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Original article

# Virulence characterization of *Escherichia coli* sequence type 131 clone isolated from hospital-acquired and community-acquired urinary tract infections, Egypt

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**ABSTRACT**

**Background:** The recently emerged *E. coli* ST131 clone is now a predominant cause of UTIs. There is a need to understand the virulence characteristics of this clone to limit its dissemination. **Aim of work:** We aimed to detect the presence of ST131 clones among *E. coli* strains isolated from UTI infections and compare the virulence carriage capacity between ST131 and non-ST131 strains. **Methods**: A total of 89 (63.5%) *E. coli* strains isolated from 140 patients with UTI were investigated for six different virulence genes using PCR-based assays. **Results:** Overall, 65 of 89 (73%) *E. coli* isolates belonged to the ST131 clone. Of these, 61 isolates (93.8%) were of the O25-ST131 clone, and only 4 isolates (6.2%) were of the O16-ST131 clone. From 24 catheterized patients, 20 (83.3%) were positive for the ST131 clone. Regarding the adhesin virulence genes, the *fim*H gene was detected in all studied isolates. Then, *pap*EF, *sfa*(sfa/foc), and *pap*A genes were found in 59.6%, 53.9%, and 30.3% of isolates, respectively. However, the *iro*N and *hylA* genes were found in 52.8% and 16.9%, respectively. **Conclusion**: *E. coli* ST131 isolates exhibited a higher prevalence among hospital-acquired UTIs than community-acquired UTIs, raising concern that this emerging clone has become a threat to nosocomial infections. The high virulence potential among *E. coli* ST131 isolates suggests that the virulence characterizations of this clone are in a continuous step-up evolution. More importantly, increased carriage of adhesin genes among ST131 isolates was demonstrated and can be targeted to develop new management strategies for UTI infections.

## Introduction

Extraintestinal pathogenic *Escherichia coli* (ExPEC) strains cause a wide range of infections, particularly urinary tract infections (UTIs) [1]. These strains are suggested to originate from the intestinal microbiota of humans and animals. From these reservoirs, strains may have acquired virulence factors to be able to migrate and cause different extraintestinal infections [2]. UTIs are the most common global bacterial infection. About 150 million cases of UTI have been estimated annually throughout the world, with high medical costs. ExPECs are responsible for more than 90% of community-acquired and 50% of nosocomial UTIs [3].

The emerging *E. coli* sequence type (ST131) is now considered a global pandemic strain [4,5]. In the last decade, this clone has become a predominant cause of UTIs, resulting in high

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morbidity and increased healthcare costs [6]. The high convergence of virulence with the antibiotic resistance of the prevalent *E. coli* ST131 clone is the most likely explanation for its successful dissemination in the last few years [7].

*E. coli* sequence type (ST131) can be transmitted in all ages and in both the community and hospital settings [4]. Rates of asymptomatic faecal carriage of this strain are increasing among the community worldwide, they are transmitted rapidly between hosts and become persistent gut colonizers [8]. During hospitalization, having a urinary catheter was associated with colonization [9].

ExPEC strains, including the *E. coli* ST131 clone, have a diverse repertoire of virulence factors that facilitate the colonization and establishment of UTI [10]. These virulence factors are divided into adhesins, iron-acquisition molecules, toxins, and others. Surface adhesins are important virulence factors for pathogenic *E. coli* that mediate the attachment and contact of bacteria to host cells [1]. Type 1 fimbria encoded by the *fim*H gene is a mannose-specific adhesin. It is considered an important factor in colonization and biofilm formation in UTIs and has been detected in most *E. coli* ST131 [6]. A fimbrial adhesin encoded by *afa* gene invades epithelial cells lining the host urinary tract by binding to specific receptors on the surface of these cells, and it has a hemagglutination capacity. The *sfa* gene encodes S fimbria which mediates adhesion to both intestinal and urinary tract epithelial cells and the penetration of bacteria. The high prevalence of genes encoding these adhesins in *E. coli* isolates in patients with UTI confirms the necessity of these structures to cause infection [11]. P fimbriae play a role in cytokine production in addition to its colonizing function, particularly in the pathogenesis of pyelonephritis [12].

Siderophores, or iron-uptake molecules, enable ExPECs to capture iron for enhancing bacterial growth and development. Salmochelin, encoded by the *iro*N gene, is a characteristic virulence factor of ExPEC strains [13]. Alpha (α haemolysin) is one of the toxins produced by ExPECs that causes cell lysis by creating pores in the membranes of host cells [14].

Recently, the successful worldwide spread of the *E. coli* ST131 clone was mostly related to its persistent ability to colonize the gut without antibiotic exposure history and to the easy transmission of this clone between healthy hosts [4]. So, there is an urgent need to understand the bacterial factors and virulence characteristics of this emerging clone to limit its dissemination, as the standard antimicrobial stewardship policies are not enough to control this clone. The emerging anti-virulence alternative therapeutic strategies targeting uropathogens as anti-adhesive molecules highlight the necessity of focusing on the virulence factors of this clone [15].

