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Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* as a Cause of Pediatric Infections: Report of a Neonatal Intensive Care Unit Outbreak Due to a CTX-M-14-Producing Strain

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Little information is available about pediatric infections caused by extended-spectrum-β-lactamase (ESBL)-producing *Escherichia coli*. We characterized an outbreak caused by a CTX-M-14-producing *E. coli* isolate in a neonatal intensive care unit (NICU) and studied other infections caused by ESBL-producing *E. coli* in non-NICU pediatric units. All children ≤4 years old who were infected or colonized by ESBL-producing *E. coli* isolates between January 2009 and September 2010 were included. Molecular epidemiology was studied by phylogroup analysis, pulsed-field gel electrophoresis (PFGE), and multilocus sequence typing. Antibiotic resistance genes were analyzed by PCR and sequencing. Plasmids were studied by PFGE with S1 nuclease digestion and by incompatibility group analysis using a PCR-based replicon-typing scheme. Of the ESBL-producing *E. coli* isolates colonizing or infecting the 30 newborns, identical PFGE results were observed for 21 (70%) isolates, which were classified as CTX-M-14-producing *E. coli* of ST23 phylogroup A. $bla_{\text{CTX-M-14a}}$ was linked to IS*Ecp1* and was carried on an ~80-bp IncK plasmid. A smaller ongoing outbreak due to SHV-12-producing ST131 *E. coli* was also identified in the same NICU. Fifteen additional infections with ESBL-producing *E. coli* were identified in non-NICU pediatric units, but none was caused by the CTX-M-14-producing *E. coli* epidemic clone. Overall, CTX-M-14 (71.1%), CTX-M-15 (13.3%), and SHV-12 (13.3%) were the most important ESBLs causing pediatric infections in this study. Infections of newborns with CTX-M-14-producing *E. coli* were caused by both clonal and nonclonal isolates.

Most countries have experienced rapid dissemination of extended-spectrum-β-lactamase (ESBL)-producing *Enterobacteriaceae* isolates, particularly *Escherichia coli* and *Klebsiella pneumoniae* isolates. This increase is due mainly to the dissemination of the CTX-M type, particularly CTX-M-14 and CTX-M-15 ESBLs (12).

The spread of ESBLs is frequently due to the dissemination of mobile genetic elements among a genetically diverse *E. coli* population. Although a community outbreak of clonally related CTX-M-14-producing *E. coli* in Canada has been reported (13), the dissemination of CTX-M-14 in *E. coli* is usually nonclonal (11).

In neonatal intensive care units (NICUs), few cases of clonal spread of ESBL-producing *E. coli* have been reported (8, 16), and thus far, significant nosocomial outbreaks among newborns due to the clonal dissemination of CTX-M-14-producing *E. coli* have not been described.

The aims of this study were (i) to characterize an outbreak caused by a CTX-M-14-producing *E. coli* strain in an NICU and (ii) to study other infections due to ESBL-producing *E. coli* in pediatric units other than the NICU.

MATERIALS AND METHODS

Study design and bacterial isolates. During 2010, an unexpected increase in the frequency of isolation of ESBL-producing *E. coli* was observed in the NICU of the University Hospital Gregorio Marañón (UHGM), Madrid, Spain. This observation prompted the present investigation. All children admitted to this NICU, either infected or colonized by ESBL-producing *E. coli* isolates between January 2009 and September 2010, were included in the study. Additionally, all cases of infection due to ESBL-producing *E. coli* occurring in children ≤4 years old who were admitted to other, non-neonatal pediatric units during the study period were also studied.

Antibiotic susceptibility testing and ESBL detection were performed by using the MicroScan microdilution (Siemens Healthcare Diagnostics, Deerfield, IL) and Etest (AB Biodisk, Solna, Sweden) methods according to the Clinical and Laboratory Standards Institute guidelines (4). Antibiotic-susceptible *E. coli* ATCC 25922 and CTX-M-15-producing *E. coli* 222 (10) were used as quality control strains.

Molecular epidemiology. The genetic relationships between the ESBL-producing *E. coli* isolates were determined by pulsed-field gel electrophoresis (PFGE) after digestion of total chromosomal DNA with XbaI (10).

