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Characterization of Extended-Spectrum-Beta-Lactamase-Producing *Escherichia coli* Strains Involved in Maternal-Fetal Colonization: Prevalence of *E. coli* ST131

André Birgy,[a] Patricia Mariani-Kurkdjian,[a,b] Philippe Bidet,[a,b] Catherine Doit,[a,b] Nathalie Genel,[c] Céline Courroux,[a] Guillaume Arlet,[c,d] Edouard Bingen[a,b]

Laboratoire de Microbiologie, Hôpital Robert Debré, Service de Microbiologie, Assistance Publique des Hôpitaux de Paris, Paris, France[a]; Université Paris Diderot, Sorbonnes Paris Cité, Paris, France[b]; Université Pierre et Marie Curie-Paris 6, Faculté de Médecine, Site Saint-Antoine, Laboratoire de Bactériologie, Paris, France[c]; Hôpital Tenon, Service de Bactériologie-Hygiène, Assistance Publique des Hôpitaux de Paris, Paris, France[d]

Maternal-fetal *Escherichia coli* infections, such as neonatal bacteremia and meningitis, are important causes of morbidity and mortality. From 2006 to 2010, we studied newborns and their mothers who were colonized with *E. coli* in a French hospital in order to document (i) the epidemiology and genetic characteristics of extended-spectrum-beta-lactamase (ESBL)-producing *E. coli* strains, (ii) the prevalence of associated virulence genes, (iii) the prevalence of clone sequence type 131 (ST131), and (iv) the genetic relationship among ESBL-producing strains. Among the 2,755 *E. coli* cultures recovered from vaginal or neonatal samples, 68 were ESBL producers (2.46%). We found a wide diversity of ESBL genes, with the majority being $bla_{CTX\text{-}M\text{-}14}$, $bla_{CTX\text{-}M\text{-}1}$, and $bla_{CTX\text{-}M\text{-}15}$, distributed among the 4 main phylogenetic groups. Genes encoding virulence factors were found in 90.7% of the isolates, with ≥2 virulence genes present in 76% of cases. The prevalence of ST131 among ESBL-producing *E. coli* isolates was 9.4% (6/64). Five of these 6 ST131 isolates possessed $bla_{CTX\text{-}M\text{-}15}$ enzymes (and also were resistant to quinolones), and one possessed $bla_{CTX\text{-}M\text{-}2}$ enzymes. Two possessed virulence genes, suggesting the presence of pathogenicity island II_{J96} ($PAI\ II_{J96}$)-like domains. Pulsed-field gel electrophoresis (PFGE) revealed a high level of genomic diversity overall, except for 3 closely related isolates belonging to clonal group ST131. Repetitive PCR showed that the six ST131 isolates were closely related to ST131 control strains (>95% similarity). This study shows a high prevalence of ESBL-producing *E. coli* strains and clonal group ST131 in the French maternal-fetal population. These results suggest a widespread distribution of ESBL enzymes in the community and highlight the early transmission between mothers and neonates. These findings are worrisome, especially for this particularly vulnerable population.

In the past decade, there has been an alarming increase in antibiotic-resistant *Enterobacteriaceae* producing extended-spectrum beta-lactamases (ESBLs), due at least in part to the overuse of broad-spectrum cephalosporins.

The majority of ESBLs identified in clinical isolates during the 1980s to 1990 were of the SHV or TEM type (1). However, since the mid-2000s, CTX-M ESBLs, originating from environmental *Kluyvera* spp., have gained prominence and are considered pandemic enzymes. CTX-M has been identified in several members of the *Enterobacteriaceae* family, and especially in *Escherichia coli*, which has replaced *Klebsiella* spp. as the principal ESBL-producing member of the *Enterobacteriaceae* (2–4).

ESBL enzymes are important causes of cephalosporin treatment failure. *E. coli* strains carrying these enzymes were initially responsible mainly for nosocomial infections, but they have now disseminated into the community. One of the best examples of this trend is the global spread of *E. coli* clone sequence type 131 (ST131), expressing CTX-M enzymes (5). Maternal-fetal *E. coli* infections, such as neonatal bacteremia and meningitis, are important causes of morbidity and mortality (6).