Data about the *E. coli* ST131 clone in Egypt is still limited. In a previous study conducted in Upper Egypt, we found a high prevalence of this clone and high antimicrobial resistance, as well as a significant carriage rate of the CTX-M-15 gene [16]. The aim of this study was to identify *E. coli* strains isolated from UTIs and study the virulence characteristics of isolated strains, screening for the O25/O16-ST131 clone, and comparing the virulence capacity between ST131 and non-ST131 strains.

## Patients and methods

### Study design

This retrospective study was performed from October 2022 to June 2023 in the Department of Medical Microbiology and Immunology, Faculty of Medicine, Minia University, Egypt. A total of 89 *E. coli* isolates from urine specimens from 140 UTI patients were included in this study. All participants in this study were adults (>18 years). The study included both outpatients (from Urology and Internal Medicine outpatients' clinics) and inpatients after 48 hours of admission in the ICU and different departments of Minia University Hospitals. UTI was diagnosed by having one symptom or more of the following: dysuria, frequency (> 8 times per day), suprapubic pain, or fever in association with pyuria and significant bacteriuria (*E. coli* $>10^5$ colony-forming units (CFU)/mL). A history of catheterization, recurrent UTI, and risk factors such as diabetes and urogenital abnormalities was taken. This study was conducted in agreement with the guidelines of the Declaration of Helsinki. The Ethics Committee of Minia University, Faculty of Medicine, Institutional Review Board has approved the study protocol (MUFMIRB Approval No. 490:10/2022).

### Bacterial isolation and identification

Urine samples were collected under complete aseptic precautions in sterile containers

and then transported within two hours to the microbiology laboratory for immediate examination. Urine samples with pus cells > 5 CFU/mL were streaked by the semi-quantitative streaking method onto chromogenic media (CHROMagar™ Orientation, Paris, France) and by the calibrated loop technique on MacConkey and Eosin Methylene Blue (EMB) agar (Oxoid, UK). After overnight incubation at 37°C, isolated colonies were then identified biochemically by indole, methyl red, citrate, Voges-Proskauer, urease, and sugar fermentation tests. Strains identified and confirmed as *E. coli* were inoculated in Trypticase soy broth (Oxoid, UK), incubated for 24 hours, mixed with sterilized glycerol (20%), and stored at -20°C for further tests. A total of 89 non-repetitive *E. coli* isolates were recovered from all urine samples.

**DNA extraction**

The DNA of 89 *E. coli* isolates was extracted using a modified boiling technique by the heat shock method [17]. First, centrifugation of 1.5 ml of overnight bacterial culture at 13,000 rpm was done for 5 min at 4°C, and then the supernatant was removed carefully. The suspended pellet in 200 µl of sterile distilled water was then put for 15 min in a water bath and immediately shocked by ice-cooling for 10 min. Lastly, centrifugation at 13,000 rpm for 5 min at 4°C was done, and the supernatant transferred, containing genomic DNA, into a new tube for subsequent PCR amplification. Extracted DNA was used immediately or stored at -20 ˚C until used.

**Detection of ST131 clones**

Screening of *E. coli* isolates from urine specimens was done for the detection of *E. coli* ST131 clones using primers to amplify *pap*B and *trp*A genes for the O25-ST131 and O16-ST131 clades, respectively **(Table 1)**. The following optimized cycling conditions were used: Initial denaturation for 4 min at 94°C was followed by 30 amplification cycles (denaturation for 5s at 94°C, annealing for 20s at 63°C, and extension for 30s at 72°C), and lastly, a final extension step at 72°C for 5 min [12,18]. The PCR products were resolved on 1.5% agarose gel electrophoresis. We used strains of O25 and O16-ST131 *E. coli* from our laboratory from a previous study as positive controls in PCR reactions for the ST131 clone [16,19].

**Detection of virulence genes**

The presence of six different virulence genes for adhesins, iron acquisition, and toxin was examined in all isolates using PCR-based assays. The amplification reactions were carried out under the following conditions: initial denaturation at 95 ˚C for 5 min, then 35 amplification cycles (30s at 94 ˚C, 30s at 63 ˚C, and 30s at 72 ˚C), and a final extension at 72 ˚C for 5 min. All the amplification reactions were carried out using Biometra UNO II thermal cycler (Goettingen, Germany). Each single PCR reaction was performed in a total volume of 25µL (12.5µL of Hot Start Green PCR Master Mix, 3µL (100 ng/mL) of DNA template, 1µL (10 pmol) of each primer, and 7.5µL of nuclease-free water). The virulence scores of isolates were calculated as the sum of the genes carried. All primer sequences used in this study and the predicted PCR products' sizes are shown in **Table 1**. Positive strains from a previous study on virulence genes were used as positive quality controls in PCR reactions [24].

