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Escherichia coli causing bloodstream infections in Mexican paediatric patients: molecular typing, antimicrobial resistance, virulence factors, and clinical features

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Abstract

Background *Escherichia coli* is one of the main pathogens causing bloodstream infections (BSIs) in paediatric patients. It is classified into pathogenic (B2, D and F) and commensal (A, B1 and C) phylogroups, with virulence mainly attributed to adhesins, toxins and iron acquisition systems. In recent years, the global spread of high-risk clones such as ST131 and ST405, often associated with extended-spectrum beta-lactamases (ESBLs), has contributed to increased resistance and limited treatment options. The BSI mortality rate in children varies from 14 to 21.6%. This study aimed to describe resistant mechanisms; virulence factors and clonal distribution of *E. coli* isolates that cause BSIs in children in Mexico and clinical features.

Methods Thirty-eight ceftriaxone (CRO)-resistant *E. coli* isolates were included. Beta-lactamase and virulence genes were detected by PCR. Molecular typing included phylogroup determination, sequence types (ST), and pulsed-field gel electrophoresis (PFGE). Clinical information was acquired.

Results CTX-M was the most frequently identified beta-lactamase (82%) and *aac*(6')-Ib-cr was present in 45%. Phylogroup distribution was A (21.1%), C (7.9%), D (28.9%), B2 (23.7%), and F (18.4%). The most common virulence factor was *fim*H (71%), while *pap*C, *sat* and *irp*2 were significantly more frequently in the pathogenic phylogroups ($P=0.029$, 0.011 and 0.006, respectively). PFGE identified 5 clusters, 20 non-related isolates and 4 non-typeable. Predominant clonal complexes (CC) were CC405 (23.7%) and CC131 (21.1%), with 82% of isolates belonging to high-risk clones. Survival rates differed significantly with moderate high-grade fever ($P=0.022$). All patients who died had complications, compared to 34.8% of survivors ($P<0.0001$). Mortality was higher in adolescents (53.3%), patients with leukaemia or lymphoma (40%), those with hospital-acquired infections (86.8%), those with an abdominal or pulmonary focus (33.3% each). No significant differences were found in of haematological parameters.

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Conclusions Both commensal and pathogenic *E. coli* strains cause BSIs in paediatric patients with underlying diseases. Resistance to 3GCs and 4GCs is mainly mediated by CTX-M, hence treatment with carbapenems was used. Infection-related deaths were more frequent in patients infected by pathogenic phylogroups, where *papC*, *sat*, and *irp2* were more prevalent. High-risk clones were widely distributed among isolates.

Keywords BSI, *E. coli*, ESBL, ExPEC, Virulence factors, Phylogenetic groups, Paediatric patients, High-risk clone, Mexico

Introduction

It is estimated that infectious diseases caused 13.7 million deaths in 2019, of which 4.9 million were associated with antimicrobial resistance (AMR) and 1.27 million were attributed to AMR. In total, 45.3% of the deaths attributable to AMR occurred due to infections caused by gram-negative bacteria, 219,000 of which were attributed to *E. coli* [1, 2]. Another estimate indicates that 2.68 million deaths from sepsis that occurred among children under 5 years of age in 2021, were associated with AMR [3]. From 2013–2016, the main pathogen that caused bloodstream infections (BSIs) in Latin America was *E. coli*, which was responsible for 18.3% of the cases [4]. The mortality rate from BSIs in children is 14% in the United States and 21.6% in Brazil [5].

E. coli strains that cause infections outside the intestine are known as extraintestinal pathogens (ExPECs) as meningitis and urinary tract infections (UTIs), in addition to BSIs [6].

The virulence of ExPECs is associated with genes that encode adhesins, such as type 1 fimbriae (*fim*H*)* and S fimbriae adhesins (*sfa*); toxins, such as α-haemolysin (*hly*A), cytotoxic necrotizing factor 1 (*cnf* 1), secreted autotransporter toxin (*sat*), plasmid-encoded toxin (*pet*), and vacuolating autotransporter toxin (*vat*); and proteins involved in intestinal colonization (*pic*) and iron acquisition, such as the siderophores aerobactin siderophore receptor (*iut*A), yersiniabactin siderophore (*irp*2), aerobactin (*iuc*C), and salmochelin siderophore receptor (*iro*N) [7]. Together, these virulence factors facilitate host colonization and allow the pathogen to invade the bloodstream [8, 9].

ESBLs are a group of enzymes that hydrolyse beta-lactam antibiotics, including third- and/or fourth-generation cephalosporins (3GCs and 4GCs, respectively) and monobactams, which has led to carbapenems being the main treatment option for infections caused by ESBL-producing bacteria. The most important ESBL family members are Temoneira beta-lactamase (TEM), sulfhydryl variable beta-lactamase (SHV), cefotaximase-Munich beta-lactamase (CTX-M), oxacillinase (OXA) and Guiana extended-spectrum beta-lactamase (GES), each with a different level of activity against beta-lactams [10].

E. coli can be classified into pathogenic (B2, D, and F) and commensal (A, B1, and C) phylogroups [11]). PFGE is considered as the molecular gold standard in local epidemiological studies and allows identify clonal relationships and potential outbreaks [12]. ExPECs belong mainly to the STs 131, 69, 73 and 95; among them, ST131 strains are considered high-risk clones because they are carriers of the ESBL CTX-M and are resistant to aminoglycosides and fluoroquinolones [13, 14].

Hospital-acquired infections (HAIs) caused by ESBL-producing *E. coli* in intensive care units (ICUs) remain significant contributors to morbidity and mortality, often leading to prolonged hospital stays and higher healthcare costs compared with infections caused by susceptible *E. coli* strains [12]. However, data on comorbidities among paediatric patients from Latin America with *E. coli* causes BSIs, as well as information on their virulence profiles and molecular typing is limited [15–17]. The aim of this study was to describe resistant mechanisms, virulence factors and clonal distribution of *E. coli* isolates that cause BSIs in children in Mexico and clinical features.

Methods

Identification and susceptibility profiling

This study was performed at the National Institute of Paediatrics (INP), a tertiary hospital with 254 beds, located at south of Mexico City. During a two-year period from February 2013-January 2015, consecutive CRO- resistant *E. coli* isolates that caused BSIs in paediatric patients (aged 0–18 years) were included. After the isolates were identified, their susceptibility profiles were obtained using the Microbiology Phoenix® automated system (Becton Dickinson, NJ, USA) according to the manufacturer's instructions. Antibiotic categorization was performed according to the M100 document of the Clinical Laboratory Standards Institute (CLSI) [18].

