JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Detection of O25b-ST131 clone in extended spectrum beta-lactamase-producing *E. coli* from urinary tract infections in Mexico

Josefina Duran-Bedolla[1], Juan Téllez-Sosa[1], Ana María González-Villoria[2], Humberto Barrios-Camacho[1]

[1] Instituto Nacional de Salud Pública (INSP), Centro de Investigación Sobre Enfermedades Infecciosas (CISEI), Departamento de Diagnóstico Epidemiológico. Cuernavaca, Morelos, México
[2] División de Estudios de Posgrado. Universidad de la Sierra Sur, Oaxaca, México

Abstract

Introduction: *Escherichia coli* has emerged as an important pathogen in urinary tract infections (UTIs) due to the rapid acquisition of antibiotic resistance genes. This enhances the ability of *E. coli* to colonize and creates therapeutic challenges within the healthcare system. This study aimed to identify the extended spectrum beta-lactamase (ESBL) and O25b-ST131 pandemic clones in *E. coli* isolated from two hospitals in Mexico.

Methodology: Bacterial identification and antibiotic susceptibility tests were conducted using the VITEK 2 system. The ESBL and plasmid-mediated quinolone resistance (PMQR) genes were identified by polymerase chain reaction (PCR). *E. coli* genotyping was carried out by the phylogenetic group analysis and O25b-ST131 identification.

Results: A total of 103 unique *E. coli* clinical isolates were analyzed from a pool of 1,002 strains; 75% obtained from UTIs and vaginal secretions. Multi-resistant antibiotic profiles were observed. Notably, the presence of the *aac(6')lb-cr* and *qnr* genes was associated with 100% ciprofloxacin resistance, when ESBL was present. Additionally, the B2 phylogenetic group was identified, with 23% of isolates belonging to the O25b-ST131 clone.

Conclusions: Our research revealed a 10% prevalence of ESBL, in contrast to global prevalence rates. The resistance profiles suggest that the effectiveness of these commonly used antibiotics in treating *E. coli*-associated UTIs or vaginal infections has decreased significantly. Excessive use of antimicrobial agents contributes to the regional variation. Our results underscore the importance of monitoring the molecular epidemiology, antibiotic resistance, and transmission dynamics of the O25b-ST131 *E. coli* clone.

Key words: UTI; ESBL; *Escherichia coli*; ST131.

J Infect Dev Ctries 2024; 18(12):1861-1866. doi:10.3855/jidc.18854

(Received 07 July 2023 – Accepted 26 April 2024)

Introduction

Urinary tract infections (UTIs) are one of the most widespread infections globally and represent a significant threat to global health. *Escherichia coli* (*E. coli*), a predominant bacterium in the intestinal microbiome, is the leading cause of UTIs. Annually, a significant number of UTI cases are reported, with 80–90% occurring in community settings and approximately 30–50% within healthcare systems [1]. Over the last decade, there has been a global increase in morbidity and mortality rates associated with UTIs. The incidence of UTIs is notably three times higher in women than in men, and it is anticipated that at least 60% of women and 12% of men will experience an acutely symptomatic UTI at some point in their lives. Additionally, a significant majority of cases are characterized by the presence of asymptomatic bacteriuria [2].

The excessive and improper use of empirical antibiotics has significantly accelerated the development of bacterial resistance. The therapeutic application of quinolones has diminished due to the emergence of plasmid-mediated quinolone resistance reported in recent years [3]. In 2019, it was reported that UTI isolates from Latin American patients exhibited a high prevalence of *E. coli* resistance to ciprofloxacin [4]. Consequently, cephalosporins have become an alternative treatment; however, their use has led to a rise in resistance rates.

