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High prevalence of B2-ST131 clonal group among extended-spectrum β-lactamase-producing *Escherichia coli* isolated from bloodstream infections in Quito, Ecuador★

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ABSTRACT

Objectives: The purpose of this study was to describe the clonal relationships and phylogroups of extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-Ec) isolated from patients with bacteraemia in three hospitals in Quito, Ecuador.
Methods: Between June 2013 and September 2014, a total of 4354 blood cultures were performed in three hospitals located in different areas of Quito. A BACTEC™ system was used for blood culture, and the VITEK® 2 system was used for species identification and in vitro antimicrobial susceptibility testing. The ESBL genotype, presence of the $bla_{\text{CTX-M}}$, bla_{TEM} and bla_{SHV} genes, and the phylogenetic group of *E. coli* isolates was determined by PCR. Clonal groups were established by multilocus sequence typing (MLST).
Results: Of 929 blood cultures positive for Gram-negative bacilli, 181 (19.5%) were positive for *E. coli*, representing the most frequent bacteraemia isolates in each hospital. Of the 181 *E. coli* isolates, 57 (31.5%) were ESBL-Ec. The main sources of ESBL-Ec bacteraemia were urinary tract infection (40; 70.2%), biliary tract infection (10; 17.5%) and other infections (7; 12.3%). The majority of ESBL-Ec isolates (39; 68.4%) from the three hospitals belonged to the virulent phylogenetic group B2, of which 36/39 (92.3%) were ST131 and 33/36 (91.7%) carried the $bla_{\text{CTX-M-15}}$ gene.
Conclusion: These results provide knowledge of the phylogenetic relationships of *E. coli* from bacteraemia in Ecuadorian patients. ST131 has emerged in ESBL-Ec, representing an important public-health problem because this multiresistant clone is considered to be a vehicle for the propagation of antimicrobial resistance genes and is a highly virulent, well-adapted human pathogen.

1. Introduction

Escherichia coli is an extraordinarily versatile bacterial species with the ability to colonise numerous animal hosts, the environment and food [1]. Most *E. coli* strains are non-pathogenic commensal bacteria; however, *E. coli* variants have the ability to cause infections inside and outside the gastrointestinal system, with the most frequent being bloodstream infections (BSIs) and urinary tract infections (UTIs) [2]. Antimicrobial resistance rates in *E. coli* are rising rapidly, especially resistance to fluoroquinolones and third- and fourth-generation cephalosporins. Furthermore, extended-spectrum β-lactamases (ESBLs) are a major cause of antimicrobial resistance among these bacterial strains, and the production of ESBLs confers resistance to most β-lactam antibiotics [3]. Infections caused by ESBL-producing *E. coli* (ESBL-Ec) are increasing worldwide both in hospital [4] and community [5] settings. This phenomenon is related to the emergence of hyperepidemic clones, primarily *E. coli* sequence type 131 (ST131) and phylogroup B2; these clones cause UTIs and are a source of bacteraemia in humans [6].

Escherichia coli ST131 was initially described among clinical isolates producing the CTX-M-15 ESBL enzyme and resistant to fluoroquinolones in several countries [7]. Currently, *E. coli* ST131 has a global distribution that is associated with various antimicrobial resistance determinants [8]. In Latin America, *E. coli* ST131 was

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initially detected in hospitals in Colombia and Brazil in 2008 and subsequently in the Colombian community setting in 2010 [9].

In Ecuador, there is scarce information regarding *E. coli* ST131 [10] and no information about bacteraemia isolates. To contribute to our knowledge of the molecular epidemiology of ESBL-Ec in the region, the aim of this study was to identify the clonal relationships and phylogroups among the ESBL-Ec isolates from patients with bacteraemia in three hospitals in Quito, Ecuador.

