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RESEARCH ARTICLE



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Distribution of virulence determinants among *Escherichia coli* ST131 and its H30/H30-Rx subclones in Turkey

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ABSTRACT

Extraintestinal pathogenic *Escherichia coli* (ExPEC) is the leading pathogen in urinary tract infection. In recent years multidrug-resistant B2-ST131 *E. coli* clonal group has disseminated worldwide. The ST131 and its subclones H30 and H30-Rx have been identified only in a few studies from Turkey. The aim of this study is to investigate the presence of ST131 and its subclones and to analyze their adhesin virulence genes and antimicrobial resistance. A total of 250 urinary ExPEC isolates were included in the study. Resistance rates of 16 antimicrobial agents were determined by disk-diffusion. Multidrug-resistance and ESBL production were analyzed. Altogether 8 adhesin genes were investigated namely, *papAH*, *fimH*, *sfa/focDE*, *focG*, *afa/draBC*, *iha*, *bmaE* and *gafD*. A total of 39 ST131 isolate were determined and 33 (84.6%) were multidrug-resistant. ESBL production was detected in 34 (87.2%) ST131 and 61 (28.9%) of non-ST131 strains. In our study, we found a strong correlation between ST131 strains and *fimH*, *iha*, *afa/draBC*, *papAH* virulence determinants. Twenty-nine (85.3%) of 34 ST131-O25b-H30 isolates were identified as H30-Rx. All the *papAH* gene positive isolates were identified within ST131-O25b-H30-Rx lineage. Non-H30-Rx isolates within H30 isolates were identified as pattern 2. Almost 16% of the isolates were identified as ST131 regardless of clinical syndrome and approximately 34% of the multidrug-resistant isolates were H30-Rx subclone. We report H30-Rx as the dominant subclone of ST131 in our study. Imipenem, fosfomycin and nitrofurantoin proved to be the most effective agents according to antibiotic resistance patterns of both ST131 and non-ST131 *E. coli* strains.

KEYWORDS

ST131, H30, H30-Rx, *Escherichia coli*, urinary tract infection

INTRODUCTION

Urinary tract infection (UTI) is one of the most common infections worldwide both in the community and hospital setting [1]. *Escherichia coli* (*E. coli*) is the leading causative agent of the UTI [2]. Extraintestinal pathogenic *E. coli* (ExPEC) strains possess various virulence traits and the most important ones are adhesins (P fimbriae, S fimbriae, F1C fimbriae, G fimbriae, Dr antigen-specific adhesins, type 1 fimbriae, blood group M-specific adhesin and iron regulated gene-homologue adhesin) which are responsible for colonization and the onset of infection [3].

Sequence type 131 (ST131) of *E. coli* has become a public health problem based on its hypervirulent activity, pandemic feature and multidrug-resistant extraintestinal infection

capacity [2]. ST131 *E. coli* isolates belong to B2 phylogenetic group, which is the dominant phylogenetic group in UTI. Also, *E. coli* ST131 mostly belongs to O25b serogroup, and O16 serogroup is less frequently encountered. In UTI caused by *E. coli*, ST131 was identified as the predominant strain [4].

The lately emerged allele 30 of *fimH*; H30 and H30-Rx subclones of *E. coli* O25b-ST131 are predominant subclones in UTI and these subclones are frequently multidrug-resistant. The ST131 has been identified in only a few studies from our country [5] and the prevalence and interactions between patient characteristics with subclones are not well known.

The aim of this study is to investigate the ST131 status, presence of H30 and H30-Rx subclones of ST131 *E. coli* and to analyze their adhesin virulence types in the insight of antimicrobial resistance and patient characteristics.

MATERIAL AND METHODS

Patients and bacterial strains

A total of 250 non-duplicate UTI *E. coli* isolates were prospectively collected between 2015 and 2016 in the Laboratory of Clinical Microbiology at Kocaeli University Hospital, Turkey.

