

Comparative Study of CTX-M-15 Producing *Escherichia coli* ST131 Clone Isolated from Urinary Tract Infections and Acute Diarrhoea

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Background and Purpose: The alarming increase in the prevalence of CTX-M-15 extended-spectrum β -lactamase (ESBL) producing *E. coli* has been significantly linked to the clonal expansion of emerging sequence type (ST131). This study aimed to screen for the O16/O25-ST131 clones among different phylogenetic types of *E. coli* strains isolated from urinary and diarrhoeal samples.

Methods: A total of 205 *E. coli* strains isolated from patients with UTI and acute diarrhoea were investigated by phenotypic and genotypic methods for ESBL identification. Molecular methods were used for identification of O25/O16-ST131 clone and phylogenetic typing of *E. coli* isolates.

Results: O25-ST131 clone was detected in 89/105 (84.8%) and 47/100 (47%) of urinary and intestinal *E. coli* isolates, respectively, with a significant difference (P -value<0.001). There was a significant high rate of occurrence of ESBLs, MDR, and antibiotic resistance to most antibiotic classes among O25-ST131 than non-O25-ST131 isolates. CTX-M-15 gene was detected in 64/71 (90%) of ESBLs producing intestinal isolates and 54/79 (68.4%) of urinary ESBLs producing isolates. The O25-ST131 clone was reported among all phylogenetic groups. The O16-ST131 clone serotype was not detected in the study isolates.

Conclusion: High prevalence of the O25-ST131 clone was reported among extraintestinal and intestinal *E. coli* isolates. First detection of the O25-ST131 clone among phylogenetic groups other than group B2 draws attention of the ability of this clone to transfer among commensal groups. An increasing in the prevalence of CTX-M-15 among *E. coli* strains especially of intestinal origin is alarming as the intestine is the main reservoir for ExPEC strains causing UTI.

Keywords: *E. coli*, O25-ST131 clone, ESBLs, UTI, diarrhoea

Introduction

Escherichia coli (*E. coli*) is one of the common causes of bacterial infections in both digestive and urinary tracts.¹ *E. coli* is a member of the gut flora. Different species of bacteria interact in the gut and can gain virulence and antibiotic resistance genes developing pathogenic *E. coli* strains.² Pathogenic *E. coli* strains cause either diarrhoea by intestinal pathogenic *E. coli* (IPEC) or extra-intestinal infections by extra-intestinal pathogenic *E. coli* (ExPEC). Urinary tract infection (UTI) is the commonest extra-intestinal infection as uropathogenic *E. coli* (UPEC) was found to be associated with more than 80% of UTI.^{3,4}

The emerging *E. coli* ST131 was identified in 2008 as a multidrug resistant clone producing ESBLs. CTX-M-15 is the most dominant ESBL reported among *E. coli* ST131 strains.⁵ The worldwide alarming increase in CTX-M-15 ESBL producing

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Enterobacteriaceae, especially *E. coli* strains, represents a major challenge to public health.⁶ This increased isolation of CTX-M-15 producing *E. coli* strains was linked to the rapid clonal expansion of ST131 during the last 20 years.² The success of dissemination of *E. coli* ST131 clone is explained by carrying ESBL resistance genes and different virulence factors⁷ and also by its predominance in the human gut.¹ *E. coli* O25-ST131 is the predominant clone serotype, while O16-ST131 was reported in a very small percentage in some studies.² Misuse of antibiotics in Middle East countries due to lack of policies controlling for sale of antibiotics plays an important role in the emergence of resistant bacterial strains. The relationships between use of third generation cephalosporins and increasing ESBL producing strains have been reported in several studies.⁸

E. coli is classified phylogenetically into four main group; A, B1, D, and B2.⁹ Most previous studies restrict the *E. coli* ST131 clone to phylogenetic group B₂ but recently some studies reported ST131 isolates in other phylogenetic groups which may be an index of risk.^{10,11} Increasing rates of intestinal colonization with CTX-M-15 ESBL producing *E. coli* ST131 is particularly worrisome as the intestine is the main reservoir for ExPEC strains causing UTI and it was found that most UTIs were caused by *E. coli* in feces of the patients.¹² The information about the *E. coli* ST131 clone in Egypt is very sparse. Therefore, the current study aimed to identify the prevalence of O16/O25-ST131 clones among different phylogenetic types of *E. coli* strains isolated from UTIs and diarrhoeal samples. The study also aimed to identify the association between this clone and antibiotic resistance patterns, ESBL production, and the presence of the CTX-M-15 gene.

Patients and Methods

Study Design

This study was conducted in the period from July 2020 to February 2021 in the department of Microbiology and Immunology, Faculty of medicine, Minia University, Egypt. A total of 205 *E. coli* clinical isolates (104 from urine of UTI patients and 100 from stool of patients with acute diarrhoea) were included in this study. All study participants were adults attending Outpatient clinics of Minia University Hospitals. UTI was diagnosed by dysuria, frequency, urgency with pyuria, and significant bacteriuria *E. coli* >10⁵ CFU/ mL. Acute diarrhoea was diagnosed clinically by passage of loose or watery stools more than 3-times daily with any of the symptoms

vomiting, abdominal cramps, or dysentery. Patients with any history of antibiotic use or hospitalization 2 weeks before specimen collection or any history of chronic disease were excluded. The study was approved by the Medical Ethics of Minia University hospital and written informed consent was taken from each participant for the use of the samples. This study was performed in agreement with the guidelines of the Declaration of Helsinki.