2. Materials and methods

2.1. Study design, population and antimicrobial susceptibility profile

This study was conducted between June 2013 and September 2014 in three high-complexity teaching hospitals in Quito, the capital city of Ecuador, namely: Hospital Vozandes Quito (H1), a private hospital located in the north (68 beds; 5750 hospital discharges); Hospital de Especialidades de las Fuerzas Armadas (H2), a public hospital located in the central part of the city (236 beds; 10 322 hospital discharges); and Hospital Enrique Garcés (H3), a public hospital located in the south (323 beds; 18 506 hospital discharges). Data regarding age, sex and source of bacteraemia were recorded for each patient. Infections were also classified as community-acquired or hospital-acquired. A community-acquired infection was defined as an infection in a patient who had no history of being in a hospital or long-term care facility in the past 3 months [11]. A hospital-acquired infection was defined as an infection that occurred >48 h after hospital admission.

Participant hospitals were asked to collect consecutive, non-duplicate BSI isolates with minimum inhibitory concentrations (MICs) of $\geq 1\ \mu g/mL$ for ceftriaxone (phenotypically ESBL-Ec isolates). All microbiology laboratories in the participating hospitals used automated systems. The BACTEC™ system (Becton Dickinson & Co., Franklin Lakes, NJ, USA) was used for blood culture, and the VITEK®2 Compact System (bioMérieux, Marcy-l'Étoile, France) was used for species identification and in vitro antimicrobial susceptibility testing. Colistin susceptibility was determined by broth microdilution (Thermo Scientific™ Sensititre™ AST System; Thermo Fisher Scientific, Waltham, MA, USA). MICs were interpreted according to Clinical and Laboratory Standards Institute (CLSI) breakpoints [12].