Isolates were identified by VITEK-2 and VITEK-MS (bioMérieux, France) automatized systems. Clinical characteristics including sex, age, complication (complicated-uncomplicated), infection origin (community acquired - healthcare associated) and clinical syndrome (cystitis-pyelonephritis) were obtained by medical record review for each voluntary patient [6] and informed written consent was obtained. The study was approved by Ethical Committee of Kocaeli University, Turkey (KOU KAEK 2015/168).

Detection of *E. coli* phylogenetic groups

Phylogenetic groups including A, B1, B2 and D were screened by triplex-PCR based method as previously described with minor modifications [6, 7]. All group B2 isolates were investigated for ST131 clonal status with ST131-specific single nucleotide polymorphism in *gyrB* and *mdh* genes [6, 8]; and O serogroups including O25 and O16 were identified by another multiplex PCR [6, 9]. Allele 30 of *fimH*; H30 [10] and H30-Rx [11] were investigated as previously described.

Detection of virulence determinants

Adhesin virulence associated genes (VAGs) (*papAH*, *fimH*, *focG*, *gafD*, *sfa/focDE*, *iha*, *afa/draBC* and *bmaE*) were identified by multiplex PCR [6].

VAG patterns were determined as all unique VAG assemblages and VAG score was calculated according to the number of VAGs possessed by the isolates in the ST131 *E. coli* population.

Antimicrobial susceptibility testing

Antimicrobial susceptibilities to ampicillin, amoxicillin/clavulanic acid (AMC), amikacin, fosfomicin, gentamicin,

imipenem, nalidixic acid, nitrofurantoin, norfloxacin, cefazolin, cefuroxime, ceftriaxone, cefotaxime, ciprofloxacin, tetracycline, and trimethoprim/sulfamethoxazole (SXT) of the isolates were investigated by Kirby-Bauer disk diffusion method. Isolates that were resistant to ceftriaxone and cefotaxime were investigated for extended-spectrum- β -lactamase (ESBL) production by ceftazidime and cefotaxime, alone and in combination with clavulanate disk diffusion method based on Clinical and Laboratory Standards Institute (CLSI) [12]. The results of antimicrobial susceptibility and ESBL production were interpreted according to CLSI criteria [12]. Multidrug-resistance was identified as resistance to ≥ 3 of the 5 antimicrobial agents (AMC, gentamicin, cefuroxime, ciprofloxacin and SXT) which are representing distinct antibiotic classes [13].

Statistical analysis

Comparisons of proportions were investigated by Pearson chi-square test or Fisher's exact test based on expected numbers. When expected numbers were less than 5, Fisher's exact test (two-tailed) was applied. A *P*-value < 0.05 was considered statistically significant. All statistical tests were performed using SPSS software for Windows, version 21 (IBM Corp., USA).

RESULTS

Of the 250 patients, 74 (29.6%) were male, 176 (70.4%) were female. According to clinical syndrome 183 patients (73.2%) were diagnosed with cystitis and 67 (26.8%) with pyelonephritis. Complicated and uncomplicated infection rates were 186 (74.4%) and 64 (25.6%), respectively. Of the patients, 186 (74.4%) had community acquired and 64 (25.6%) had healthcare associated infection. Median age of the patients was 56 (ranged 1–96).

The prevalence of the B2, D, A and B1 were 78 (31.2%), 71 (28.4%), 61 (24.4%) and 40 (16%), respectively.

Of the 78 B2 isolates, 39 (50%) were belonging to ST131 clonal group. Among all isolates, 15.6% were determined as ST131 *E. coli*. O25b was the predominant serogroup in ST131 isolates; 34 (87.2%) of the ST131 isolates were belonging to O25b whereas 5 (12.8%) of them were identified as O16 serogroup. All of the 34 (100%) ST131-O25b isolates were belonging to H30 lineage and 29 (85.3%) of the ST131-O25b-H30 isolates were identified as H30-Rx. None of the ST131-O16 isolates were harbouring *fimH* 30 allele nor H30-Rx.