Confirmatory ESBL test

ESBL activity detection was performed by the double disk diffusion method using cefotaxime and ceftazidime SensiDisks alone and in combination with clavulanic acid following CLSI guidelines [18].

Molecular detection of beta-lactamase and quinolone resistance genes

Total DNA was extracted using the QIAamp® DNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. PCR was used to detect the beta-lactamase genes $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M8/25}}$, bla_{LAT}, bla_{DHA}, bla_{TEM}, bla_{SHV} and quinolone resistance genes *qnr*A, *qnr*B, *qnr*S and *aac(6)-Ib-cr* using previously published primers (Table S1) [19–21]. PCR was performed with an AB9700 thermal cycler (Applied Biosystems Foster City, CA, USA) with AmpliTaq Gold® 360 MasterMix (Applied Biosystems, Foster City CA, USA). The fragments obtained were purified with a QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) and sequenced on a 3500 XL system (Applied Biosystems) exclusively for beta-lactamase genes. The sequences were analysed with the BLASTN program [22, 23]. Multiple sequence alignments were performed with BioEdit v.7.7.1 (Ibis Biosciences, Carlsbad CA, USA) to determine the enzyme subtypes and for comparison with sequences from the Beta-Lactamase Data Resources of the National Center for Biotechnology Information (NCBI).

Identification of virulence genes

Thirteen virulence genes were detected by multiplex PCR. Amplifications were carried out in 25 µL reaction mixtures containing 0.1 µg of template DNA and AmpliTaq Gold® 360 Master Mix (Applied Biosystems, Foster City, CA, USA). Four multiplex PCR assays were performed as follows: PCR 1 included *iroN* (665 bp), *cnf*1 (498 bp), and *pap*C (328 bp), with cycling conditions of initial denaturation at 95 °C for 2 min; 30 cycles of denaturation at 95 °C for 30 s, annealing at 64 °C for 30 s, and extension at 72 °C for 1 min; and a final extension at 72 °C for 7 min. PCR 2 targeted *pic* (1011 bp), *iuc*C (269 bp), and *sat* (501 bp), using an annealing temperature of 56 °C and a final extension of 10 min at 72 °C. PCR 3 included *irp*2 (287 bp) and *fim*H (508 bp), with an annealing temperature of 63 °C and final extension of 7 min. PCR 4 targeted *pet* (302 bp) and *vat* (420 bp), with an annealing temperature of 58 °C and final extension of 7 min. Additionally, *hly*CA (556 bp), *sfa* (410 bp), and *iut*A (302 bp) were individually amplified under standard PCR conditions, using an annealing temperature of 55 °C. The sequence of the primers used are available in Table S1 [24–30]. *E. coli* CFT073 was used as a positive control.

Molecular typing

Determination of phylogenetic group

A multiplex PCR was performed to determine the phylogenetic group of each strain, based on the presence or absence of four genetic markers (*arp*A, *chu*A, *yja*A, TspE4.C2). using specific primers (Table S1). PCR conditions included an initial denaturation at 94 °C for 4 min; 30 cycles of 94 °C for 5 s, 59 °C for 20 s, and 72 °C for 1 min; followed by a final extension at 72 °C for 5 min. Phylogenetic groups (A, B1, B2, C, D, E, F, or clade I) were determined according to the genotypic profiles obtained using the Clermont method [11].

Pulsed-field gel electrophoresis (PFGE)

Clonal relatedness was assessed by PFGE using XbaI (Invitrogen, Life Technologies, Waltham, MA, USA), following the PulseNet protocol [31]. *Salmonella enterica* serovar Braenderup ATCC BAA-664 was used as a molecular size marker. PFGE gels were stained with ethidium bromide (10 mg/mL) and visualized under UV light. Banding patterns were interpreted according to Tenover criteria [32] using Image Lab Software v6.1 (Bio-Rad, Hercules, CA, USA). The resulting dendrogram from PFGE analysis were visualized using the GGTREE v3.16.0 package in Rv4.5.0 [33], enabling detailed annotation and graphical display of the clonal relationships among *E. coli* isolates.

Genotyping by MLST

Seven housekeeping genes (*adk*, *fum*C, *gyr*B, *icd*, *mdh*, *pur*A, and *rec*A) were amplified by PCR using specific primers (Table S1). Each amplification reaction was performed in a 25 µL volume using a Veriti Thermal Cycler (Applied Biosystems) under the following conditions: initial denaturation at 95 °C for 2 min; 30 cycles of denaturation at 95 °C for 15 s, annealing at 54–60 °C for 30 s, and extension at 72 °C for 1 min; followed by a final extension at 72 °C for 7 min. PCR products were confirmed by agarose gel electrophoresis and purified using the QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany). Purified amplicons were sequenced by an external service (Macrogen Inc., Korea), and the resulting sequences were analyzed using the *E. coli* multilocus sequence typing (MLST) database (https://pubmlst.org/organisms) [34, 35]. The closest ST or clonal complex (CC) was assigned based on the concordance of at least four loci from the MLST scheme and classified as a high-risk clone according to previously published data [36].

Clinical information

The clinical records of the patients were reviewed to obtain information such as age, sex, underlying diseases, history of previous surgery, HAI or community-acquired infection (CAI), focus of bacteraemia, presence of fever, respiratory rate (RR), blood counts, treatments, admission to ICU, use of mechanical ventilation or central venous catheter (CVC), bacteriemia complications

(sepsis, septic shock, multiple organ failure), outcome (cure or death), and, in the case of death, if it was associated with the infection also were recorded.

We used the following age categories: neonatal (birth–27 days), infant (28 days–12 months), toddler (13 months–2 years), early childhood (2–5 years), middle childhood (6–11 years), and early adolescence (12– < 18 years) [37]. Underlying diseases were defined as medical conditions that involved any organ or system that required specialized care and were classified into the following categories: neurological, renal, gastrointestinal, congenital, leukaemia and lymphoma, solid tumours, nonneoplastic haematological disease and malnutrition [38]. Surgeries performed (14-days and 1-year) before the onset of symptoms were considered.

BSIs were classified as HAIs if they developed on or after the third day of hospitalisation, while CAIs were defined as those diagnosed in an outpatient setting or detected by culture within 48 h of hospital admission [39]. Primary BSI was defined as a laboratory confirmed bloodstream infection without a secondary site of infection [39], whereas a secondary was defined as a BSI that is thought to be seeded from a site-specific infection at another body site. The focus of bacteraemia was classified as pulmonary, abdominal, skin and soft tissue, urinary, catheter-related, or without a determined focus [39].

The presence of low-grade fever was considered 37.3 to 38.0 °C; moderate-grade fever: 38.1 to 39.0 °C; high-grade fever: 39.1 to 41 °C and hyperthermia: greater than 41 °C [40].

