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 a 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 subtype 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.

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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 *fimH*30 is the most common *fimH* type (Nicolas-Chanoine et al., 2014). ST131 H30 subtype involves nearly all fluoroquinolone resistant ST131 isolates, and it has two important MDR subsets, H30R and H30Rx. H30Rx 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 H30R or non-Rx subtype (Nicolas-Chanoine et al., 2014).
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 ≤0.05 was used as threshold for statistical significance.

| Isolate ID | MDRa | 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 | – | NAa | – | – |
| 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           | +     |           |

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a MDR phenotype refers to displaying resistance to at least 1 antibiotic in ≥ 3 antibiotic groups (Magiorakos et al., 2012).

b NA: Not applicable.
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

| 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                       | MAN          |
| 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          |

Table 2
List of biochemical tests used in this study.
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 H30Rx 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, H30Rx was higher in 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 valuea |
|------|-----------------------------|---------------------------------|-----------------------------|----------|
| 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    |
| β-MG | 0 (0%)                      | 0 (0%)                          | 0 (0%)                      | 1.000    |
| CEL  | 0 (0%)                      | 1 (5%)                          | 1 (2.5%)                    | 1.000    |
| MEZ  | 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 scoreb (range) | 20 (9.16–23)                    | 22 (17–27)                     | 21 (16–27)                  | 0.07     |

* 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 valuea |
|------|-------------------------------------------|-----------------------------------------------|-----------------------------|----------|
| LAC  | 20 (100%)                                 | 6 (100%)                                      | 26 (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  | 20 (100%)                                 | 6 (100%)                                      | 26 (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 | 17 (85%)                                  | 11 (55%)                                      | 28 (70%)                    | 0.08     |
| MNE  | 14 (100%)                                 | 6 (100%)                                      | 20 (100%)                   | 1.000    |
| INU  | 0 (0%)                                    | 2 (33.3%)                                     | 2 (10%)                     | 0.08     |
| SG   | 11 (69.3%)                                | 6 (100%)                                      | 17 (90%)                    | 0.08     |
| GLY  | 14 (100%)                                 | 6 (100%)                                      | 20 (100%)                   | 1.000    |
| SAL  | 0 (0%)                                    | 0 (0%)                                        | 0 (0%)                      | 1.000    |
| DUL  | 1 (7.1%)                                  | 1 (6.7%)                                      | 2 (10%)                     | 0.52     |
| INO  | 0 (0%)                                    | 0 (0%)                                        | 0 (0%)                      | 1.000    |
| SOR  | 17 (85%)                                  | 11 (55%)                                      | 28 (70%)                    | 0.08     |
| MAN  | 1 (7.1%)                                  | 0 (0%)                                        | 1 (5%)                      | 1.000    |
| ADO  | 0 (0%)                                    | 0 (0%)                                        | 0 (0%)                      | 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    |
| β-MG | 0 (0%)                                    | 0 (0%)                                        | 0 (0%)                      | 1.000    |
| CEL  | 0 (0%)                                    | 0 (0%)                                        | 0 (0%)                      | 1.000    |
| MEZ  | 0 (0%)                                    | 0 (0%)                                        | 0 (0%)                      | 1.000    |
| Median bio scoreb (range) | 20 (9.16–23)                    | 22 (17–27)                     | 21 (16–27)                  | 0.07     |