ST131 *E. coli* was much more observed in complicated infection ($P = 0.017$). Also, when compared in the B2 phylogenetic group, ST131 isolates were much more identified in the patients with ≥ 65 years old ($P = 0.026$).

A total of 39 ST131 *E. coli* isolates were investigated for adhesin VAGs. Prevalence of the 8 adhesin VAGs were *fimH*, 39 (100%); *iha*, 35 (89.7%); *afa/draBC*, 14 (35.9%); *papAH*, 10 (25.6%) and *sfa/focDE*, 1 (2.6%), respectively. None of the ST131 *E. coli* isolates harbored *bmaE*, *gafD* and *focG* genes. Six different VAG patterns were identified (Table 1).



Table 1. ST131 *E. coli* VAG patterns

Pattern	ST131 <i>E. coli</i> <i>n</i> = 39 (%)	H30 <i>n</i> = 34 (%)	H30-Rx <i>n</i> = 29 (%)	<i>papAH</i>	<i>fimH</i>	<i>sfa/focDE</i>	<i>focG</i>	<i>afa/draBC</i>	<i>iha</i>	<i>bmaE</i>	<i>gafD</i>
1	13 (33.3)	10 (29.4)	10 (34.5)	–	+	–	–	+	+	–	–
2	13 (33.3)	12 (35.3)	7 (24.2)	–	+	–	–	–	+	–	–
3	8 (20.6)	8 (23.6)	8 (27.6)	+	+	–	–	–	+	–	–
4	2 (5.1)	2 (5.9)	2 (6.9)	+	+	–	–	–	–	–	–
5	2 (5.1)	1 (2.9)	1 (3.4)	–	+	–	–	–	–	–	–
6	1 (2.6)	1 (2.9)	1 (3.4)	–	+	+	–	+	+	–	–

Each isolate carried at least one VAG and the highest VAG score was 4 (Median, 3). Prevalence of VAG score was as follow; VAG score 1, 2 (5.2%) isolates; VAG score 2, 15 (38.4%) isolates, VAG score 3, 21 (53.8%) isolates and VAG score 4, 1 (2.6%) isolate. All of the *papAH* positive isolates were belonging to ST131-O25b-H30-Rx lineage ($P = 0.040$). Non-H30-Rx isolates within H30 isolates were clustered in pattern 2 (Table 1).

Of the 250 isolates, 86 (34.4%) were identified as multidrug-resistant. ST131 *E. coli* was predominant in multidrug-resistant strains; of the 86 multidrug-resistant isolates, 33 were belonging to ST131 clonal group ($P < 0.001$). Also, ESBL production rate in ST131 isolates were too high and all of the ST131 isolates were resistant to nalidixic acid. Interactions between antimicrobial drug resistance, ESBL production and ST131 status were shown in Table 2.

ESBL production and multidrug-resistance was common within H30-Rx subclones; 28 ESBL positive ($P = 0.011$) and 27 multidrug resistant ($P = 0.028$) ST131-H30-Rx strain were identified.

DISCUSSION

In the present study, a total of 250 well-characterized UTI *E. coli* isolates were analyzed for ST131 status, ST131 subclones, adhesin virulence genotypes, resistance patterns and phylogenetic groups in terms of clinical features.

The rising prevalence of resistance to first line antibiotics among *E. coli* isolated from UTI leads to treatment failure and higher treatment costs. Furthermore, ST131 *E. coli* clonal group occupies an important place in treatment failure based on its multidrug-resistant potential and its prevalence in UTI is increasing worldwide. Therefore, *E. coli* ST131 clonal group has become a public health problem; it is responsible for pandemic and multidrug resistance-related infections. In the current study, ST131 *E. coli* isolates were more resistant than non-ST131 isolates to the antibiotics tested except cefazolin, tetracycline, gentamicin and nitrofurantoin. Although fosfomycin resistance rate is low, ST131 *E. coli* were much more resistant to fosfomycin compared with non-ST131 isolates ($P = 0.013$). In a study conducted