E. coli isolates representing the different clusters detected by PFGE were studied further by multilocus sequence typing (MLST) according to the University College Cork (Cork, Ireland) scheme for *E. coli* (http://mlst.ucc.ie/mlst/dbs/Ecoli [date last accessed, 20 May 2011]).

E. coli phylogenetic groups were determined by a multiplex PCR assay as described previously (2).

To search for the ST131 phylogroup B2 serotype O25b *E. coli* clone, O25b type detection was performed using allele-specific PCR (3).

Characterization of antibiotic resistance genes. Multiplex PCR conditions were used to amplify genes encoding ESBLs belonging to the CTX-

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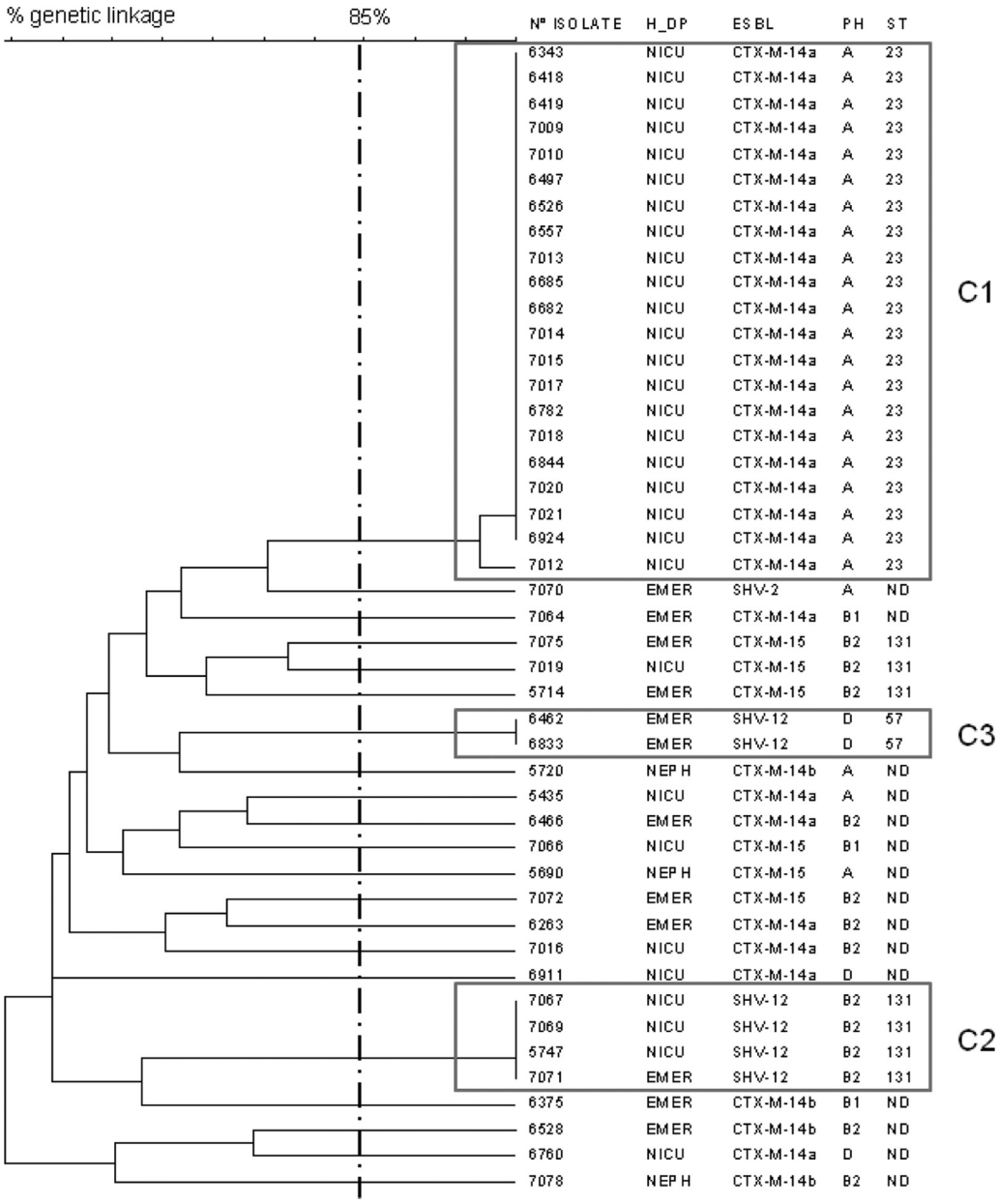

