

Original Article

IncFIB plasmids carrying the resistance gene *bla*_{CTX-M-15} in ESBL-producing *Escherichia coli* clones from pediatric patients

Rosa del Carmen Rocha-Gracia¹, Patricia Lozano-Zarain¹, Zita Gutiérrez Cázarez², Carla Andrea Alonso³, Eduardo Brambila⁴, Carmen Torres³, Gerardo Cortés-Cortés¹

¹ Benemérita Universidad Autónoma de Puebla, Posgrado en Microbiología, Centro de Investigaciones en Ciencias Microbiológicas, Instituto de Ciencias, Ciudad Universitaria, Puebla, Mexico

² Hospital para el Niño Poblano, Laboratorio de Microbiología, Puebla, Mexico

³ Universidad de La Rioja, Área Bioquímica y Biología Molecular, Logroño, Spain

⁴ Benemérita Universidad Autónoma de Puebla, Facultad de Ciencias Químicas, Puebla, Mexico

Abstract

Introduction: The emergence of extended-spectrum β -lactamases (ESBLs)-producing *Escherichia coli* clones are a public health concern worldwide. Scarce information does exist about the spread of ESBLs-producing *E. coli* in pediatric patients from developing countries.

Methodology: *E. coli* strains were analyzed by multilocus-sequence-typing, pulsed-field-gel-electrophoresis and phylogenetic group. The antimicrobial-resistance genes were detected by PCR, and plasmid content by the PCR-based replicon-typing. Horizontal transfer was tested by conjugation and the location of the *bla*_{CTX-M-15} gene by Southern blot hybridization.

Results: Thirty-two cefotaxime-resistant *E. coli* were recovered. Eleven of them were ESBL-producing isolates, which were well characterized and ascribed to seven sequence types and five phylogroups. The ESBL CTX-M-15 was the most prevalent enzyme (9 of 11). Plasmids of variable sizes (40-220 kb) were visualized, and the incompatibility (Inc) group FIB plasmid-replicon was detected in the ESBL strains and transferred by conjugation in 45.45% of them. Plasmid-borne toxin-antitoxin systems were the most frequently detected systems, strongly associated to IncF plasmids. The CTX-M-15-encoding gene was located on IncFIB plasmids.

Conclusions: Even though a small number of ESBL-producing strains was recovered, we evidenced that IncFIB plasmids carry the *bla*_{CTX-M-15} gene, highlighting the role of IncF-type plasmids in facilitating the spread and maintenance of ESBL-encoding genes, which further favors the rapid increase of the antimicrobial resistance dissemination in disease-causing *E. coli* strains in pediatric patients.

Key words: Anti-bacterial agents; child; drug resistance; *Escherichia coli*; plasmids.

J Infect Dev Ctries 2022; 16(3):500-506. doi:10.3855/jidc.15080

(Received 23 March 2021 – Accepted 21 January 2022)

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Introduction

Many pediatric infections are caused by extended-spectrum β -lactamases (ESBLs)-producing *Enterobacteriaceae* [1,2]. These organisms are resistant to broad-spectrum β -lactam antibiotics, including penicillins, third and fourth generation cephalosporins, and monobactams. β -lactams are the most used antibiotics in pediatrics because of their bactericidal action, low toxicity and diverse spectrum [3]. Epidemiologically, CTX-M-15 is the most prevalent ESBL in *Escherichia coli* clones worldwide in both community and hospital environments [4], which generally confers resistance against third and fourth generation cephalosporins and aztreonam, but do not confer resistance against carbapenems or cephamycins. There are reports indicating differences in the prevalence of ESBL types in *E. coli* strains isolated from adult and pediatric patients in some geographical

regions, which could indicate the participation of mobile genetic elements such as plasmids, mobilizing different ESBLs [5]. Bacterial plasmids are dispensable extra-chromosomal DNA with autonomous replication ability, and they can be transferred horizontally from one cell to another cell through by the mechanism of conjugation. CTX-M-15 is usually associated with IncF-type plasmids, a predominant group of large conjugative plasmids that carry and mobilize multiple resistance and virulence determinants [6]. IncF plasmids use postsegregational killing and addiction systems to ensure their propagation and maintenance among high-risk bacterial populations [7]. IncF plasmids encoding CTX-M-15 are strongly associated with the emerging worldwide *E. coli* sequence types as ST131 [6]. ST131-O25b:H4 lineage is being rapidly spread as an emerging problem in community-acquired and hospital infections.