The interpretation of the haematological results was performed according to the age of the patients and were categorized into anaemia, leukocytosis, leukopenia, neutrophilia, neutropenia, lymphocytosis, lymphopenia, thrombocytosis, and thrombocytopenia [41]. Neutropenia was defined as mild (≥ 1000 to < 1500 cells/μL), moderate (≥ 500 to < 1000 cells/μL) and severe (< 500 cells/μL) [42].

Previous treatment was considered the use of antibiotic in the last 3 months; empiric treatment was defined as any antimicrobial administered from the day of symptoms until date of susceptibility profile report day (SPRD); definitive treatment was the antibiotics administered after the SPRD, monotherapy or combined therapy. To standardize the duration of treatment in all cases, the day of symptoms was considered Day 0.

Microbiological cure was defined as obtaining negative culture results following antimicrobial treatment. General mortality was considered when a patient died during hospitalisation with *E. coli* BSI, whereas infection-related mortality was defined as death directly attributable to the BSI or resulting from the exacerbation of a pre-existing condition within three months of the onset of BSI symptoms [39, 43].

Statistical analysis

The Kolmogorov–Smirnov test was used to evaluate the distribution of the variables. Continuous variables are presented as the median [25–75 th percentile]. Qualitative variables are expressed as frequencies (%). The Mann–Whitney U test was used to evaluate the differences between continuous quantitative variables between two groups. The χ^2 test for trends and Fisher's exact test were used to evaluate the proportional differences among the qualitative variables. All analyses were performed using SPSS version 21 (IBM). A value of $P < 0.05$ was considered to indicate statistical significance.

Results

In total, 38 *E. coli* isolates obtained from paediatric patients with BSIs were included. All isolates were resistant to ampicillin-sulbactam (SAM), cefazolin (CFZ), cefuroxime (CXM), and ceftriaxone (CRO). Furthermore, 3% were susceptible to ceftazidime (CAZ), 5% aztreonam (AZT) and cefepime (FEP); 13% to ciprofloxacin (CIP); 18% tobramycin (TOB); 24% to levofloxacin (LEV); 37% to gentamicin (GE), 53% to piperacillin-tazobactam (PTZ); 13% to trimethoprim-sulfamethoxazole (SXT), 95% to amikacin (AK), and 97% to MEM and IPM (Fig. 1).

Enzymes from the CTX-M family were the most identified ESBLs (n = 31, 81.6%). SHV-ESBL was detected in two isolates (P17 and P19). No ESBLs were identified in five isolates, among which three had CMY-type enzymes (P23, P32 and P36). In 17 (45%) isolates, *aac*(6')-Ib-cr gene was detected. None isolate carried *qnr*A, *qnr*B and *qnr*S genes (Fig. 1).

The most identified virulence factor was *fim*H (71%), followed by *irp*2 (63%), *iuc*C (55%) and *iut*A (40%). The isolates from the pathogenic phylogroups B2, D, and F contained a higher number of virulence genes compared to those from other phylogroups (Fig. 1, Table 1). Moreover, the genes *pap*C, *sat*, and *irp*2 were significantly more frequent in the pathogenic phylogroups ($P = 0.029$, 0.011, and 0.006, respectively). None of the isolates harbored the *sfa*, *pic*U, or *pet* gene (Fig. 1, Table 1).

A total of 28.9% of the isolates were classified into commensal phylogroups A (21.1%) and C (7.9%), and 71.1% were classified as pathogens (D, 28.9%; B2, 23.7%; and F, 18.4%). None isolate was classified in B1 phylogroup. No relationship was observed between phylogroup and mortality ($P = 0.455$). The virulence factors did not show statistical difference with the outcome (Table 1, Table S3).

The analysis of PFGE profiles grouped 14 isolates into 5 clusters, 20 isolates were non-related (Fig. 2). Four isolates (P25, P32, P36 and P38) were non-typeable by this

