Research Article
Comparative Metabolic Characterization of Extraintestinal Pathogenic Escherichia coli Blood Isolates from Saudi Arabia

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Background. The prevalence of bloodstream infections caused by extraintestinal pathogenic Escherichia coli (ExPEC) has increased substantially. E. coli ST131 is one of the dominant ExPEC clones among E. coli bacteremia population. Metabolism can trigger the pathogenesis of some bacterial isolates, and here we evaluated and compared the metabolic traits of E. coli bacteremia isolates including β-lactamase (BL)/extended-spectrum β-lactamase (ESBL)-positive and ESBL-negative isolates and ST131 and non-ST131 isolates.

Methods. The metabolic profiles of thirty E. coli isolates, obtained from blood samples for hospitalized individuals at a tertiary healthcare facility in Riyadh, were determined using HiMedia carbohydrate test strips. The difference in the utilization ability between isolate groups was then statistically assessed. Results. Our data found that non-BL/ESBL producers were of low metabolic capacity compared with ESBL-positive isolates although the difference remained insignificant. Higher levels of utilization for some carbohydrates, such as fructose and trehalose, were detected among ST131 isolates when compared with non-ST131, and ST131 was also significantly associated with metabolizing rhamnose. The mean bio-score of both isolate groups was insignificant. We showed no link between metabolism and antimicrobial susceptibility profiles among tested blood isolates.

Conclusion. ST131 blood isolates were slightly higher in their carbohydrate utilization activity than non-ST131. More importantly, ST131 isolates were significantly capable of metabolizing rhamnose. Future research should focus on the factors that might drive the success of major ExPEC clones such as ST131.

1. Introduction

Bloodstream infections (BSIs), such as bacteremia, are predominantly caused by extraintestinal pathogenic Escherichia coli (ExPEC) [1, 2]. The recent escalating number of ExPEC bacteremia cases presents a challenge to global healthcare systems [3, 4]. A previous study found that mortality rates of bacteremia can be as high as 33% in elderly hospitalized patients [5].

The spectrum of ExPEC resistance to several antibiotics, such as trimethoprim-sulfamethoxazole and fluoroquinolones, has increased [6–8]. More recently, many studies have demonstrated the increase in the levels of ExPEC resistance to more powerful agents such as carbapenems and polymyxins [9–12], which can complicate the management of patients. Additionally, some β-lactamases (BLs), such as TEM-1 and OXA-1, and extended-spectrum β-lactamases (ESBLs), particularly CTX-M family, have highly been detected among ExPEC [13, 14].

E. coli sequence type 131 (E. coli ST131), discovered in 2008, has been considered the major driver of the current high prevalence of multidrug resistance (MDR) globally [15, 16]. E. coli ST131 isolates show insusceptibility to fluoroquinolone (FQ) and carry CTX-M genes, predominantly
CTX-M-15 [17–21]. Moreover, it has been shown that ST131 has higher virulence capabilities than other important ExPEC clones [22, 23].

Metabolism is one of many factors that can mediate most of the physiological processes and trigger bacterial pathogenesis. In addition to all types of virulence determinants, such as toxins and adhesions, bacterial pathogens possess specific metabolic traits that allow them to overcome host immune defenses and antimicrobial killing aiding their survival, replication, and colonization [24]. For example, carbohydrate utilization [25] and possessing specific metabolic enzymes [26] can enhance bacterial virulence. A previous study has demonstrated that increased catabolism of D-serine by the E. coli ST73 strain, CFT073, can enhance its virulence gene expression during the urinary tract infection process [27]. Additionally, it has been proposed that antimicrobial resistance (AMR) is associated with a high metabolic activity of ExPEC isolates [28]. Another study found that MDR E. coli isolates have also been linked with a high ability to utilize particular substrates, such as citrate, as a sole source of carbon [29, 30].