Bacterial Isolation and Identification

Both urine and stool samples were collected under aseptic precautions in sterile containers and transported within 2 hours to the microbiology laboratory for immediate examination. Urine samples with pus >5 cells/HPF were cultured on chromogenic media (CHROMagar™ Orientation, Paris, France). Stool samples were inoculated into enrichment broth and then sub-cultured on MacConkey and Eosin Methylene Blue (EMB) agar (Oxoid, UK). 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 grown in Trypticase soy broth (Oxoid, UK), mixed with sterilized glycerol 20% and stored at -20°C for further testing.

Antimicrobial Susceptibility Testing

All isolated *E. coli* strains were subjected to antimicrobial susceptibility testing using Kirby Bauer disc diffusion method and identified according to Clinical and Laboratory Standards Institute (CLSI).¹³ The following antibiotic discs were used; amoxicillin/clavulanic acid (AMC, 30 µg), ceftriaxone (CRO, 30 µg), ceftazidime (CAZ, 30 µg), imipenem (IPM, 10 µg), amikacin (AMK, 30 µg), sulphamethoxazole/trimethoprim (SXT, 25 µg), and Ciprofloxacin (CIP, 5 µg) (Thermo Scientific™ Oxoid, UK). Resistance to three or more different antimicrobial groups is identified as multiple drug resistance (MDR).¹⁴ ESBL-production is suggested in isolates giving inhibition zone size ≤22 mm with ceftazidime and ≤25 mm with ceftriaxone. Double disk synergy test (DDST) was used to confirm ESBL producers.¹⁵ *E. coli* isolate from a previous study was used as quality control in antimicrobial susceptibility testing.¹⁶

DNA Extraction

The DNA of 205 *E. coli* isolates was extracted using GeneJET genomic DNA purification kit (Thermo Scientific, USA) according to manufacturer's manual procedures.

Phylogenetic Analysis

E. coli isolates were classified into four phylogenetic groups using the multiplex PCR procedure described by Doumith and his colleagues for *gadA*, *chuA*, *yjaA* genes, and the TSPE4.C2 DNA fragment.¹⁶ The glutamate decarboxylase-alpha (*gadA*) gene of *E. coli* was used as an internal control. It was performed with the following optimized cycling conditions: initial denaturation at 94°C for 4 minutes; then 30 cycles (94°C for 30 seconds, annealing at 65°C for 30 seconds, and extension at 72°C for 30 seconds) and a final extension step of 5 minutes at 72°C. On the basis of the results of multiplex PCR, detection of *chuA*–*yjaA* – TSPE4.C2–, *chuA*–*yjaA* – TSPE4.C2+, *chuA*+*yjaA* – TSPE4.C2+, and *chuA*+*yjaA*+ TSPE4.C2+ were belonging to groups A, B1, D, and B2, respectively, with *gadA* detected in all isolates as internal control.¹⁷ *E. coli* isolate from a previous study, belonging to group B2 with four amplified bands, was used as a positive control in each PCR run.¹⁶

Detection of *bla*_{CTX-M-15} Gene and ST131 Clones

Screening of *E. coli* isolates from urine and stool specimens were first done for CTX-M-15. The thermal cycling conditions were: initial denaturation at 95°C for 10 minutes, then 35 cycles (95°C for 1 minute, annealing at 55°C for 45 seconds, extension at 72°C for 1 minute) and final extension at 72°C for 10 minute.¹⁸ Secondly, isolates were screened for detection of *E. coli* ST131 clones using primers in Table 1 to amplify *pabB*

and *trpA* genes for O25-ST131 and O16-ST131 clades, respectively. The following amplification conditions: Initial denaturation for 4 minutes at 94°C then 30 cycles of denaturation for 5 seconds at 94°C, annealing for 20 seconds at 63°C and extension for 30 seconds at 72°C) and lastly final extension at 72°C for 5 minutes.^{19,20} The PCR products were identified by 1.5% Agarose gel electrophoresis (Biometra Goettingen, Germany). Positive O25 and O16-ST131 *E. coli* isolates, from previous studies done in the same laboratory, were used as positive control in PCR reactions for the ST131 clone.²¹ All primers sequences used in this study and amplification band sizes are described in Table 1.

Statistical Analysis

Demographic, clinical, and laboratory data analyses were carried out using SPSS program for windows version 20.0 (IBM, USA). $P \leq 0.05$ were considered statistically significant.

Results

Demographic Characteristics of the Study Subjects

A total of 205 *E. coli* strains were isolated from patients with UTI and acute diarrhoea. Out of 105 urinary isolates, 71 (67.6%) were from female patients and 34 (32.4%) were from male patients. The mean age of all UTI patients was 36.9±11.8. The mean age of female patients was 33.8±11.00, but the mean age of males was 43.2±10.9. Clinically, 89/105 (84.7%) of UTI patients complained from dysuria and

Table 1 PCR Primers Used in the Study

Primer	Primers (5_ to 3_)	Amplicon Size (bp)	Annealing Temperature (°C)	Reference
<i>gadA</i>	F-GATGAAATGGCGTTGGCGCAAG R-GGCGGAAGTCCCAGACGATATCC	373	65	[17]
<i>chuA</i>	F- ATGATCATCGCGGCTGCTG R-AAACGCGCTCGCGCCTAAT	281		
<i>yjaA</i>	F-TGTTGCGGATCTTGAAAGCAAACGT R-ACCTGTGACAAACCGCCCTCA	216		
TSPE4.C2	F-GCGGGTGAGACAGAAACGCG R- TTGTCGTGAGTTGCGAACCCG	152		
CTX-M-15	F-CACACGTGGAATTTAGGGACT R-GCCGTCTAAGGCCATAACA	996	55	[18]
<i>pabB</i> _{ST131-O25b}	F- TCCAGCAGGTGCTGGATCGT R- GCGAAATTTTCGCCGTACTGT	347	63	[19]
<i>trpA</i> _{ST131-O16}	F- AAAACCGCGCCGCGTTACCT R- CCAGAAATCGCGCCCGCATT	145	63	[20]

increased frequency and only 41/105 (39%) suffered from fever.