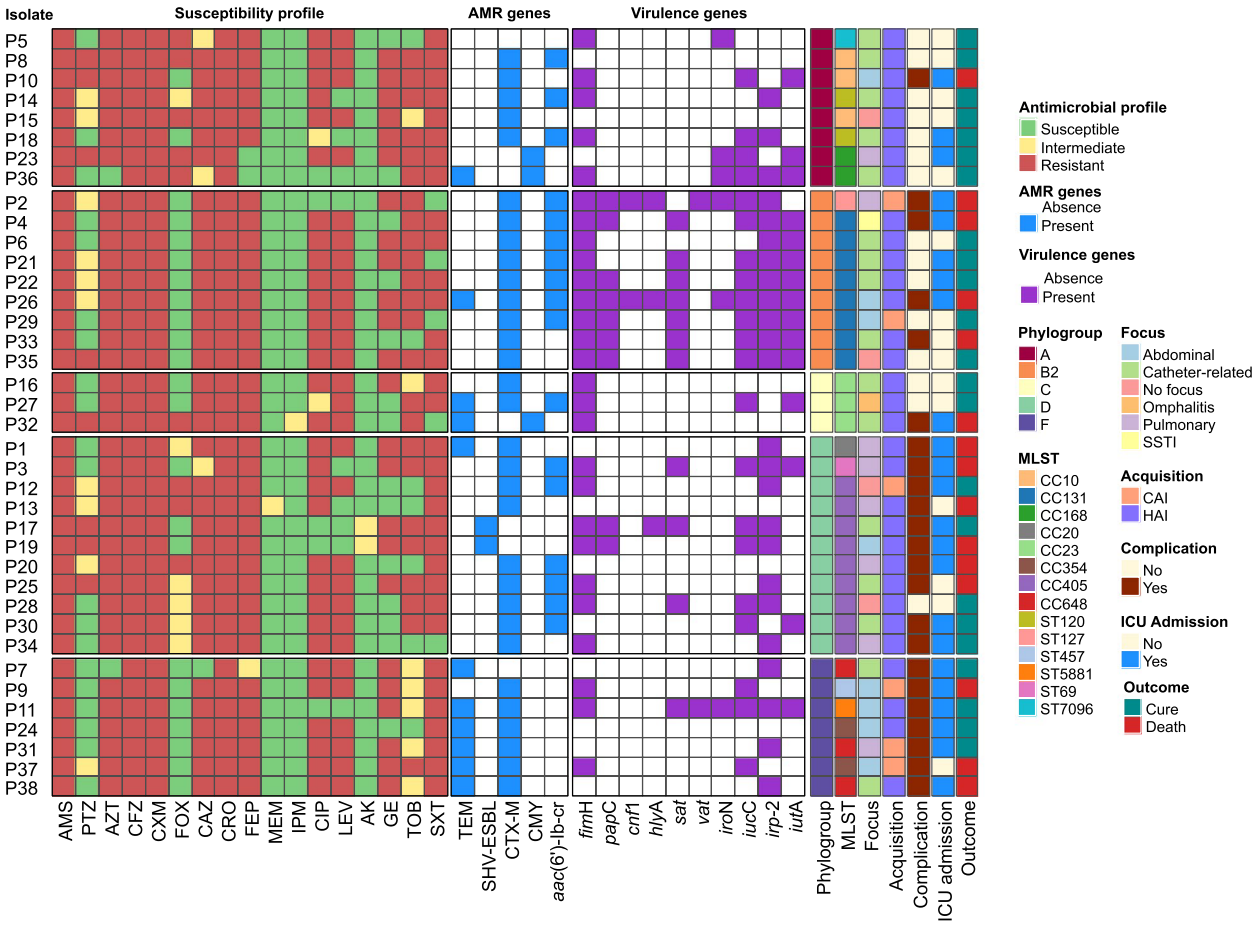

Fig. 1 Characteristics of the *E. coli* caused BSI in paediatric patients

MLST: multilocus sequence typing, AMS: ampicillin-sulbactam, PTZ: piperacillin-tazobactam, AZT: aztreonam, CFZ: cefazolin, CXM: cefuroxime, FOX: cefoxitin, CAZ: ceftazidime, CRO: ceftriaxone, FEP: cefepime, MEM: meropenem, IPM: imipenem, CIP: ciprofloxacin, LEV: levofloxacin, AK: amikacin, GE: gentamicin, TOB: tobramycin, SXT: trimethoprim-sulfamethoxazole, CC: clonal complex, ST: sequence typing, CAI: community-acquired infection, HAI: hospital-acquired infection, SSTI: skin and soft tissue infections, ICU: intensive care unit, AMR: antimicrobial resistance. Data visualization was performed using R software (v4.5.0) with the ComplexHeatmap package (v2.24.0)

Table 1 Virulence gene distribution among the commensal and pathogenic phylogroups

Phylogroup (*n*)	Virulence gene									
	Adhesins		Toxins				Siderophores			
	***fim*H**	***pap*C**	***cnf* 1**	***hly* A**	***sat***	***vat***	***iro*N**	***iuc*C**	***irp*2**	***iut*A**
Commensal (11)	8	0	0	0	0	0	3	5	3	4
A (8)	5	0	0	0	0	0	3	4	3	3
C (3)	3	0	0	0	0	0	0	1	0	0
Pathogenic (27)	19	9	2	3	11	2	3	16	21	11
B2 (9)	9	7	2	2	7	1	2	8	9	8
D (11)	7	2	0	1	3	0	0	5	8	2
F (7)	3	0	0	0	1	1	1	3	4	1
Total (38)	27	9	2	3	11	2	6	21	24	15
P value	0.604	**0.029**	0.499	0.347	**0.011**	0.499	0.221	0.438	**0.006**	0.55

P values that indicate statistical significance, as determined by Fisher's exact test upon comparison of the virulence genes and the total number of commensal ($n = 11$) and pathogenic ($n = 27$) isolates, are shown in **bold**

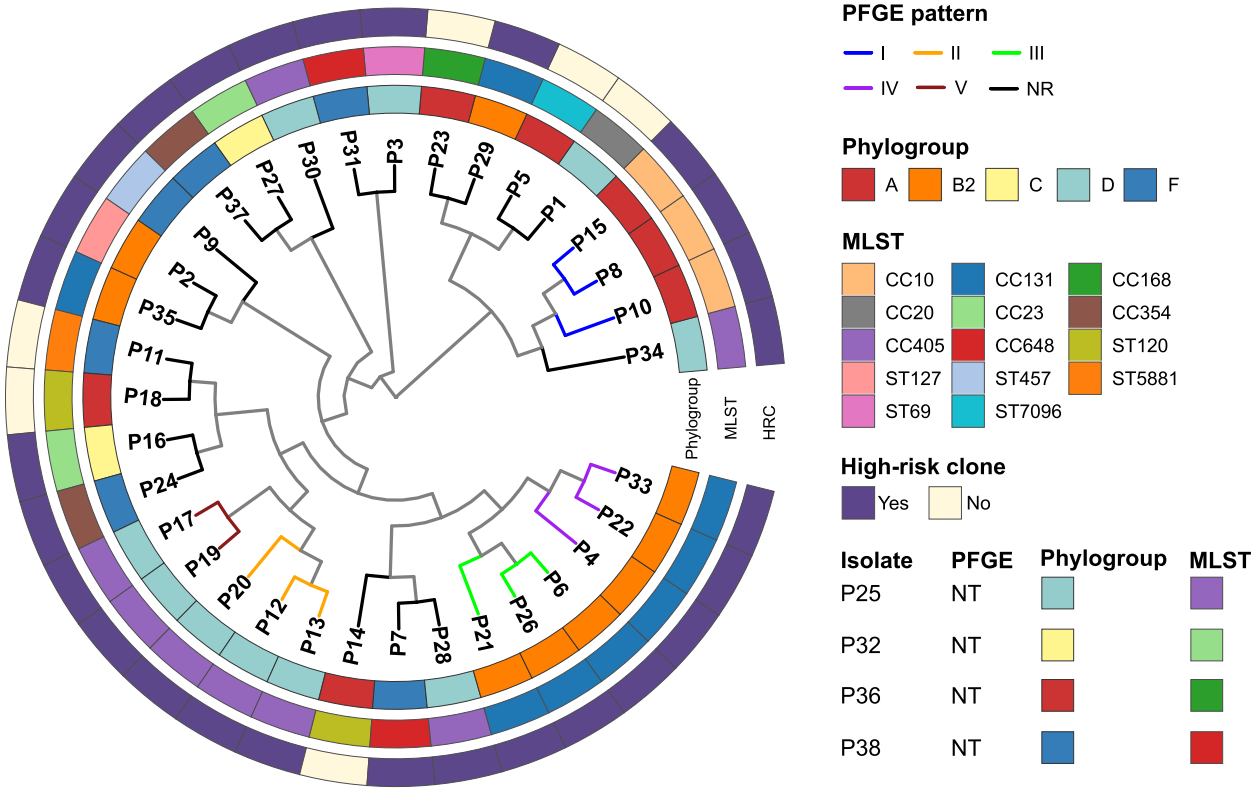

Fig. 2 Clonal relationship of the *E. coli* isolates associated with bacteraemia

Dendrogram based on PFGE pattern analysis showing clonal relationships among isolates. Sequence types (ST), phylogroup and high-risk clones or clonal complex (HRC)/HRCC are indicated for each isolate. Four isolates were non-typeable (NT) by PFGE. MLST: multilocus sequence typing, CC: clonal complex, PFGE: pulsed-field gel electrophoresis and NR: non-related

method. *E. coli* CC405 were the most frequent (n = 9, 23.7%) followed by CC131(n = 8, 21.1%), and one isolate was classified as ST69 (P3). We detected CC10, CC23 y CC648 with three isolates each one (7.9%) (Fig. 2). Eighty-two percent of the isolates belonged to high-risk clones or high-risk CC (CC405, CC131, CC648, CC10, CC23, CC354, ST127, ST457 and ST69).