**Statistical analysis**

IBM SPSS software (version 20.0) was employed for the analysis of demographic, clinical, and laboratory data. A P value of $< 0.05$ is used as an indication of statistical significance.

**Results**

**Demographic characteristics of the study subjects**

A total of 89 *E. coli* strains were isolated from 140 patients with UTI (65.5%). Out of 89 urinary isolates, 53 (59.6%) were from female patients and 36 (40.4%) were from male patients. The mean age of all UTI patients was 39.6 ± 14.4. Of the 89 patients, 47 (52.8%) were inpatients, representing hospital-acquired UTIs, and 42 (47.2%) were outpatients, representing community-acquired UTIs. The clinical characteristics of the ST131 group differ significantly from those of the non-ST131 group for fever (84.1% vs. 15.9%, P value = 0.02) and frequency (67.6% vs. 32.4%, P value = 0.039). ST131 clone was significantly higher among isolates from inpatients (hospital-acquired UTI) (39/47, 83%) than that from outpatients (community-acquired UTI) (26/42, 61.9%), with a P value of 0.025. From 24 catheterized patients, 20 (83.3%) were positive for the ST131 clone. More than 65% of *E. coli* strains isolated from patients with a history of recurrent UTIs were positive for ST131. Also, *E. coli* strains isolated from patients with risk factors (diabetes and urogenital abnormalities) showed high rates of ST131 clone carriage, with no significant difference between ST131 and non-ST131 patient groups **(Table 2)**.

**Detection of O25-ST131, O16-ST131 clones**

The overall prevalence of ST131 clones among all *E. coli* isolates was 73% (65 of 89). From these, 61 isolates (93.8%) were positive for *pab*B gene of the O25-ST131 clone and only 4 isolates (6.2%) were positive for *trp*A gene of the O16-ST131 clone. There was a higher rate of O25-ST131 clones than O16-ST131 clones among all *E. coli* isolates **(Figure 1)**.

**Detection of virulence genes and different virotypes**

The frequencies of the six studied virulence genes among all *E. coli* isolates are shown in **Figure 2**. Regarding the adhesin virulence genes, the *fim*H gene was detected in all studied isolates. Then, *pap*EF and *sfa(sfa/foc)* were found in 59.6% and 53.9% of isolates, respectively. The *pap*A gene was the least detected adhesin gene (30.3%), with only one non-ST131 isolate being positive for the *pap*A gene. A high prevalence of the iron chelating gene (*iro*N) was found in all *E. coli* isolates (52.8%). Finally, the *hyl*A gene was positive in only 16.9% of the studied isolates, and all positive isolates were of the ST131 *E. coli* clone. The rates of the six virulence genes in ST131 isolates were higher than those in non-ST131 isolates. This difference was significant for *pap*A, *pap*EF, *sfa(sfa/foc)*, *iro*N, and *hyl*A **(Table 3)**.

The studied *E. coli* strains exhibited 18 different virulence gene patterns, referred to as EC. The ST131 isolates showed more virulence genotype patterns (17 EC patterns) than non-ST131 isolates, which showed only seven EC patterns. The most detected pattern was EC18, which carried only the *fim*H gene, and of these, 14/17 (82.4%) belonged to the non-ST131 clone. The most frequently detected combined virulence patterns were EC4 which carries four genes (*fim*H, *sfa*, *pap*EF, and *iro*N), and EC2 which carries five genes (*fim*H, *sfa*, *pap*A, *pap*EF, and *iro*N). All isolates belonging to the EC2 and EC4 patterns were positive for ST131 clones **(Table 4)**.

Regarding virulence capacity, *E. coli* ST131 isolates showed a higher virulence score (≥4 genes) when compared to non-ST131 isolates (1-3 genes), which was significant (P value =<0.0001). More than 50% of non-ST131 isolates carried one gene (*fim*H), and the remaining isolates carried 2 or 3 genes. The median and range of virulence scores were 4 (1–6) for ST131 isolates and 1 (1–3) for non-ST131 isolates. For ST131 *E. coli* isolates, the median virulence scores (ranges) of the O16-ST131 clone were 5.5 (4–6) and those of the O25-ST131 clone were 4 (1–6). However, the higher virulence scores of the O16-ST131 clone strains, but the too-low number of isolates (4), limited the statistical differentiation between the O16 and O25 clones based on virulence.