One hundred *E. coli* strains were isolated from the stool of patients with acute diarrhoea. The age range of patients was from 20–63 years old (mean=43.2±12). Out of 100 patients with diarrhoea, 53 were males and 47 were females. Clinically, all cases passed more than three loose stools daily. Blood and mucus were detected in 16/100 and 69/100 of samples, respectively.

Antimicrobial Susceptibility Testing

All *E. coli* isolates were tested for their resistance to seven antibiotics belonging to different classes (Figure 1). High resistance rates were detected against AMC, CRO, SXT, and CAZ in both urinary and intestinal *E. coli* isolates and the rates were higher in urinary isolates with no significant difference. Moderate resistance rates against CIP and AMK were detected in both groups. Imipenem showed very low resistance (1.9 and 4% in urinary and intestinal isolates, respectively).

ESBLs production was identified phenotypically using DDST in 75.2% of urinary isolates and 71% of intestinal isolates. MDR was found in 65/105 (61.9%) and 56/100

(56%) of urinary and intestinal *E. coli* isolates, respectively (Table 2).

Phylogenetic Grouping

The phylogenetic group distribution among the urinary isolates showed that 30.5%, 28.6%, 24.8, and 16.2% of isolates belonged to group B2, B1, A, and D, respectively. The B2 group was the predominant among urinary isolates. However, the phylogenetic group distribution among IPEC isolates was different, with 44, 39, 12, and 5% belonging to groups A, B1, D, and B2, respectively. There was a predominance of phylogenetic groups A and B2 among intestinal isolates. There was a significant difference between the urinary and intestinal isolates for phylogenetic groupings B2 & A (Table 2).

Detection of O25-ST131, O16-ST131 Clones

The *pabB* gene for the O25-ST131 clone (Figure 2) was detected in 89/105 (84.8%) and 47/100 (47%) of urinary and intestinal *E. coli* isolates. There was a significantly higher rate of this clone among urinary than intestinal isolates (Table 2). The distribution of phylogenetic groups among all O25-ST131

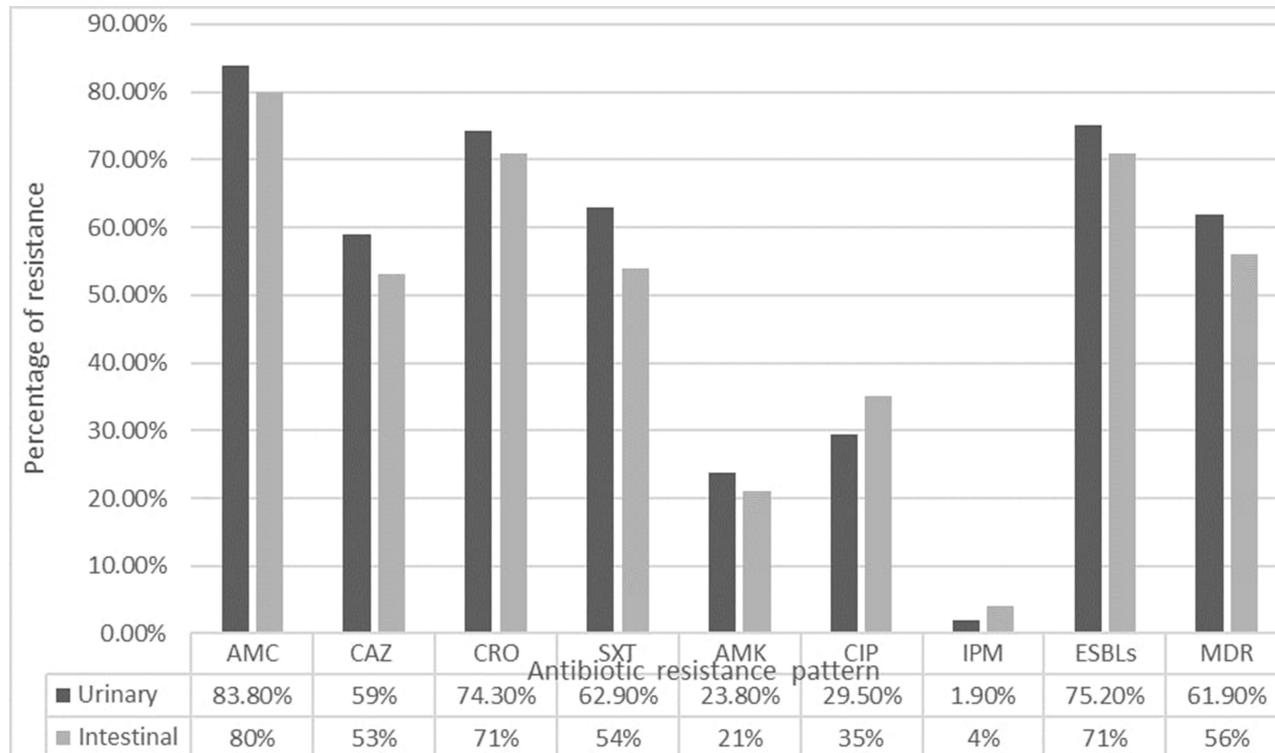


Figure 1 Antibiotic resistance, ESBLs, and MDR distributions among urinary and intestinal isolates.