Table 2. Antibiotic resistance among ST131 and non-ST131 *E. coli*

Drug (no. resistant, no. susceptible)	No. (%) of isolates that were resistant		* <i>P</i>
	ST131 <i>E. coli</i> <i>n</i> = 39	Non-ST131 <i>E. coli</i> <i>n</i> = 211	
Cefazolin (171; 79)	31 (79.5)	140 (66.4)	0.122
Ampicillin (168; 82)	37 (94.9)	131 (62.1)	<0.001
Norfloxacin (134; 115)	36 (92.3)	98 (46.4)	<0.001
Nalidixic acid (131; 119)	39 (100)	92 (43.6)	<0.001
Tetracycline (129; 121)	22 (56.4)	107 (50.7)	0.356
Ciprofloxacin (122; 128)	35 (89.7)	87 (41.2)	<0.001
SXT (120; 130)	29 (74.4)	91 (43.1)	<0.001
AMC (113; 137)	29 (74.4)	84 (39.8)	<0.001
Cefuroxime (111; 139)	35 (89.7)	76 (36.0)	<0.001
Cefotaxime (95; 155)	30 (76.9)	65 (30.8)	<0.001
ESBL (95; 155)	34 (87.2)	61 (28.9)	<0.001
Ceftriaxone (94; 156)	32 (82.1)	62 (29.4)	<0.001
Gentamicin (49; 201)	11 (28.2)	38 (18.0)	0.186
Amikacin (40; 210)	18 (46.2)	22 (10.4)	<0.001
Nitrofurantoin (18; 232)	1 (2.6)	17 (8.1)	0.322
Fosfomycin (7; 243)	4 (10.3)	3 (1.4)	0.013
Imipenem (0; 250)	0 (0)	0 (0)	–
MDR (86; 164)	33 (84.6)	53 (25.1)	<0.001

ESBL: extended-spectrum beta-lactamase, SXT: trimethoprim/sulfamethoxazole, AMC: amoxicillin/clavulanic acid, MDR: multidrug-resistance.

**P* values are for comparisons of isolates susceptible and resistant to the indicated drug.

P-values less than 0.05 were considered significant, and are shown in bold.



in Spain, fosfomycin consumption may increase fosfomycin resistance in ST131 *E. coli* [14]. Also, fosfomycin is used in the last few years in Turkey; therefore, non-ST131 isolates may exhibit lower resistance to this antimicrobial agent [15]. In the case of nitrofurantoin, only one resistant ST131 isolate was identified. None of the isolates were resistant to imipenem. Nitrofurantoin is one of the recommendations of the Infectious Diseases Society of America and the European Society of Microbiology and Infectious Diseases [16]. Overall, according to our findings, nitrofurantoin is recommended as the drug of choice for the treatment of uncomplicated cystitis due to ST131 *E. coli*.

ST131 clonal group corresponds to almost 16% of all isolates in the current region and the prevalence of ST131 is similar to the rates reported previously in France, China and Mexico [4, 17–19]. Based on its truly pathogenic nature, ST131 *E. coli* was more common in complicated infections in the study ($P = 0.017$) [20]. Previous studies have shown that the O25b is the predominant serotype and O16 serotype is less frequently detected [21–23]. This is particularly important since H30-Rx subclone belongs to O25b serogroup. This finding is supported in our study; 34 (87.2%) of the ST131 isolates were identified as O25b whereas 5 (12.8%) of them were belonging to O16 serogroup.