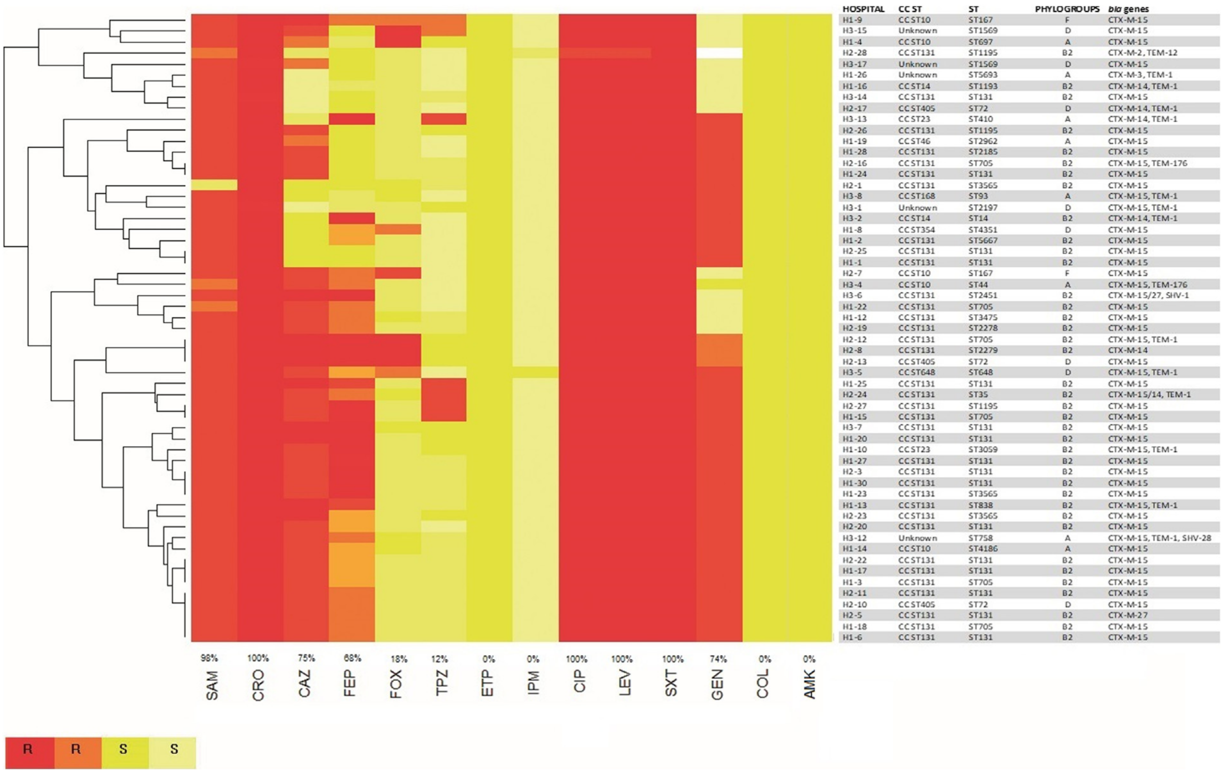
2.2. Molecular characterisation

Total DNA of the isolates was obtained using a High Pure PCR Template Preparation Kit (Roche Diagnostics, Rotkreuz, Switzerland). To determine the ESBL genotype, the bla_{CTX}, bla_{TEM} and bla_{SHV} genes were amplified and sequenced using previously described conditions [13]. The phylogenetic group of the *E. coli* isolates was determined by multiplex PCR [14].

Clonal groups were established by multilocus sequence typing (MLST) using the Achtman scheme [15]. Allelic profiles of seven gene sequences as well as the sequence types (STs) were obtained from the database of the Center for Genomic Epidemiology [16].

2.3. Statistical analysis

Categorical variables were evaluated using the χ^2 test with Fisher's exact test. A two-tailed *P*-value of <0.05 was considered statistically significant. A heatmap was constructed using the MICs of the isolates. All statistical analyses were performed in R 3.5.0.



HOSPITAL	CC ST	ST	PHYLOGROUPS	*bla* genes
H1-9	CCST10	ST167	F	CTX-M-15
H3-15	Unknown	ST1569	D	CTX-M-15
H1-4	CCST10	ST697	A	CTX-M-15
H2-28	CCST131	ST1195	B2	CTX-M-2, TEM-12
H3-17	Unknown	ST1569	D	CTX-M-15
H1-26	Unknown	ST5693	A	CTX-M-3, TEM-1
H1-16	CCST14	ST1193	B2	CTX-M-14, TEM-1
H3-14	CCST131	ST131	B2	CTX-M-15
H2-17	CCST405	ST72	D	CTX-M-14, TEM-1
H3-13	CCST23	ST410	A	CTX-M-14, TEM-1
H2-26	CCST131	ST1195	B2	CTX-M-15
H1-19	CCST46	ST2962	A	CTX-M-15
H1-28	CCST131	ST2185	B2	CTX-M-15
H2-16	CCST131	ST705	B2	CTX-M-15, TEM-176
H1-24	CCST131	ST131	B2	CTX-M-15
H2-1	CCST131	ST3565	B2	CTX-M-15
H3-8	CCST168	ST93	A	CTX-M-15, TEM-1
H3-1	Unknown	ST2197	D	CTX-M-15, TEM-1
H3-2	CCST14	ST14	B2	CTX-M-14, TEM-1
H1-8	CCST354	ST4351	D	CTX-M-15
H1-2	CCST131	ST5667	B2	CTX-M-15
H2-25	CCST131	ST131	B2	CTX-M-15
H1-1	CCST131	ST131	B2	CTX-M-15
H2-7	CCST10	ST167	F	CTX-M-15
H3-4	CCST10	ST44	A	CTX-M-15, TEM-176
H3-6	CCST131	ST2451	B2	CTX-M-15/27, SHV-1
H1-22	CCST131	ST705	B2	CTX-M-15
H1-12	CCST131	ST3475	B2	CTX-M-15
H2-19	CCST131	ST2278	B2	CTX-M-15
H2-12	CCST131	ST705	B2	CTX-M-15, TEM-1
H2-8	CCST131	ST2279	B2	CTX-M-14
H2-13	CCST405	ST72	D	CTX-M-15
H3-5	CCST648	ST648	D	CTX-M-15, TEM-1
H1-25	CCST131	ST131	B2	CTX-M-15
H2-24	CCST131	ST35	B2	CTX-M-15/14, TEM-1
H2-27	CCST131	ST1195	B2	CTX-M-15
H1-15	CCST131	ST705	B2	CTX-M-15
H3-7	CCST131	ST131	B2	CTX-M-15
H1-20	CCST131	ST131	B2	CTX-M-15
H1-10	CCST23	ST3059	B2	CTX-M-15, TEM-1
H1-27	CCST131	ST131	B2	CTX-M-15
H2-3	CCST131	ST131	B2	CTX-M-15
H1-30	CCST131	ST131	B2	CTX-M-15
H1-23	CCST131	ST3565	B2	CTX-M-15
H1-13	CCST131	ST838	B2	CTX-M-15, TEM-1
H2-23	CCST131	ST3565	B2	CTX-M-15
H2-20	CCST131	ST131	B2	CTX-M-15
H3-12	Unknown	ST758	A	CTX-M-15, TEM-1, SHV-28
H1-14	CCST10	ST4186	A	CTX-M-15
H2-22	CCST131	ST131	B2	CTX-M-15
H1-17	CCST131	ST131	B2	CTX-M-15
H1-3	CCST131	ST705	B2	CTX-M-15
H2-11	CCST131	ST131	B2	CTX-M-15
H2-10	CCST405	ST72	D	CTX-M-15
H2-5	CCST131	ST131	B2	CTX-M-27
H1-18	CCST131	ST705	B2	CTX-M-15
H1-6	CCST131	ST131	B2	CTX-M-15