Abbreviations: AMC, Amoxicillin-clavulanate; CAZ, Cefazidime; CRO, Ceftriaxone; SXT, sulfamethoxazole-Trimethoprim; AMK, Amikacin; IPM, Imipenem; CIP, Ciprofloxacin.

Table 2 Differentiation between *E. coli* Isolates from Urinary and Intestinal Origin

		No. (%) of Strains		P-value
		Urinary Isolates (n=105)	Intestinal Isolates (n=100)	
Phylogenetic group	B ₂	32 (30.5%)	5 (5%)	<0.001*
	B ₁	30 (28.6%)	39 (39%)	0.114
	A	26 (24.8%)	44 (44%)	0.004*
	D	17 (16.2%)	12 (12%)	0.398
O25-ST131 (pabB positive)		89 (84.8%)	47 (47%)	<0.001*
MDR		65 (61.9%)	56 (56%)	0.39
ESBL positive (synergy test)		79 (75.2%)	71 (71%)	0.494
CTX-M-15 positive		57 (54.3%)	66 (66%)	0.087
O16-ST131 (trpA)		0	0	–

Note: *P-value is significant.

Abbreviation: n, number.

and non O25-ST131 isolates was as shown in Figure 3 and its distribution among urinary and intestinal isolates is shown in Table 3. There was a significant difference between O25-ST131 and non-O25-ST131 isolates in the percentage of ESBLs, MDR, and antibiotic resistance to most antibiotic classes (Table 3). No O16-ST131 clone was detected as the trpA gene was negative in all studied isolates.

Detection of *bla*_{CTX-M-15} Gene

All *E. coli* isolates were examined for *bla*_{CTX-M-15} gene and it was positive in 66 and 54.3% of intestinal and urinary isolates, respectively, with a higher rate among intestinal over urinary isolates that was not significant (Table 2). CTX-M-15 was positive in 64/71 (90%) of ESBLs producing intestinal isolates. However, 54/79 (68.4%) of urinary ESBLs producing isolates were positive for CTX-M-15 and this difference was significant (Table 4).

Determination of Different *E. coli* Patterns among ESBLs Producing Strains According to Source, Antibiotype, Phylogenetic Group, CTX-M-15, and O25-ST131 Clone

All isolated ESBLs producing *E. coli* strains were classified according to source, antibiotype pattern, phylogenetic

group, CTX-M-15, and O25-ST131 clone detection into 17 different patterns. Nine patterns “EC 1, 2, 3, 4, 5, 6, 8, 10, & 13” were found in both urinary and intestinal studied groups. Four patterns “EC 7, 9, 11, & 12” were only found among intestinal isolates only and four different patterns “EC 14, 15, 16, & 17” were found among urinary isolates only. EC1 is the most prevalent pattern in both studied groups and its antibiotype is MDR to AMC, CAZ, CRO, SXT, and CIP (Table 4).

Discussion

There is a dramatic increase in the prevalence of ESBL producing *E. coli*, where CTX-M is the commonest detected type.²² This increased prevalence was significantly linked to the clonal expansion of *E. coli* ST131.²³ Most previous studies reported this emerging clone in extraintestinal infections, especially UTI.² However, intestinal colonization by the *E. coli* ST131 clone has been reported by recent studies.¹ Data about the *E. coli* ST131 clone in Egypt is still scarce. To the best of our knowledge, this study is the first to investigate the prevalence of *E. coli* ST131 clone in both extra-intestinal (urinary) and intestinal isolates in Upper Egypt. The study demonstrated that the prevalence of the O25-ST131 clone was very high in urinary isolates (83.4%) and the prevalence among intestinal isolates was less (47%), with a significant difference (P -value<0.001). Our results were similar to a study from India that detected the O25-ST131 clone in 87% of extra-intestinal clinical *E. coli* isolates.¹¹ However, our results were high compared to other studies from different countries in the Middle East region and across the world: Iraq (15%), Iran (24.7%), Turkey (22%), Pakistan (46%), China (12.5%), and Mexico (11%).^{8,10,24-27} This low prevalence of the ST131 clone in most of these studies can be explained by using different molecular techniques, but the main reason of this variation is that most of these studies investigated only phylogenetic group B2 so the percentage of the clone was dependent on the distribution of phylogenetic group B2. Lately, different studies investigated the ST131 clone among the different phylogenetic groups.^{10,11}

No isolates of the O16-ST131 clone were detected among the studied isolates. Our finding agrees with the worldwide prevalence of O16-ST131 serotype, where it was reported in a very small percentage in some countries (1% in Australia, 4.3% in Spain, 8% in France).²

Regarding the phylogenetic distribution of the studied isolates, the current study showed that the phylogenetic