The epidemiology of ESBL-producing *E. coli* strains in adults is well documented (1, 7). In the neonatal period, most studies have focused on hospital-acquired colonization or infection with ESBL-producing *E. coli* strains (8), leaving aside the epidemiology of community carriage (9). It is necessary to know more about community-acquired infections and particularly about maternal-neonatal transmission to implement empirical therapy (e.g., use of carbapenems in maternity wards where the prevalence of ESBL-producing *Enterobacteriaceae* is high).

Here, we studied colonized newborns and their mothers who were hospitalized at Robert-Debré Hospital (Paris, France) from 2006 to 2010 in order to document (i) the epidemiology and genetic characteristics of ESBL-producing *E. coli* strains, (ii) the prevalence of associated virulence genes, (iii) the prevalence of ST131, and (iv) the genetic relation among ESBL-producing strains.

MATERIALS AND METHODS

Setting. We studied samples collected from newborns and/or their mothers at Robert-Debré Pediatric Hospital in Paris, France, over a 5-year period (February 2006 to December 2010). On average, 3,000 women gave birth in our institution each year between 2006 and 2010. Clinically indicated vaginal swabs were obtained from women during the period of childbirth. Birth samples (from gastric fluid, the outer ear, meconium, or the placenta) were taken routinely (<3 h after birth) to detect colonization/infection. This study included all *E. coli*-positive samples collected

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Address correspondence to Guillaume Arlet, guillaume.arlet@tnn.aphp.fr.

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from a mother or neonate. When *E. coli* was isolated on both the mother and child samples, antibiotic susceptibility testing was performed on both of them. Zone diameter inhibition and phenotypes were compared. When they were equal, only one sample per mother-child pair was studied. Some of the isolates studied here have also been used to test the interaction between cefixime and clavulanic acid *in vitro* (22 isolates) (10).

Isolation, identification, and antibiotic susceptibility testing. All the isolates were routinely cultured at 37°C on Trypticase soy agar, Drigalski agar, and URI4 agar (Bio-Rad, Marnes-la-Coquette, France). Isolates were identified with the API 20E system (bioMérieux, Marcy l'Etoile, France).

Antibiotic susceptibility was determined with the standard disk diffusion method on Mueller-Hinton agar (Bio-Rad, Marnes-la-Coquette, France) according to CLSI guidelines (11). All the isolates were tested for susceptibility to amoxicillin, ticarcillin, cefepime, ticarcillin-clavulanic acid, cephalothin, ceftazidime, amoxicillin-clavulanic acid, cefotaxime, ertapenem, cefoxitin, moxalactam, imipenem, ofloxacin (OFLO), gentamicin (GEN), and trimethoprim-sulfamethoxazole (SXT). ESBL detection was performed by using the double-disk synergy test between clavulanic acid and extended-spectrum cephalosporins (ceftazidime and cefotaxime) on Mueller-Hinton agar (12).

Molecular characterization of beta-lactamases. Strains with a positive double-disk synergy test result were further studied by specific PCR amplification and sequencing of ESBL genes. Lysates were obtained by boiling under standard conditions. The PCR primers and assay methods are described in detail elsewhere (13). CTX-M-type beta-lactamases were checked using the method of Eckert et al. (14).

Phylogenetic background and virulence gene analysis. The *E. coli* strains were assigned to one of the four main phylogenetic groups (A, B1, B2, and D) by using a previously described multiplex PCR method (15) that uses a combination of 3 DNA markers (*chuA*, *yjaA*, and TSPE4.C2). Using a PCR-based method, the strains were also screened for 11 genes encoding the following putative virulence factors (VFs): *fyuA*, iron uptake; *hly*, hemolysin; *sfa*/*foc*, S or F1C fimbriae; *papC* genes of P fimbriae operons; *iucC*, aerobactin; *papG* (II and III alleles); *cnf1*, cytotoxic necrotizing factor; *iroN*, salmochelin receptor; *ibeA*, putative invasin; *hra*, heat-resistant agglutinin; and the capsular antigen K1 (16, 17).