FIG 1 Dendrogram illustrating the genetic relationships among 45 ESBL-producing *Escherichia coli* isolates obtained from children ≤4 years old. H_DP, hospital department; PH, phylogroup; ST, sequence type by MLST; EMER, Emergency Unit; NEPH, Nephrology Unit; ND, not determined.

M-1, CTX-M-9, SHV, and TEM groups. Entire ESBL genes were amplified and sequenced with primers specific for bla_{SHV} and $bla_{CTX\text{-}M}$ (10). In addition, the *aac*(3)-*IIa* aminoglycoside resistance gene and the *aac*(6′)-*Ib-cr* aminoglycoside-quinolone resistance gene were characterized by PCR amplification with specific primers and DNA sequencing.

Furthermore, the genetic environment of $bla_{CTX\text{-}M\text{-}14a}$ was analyzed using specific primers to detect the linkage of $bla_{CTX\text{-}M\text{-}14}$ alleles with IS*Ecp1*-like or IS*903*-like elements (6).

Conjugation assay and plasmid characterization. Conjugation experiments were performed using the kanamycin-azide-resistant *E. coli* strain BM101 as a recipient. Putative transconjugants were selected on Mueller-Hinton agar plates containing kanamycin (100 mg/liter) and cefotaxime (4 mg/liter).

The number and size of plasmids were determined by PFGE after S1 nuclease digestion of whole genomic DNA (7). Plasmids were classified according to their incompatibility groups by using a PCR-based replicon-typing scheme (1).

RESULTS AND DISCUSSION

Patients and bacterial isolates. Between January 2009 and September 2010, 30 newborns admitted to the NICU were either infected ($n = 14$ [46.7%]) or colonized ($n = 16$ [53.3%]) by ESBL-producing *E. coli*. Their clinical diagnostics were as follows: blood infections, 6 cases (42.9%); wound infections, 3 cases (21.4%); urinary tract infections (UTIs), 2 cases (14.3%); respiratory tract infections, 2 cases (14.3%); and ear infection, 1 case (7.1%). For the 16 colonized children, ESBL-producing *E. coli* was isolated mainly from rectal swabs ($n = 14$ [87.5%]).

The NICU has a total of 66 beds, with average occupancy rates

TABLE 1 ESBL types and molecular epidemiology markers of 45 ESBL-producing *Escherichia coli* isolates obtained from children ≤4 years old

Pediatric unit[a]	No. of cases	PFGE cluster[b]	MLST type[c]	Phylogroup	ESBL
NICU	21	C1	23	A	CTX-M-14a
NICU	3	C2	131	B2	SHV-12
NICU	1	NR	ND	D	CTX-M-14a
NICU	1	NR	ND	D	CTX-M-14a
NICU	1	NR	ND*	B2	CTX-M-14a
NICU	1	NR	ND	A	CTX-M-14a
NICU	1	NR	131	B2	CTX-M-15
NICU	1	NR	ND	B1	CTX-M-15
EMER	2	C3	57	D	SHV-12
EMER	1	C2	131	B2	SHV-12
EMER	1	NR	ND*	B2	CTX-M-14a
EMER	1	NR	ND*	B2	CTX-M-14a
EMER	1	NR	ND*	B2	CTX-M-14b
NEPH	1	NR	ND*	B2	CTX-M-14b
NEPH	1	NR	ND	A	CTX-M-14b
EMER	1	NR	ND	B1	CTX-M-14b
EMER	1	NR	ND	B1	CTX-M-14a
EMER	1	NR	131	B2	CTX-M-15
EMER	1	NR	131	B2	CTX-M-15
EMER	1	NR	ND*	B2	CTX-M-15
NEPH	1	NR	ND	A	CTX-M-15
EMER	1	NR	ND	A	SHV-2