**Table 1.** PCR primers used in the study

| Primer | Primers (5_ to 3_) | Amplicon size (bp) | Reference |
|---|---|---|---|
| *pab*B ST131-O25b | F- TCCAGCAGGTGCTGGATCGT<br>R- GCGAAATTTTTCGCCGTACTGT | 347 | [18] |
| *trp*A ST131-O16 | F- AAAACCGCGCCGCGTTACCT<br>R- CCAGAAATCGCGCCCGCATT | 145 | [12] |
| *fim*H | F: TGCAGAACGGATAAGCCGTGG<br>R: GCAGTCACCTGCCCTCCGGTA | 506 | [20] |
| *pap*A | F: ATGGCAGTGGTGTTTTGGTG<br>R: CGTCCCACCATACGTGCTCTTC | 720 | [17] |
| *sfa(sfa/foc)* | F: CTCCGGAGAACTGGGTGCATCTTAC<br>R: CGGAGGAGTAATTACAAACCTGGCA | 410 | [21] |
| *pap*E/F | F: GCAACAGCAACGCTGGTTGCATCAT<br>R: AGAGAGAGCCACTCTTATACGGACA | 336 | [22] |
| *hly*A | F: AACAAGGATAAGCACTGTTCTGGCT<br>R: ACCATATAAGCGGTCATTCCCGTCA | 1170 | [22] |
| *iro*N | F AAGTCAAAGCAGGGGTTGCCCG<br>R GACGCCGACATTAAGACGCAG | 665 | [23] |

Abbreviations: bp (base pair)

**Table 2.** Clinical data and risk factors among UTI patients in the study

| Characteristic | | Total 89 | ST131 *E. coli* isolates (65) | Non-ST131 *E. coli* isolates (24) | P value |
|---|---|---|---|---|---|
| Type of visit | Inpatients | 47 | 39(83%) | 8(17%) | **0.025*** |
| | Outpatients | 42 | 26(61.9%) | 16(38.1%) | |
| Fever | Positive | 44 | 37(84.1%) | 7(15.9%) | **0.02*** |
| | Negative | 45 | 28(62.2%) | 17(37.8%) | |
| Dysuria | Positive | 79 | 57(72.2%) | 22(27.8%) | 0.59 |
| | Negative | 10 | 8(80%) | 2(20%) | |
| Frequency | Positive | 68 | 46(67.6%) | 22(32.4%) | **0.039*** |
| | Negative | 21 | 19(90.5%) | 2(9.5%) | |
| Suprapubic pain | Positive | 64 | 45(70.3%) | 19(29.7%) | 0.35 |
| | Negative | 25 | 20(80%) | 5(20%) | |
| Catheterization | Yes | 24 | 20(83.3%) | 4(16.7%) | 0.18 |
| | No | 65 | 45(69.2%) | 20(30.8%) | |
| Recurrent UTI | Yes | 48 | 32(66.7%) | 16(33.3%) | 0.14 |
| | No | 41 | 33(80.5%) | 8(19.5%) | |
| Diabetes | Yes | 25 | 16(64%) | 9(36%) | 0.23 |
| | No | 64 | 49(76.6%) | 15(23.4%) | |
| Urogenital abnormality | Yes | 19 | 14(73.7%) | 5(26.3%) | 0.94 |
| | No | 70 | 51(72.9%) | 19(27.1%) | |

* P value is significant

**Table 3.** Virulence genes distributions among ST131 and non-ST131 *E. coli* isolates

| Virulence gene | | ST131 *E. coli* isolates (65) | Non-ST131 *E. coli* isolates (24) | P value |
|---|---|---|---|---|
| *iro*N | Positive | 44 (67.7%) | 3 (12.5%) | **<0.0001*** |
| | Negative | 21 (32.3%) | 21 (87.5%) | |
| *sfa(sfa/foc)* | Positive | 42 (64.6%) | 6 (25%) | **0.001*** |
| | Negative | 23 (35.4%) | 18 (75%) | |
| *pap*EF | Positive | 49 (75.4%) | 4 (16.7%) | **<0.0001*** |
| | Negative | 16 (24.6%) | 20 (83.3%) | |
| *pap*A | Positive | 26 (40%) | 1 (4.2%) | **0.001*** |
| | Negative | 39 (60%) | 23 (95.8%) | |
| *hyl*A | Positive | 15 (23.1%) | 0 (0%) | **0.01*** |
| | Negative | 50 (76.9%) | 24 (100%) | |
| *fim*H | Positive | 65(100%) | 24 (100%) | - |
| | Negative | 0 | 0 | |