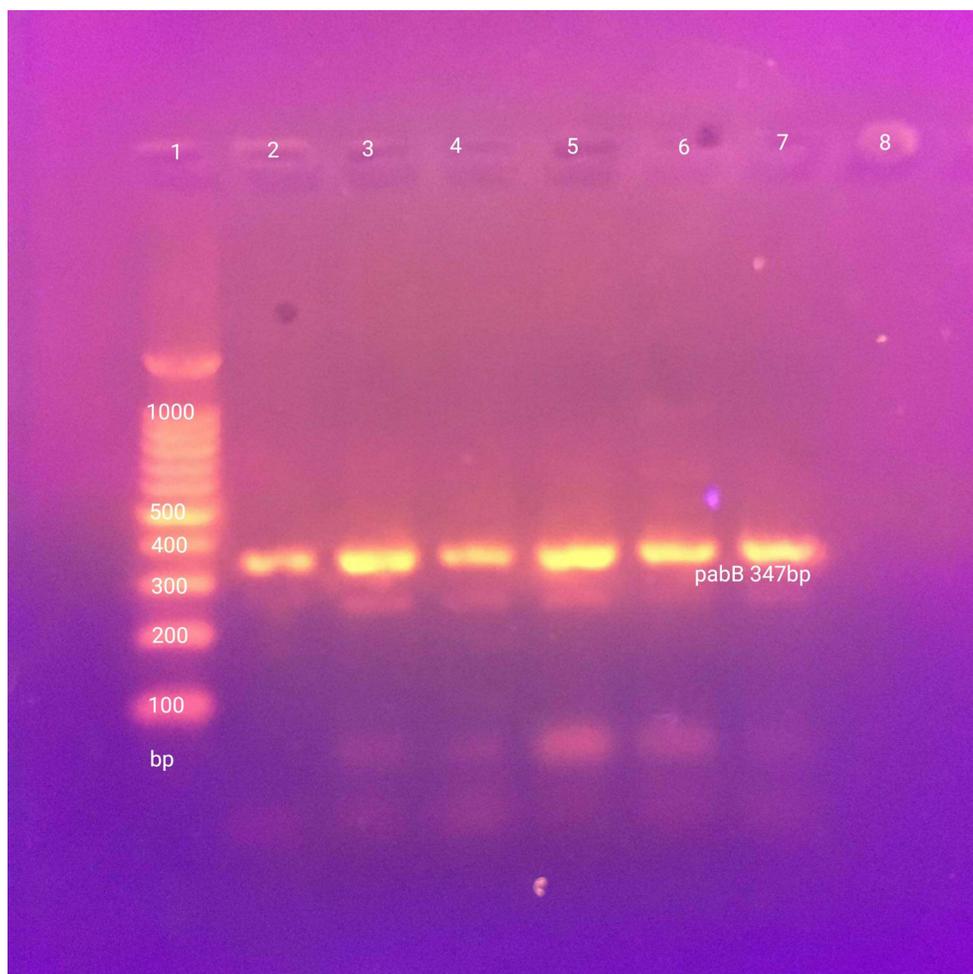


Figure 2 Screening of pabB gene for O25-ST131 in *E. coli* isolates. Lane 1, 100 bp molecular marker (iNtRON Biotechnology, South Korea); Lane 2–6 show (pabB gene) 347 bp; Lane 7 (positive control); Lane 8 (negative control).

group B2 was more predominant among the urinary isolates (30.5%) than intestinal isolates (5%). However, most of the studied intestinal isolates were belonging to phylogenetic groups A and B1 (45%, 39%). These findings were in agreement with many previous reports, considered groups B2 and D as the commonest in UPEC isolates, while groups A and B1 are commensal phylogenetic groups.^{28,29} For the prevalence of O25-ST131 clone among different phylogenetic groups, we found a high prevalence of this clone among group B2 (94.6%) that agrees with several previous studies.^{11,30,31} The current study is the first study in Egypt to report the ST131 clone among phylogenetic groups other than group B2. There was a significant difference of O25-ST131 clone prevalence in different phylogenetic groups among urinary and intestinal isolates. Most of the positive O25-ST131 clone isolates in phylogenetic groups A, B1, and D were from urinary origin and most intestinal isolates were non-

O25-ST131. However, all intestinal isolates of the phylogenetic group B2 carry this clone gene. The first study of this clone in Egypt was by Fam and his colleagues in 2011, who found the *E. coli* ST131 clone was representing 75% of phylogenetic group B₂ *E. coli* clinical isolates and this group was representing 26% of all isolates.³⁰ We found a high prevalence of ST131 clones among group B2 (94.6%). This indicated that there is an increasing rate of prevalence of ST131 clones in the last decade and it is in agreement with results of studies from Saudi Arabia that reported increasing the ST131 clone prevalence from 17.3 to 61.7% from UPEC isolates in the last few years.^{4,32} The detection of the ST131 clone in different groups other than B2 was reported also in other studies in India and Mexico.^{10,11} These findings draw attention about the ability of this clone to transfer among commensal groups and so the increased risk of this clone dissemination.