The adhesin virulence gene prevalence was alike earlier studies [24]. Similar to previous studies, none of the ST131 isolates harbor *gafD*, *focG* and *bmaE* virulence genes [24, 25]. The ST131 isolate harboring *sfa/focDE* gene is rarely encountered and documented previously [6, 26]. In the present study, only one *sfa/focDE* positive isolate was identified. In a study we conducted earlier, B2-ST131 and non-B2-ST131 isolates were compared according to VAG scores and there was no significant difference observed [6]. Similar to our previous study, ST131 median VAG score was determined as 3. It is indicated that hypervirulence and dominance feature of ST131 isolates were coming from its enhanced metabolic capacity and colonization capability; based on *in vivo* studies, it was found that ST131 isolates were not more virulent than non-ST131 isolates [27–29]. Virulence characteristics that are not defined yet may also be related to hypervirulent properties of this clonal group.

It is well-known that H30-Rx subclone is predominant within ST131 *E. coli* isolates and H30 and H30-Rx lineages are associated with multidrug-resistance and ESBL production. H30-Rx subclones are much more associated with resistance compared with H30 lineage [11]. In our study, of the 29 H30-Rx positive isolates, only one non-ESBL producer and two non-multidrug resistant isolates were identified. In the 10 H30-Rx negative isolates, four non-ESBL producer and non-multidrug resistant isolates were detected. Also, in the multidrug-resistant strains, 33.7% of the isolates were belong to H30-Rx subgroup. In a previous study including 107 multidrug-resistant *E. coli* isolates, it is reported that approximately 20% of the isolates were belong to H30-Rx subclone [5]. These findings suggest that isolates which belong to H30-Rx lineage are particularly associated with multidrug-resistance and ESBL producing. Price et al. have defined these ancestral interactions between the lineages as “Russian-doll-like” configuration [30].

A study limitation is only urine isolates were included in this study. Bacteremia and/or meningitis associated ST131-ExPEC isolates are available in the literature. Thus, results of this study may not be generalizable to these clinical syndromes. Despite this limitation, the current study was able to obtain important data about ST131 and its H30 and H30-Rx subclones.

To the best of our knowledge, this is the first report which was investigated the ST131 clonal group and its H30 and H30-Rx subclones in terms of adhesin virulence genes, patient characteristics, and antimicrobial resistance in Turkey.

In conclusion, almost 16% of the isolates were belonging to ST131 clonal group regardless of clinical syndrome and approximately 34% of the multidrug-resistant isolates were H30-Rx subclone. Also, H30-Rx is the predominant subclone in ST131 *E. coli* which is the main subclone for multidrug-resistant. Considering that H30-Rx is the dominant subclone among the ExPEC isolates, we recommend nitrofurantoin, which is already recommended by international guidelines as drug of choice for uncomplicated cystitis treatment.

Conflict of interest: The authors have no relevant financial or non-financial interests to disclose.

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REFERENCES

1. Tandogdu Z, Wagenlehner FM. Global epidemiology of urinary tract infections. *Curr Opin Infect Dis* 2016; 29: 73–9. <https://doi.org/10.1097/QCO.0000000000000228>.
2. Rogers BA, Sidjabat HE, Paterson DL. *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. *J Antimicrob Chemother* 2011; 66: 1–14. <https://doi.org/10.1093/jac/dkq415>.
3. Johnson JR, Russo TA. Molecular epidemiology of extraintestinal pathogenic (uropathogenic) *Escherichia coli*. *Int J Med Microbiol* 2005; 295: 383–404. <https://doi.org/10.1016/j.ijmm.2005.07.005>.
4. Nicolas-Chanoine MH, Bertrand X, Madec JY. *Escherichia coli* ST131, an intriguing clonal group. *Clin Microbiol Rev* 2014; 27: 543–74. <https://doi.org/10.1128/CMR.00125-13>.
5. Can F, Kurt-Azap O, Ispir P, Nurtop E, Seref C, Loclar I, et al. The clinical impact of ST131 H30-Rx subclone in urinary tract infections due to multidrug-resistant *Escherichia coli*. *J Glob Antimicrob Resist* 2016; 4: 49–52. <https://doi.org/10.1016/j.jgar.2015.10.006>.