Fig. 1. Heatmap of antimicrobial resistance profiles. The heatmap denotes the relationship between the antimicrobial resistance pattern, phylogroup, clonal complex sequence type (CC ST) and extended-spectrum β-lactamase (ESBL) genes. Each row represents one isolate tested. SAM, ampicillin/sulbactam; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; FOX, cefoxitin; TPZ, piperacillin/tazobactam; ETP, ertapenem; IPM, imipenem; CIP, ciprofloxacin; LEV, levofloxacin; SXT, trimethoprim/sulfamethoxazole; GEN, gentamicin; COL, colistin; AMK, amikacin. The different tones indicating resistance and susceptibility depend on the MIC range obtained and are in accordance with the Clinical and Laboratory Standards Institute (CLSI) breakpoints [12]. Intermediate susceptibility was considered resistance. MIC, minimum inhibitory concentration; R, resistant; S, susceptible.

Allelic profiles of STs were used to generate a global optimal eBURST (goeBURST) diagram using PHYLOViZ software [17].

3. Results

During the study, a total of 4354 blood cultures were performed, including 1798 samples (41.3%) from H1, 1409 samples (32.4%) from H2 and 1147 samples (26.3%) from H3. Gram-negative bacilli were detected in 929 blood cultures, of which 181 (19.5%) were *E. coli*. Of the 181 *E. coli* bacteraemia isolates, 57 (31.5%) were ESBL-Ec, comprising 35.7% (25/70) from H1, 31.3% (20/64) from H2 and 25.5% (12/47) from H3.

Patients with ESBL-Ec-positive blood cultures had a median age of 65.4 years (range 25–94 years). A similar percentage of males and females was observed ($P = 0.7048$). Of the 57 bacteraemia cases caused by ESBL-Ec, only 4 (7.0%) were of hospital origin, whereas there was a high association with infections from community sources (93.0%). The main sources of bacteraemia was UTIs (40; 70.2%), followed by biliary tract infections (10; 17.5%), respiratory tract infections (4; 7.0%), skin and soft-tissue infections (2; 3.5%) and central nervous system infections (1; 1.8%).