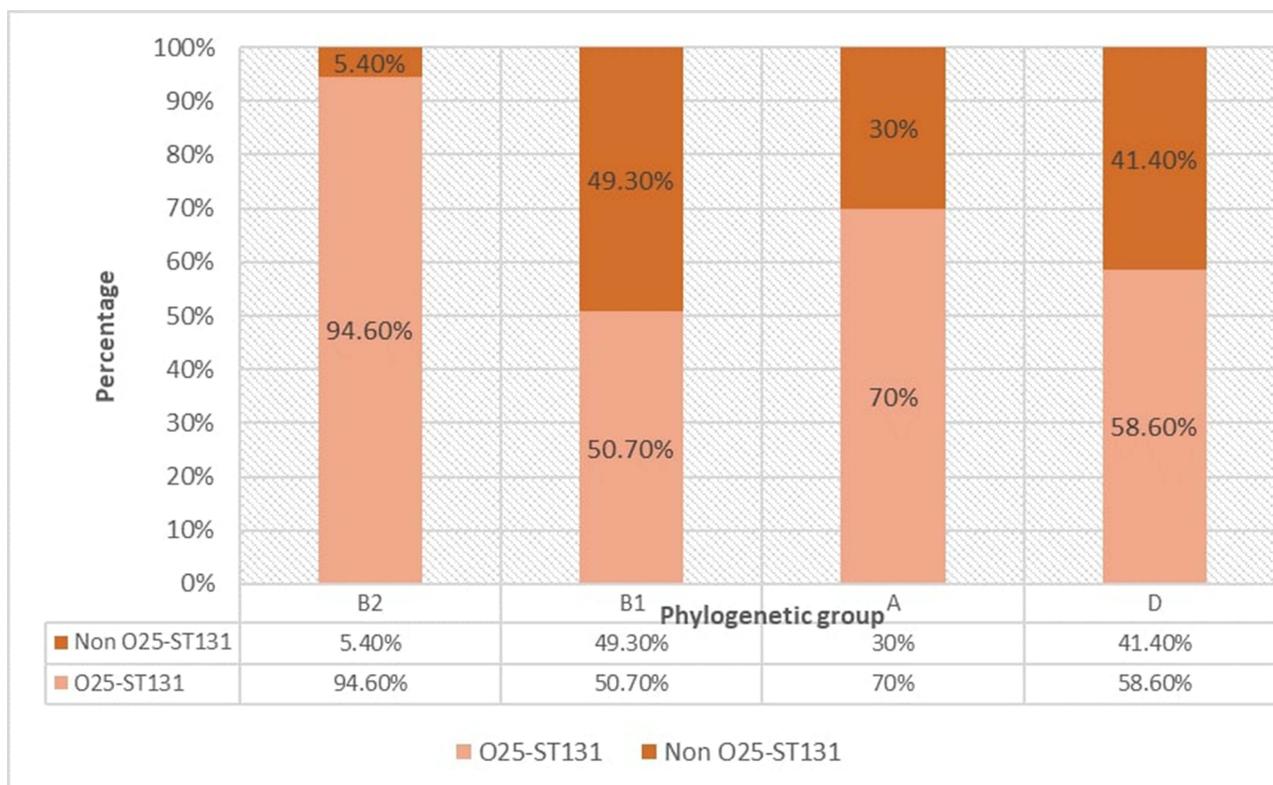


Figure 3 Phylogenetic distribution of O25-ST 131 and non O25-ST131 *E. coli* isolates.

ESBL producing *E. coli* strains were detected among clinical and commensal isolates worldwide.³³ Globally, CTX-M-15 is now the most prevalent ESBL type in both community and hospital setting.²³ In Egypt, CTX-M-15 is the most predominant ESBL producing type among uropathogenic and diarrheagenic *E. coli* strains.^{22,30,34} In this

Table 3 Differentiation between O25-ST131 and Non-O25-ST131 *E. coli* Isolates

n= (Urinary+Intestinal)		O25-ST131		Non O25-ST131		P-value
		Urinary Isolates	Intestinal Isolates	Urinary Isolates	Intestinal Isolates	
Phylogenetic group	B ₂ , n=(32+5)	30/32 (93.8%)	5/5 (100%)	2/32 (6.2%)	0/5 0	1.000
	B ₁ , n=(30+39)	23/30 (76.7%)	12/39 (30.8%)	7/30 (23.3%)	27/39 (69.2%)	<0.001*
	A, n=(26+44)	24/26 (92.3%)	25/44 (56.8%)	2/26 (7.7%)	19/44 (43.2%)	0.002*
	D, n=(17+12)	12/17 (70.6%)	5/12 (41.7%)	5/17 (29.4%)	7/12 (58.3%)	0.148
MDR n= (65+56)		58 (89.2%)	42 (75%)	7 (10.8%)	14 (25%)	0.039*
ESBLs positive (synergy test) n=(79+71)		66 (83.5%)	41 (57.7%)	13 (16.5%)	30 (42.3%)	<0.001*
CTX-M-15, n=(57+66)		45 (78.9%)	35 (53%)	12 (21.1%)	31 (47%)	0.004*
Antibiotic resistance	AMC (88+80)	74 (84.1%)	45 (56.2%)	14 (15.9%)	35 (43.8%)	<0.001*
	CAZ (62+53)	53 (85.5%)	38 (71.7%)	9 (14.5%)	15 (28.3%)	0.07
	CRO (78+71)	66 (84.6%)	42 (59.2%)	12 (15.4%)	29 (40.8%)	0.001*
	STX (66+54)	59 (89.4%)	41 (75.9%)	7 (10.6%)	13 (24.1%)	0.049*
	AMK (25+21)	24 (96%)	14 (66.7%)	14 (4%)	7 (33.3%)	0.016*
	CIP (31+35)	25 (80.6%)	29 (82.9%)	6 (19.4%)	6 (17.1%)	0.816
	IPM (2+4)	2 (100%)	4 (100%)	0	0	

Note: *P-value is significant.