A total of 31.6% of the patients were female, and adolescents composed the predominant group (36.8%). Leukaemia and lymphoma and congenital diseases were the most common underlying diseases among children with BSIs (42.1% and 21.1%, respectively). Most of the BSI were secondary (86.4%), catheter-associated infections were the most prevalent with 42.1% of the cases, followed by abdominal and pulmonary infections (21.1% each). Twenty-five (65.8%) patients had a previous surgery in the last year; 29 (76.3%) received at least one antimicrobial in last 3 months and 34 (89.5%) in the last year. Sixty-five per cent of the patients presented fever, 57.9% polypnea. Anaemia was observed in 76.3% and leukopenia in 65.7% (Table S2).

A total of 57.9% of the patients received empirical monotherapy; 36.8% combined therapy and 5.3% did not receive empirical treatment (P = 0.151). A total of 75% of the patients received carbapenems as empirical therapy (P = 0.272). By other side, A total of 55.3% of the patients received definitive monotherapy; 34.2% combined therapy and 10.5% did not receive definitive treatment (P = 0.294). A total of 81.6% of the patients received carbapenem as definitive therapy (P = 0.261) (Table 2, Table S3).

Sixty-five percent of the patients were admitted to the ICU and 63% required mechanical ventilation (Table S2). The BSI was resolved in 60.5% of the patients, and 34.5% of the deaths were considered related to the infection (Table 2, Table S3). Mortality did not differ significantly with respect to sex (P = 0.337), age (P = 0.471), underlying condition (P =0.506) and site of bacteraemia (P = 0.105) and haematological parameters. However, the presence of fever (P = 0.038) and grade of fever (moderate and high-grade fever, P= 0.022) were related with cure. A total of 60.5% of patients had complications, the most common was septic shock (52%) followed by multiple organ failure

Table 2. Antimicrobial treatment in paediatrics patients with BSI

Patient	SPRD	CTX	CRO	PTZ	FEP	MEM	ERT	COL	AK	SXT	CIP	Outcome, days
P1	11					*0 to 9*						**Dead,9**
P2	9											**Dead,2**
P3	3				**3 to 8**							**Dead,8**
P4	5		*0 to 3*			***4 to 55***						Dead, 56
P5	3				-9 to -7	***1 to 14***						Alive
P6	4		*-5 to 0*			**1 to 45**						Alive
P7	4					***1 to 15***						Alive
P8	5		*1 to 3*			***2 to 17***						Alive
P9	14		*9 to 10*	***12 to 14***		***11 to 14***						**Dead,14**
P10	3					***1 to 5***						**Dead,5**
P11	3		*1 to 3*			**3 to 28**				***-5 to 4***		Alive
P12	16		*0 to 7*			**16 to 29**						Alive
P13	2					***1 to 8***			**2 to 8**			**Dead,8**
P14	4				*-5 to 0*	***1 to 20***						Alive
P15	14				*3 to 9*	***10 to 18***						Alive
P16	3					***1 to 6***					**11 to 28**	Alive
P17	9					***1 to 14***						Alive
P18	2		*-8 to 0*			***0 to 71***		**54 to 71**				Alive
P19	3						*0 to 2*					**Dead,2**
P20	2					***0 to 2***						Dead,1
P21	4					***3 to 9***						Alive
P22	7		*-5 to 2*			***3 to 14***	***4 to 19***					Alive
P23	4					***0 to 16***						Alive
P24	3			***2 to 6***		***1 to 17***						Alive
P25	7			**13 to 15**	*0 to 6*	**7 to 15**		**14 to 15**		**13 to 15**		**Dead,15**
P26	5	*-4 to 1*				***0 to 5***						**Dead,5**
P27	4	*-22 to -5*										Alive
P28	3				***1 to 4***	**5 to 11**						Alive
P29	4		***1 to 11***									Alive
P30	6		*1 to 2*			***1 to 13***				1 to 8		Alive
P31	5			**7 to 21**	*2 to 4*	***3 to 36***						Alive
P32	5			**18 to 24**		***1 to 24***		**23 to 24**				**Dead,24**
P33	4		*0 to 4*			5 to 8						**Dead,8**
P34	10	*0 to 7*		**11 to 30**	*6 to 7*	***7 to 30***				***9 to 30***		Alive
P35	1				***0 to 2***	**1 to 10**						Alive
P36	2		*0 to 1*		**1 to 16**							Alive
P37	8					***2 to 9***						**Dead,9**
P38	17					***1 to 21***		**17 to 21**		**18 to 21**		**Dead,21**

Colour coding for antimicrobial susceptibility: red, resistant; orange, intermediate; green, susceptible; white, not determined. Numbers indicate duration of treatment in days. Treatment type: italics: empirical, bold: definitive, bold and italics: empirical and definitive. SPRD: susceptibility profile report days. The day of symptoms was considered Day 0.*CTX* cefotaxime, *CRO* ceftriaxone, *PTZ* piperacillin-tazobactam, *FEP* cefepime,*MEM* meropenem, *ERT* ertapenem, *COL* colistin, *AK* amikacin, *SXT* trimethoprim-sulfamethoxazole, *CIP* ciprofloxacin

(22%); the complications were statistically significant with mortality ($P = < 0.0001$) (Table S3.)

Moreover, the mortality rate was higher among adolescents (53.3%), those with leukaemia or lymphoma (40%), those with HAIs (86.8%), those with BSIs with an abdominal or pulmonary focus (33.3% each) and those with *E. coli* from a pathogenic phylogroup as the causative agent (86.7%), but none showed statistically significant ($P = 0.455$) (Table S2 and S3).

Discussion

In this study, 38 isolates of *E. coli* causing BSIs were characterized in paediatric patients, among which more than 85% were resistant to 3GCs and 4GCs. Among other BSI-related isolates collected from children, 33% to 57.8% exhibited resistance to these antimicrobials [44, 45]. None of the isolates in this study were resistant to carbapenems.