6. Er DK, Dundar D, Uzuner H, Osmani A. Relationship between phylogenetic groups, antibiotic resistance and patient characteristics in terms of adhesin genes in cystitis and pyelonephritis isolates of *Escherichia coli*. Microb Pathog 2015; 89: 188–94. <https://doi.org/10.1016/j.micpath.2015.10.014>.
7. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol 2000; 66: 4555–8. <https://doi.org/10.1128/aem.66.10.4555-4558.2000>.
8. Johnson JR, Menard M, Johnston B, Kuskowski MA, Nichol K, Zhanel GG. Epidemic clonal groups of *Escherichia coli* as a cause of antimicrobial-resistant urinary tract infections in Canada, 2002 to 2004. Antimicrob Agents Chemother 2009; 53: 2733–9. <https://doi.org/10.1128/AAC.00297-09>.
9. Clermont O, Johnson JR, Menard M, Denamur E. Determination of *Escherichia coli* O types by allele-specific polymerase chain reaction: application to the O types involved in human septicemia. Diagn Microbiol Infect Dis 2007; 57: 129–36. <https://doi.org/10.1016/j.diagmicrobio.2006.08.007>.
10. Colpan A, Johnston B, Porter S, Clabots C, Anway R, Thao L, et al. *Escherichia coli* sequence type 131 (ST131) subclone H30 as an emergent multidrug-resistant pathogen among US veterans. Clin Infect Dis 2013; 57: 1256–65. <https://doi.org/10.1093/cid/cit503>.
11. Banerjee R, Robicsek A, Kuskowski MA, Porter S, Johnston BD, Sokurenko E, et al. Molecular epidemiology of *Escherichia coli* sequence type 131 and its H30 and H30-Rx subclones among extended-spectrum-beta-lactamase-positive and -negative *E. coli* clinical isolates from the Chicago Region, 2007 to 2010. Antimicrob Agents Chemother 2013; 57: 6385–8. <https://doi.org/10.1128/AAC.01604-13>.
12. CLSI. Performance Standards for antimicrobial susceptibility testing. 28th ed CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
13. Johnson JR, Johnston B, Clabots C, Kuskowski MA, Castanheira M. *Escherichia coli* sequence type ST131 as the major cause of serious multidrug-resistant *E. coli* infections in the United States. Clin Infect Dis 2010; 51: 286–94. <https://doi.org/10.1086/653932>.
14. Oteo J, Orden B, Bautista V, Cuevas O, Arroyo M, Martinez-Ruiz R, et al. CTX-M-15-producing urinary *Escherichia coli* O25b-ST131-phylogroup B2 has acquired resistance to fosfomycin. J Antimicrob Chemother 2009; 64: 712–7. <https://doi.org/10.1093/jac/dkp288>.
15. Baylan O. Fosfomycin: past, present and future. Mikrobiyol Bul 2010; 44: 311–21.
16. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis 2011; 52: e103–20. <https://doi.org/10.1093/cid/ciq257>.
17. Li B, Lu Y, Lan F, He Q, Li C, Cao Y. Prevalence and characteristics of ST131 clone among unselected clinical *Escherichia coli* in a Chinese university hospital. Antimicrob Resist Infect Control 2017; 6: 118. <https://doi.org/10.1186/s13756-017-0274-0>.
18. Lafolie J, Nicolas-Chanoine MH, Grenouillet F, Hocquet D, Bertrand X. Prevalence of *Escherichia coli* sequence type 131 and its H30 subclone among *E. coli* isolates in a French hospital. Int J Antimicrob Agents 2014; 44: 466–8. <https://doi.org/10.1016/j.ijantimicag.2014.07.016>.
19. Luna-Pineda VM, Ochoa SA, Cruz-Cordova A, Cazares-Dominguez V, Reyes-Grajeda JP, Flores-Oropeza MA, et al. Features of urinary *Escherichia coli* isolated from children with complicated and uncomplicated urinary tract infections in Mexico. PLoS One 2018; 13: e0204934. <https://doi.org/10.1371/journal.pone.0204934>.