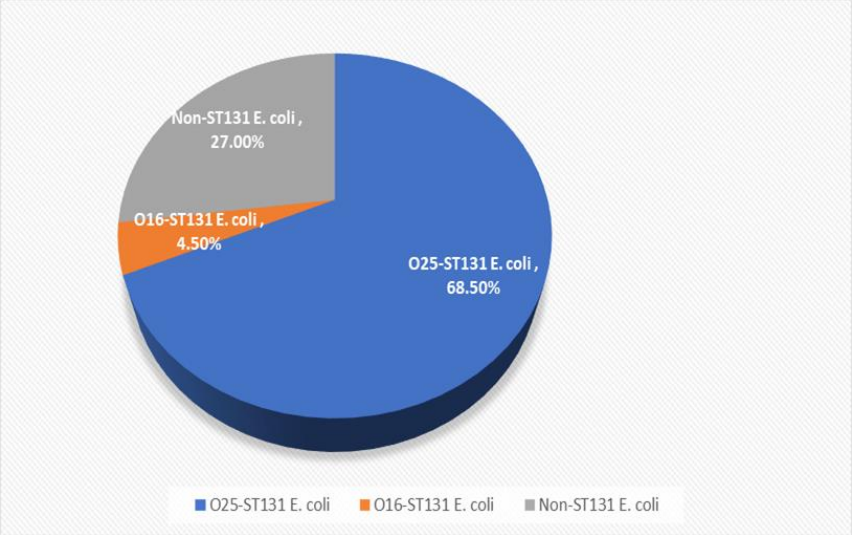
* P value is significant

**Table 4.** Distribution of different virulence gene patterns among ST131 and Non-ST131 *E. coli* isolates

| *E. coli* virulence pattern (EC) | | *fim*H | *sfa (sfa/ foc)* | *pap*A | *pap*EF | *iro*N | *hyl*A | No. of isolates | ST131 | | Non-ST131 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | 025-ST131 | O16-ST131 | |
| 6 genes | EC1 | + | + | + | + | + | + | 7 | 5 | 2 | 0 |
| 5 genes | EC2 | + | + | + | + | + | - | 9 | 8 | 1 | 0 |
| | EC3 | + | + | - | + | + | + | 2 | 2 | 0 | 0 |
| 4 genes | EC4 | + | + | - | + | + | - | 11 | 11 | 0 | 0 |
| | EC5 | + | + | - | + | - | + | 4 | 4 | 0 | 0 |
| | EC6 | + | + | + | - | + | - | 2 | 2 | 0 | 0 |
| | EC7 | + | - | - | + | + | + | 2 | 2 | 0 | 0 |
| | EC8 | + | + | + | + | - | - | 1 | 0 | 1 | 0 |

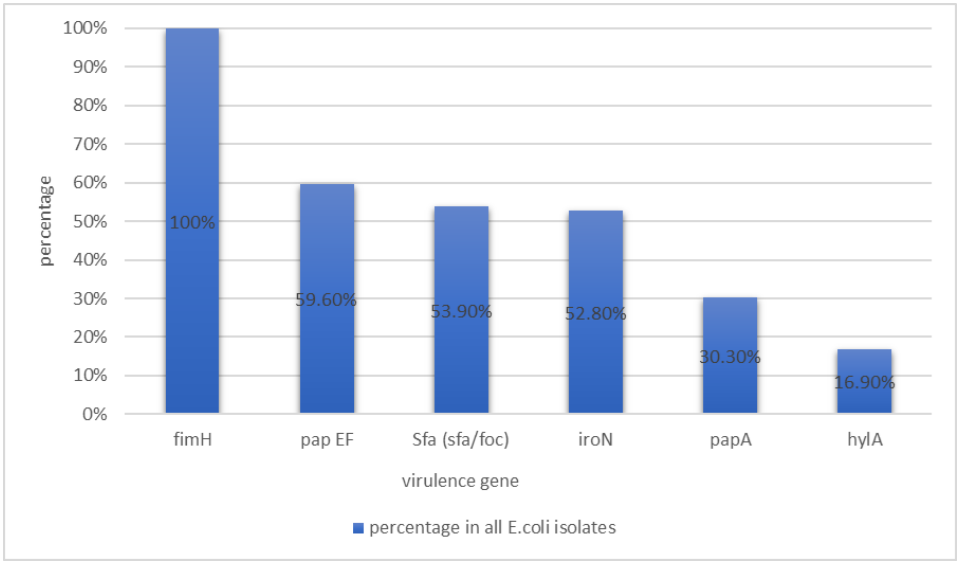
| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 genes | EC9 | + | - | - | + | + | - | 8 | 7 | 0 | 1 |
| | EC10 | + | - | + | + | - | - | 3 | 3 | 0 | 0 |
| | EC11 | + | + | - | + | - | - | 4 | 2 | 0 | 2 |
| | EC12 | + | + | - | - | + | - | 1 | 1 | 0 | 0 |
| | EC13 | + | - | + | - | + | - | 1 | 0 | 0 | 1 |
| 2 genes | EC14 | + | + | - | - | - | - | 7 | 3 | 0 | 4 |
| | EC15 | + | - | - | - | + | - | 4 | 3 | 0 | 1 |
| | EC16 | + | - | + | - | - | - | 4 | 4 | 0 | 0 |
| | EC17 | + | - | - | + | - | - | 2 | 1 | 0 | 1 |
| 1 gene | EC18 | + | - | - | - | - | - | 17 | 3 | 0 | 14 |

**Figure 1.** Prevalence of O25-ST131 and O16-ST131 clones among studied *E. coli* isolates




ST131 clones among all *E. coli* isolates was 73% with 61 isolates (68.5%) were O25-ST131 clone and only 4 isolates (4.5%) were O16-ST131 clone. However, 27% were negative for ST131 clones

