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Original article

Comparative phenotypic characterization identifies few differences in the metabolic capacity between *Escherichia coli* ST131 subclones

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ABSTRACT

Background: Extraintestinal pathogenic *Escherichia coli* (ExPEC) is responsible for causing many infections such as urinary tract infections (UTIs). The current dissemination of the multidrug resistant (MDR) ExPEC clone, *Escherichia coli* sequence type 131 (*E. coli* ST131), poses a real threat to public health worldwide. This study aimed to determine and compare the metabolic capacity of a collection of ExPEC isolates including ST131, non-ST131 and various ST131 subclones, and sought to assess the association between antimicrobial resistance and metabolic capacity of ST131 isolates.

Methods: The metabolic activity of forty urine *E. coli* isolates, collected from in-patients hospitalized at tertiary hospital in Riyadh, was tested using KB009 Hi carbohydrate kit, and then statistically analysed to assess the difference in the metabolic profiles between ST131 and non-ST131 isolates, and between ST131 subclones.

Results: The data of this study found almost similar metabolic profiles between ST131 and non-ST131, suggesting that ST131 is not a metabolically unique clone of ExPEC. There was also no link between antimicrobial susceptibility profiles and high metabolic capacity of ST131 isolates. Testing the biochemical activity of isolates belonging to ST131 subclones found higher activity of *H30* subclone than non-*H30* isolates, however it revealed few significant differences between these subclones.

Conclusion: This study demonstrated no difference in the metabolism of ST131 and non-ST131, although it uncovered the presence of few significant differences in the metabolic capacity between ST131 subclones. Carrying out whole-genome based studies on ST131 and its main subclones is essential to elucidate the genetic factors responsible for the success of particular ST131 subclones.

1. Introduction

Escherichia coli (*E. coli*) is the leading cause of many serious illnesses such as urinary tract infections (UTIs), and approximately 80% of UTIs are due to extraintestinal pathogenic *E. coli* (ExPEC) isolates (Kaper et al., 2004). The antimicrobial resistance of ExPEC to several first-line agent, such as cephalosporins and fluoroquinolones, has increased globally (Foxman, 2010). Additionally, the prevalence of extended spectrum β-lactamases (ESBLs), particularly CTX-M family, in ExPEC has increased substantially (Pitout et al., 2005).

E. coli sequence type 131 (*E. coli* ST131) has now been the most prevalent ExPEC clone that is responsible for the global widespread multidrug resistance (MDR) (Nicolas-Chanoine et al., 2014). *E. coli* ST131 is frequently resistant to fluoroquinolone (FQ) and commonly associated with CTX-M production, particularly CTX-M-15 ESBL variant (Nicolas-Chanoine et al., 2008; Peirano and Pitout, 2010). Furthermore, recent studies have demonstrated high virulence gene carriage of ST131 compared to other main ExPEC STs (Alqasim et al., 2020a, 2020b). ST131 isolates possess the *fimH* gene, which is associated a great allelic diversity, and *fimH30* is the most common *fimH* type (Nicolas-Chanoine et al., 2014). ST131 *H30* subclone involves nearly all fluoroquinolone resistant ST131 isolates, and it has two important MDR subsets, *H30*R and *H30*Rx. *H30*Rx accounts for approximately all CTX-M-15-producing ST131 isolates. However, isolates that harbour allele *H30* but do not carry CTX-M-15 are frequently grouped in the *H30*R or non-Rx subclone (Nicolas-Chanoine et al., 2014).

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With regard to the relationship between metabolism and pathogenesis, previous reports have proposed the role of high metabolic capacity in enhancing bacterial fitness, leading to increased pathogenesis. For instance, it has been found that sugar metabolism (Le Bouguénec and Schouler, 2011) and specific metabolic enzymes (Pancholi and Chhatwal, 2003) may enhance bacterial virulence.

Previous work by our research group has demonstrated that ST131 accounted for 61.7% of all *E. coli* urine isolates, 64.9% of which were ESBL-producing, and that the virulence capacity of ST131 was higher compared to non-ST131 isolates (Alqasim et al., 2020b). Given that information on the metabolic traits of ExPEC isolates in Saudi Arabia is very scarce, this study aimed to determine and compare the metabolic potential of ExPEC isolates, including ESBL-producing and non ESBL-producing isolates, ST131 and non-ST131 isolates and those belonging to different ST131 subclones. It also sought to assess the association between antimicrobial resistance and metabolic capacity of ST131 isolates.