3.1. Antimicrobial resistance

The isolates presented high resistance rates to ampicillin/sulbactam (98.2%), ciprofloxacin (100%), levofloxacin (100%), trimethoprim/sulfamethoxazole (100%), gentamicin (73.7%), cefoxitin (17.5%) and piperacillin/tazobactam (12.3%). No resistance was observed to carbapenems, amikacin or colistin (Fig. 1).

3.2. Molecular characterisation

Phylogenetic group B2 ($n = 39$; 68.4%) was the most common among ESBL-Ec, followed by phylogroups A ($n = 10$; 17.5%), D ($n = 7$; 12.3%) and F ($n = 1$; 1.8%). The most common ESBLs identified were $bla_{\text{CTX-M-15}}$ (49/57) and $bla_{\text{CTX-M-14}}$ (6/57). Phylogenetic group B2 was principally associated with $bla_{\text{CTX-M-15}}$ (34/49), followed by phylogroup A (8/49), phylogroup D (6/49) and phylogroup F (1/49). Of the six $bla_{\text{CTX-M-14}}$-positive isolates, four were phylogroup B2, one was phylogroup A and one was phylogroup D (Table 1).

MLST analysis identified 29 different sequence types. eBURST software clustered the types into the four following clonal complexes (CCs): CC ST10 (ST167, ST4186, ST44 and ST697); CC ST23 (ST410 and ST3059); CC ST14 (ST14 and ST1193); and CC ST131 (ST131, ST705, ST1195, ST2185, ST2278, ST2279, ST2451, ST3475, ST3565, ST838, ST35 and ST5667). In addition, nine singletons were identified. ST131 was identified as the most frequent sequence type and was the founding group of the largest clonal complex, which contained 12 different STs. *Escherichia coli* ST131 was isolated from BSIs in all three hospitals (Fig. 2).

A strong association between ST and phylogenetic group was found ($P = 0.03703$); the most frequent ST was the ST131 complex (36/57; 63.2%), which was assigned to the virulent extraintestinal phylogroup B2. Most *E. coli* ST131 (33/36; 91.7%) strains were associated with $bla_{\text{CTX-M-15}}$. Other ESBL types found among the ST131 isolates were $bla_{\text{CTX-M-27}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-2}}$ and $bla_{\text{TEM-12}}$ (Table 1).

4. Discussion

Here we describe the clonal relationships and phylogroups of ESBL-Ec isolated from patients with bacteraemia in three hospitals in Quito, Ecuador. These data provide insights into the molecular epidemiology of bloodstream-associated *E. coli* in Ecuador. The data show a high prevalence of *E. coli* ST131, with a higher incidence of acquisition in the community than in a hospital setting [18]. In this study, the main type of bacteraemia caused by ST131 *E. coli* was from UTI, followed by abdominal infections, respiratory tract infections and skin and soft-tissue infections. This finding suggests the ability of this competitive phylogroup B2 clone to colonise the human digestive tract and to cause human UTIs owing to its ability to adhere to the intestinal, bladder and kidney epithelial cells [18].

Currently, ST131 has a worldwide distribution; however, the reasons for its ability to spread, to be maintained within the community and to adapt so well to humans are not clear. It is believed that its ability to survive in different environments, the capacity to transmit between humans, and its multidrug resistance pattern and virulence factors play a role [18]. Kallonen et al. stated that the main advantage is that *E. coli* ST131 causes infections in the bloodstream and occupies a huge commensal niche in the human population. Thus, *E. coli* does not form a discrete population but extends into the intestine, so it has a high probability to enter the bloodstream and cause bacteraemia [19]. Data in the current study showed that 63% of bacteraemia cases caused by *E. coli* ST131 were recovered from UTIs. This clone is likely the most frequent in the intestines of the studied population and is likely the cause of UTIs and, thereafter, bacteraemia. Therefore, surveillance of this clone in the human population is essential to understanding the dynamics and structure of invasive *E. coli* [19].