Scarce information does exist about the spread of ESBLs-producing *E. coli* clones in pediatric patients and the horizontal transfer of resistance determinants. In this study, we determined the molecular typing, plasmid characterization and horizontal transfer of antimicrobial resistance determinants in ESBL-producing *E. coli* isolated from a pediatric hospital in Mexico.

Methodology

Ethical approval

Approval of this study was obtained from the institutional review board of the pediatric “Hospital para el Niño Poblano” (HNP) (HNP/ENS/177/2016). This study does not involve human subjects directly, samples were obtained by routine procedures and did not affect the patients. Informed consent of patients was not required. Verbal informed consent was granted by parents of patients and sampling was made as part of diagnostic tests with the aid of nurses or doctors in the health facilities of the HNP.

Demographic data of the hospital

The HNP is a pediatric intensive and critical care hospital located southwest of Puebla City in Mexico; there are 160 beds in total, 10 of them are situated in the intensive care unit. The average number of hospital admissions is 3,456 patients per year, and from 250 to 300 of them are hospitalized at the intensive care unit every year.

Recovery of *E. coli* strains

Cefotaxime-resistant (CTX^R) *E. coli* isolates were obtained from pediatric patients with a critical illness in the Hospital HNP, Puebla City, Mexico, over a two-year period (April 2009 to January 2011). Those strains were recovered from biological samples (fluids and organs) of patients at internal medicine unit, intensive care unit, pathology and surgery services. For convenience, those eleven characterized strains were chosen to take into account: a) the severity and outcomes of patients hospitalized with infectious diseases (in one of them from post-mortem examination), and b) previous phenotypic resistance to cefotaxime as a filter to obtain potential ESBL-positive strains. Isolates were identified by the automated equipment Vitek® 2 (Vitek 2 Compact, bioMérieux; 100 Rue Louis Pasteur, 69280 Marcy-l'Étoile, France) and by PCR using the species-specific *uidA* gene detection. *E. coli* isolates obtained from the same patient were included in this study only if they were isolated from different specimens or in different periods of time. Data related to the age and gender of patients were recorded (Table 1).

Phylogenetic and clonal typing

Phylogenetic groups were assigned according to the Clermont phylotyping method [8], and sequence types (STs) by the multilocus-sequence-typing technique [9].

Pulsotypes were also assigned to ST-selected *E. coli* strains by pulsed-field-gel-electrophoresis (PFGE).

Table 1. Origin, molecular typing, β-lactamases and plasmid typing of ESBL-producing *E. coli* isolated from pediatric patients.