[a] NICU, neonatal intensive care unit; EMER, Emergency Unit; NEPH, Nephrology Unit.
[b] NR, nonrelated.
[c] ND, not determined. *, phylogroup B2 isolate negative for serotype O25b.

of 83.64% and 79.7% in 2009 and 2010, respectively. During the period of study, 3,114 newborns were admitted to this unit: 1,762 newborns in 2009 and 1,352 in 2010. The prevalence of ESBL-producing *E. coli* infection in the NICU was 0.3% (5 cases) in 2009 and 1.8% (25 cases) in 2010, a 6-fold increase. The highest prevalence was detected in August 2010 (6 cases out of 139 new admissions [4.3%]).

During the study period, ESBL-producing *E. coli* caused infections in 15 children ≤4 years old who were admitted to non-NICU pediatric units: 12 (80%) in the Emergency Unit and 3 (20%) in the Nephrology Unit. Most of these isolates (13 [86.7%]) caused UTIs.

ESBL types and molecular epidemiology of ESBL-producing *E. coli* from the NICU. Of the 30 ESBL-producing *E. coli* specimens isolated from newborns, 25 (83.3%), 3 (10%), and 2 (6.7%) were identified as producing CTX-M-14, SHV-12, and CTX-M-15, respectively. All CTX-M-14-producers had the $bla_{\text{CTX-M-14a}}$ gene.

PFGE profiles consistently revealed two different clusters (Fig. 1). Cluster 1 (C1) included 21 indistinguishable CTX-M-14-producing isolates, and cluster 2 (C2) included 3 SHV-12-producing *E. coli* isolates. The remaining 6 isolates (4 with CTX-M-14 and 2 with CTX-M-15) were nonrelated according to PFGE (Fig. 1; Table 1).

According to MLST and phylogroup characterization, C1 isolates producing CTX-M-14 were identified as ST23 phylogroup A, whereas SHV-12-producing C2 isolates were classified as ST131 phylogroup B2 serotype O25b.

Very few outbreaks due to ESBL-producing *E. coli* have been reported in NICUs. Recently, two outbreaks caused by *E. coli* producing TEM-12 and TEM-52 in the NICUs of hospitals in Switzerland (16) and France (8), respectively, have been reported. To our knowledge, this is the first documented report of the clonal spread of *E. coli* ST23 phylogroup A harboring CTX-M-14 in newborns. CTX-M-14 is one of the most prevalent ESBL types produced by *E. coli* (12) and usually belongs to a wide variety of clones (9, 11). Only a few CTX-M-14-producing *E. coli* strains have caused community outbreaks in the general population, as reported in Canada and Japan (13, 15).

Four isolates of CTX-M-14-producing *E. coli* belonging to the ST23 phylogroup A clone were previously identified in two different Spanish studies (11, 17), but this clone was not detected in a recent study in northwest Spain (9).

In addition, SHV-12-producing ST131 *E. coli* outbreaks have not been described previously in newborns, either.

Identification of ESBL-producing *E. coli* in non-NICU pediatric units. Among the 15 children ≤4 years old who were infected by ESBL-producing *E. coli* and were admitted to non-NICU pediatric units, 7 isolates (46.7%) produced CTX-M-14 (3 produced $bla_{\text{CTX-M-14a}}$, and 4 produced $bla_{\text{CTX-M-14b}}$), whereas 4 (26.7%), 3 (20%), and 1 (6.7%) isolate produced CTX-M-15, SHV-12, and SHV-2, respectively (Table 1).

None of these 15 isolates belonged to the C1 ST23 epidemic clone, but 1 additional SHV-12-producing isolate belonging to the C2 ST131 phylogroup B2 cluster was detected. In addition, two SHV-12-producing *E. coli* isolates had a new common profile (cluster C3 [Fig. 1]), further identified as ST57 phylogroup D (Fig. 1).