Resistance to 3GCs and 4GCs among *E. coli* isolates in our study was mainly due to CTX-M-type ESBLs (81.6%), which are the most widely distributed ESBLs globally [46], similar findings were reported from Nepalese children (84.6%) and Shanghai, China (97.5%) [47].

The enzyme SHV-ESBL, predominant a few decades ago but later displaced by CTX-M [48, 49], was detected in two isolates; however, it is possible that resistance to 3GCs and 4GCs can be attributed to these enzymes, as shown by Patil et al. in a collection of *E. coli* ST410 isolates, 62% of which contained the bla_{SHV} gene [50].

AmpC-type beta-lactamases were identified in three isolates (CMY in three isolates); these enzymes can hydrolyse 3GCs and 4GCs [51]. Therefore, detecting these beta-lactamases is important if antibiotics such as ceftazidime-avibactam or ceftolozane-tazobactam are available [52] so that escalation to carbapenem use can be avoided to reduce selection pressure. However, CMY-2 has been reported to coexist with CTX-M-15 and NDM-5 in children with BSIs and underlying conditions [53].

FimH is essential for the translocation of bacteria, their invasion of the urinary epithelium, and the formation of intracellular bacterial communities [54]. In this study, *fim*H was the most frequently identified virulence gene (71%); this gene was also identified in BSI collections in other countries, such as Lithuania (98.4%) and Iran (85%) [10, 55]. Unlike the *pap*C gene, which is detected only in pathogenic strains, the *fim*H gene is found in both pathogenic and commensal phylogroups. This gene is associated mainly with UTIs but is also involved in kidney damage and pyelonephritis; however, its role in bacteraemia has yet to be fully explored.

Siderophores capture ferric iron from media by competing with other chelating compounds from the host, such as transferrin or lactoferrin [56]. The *iuc*C and *iro*N genes were previously identified in ExPEC isolates from patients with neonatal meningitis, UTIs, and prostatitis and in avian pathogenic *E. coli* (APEC) [57]. In this study, siderophores were mainly found in the pathogenic phylogroups B2, D and F, as reported in Brazil, where the genes *pap*C, *sat* and *irp*2 were found in isolates of phylogroups B2 and D [58]. In studies carried out in Iran and Turkey, *iut*A was detected in 69% of the isolates; *iuc*C was detected in 79%; *iut*A was detected in 45.3%; and *iro*N was detected in 23.7% [10, 59]. In a study from Belgium, the *irp*2 gene was also identified as encoding the main siderophore [60].

In this study, the isolates that caused BSIs presented genes mainly related to iron acquisition *(irp*2 and *iut*A) and adherence (*fim*H), which are essential for successful BSI and can lead to bacteraemia. Pathogenic phylogroups often contain more virulence genes than commensal groups do.

In this work, the pathogenic phylogroups B2 and D and the commensal phylogroup A were detected in relatively high proportions, whereas groups E and B1 were not detected. A higher frequency of phylogroup A (21%) was detected in this study than that reported by Hemati et al. among Iranian patients with bacteraemia (9%) [10]. All the children had an underlying condition that led to some degree of immunosuppression, which could facilitate the development of BSIs with commensal *E. coli* strain, like what occurs in UTIs [54]. Finally, 87.5% of these isolates belonged to a pathogenic phylogroup. Information on the characteristics of commensal strains is limited [61]. However, isolates from phylogroups A and C presented resistance to antibiotics, ESBLs and virulence factors such as *fim*H and siderophores. Moreover, an isolate of phylogroup A carrying NDM-5 and *mcr*−1.1 was recently reported [62]. These findings highlight the importance of expanding surveillance of commensal *E. coli* strains in healthy individuals. Although these bacteria form part of the intestinal microbiota, they have the potential to become pathogenic and cause infections in other organ sites, resulting in invasive diseases such as UTIs, BSIs, pneumonia and meningitis.

PFGE analysis identified five cluster and 20 non-related unique patterns, revealing substantial clonal heterogeneity suggestive of multiple origins rather than a single outbreak. This clonal diversity was confirmed by MLST, which detected 14 distinct CC/ST. Notably, we observed high frequencies of HRCC CC405 (23.8%) and CC131 (21%). ST131 has been reported with a frequency ranging from 8–50.8% in similar studies, confirming its status as one of the most widespread clones worldwide [10, 63, 64]. In contrast to our study, ST405 has been observed at a lower frequency ranging from 4.1–8.8% [47, 65, 66]. Additional HRCC detected included CC10, CC23, CC648, CC354, ST69, ST127 and ST457, all of them contribute to spread ESBLs, mainly CTX-M enzymes [36]. Some of these clones such as ST131, ST167 (which belongs to CC10), ST354 and ST648 are also reported to harbour carbapenemases [67].

The distribution of the *E. coli* STs causing BSIs varies according to factors such as the genetic diversity of strains, geographic distribution, host characteristics, associated clinical conditions, and hospital type [68–70]. Other ExPEC high-risk clones, such as ST69, ST73, ST95, ST410 and ST1193, have been increasingly in recent years [71–73]. In this study, only one ST69 isolate was detected; previous reports have shown a frequency for this clone ranging from 5.6% to 20% [66, 74]. ST73 and ST95 were not detected in this collection. Notably, this 2013–2015 collection predates the first reported carbapenemase-producing Enterobacterales (CPE) at INP [75]; suggesting that these high-risk clones may have facilitated the initial acquisition and dissemination of CRE within INP.

A total of 31.6% of the patients in this study were female, a proportion similar to that described in a report from Spain, where 45% of the patients were female, and an Iranian study involving patients with leukaemia, where 35% of the BSI patients were female [76, 77].

E. coli is the first gram-negative bacterium causing hospital-acquired BSIs in high-income countries (HICs), and the second in middle- and low-income countries (MICs

and LICs) [78]. Among children under three months of age, *E. coli* is also the principal cause of BSIs [79]. However, in MICs and LICs, the predominant species causing community-acquired BSIs are *Salmonella Typhi*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* [80, 81]. In our study, 13.1% (n = 5) of BSIs were classified as CAIs.

Eighty percent of the patients had leukaemia or lymphoma as the underlying condition, which has been reported as a risk factor for BSI complications [79]. Importantly, none of the infections in this series were associated with UTIs.

In this study, most of the BSI were secondary (86.4%), catheter-associated infections were the most prevalent with 42.1% of the cases. However, no statistically significant differences were observed between primary and secondary infections (P = 0.12) or between different source of BSI (P = 0.105) in relation to the clinical outcome (death or cure), suggesting that, in our population, neither the type nor the focus of infection were determining factors for mortality. These results partially coincide with the EUROBACT-2 study conducted in adults, where secondary BSIs represented 83.7% of cases. However, in that study, catheter-associated infections were the second most common cause (26.4%) and were confirmed not to be a risk factor for mortality (OR =1, 95%CI, P= 0.027) [82]. In contrast, our analysis could not establish a definitive association between infection source and clinical outcome.