We previously characterized a collection of E. coli blood isolates from Saudi Arabia and demonstrated that ST131 accounted for 54.8% of all isolates, 88.2% of which were ESBL-producing [23]. Many previous local and international studies have focused on determining and comparing the metabolic potential of uropathogenic E. coli (UPEC) clones [28, 31, 32]. For instance, our research group has recently published a comparative metabolic analysis, on a panel of E. coli isolates from urine population, showing no unique metabolic potential of ST131 [31]. However, little is known about metabolic traits of E. coli bacteremia population, particularly those for ST131. This comparative study sought to determine the metabolic traits of E. coli blood isolates, including BL/ESBL-positive and BL/ESBL-negative and ST131 and non-ST131. It also assessed the relationship between metabolic capacity and antimicrobial resistance of these isolates.

2. Materials and Methods

2.1. Bacterial Isolates. This study involved using thirty clinical E. coli blood isolates. They were part of a larger E. coli strain set collected, between January 2018 and March 2018, from bacteremia samples of hospitalized individuals at a main hospital in Riyadh. The isolates were identified as E. coli by conventional cultural and biochemical methods. VITEK 2 identification system (VITEK 2-1D-GNB, bio- Merieux) was then used for confirmation of preliminary identification and was fully characterized for their antimicrobial sensitivity, BL/ESBL carriage, virulence potential, and ST131 status [22]. Table 1 shows the information on the E. coli isolates used in this study.

2.2. Metabolic Profiling Assays. KB009 test strips provided on the Hi-Carbohydrate Kit (HiMedia, India) were used to carry out the metabolic profiling of all isolates, according to the manufacturer’s instructions. The bacterial metabolic activity was determined by measuring the utilization level of 35 substrates (Table 2). The experiments were carried out in triplicate on two independent occasions showing completely similar results.

2.3. Statistical Analysis. IBM SPSS (version 21.0) software was employed to carry out statistical analysis. The metabolism results of different isolate groups were compared using Fisher’s exact test (FET). The Mann–Whitney U test was used to calculate the mean biochemical scores (mean bio-scores) of isolate groups. The bio-score (BS) was calculated as the sum of all substrates that tested positive for each isolate. The sum of all the BSs of the isolates was then calculated, and finally, this sum was divided by the number of isolates to give the mean bio-score. p value of ≤0.05 was used as threshold for statistical significance.

3. Results

3.1. Metabolic Activity of All E. coli Blood Isolates. The metabolic profiling data, obtained from using 35 biochemical substrates, showed that esculin was the only substrate to be utilized by all the tested 30 E. coli blood isolates (Figure 1). Nonetheless, all isolates failed to utilize 14 substrates: salicin, dulcitol, inositol, sorbitol, adonitol, arabitol, erythritol, alpha-methyl-D-glucoside, cellobiose, melezitose, alpha-methyl-D-mannoside, xylitol, malonate, and sorbose. Variable utilization levels were found for the remaining 20 substrates such as glycerol, ortho-nitrophenyl-β-galactoside, and sucrose (Figure 1). For example, 28 (93.3%) blood isolates were ortho-nitrophenyl-β-galacto-side-positive, while 2 (6.7%) isolates failed to utilize ortho- nitrophenyl-β-galactoside; however, glycerol was utilized by only 4 (13.3%) isolates, whereas 26 (86.7%) isolates did not show activity against glycerol (Figure 1).

3.2. The Metabolic Activity of BL/ESBL-Positive and BL/ESBL-Negative E. coli Isolates. Our metabolic profiling data showed a complete similarity in the capability of BL/ESBL-positive and BL/ESBL-negative isolates to utilize 21 substrates. Nevertheless, their metabolic activity was variable for the remaining 14 substrates (Table 3). ESBL-producing isolates were higher than non-BL/ESBL producers in metabolizing 9 substrates (fructose, dextrose, trehalose, melibiose, sucrose, mannitol, D-arabinose, citrate, and rhamnose). Additionally, D-arabinose was highly utilized by BL/ESBL-producing members (Table 3). However, BL/ ESBL-negative isolates were higher than BL/ESBL producers in utilizing only 5 substrates (xylose, lactose, maltose, raffinose, and mannose).