Until the 1990s, beta-lactam resistance was associated with *blaTEM* and *blaSHV*, along with their different alleles. However, since 2000, *blaSHV* has surpassed *blaCTX-M* as the most frequently reported extended-spectrum beta-lactamase (ESBL). The coexistence of both is associated with multidrug-resistant phenotypes, including resistance to beta-lactams, fluoroquinolones, and aminoglycosides [5]. Quinolone resistance is primarily associated with

chromosomal mutations, efflux pumps, and horizontal transfer via plasmids. Additional mechanisms contributing to this resistance include plasmid-mediated quinolone resistance (PMQR). PMQR includes the Qnr protein family (QnrA, QnrB, QnrC, QnrD, and QnrS) [3], the aminoglycoside-acetyltransferase modifying enzyme AAC(6′)-Ib-cr [6]; and the efflux pumps such as QepA and OqxAB [7].

The *E. coli* O25b-ST131 clone is considered a high-risk entity, and has emerged as one of the most significant pandemic clones globally. It is characterized by drug resistance, virulence, and community-acquired pathogens [8–9]. ST131 is associated with *CTX-M-15*, conferring resistance to cephalosporins and monobactams, along with common resistance to fluoroquinolones and trimethoprim/sulfamethoxazole [8]. The presence of PMQR genes (*qnrA*, *qnrB*, and *qnrS*), and the *aac(6)-Ib-cr*, contributes to quinolone resistance. Members of the O25b-ST131 clone belong to the phylogenetic group B2, which represents virulent extraintestinal strains. In medical practice, ST131 is recognized as a highly virulent clone that can cause septic shock. Thus, the O25b-ST131 clone represents a serious public health concern due to its extensive multidrug resistance profile, high virulence potential, and global dissemination [9]. This study aims to use molecular methods to characterize and identify the O25b-ST131 pandemic clone in ESBL-producing *E. coli* isolated from two hospitals in northern Mexico.

Methodology

Bacterial isolates and antimicrobial susceptibility testing

A total of 1,002 *E. coli* isolates were obtained from Unidad Medica Familiar #33 (835/1002) and Hospital General Regional IMSS #270 (167/1002) during January–December 2018 in Tamaulipas, Mexico. These isolates were collected from patients of all age groups exhibiting symptoms suggestive of UTIs, or from vaginal secretions. Bacterial species identification and antibiotic susceptibility tests were conducted using the VITEK 2 compact system (BioMérieux, Durham, USA). The isolates were identified as ESBL-producing by the double-disk synergism method according with the guidelines of the Clinical and Laboratory Standards Institute [10].

Polymerase chain reaction (PCR) amplification of genes

DNA extraction from colonies was performed via heat shock. The *blaSHV*, *blaCTX-M*, *qnrA*, *qnrB*, *qnrS*, and *aac(6′)lb-cr* genes were screened using specific primers for each gene by PCR [11–16]. The primer sets are described in Table 1.

E. coli genotyping

Phylogenetic group analysis was conducted by PCR amplification of the *chuA*, *yjaA*, and *tspE4C2* fragments [17]. The amplification of the *pabB* gene using primers O25pabBspe.F and O25pabBspe.R facilitated the identification of isolates belonging to the O25b-ST131 clone. The identification of O25b typing was performed by PCR as previously described, using the rfbO25b.r and rfb1bis.f primers [14,15].

Results

A total of 103 *E. coli* clinical isolates were collected from a pool of 1,002 strains. The majority of the isolates, 76% (79/103), were obtained from clinic #33, while the remaining 24% (24/103) were from hospital #270. Identification of the isolates was conducted using the VITEK 2 compact system (BioMérieux, Durham,

Table 1. Primer sequences of ESBL and PMQR.

Gene	Primer	5′sequences 3′	Amplicon	Reference
*bla*SHV	P1 P2	ACTGAATGAGGCGCTTCC TCC CGC AGA TAA ATC ACC	291	[11]
*bla*CTX-M	CTX-MF CTX-MR	GCTGTTGTTAGGAAGTGTG GGTGACGATTTTAGCCGCC	811	[16]
qnrA	QnrAm-F QnrAm-R	AGAGGATTTCTCACGCCAGG TGCCAGGCACAGATCTTGAC	580	[13]
qnrB	QnrBm-F QnrBm-R	GGMATHGAAATTCGCCACTG TTTGCYGYYCGCCAGTCGAA	264	[13]
qnrS	QnrSm-F QnrSm-R	GCAAGTTCATTGAACAGGGT TCTAAACCGTCGAGTTCGGCG	428	[13]
aac(6′)-lb-cr	aac-lb-cr-F aac-lb-cr-R	TTGCGATGCTCTATGAGTGGCTA TCGAATGCCTGGCGTGTTT	482	[12]
O25b	rfb.1bis rfbO25b.r	ATACCGACGACGCCGATCTG TGCTATTCATTATGCGCAGC	300	[14]
pabB	O25pabBspe.F O25pabBspe.R	TCCAGCAGGTGCTGGATCGT GCGAAATTTTTCGCCGTACTGT	347	[15]