2. Materials and methods

2.1. Bacterial isolates

A total of forty *E. coli* isolates, obtained from urine specimens of in-patients hospitalized at a tertiary healthcare facility in Riyadh, Saudi Arabia, were included in this study. Antimicrobial susceptibility profiles, ESBL carriage, phylogenetic grouping, ST131 status and virulence capacity of these isolates have previously been described (Alqasim et al., 2018, 2020b). Table 1 shows details on these *E. coli* isolates.

2.2. Metabolic profiling assays

KB009 Hi carbohydrate kit (HiMedia, India), that comprises thirty-five biochemical tests (Table 2), was used to for metabolic profiling of *E. coli* isolates. This kit allows the identification of bacterial metabolic activity through measurement of carbohydrate utilization. The preparation of bacterial suspensions and inoculation of test cupules were carried out according to the manufacturer's instructions. The assays were performed in duplicate on two independent occasions giving entirely similar results.

2.3. Statistical analysis

IBM SPSS (version 21.0) software was employed to carry out statistical analysis. Comparisons of different groups were conducted using Fisher's exact test (FET) and mean biochemical scores (mean bio scores) were determined by Mann-Whitney *U* test. *P* value of $\leq$0.05 was used as threshold for statistical significance.

Table 1
Information on the *E. coli* isolates used in this study.

Isolate ID	MDR[a]	ESBL	ESBL type(s)	ST131	Reference
U1	MDR	+	CTX-M-15	–	Alqasim et al. (2018, 2020b)
U4	MDR	+	CTX-M-15	+	
U7	MDR	+	CTX-M-15	+	
U9	MDR	+	CTX-M-15	+	
U10	MDR	+	CTX-M-15	+	
U12	MDR	+	CTX-M-15	+	
U15	MDR	+	CTX-M-15 & OXA	+	
U16	MDR	+	CTX-M-15	–	
U20	MDR	+	CTX-M-15	+	
U24	MDR	+	CTX-M-15	+	
U27	MDR	+	CTX-M-15	+	
U28	MDR	+	CTX-M-15 & OXA	+	
U46	MDR	+	CTX-M-15 & OXA	+	
U55	MDR	+	CTX-M-15, OXA & TEM	+	
U57	MDR	+	CTX-M-15	+	
U68	MDR	+	CTX-M-15	–	
U75	MDR	+	TEM	–	
U78	MDR	+	CTX-M-15	+	
U93	MDR	+	CTX-M-15 & TEM	–	
U98	MDR	+	CTX-M-15 & TEM	–	
U3	Non-MDR	–	NA[b]	–	
U6	Non-MDR	–	NA	–	
U19	Non-MDR	–	NA	+	
U22	MDR	–	NA	–	
U23	Non-MDR	–	NA	–	
U25	MDR	–	NA	–	
U29	Non-MDR	–	NA	–	
U30	Non-MDR	–	NA	–	
U32	Non-MDR	–	NA	–	
U33	MDR	–	NA	–	
U34	MDR	–	NA	–	
U35	Non-MDR	–	NA	–	
U36	MDR	–	NA	–	
U37	Non-MDR	–	NA	–	
U38	Non-MDR	–	NA	+	
U42	Non-MDR	–	NA	–	
U43	Non-MDR	–	NA	+	
U56	Non-MDR	–	NA	+	
U61	MDR	–	NA	+	
U67	MDR	–	NA	+	

[a] MDR phenotype refers to displaying resistance to at least 1 antibiotic in $\geq$3 antibiotic groups (Magiorakos et al., 2012).
[b] NA: Not applicable.

Table 2
List of biochemical tests used in this study.

Test	Abbreviation
Lactose	LAC
Xylose	XYL
Maltose	MAL
Fructose	FRU
Dextrose	DEX
Galactose	GAL
Raffinose	RAF
Trehalose	TRE
Melibiose	MEL
Sucrose	SUC
L-Arabinose	LARA
Mannose	MNE
Inulin	INU
Sodium gluconate	SG
Glycerol	GLY
Salicin	SAL
Dulcitol	DUL
Inositol	INO
Sorbitol	SOR
Mannitol	MAN
Adonitol	ADO
Arabitol	ARA
Erythritol	ERY
Alpha-Methyl-D-glucoside	α-MG
Rhamnose	RHA
Cellobiose	CEL
Melezitose	MEZ
Alpha-Methyl-D-mannoside	α-MN
Xylitol	XYT
Ortho-Nitrophenyl-β-galactoside	ONPG
Esculin	ESC
D-Arabinose	DARA
Citrate	CIT
Malonate	MNT
Sorbose	SOR

3. Results

3.1. Metabolic activity of all E. coli isolates

All *E. coli* isolates were capable of utilizing nine substrates: lactose, fructose, dextrose, galactose, trehalose, mannose, glycerol, mannitol and esculin. However, all isolates failed to utilize three substrates: inositol, erythritol and α-Methyl-D-glucoside, while different levels of utilization were found for the remaining twenty-three substrates such as ONPG, citrate and dulcitol (Fig. 1). For example, 31 (77.5%) isolates were able to utilize citrate while 9 (22.5%) isolates were citrate negative.

