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Prevalence of day-care centre children (France) with faecal CTX-M-producing *Escherichia coli* comprising O25b:H4 and O16:H5 ST131 strains

Véronique Blanc[1], Véronique Leflon-Guibout[2], Jorge Blanco[3], Marisa Haenni[4], Jean-Yves Madec[4], Gwenaële Rafignon[2], Pascale Bruno[5], Azucena Mora[3], Cecilia Lopez[3], Ghizlane Dahbi[3], Brigitte Dunais[5], Magali Anastay[1], Catherine Branger[6], Richard Moreau[7], Christian Pradier[5] and Marie-Hélène Nicolas-Chanoine[2,7,8]*

[1]Service de Biologie, Centre Hospitalier d'Antibes-Juan les Pins, Antibes, France; [2]Service de Microbiologie, Hôpital AP-HP Beaujon, Clichy, France; [3]Laboratorio de Referencia de E. coli (LREC), Departamento de Microbioloxía e Parasitoloxía, Facultade de Veterinaria, Universidade de Santiago de Compostela (USC), Lugo, Spain; [4]Agence Nationale de Sécurité Sanitaire (Anses), Unité Antibiorésistance et Virulence Bactériennes, Lyon, France; [5]Département de Santé Publique, Centre Hospitalier Universitaire de Nice, Nice, France; [6]Service de Microbiologie, Hôpital AP-HP L Mourier, Colombes, France; [7]Institut National de la Santé et de la Recherche Médicale, U773, Centre de Recherche Biomédicale Bichat-Beaujon (CRB3), Université Paris Diderot, Paris, France; [8]Faculté de Médecine D. Diderot, Paris, France

*Corresponding author. Service de Microbiologie, Hôpital Beaujon, 92110 Clichy, France. Tel: +33-1-40-87-56-06; Fax: +33-1-40-87-05-50; E-mail: mhn.chanoine@bjn.aphp.fr

Objectives: Determining the prevalence of children in day-care centres (DCCs) carrying faecal extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae and molecularly characterizing those belonging to the *Escherichia coli* species.

Methods: Stools were collected from children's diapers (January–April 2012) in randomly chosen DCCs and plated onto ChromID™ ESBL. Colonies growing on this medium were identified by the Vitek 2® system and tested for antibiotic susceptibility and for ESBL production by the double-disc synergy test. ESBL genotypes were determined as well as phylogenetic groups, ERIC-2 (enterobacterial repetitive intergenic consensus) PCR profiles and sequence types (STs) for the *E. coli* isolates. Serotypes, virotypes, *fimH* alleles, ESBL-carrying plasmids and PFGE patterns were determined for the ST131 *E. coli* isolates.

Results: Among 419 children from 25 participating DCCs, 1 was colonized by CTX-M-15-producing *Klebsiella pneumoniae* and 27 (6.4%) by *E. coli*, which all produced CTX-M enzymes [CTX-M-15 (37%), CTX-M-1 (26%), CTX-M-14 (22%), CTX-M-27 (11%) and CTX-M-22 (4%)]. The 27 *E. coli* isolates, 55.5% belonging to group B2, displayed 20 ERIC-2 PCR profiles and 16 STs. The ST131 *E. coli* isolates were dominant (44%), displayed serotypes O25b:H4 and O16:H5, *fimH* alleles 30 and 41 and virotypes A and C. According to the PFGE patterns, one strain of *E. coli* ST131 producing a CTX-M-15 enzyme carried by an IncF F2:A1:B− plasmid had spread within one DCC.

Conclusions: This study shows a notable prevalence (6.4%) of DCC children with faecal CTX-M-producing *E. coli* isolates comprising a high proportion of *E. coli* ST131 isolates, suggesting that these children might be a reservoir of this clone.