ESBL: extended spectrum beta-lactamase; PMQR: plasmid-mediated quinolone resistance.

USA), and confirmation of ESBL production was achieved through a double disk synergism test.

The primary sources of ESBL *E. coli* isolates were UTIs (71%, 73/103), and vaginal secretions (22%, 23/103). Women were the predominant demographic for UTIs, accounting for 71% of these cases, while men represented the remaining 29%. ESBL-producing *E. coli* isolates from UTIs showed a multi-resistant profile: 100% were resistant to ampicillin; 97% to cefazolin, ceftriaxone, and ceftazidime; 41% to cefepime; 95% to aztreonam; 89% to ciprofloxacin; and 53% to gentamicin.

Isolates from vaginal secretions displayed resistance as follows: 95% were resistant to ampicillin, cefazolin, ceftriaxone, and ceftazidime; 73% to ciprofloxacin; 65% to gentamicin; and 30% to cefepime. Notably, the isolates did not have resistance to amikacin, nitrofurantoin, or tigecycline.

Regarding genotypic analysis, the most commonly identified ESBL and PMQR genes were *blaCTX-M* in 48% (49/103), *blaSHV* in 28% (29/103), and *blaCTX-M/SHV* in 24% (25/103). Additionally, *aac(6')lb-cr* was identified in 42% (43/103), and the *qnr* genes were found in 7% (7/103), distributed as follows: *qnrA* in 2.9% (3/103), *qnrB* in 3.8% (4/103), and *qnrS* in 1.9% (2/103).

A clear correlation between various genotypic profiles and antibiotic resistance in ESBL *E. coli* isolates was identified. For instance, the *blaCTX-M* genotype was present in 26% (27/103) of the isolates and exhibited 100% resistance to ampicillin, cefazolin, ceftriaxone, and ceftazidime; 96% to aztreonam; 40% to cefepime; 70% to ciprofloxacin; and 40% to gentamicin. The *blaCTX-M/aac(6´)lbcr* genotype was present in 18% (18/103) of the isolates, with a similar resistance profile to the previously described genotype, along with an increase in ciprofloxacin resistance from 70% to 94%.

The *blaSHV* genotype was detected in 16% (17/103) of the isolates, and exhibited 94% resistance to ampicillin; 64% to aztreonam; 88% to cefazolin, ceftriaxone, and ceftazidime; 11% to cefepime; 70% to ciprofloxacin; and 52% to gentamicin.

The *blaCTX-M/SHV* genotype was found in 11% (12/103) of the isolates, with 100% resistance to ampicillin, aztreonam, cefazolin, ceftriaxone, and ceftazidime. It also exhibited 41% resistance to cefepime, 91% to ciprofloxacin, and 75% to gentamicin. The *blaCTX-M/SHV/aac(6´)lbcr* genotype was present in 10% (11/103) of the isolates, with a similar resistance profile as the *blaCTX-M/SHV* genotype, but demonstrated a complete (100%) resistance to ciprofloxacin. The remaining genotypes all had a similar resistance profile, and together they constituted about 18% of all the isolates (Table 2).

The B2 phylogroup was the most prominent, comprising 42% (43/103) of the isolates, followed by group A with 34% (35/103), group B1 with 14% (14/103), and group D with 11% (11/103). Of the 43 ESBL *E. coli* isolates belonging to the B2 phylogroup, 23% (10/43) were identified as the global pandemic O25b-ST131 clone.