CTX-M-15-producing *E. coli* ST131 in children ≤4 years old. Overall, six CTX-M-15 isolates from children were identified: two among the NICU patients and four among the non-NICU pediatric patients (Table 1). According to PFGE data, these six isolates were genetically unrelated (Fig. 1), although three of them belonged to the international ST131 phylogroup B2 serotype O25b clone (12). According to PFGE, the genetic linkage of these three ST131 CTX-M-15 isolates (one from the NICU and two from the Emergency Room) was approximately 70%.

Four *E. coli* clones producing CTX-M-15, according to repetitive element-based PCR results, in the NICU of an Indian hospital have been described recently, although their MLST types were not reported (14).

Genetic environment and characterization of the plasmid carrying $bla_{\text{CTX-M-14a}}$. All 25 CTX-M-14-producing *E. coli* specimens isolated in the NICU neonates carried $bla_{\text{CTX-M-14a}}$ linked to IS*Ecp1*, which was detected 42 nucleotides upstream of this ESBL gene.

PFGE after S1 nuclease digestion of whole genomic DNA obtained from clinical isolates revealed two plasmids of approximately 80 and 100 kb, but only the 80-kb plasmid was detected in the cefotaxime-resistant transconjugants. This plasmid belonged to incompatibility group K and carried $bla_{\text{CTX-M-14a}}$.

Both mobile genetic elements—IncK conjugative plasmids and the IS*Ecp1* insertion sequence—were described previously as responsible for the spread of CTX-M-14a (17).

Antibiotic susceptibility of the C1 ST23 clone. All isolates of the C1 ST23 clone had identical susceptibility patterns, including resistance to ampicillin (MIC, >16 μg/ml), cefazolin (MIC, >16 μg/ml), cefuroxime (MIC, >16 μg/ml), cefotaxime (MIC, 64 to 256 μg/ml), cefepime (MIC, 16 to >16 μg/ml), and gentamicin (MIC, >8 μg/ml) and susceptibility to ceftazidime (MIC, ≤1 μg/ml), amoxicillin-clavulanic acid (MIC, ≤4 to 8 μg/ml),

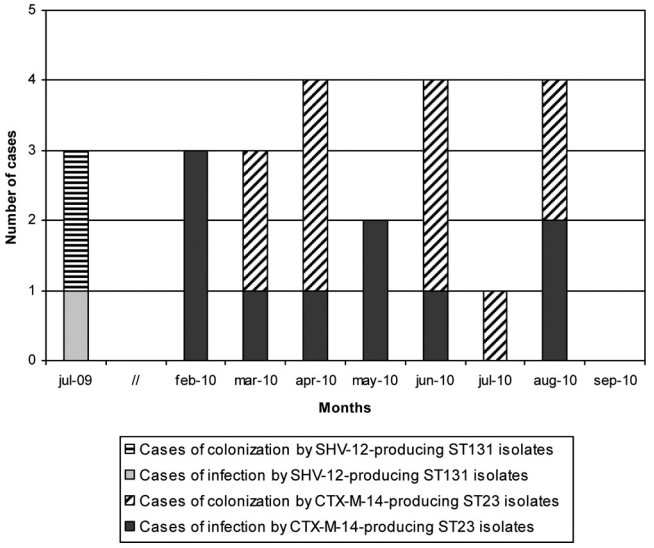

FIG 2 Monthly evolution of cases of infection or colonization of newborns by CTX-M-14- or SHV-12-producing *Escherichia coli* isolates belonging to C1 ST23 or C2 ST131, respectively.

piperacillin-tazobactam (MIC, ≤8 μg/ml), imipenem (MIC, ≤1 μg/ml), cotrimoxazole (MIC, ≤2 μg/ml), and ciprofloxacin (MIC, ≤0.12 μg/ml). Tobramycin and amikacin MICs ranged from ≤2 to >8 μg/ml and from ≤8 to 16 μg/ml, respectively.

Additionally, in all isolates of the C1 ST23 *E. coli* clone, the *aac*(3)-*IIa* aminoglycoside resistance gene was positively identified by PCR, whereas none of the isolates expressed the *aac*(6′)-*Ib-cr* aminoglycoside-quinolone resistance gene.