3.2. Comparative metabolic activity of ESBL-producing and non ESBL-producing E. coli isolates

Metabolic profiling results for twenty ESBL-producing and twenty non ESBL-producing *E. coli* isolates showed that there was a similarity in their capability of utilizing fifteen substrates. However, slight differences in their metabolic activity were detected for the remaining twenty substrates (Table 3). Non ESBL-producing isolates were more metabolically active than ESBL-producers, and they were more able to utilize thirteen substrates such as dulcitol and ONPG. Additionally, our data found seven substrates, inulin, salicin, arabitol, cellobiose, melezitose, alpha-Methyl-D-mannoside and xylitol, which were utilized by at least one of the non ESBL-producing isolates (Table 3). However, ESBL-producing isolates were more able to utilize only seven substrates, such as malonate and sorbose, than non ESBL-producers. The difference in the mean bio score between the two isolate groups was insignificant (P = 0.59), and there was no association between ESBL carriage and specific metabolic profile (Table 3).

3.3. Comparative metabolic activity of E. coli ST131 and non-ST131 isolates

Metabolic profiling results for twenty *E. coli* ST131 and twenty non-ST131 isolates found a comparable capability of utilizing thirteen substrates, while slight differences in their metabolic activity were found for the remaining twenty-two substrates (Table 4). Non-ST131 isolates were more metabolically active than ST131 isolates, and they were more able to utilize fifteen substrates. Nonetheless, ST131 isolates were of higher capability of metabolizing seven substrates, such as sucrose and malonate. Our data found six substrates: salicin, arabitol, cellobiose, melezitose, xylitol and Alpha-Methyl-D-mannoside that were only utilized by members of non-ST131 isolates (Table 4). However, we did not find any substrate that was exclusively utilized by ST131 isolates, and there was a significant negative association between ST131 and dulcitol utilization (P = 0.0001). The difference in the mean bio score between the two isolate groups was insignificant (P = 0.07), and

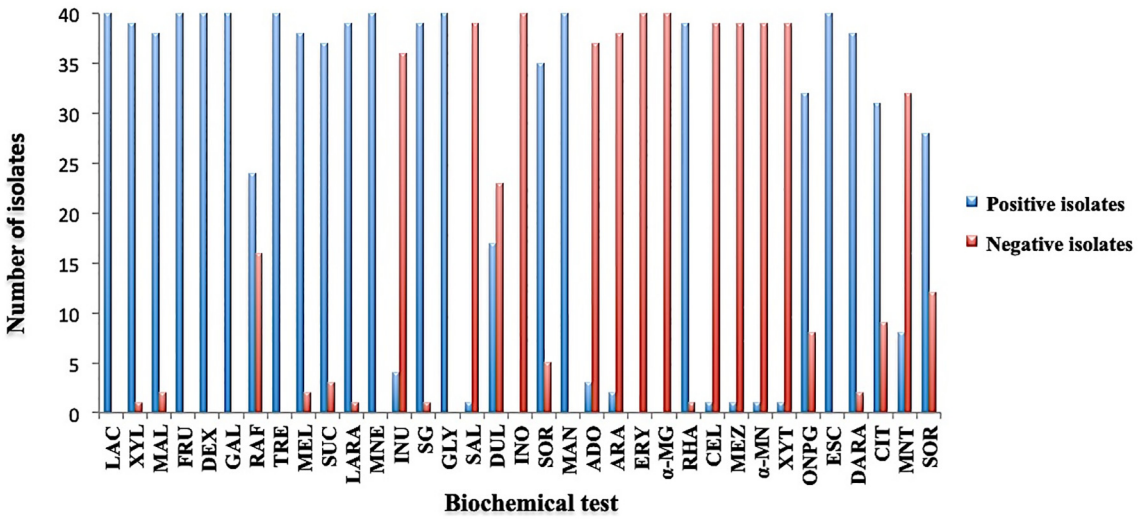

Fig. 1. Metabolic activity of all *E. coli* isolates.

Table 3
The metabolic activity of ESBL-producing and non ESBL- producing isolates.