Discussion

E. coli has been identified as the primary pathogen involved in UTIs. Its rapid acquisition of antibiotic resistance and virulence genes has not only enhanced *E. coli*'s colonization capabilities but also complicated therapeutic strategies [18]. Consequently, the World Health Organization (WHO) has recognized *E. coli* as a critical research priority.

Our study found that 71% of *E. coli* isolates were obtained in samples from UTIs and vaginal secretions,

Table 2. Presence of ESBL - PMQR and the antimicrobial susceptibility. Genotypes identified and antimicrobial resistance percentages of *E. coli* clinical isolates.

ESBL and PMQR genotype	No. of Isolates (%)	Resistance (%) to:							
		AM	ATM	CZ	CRO	CAZ	FEP	CIP	GM
CTX-M	27 (26.2)	100	96.2	100	100	100	40.7	70.3	40.7
CTX-M + aac(6´)lb-cr	18 (18.4)	100	94.4	100	100	100	50	94.4	66.6
SHV	17 (16.5)	94.1	64.7	88.2	88.2	82.3	11.7	70.5	52.9
CTX-M + SHV	12 (11.6)	100	100	100	100	100	41.6	91.6	75
CTX-M + SHV + aac(6´)lb-cr	11 (10.6)	100	100	100	100	100	58.3	100	75
SHV + aac(6´)lb-cr	11 (10.6)	100	90.9	90.9	90.9	72.7	45.4	100	72.7
CTX-M + qnr	3 (2.9)	100	66.6	100	66.6	66.6	33.3	100	33.3
CTX-M + SHV + qnr	1 (0.9)	100	100	100	100	100	0	100	100
CTX-M + aac(6´)lb-cr + qnr	1 (0.9)	100	100	100	100	100	100	100	100
CTX-M + SHV + aac(6´)lb-cr + qnr	1 (0.9)	100	100	100	100	100	0	100	100
SHV + aac(6´)lb-cr + qnr	1 (0.9)	100	100	100	100	100	0	100	100

ESBL: extended spectrum beta-lactamase; PMQR: plasmid-mediated quinolone resistance. AM: ampicillin; ATM: aztreonam; CZ: cefazolin; CRO: ceftriaxone; CAZ: ceftazidime; FEP: cefepime; CIP: ciprofloxacin; GM: gentamicin. All isolates showed 0% resistance to meropenem: amikacin: tigecycline: and nitrofurantoin.

predominantly in women. This observation aligns with several studies confirming *E. coli* as the principal cause of the majority of UTIs, which mainly affect women. Both age and gender emerged as important factors to consider when examining the recurrence of infections and potential sexual transmission [19].

We observed antibiotic resistance towards ampicillin, aztreonam, cefazolin, ceftriaxone, and ceftazidime (> 95%); along with resistance to ciprofloxacin (90%), which is commonly used as the first-choice treatment for UTIs.

These findings suggest a significant reduction in the efficacy of these antibiotics for treating *E. coli*-associated UTIs or vaginal infections. Additionally, we detected resistance rates of 40% and 50% towards fourth-generation cephalosporins and gentamicin, respectively, which could potentially compromise their effectiveness in treating patients diagnosed with UTIs.

On the other hand, amikacin and nitrofurantoin continue to demonstrate significant success in the treatment of *E. coli* UTIs, consistent with reports from Swedish hospitals where nitrofurantoin is the main treatment choice for UTIs, independent of the gender of the patient [20].

In our study, we identified a 10% prevalence of ESBL, contrasting with the prevalence rates reported globally: 40% in Nepal, 19% in Korea, 41% in Iran and Djibouti, 56% in Egypt, and 33% in Saudi Arabia [21–26]. This variability in the prevalence of β-lactam resistance across different countries and hospitals can be attributed to multiple factors, with the excessive use of these antimicrobial agents being a significant one [27,28].

Several studies have reported a transition from *blaSHV* to *blaCTX-M*; however, we identified *blaCTX-M-15*, *blaSHV*, and *blaCTX-M15/SHV* in 28%, 24%, and 47% of isolates, respectively. This prevalence significantly differs from that reported in Korea and Djibouti, with 83% and 97% respectively [22,24,29].