**Figure 2.** Frequencies of virulence genes among all studied *E. coli* isolates were (*fim*H 100%, *pap*EF 59.6%, sfa(sfa/foc) 53.9%, iroN 52.8%, *pap*A 30.3%, *hyl*A 16.9%)



**Discussion**

The increased prevalence of the worldwide pandemic *E. coli* ST131 clone highlights the necessity of studying its bacterial characteristics. The *E. coli* ST131 clone is considered an ideal model for the co-evolution of virulence and antibiotic resistance [7]. In the current study, the O25/O16 ST131 clones were detected in 73% of urinary *E. coli* isolates. This high detected prevalence agrees with recent reports in Egypt [13, 16]. Also, a high prevalence of this clone was found in the Middle East region (KSA 61.7%, Iran 84.4%) [25,26]. The O25-ST131 clone was the predominant demonstrated clone (68.5%), and the O16-ST131 clone was detected in a very low percentage (4.5%) of all urinary *E. coli* isolates. These results are similar to most worldwide reports that the O25-ST131 serotype is the dominant clone and that the O16-ST131 serotype accounts for only 1-5% of overall *E. coli* stains [27]. However, studies on ST131 clones in Egypt are scarce, the O25-ST131 clone was also more predominant than the O16-ST131 clone in the few previous studies done in Egypt [19]. No isolates of the O16-ST131 clone were detected in the most recent study done in Upper Egypt [16]. On the other hand, the O16-ST131 clone was detected at a higher rate in a previous study done on the fluoroquinolone-resistant *E. coli* strains. The relatively increased rate among fluoroquinolone resistance strains suggests the higher resistance of the O16-ST131 serotype [19].

The ST131 clone was significantly higher among isolates from inpatients (hospital-acquired UTI) (39/47, 83%) than that from outpatients (community-acquired UTI) (26/42, 61.9%), raising concern that this emerging clone became a threat for nosocomial infections. To the best of our knowledge, this is new in this study to compare the prevalence of the *E. coli* ST131 clone between hospital-acquired and community-acquired UTIs in Egypt. Also, we found a higher rate of this clone among catheterized patients (20/24, 83.3%). In agreement with these findings, Zou and his colleagues found an association between the ST131 clone and catheter-associated UTIs. They also explained the possibility that these ST131 strains arose from coevolution with catheterized hosts and the carriage of distinctive combinations of virulence-associated genes [28].

The pathogenic potential of any bacterial strain relies on both host and bacterial characteristics [29]. This study shows some risk factors such as catheterization, hospitalization, history of recurrent UTI, diabetes mellitus, and urogenital abnormalities. This study detected a high rate of the ST131 clone in most patients with risk factors that were only significantly higher among hospitalized inpatients than outpatients. Lindblom and his colleagues detected ST131 in more than half of the patients with recurrent UTIs, and this was close to our findings (65% with recurrent UTIs) [30]. In a meta-analysis study on risk factors associated with ST131, it was difficult to identify common risk factors associated with this pandemic clone. The authors suggested that the success of the ST131 clone relies more on bacterial factors than host characteristics [31].

The rapid step-up in *E. coli* ST131 prevalence is possibly related to the increased virulence potential of this clone [31]. Studies on the virulence characteristics of the *E. coli* ST131 clone have yielded conflicting results. Most earlier studies suggested that ST131 isolates were less virulent than other UPECs [33]. The initial findings about the virulence of the *E. coli* ST131 clone proved the absence of *pap* genes encoding P fimbriae [6]. Recently, many studies have detected contrary findings, and the number of virulence gene profiles detected in *E. coli* ST131 isolates is increasing [32]. Ben Zakour and his colleagues mapped the evolution storm of this clone by sequential acquisition of different virulence gene determinants, followed by the development of antibiotic resistance as fluoroquinolones. They proposed that virulence factors were the precursors that gave this clone the ability to colonize and then acquire mobile genetic elements and mutations that caused resistance [34]. This was against the old, long-established view of studies that found that high antibiotic-resistant strains are associated with fitness costs and possess fewer virulence genes than other susceptible strains [35].

Despite the high number of studies on the *E. coli* ST131 clone, a lot is still unknown about the virulence characteristics of this organism that promoted its dramatic global spread. The key difference between UPECs and commensal *E. coli* stains is their ability to express virulence factors, particularly adhesins. These adhesins are considered necessary steps for the entry of organisms and to enhance colonization and biofilm formation. Toxins and iron uptake systems also differentiate pathogenic from commensal *E. coli* strains [36].

Concerning the virulence capacity of the studied *E. coli* ST131 isolates, the *fim*H gene encoding pilus one was demonstrated in 100% of the isolates. This was like most previous studies that detected this gene in all ST131 strains [27, 37]. Type one fimbriae encoded by *fim* genes are the key virulence factor for *E. coli* ST131 strains. Totsika and his colleagues used a mouse model and found the ability of *E. coli* ST131 isolates colonizing mouse bladders to switch on the expression of type one fimbriae [38]. The *sfa(sfa/foc)* gene encoding S fimbria was demonstrated in 53.9% of all isolates and 67.6% of ST131 isolates with significant association with the ST131 clone (P value = 0.001). This percent was higher than that detected by Ali and his colleagues in Pakistan, who found this gene in only 18%, with a significant association with ST131 isolates [37]. However, no *sfa* gene was detected in some studies [25, 27].