Pulsed-field gel electrophoresis. All *E. coli* isolates were typed by pulsed-field gel electrophoresis (PFGE) on the CHEF DRII system (Bio-Rad, Marnes-la-Coquette, France) using genomic DNA digested with NotI (18). A dendrogram was constructed using the Dice similarity coefficient, and the unweighted-pair group method using average linkages (UPGMA) algorithm was used to cluster the strains.

Detection of ST131. Allele-specific PCR of the *pabB* and *trpA* alleles was used to detect B2 strains belonging to the pandemic clone O25b:H4-ST131 (19). Four strains known to belong to clone ST131 were used as positive controls (e.g., strain TN03 [20]).

Repetitive PCR. For strains belonging to clone ST131 (as determined by PCR), clonal relatedness was determined by using the DiversiLab semi-automated repetitive-sequence-based PCR (rep-PCR) typing method as described previously (21, 22). Four strains known to belong to clone ST131 were used as controls.

RESULTS

During the 5-year study period, 2,755 *E. coli* isolates were recovered from neonatal samples (about 2,000 samples were obtained each year, each collected <3 h after birth) or from maternal vaginal samples (about 2,500 samples each year). A total of 68 ESBL-producing *E. coli* isolates were identified (2.46%). Four strains did not grow in culture after being frozen. Twenty-eight (44%) of the ESBL-producing strains were obtained from vaginal swabs and 36 (56%) from neonatal samples (from gastric fluid or the outer ear). When inhibition zone diameters of antibiotics and antibiotypes were equal for both isolates from the mother and neonate, only one sample per mother-child pair was studied; the inhibition zone diameters were found to be the same in each case.

No significant variation in the total number of *E. coli* isolates was observed between 2006 and 2010. In contrast, the prevalence of ESBL-producing *E. coli* of all *E. coli* isolates rose from 1.1% (6/523) in 2006 to 4.1% (22/543) in 2010 ($P < 0.05$).

Phylogenetic diversity. The ESBL-producing *E. coli* isolates belonged to phylogenetic group A ($n = 22$), B1 ($n = 8$), B2 ($n = 15$), or D ($n = 19$).

Beta-lactamase genes. Of the 64 ESBL-producing *E. coli* isolates, 84.3% were positive for $bla_{\text{CTX-M}}$ genes, 9.4% for bla_{SHV} genes, and 6.3% for bla_{TEM} genes. The CTX-M types were as follows: 19 $bla_{\text{CTX-M-14}}$ (29.7%), 17 $bla_{\text{CTX-M-1}}$ (26.5%), 12 $bla_{\text{CTX-M-15}}$ (18.8%), 3 $bla_{\text{CTX-M-2}}$ (4.7%), 1 $bla_{\text{CTX-M-3}}$ (1.6%), 1 $bla_{\text{CTX-M-57}}$ (1.6%), and 1 $bla_{\text{CTX-M-27}}$ (1.6%). There were 4 $bla_{\text{TEM-52}}$ and 4 $bla_{\text{SHV-12}}$ (6.3% each, respectively) and 2 $bla_{\text{SHV-2a}}$ (3.1%) genes.

The CTX-M-producing *E. coli* isolates belonged to groups A (17/54, 31.5%), B1 (7/54, 13%), B2 (13/54, 24%), and D (17/54, 31.5%). We noted that 8/12 (67%) of $bla_{\text{CTX-M-15}}$ genes were found in group B2, and 8/19 (42%) $bla_{\text{CTX-M-14}}$ genes were found in group D.

Additionally, 21 (33%) of the ESBL-producing isolates also possessed $bla_{\text{TEM-1}}$, and 5 (8%) also possessed $bla_{\text{OXA-1}}$.

Thirty-one (48.4%) of the 64 isolates were resistant to SXT, 21 (32.8%) were resistant to quinolones (nalidixic acid and ofloxacin), 12 (18.8%) were resistant to gentamicin, and 7 (11%) were resistant to all 3 families of antibiotics. All the isolates were susceptible to imipenem and ertapenem, defined according to the CLSI guidelines (11).

Virulence genes. All but 6 (9.3%) of the isolates possessed at least one virulence gene, and 49/64 (76%) possessed at least two. Four of the 6 strains with no virulence gene belonged to phylogenetic group A, one to group B1, and one to group D.