We found that isolates with either the *blaCTX-M* or the *blaSHV* showed a ciprofloxacin resistance rate of 70%. However, in cases where ESBL was co-expressed with the *aac(6')lb-cr* or *qnr* genes, the resistance increased to 100%.

The horizontal transfer of PMQR determinants accelerates the selection of higher levels of quinolone resistance, facilitating bacterial survival and the subsequent generation of mutants in *GyrA* and *ParC* with higher-level quinolone resistance that lead to therapeutic failure [30].

Our study identified the B2 phylogenetic group as the most prevalent, comprising 41% of the total cases, which is consistent with several studies [24]. Within the isolates belonging to the B2 phylogroup, we found that 23% (10/43) of the isolates belong to the O25b-ST131 clone.

The ST131 clone plays a significant role in the global spread of antimicrobial resistance among *E. coli* isolates. This high-risk clone owes its widespread success to several factors, including its association with the B2 phylogroup and the O25b serotype, both known for their considerable virulence potential, and their common correlation to the *blaCTX-M-15* gene, the most widespread ESBL globally [9]. Monitoring the molecular epidemiology, including the characteristics, antibiotic resistance profiles, and transmission dynamics of these clones, can significantly improve clinical programs. This approach will enable effective control of high-risk clones, preventing their spread within the community.

Conclusions

Our findings reveal an alarming degree of antibiotic resistance against common UTI treatments, including ampicillin, aztreonam, cefazolin, ceftriaxone, ceftazidime, and ciprofloxacin. The elevated resistance rates, particularly against third-generation cephalosporins, suggest a decrease in the efficacy of these antibiotics, which could compromise their therapeutic effectiveness. However, amikacin and nitrofurantoin continue to demonstrate promising results in treating *E. coli* UTIs. The prevalence of the *blaCTX-M-15* gene in a significant portion of isolates reflects current scientific trends. Within the isolates belonging to the B2 phylogenetic group, the O25b-ST131 clone emerges as the most prevalent. This high-risk clone, recognized for its significant virulence potential and association with the *blaCTX-M-15*, has been associated with recurrent UTIs and severe clinical complications. Our study emphasizes the urgent necessity for ongoing surveillance programs to identify resistance patterns and high virulence serotypes, and thereby control antibiotic resistance.

Acknowledgements

We thank Cindy Hernández-Garcia for technical assistance and Elvia Rodríguez-Ortiz for providing the bacterial isolates.

Authors' contributions

All authors declare that they have participated in the work to take public responsibility for the content, including participation in the concept, investigation, methodology, writing, and revision of the article.

Funding

This work was supported by the grant 256927 from CONACyT (Mexican Council for Science and Technology).