3.3. The Metabolic Activity of E. coli ST131 and Non-ST131 Isolates. We found that both isolate groups were similar in metabolizing 16 carbohydrates (Table 4). However, the ability of ST131 isolates in utilizing 13 substrates (maltose, fructose, dextrose, galactose, trehalose, raffinose, sucrose, L-arabinose, mannitol, rhamnose, ortho-nitrophenyl-
β-galactoside, D-arabinose, and citrate) was higher than non-ST131. Rhamnose was highly utilized by ST131 members compared with non-ST131, and the difference in rhamnose utilization was statistically significant ($p < 0.04$). Nonetheless, non-ST131 isolates were higher than ST131 in utilizing 6 substrates (lactose, xylose, mannose, inulin, sodium gluconate, and glycerol), but this difference remained insignificant (Table 4).

### 3.4. Comparison of the Mean Bio-Scores of E. coli Blood Isolate Groups

The mean bio-scores, defined as the number of substrates tested positive for an isolate group divided by the total number of tested substrates, of BL/ESBL-positive and ESBL-negative isolates, and ST131 and non-ST131 isolates were determined and compared (Figure 2). Our data demonstrated that the mean bio-score of BL/ESBL producers was 12.73 compared with 12.13 for non-BL/ESBL producers, and the difference was found to be insignificant ($p = 0.77$) (Figure 2). ST131 isolates had a mean bio-score of 12.88, whereas it was 12.64 for non-ST131 ($p = 0.48$) (Figure 2).

### 3.5. Relating the Metabolic Capacity to Antibiotic Susceptibility Profiles of Blood Isolates

Our data found that the non-MDR isolates, B17 and B18, had similar bio-scores compared with the MDR isolates B9 and B10. Moreover, the non-MDR BL/ESBL-negative isolate, B6, had a metabolic

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

| Isolate ID | MDR | ESBL | BL/ESBL type(s) | ST131 | Reference |
|------------|-----|------|----------------|-------|-----------|
| B1         | MDR | +    | TEM-1          | +     | [23]      |
| B2         | Non-MDR | - | NA<sup>b</sup> | -     |           |
| B3         | MDR | -    | NA             | -     |           |
| B4         | MDR | -    | NA             | -     |           |
| B5         | MDR | -    | NA             | -     |           |
| B6         | Non-MDR | - | NA             | -     |           |
| B7         | MDR | +    | CTX-M-15 and OXA-1 | +    |           |
| B8         | MDR | +    | CTX-M-15 and TEM-1 | +    |           |
| B9         | MDR | +    | OXA-1          | +     |           |
| B10        | MDR | +    | CTX-M-15       | +     |           |
| B11        | MDR | -    | NA             | -     |           |
| B12        | Non-MDR | - | NA             | -     |           |
| B13        | Non-MDR | - | NA             | -     |           |
| B14        | MDR | -    | NA             | -     |           |
| B15        | MDR | -    | NA             | -     |           |
| B16        | MDR | +    | CTX-M-15 and OXA-1 | +    | [23]      |
| B17        | Non-MDR | - | NA             | -     |           |
| B18        | Non-MDR | - | NA             | -     |           |
| B19        | Non-MDR | - | NA             | -     |           |
| B20        | MDR | +    | CTX-M-15 and OXA-1 | +    |           |
| B21        | MDR | +    | CTX-M-14       | -     |           |
| B22        | MDR | +    | CTX-M-15 and OXA-1 | +    |           |
| B23        | MDR | +    | CTX-M-14 and TEM-1 | +    |           |
| B24        | MDR | +    | CTX-M-15 and TEM-1 | +    |           |
| B25        | MDR | -    | NA             | +     |           |
| B26        | MDR | +    | TEM-1          | +     |           |
| B27        | Non-MDR | - | NA             | -     |           |
| B28        | MDR | +    | CTX-M-15 and TEM-1 | +    |           |
| B29        | MDR | +    | CTX-M-15 and TEM-1 | +    |           |
| B30        | MDR | +    | CTX-M-15 and TEM-1 | +    |           |

<sup>a</sup> MDR phenotype refers to displaying resistance to at least 1 antibiotic in ≥3 antibiotic groups. <sup>b</sup> NA: not applicable.

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**Table 2:** List of substrates used in this study.

| Substrates                  | 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          |

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*a MDR phenotype refers to displaying resistance to at least 1 antibiotic in ≥3 antibiotic groups. <sup>b</sup> NA: not applicable.
Figure 1: Metabolic activity of all E. coli blood isolates.