C1 ST23 outbreak evolution. The monthly evolution of the 21 cases of infection ($n = 10$) or colonization ($n = 11$) caused by the C1 ST23 clone in the NICU is shown in Fig. 2. All neonates were admitted to the NICU between February and August 2010; during the same period, three additional newborns admitted to the NICU had infections due to CTX-M-14a-producing *E. coli*, but the isolates were genetically unrelated (Table 1).

The first case identified was that of a 7-day-old male who was transferred from another Spanish hospital to the NICU of UHGM because of congenital heart disease. Previously, from January 2009 to February 2010, only five cases of ESBL-producing *E. coli* infection or colonization had been detected in this NICU: four in July 2009 (two colonizations and one infection by SHV-12-producing ST131 isolates, and one colonization by a nonrelated CTX-M-15-producing isolate) and one in January 2009 (infection by a nonrelated CTX-M-14a-producing isolate).

Clinical risk factors, treatment, and outcome. All 10 newborns infected by C1 ST23 isolates had underlying predisposing conditions, including congenital heart disease and prematurity (5) (Table 2). All 10 of these patients had received previous antibiotic treatment, and 7 (70%) had been treated with more than one antibiotic (Table 2). These 10 patients were mostly treated with meropenem as a monotherapy ($n = 6$ [60%]) or in combination with amikacin ($n = 1$ [10%]); 9 patients were cured with these treatments, but 1 newborn with bacteremia, who was treated with meropenem monotherapy, died (Table 2).

Infection control measures. All children with ESBL-producing *E. coli* recovered from any clinical specimen according to the microbiology laboratory reports were identified. Active surveillance of all patients admitted to the NICU was performed at least once a week; rectal swabs were cultured for detection of ESBL-producing *E. coli*. Patients harboring ESBL-producing *E. coli* were assigned to contact precautions, including the use of disposable gowns and gloves. In addition, standard precautions were reinforced for all patients admitted to the NICU, including improvement of hand hygiene compliance by the use of alcohol rubs before and after the care of patients. None of the environmental cultures was positive for CTX-M-14-producing *E. coli*. Between September and December 2010, no cases of infection or colonization by ESBL-producing *E. coli* isolates were detected in the NICU.

Concluding remarks. We report the characterization of an NICU outbreak caused by a CTX-M-14-producing *E. coli* isolate belonging to the ST23 phylogroup A clone, an ESBL type associated mainly with polyclonal community acquisition in adults. An additional, smaller outbreak, due to SHV-12-producing *E. coli*, was also identified in the same unit. Overall, CTX-M-14, CTX-M-15, and SHV-12 were the most important ESBLs causing pediatric infections in this study. Infections of newborns with CTX-M-14-producing *E. coli* were caused by both clonal and nonclonal isolates.

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TABLE 2 Clinical characteristics of patients infected with CTX-M-14-producing isolates belonging to the C1 ST23 clone

Gender[a]	Localization of infection[b]	Underlying condition(s)[c]	Previous antibiotic treatment(s)	Antimicrobial therapy	Outcome
M*	UTI	CC	Amikacin	Meropenem	Cure
M	LRTI	CC	Cefazolin	Meropenem	Cure
F	Wound infection	CC	Cefazolin	Meropenem	Cure
F	Bacteremia	PNI	Amikacin + vancomycin	Meropenem	Death
M	Wound infection	CC	Ampicillin + gentamicin + cefazolin	Amikacin + chlorhexidine washings	Cure
M	Bacteremia	PNI	Ampicillin + gentamicin	Meropenem + amikacin	Cure
M	LRTI	CC	Amikacin + vancomycin	Cotrimoxazole	Cure
F	Bacteremia	PNI	Ampicillin + gentamicin; amikacin + vancomycin	Meropenem	Cure
M	LRTI	PNI + MCM	Ampicillin + gentamicin; amikacin + vancomycin	Amikacin	Cure
F	Bacteremia	PNI	Ampicillin + gentamicin	Meropenem	Cure

[a] M, male; F, female. *, index case.
[b] LRTI, lower respiratory tract infection.
[c] CC, congenital cardiopathy; PNI, preterm newborn infant; MCM, multiple congenital malformations.

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