 among UPEC isolates locally. More importantly, it also demonstrated the presence of distinctive virulence genotypes and antimicrobial resistance phenotypes among different ST131 subclones, with ST131 *H30* isolates showing the highest virulence potential, ESBL carriage and FQ resistance. Given that the ST131 *H30* subclone has currently disseminated as a major cause of MDR infections, it would be essential to use rapid diagnostic tests to identify *E. coli* ST131 and its important subclones in the future. These tests can provide results in <1 h, therefore allowing the guidance for empirical antibiotic selection. Additionally, carrying out comparative whole genome sequencing-based studies on ST131 and its subclones will significantly enhance our knowledge of factors that drive the success of these organisms.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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