Test	Positive ESBL-producing isolates (%)	Positive non ESBL-producing isolates (%)	Total positive isolates (%)	*P* value[a]
LAC	20 (100%)	20 (100%)	40 (100%)	1.000
XYL	20 (100%)	19 (95%)	39 (97.5%)	1.000
MAL	18 (90%)	20 (100%)	38 (95%)	0.48
FRU	20 (100%)	20 (100%)	40 (100%)	1.000
DEX	20 (100%)	20 (100%)	40 (100%)	1.000
GAL	20 (100%)	20 (100%)	40 (100%)	1.000
RAF	12 (60%)	12 (60%)	24 (60%)	1.000
TRE	20 (100%)	20 (100%)	40 (100%)	1.000
MEL	19 (95%)	19 (95%)	38 (95%)	1.000
SUC	20 (100%)	17 (85%)	37 (92.5%)	0.23
LARA	19 (95%)	20 (100%)	39 (97.5%)	1.000
MNE	20 (100%)	20 (100%)	40 (100%)	1.000
INU	0 (0%)	4 (20%)	4 (10%)	0.11
SG	19 (95%)	20 (100%)	39 (97.5%)	1.000
GLY	20 (100%)	20 (100%)	40 (100%)	1.000
SAL	0 (0%)	1 (5%)	1 (2.5%)	1.000
DUL	6 (30%)	11 (55%)	17 (42.5%)	0.20
INO	0 (0%)	0 (0%)	0 (0%)	1.000
SOR	18 (90%)	17 (85%)	35 (87.5%)	1.000
MAN	20 (100%)	20 (100%)	40 (100%)	1.000
ADO	1 (5%)	2 (10%)	3 (7.5%)	1.000
ARA	0 (0%)	2 (10%)	2 (5%)	0.48
ERY	0 (0%)	0 (0%)	0 (0%)	1.000
α-MG	0 (0%)	0 (0%)	0 (0%)	1.000
RHA	20 (100%)	19 (95%)	39 (97.5%)	1.000
CEL	0 (0%)	1 (5%)	1 (2.5%)	1.000
MEZ	0 (0%)	1 (5%)	1 (2.5%)	1.000
α-MN	0 (0%)	1 (5%)	1 (2.5%)	1.000
XYT	0 (0%)	1 (5%)	1 (2.5%)	1.000
ONPG	15 (75%)	17 (85%)	32 (80%)	0.69
ESC	20 (100%)	20 (100%)	40 (100%)	1.000
DARA	19 (95%)	19 (95%)	38 (95%)	1.000
CIT	16 (80%)	15 (75%)	31 (77.5%)	1.000
MNT	6 (30%)	2 (10%)	8 (20%)	0.24
SOR	15 (75%)	13 (65%)	28 (70%)	0.73
Median bio score[b] (range)	21.2 (16–24)	21.6 (17–27)	21.4 (16–27)	0.59

[a] *P* values for 2-group comparison: ESBL and non ESBL.
[b] Median number of substrates utilized by isolates (range).

there was no association between ST131 and specific metabolic profile (Table 4).

3.4. Relating the metabolic capacity of ST131 to antimicrobial susceptibility profiles

The results of testing the metabolic activity of twenty *E. coli* ST131 isolates, 14 ESBL-producing and 6 non ESBL-producing isolates, are shown in Table 5. With very few exceptions, the substrate utilization capacity of all isolates was almost similar between ESBL-producing and non ESBL-producing ST131 isolates. However, inulin utilization was only observed among non ESBL-producing ST131 isolates, while malonate was only metabolized by ESBL-producing ST131 isolates. The difference in the mean bio scores between the two isolate groups was insignificant ($P = 0.77$), and there was no correlation between ESBL carriage and specific metabolic profile among ST131 isolates. Additionally, we found a general similarity in the metabolic activity between MDR and non-MDR ST131 isolates (Table 6). However, very few differences in their metabolic traits were detected, although these differences remained insignificant.

3.5. Comparative metabolic activity of E. coli ST131 subclones

Metabolic profiling results for twenty *E. coli* ST131 isolates belonging to different subclones are shown in Table 7. Of these, 17 belonged to *H30* subclone (7 *H30*Rx and 10 *H30* non-Rx) while 3 isolates were from non-*H30* subclones. All isolates were positive for utilizing twelve substrates, however they were all negative for metabolizing nine substrates. We found variability between ST131 subclones in the ability to utilize fourteen substrates. Generally, *H30* isolates were slightly higher in their utilization ability for some substrates, such as raffinose, citrate and sorbose, than non-*H30* isolates. Within *H30* subclone, *H30*Rx was of higher metabolic ability compared to *H30* non-Rx. However, we found that dulcitol was only utilized by non-*H30* isolates while adonitol was exclusively metabolized by members of *H30* non-Rx. Few significant differences between ST131 subclones in terms of metabolic activity were detected, such as that between non-*H30* and *H30* non-Rx isolates for dulcitol utilization ($P = 0.04$), and that between *H30* non-Rx and non-*H30* for D-Arabinose ($P = 0.04$).