Table 3: Metabolic activity of BL/ESBL-producing and non-producing isolates.

| Substrates | Positive BL/ESBL-producing isolates (%) | Positive non-BL/ESBL-producing isolates (%) | Total positive isolates (%) | p value\(^a\) |
|------------|----------------------------------------|---------------------------------------------|-----------------------------|---------------|
| LAC        | 13 (86.7%)                             | 15 (100%)                                  | 28 (93.3%)                  | 0.48          |
| XYL        | 11 (73.3%)                             | 12 (80%)                                   | 23 (76.7%)                  | 1.00          |
| MAL        | 10 (66.7%)                             | 12 (80%)                                   | 22 (73.3%)                  | 0.68          |
| FRU        | 15 (100%)                              | 14 (93.3%)                                 | 29 (96.7%)                  | 1.00          |
| DEX        | 15 (100%)                              | 14 (93.3%)                                 | 29 (96.7%)                  | 1.00          |
| GAL        | 13 (86.7%)                             | 13 (86.7%)                                 | 26 (86.7%)                  | 1.00          |
| RAF        | 2 (13.3%)                              | 3 (20%)                                    | 5 (16.7%)                   | 1.00          |
| TRE        | 14 (93.3%)                             | 12 (80%)                                   | 26 (86.7%)                  | 0.59          |
| MEL        | 8 (53.3%)                              | 7 (46.7%)                                  | 15 (50%)                    | 1.00          |
| SUC        | 4 (26.7%)                              | 3 (20%)                                    | 7 (23.3%)                   | 1.00          |
| LARA       | 6 (40%)                                | 6 (40%)                                    | 12 (40%)                    | 1.00          |
| MNE        | 13 (86.7%)                             | 14 (93.3%)                                 | 27 (90%)                    | 1.00          |
| INU        | 9 (60%)                                | 9 (60%)                                    | 18 (60%)                    | 1.00          |
| SG         | 9 (60%)                                | 9 (60%)                                    | 18 (60%)                    | 1.00          |
| GLY        | 2 (13.3%)                              | 2 (13.3%)                                  | 4 (13.3%)                   | 1.00          |
| SAL        | 0 (0%)                                 | 0 (0%)                                     | 0 (0%)                      | 1.00          |
| DUL        | 0 (0%)                                 | 0 (0%)                                     | 0 (0%)                      | 1.00          |
| INO        | 0 (0%)                                 | 0 (0%)                                     | 0 (0%)                      | 1.00          |
| SOR        | 0 (0%)                                 | 0 (0%)                                     | 0 (0%)                      | 1.00          |
| MAN        | 7 (46.7%)                              | 6 (40%)                                    | 13 (43.3%)                  | 1.00          |
| ADO        | 0 (0%)                                 | 0 (0%)                                     | 0 (0%)                      | 1.00          |
| ARA        | 0 (0%)                                 | 0 (0%)                                     | 0 (0%)                      | 1.00          |
| ERY        | 0 (0%)                                 | 0 (0%)                                     | 0 (0%)                      | 1.00          |
| α-MG       | 0 (0%)                                 | 0 (0%)                                     | 0 (0%)                      | 1.00          |
| RHA        | 4 (26.7%)                              | 1 (6.7%)                                   | 5 (16.7%)                   | 0.33          |
| CEL        | 0 (0%)                                 | 0 (0%)                                     | 0 (0%)                      | 1.00          |
| MEZ        | 0 (0%)                                 | 0 (0%)                                     | 0 (0%)                      | 1.00          |
| α-MN       | 0 (0%)                                 | 0 (0%)                                     | 0 (0%)                      | 1.00          |
| XYT        | 0 (0%)                                 | 0 (0%)                                     | 0 (0%)                      | 1.00          |
| ONPG       | 14 (93.3%)                             | 14 (93.3%)                                 | 28 (93.3%)                  | 1.00          |
| ESC        | 15 (100%)                              | 15 (100%)                                  | 30 (100%)                   | 1.00          |
| DARA       | 4 (26.7%)                              | 0 (0%)                                     | 4 (13.3%)                   | 0.09          |
| CIT        | 3 (20%)                                | 1 (6.7%)                                   | 4 (13.3%)                   | 0.60          |
| MNT        | 0 (0%)                                 | 0 (0%)                                     | 0 (0%)                      | 1.00          |
| SOR        | 0 (0%)                                 | 0 (0%)                                     | 0 (0%)                      | 1.00          |