The prevalences of the virulence genes were as follows: *fyuA*, 70%; *iucC*, 65%; *iroN*, 37%; *papC*, 34%; *papGII*, 26%; *hra*, 25%; *hly*, 11%; *cnf1*, 6%; *papGIII*, 5%; and *sfa*, 5%. The gene of the capsular antigen K1 was possessed by 14/64 isolates (22%) (Table 1).

The most frequent combination of virulence genes was *fyuA*, *papC*, *iucC*, and *papGII*, which was found in 8 isolates, of which 6 belonged to phylogenetic group D.

PFGE revealed a high level of genomic diversity, except for 3 isolates (*E. coli* ST131 31342, 29369, and 30151) belonging to phylogenetic group B2 ST131, which were all closely related according to Tenover's criteria (23). As an example, a dendrogram showing the genetic relation within group B2 is provided in Fig. 1.

The PFGE pulsotype could not be obtained for 7 isolates, despite several attempts.

ST131. The prevalence of ST131 was 9.4% (6/64) overall and 43% among B2 isolates. Five ST131 strains possessed $bla_{\text{CTX-M-15}}$ and one possessed $bla_{\text{CTX-M-2}}$. The 5 strains possessing $bla_{\text{CTX-M-15}}$ were resistant to quinolones, and 4 were resistant to trimethoprim-sulfamethoxazole.

All six ST131 strains possessed the *fyuA* and *iucC* virulence genes.

Repetitive PCR. DiversiLab analysis showed that the six *E. coli* ST131 strains (isolates 26601, 27144, 28678, 29369, 30151, and 31342) were closely related to the ST131 control strains (>95% similarity) (Fig. 2).

TABLE 1 Characteristics of isolates obtained through mother and newborn samples in this study