4. Discussion

Metabolism is a key factor that enhances bacterial colonization of human hosts (Rohmer et al., 2011). With regard to the role of metabolism in triggering the virulence of ExPEC, a previous report demonstrated that the increased catabolism of the amino acid D-serine by the *E. coli* CFT073 strain during UTI can enhance its colonization and virulence gene expression (Anfora et al., 2007).

In the present study, the metabolic traits of all isolates were generally similar to those typically known for *E. coli*, and this was shown by their complete ability to utilize substrates such as lactose and mannose. Additionally, all isolates were negatively associated with utilizing inositol and erythritol. Interestingly, our data demonstrated very high ability of *E. coli* isolates to utilize citrate. *E. coli* is typically citrate negative (Reynolds and Silver,

Table 4
The metabolic activity of ST131 and non-ST131 isolates.

Test	Positive ST131 isolates (%)	Positive non ST131 isolates (%)	Total positive isolates (%)	*P* value[a]
LAC	20 (100%)	20 (100%)	40 (100%)	1.000
XYL	20 (100%)	19 (95%)	39 (97.5%)	1.000
MAL	18 (90%)	20 (100%)	38 (95%)	0.48
FRU	20 (100%)	20 (100%)	40 (100%)	1.000
DEX	20 (100%)	20 (100%)	40 (100%)	1.000
GAL	20 (100%)	20 (100%)	40 (100%)	1.000
RAF	9 (45%)	15 (75%)	24 (60%)	0.11
TRE	20 (100%)	20 (100%)	40 (100%)	1.000
MEL	18 (90%)	20 (100%)	38 (95%)	0.48
SUC	20 (100%)	17 (85%)	37 (92.5%)	0.23
LARA	19 (95%)	20 (100%)	39 (97.5%)	1.000
MNE	20 (100%)	20 (100%)	40 (100%)	1.000
INU	2 (10%)	2 (10%)	4 (10%)	1.000
SG	19 (95%)	20 (100%)	39 (97.5%)	1.000
GLY	20 (100%)	20 (100%)	40 (100%)	1.000
SAL	0 (0%)	1 (5%)	1 (2.5%)	1.000
DUL	2 (10%)	15 (75%)	17 (42.5%)	**<0.0001**
INO	0 (0%)	0 (0%)	0 (0%)	1.000
SOR	18 (90%)	17 (85%)	35 (87.5%)	1.000
MAN	20 (100%)	20 (100%)	40 (100%)	1.000
ADO	1 (5%)	2 (10%)	3 (7.5%)	1.000
ARA	0 (0%)	2 (10%)	2 (5%)	0.48
ERY	0 (0%)	0 (0%)	0 (0%)	1.000
α-MG	0 (0%)	0 (0%)	0 (0%)	1.000
RHA	20 (100%)	19 (95%)	39 (97.5%)	1.000
CEL	0 (0%)	1 (5%)	1 (2.5%)	1.000
MEZ	0 (0%)	1 (5%)	1 (2.5%)	1.000
α-MN	0 (0%)	1 (5%)	1 (2.5%)	1.000
XYT	0 (0%)	1 (5%)	1 (2.5%)	1.000
ONPG	15 (75%)	17 (85%)	32 (80%)	0.69
ESC	20 (100%)	20 (100%)	40 (100%)	1.000
DARA	18 (90%)	20 (100%)	38 (95%)	0.48
CIT	16 (80%)	15 (75%)	31 (77.5%)	1.000
MNT	5 (25%)	3 (15%)	8 (20%)	0.69
SOR	17 (85%)	11 (55%)	28 (70%)	0.08
Median bio score[b] (range)	20.9 (16–23)	22 (17–27)	21.4 (16–27)	0.07

[a] *P* values for 2-group comparison: ST131 and non-ST131, the bold numbers refer to presence of significant difference between groups for some traits.
[b] Median number of substrates utilized by isolates (range).

Table 5
The metabolic activity of ESBL-producing ST131 and non ESBL-producing ST131 isolates.