\(^a\) p values for 2-group comparison: ESBL and non-ESBL.
Table 4: Metabolic activity of ST131 and non-ST131 isolates.

| Substrates | Positive ST131 isolates (%) | Positive non-ST131 isolates (%) | Total positive isolates (%) | p value* |
|------------|-----------------------------|---------------------------------|-----------------------------|----------|
| LAC        | 14 (87.5%)                  | 14 (100%)                       | 28 (93.3%)                  | 1.000    |
| XYL        | 12 (75%)                    | 11 (78.6%)                      | 23 (76.7%)                  | 1.000    |
| MAL        | 12 (75%)                    | 10 (71.4%)                      | 22 (73.3%)                  | 1.000    |
| FRU        | 16 (100%)                   | 13 (92.9%)                      | 29 (96.7%)                  | 0.47     |
| DEX        | 16 (100%)                   | 13 (92.9%)                      | 29 (96.7%)                  | 0.47     |
| GAL        | 14 (87.5%)                  | 12 (85.7%)                      | 26 (86.7%)                  | 1.000    |
| RAF        | 3 (18.9%)                   | 2 (14.3%)                       | 5 (16.7%)                   | 1.000    |
| TRE        | 15 (93.8%)                  | 11 (78.6%)                      | 26 (86.7%)                  | 0.32     |
| MEL        | 8 (50%)                     | 7 (50%)                         | 15 (50%)                    | 1.000    |
| SUC        | 6 (37.5%)                   | 1 (7.1%)                        | 7 (23.3%)                   | 0.08     |
| LARA       | 7 (43.8%)                   | 5 (35.7%)                       | 12 (40%)                    | 0.72     |
| MNE        | 14 (87.5%)                  | 13 (92.9%)                      | 27 (90%)                    | 1.000    |
| INU        | 9 (56.3%)                   | 9 (64.3%)                       | 18 (60%)                    | 0.72     |
| SG         | 9 (56.3%)                   | 9 (64.3%)                       | 18 (60%)                    | 0.72     |
| GLY        | 1 (6.3%)                    | 3 (21.4%)                       | 4 (13.3%)                   | 0.33     |
| SAL        | 0 (0%)                      | 0 (0%)                          | 0 (0%)                      | 1.000    |
| DUL        | 0 (0%)                      | 0 (0%)                          | 0 (0%)                      | 1.000    |
| INO        | 0 (0%)                      | 0 (0%)                          | 0 (0%)                      | 1.000    |
| SOR        | 0 (0%)                      | 0 (0%)                          | 0 (0%)                      | 1.000    |
| MAN        | 8 (50%)                     | 5 (35.7%)                       | 13 (43.3%)                  | 0.48     |
| 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    |
| RHA        | 5 (31.3%)                   | 0 (%)                           | 5 (16.7%)                   | 0.04     |
| 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       | 15 (93.4%)                  | 13 (92.9%)                      | 28 (93.3%)                  | 1.000    |
| ESC        | 16 (100%)                   | 14 (100%)                       | 30 (100%)                   | 1.000    |
| DARA       | 3 (18.8%)                   | 1 (7.1%)                        | 4 (13.3%)                   | 0.60     |
| CIT        | 3 (18.8%)                   | 1 (7.1%)                        | 4 (13.3%)                   | 0.60     |
| MNT        | 0 (0%)                      | 0 (0%)                          | 0 (0%)                      | 1.000    |
| SOR        | 0 (0%)                      | 0 (0%)                          | 0 (0%)                      | 1.000    |

*p values for 2-group comparison: ST131 and non-ST131, and the bold numbers refer to the presence of significant difference between groups for some traits.