Abbreviations: MDR, Multi-Drug Resistant; ESBLs, Extended Spectrum B-Lactamases; AMC, Amoxicillin-clavulanate; CAZ, Cefazidime; CRO, Ceftriaxone; SXT, sulfamethoxazole -Trimethoprim; AMK, Amikacin; IPM, Imipenem; CIP, Ciprofloxacin; n, number.

Table 4 Distribution of Phenotypic ESBLs Producing *E coli* Isolates According to O25-ST131, CTX-M-15, Phylogenetic Group, and Antibiotypes

<i>E coli</i> Pattern (EC)	Source	No of Isolates	O25-ST131	Non O25-ST131	CTX-M-15 +ve	CTX-M-15 -ve	Phylogenetic Group				Antibiotype
							A	B1	D	B2	
EC1	Urinary	21	15	6	12	9	2	9	4	6	AMC, CAZ, CRO, SXT, CIP
	Intestinal	17	15	2	15	2	8	7	1	1	
EC2	Urinary	18	18	0	13	5	3	4	4	7	AMC, CAZ, CRO, SXT, AMK
	Intestinal	5	3	2	5	0	4	1	0	0	
EC3	Urinary	7	3	4	7	0	0	1	4	2	AMC, CRO
	Intestinal	12	0	12	12	0	3	9	0	0	
EC4	Urinary	9	9	0	6	3	2	4	0	3	AMC, CAZ, CRO, SXT
	Intestinal	11	7	4	11	0	8	1	2	0	
EC5	Urinary	9	7	2	8	1	4	0	0	5	AMC, CAZ, CRO
	Intestinal	5	1	4	4	1	4	1	0	0	
EC6	Urinary	4	4	0	2	2	1	1	1	1	AMC, CRO, SXT
	Intestinal	4	3	1	4	0	4	0	0	0	
EC7	Urinary	3	3	0	2	1	2	1	0	0	AMC, CRO, CIP
	Intestinal	0	0	0	0	0	0	0	0	0	
EC8	Urinary	3	3	0	1	2	2	0	0	1	AMC, CRO, SXT, AMK
	Intestinal	1	0	1	1	0	1	0	0	0	
EC9	Urinary	1	1	0	0	1	0	0	0	1	AMC, CAZ, AMK
	Intestinal	0	0	0	0	0	0	0	0	0	
EC10	Urinary	1	1	0	1	0	0	0	0	1	AMC, CAZ, CRO, CIP
	Intestinal	2	1	1	2	0	0	1	1	0	
EC11	Urinary	1	0	1	1	0	0	0	0	1	AMC, CAZ, SXT, AMK
	Intestinal	0	0	0	0	0	0	0	0	0	
EC12	Urinary	1	1	0	0	1	0	0	1	0	AMC, CAZ, CRO, SXT, AMK, IPM
	Intestinal	0	0	0	0	0	0	0	0	0	
EC13	Urinary	1	1	0	1	0	0	0	0	1	AMC, CAZ, CRO, SXT, CIP, IPM
	Intestinal	3	3	0	3	0	0	0	0	3	
EC14	Urinary	0	0	0	0	0	0	0	0	0	AMC, CAZ, CRO, SXT, AMK, CIP
	Intestinal	7	6	1	5	2	0	4	2	1	
EC15	Urinary	0	0	0	0	0	0	0	0	0	AMC, CAZ, CRO, AMK, CIP
	Intestinal	2	1	1	0	2	0	1	1	0	
EC16	Urinary	0	0	0	0	0	0	0	0	0	AMC, CAZ, CRO, AMK, CIP
	Intestinal	1	0	1	1	0	0	1	0	0	
EC17	Urinary	0	0	0	0	0	0	0	0	0	AMC, CAZ, CRO, SXT, AMK, CIP, IPM
	Intestinal	1	1	0	1	0	0	0	1	0	

(Continued)

Table 4 (Continued).

E coli Pattern (EC)	Source	No of Isolates	O25-ST131	Non O25-ST131	CTX-M-15 +ve	CTX-M-15 -ve	Phylogenetic Group				Antibiotype
							A	B1	D	B2	
Total (percent to ESBLs)	Urinary	79	66 (83.5%)	13 (16.5%)	54 (68.4%)	25 (31.1%)	16 (20.3%)	20 (25.3%)	14 (17.7%)	29 (36.7%)	
	Intestinal	71	41 (57.7%)	30 (43.3%)	64 (90.1%)	7 (9.9%)	32 (45.1%)	26 (36.6%)	8 (11.3%)	5(7%)	
P-value			<0.001*		0.001*		<0.001*				

Note: *P-value is significant.

Abbreviations: AMC, Amoxicillin-clavulanate; CAZ, Cefazidime; CRO, Ceftriaxone; SXT, sulfamethoxazole-Trimethoprim; AMK, Amikacin; IPM, Imipenem; CIP, Ciprofloxacin.

study, the prevalence of ESBL production among both urinary and intestinal isolates were 75.2% and 71%, respectively. These results are higher than reports of previous studies in Egypt, with ESBL prevalence range from 52–69.6%.^{16,22,34,35} In the same context as our results, Valverdi et al³⁶ documented that acute diarrhoea is a frequent reason for intestinal colonization by ESBL producers that reached to 55.5% in acute diarrhoea. This can be explained by the change in the gut flora that occurs in acute diarrhoea, causing predominance of pathogenic bacterial strains.