Although limited data exist on children, recent studies have reported that paediatric patients with catheter-related BSI have a four-fold increased risk of mortality (OR =4.29, 95%CI =1.28–14.36, P= 0.018) [83], highlighting the importance of considering the differences between paediatric and adult populations in BSI management.

Our study found that 66% of the patients had previous surgery (within 1 year) and 39% had surgery before BSI onset (within ≤14 days) with no statistical significance (P = 0.332 and P= 0.418, respectively). This contrasts with findings from a Chinese retrospective study which demonstrated that surgery and/or trauma (within 3 months) was associated with significantly higher mortality in children with BSI caused by *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanni*, *Pseudomonas aeruginosa*, *Enterobacter* spp. and *E. coli* (ESKAPEEc) pathogens [OR =7, P= 0.006, 95 CI (1.761–27.876)] [84]. An association between previous surgical interventions and BSI caused by Enterobacterales has also been reported. In a Turkish paediatric tertiary university hospital, previous surgery interventions (3 months) was a predisposing factor for BSI caused by carbapenem resistant Enterobacterales (CRE) [85]. Similarly, Ruvinsy et al. found that invasive surgeries or procedures were major risk factors for CRE-bacteraemia development [OR =3, 95% CI (1–7), P= 0.008] in an Argentine paediatric referral hospital [86].

Fever, however, demonstrated a notable relationship with patient outcomes. Survivors presented with significantly higher median temperatures compared to non-survivors (38.4 °C vs. 37.4 °C, P= 0.038), and moderate to high-grade fever was more frequent among those who survived (62.5% vs. 26.7%, P= 0.022). These findings suggest that a febrile response may be linked to better outcome, possibly reflecting a stronger inflammatory reaction. Similarly, previous studies have shown that fever is a key early marker for bacteremia in pediatric patients, supporting its importance in both diagnosis and prognosis [76].

RR is an important indicator in evaluating febrile children, with some studies suggesting its potential to predict serious bacterial infections. However, in our analysis, no significant differences in RR patterns (such as polypnea and tachypnea) were found between the deceased and cured groups (P > 0.05). This indicates that RR alone may not reliably predict outcomes, and other clinical factors likely play a more important role in assessing infection severity [87].

Hematological abnormalities such as anemia, leukopenia, severe neutropenia, and thrombocytopenia were common but did not show a significant association with mortality. This finding is consistent with previous observations that hematologic parameters alone may not reliably predict outcomes, particularly in children with underlying malignancies or immunosuppression, where baseline hematologic dysfunctions are frequent [88]. These results highlight the complexity of mortality risk in this population and suggest that isolated blood count alterations must be interpreted within a clinical context.

In our study, prior antimicrobial treatment was common among patients with BSIs, and no significant differences were observed between cured and death. These findings are consistent with previous reports suggesting that, although previous antibiotic exposure is frequent and may increase the risk of hospital-acquired infections, it does not independently predict mortality. Instead, factors such as the severity of the underlying condition are likely to have a greater influence on patient outcomes [89].

Empirical antimicrobial treatment was initiated in most patients, with 86.7% of deceased patients and 100% of survivors receiving empirical antibiotics. However, monotherapy was the most common initial strategy, and carbapenems were frequently used both empirically and definitively. Despite these efforts, no significant differences were observed between survivors and

non-survivors regarding empirical or definitive treatment regimens, including the use of carbapenems. Our findings are consistent with previous studies showing that while prior and current antibiotic use, including carbapenem administration, are frequent in children with *E. coli* BSIs, they are not independently associated with mortality. This suggests that disease severity and underlying conditions may have a greater influence on clinical outcomes [81].

This work provides valuable information regarding the epidemiology of resistance to 3GCs and 4GCs, and the results indicate that these antibiotics should not be used as empirical therapies for *E. coli* bacteraemia in this paediatric hospital. This situation may be similar in other hospitals, as reported by the Network for the Research and Surveillance of Drug Resistance (Red Temática de Investigación y Vigilancia de la Farmacorresistencia in Spanish; INVIFAR) [90]. Clinical guidelines suggest that a carbapenem should be used for the treatment of ESBL-producing Enterobacterales strains [91]. A total of 87% of the patients received carbapenems as empirical monotherapy or in combination with AK or CIP. Ninety-five percent of the isolates were susceptible to AK and 13% to CIP. Resistance to 3GCs and 4GCs and susceptibility to AK and carbapenems has also been reported in isolates from Chinese patients [45].

Our study found a survival rate of 52.2% among patients admitted to the ICU, suggesting that the care protocols in place may have positively influenced patient outcomes. Statistical analysis revealed no significant differences between survivors and non-survivors with respect to ICU admission rates (73.3% vs. 52.2%, $P=$ 0.168) or ICU length of stay (median 4 [2–17] days vs. 12 [6–16] days, $P=$ 0.134). These findings are consistent with those reported by previous studies, who, in a cohort of 6,487 admissions, found no association between PICU stay duration and mortality, despite observing markedly elevated standardized mortality ratios (SMRs) in patients with cardiovascular (SMR 191) and oncological/hematological (SMR 179) diagnoses. Notably, these studies also reported a median follow-up of 7.2 years and highlighted a significantly higher long-term mortality risk among patients with repeated ICU admissions, particularly those with oncological, hematological, or neurological conditions [92].

The length of hospital stay serves as indicator of the severity of BSI. In a study from the Second Western China University Hospital, patients infected with ESKAPEEc pathogens, including *E. coli,* experienced significantly longer hospital stays compared to those with non-ESKAPEEc infections, with a median of 20.5 days versus 14.0 days ($P = 0.023$). Although our findings did not reveal significant differences in ICU stay duration between death and cured (4 days [2–17] vs. 12 days [6–16], $P=$ 0.134), the tendency toward longer stays in the ICU for deaths suggests that infection severity plays a significant role in the need for prolonged critical care [84].

Mechanical ventilation was more frequently required among patients who died compared with those who survived (73.3% vs. 56.5%); however, this difference was not statistically significant ($P = 0.242$). Studies in similar settings have shown that the need for mechanical ventilation in children with sepsis nearly triples the risk of death, and that prolonged ventilation lasting 96 h or more tends to worsen clinical outcomes. While our findings did not reveal a significant association, the proportion of ventilated patients among those who died suggests that respiratory support remains an important indicator of illness severity in paediatric BSIs. [93, 94]. In our study, 60.5% of patients had complications, the most frequent being septic shock (52%). In Chinese children, this complication was also the most common and was associated with mortality ($P < 0.001$) [95]. On the other hand, 22% for our patients had multiple organ failure, in a South African study, this was reported in only 1.3% [96].