Table 1
Association between the phylogenetic groups and extended-spectrum β-lactamase (ESBL) and non-ESBL genotypes.

Genotype (no. of isolates)	Phylogenetic group			
	A	B2	D	F
$bla_{\text{CTX-M-15}}$ (37)	ST10 Cplx (4) ST46 Cplx (1)	ST131 Cplx (27)	ST354 Cplx (1) ST405 Cplx (2) Unknown (2)	
$bla_{\text{CTX-M-15}}/bla_{\text{TEM-1}}$ (6)	ST168 Cplx (1)	ST23 Cplx (1) ST131 Cplx (2)	Unknown (1)	ST648 Cplx (1)
$bla_{\text{CTX-M-15}}/bla_{\text{SHV-1}}$ (1)		ST131 Cplx (1)		
$bla_{\text{CTX-M-15}}/bla_{\text{TEM-176}}$ (2)	ST10 Cplx (1)	ST131 Cplx (1)		
$bla_{\text{CTX-M-15}}/bla_{\text{SHV-28}}/bla_{\text{TEM-1}}$ (1)	Unknown (1)			
$bla_{\text{CTX-M-14}}$ (1)		ST131 Cplx (1)		
$bla_{\text{CTX-M-14}}/bla_{\text{TEM-1}}$ (4)	ST23 Cplx (1)	ST14 Cplx (2)	ST405 Cplx (1)	
$bla_{\text{CTX-M-2}}/bla_{\text{TEM-12}}$ (1)		ST131 Cplx (1)		
$bla_{\text{CTX-M-27}}$ (1)		ST131 Cplx (1)		
$bla_{\text{CTX-M-3}}/bla_{\text{TEM-1}}$ (1)	Unknown (1)			
$bla_{\text{CTX-M-15}}/bla_{\text{CTX-M-14}}/bla_{\text{TEM-1}}$ (1)		ST131 Cplx (1)		
$bla_{\text{CTX-M-15}}/bla_{\text{CTX-M-27}}/bla_{\text{SHV-1}}$ (1)		ST131 Cplx (1)		

Cplx, ST clonal complex.