* P values for 2-group comparison: ESBL-producing and non ESBL-producing, 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 (continued)

| Test | Positive ESBL-producing ST131 isolates (%) | Positive non ESBL-producing ST131 isolates (%) | Total positive isolates (%) | P valuea |
|------|------------------------------------------|------------------------------------------------|-----------------------------|----------|
| 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 scoreb (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   |
| DJUL | 12 (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   |
| ADD  | 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 |
|------|------------------------------|---------------------------------|-------------------------------|---------|
| LAC  | 7 (100%)                     | 10 (100%)                       | 3 (100%)                      | 1.000   |
| XYL  | 7 (100%)                     | 10 (100%)                       | 3 (100%)                      | 1.000   |
| MAL  | 5 (71.4%)                    | 10 (100%)                       | 3 (100%)                      | 1.000   |
| FRU  | 7 (100%)                     | 10 (100%)                       | 3 (100%)                      | 1.000   |
| DEX  | 7 (100%)                     | 10 (100%)                       | 3 (100%)                      | 1.000   |
| GAL  | 7 (100%)                     | 10 (100%)                       | 3 (100%)                      | 1.000   |
| RAF  | 1 (6.3%)                     | 4 (25%)                         | 2 (10%)                       | 0.83   |
| TRE  | 7 (100%)                     | 10 (100%)                       | 3 (100%)                      | 1.000   |
| MEL  | 7 (100%)                     | 9 (90%)                         | 2 (66.7%)                     | 1.000   |
| SUC  | 7 (100%)                     | 10 (100%)                       | 3 (100%)                      | 1.000   |
| LARA | 7 (100%)                     | 9 (90%)                         | 3 (100%)                      | 1.000   |
| MNE  | 7 (100%)                     | 10 (100%)                       | 3 (100%)                      | 1.000   |

(continued on next page)
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 previous data showing no association between ESBL producers, although this difference was insignificant. This may not reflect the metabolic capacity of isolates 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

### 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*     |         |
|      | RX vs non-Rx                  | RX vs non-Rx                     | non-Rx vs non-Rx               |         |
| INU  | 1 (14.3%)                     | 1 (10%)                          | 0 (0%)                         | 1.000   |
| SG   | 1 (100%)                      | 2 (66.7%)                        | 1.000                          | 1.000   |
| GLY  | 1 (100%)                      | 3 (100%)                         | 1.000                          | 1.000   |
| SAL  | 0 (0%)                        | 0 (0%)                           | 0.04                           | 1.000   |
| DUL  | 0 (0%)                        | 2 (66.7%)                        | 1.000                          | 0.08    |
| INO  | 0 (0%)                        | 0 (0%)                           | 1.000                          | 1.000   |
| SOR  | 6 (85.7%)                     | 2 (66.7%)                        | 0.41                           | 1.000   |
| MAN  | 7 (100%)                      | 3 (100%)                         | 1.000                          | 1.000   |
| ADO  | 0 (0%)                        | 0 (0%)                           | 1.000                          | 1.000   |
| ARA  | 0 (0%)                        | 0 (0%)                           | 1.000                          | 1.000   |
| ERY  | 0 (0%)                        | 0 (0%)                           | 1.000                          | 1.000   |
| α-MG | 0 (0%)                        | 0 (0%)                           | 1.000                          | 1.000   |
| RHA  | 7 (100%)                      | 3 (100%)                         | 0.04                           | 1.000   |
| CEL  | 0 (0%)                        | 0 (0%)                           | 1.000                          | 1.000   |
| MEZ  | 0 (0%)                        | 0 (0%)                           | 1.000                          | 1.000   |
| MN   | 0 (0%)                        | 0 (0%)                           | 1.000                          | 1.000   |
| XYT  | 0 (0%)                        | 0 (0%)                           | 1.000                          | 1.000   |
| ONPG | 6 (85.7%)                     | 3 (100%)                         | 0.34                           | 1.000   |
| ESC  | 7 (100%)                      | 3 (100%)                         | 1.000                          | 1.000   |
| DARA | 7 (100%)                      | 1 (33.3%)                        | 0.07                           | 0.04    |
| CIT  | 6 (85.7%)                     | 2 (66.7%)                        | 1.000                          | 1.000   |
| MNT  | 1 (14.3%)                     | 1 (33.3%)                        | 0.60                           | 1.000   |
| SOR  | 7 (100%)                      | 2 (66.7%)                        | 0.49                           | 0.30    |

*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.
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 a 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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