Figure 2: (a) Median bio-scores of BL/ESBL and non-BL/ESBL isolates. The difference between the two isolate groups was insignificant (p = 0.77). (b) Median bio-scores of ST131 and non-ST131 isolates. The difference between these isolate groups was insignificant (p = 0.48).
bio-score of 6, which was almost comparable to bio-score of the MDR BL/ESBL-producing B29 isolate (Table 5). Additionally, carrying more than one BL/ESBL genotype was not linked to superior metabolic potential in comparison with harboring only one BL/ESBL type. For example, B7, associated with carrying 2 BL/ESBL variants: CTX-M-15 and OXA, had a bio-score of 15, which was lower than that of CTX-M-14-producing isolate B21. Therefore, MDR phenotype and ESBL production were not associated with an elevated metabolic activity among isolates.

4. Discussion

Bacteremia due to ExPEC has increasingly been reported in the healthcare and community settings [33, 34]. Metabolism is an important factor used by bacteria to enhance their colonization of hosts [35], and it has been shown that humans could be exploited by many bacterial pathogens as a rich source of nutrients to aid their survival and growth [36]. Given the rapid global dissemination of particular MDR ExPEC clones, such as ST131, previous studies have explored the role of metabolism in driving the success of these clones [28, 31, 32]. However, these reports were limited to UPEC population and the metabolic traits of ExPEC isolates from the bacteremia population have poorly been studied. Here, we determined the metabolic profiles of 30 E. coli bacteremia isolates and compared ESBL-positive and ESBL-negative and ST131 and non-ST131 isolates in terms of their metabolic capacity.

Our study found that almost all E. coli blood isolates were capable of metabolizing many substrates, such as fructose and lactose. Nonetheless, all isolates entirely failed to utilize many substrates, such as inositol and alpha-methyl-D-glucoside, which is comparable to the typical biochemical activity of E. coli for these tests [32]. Interestingly, we reported a reduced utilization activity of our isolates for some substrates in comparison with what is typically known for E. coli, particularly those isolated from urine samples. For example, only 43% and 17.6% of these blood isolates were capable of utilizing mannitol and rhamnose, respectively. We previously found very high utilization levels for these two substrates among E. coli urine isolates [31]. In this regard, previous comparative phenotypic microarrays analysis of 10 isolates representing the major ExPEC STs, including 190 different substrates, found that bacteremia isolates were associated with lower metabolic activity compared with those isolated from urine [32]. However, given the small sample size used in that study in addition to the very limited number of studies on the metabolic traits of E. coli blood isolates, we believe that performing large-scale studies on the metabolic capacity of various ExPEC populations would be essential for providing an accurate comparison between urine and blood isolates in terms of their carbohydrate utilization capabilities.

Table 5: Relating the antibiotic susceptibility profiles to the biochemical activity of all isolates.

| Isolate ID | MDR | ESBL | BL/ESBL type(s) | ST131 | Bio-score |
|------------|-----|------|-----------------|-------|----------|
| B1         | MDR | +    | TEM-1           | +     | 9        |
| B2         | Non-MDR | −   | NA             | −     | 11       |
| B3         | MDR | −    | NA             | −     | 14       |
| B4         | MDR | −    | NA             | +     | 15       |
| B5         | MDR | −    | NA             | −     | 9        |
| B6         | Non-MDR | −   | NA             | −     | 6        |
| B7         | MDR | +    | CTX-M-15 and OXA-1 | +     | 15       |
| B8         | MDR | +    | CTX-M-15 and TEM-1 | +     | 14       |
| B9         | MDR | +    | OXA-1          | +     | 14       |
| B10        | MDR | +    | CTX-M-15       | +     | 13       |
| B11        | MDR | −    | NA             | −     | 15       |
| B12        | Non-MDR | −   | NA             | −     | 14       |
| B13        | Non-MDR | −   | NA             | −     | 12       |
| B14        | MDR | −    | NA             | −     | 7        |
| B15        | Non-MDR | −   | NA             | −     | 13       |
| B16        | MDR | +    | CTX-M-15 and OXA-1 | +     | 10       |
| B17        | Non-MDR | −   | NA             | −     | 14       |
| B18        | Non-MDR | −   | NA             | −     | 13       |
| B19        | Non-MDR | −   | NA             | −     | 11       |
| B20        | MDR | +    | CTX-M-15 and OXA-1 | +     | 11       |
| B21        | MDR | +    | CTX-M-14        | −     | 17       |
| B22        | MDR | +    | CTX-M-15 and OXA-1 | +     | 12       |
| B23        | MDR | +    | CTX-M-14 and TEM-1 | +     | 15       |
| B24        | MDR | +    | CTX-M-15 and TEM-1 | +     | 11       |
| B25        | MDR | −    | NA             | +     | 17       |
| B26        | MDR | +    | TEM-1           | +     | 12       |
| B27        | Non-MDR | −   | NA             | −     | 11       |
| B28        | MDR | +    | CTX-M-15 and TEM-1 | +     | 19       |
| B29        | MDR | +    | CTX-M-15 and TEM-1 | +     | 7        |
| B30        | MDR | +    | CTX-M-15 and TEM-1 | +     | 12       |
We also found 4 citrate-positive isolates, and this is surprising given that E. coli is usually citrate-negative [37]. This might be ascribed to that some MDR E. coli are associated with showing positive citrate reaction [29, 30], which is the case of our blood isolates. In this regard, we have previously demonstrated that 31 of 40 (77.5%) E. coli urine isolates were citrate-positive, the majority of which were exhibiting MDR phenotype [31]. Citrate utilization detected among some MDR ExPEC isolates highlights the need to further investigate the factors leading to this unexpected finding.