Test	Positive ESBL-producing ST131 isolates (%)	Positive non ESBL-producing ST131 isolates (%)	Total positive isolates (%)	*P* value[a]
LAC	14 (100%)	6 (100%)	20 (100%)	1.000
XYL	14 (100%)	6 (100%)	20 (100%)	1.000
MAL	12 (85.7%)	6 (100%)	18 (90%)	1.000
FRU	14 (100%)	6 (100%)	20 (100%)	1.000
DEX	14 (100%)	6 (100%)	20 (100%)	1.000
GAL	14 (100%)	6 (100%)	20 (100%)	1.000
RAF	6 (42.9%)	3 (50%)	9 (45%)	1.000
TRE	14 (100%)	6 (100%)	20 (100%)	1.000
MEL	13 (92.9%)	5 (83.3%)	18 (90%)	0.52
SUC	14 (100%)	6 (100%)	20 (100%)	1.000
LARA	13 (92.9%)	6 (100%)	19 (95%)	1.000
MNE	14 (100%)	6 (100%)	20 (100%)	1.000
INU	0 (0%)	2 (33.3%)	2 (10%)	0.08
SG	13 (92.9%)	6 (100%)	19 (95%)	1.000
GLY	14 (100%)	6 (100%)	20 (100%)	1.000
SAL	0 (0%)	0 (0%)	0 (0%)	1.000
DUL	1 (7.1%)	1 (16.7%)	2 (10%)	0.52
INO	0 (0%)	0 (0%)	0 (0%)	1.000
SOR	13 (92.9%)	5 (83.3%)	18 (90%)	0.52
MAN	14 (100%)	6 (100%)	20 (100%)	1.000
ADO	1 (7.1%)	0 (0%)	1 (5%)	1.000
ARA	0 (0%)	0 (0%)	0 (0%)	1.000
ERY	0 (0%)	0 (0%)	0 (0%)	1.000
α-MG	0 (0%)	0 (0%)	0 (0%)	1.000
RHA	14 (100%)	6 (100%)	20 (100%)	1.000
CEL	0 (0%)	0 (0%)	0 (0%)	1.000
MEZ	0 (0%)	0 (0%)	0 (0%)	1.000
α-MN	0 (0%)	0 (0%)	0 (0%)	1.000

Table 5 (*continued*)

Test	Positive ESBL-producing ST131 isolates (%)	Positive non ESBL-producing ST131 isolates (%)	Total positive isolates (%)	*P* value[a]
XYT	0 (0%)	0 (0%)	0 (0%)	1.000
ONPG	10 (71.4%)	5 (83.3%)	15 (75%)	1.000
ESC	14 (100%)	6 (100%)	20 (100%)	1.000
DARA	13 (92.9%)	5 (83.3%)	18 (90%)	0.52
CIT	11 (78.6%)	5 (83.3%)	16 (80%)	1.000
MNT	5 (35.7%)	0 (0%)	5 (25%)	0.26
SOR	11 (78.6%)	6 (100%)	17 (85%)	0.52
Median bio score[b] (range)	20.8 (16–23)	21 (19–22)	20.9 (16–23)	0.77

[a] *P* values for 2-group comparison: ESBL producing ST131 and non ESBL-producing ST131.
[b] Median number of substrates utilized by isolates (range).

Table 6
The metabolic activity of MDR ST131 and non-MDR ST131 isolates.

Test	Positive MDR ST131 isolates (%)	Positive non MDR ST131 isolates (%)	Total positive isolates (%)	*P* value
LAC	16 (100%)	4 (100%)	20 (100%)	1.000
XYL	16 (100%)	4 (100%)	20 (100%)	1.000
MAL	14 (87.5%)	4 (100%)	18 (90%)	1.000
FRU	16 (100%)	4 (100%)	20 (100%)	1.000
DEX	16 (100%)	4 (100%)	20 (100%)	1.000
GAL	16 (100%)	4 (100%)	20 (100%)	1.000
RAF	8 (50%)	1 (25%)	9 (45%)	0.58
TRE	16 (100%)	4 (100%)	20 (100%)	1.000
MEL	14 (87.5%)	4 (100%)	18 (90%)	1.000
SUC	16 (100%)	4 (100%)	20 (100%)	1.000
LARA	15 (93.8%)	4 (100%)	19 (95%)	1.000
MNE	16 (100%)	4 (100%)	20 (100%)	1.000
INU	1 (6.3%)	1 (25%)	2 (10%)	1.000
SG	15 (93.8%)	4 (100%)	19 (95%)	1.000
GLY	16 (100%)	4 (100%)	20 (100%)	1.000
SAL	0 (0%)	0 (0%)	0 (0%)	1.000
DUL	1 (6.3%)	1 (25%)	2 (10%)	0.41
INO	0 (0%)	0 (0%)	0 (0%)	1.000
SOR	15 (93.8%)	3 (75%)	18 (90%)	0.37
MAN	16 (100%)	4 (100%)	20 (100%)	1.000
ADO	1 (6.3%)	0 (0%)	1 (5%)	1.000
ARA	0 (0%)	0 (0%)	0 (0%)	1.000
ERY	0 (0%)	0 (0%)	0 (0%)	1.000
α-MG	0 (0%)	0 (0%)	0 (0%)	1.000
RHA	16 (100%)	4 (100%)	20 (100%)	1.000
CEL	0 (0%)	0 (0%)	0 (0%)	1.000
MEZ	0 (0%)	0 (0%)	0 (0%)	1.000
α-MN	0 (0%)	0 (0%)	0 (0%)	1.000
XYT	0 (0%)	0 (0%)	0 (0%)	1.000
ONPG	11 (68.8%)	4 (100%)	15 (75%)	0.53
ESC	16 (100%)	4 (100%)	20 (100%)	1.000
DARA	15 (93.8%)	3 (75%)	18 (90%)	0.37
CIT	12 (75%)	4 (100%)	16 (80%)	1.000
MNT	5 (31.3%)	0 (0%)	5 (25%)	0.53
SOR	12 (75%)	4 (100%)	17 (85%)	0.54