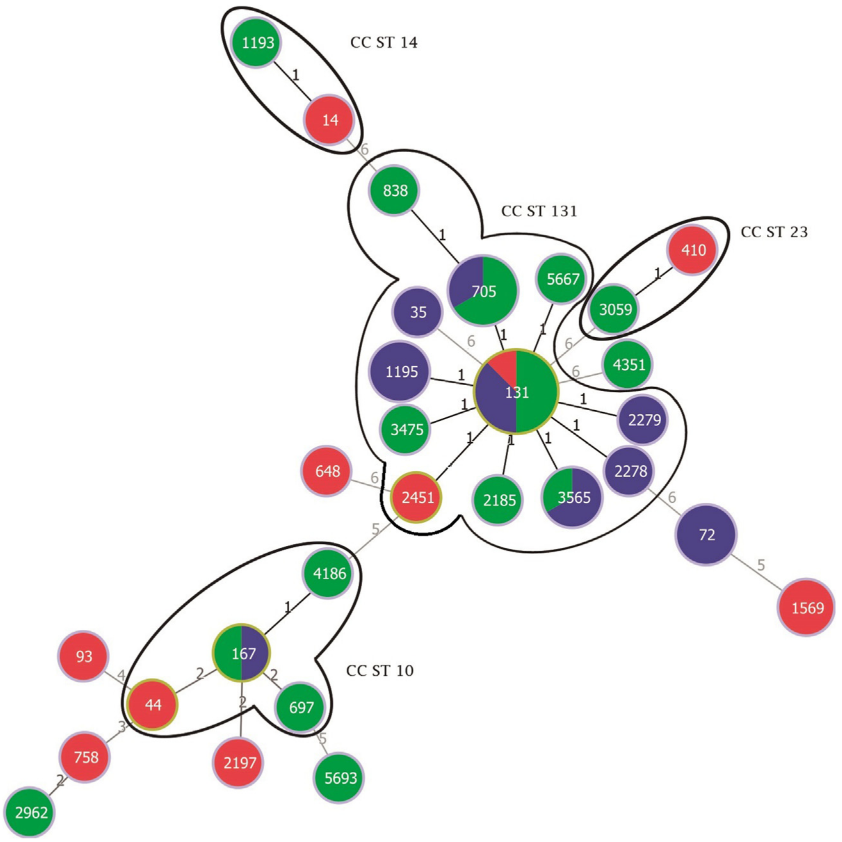


Fig. 2. eBURST diagram with strict group definition (default). The chart represents 57 *Escherichia coli* isolates from bloodstream infections, including 9 singletons and four clonal complexes as follows: CC ST10 (ST167, ST4186, ST44 and ST697); CC ST23 (ST410 and ST3059); CC ST 14 (ST14 and ST1193); and CC ST131 (ST131, ST705, ST1195, ST3565, ST2451, ST3475, ST5667, ST2185, ST2279, ST2278, ST35 and ST838). Each hospital is identified by a colour as follows: green, H1; blue, H2; and red, H3. The area of each circle corresponds to the sequence type (ST) prevalence in the multilocus sequence typing (MLST) data of this study (number of isolates per strain).

In this study, ST131 was found at a higher prevalence in adults aged >65 years with UTIs. The susceptibility to infectious diseases and the spread of this strain in the elderly population is increased due to several factors, including the presence of extraintestinal infections, previous use of antibiotics and living in long-term healthcare facilities [20]. Previous studies in Europe showed that the strain ST131 was present in all isolates obtained from nursing homes, suggesting that elderly patients have a greater susceptibility to acquire infections with antimicrobial resistance because of these strains [21]. In Ecuador, the elderly are still cared for by their family, and nursing homes and long-term care facilities are not common. There are no statistics (neither the rate of self-medication nor antibiotic treatment compliance) in Ecuador regarding the use of antibiotics in this population group. Therefore, it was not possible to conclude whether nursing homes play an important role as a risk factor in Ecuador.

The frequency of *E. coli* ST131 has increased in the population and it has become an important clonal complex [8]. *Escherichia coli* ST131-related isolates represented 63% of the isolates found in this study, including single-locus variant and double-locus variant isolates of ST131, showing a local variation of ST131 clones. In Latin America, *E. coli* ST131 isolates harbouring the $bla_{\text{CTX-M-15}}$ gene have

Table 2
Escherichia coli ST131 among clinical isolates described in Latin America countries.

Country	Source	Total *E. coli*	ESBL-positive *E. coli*	$bla_{\text{CT-XM-15}}$-positive *E. coli*	*E. coli* ST131	Period	Reference
Argentina	Clinical isolates	1120	1.3% (14/1120)	50% (7/14)	57% (4/7)	1 week during October 2010	[22]
Brazil	Blood/urine/rectal swab/ascitic fluid/wound	ND	25	64% (16/25)	12.5% (2/16)	February 2008–June 2009	[23]
Colombia	UTI, community	ND	33	42.4% (14/33)	14.3% (2/14)	January–May 2002	[11]
Colombia	UTI, community	431	12.5% (54/431)	53.7% (29/54)	65.5% (19/29)	August–December 2012	[24]
Ecuador	UTIs, nosocomial/healthcare-acquired or community	ND	35	17.1% (6/35)	100% (6/6)	July–December 2012	[10]
Mexico	ND	70	52.9% (37/70)	37.8% (14/37)	92.9% (13/14)	Between 2011 and 2014	[25]
Mexico	UTI, community	4735	10.56% (500/4735)	25% (14/56[a])	100% (14/14)	November 2006–August 2007	[26]
Mexico	UTI	119	30.3% (36[b]/119)	ND	36.1% (13/36)	Between 2004 and 2007	[27]
Uruguay	Urine/blood/skin lesions/abscesses/ respiratory/peritoneal fluid	ND	55	67.3% (37/55)	54.1% (20/37)	March 2010 and February 2011	[28]

ST, sequence type; ESBL, extended-spectrum β-lactamase; ND, data not mentioned in the article; UTI, urinary tract infection.