Strain	Clinical Origin	Patient Gender ^a /Age ^b	Phylogenetic group	ST/ST Complex	Detected β-lactamases	Plasmid characterization		
						Replicon typing	Number: size (kb)	Addiction systems
C4428	Spleen (post-mortem)	F/2	A	44/10	CTX-M-15, OXA-1	FIA, FIB ^c	2: 40, 140 ^e	VagCD, CcdAB, PemKI, SrnBC
C4429	left lung (post-mortem)							FIB ^c
C4433 ^c	Intra-abdominal abscess	M/15	A	167/10	CTX-M-15 ^{c,e} , OXA-1 ^c	FIB ^{c,e} , N	2: 140, 175 ^{c,e}	Hok-sok ^c , VagCD ^c , RelBE, CcdAB, PemKI ^c , SrnBC ^c
C4436 ^c	Peritoneal fluid	M/2	B1	58/155	CTX-M-15 ^{c,e} , OXA-1 ^c , TEM-1b	FIB ^{c,e} , I1	2: 100, 160 ^{c,e}	PndAC, RelBE, CcdAB, SrnBC
C4435	Peritoneal fluid	F/0.1	B2	131/131 ^d	CTX-M-15 ^c , OXA-1	FIB ^c	1: 140 ^c	Hok-sok, PndAC, VagCD, RelBE, CcdAB, PemKI
C4432	Vaginal discharge	F/8	C	90/23	CTX-M-15 ^c , OXA-1	I1, FIA, FIB ^c , N	2: 140, 150 ^c	PndAC, VagCD, RelBE, CcdAB, PemKI, SrnBC
C4425			D		CTX-M-15 ^c , OXA-1	FIA, FIB ^c	1: 135 ^c	VagCD, RelBE, CcdAB, PemKI, SrnBC
C4426 ^c	Peritoneal dialysis	F/17.1	D	69/69	CTX-M-15 ^c , OXA-1 ^c	FIA ^c , FIB ^c	1: 130 ^{c,e}	VagCD ^c , RelBE, CcdAB ^c , PemKI ^c , SrnBC ^c
C4427 ^c			D		CTX-M-15 ^{c,e} , OXA-1	FIB ^{c,e}	1: 135 ^{c,e}	VagCD, RelBE, CcdAB ^c , PemKI ^c , SrnBC ^c
C4430	Peritoneal dialysis	M/2.8	A	656/10	OXA-1, TEM-1b, SHV-2a	FIB	8: 55, 140, 165, 180, 195, 205, 215, 220	Hok-sok, VagCD, RelBE, CcdAB
C4431 ^c					OXA-1, TEM-1b ^c , SHV-2a ^c	FIA, FIB ^{c,e}	8: 55, 140 ^{c,e} , 165, 180, 195, 205, 215, 220	Hok-sok, VagCD ^c , RelBE, CcdAB ^c

^a: F, female; M: male; ^b: Age in years; ^c: Conjugative strains, β-lactamases and plasmid determinants transferred by conjugation to the recipient strain *E. coli* J53^{RifR}; ^d: Somatic (O) and flagellar (H) antigens as well as the *fimH* allele variant were only determined for the ST131 isolate. Serotype/*fimH* allele: O25b:H4/35; ^e: *bla*_{CTX-M-15} gene and associated plasmids detected by Southern blot hybridization.

ST131 strains were serotyped by the O and H antigens determination, using reference sera [9].

Susceptibility testing and ESBL production

Antimicrobial susceptibility testing was carried out in the *E. coli* isolates by the disk diffusion method for 21 antimicrobial agents: ampicillin (AM), amoxicillin-clavulanic acid (AMC), cefazolin (CZ), cefoxitin (FOX), cefotaxime (CTX), ceftriaxone (CRO), ceftazidime (CAZ), cefepime (FEP), aztreonam (ATM), imipenem (IMP), meropenem (MEM), gentamicin (GM), tobramycin (NN), amikacin (AN), kanamycin (K), streptomycin (S), tetracycline (TE), ciprofloxacin (CIP), nalidixic acid (NA), sulfamethoxazole-trimethoprim (SXT), and chloramphenicol (C). Susceptibility was interpreted according to the Clinical Laboratory Standards Institute [10]. Phenotypic ESBL production was screened by the double-disk synergy test with CTX, CAZ, FEP, and ATM discs in combination with AMC. *E. coli* ATCC 25922 was used as control strain. Additionally, all strains were tested to verify the presence of ESBL-encoding genes by PCR.

Antimicrobial resistance genes detection

Genes encoding β-lactam, quinolone, aminoglycoside, tetracycline, sulphonamide and chloramphenicol resistance were screened by PCR and sequencing, as well as mutations in the chromosomal

ampC, *gyrA* and *parC* genes. Gene cassette arrays of class 1 and 2 integrons were analyzed by primer-walking.

Plasmid characterization

Plasmids were visualized by DNA digestion with S1 nuclease and PFGE analysis [9]. Subsequently, plasmids were classified according to their incompatibility group by the PCR-based replicon-typing method [11]. Toxin/antitoxin plasmid addiction systems were also tested by PCR [12].

Conjugation and hybridization assays

Transference of resistance genes and plasmids was analyzed by conjugation using the recipient strain rifampicin-resistant *E. coli* J53 (J53^{Rif-R}). Transconjugants were tested for resistance to antimicrobial agents, phenotypic ESBL-production, detection of antimicrobial resistance genes, integrons, and plasmid characterization.