[a]*P* values for 2-group comparison: MDR ST131 and non MDR ST131.

Table 7
The metabolic activity of isolates belonging to ST131 subclones.

Test	Positive *H30 Rx* isolates (%)	Positive *H30 non-Rx* isolates (%)	Positive non *H30* isolates (%)	*P* value		
				H30 Rx vs *H30* non-Rx	*H30* Rx vs non *H30*	*H30* non-Rx vs non *H30*
LAC	7 (100%)	10 (100%)	3 (100%)	1.000	1.000	1.000
XYL	7 (100%)	10 (100%)	3 (100%)	1.000	1.000	1.000
MAL	5 (71.4%)	10 (100%)	3 (100%)	0.15	1.000	1.000
FRU	7 (100%)	10 (100%)	3 (100%)	1.000	1.000	1.000
DEX	7 (100%)	10 (100%)	3 (100%)	1.000	1.000	1.000
GAL	7 (100%)	10 (100%)	3 (100%)	1.000	1.000	1.000
RAF	4 (57.1%)	4 (40%)	1 (33.3%)	0.64	1.000	1.000
TRE	7 (100%)	10 (100%)	3 (100%)	1.000	1.000	1.000
MEL	7 (100%)	9 (90%)	2 (66.7%)	1.000	0.30	0.42
SUC	7 (100%)	10 (100%)	3 (100%)	1.000	1.000	1.000
LARA	7 (100%)	9 (90%)	3 (100%)	1.000	1.000	1.000
MNE	7 (100%)	10 (100%)	3 (100%)	1.000	1.000	1.000

(*continued on next page*)

Table 7 (*continued*)

Test	Positive *H30 Rx* isolates (%)	Positive *H30 non-Rx* isolates (%)	Positive non *H30* isolates (%)	*P* value		
				H30 Rx vs *H30* non-Rx	*H30* Rx vs non *H30*	*H30* non-Rx vs non *H30*
INU	1 (14.3%)	1 (10%)	0 (0%)	1.000	1.000	1.000
SG	7 (100%)	10 (100%)	2 (66.7%)	1.000	0.30	0.23
GLY	7 (100%)	10 (100%)	3 (100%)	1.000	1.000	1.000
SAL	0 (0%)	0 (0%)	0 (0%)	1.000	1.000	1.000
DUL	0 (0%)	0 (0%)	2 (66.7%)	1.000	0.08	**0.04**
INO	0 (0%)	0 (0%)	0 (0%)	1.000	1.000	1.000
SOR	6 (85.7%)	10 (100%)	2 (66.7%)	0.41	1.000	0.23
MAN	7 (100%)	10 (100%)	3 (100%)	1.000	1.000	1.000
ADO	0 (0%)	1 (10%)	0 (0%)	1.000	1.000	1.000
ARA	0 (0%)	0 (0%)	0 (0%)	1.000	1.000	1.000
ERY	0 (0%)	0 (0%)	0 (0%)	1.000	1.000	1.000
α-MG	0 (0%)	0 (0%)	0 (0%)	1.000	1.000	1.000
RHA	7 (100%)	10 (100%)	3 (100%)	1.000	1.000	1.000
CEL	0 (0%)	0 (0%)	0 (0%)	1.000	1.000	1.000
MEZ	0 (0%)	0 (0%)	0 (0%)	1.000	1.000	1.000
α-	MN	0 (0%)	0 (0%)	0 (0%)	1.000	1.000
1.000						
XYT	0 (0%)	0 (0%)	0 (0%)	1.000	1.000	1.000
ONPG	6 (85.7%)	6 (60%)	3 (100%)	0.34	1.000	0.50
ESC	7 (100%)	10 (100%)	3 (100%)	1.000	1.000	1.000
DARA	7 (100%)	10 (100%)	1 (33.3%)	1.000	0.07	**0.04**
CIT	6 (85.7%)	8 (80%)	2 (66.7%)	1.000	1.000	1.000
MNT	1 (14.3%)	3 (30%)	1 (33.3%)	0.60	1.000	1.000
SOR	7 (100%)	8 (80%)	2 (66.7%)	0.49	0.30	1.000