20. Banerjee R, Johnston B, Lohse C, Chattopadhyay S, Tchesnokova V, Sokurenko EV, et al. The clonal distribution and diversity of extraintestinal *Escherichia coli* isolates vary according to patient characteristics. Antimicrob Agents Chemother 2013; 57: 5912–7. <https://doi.org/10.1128/AAC.01065-13>.
21. Matsumura Y, Yamamoto M, Nagao M, Hotta G, Matsushima A, Ito Y, et al. Emergence and spread of B2-ST131-O25b, B2-ST131-O16 and D-ST405 clonal groups among extended-spectrum-beta-lactamase-producing *Escherichia coli* in Japan. J Antimicrob Chemother 2012; 67: 2612–20. <https://doi.org/10.1093/jac/dks278>.
22. Ciesielczuk H, Doumith M, Hope R, Woodford N, Wareham DW. Characterization of the extra-intestinal pathogenic *Escherichia coli* ST131 clone among isolates recovered from urinary and blood-stream infections in the United Kingdom. J Med Microbiol 2015; 64: 1496–503. <https://doi.org/10.1099/jmm.0.000179>.
23. Banerjee R, Johnson JR. A new clone sweeps clean: the enigmatic emergence of *Escherichia coli* sequence type 131. Antimicrob Agents Chemother 2014; 58: 4997–5004. <https://doi.org/10.1128/Aac.02824-14>.
24. Blanco J, Mora A, Mamani R, Lopez C, Blanco M, Dahbi G, et al. Four main virotypes among extended-spectrum-beta-lactamase-producing isolates of *Escherichia coli* O25b:H4-B2-ST131: bacterial, epidemiological, and clinical characteristics. J Clin Microbiol 2013; 51: 3358–67. <https://doi.org/10.1128/JCM.01555-13>.
25. Coelho A, Mora A, Mamani R, Lopez C, Gonzalez-Lopez JJ, Larrosa MN, et al. Spread of *Escherichia coli* O25b:H4-B2-ST131 producing CTX-M-15 and SHV-12 with high virulence gene content in Barcelona (Spain). J Antimicrob Chemother 2011; 66: 517–26. <https://doi.org/10.1093/jac/dkq491>.
26. Wang LH, Liu PP, Wei DD, Liu Y, Wan LG, Xiang TX, et al. Clinical isolates of uropathogenic *Escherichia coli* ST131 producing NDM-7 metallo-beta-lactamase in China. Int J Antimicrob Agents 2016; 48: 41–5. <https://doi.org/10.1016/j.ijantimicag.2016.03.009>.
27. Yair Y, Gophna U. Pandemic bacteremic *Escherichia coli* strains: evolution and emergence of drug-resistant pathogens. In: Frankel G, Ron EZ, editors. *Escherichia coli*, a versatile pathogen. Springer International Publishing; 2018.
28. Merino I, Porter SB, Johnston BD, Clabots C, Shaw E, Horcajada JP, et al. Virulence genes and subclone status as markers of experimental virulence in a murine sepsis model among *Escherichia coli* sequence type 131 clinical isolates from Spain. PLoS One 2017; 12: e0188838. <https://doi.org/10.1371/journal.pone.0188838>.
29. Johnson JR, Porter SB, Zhanel G, Kuskowski MA, Denamur E. Virulence of *Escherichia coli* clinical isolates in a murine sepsis model in relation to sequence type ST131 status, fluoroquinolone resistance, and virulence genotype. Infect Immun 2012; 80: 1554–62. <https://doi.org/10.1128/IAI.06388-11>.
30. Price LB, Johnson JR, Aziz M, Clabots C, Johnston B, Tchesnokova V, et al. The epidemic of extended-spectrum-beta-lactamase-producing *Escherichia coli* ST131 is driven by a single highly pathogenic subclone, H30-Rx. mBio 2013; 4: e00377–13. <https://doi.org/10.1128/mBio.00377-13>.