Southern blot hybridization experiments were performed using radioactive labeling (³²P) to investigate the bla_{CTX-M-15} gene location in donors and transconjugants.

Results

***E. coli* isolates and antimicrobial susceptibility**

Thirty-two *E. coli* CTX^R were recovered from 26 patients. Eleven CTX^R strains from seven patients

Table 2. Acquired and chromosomal antimicrobial resistance mechanisms among the ESBL-producing *E. coli*.

Strain	Resistance to non-β-lactam agents ^a	Resistance genes for non-β-lactam agents	Class 1 integron		Class 2 integron	Mutations in chromosomal <i>ampC</i> gene	Amino acid changes ^b	
			3' conserved region	Genetic array	Genetic array		GyrA	ParC
C4428	NA, CIP, NN, C, TE, SXT	<i>aac(6)-Ib-cr</i> , <i>aac(3)-II</i> , <i>tet(B)</i>	<i>qacEΔ1</i> + <i>sul1</i>	<i>dfrA17-aadA5</i>	- ^d	Wild	S83L, D87N	S80I
C4429	NA, CIP, AN, GM, S, NN, TE, SXT	<i>aac(6)-Ib-cr</i> , <i>aac(3)-II</i> , <i>tet(B)</i>	<i>qacEΔ1</i> + <i>sul1</i>	<i>dfrA17-aadA5</i>	-	Wild	S83L, D87N	S80I
C4433 ^c	NA, CIP, GM, S, NN, C, TE, SXT	<i>aac(6)-Ib-cr^c</i> , <i>aac(3)-IIF</i> , <i>tet(A)^c</i> , <i>tet(B)</i>	<i>qacEΔ1</i> + <i>sul1</i>	<i>dfrA17-aadA5</i>	-	Wild	S83L, D87N	S80I
C4436 ^c	NA, CIP, AN, GM, S, NN, TE	<i>aac(6)-Ib-cr^c</i> , <i>aac(3)-IIF</i>	<i>qacEΔ1</i> + <i>sul1</i>	<i>dfrA17-aadA5</i>	<i>dfrA1-sat2-aadA1</i>	-18, -1, +58	S83L, D87N	S80I, E84V
C4435	AN, GM, NN, TE	<i>tet(A)</i>	<i>qacEΔ1</i> + <i>sul1</i>	<i>dfrA17-aadA5</i>	<i>dfrA1-sat2-aadA1</i>	Wild	Wild	Wild
C4432	NA, CIP, AN, GM, NN, C, TE, SXT	<i>aac(6)-Ib-cr</i> , <i>tet(A)</i> , <i>tet(B)</i>	<i>qacEΔ1</i> + <i>sul1</i>	<i>dfrA17-aadA5</i>	-	-18, -1, +58	Wild	Wild
C4425	NA, CIP, AN, GM, NN, TE	<i>aac(6)-Ib-cr</i> , <i>aac(3)-II</i> , <i>tet(A)</i>	<i>qacEΔ1</i> + <i>sul1</i>	<i>dfrA17-aadA5</i>	-	Wild	S83L	Wild
C4426 ^c	NA, CIP, AN, GM, NN, TE	<i>aac(6)-Ib-cr^c</i> , <i>aac(3)-IIF</i> , <i>tet(A)^c</i>	<i>qacEΔ1</i> + <i>sul1</i>	<i>dfrA17-aadA5</i>	-	Wild	S83L	D111N
C4427 ^c	NA, CIP, AN, GM, NN, TE	<i>aac(6)-Ib-cr^d</i> , <i>aac(3)-IIF</i> , <i>tet(A)^c</i>	<i>qacEΔ1</i> + <i>sul1</i>	<i>dfrA17-aadA5</i>	-	Wild	S83L	D111N
C4430	NA, CIP, AN, S, NN, TE, SXT	<i>aac(3)-II</i> , <i>tet(B)</i>	<i>qacEΔ1</i> + <i>sul1</i>	<i>dfrA17-aadA5</i>	-	Wild	S83L, D87N	Wild
C4431 ^c	NA, CIP, S, TE, SXT	<i>tet(B)^c</i>	<i>qacEΔ1</i> + <i>sul1</i>	<i>dfrA17-aadA5</i>	-	Wild	S83L, D87N	Wild