E. coli ST131 isolate	Patient	Specimen source	Gene(s) encoding beta-lactamases	Antimicrobial, nonsusceptibility associated (intermediate or resistant)	Phylogenetic group	ST131 status	K1 status	Gene(s) encoding virulence factors
22328	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}14}$, $bla_{TEM\text{-}1}$	SXT, OFLO, GEN	D	ND[a]	−	*fyuA*, *papC*
22873	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}2}$, $bla_{TEM\text{-}1}$	SXT	A	ND	−	*hra*, *fyuA*, *papC*, *iucC*, *iroN*
23542	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}14}$		A	ND	+	*fyuA*, *papC*, *iucC*, *papGII*
23805	Newborn	Gastric	$bla_{TEM\text{-}52}$		B2	−	+	*fyuA*, *papC*, *iucC*, *papGII*
23887	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}14}$, $bla_{TEM\text{-}1}$	SXT, OFLO	A	ND	+	*fyuA*, *papC*, *iucC*, *papGIII*
24213	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}3}$		D	ND	−	*fyuA*, *papC*, *iucC*, *papGII*
24607	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}1}$	SXT, OFLO	A	ND	−	*hra*, *iucC*
24949	Newborn	Ear	$bla_{CTX\text{-}M\text{-}1}$		D	ND	−	*fyuA*, *iucC*, *iroN*
24952	Mother	Vaginal	$bla_{TEM\text{-}52}$	OFLO	A	ND	−	No virulence factors
25195	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}14}$		B1	ND	−	*sfa*, *iroN*
25531	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}1}$, $bla_{TEM\text{-}1}$		B1	ND	−	*hra*, *fyuA*, *hly*, *papC*, *iucC*
25556	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}14}$, $bla_{TEM\text{-}1}$	SXT, OFLO	D	ND	−	*fyuA*
25798	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}14}$		D	ND	−	*fyuA*, *iucC*
25834	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}1}$		D	ND	−	*iucC*, *iroN*
25930	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}14}$, $bla_{TEM\text{-}1}$	SXT	B1	ND	−	*fyuA*, *iucC*, *iroN*
25982	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}14}$		A	ND	+	*fyuA*, *hly*, *papC*, *iucC*
26240	Newborn	Gastric	$bla_{TEM\text{-}52}$		B2	−	−	*fyuA*, *iucC*, *ibeA*, *iroN*
26601	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$	SXT, OFLO, GEN	B2	+	−	*fyuA*, *iucC*
26777	Mother	Vaginal swab	$bla_{TEM\text{-}52}$	SXT	A	ND	−	*fyuA*
27048	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}1}$, $bla_{TEM\text{-}1}$	SXT	A	ND	−	*hra*, *fyuA*, *papC*, *iucC*, *iroN*
27139	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}14}$	SXT, OFLO	A	ND	+	*fyuA*, *papC*, *iucC*, *papGIII*
27144	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}2}$, $bla_{TEM\text{-}1}$	SXT	B2	+	+	*fyuA*, *iucC*, *ibeA*, *iroN*
27487	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}15}$	SXT, OFLO	B2	−	+	*fyuA*, *iucC*
27915	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}15}$	SXT, OFLO, GEN	A	ND	−	No virulence factors
27959	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}2}$, $bla_{TEM\text{-}1}$	GEN	B1	ND	−	*iroN*
28130	Newborn	Ear	$bla_{CTX\text{-}M\text{-}15}$	SXT	A	ND	−	*hra*, *fyuA*, *papC*
28322	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}1}$		B2	−	+	*hra*, *fyuA*, *hly*, *sfa*, *papC*, *papGIII*, *cnf1*, *iroN*
28678	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}15}$	SXT, OFLO	B2	+	−	*fyuA*, *iucC*
28936	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}1}$	SXT	D	ND	−	*hra*
28949	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}1}$		B1	ND	−	*iroN*
29047	Newborn	Gastric	$bla_{SHV\text{-}12}$		D	ND	−	No virulence factors
29081	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}15}$	SXT, GEN	B2	−	−	*fyuA*
29191	Mother	Vaginal swab	$bla_{SHV\text{-}12}$, $bla_{TEM\text{-}1}$	SXT	A	ND	−	*iroN*
29228	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}1}$, $bla_{TEM\text{-}1}$		D	ND	−	*fyuA*, *iroN*
29229	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}1}$, $bla_{TEM\text{-}1}$		D	ND	−	*fyuA*, *iroN*
29245	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}14}$		B1	ND	−	*fyuA*, *papGII*
29273	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}14}$		B1	ND	−	*fyuA*, *papGII*
29369	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$	SXT, OFLO, GEN	B2	+	−	*hra*, *fyuA*, *hly*, *papC*, *iucC*, *papGII*, *cnf1*
29386	Mother	Vaginal	$bla_{CTX\text{-}M\text{-}57}$		A	ND	−	No virulence factors
29512	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$	SXT, OFLO	A	ND	−	*fyuA*, *iucC*
29560	Mother	Vaginal swab	$bla_{SHV\text{-}12}$		D	ND	−	*fyuA*, *iucC*, *iroN*
29724	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}14}$	SXT, OFLO, GEN	D	ND	+	*fyuA*, *papC*, *iucC*, *papGII*
29749	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}14}$, $bla_{TEM\text{-}1}$	SXT, GEN	D	ND	+	*fyuA*, *papC*, *iucC*, *papGII*
29752	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}14}$, $bla_{TEM\text{-}1}$		B2	−	+	*fyuA*, *iucC*, *ibeA*, *iroN*
29905	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}14}$, $bla_{TEM\text{-}1}$	SXT	D	ND	−	*hra*, *fyuA*, *hly*, *papC*, *iucC*, *papGII*
30064	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}1}$		D	ND	−	*fyuA*, *papC*, *iucC*, *papGII*
30151	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$	OFLO, GEN	B2	+	−	*hra*, *fyuA*, *hly*, *papC*, *iucC*, *papGII*, *cnf1*
30204	Newborn	Gastric	$bla_{SHV\text{-}12}$		B1	ND	−	No virulence factors
30363	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}15}$, $bla_{TEM\text{-}1}$	SXT, GEN	B2	−	−	*fyuA*, *iucC*
30525	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}27}$, $bla_{TEM\text{-}1}$	SXT, OFLO, GEN	B21	−	+	*fyuA*, *hly*, *papC*, *iucC*, *papGII*, *iroN*
30546	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}1}$, $bla_{TEM\text{-}1}$	SXT	A	ND	−	*fyuA*, *iucC*, *iroN*
30576	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}14}$		B2	−	−	*hra*, *fyuA*, *sfa*, *iucC*, *iroN*
30661	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}1}$		A	ND	+	*hra*
30679	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}15}$, $bla_{TEM\text{-}1}$	SXT, OFLO, GEN	D	ND	−	*fyuA*, *papC*, *iucC*, *papGII*
30705	Mother	Vaginal swab	$bla_{SHV\text{-}2a}$	OFLO	A	ND	−	*iucC*, *iroN*
30720	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}1}$	OFLO	A	ND	−	*hra*, *iucC*, *iroN*
31059	Newborn	Gastric	$bla_{SHV\text{-}2a}$		A	ND	−	*hra*, *fyuA*, *iucC*, *iroN*
31085	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}14}$	SXT, OFLO	D	ND	+	*fyuA*, *papC*, *iucC*, *papGII*
31096	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}1}$	SXT	D	ND	−	*iucC*, *iroN*
31254	Mother	Vaginal swab	$bla_{CTX\text{-}M\text{-}1}$, $bla_{TEM\text{-}1}$		A	ND	−	*iucC*, *iroN*
31289	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}1}$, $bla_{TEM\text{-}1}$	SXT	A	ND	−	*hra*, *iucC*
31342	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$	SXT, OFLO	B2	+	−	*hra*, *fyuA*, *papC*, *iucC*, *papGII*
31385	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}14}$		A	ND	−	No virulence factors
31776	Newborn	Gastric	$bla_{CTX\text{-}M\text{-}14}$	OFLO	D	ND	−	*fyuA*