For the pap genes, *pap*EF and *pap*A genes were demonstrated in 59.6% and 30.3% of all isolates, with a significantly higher rate among ST131 isolates than non-ST131 isolates (P values <0.0001 and 0.001, respectively). The *pap*EF gene prevalence was markedly higher than in previous reports [25, 27]. For the *pap*A gene, it was similarly detected in a study in KSA (35%) [23], but it was found at a higher rate (95% of ST131 isolates) in a study in China [27] and detected at a lower rate (17%) in another study in Pakistan [37]. This variable virulence distribution suggests that the virulence profile may depend on the geographical region or other factors.

For the virulence genes *iro*N and *hyl*A, the prevalence in our study was 67.7% and 23.1% among ST131 isolates, respectively. The *iro*N gene was the most detected in this study and was markedly higher than most previous studies that detected this gene in a low percentage ranging from 2.6 to 16.5% [8,25,27]. However, the *Iro*N gene was detected previously among UPEC isolates in Egypt in 36%, suggesting that this gene is prevalent in *E. coli* isolates in our country [23]. The toxin *hyl*A gene prevalence was detected in similar proportions (27%) in Iran and KSA [25,26], but lower than that detected recently by Kim and his colleagues (53%) in Korea [10]. The toxin *hyl*A gene was the least detected gene, indicating the low toxicity of the studied ST131 isolates.

An earlier study on ST131 *E. coli* in Egypt done in 2011 displayed few virulence genes with no significant difference in virulence patterns between ST131 and non-ST131 isolates, and no *pap* genes were detected [39]. Our results demonstrated an increased association of adhesin genes, including *pap* genes, with *E. coli* ST131 isolates in Egypt, suggesting a changing virulence of this clone in the last decade. These results were in accordance with a recent Egyptian study that characterized five pathogenicity islands in the chromosome of the Egyptian UPEC strain of the O25-ST131 clone. The strain was found to carry different adhesins (*fim*H, *pap*A, *pap*C, and *sfa* operon) [36].

By comparing these results with the previous data and local reports [19, 39] about the virulence of the *E. coli* ST131 clone, an increased virulence potential of this clone was identified, particularly for the adhesin genes and *iro*N gene. This also agreed with the recent results of Alqasim and his colleagues in 2020 in KSA [26]. They detected a higher virulence potential in uropathogenic ST131 isolates in comparison to the moderate virulence detected by Alghoribi and his colleagues in 2015 [40]. These findings suggest that the virulence characterizations of this clone are in a continuous step-up evolution. In accordance with most recent studies [26, 41], *E. coli* ST131 isolates in our study showed a significantly higher virulence score when compared to non-ST131 isolates that most of them carried only one gene. Also, higher virulence scores for the O16-ST131 clone strains were demonstrated. This agrees with previous studies proving that the O16-ST131 clade is highly virulent [18]. Johnson and his colleagues provided evidence that the O16-ST131 clone is virulent in a mouse model [12]. The very low number of O16-ST131 isolates (4 isolates) in our study limited the statistical differentiation between O16 and O25 clones based on virulence.

The *E. coli* ST131 isolates showed more diverse virulence genotype patterns than non-ST131 isolates. The most frequently detected combined virulence patterns (EC2 and EP4) belonged to ST131 clones. We detected that more than 50% of ST131 isolates have virulence patterns that carry the *fim*H, *sfa*, and *pap* genes. In agreement with our study, Rahdar and his colleagues found that 36% of UPEC isolates carry the *fim*, *sfa*, and *pap* virulence genes [42]. The combination of these genes is explained by the presence of these genes on the same pathogenicity island of UPEC [43]. Also, a recent Egyptian study characterized the pathogenicity islands in the chromosome of the UPEC strain of the

O25-ST131 clone carrying the following adhesins (*fim*H, *pap*A, *pap*C, and *sfa* operon) [36]. These findings explain our findings of increased virulence profile patterns carrying these genes.