^a: GM gentamicin; NN: tobramycin; AN: amikacin; S: streptomycin; NA: nalidixic acid; CIP: ciprofloxacin; C: chloramphenicol; SXT: trimethoprim/sulfamethoxazole; TE: tetracycline. The breakpoints for the antimicrobial agents were compared with CLSI, 2018. ^b: Amino acid substitutions; S: serine; L: leucine; D: aspartic acid; N: asparagine; I: isoleucine; V: valine; E: glutamic acid. ^c: Conjugative strains and resistance genes transferred by conjugation to the recipient strain *E. coli* J53^{Rif-R}; ^d: Negative.

showed phenotypic ESBL production, and some of them were isolated from the same patient but in different specimens or periods of time (Table 1). The 11 ESBL-producing strains were fully characterized. All strains were multidrug-resistant and showed resistance to β -lactam, quinolone, aminoglycoside, tetracycline, or sulphonamide agents (Table 2).

Phylogenetic and clonal typing

ESBL-producing *E. coli* strains were ascribed to phylogroups A (n = 5), B1 (n = 1), B2 (n = 1), C (n = 1), and D (n = 3), and they were also assigned to seven STs: ST44, ST58, ST69, ST90, ST131, ST167 and ST656 (Table 1, Figure 1). Isolates (one of each ST) were classified by PFGE in 7 different pulsotypes by the cluster analysis of Dice similarity index (value > 85%). Finally, a dendrogram was generated with the clustering Unweighted Pair Group Method using Arithmetic averages (UPGMA) and the NTSYSpc 2.21q software (Figure 1).

Strain C4435 of phylogroup B2 was assigned to the ST131 clonal group, identifying the serogroup O25:H4 and the *fimH35* allele variant (Table 1).

Antimicrobial resistance genes and interns

The *bla*_{CTX-M-15} gene was identified in 9 out of the 11 ESBL positive strains (associated with *bla*_{OXA-1} in all of them); the *bla*_{SHV-2a} gene was found in the remaining two ESBL *E. coli* strains (in addition to *bla*_{OXA-1} and *bla*_{TEM-1b}) (Table 1).

Table 2 shows the resistance genes for non- β -lactam agents identified among the ESBL-producing *E. coli* strains. Additionally, mutations in the *ampC*-regulatory region, GyrA and ParC proteins were also identified. Classic class 1 integrons were detected in all strains carrying the *dfrA17-aadA5* cassette array. Two strains also harbored a class 2 integrons with the *dfrA1-sat2-aadA1* array.

Plasmid analysis and gene transfer

Plasmids of variable sizes were visualized in all strains with a wide range of sizes (40-220 kb) (Table 1). IncFIA and IncFIB were the most prevalent replicon types, highlighting the presence of IncFIB in all ESBL strains. At least four plasmid-borne toxin-antitoxin addiction systems were detected, being the combination *vagCD*, *ccdAB*, *pemKI* and *srnBC* present in all the cases (Table 1).

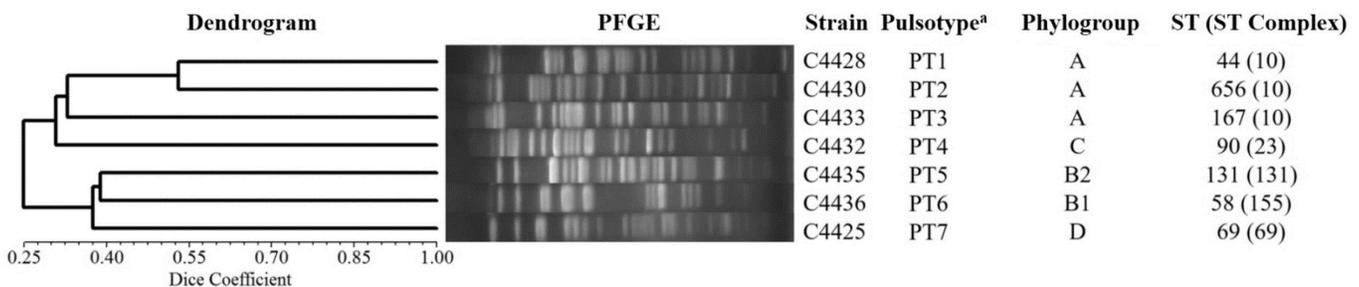
Five out of the 11 strains were conjugative. *bla*_{CTX-M-15}, *aac(6)-Ib-cr*, *aac(3)-II*, *tet(A)* or *tet(B)* resistance genes were transferred to the recipient J53^{Rif^R}, as well as IncFIA or IncFIB plasmids and different plasmid addiction systems (Tables 1 and 2).