[a] ND, not determined.

DISCUSSION

This study shows an increase in the prevalence of ESBL-producing strains among *E. coli* isolates recovered from neonates or their mothers in a French hospital between 2006 and December 2010, which rose from 1.15% (6/523) in 2006 to 4.1% (22/543) in 2010. This increase is in keeping with the global rise in ESBL-producing *E. coli* carriage in the general population (3, 24, 25).

Note that the prevalence of ESBL-producing *E. coli* carriage might have been underestimated in this study, because not all mothers or neonates were sampled. $bla_{CTX\text{-}M\text{-}14}$, $bla_{CTX\text{-}M\text{-}1}$, and

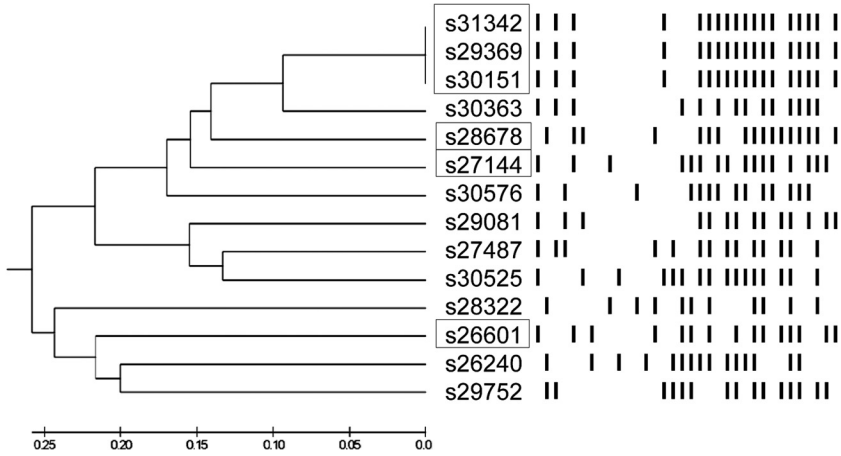

FIG 1 Genetic relation among the 13 ESBL-producing *E. coli* strains in group B2, as determined by PFGE (the pulsotype could not be obtained for 1 strain). The framed isolates are those belonging to clonal group ST131. These 6 isolates yielded 4 different profiles.