E. coli is the most common cause of BSIs, and a significant cause of morbidity and mortality in hospitalized children [84]. When compared to a study from a tertiary pediatric hospital in China, where a 28-day mortality rate of 36.9% was reported among PICU patients with BSI [95], our data demonstrated a higher survival rate. Differences in patient profiles, infection severity, and critical care resources likely contribute to these variations across centers.

The overall estimate for case-fatality rate among 8 studies was 12.7% [6.6–20.2%]. Underlying conditions, such as malnutrition or HIV infection were assessed as a factor associated with bacteraemia in 4 studies each [77].

Sepsis is the second leading cause of death in paediatric patients, with a frequency of 7%. In MICs and LICs, the mortality rate from community-acquired BSIs is estimated to be 12.7% (6.6–20.2%) [79], whereas the rate of mortality attributable to infection is estimated to be 13.6% [97]. In our study, 34.5% of the deaths were considered related to infection. It is estimated that 3.02 million deaths from sepsis occurred in children under 14 years of age and that the pathogens that caused the deaths attributable to AMR were *S. aureus*, *Acinetobacter baumannii* and *E. coli* [3].

Adolescents were the predominant group in this study ($n = 14$, 36.8%), 57.1% of whom died. Deaths occurred between days 1 and 56 after the onset of symptoms. Only one case was considered a CAI.

This single-centre study does not reflect the epidemiology of all *E. coli* isolates causing BSIs in the paediatric

population in Mexico; however, it provides important insights into the commensal phylogroups, and pathogenic strains associated with BSIs.

Conclusion

E. coli is a major cause of BSIs in paediatric patients in Mexico. High resistance to 3GCs and 4GCs was predominantly mediated by CTX-M-type ESBLs, leading to increased reliance on carbapenems. High-risk clones such as CC131, CC405, and CC10 were frequently identified, demonstrating significant genetic diversity among the isolates. Virulence factors associated with adhesion and iron acquisition were common across both pathogenic and commensal phylogroups. Clinically, the presence of fever at admission was linked to better outcomes, while complications were associated with increased disease severity.

Abbreviations

AK	Amikacin
AMR	Antimicrobial resistance
APEC	Avian pathogenic *E. coli*
AZT	Aztreonam
BSI/BSIs	Bloodstream infection/s
CAI/CAIs	Community-acquired infection/s
CAZ	Ceftazidime
CC	Clonal complex
CFZ	Cefazolin
CIP	Ciprofloxacin
cnf 1	Cytotoxic necrotizing factor 1
CLSI	Clinical Laboratory Standards Institute
CPE	Carbapenemase-producing Enterobacterales
CRE	Carbapenem resistant Enterobacterales
CRO	Ceftriaxone
CTX-M	Cefotaximase-Munich beta-lactamase
CVC	Central venous catheter
CXM	Cefuroxime
ESBL	Extended-spectrum beta-lactamase
ESKAPEEc	*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp. and *E. coli*
ExPEC	Extraintestinal pathogenic *E. coli*
FEP	Cefepime
GE	Gentamicin
GES	Guiana extended-spectrum beta-lactamase
HAI	Hospital-acquired infection
HICs	High-income countries
HRC	High-risk clones
HRCC	High-risk clonal complexes
*hly*A	α-haemolysin
ICU	Intensive care unit
*fim*H	Type 1 fimbriae
IPM	Imipenem
INP	National Institute of Pediatrics
INVIFAR	Network for the Research and Surveillance of Drug Resistance
*iro*N	Salmochelin siderophore receptor
*iut*A	Aerobactin siderophore receptor
*irp*2	Yersiniabactin siderophore
*iuc*C	Aerobactin
LEV	Levofloxacin
LICs	Low-income countries
MEM	Meropenem
MICs	Middle- income countries
MLST	Multilocus sequence typing
NCBI	National Center for Biotechnology Information
NR	Non-related
OXA	Oxacillinase
pet	Plasmid-encoded toxin
PFGE	Pulsed-field gel electrophoresis
pic	Proteins involved in intestinal colonization
PTZ	Piperacillin–tazobactam
RR	Respiratory rate
SAM	Ampicillin-sulbactam
sat	Secreted autotransporter toxin
sfa	S fimbriae adhesins
SHV	Sulfhydryl variable beta-lactamase
SMR	Standardized mortality ratios
SPRD	Susceptibility profile report day
SSTI	Skin and soft tissue infections
ST	Sequence type
SXT	Trimethoprim-sulfamethoxazole
TEM	Temoneira beta-lactamase
TOB	Tobramycin
vat	Vacuolating autotransporter toxin
HIV	Human immunodeficiency virus
UTI/UTIs	Urinary tract infection/s
3GCs	Third-generation cephalosporins
4GCs	Fourth-generation cephalosporins

Supplementary Information

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Supplementary Material 1. Table S1: Primers used in this Study.

Supplementary Material 2. Table S2: Clinical features of patients with BSI by E. coli.

Supplementary Material 3. Table S3: Statistical Analysis of Clinical Variables Between deaths and cured.

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Clinical trial

Not applicable.

Authors' contributions

AAA designed the study, JMV, LBM, ACR and AAA analysed and interpreted the data; LBM, JMV, JAMA, RBH, EEJM and AAA conducted the experiments; AAA, IMV and ACR collected the clinical information; AAA, JAMA and IMV created the database and performed the statistical analysis; RBH: bioinformatic analysis, AAA, LBM and JMV wrote the first version of the manuscript. All the authors reviewed and approved the final version of the manuscript.

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Data availability

The nucleotide sequences generated in this study have been submitted to the GenBank database and are openly accessible at https://www.ncbi.nlm.nih.gov/genbank/. Accession numbers for ESBL-related genes range from PV494929 to PV494961. Multilocus sequence typing gene sequences were deposited under the following ranges PV564256–PV564431 and PV575742-PV575766. All data supporting the findings of this study are available in the main text and listed as Supplementary Material.

Declarations

Ethics approval and consent to participate

This study was approved by the research, ethics, and biosafety committees of Instituto Nacional de Pediatria (IRB: 00008064 and IRB: 00008065) under

registration INP 2013/066. The ethics committee of Instituto Nacional de Pediatria waived informed consent because the samples obtained were part of the standard care for hospitalized patients, and the isolates were obtained retrospectively. The patient data were deidentified. This study adhered to the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
The authors have no conflicts of interest to declare.

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