The *bla*_{CTX-M-15} gene was located by hybridization on FIB plasmids in 8 of 9 CTX-M-15-positive strains. We observed that plasmids FIB mobilized the *bla*_{CTX-M-15} gene in 3 conjugative strains (Table 1). *bla*_{SHV-2} and *bla*_{TEM-1b} genes were co-transferred by an FIB plasmid in one more transconjugant (Table 1).

Discussion

A high incidence of ESBLs was detected in this study, being CTX-M-15, the most prevalent in combination with oxacillinase OXA-1, which are involved in resistance to cephalosporines from first to fourth generation and penicillin-type β -lactams, respectively. These findings are consistent with reports around the world showing that CTX-M enzymes are largely the most prevalent ESBLs in children, with a predominance of CTX-M-15 [13,14], also with previous reports in Mexico, highlighting the presence of CTX-M-15 (CTX-M group 1) in *E. coli* from adults and child [15-17]. A combination of ESBLs TEM-1 and SHV2a was also detected in this study, which confers resistance against penicillins and ceftazidime, respectively. These findings are important due to β -lactam agents are recommended as first-line therapy by the World Health Organization [18], and they are widely prescribed treating infections caused by *E. coli* strains in children, limiting their therapeutic options.

Figure 1. UPGMA dendrogram, PFGE patterns and molecular typing from seven selected ESBL-producing *E. coli*. a Classification based on the cluster analysis of Dice similarity index (value > 85%).



Even selective pressure is a clear phenomenon in the clinical setting, some emerging ESBL-producing *E. coli* clones, such as ST131, have contributed to the spread of global multidrug resistance [19]. *E. coli* ST131-B2 clone usually belongs to the serotype O25b:H4 with *fimH* 30 variant (ciprofloxacin-resistant) and it is reported to harbor IncF plasmids [7]. The CTX-M-15-producing *E. coli* ST131-B2-O25b:H4 clone identified in this study, contained the *fimH* 35 allele, a specific lineage that is usually not associated with ciprofloxacin resistance, which is consistent with other reports due to the infrequent fluoroquinolone use in pediatric patients [20,21]. However, this strain showed the presence of class 1 and 2 integrons and harbored an FIB plasmid of 140 kb carrying the *bla*_{CTX-M-15} gene. Clone CTX-M-15-producing *E. coli* ST131-B2-O25:H4 is associated with gene cassettes involved in resistance to other antibiotic families, such as aminoglycosides and quinolones [6]. In some complicated pediatric infections, aminoglycoside agents are a convenient election [22,23]; contrary, quinolones are not the first-line systemic therapy in children due to their serious adverse effects; however, some fluoroquinolones, like ciprofloxacin, may be used in children as alternative agents in life-threatening and difficult-to-treat infections [24,25]. It is relevant that even quinolones are not commonly used in the pediatric population, CTX-M-15-producing *E. coli* are likely to be more resistant to ciprofloxacin due to co-transfer of resistance determinants carried in the same plasmid, what could explain the phenotype of multidrug resistance in these pediatric strains. Our results are similar to some reports in which high percentages of pediatric *E. coli* isolates were fluoroquinolone resistant and/or ESBL producers [26], due to our strains showed several mechanisms of resistance to quinolones (Table 2). According to medical data, these pediatric patients were not treated with fluoroquinolones (data not shown), then a selective pressure with quinolone-treatment is discarded; however, a previous exposition to fluoroquinolones in the community setting could be a possibility, since there are reports demonstrating prevalent colonization of healthy, community-based infants with ESBL and carbapenemase-producing Gram-negative bacteria [27]. Co-transfer of resistance determinants might be implicated in some of these cases since the *aac(6)-Ib-cr* gene was transferred with *bla*_{CTX-M-15} by IncFIB plasmids in four strains that contained multiple plasmid addiction systems, which could be responsible for resistance gene maintenance. Moreover, class 1 integrons were identified in all the studied strains, reflecting a rapid gene cassette mobilization and

the consequent high level of adaptation to other antimicrobial agents commonly used in the clinical settings.