The key factor in the evolution of the pandemic *E. coli* ST131 clone is mostly related to its ability to colonize, depending on the complement of its virulence factors. The co-evolution of virulence and antibiotic resistance of the *E. coli* ST131 clone is due to its high ability to acquire transmissible genetic elements and its dynamic accessory genome [7]. Zakaria and his colleagues found that the problem of the global dissemination of the *E. coli* ST131 clone is the acquisition of large genomic regions (pathogenicity islands) that encode a wide range of virulence factors. These pathogenicity islands can undergo spontaneous excision and integration by horizontal transfer, resulting in the evolution of this organism [36]. The virulence genes are also acquired through mobile genetic elements and chromosomal pathogenicity islands. Plasmids are classified into the incompatibility (Inc) groups. Most plasmids associated with the ST131 clone belong to the Inc F group, which is named after the F-type pili [44]. In addition to carrying resistance extended-spectrum beta-lactamase genes, IncF plasmids can carry virulence genes associated with iron homeostasis and toxin production [45]. This can explain the high prevalence of the *iro*N gene among the studied ST131 isolates. Also, there is suggested evidence that plasmids carried by resistant ST131 strains can also interact with and regulate chromosomally encoded pathogenic factors [46].

The bacterial factors involved in the dramatic global spread of the *E. coli* ST131 clone are still an enigma. The easy transmission of this clone between hosts and its persistent colonization of the gut even in the absence of antibiotic use are major factors in its dissemination. This created challenges in the management of infections caused by the ST131 clone, as UTI and antimicrobial stewardship programs became insufficient to stop this pandemic clone. Studying the virulence-colonizing characteristics of this clone became critical to gain more insights that can help developing new management strategies for UTI infections. The focus of some investigations is on the role of mannosides in decreasing gut colonization by ST131 strains [47]. The increasing number of virulence factors involved in the adhesion and colonization of *E. coli* ST131 strains offers the possibility to develop specifically targeted anti-adhesive therapeutic strategies. *Fim*H-encoding type 1 pilus is an important bacterial adhesin found in more than 95% of UPEC and almost all *E. coli* ST131 strains [48]. This pilus is mannose-sensitive, as it can bind to mannosylated receptors expressed by uroepithelial cells. This mediates stable bacterial adhesion and plays a role in biofilm formation, proliferation, and invasion into cells, with the formation of intracellular bacterial communities [49]. Several *fim*H inhibitor molecules have been developed recently as emerging anti-virulence alternative therapeutic strategies targeting uropathogens. These molecules are considered valuable and cost-effective promising approaches to treat UTIs by highly resistant UPEC.

## Conclusion

This study provides updated data about the prevalence and the genetic virulence patterns of the *E. coli* ST131 clone in Egypt. These data can help to understand the bacterial factors and virulence characteristics of this emerging clone to limit its dissemination through new emerging anti-virulence alternative therapeutic strategies. A high prevalence of ST131 clones (73%) was detected among uropathogenic *E. coli* isolates, with a significantly higher rate among hospital-acquired UTIs than community-acquired UTIs. The O25 serotype was the most prevalent among isolates, and the O16 serotype was found in only 4.5% of ST131 isolates. ST131 isolates exhibited high virulence potential compared to non-ST131 and demonstrated different virulence virotypes. More importantly, increased carriage of adhesin genes (*fim*H and *pap*) and the iron chelating gene (*iro*N) was detected among ST131 *E. coli* isolates. No *hyl*A gene was detected among non-ST131 isolates. The increasing number of virulence factors involved in the adhesion and colonization of *E. coli* ST131 strains offering the possibility of developing specifically targeted anti-adhesive therapeutic strategies. We recommend the need for further studies to assess the association of virulence with the clinical severity of UTI infection by this highly disseminating clone. Also, additional studies are needed to investigate the risk factors for the evolution and alarming increase in virulence characteristics of this clone.

## Abbreviations

CFU: Colony forming unit

DNA: Deoxyribonucleic acid

EC pattern: *Escherichia coli* pattern

*E. coli* ST131: *Escherichia coli* sequence type 131

ExPEC: Extra-intestinal pathogenic *E. coli*

*fim*H gene: fimbrial adhesion related gene

HPF: High power field

*hyl*A: Haemolysin alpha toxin encoding gene

IncF: Incompatibility F

*Iro*N gene: iron uptake gene

*pap genes:* Pyelonephritis–associated pilus encoding gene

PCR: Polymerase Chain Reaction

*sfa(sfa/foc)*: S fimbrial adhesins (*sfa*) and F1C ("pseudotype I") fimbriae (*foc*) gene

UPEC: Uropathogenic *E. coli*

UTIs: Urinary Tract infections

**Declarations**

**Ethics declarations**

**Ethics approval and consent to participate**

The study protocol was approved by the Research Ethics Committee, Minia University, Faculty of Medicine Institutional Review Board (MUFMIRB Approval No.490:10/2022). MUFMIRB is constituted and operates according to the guidelines of the Declaration of Helsinki and CIOMS (Council for International Organizations of Medical Sciences). Informed consents were obtained from all participants in the study for the use of the samples.

**Consent for publication**

Not applicable.

**Availability of data and materials**

All data generated or analysed during this study are included in this article.

**Competing interests**

The authors declare that they have no competing interests.

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SSA designed the study and drafted the manuscript. SSA, SZ, and DNK performed the experimental work and analyzed the data. HA analyzed the clinical data. All authors have approved the final manuscript.

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