$bla_{\text{CTX-M-15}}$ were the principal ESBLs found in this study, representing 29.7%, 26.5%, and 18.8% of all ESBLs, respectively. These results are similar to those reported in adults (26, 27). In contrast, they differ from those obtained in children from Asia and South America (28, 29) and particularly from those obtained in the North American pediatric study by Chandramohan et al. (30), who found 60% $bla_{\text{CTX-M-15}}$, 14.3% $bla_{\text{CTX-M-14}}$, and 2.1% $bla_{\text{CTX-M-27}}$ ESBLs. In addition, CTX-M diversity was higher in our study, with the presence of CTX-M-1, -14, -15, -3, -2, -27, and -57 (28–30).

$bla_{\text{CTX-M-15}}$ has been reported worldwide, whereas $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-14}}$ seem to have specific geographic distributions in adults. A recent epidemiological study showed the presence of these enzymes in adults in France (31).

Virulent extraintestinal strains mainly belong to groups B2 and D, whereas commensal strains mainly belong to groups A and B1 (32). The majority of the strains studied here belonged to groups A and D, followed by groups B2 and B1. Overall, roughly half the strains belonged to groups B2/D and half to groups A/B1. Strains with $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-14}}$, and $bla_{\text{CTX-M-15}}$ belonged to phylo-

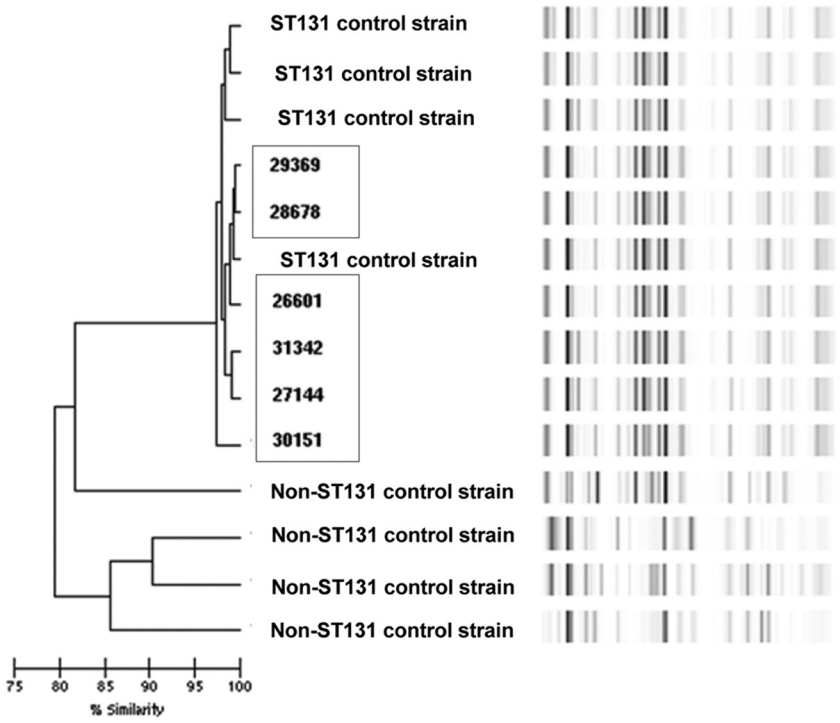

FIG 2 Genetic relatedness of *E. coli* strains belonging to clonal group O25b:H4-ST131 and control strains, as determined with DiversiLab semiautomated rep-PCR typing. There are 4 control strains known to belong to ST131 and 4 unrelated control strains that do not belong to clonal group ST131. *E. coli* ST131 isolates 29369, 28678, 26601, 31342, 27144, and 30151 are those found in this maternal-fetal study. The six *E. coli* ST131 strains in our study were closely related to the ST131 control strains (>95% similarity) and exhibited a >15% difference with strains not belonging to this sequence type.

genetic groups A, B2, and D. Interestingly, 67% of the strains possessing $bla_{CTX\text{-}M\text{-}15}$ belonged to group B2 and, of these, 5 belonged to clone ST131. These results are consistent with a recent report on $bla_{CTX\text{-}M\text{-}15}$ (30).

Half the $bla_{TEM\text{-}52}$-possessing isolates belonged to group B2 and half to group A (4 strains in total). Over 90% of the strains in our study possessed at least one virulence gene, and 76% possessed at least two. Virulence genes were found in all the phylogenetic groups studied.