By hybridization assays, we observed that the *bla*_{CTX-M-15} gene was associated with FIB plasmids in 8 of 9 strains, evidencing a strong association between this ESBL-encoding gene and the IncF plasmid family as previously documented [7]. Even CTX-M-15 was the most prevalent ESBL in this study, there are some reports indicating that ESBLs CTX-M-27 or CTX-M-14 (CTX-M group 9) are mostly identified among clinical isolates from children internationally [5,28] and in Mexico [4].

Additionally, a heterogeneous clonality with seven STs was detected in this study. Even some *E. coli* strains recovered from the same patient shared the same ST as well as resistance genes content and plasmid addition systems, we observed small differences in replicons and sizes, suggesting that plasmid determinants could be lost or gained along the time in the clinical environment (Table 1).

We also observed differences in the horizontal transfer of genes in strains from the same patient, reflecting that each *E. coli* clone may be influenced by its genetic cargo or plasmid-host adaptation. More experiments are necessary to assess whether the conjugation mechanism is modulated by environmental conditions or the source of strains.

Finally, this study reveals the IncFIB plasmids as an efficient genetic platform to spread CTX-M-15, the most abundant enzyme in ESBL-producing *E. coli* causing human infections worldwide; underlining the need to monitor the antimicrobial resistance in pediatric patients from Mexico, where epidemiological studies are essential to control the spread of ESBL-producing bacteria and to promote countrywide surveillance programs. This report also contributes to highlighting this emerging problem in developing countries of Latin America, characterized by poor hospital-level regulation and a lack of economic incentives for new antibiotics research and a continuous surveillance of multidrug-resistant organisms. Likewise, more studies including longitudinal microbial sampling are still necessary to monitor the spread of hospital and community-associated multidrug-resistant *E. coli* strains in Mexico.

Conclusions

In conclusion, even though a small number of strains were recovered, we evidenced that IncFIB plasmids carried the *bla*_{CTX-M-15} gene in *E. coli* clones from children, highlighting the role of this IncF-type

plasmids in the spread and maintenance of ESBL-encoding genes, which is contributing to increasing multidrug-resistance rates in the hospital settings, generating treatments failure with β -lactam antibiotics in pediatric patients, and subsequent clinic complications. Additionally, we highlight that ESBL-associated *E. coli* infections are affecting the quality and efficiency of medical services, especially in pediatrics from developing countries. It is also essential to share these results with medical personnel, pediatrician, or family physician, to give a timely and targeted diagnosis and treatment, which could improve strategies for ESBL-producing *Enterobacteriaceae* control in the hospital settings.

Acknowledgements

The authors would thank Dr. Miguel Castañeda and Dr. Liliana López Pliego for their support in hybridization experiments.

Funding

This work was supported by the Vicerrectoría de Investigación y Estudios de Posgrado from the Benemérita Universidad Autónoma de Puebla [100182644-VIEP2019]; and by the Consejo Nacional de Ciencia y Tecnología [CB-2017-2018/A1-S-22136]. The Consejo Nacional de Ciencia y Tecnología also provided a fellowship [233611 to GCC]; and the Ministerio de Economía y Competitividad [BES-2013-063105 to CAA].

Authors' Contributions

LZ, RCRG and CT designed the study and analyzed the data; ZGC provided the strains; CAA and GCC acquired and interpreted the data; EB and GCC performed the hybridization experiments; GCC and RCRG wrote the manuscript. All authors read and approved the final manuscript.

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Corresponding author

Gerardo Cortés-Cortés, PhD
Centro de Investigaciones en Ciencias Microbiológicas, Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla, Ciudad Universitaria, Puebla 72570, Mexico.
Tel: + 52 221-268-0828
Fax number: (22) 229-55-00
Email: dorargercor@gmail.com

Conflict of interests: No conflict of interests is declared.