[a]*P* values for 3-group comparisons: *H30*Rx, *H30* non-Rx and non *H30*, the bold numbers refer to presence of significant difference between groups for some traits.

1983), however previous studies described some *E. coli* isolates, particularly those associated with MDR phenotype, that were citrate positive (Ishiguro et al., 1978; Smith et al., 1978). Many previous reports showed that citrate utilization in *E. coli* can be plasmid conferred (Ishiguro et al., 1978) or due to complex chromosomal mutations (Hall, 1982). The possible explanation of high citrate utilization in our isolates is that the vast majority of them are MDR, which supports the previous finding showing positive citrate phenotype among MDR isolates (Smith et al., 1978). This proposes a probable association between citrate utilization and multidrug resistance in ExPEC, and merits further investigation at a genomic level in the future. We found that the metabolic activity of non-ESBL producing isolates was slightly higher than ESBL producers, although this difference was insignificant. This concurs with a previous data showing no association between ESBL carriage and specific metabolic profile among ExPEC isolates (Alqasim et al., 2014).

This study found a slightly lower metabolic potential of ST131 than non-ST131, and there was no specific metabolic profile for ST131. This is concordant with a phenotypic microarray-based study demonstrating that ST131 isolates had a reduced metabolic activity for 5 tests, esculin, 5-keto-D-gluconate, cellobiose, D-Arabinose and dulcitol, than non-ST131 isolates with no unique metabolic profile for ST131 isolates (Alqasim et al., 2014). Additionally, the low ability of ST131 isolates to utilize dulcitol and cellobiose described by Alqasim et al. (2014) was also observed here as our ST131 isolates were negatively associated with dulcitol, and failed to utilize cellobiose. However, another study found high metabolic activity of ST131 that distinguishes them from isolates belonging to other ExPEC STs (Gibreel et al., 2012).

Additionally, our data did not show a correlation between antimicrobial resistance and high metabolic profiles among ST131 isolates, suggesting no link between antimicrobial susceptibility profiles and metabolism. This disagrees with a previous report showing an association between antimicrobial resistance and high metabolic potential in ST131 (Gibreel et al., 2012). These conflicting findings call into question the role of metabolism in driving the success of ST131 as an important pathogen currently responsible for causing serious difficult-to-treat infections globally, and highlight the need of performing large-scale studies on the metabolic capacity of ST131 to elucidate the role of metabolism in the pathogenesis of this clone.

Testing the metabolic activity of ST131 subclones showed a slightly higher metabolic activity of *H30* isolates, particularly *H30*Rx, in comparison to non-*H30* isolates. The high metabolic capacity in *H30* subclone is in agreement with a previous finding showing higher virulence potential and antimicrobial resistance among *H30* than those of non-*H30* (Alqasim et al., 2020b). We also found few significant differences in terms of metabolic potential between ST131 isolates, supporting the aforementioned variability between ST131 subclones in their antimicrobial susceptibility profiles and virulence capacity. Given these differences between ST131 subclones, it is important to test ST131 isolates individually rather than considering them as one group.

With regard to the limitations of our study, it was carried out on low sample size. Additionally, it tested the metabolic activity of *E. coli* isolates using one method that comprises only 35 substrates, and this may not be fully discriminative. It also focused on determining the metabolic activity of ExPEC isolates from Riyadh city, and this might not reflect the metabolic capacity of isolates in other local geographical parts.

In conclusion, we provided the first local comparative description of metabolic capacity of ExPEC isolates including ST131 and its main subclones. The metabolic capacity of ST131 and non-ST131 isolates was almost similar, and this supports the previous suggestion that ST131 is not associated with higher metabolic potential than other ExPEC clones. Interestingly, there were few significant differences between ST131 subclones in their metabolic

activity. It is thought that studying the gene content of ST131 and its subclones might provide a full explanation of reasons that make ST131 such successful clone.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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