Our results demonstrated that 90% of ESBL producing isolates from patients with acute diarrhoea carried the *bla*_{CTX-M-15} gene. However, only 68.4% of ESBL producing urinary isolates carried this gene with a significant difference between the two studied groups. These results showed that there is an increasing in the prevalence of CTX-M-15 among *E. coli*, especially of intestinal origin, compared to previous results in Egypt, where the CTX-M-15 gene was detected in only 37.5% of ESBL producing diarrhoeagenic *E. coli*.¹⁶ This was worrisome as the intestine is the main reservoir for ExPEC strains causing UTI. Also, the *bla*_{CTX-M-15} gene was detected in 48.8% of ESBL producing *Enterobacteriaceae* including *E. coli* from patients with UTI in Egypt.³⁴ A study in Riyadh in Saudi Arabia detected CTX-M-15 in 96.7% of ESBL producing *E. coli* from clinical isolates with the majority of samples from urine.³⁷ This was higher than our results among urinary isolates, which indicates that some of the phenotypically ESBL producing urinary isolates carry other ESBLs gene types.

About 83.5% of ESBL producing urinary isolates identified as O25-ST131 clone, while only 57.7% of intestinal isolates were O25-ST131 positive with a significant difference between the studied groups. This is in agreement with several previous studies that reported a significant

association of ESBL production with this clone.^{4,27,38} A study done in 2015 on the intestinal colonization by ESBL producing enterobacteria in Egyptian patients with liver disease found that all *E. coli* isolates of the phylogenetic group B2 were belonging to the ST131 clone and were O25-ST131 positive.³¹ This is similar to our results that all intestinal isolates of group B2 were ESBL producing and positive O25-ST131. However, another study in Egypt in 2017 detected the ST131 clone in 92.3% of ESBL producing *E. coli* from clinical isolates and 43.8% of them were O25b positive serotype.³⁹

The association between emerging *E. coli* ST131 clones with CTX-M-15 production was reported in strains isolated from the stool and urine of patients from different countries.⁴⁰ In this study, 45/57 (78.9%) of urinary and 35/66 (53%) of intestinal CTX-M-15 positive isolates were identified as O25-ST131 clone. Similar results were reported in a study in Bangladesh, where 71% of positive *bla*_{CTX-M-15} strains isolated from UTI patients were belonging to the O25-ST131 clone.⁴¹ Although the frequency of O25-ST131 was lower in intestinal than urinary study isolates, it was high compared to a previous Jordanian study investigated the intestinal colonization by CTX-M-15 producing the *E. coli* ST131 clone in infants.⁴²

Regarding antibiotic resistant patterns of the study isolates, most ESBL producing isolates were found to be MDR. Classifying *E. coli* isolates into different patterns shows that the EC1 pattern (resistant to AMC, CAZ, CRO, SXT, and CIP) was the commonest pattern in both urinary and intestinal isolates. The association of ESBL production with quinolones and Trimethoprim/Sulfamethoxazole resistance can be explained by dissemination of multidrug resistant ST131 clones and by horizontal gene transfer by large plasmids carrying resistance genes to these antibiotic classes.⁴³ More than 80% of isolates resistant to

ciprofloxacin were belonging to the ST131 clone and this is similar to other studies that associate between fluoroquinolone resistance and ST131 clone in Saudi Arabia,⁴ Pakistan,²⁶ and Taiwan.³⁸

The important predisposing factor of this high prevalence of ESBL producing isolates in our country is misuse of antibiotics as there are no policies controlling purchasing these drugs and high self-medication without prescription, especially to third generation cephalosporins, and this led to selective pressure for resistant strains and dissemination of ESBLs producing bacteria.

A limitation of this study was choosing only patients with acute diarrhoea for studying intestinal colonization of the *E. coli* ST131 clone and ESBL production and not including a healthy group. But it was preferred to compare extraintestinal pathogenic strains of urinary origin with intestinal potentially pathogenic strains in patients with acute diarrhoea.

In conclusion, we detected high prevalence of the O25-ST131 clone among *E. coli* isolates in Egypt. It was higher in isolates from patients with UTI than those with acute diarrhoea. We provided the first detection of ST131 clone in different phylogenetic groups other than B2 in Egypt. An alarming increase in prevalence of CTX-M-15 ESBL producing *E. coli* in intestinal and urinary isolates was found. O25-ST131 *E. coli* clone isolates showed high antibiotic resistance rates, ESBL, and MDR compared to non-ST131 isolates. No isolates of the O16-ST131 clone were detected in both studied groups. We recommend the need to optimize the antibiotic use by establishing antimicrobial stewardship programs and taking these results of studies as guidance in empirical antibiotic selection in treatment. Also, more additional studies are needed to evaluate this emerging clone and risk factors in our country.

Abbreviations

CFU, colony forming unit; DDST, double-disc synergy test; ESBL, extended-spectrum beta-lactamase; ExPEC, extraintestinal pathogenic *E. coli*; HPF, high power field; IPEC, intestinal pathogenic *E. coli*; MDR, multiple drug resistance; ST131, *Escherichia coli* sequence type 131; UPEC, uropathogenic *E. coli*; UTI, urinary tract infection.

Data Sharing Statement

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

Acknowledgments

We thank the health care workers in Minia university hospitals for their cooperation.

Funding

There is no funding to report.

Disclosure

The authors report no conflicts of interest in this work.

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