BL/ESBL-producing blood isolates exhibited higher utilization ability compared with non-BL/ESBL producers; however, no specific metabolic repertoire was detected among BL/ESBL-positive blood isolates. This concurs with previous finding demonstrating that BL/ESBL producing was slightly associated with higher metabolic capabilities among E. coli urine isolates [31, 32]. Although some reports have demonstrated the association between AMR and metabolism by which changes in metabolism have a role in modulating bacterial phenotypic resistance to antibiotics [38], it has been shown that AMR is not necessarily linked to metabolism, which might provide an explanation for the absence of specific metabolic profile among our BL/ESBL-producing blood isolates.

Interestingly, our analysis found that ST131 isolates were higher than non-ST131 isolates in metabolizing thirteen substrates and that ST131 was significantly associated with rhamnose utilization. Nevertheless, many studies concluded that ST131 was not the metabolically distinct clone of ExPEC and that no positive association was previously found between ST131 and particular biochemical substrates [31, 32]. However, another study has shown that ST131 urine isolates had higher metabolic profiles compared with other ExPEC ST1s and that ST131 isolates were positively associated with 5-keto-D-gluconate, beta-glucuronidase, and sucrose [28].

ExPEC isolates can survive in the host bloodstream through many mechanisms. For example, they possess a number of virulence factors, such as group 2 capsular polysaccharide K antigen [39] and increased serum survival (issA) protein [40], allowing them to resist the complement-mediated killing [41]. However, very little is known about the metabolic factors used by ExPEC to trigger their pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of porcine ExPEC pathogenesis in the bloodstream. In this regard, a recent Chinese analysis, of the mechanisms of ExPEC's fitness in the bloodstream.

We found no relationship between antimicrobial resistance and high metabolism potential of the tested blood isolates. This concurs with previous reports showing the same observation among E. coli urine population [31, 32]. Nonetheless, Gibreel and coauthors demonstrated that resistant isolates were of higher metabolic potential compared with susceptible isolates [28].

Considering the study limitations, our analysis was performed on bacteremia isolates from one city, Riyadh, which might not be reflective of the metabolic capacity of E. coli isolates from other Saudi regions. It also used a relatively low sample size. However, it identified an important finding that shows E. coli ST131 blood isolates with an increased metabolic capacity compared with non-ST131.

In conclusion, this is the first study that provides a description on the metabolic traits of E. coli blood isolates in Saudi Arabia. We reported that non-BL/ESBL producers were slightly lower in the metabolic capacity compared with ESBL-positive isolates, and this difference was found to be insignificant. Although few insignificant differences in the metabolic capacity were detected between ST131 and non-ST131 isolates, ST131 members were significantly associated with metabolizing rhamnose. Such finding is important and merits further analysis to assess the role of metabolism as a key factor in triggering the pathogenesis of this widespread ExPEC clone.

**Data Availability**

The data used to support the findings of the study can be obtained from the corresponding author upon reasonable request.

**Ethical Approval**

NA.

**Conflicts of Interest**

The authors declare that there are no conflicts of interest.

**Authors’ Contributions**

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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