References

1. Wagenlehner F, Tandogdu Z, Bartoletti R, Cai T, Cek M, Kulchavenya E, Köves B, Naber K, Perepanova T, Tenke P, Wullt B, Bogenhard F, Johansen TE (2016). The global prevalence of infections in urology study: a long-term, worldwide surveillance study on urological infections. Pathogens 5: 10. doi: 10.3390/pathogens5010010.
2. Alós JI (2005) Epidemiology and etiology of urinary tract infections in the community. Antimicrobial susceptibility of the main pathogens and clinical significance of resistance. Enferm Infecc Microbiol Clin 23 Suppl 4: 3–8. [Article in Spanish].
3. Martínez-Martínez L, Eliecer Cano M, Manuel Rodríguez-Martínez J, Calvo J, Pascual A (2008) Plasmid-mediated quinolone resistance. Expert Rev Anti Infect Ther 6: 685–711. doi: 10.1586/14787210.6.5.685.
4. Antimicrobial Resistance Collaborators (2022) Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 399: 629–655. Erratum in: Lancet 2022. 400: 1102.
5. Wangkheimayum J, Paul D, Dhar D, Nepram R, Chetri S, Bhowmik D, Chakravarty A, Bhattacharjee A (2017) Occurrence of acquired 16S rRNA methyltransferase-mediated aminoglycoside resistance in clinical isolates of Enterobacteriaceae within a tertiary referral hospital of northeast India. Antimicrob Agents Chemother 61: e01037–16. doi: 10.1128/AAC.01037-16.
6. Robicsek A, Jacoby GA, Hooper DC (2006) The worldwide emergence of plasmid-mediated quinolone resistance. Lancet Infect Dis 6: 629–640. doi: 10.1016/S1473-3099(06)70599-0.
7. Hansen LH, Johannesen E, Burmølle M, Sørensen AH, Sørensen SJ (2004) Plasmid-encoded multidrug efflux pump conferring resistance to olaquindox in *Escherichia coli*. Antimicrob Agents Chemother 48: 3332–3337. doi: 10.1128/AAC.48.9.3332-3337.2004.
8. Rogers BA, Sidjabat HE, Paterson DL (2011) *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. J Antimicrob Chemother 66: 1–14. doi: 10.1093/jac/dkq415.
9. Mathers AJ, Peirano G, Pitout JD (2015). The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. Clin Microbiol Rev 28: 565–591. doi: 10.1128/CMR.00116-14.
10. CLSI (2020) M100-S30. Performance standards for antimicrobial susceptibility testing. Twenty-Second Informational Supplement. Wayne, PA: Clinical and Laboratory Standards Institute.
11. Prodinger WM, Fille M, Bauernfeind A, Stemplinger I, Amann S, Pfausler B, Lass-Florl C, Dierich MP (1996). Molecular epidemiology of *Klebsiella pneumoniae* producing SHV-5 beta- lactamase: parallel outbreaks due to multiple plasmid transfer. J Clin Microbiol 34: 564–568. doi: 10.1128/jcm.34.3.564-568.1996.
12. Park CH, Robicsek A, Jacoby GA, Sahm D, Hooper DC (2006) Prevalence in the United States of aac(6')-Ib-cr encoding a ciprofloxacin-modifying enzyme. Antimicrob Agents Chemother 50: 3953–3955. doi: 10.1128/AAC.00915-06.
13. Cattoir V, Poirel L, Rotimi V, Soussy CJ, Nordmann P (2007) Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. J Antimicrob Chemother 60: 394–397. doi: 10.1093/jac/dkm204.
14. Clermont O, Lavollay M, Vimont S, Deschamps C, Forestier C, Branger C, Denamur E, Arlet G (2008) The CTX-M-15-producing *Escherichia coli* diffusing clone belongs to a highly virulent B2 phylogenetic subgroup. J Antimicrob Chemother 61: 1024–1028. doi: 10.1093/jac/dkn084.
15. Clermont O, Dhanji H, Upton M, Gibreel T, Fox A, Boyd D, Mulvey MR, Nordmann P, Ruppé E, Sarthou JL, Frank T, Vimont S, Arlet G, Branger C, Woodford N, Denamur E (2009) Rapid detection of the O25b-ST131 clone of *Escherichia coli* encompassing the CTX-M-15-producing strains. J Antimicrob Chemother 64: 274–277. doi: 10.1093/jac/dkp194.
16. Espinal P, Garza-Ramos U, Reyna F, Rojas-Moreno T, Sanchez-Perez A, Carrillo B, Martinez P, Mattar S, Silva-Sanchez J (2010) Identification of SHV-type and CTX-M-12 extended-spectrum beta-lactamases (ESBLs) in multiresistant Enterobacteriaceae from Colombian Caribbean hospitals. J Chemother 22: 160–164. doi: 10.1179/joc.2010.22.3.160.
17. Clermont O, Bonacorsi S, Bingen E (2000) Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol 66: 4555–4558. doi: 10.1128/AEM.66.10.4555-4558.2000.
18. Totsika M, Beatson SA, Sarkar S, Phan MD, Petty NK, Bachmann N, Szubert M, Sidjabat HE, Paterson DL, Upton M, Schembri MA (2011) Insights into a multidrug resistant *Escherichia coli* pathogen of the globally disseminated ST131 lineage: genome analysis and virulence mechanisms. PloS One 6: e26578. doi: 10.1371/journal.pone.0026578.
19. Rahn DD (2008) Urinary tract infections: contemporary management. Urol Nurs 28: 333–342.
20. Jansåker F, Boel JB, Thønnings S, Hertz FB, Hansen KH, Frimodt-Møller N, Knudsen JD (2019). Pivmecillinam compared to other antimicrobials for community-acquired urinary tract infections with *Escherichia coli*, ESBL-producing or not - a retrospective cohort study. Infect Drug Resist 12: 1691–1702. doi: 10.2147/IDR.S209255.
21. Pandit R, Awal B, Shrestha SS, Joshi G, Rijal BP, Parajuli NP (2020) Extended-spectrum β-lactamase (ESBL) genotypes among multidrug-resistant uropathogenic *Escherichia coli* clinical isolates from a teaching hospital of Nepal. Interdiscip Perspect Infect Dis 2020: 6525826. doi: 10.1155/2020/6525826.
22. Kim H, Kim YA, Park YS, Choi MH, Lee GI, Lee K (2017). Risk factors and molecular features of sequence type (ST) 131 extended-spectrum β-lactamase-producing *Escherichia coli* in community-onset bacteremia. Sci Rep 7: 14640. doi: 10.1038/s41598-017-14621-4.
23. FarajzadehSheikh, A., Veisi, H., Shahin, M., Getso, M., & Farahani, A. (2019). Frequency of quinolone resistance genes among extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* strains isolated from urinary tract infections. Trop Med Health 47: 19. doi: 10.1186/s41182-019-0147-8.
24. Mohamed HS, Houmed Aboubaker M, Dumont Y, Didelot MN, Michon AL, Galal L, Jean-Pierre H, Godreuil S (2022). Multidrug-resistant Enterobacterales in community-acquired urinary tract infections in Djibouti, Republic of Djibouti. Antibiotics 11: 1740. doi: 10.3390/antibiotics11121740.