This study highlights the high proportion of *E. coli* strains belonging to sequence type 131. Indeed, 6 of the 14 B2 strains belonged to O25b:H4-ST131, representing 9.4% of all ESBL-producing isolates (and 43% of all B2 strains). This proportion is similar to that found by Chandramohan and Revell (30) in a pediatric study, in which 10.2% of *E. coli* isolates belonged to clone ST131. This sequence type was identified with a PCR-based method (19) and confirmed with rep-PCR (21, 22). All our strains belonging to clonal group ST131 had a unique rep-PCR profile with >95% similarity, whereas PFGE divided them into 4 pulsotypes. This confirms that PFGE is more discriminatory than rep-PCR (22).

Clone ST131 has spread widely and is often reported in adults (33) but rarely in infants and children (30). To our knowledge, our study is the first to provide information about ST131 in the neonatal population, showing early transmission between mothers and neonates. *E. coli* ST131 is typically multidrug-resistant, including a resistance to fluoroquinolones. This was the case of 5 of the 6 ST131 strains in our study. In addition, 4 of them possessed $bla_{OXA\text{-}1}$. Clone ST131 is most frequently described as producing the plasmid-mediated ESBL CTX-M-15 (this was the case in 5 of our 6 strains), although the production of other ESBL types, such as SHV-12, has also been reported (34).

$bla_{CTX\text{-}M\text{-}15}$ and *E. coli* ST131 have emerged and expanded during the past decade in the United States (35) in both children and adults. One possible reason for the striking epidemiological success of $bla_{CTX\text{-}M\text{-}15}$ and ST131 might be their association. Indeed, the ST131 clone has adapted well to humans (20), and this has contributed to the worldwide dissemination of efficient resistance to third-generation cephalosporins: $bla_{CTX\text{-}M\text{-}15}$. Some studies suggest that clone ST131 is virulent: like other B2 isolates, it killed 100% of experimental mice in another study (36). Van der Bij et al. (37) also reported that ST131 had a significantly higher virulence score than did other extraintestinal pathogenic *E. coli* (ExPEC) STs. In contrast, other studies have found that ST131 is not highly virulent in infection models and that it differs from classical ExPEC group B2 strains by the absence of several virulence genes, such as *hlyA* and *cnf1* (38). It appears that few virulence genes are consistently present in this clone (39).

As stated above, ST131 isolates can be subdivided by means of PFGE (40). It is therefore possible that ST131 variants might have different virulence profiles and capacities for spread. In our study, the 3 clones that had the same pulsotype seemed to possess more virulence genes than did the others, and 2 of them harbored *papGII*, *cnfI*, *hra*, and *hly*, suggesting the presence of PAI II_{J96}-like domains (16). Interestingly, only one other strain, not belonging to the ST131 clone, possessed a putative PAI II_{J96}-like domain. Clone ST131 might thus have found an optimal balance between virulence, colonization, and resistance (20).

PFGE revealed a high genomic diversity among our bla_{TEM}-, bla_{SHV}-, and $bla_{CTX\text{-}M}$-type ESBL *E. coli* isolates. This diversity illustrates the high capacity for spread among different genetic backgrounds found in the community, accomplished via mobile conjugative elements.

Together, our results suggest that the carriage of ESBL-producing *E. coli* isolates in French children and their mothers results from community acquisition of these bacteria or their genes encoding ESBLs. The high proportion of strains possessing $bla_{CTX\text{-}M}$-type ESBLs, together with the high prevalence of clonal group ST131 in the North American pediatric study reported by Chandramohan et al. and in our neonatal French study, shows the worldwide diffusion of these CTX-M genes and this clone in pediatric populations. Although person-to-person transmission might contribute to ESBL dissemination, as suggested by Rodriguez-Baño et al. (41), the reservoir might be the family, which would explain the transmission from parents to their children as observed in our study. The spread of ESBL-producing *E. coli* strains in the community, especially during the neonatal period, is a cause for concern. Elevated carriage rates necessitate strict hygiene measures, both in the hospital and at home. More-discriminatory use of antibiotics is needed to limit the dissemination of these resistant strains.

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