[a] 56 representative samples from 500 ESBL-positive *E. coli* were chosen.

[b] 36 multidrug-resistant isolates; in this study they do not mention ESBLs.

been reported in Argentina [22], Brazil [23], Colombia [11,24], Ecuador [10], Mexico [25–27] and Uruguay [28], corroborating the expansion of this clone in this region (Table 2).

Other pandemic clones were identified in this study, such as *E. coli* ST1193, which is a new emerging clone that is virulent and resistant to fluoroquinolones; this clone is also a lactose-non-fermenting isolate belonging to phylogenetic group B2. It has been reported in Australia, Korea and China [29]. This is the first report of *E. coli* ST1193 in a bacteraemia case in Ecuador harbouring *bla*$_{\text{CTX-M-14}}$, unlike previous studies in which this clone harboured *bla*$_{\text{CTX-M-55}}$ [30].

E. coli ST410 represents a globally distributed lineage associated with various antimicrobial resistance determinants, including ESBLs, AmpC β-lactamases, carbapenemases and colistin resistance genes; this lineage causes recurrent infections in humans, including BSIs [31]. This is the first report of the presence of *E. coli* ST410 belonging to phylogroup A and associated with ESBLs (*bla*$_{\text{CTX-M-14}}$) in bacteraemia in Ecuador. This clone has been previously reported in vegetable samples in Quito in which the *bla*$_{\text{CTX-M-15}}$ gene was identified [32].

Two ST167 clones (ST10 complex) carrying the *bla*$_{\text{CTX-M-15}}$ gene and belonging to phylogroup A were identified, and *E. coli* ST167 has been related to multiresistant *E. coli*, including resistance to carbapenems and colistin; however, the two isolates in the current study were susceptible to both antibiotic groups [33].

The current results confirm that the CTX-M-15 enzyme is the most common type of ESBL in clinical *E. coli* found in Ecuador (27.1%; 49/181), similar to the study by Calva et al. in the two main public hospitals of the Loja Province (south of Ecuador), where this enzyme was isolated in 30 (38%) of 79 samples [34]. Other data from this region show that *bla*$_{\text{CTX-M-15}}$ is prevalent in different clinical samples (range 17–60%), as shown in Table 2.

Although previous studies in Ecuador have identified *bla*$_{\text{CTX-M-55}}$ and *bla*$_{\text{CTX-M-65}}$ both in clinical samples and in broilers [34–38], these variants were not identified in the current study, however *bla*$_{\text{CTX-M-27}}$ and *bla*$_{\text{CTX-M-14.}}$ were observed.

In conclusion, *E. coli* ST131 is an emerging pathogen associated with BSIs in Quito, Ecuador, and constitutes an important public-health problem. The high rate of ESBL-Ec (31.5%; 57/181) could be associated with increases in the rates of high-risk clonal groups. The high prevalence of *E. coli* ST131 (63.2%; 36/57) may be due to the spread of ESBL-Ec. The *bla*$_{\text{CTX-M-15}}$ gene represented >80% of the ESBL-Ec isolates. This is the first insight into the phylogenetic composition of *E. coli* from BSIs in Ecuador.

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Competing interests

None declared.

Ethical approval

The study protocol was approved by the Ethics Committee of Pontificia Universidad Católica del Ecuador [document CEI: 026-2015]. Permission to conduct this study at Hospital Vozandes Quito [DINVES 2013-0014], Hospital de Especialidades de las Fuerzas Armadas [013-021-HB-1-10CBE] and Hospital Enrique Garcés [488-G-HEG] was also obtained.

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