25. Hassuna NA, Khairalla AS, Farahat EM, Hammad AM, Abdel-Fattah M (2020) Molecular characterization of extended-spectrum β lactamase- producing *E. coli* recovered from community-acquired urinary tract infections in Upper Egypt. Sci Rep 10: 2772. doi: 10.1038/s41598-020-59772-z.
26. Abalkhail A, AlYami AS, Alrashedi SF, Almushayqih KM, Alslamah T, Alsalamah YA, Elbehiry A (2022) The prevalence of multidrug-resistant *Escherichia coli* producing ESBL among male and female patients with urinary tract infections in Riyadh Region, Saudi Arabia. Healthcare (Basel) 10: 1778. doi: 10.3390/healthcare10091778.
27. Corkill JE, Anson JJ, Hart CA (2005) High prevalence of the plasmid-mediated quinolone resistance determinant *qnrA* in multidrug-resistant Enterobacteriaceae from blood cultures in Liverpool, UK. J Antimicrob Chemother 56: 1115–1117. doi: 10.1093/jac/dki388.
28. Ali I, Rafaque Z, Ahmed S, Malik S, Dasti JI (2016) Prevalence of multi-drug resistant uropathogenic *Escherichia coli* in Potohar region of Pakistan. Asian Pac J of Trop Biomed 6: 60–66. doi: 10.1016/j.apjtb.2015.09.022.
29. Chong Y, Ito Y, Kamimura T (2011) Genetic evolution and clinical impact in extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*. Infect Genet Evol 11: 1499–1504. doi: 10.1016/j.meegid.2011.06.001.
30. Poirel L, Cattoir V, Nordmann P (2008) Is plasmid-mediated quinolone resistance a clinically significant problem? Clin Microbiol Infect 14: 295–297. doi: 10.1111/j.1469-0691.2007.01930.x.

Corresponding author

Barrios Camacho Humberto, PhD.
Departamento de Diagnóstico Epidemiológico, Instituto Nacional de Salud Pública,
Av. Universidad # 655, Col. Sta. Ma. Ahuacatitlán. C.P. 62100. Cuernavaca, Mor., México.
Tel: (52) 7773293000 ext: 2406
Fax: (52) 7771-01-29-25
Email: humberto.barrios@insp.mx

Conflict of interests: No conflict of interests is declared.