Keywords: *E. coli*, *fimH*-based subclones, virotypes, plasmids

Introduction

In the past decade, there has been a significant increase in the prevalence of extended-spectrum cephalosporin resistance in *Escherichia coli*, owing to the presence of extended-spectrum β-lactamase (ESBL) enzymes.[1] These organisms have become important pathogens of both community-onset and hospital-associated infections on a global scale.[2] Additionally, molecular epidemiology studies revealed that one specific *E. coli* clone, ST131 (where ST stands for sequence type), closely associated with fluoroquinolone resistance and CTX-M-15 production, has spread globally.[3,4] Two recent studies that analysed the population structure of *E. coli* clinical isolates showed that clone ST131 accounted for 9%–10% of non-ESBL producers and 36%–54% of ESBL producers.[5,6] Studies focusing on

children showed that ST131 E. coli also caused infections in children. It accounted for 8% of urinary non-ESBL-producing E. coli isolates in children from Australia and for 10.2% of urinary CTX-M-producing E. coli isolates from children attending Texas Children's Hospital (USA).[7,8] The major reservoir of human extraintestinal pathogenic E. coli is the human digestive tract. Studies to evaluate the prevalence among healthy subjects of intestinal colonization by ESBL-producing E. coli have been carried out in different parts of the world. The prevalence rate was 5.8%, 6.4% and 7.3% among healthy individuals from Switzerland, Japan and Tunisia, respectively, and 6% among healthy individuals living in the Paris area.[9–12] Much higher prevalence rates were observed in Thailand (29%–50%), China (50%) and Egypt (63%).[13–15] All of these studies involved healthy adults. Studies involving healthy children are scarce. Pallecchi et al.[16] detected 1.7% of healthy children from Peru and Bolivia with faecal carriage of ESBL-producing E. coli in 2005. Kaarme et al.[17] showed that 2.9% of healthy Swedish preschool children had ESBL-producing Enterobacteriaceae in their faeces in 2010. Relatively higher prevalence rates, i.e. 4.6% and 5.2%, were observed in children attending community paediatrician consultations in three French regions and in children hospitalized for acute diarrhoea in southern France, respectively.[18,19] The present study was also conducted in southern France, but among children attending day-care centres (DCCs). The aims of the study were to determine the prevalence of DCC children who carry ESBL-producing Enterobacteriaceae in their digestive tract and to thoroughly characterize the faecal ESBL-producing E. coli isolates detected among this particular population within the community.

Materials and methods

Participants

A cross-sectional survey was conducted on children attending a random sample of DCCs in south-eastern France between January and April 2012. After obtaining parental consent, stools were collected from children's diapers whenever available on the day of the survey and conserved at 4°C until they were analysed.

Screening of faecal ESBL-producing isolates, species identification and antibiotic susceptibility

Within 24 h following stool sampling, an aliquot of stools was spread on screening plates for selection of extended-spectrum cephalosporin-resistant isolates (ChromID™ ESBL, bioMérieux, Marcy-l'Étoile, France). Colonies growing on this medium were identified by the Vitek 2® system (bioMérieux). ESBL producers were confirmed by the double-disc synergy test.[20] Antibiotic susceptibility was determined by using both the Vitek 2® system and the agar disc diffusion method and interpreted according to the 2012 recommendations of the French Antibiogram Committee (http://www.sfm-microbiologie.org/pages/?all=accueil). Susceptibility to the following antibiotics was taken into consideration to define the resistance score to antibiotic molecules other than extended-spectrum cephalosporins (cefotaxime, ceftazidime and cefepime) for each isolate: amoxicillin/clavulanic acid, cefoxitin, ertapenem, imipenem, fosfomycin, levofloxacin, gentamicin, amikacin, trimethoprim/sulfamethoxazole and nitrofurantoin. The resistance score was the number of antibiotics to which the isolate was resistant or intermediate susceptible.

ESBL characterization

ESBLs were characterized as previously described.[21] Briefly, specific primers for the bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes were used to screen for these genes. Then, specific primers were used to sequence the amplified genes and determine those encoding an ESBL enzyme.

Molecular characterization of ESBL-producing E. coli

To characterize the population structure of the ESBL-producing E. coli isolates, phylogenetic groups, ERIC-2 (enterobacterial repetitive intergenic consensus) PCR profiles and STs following Achtman's multilocus sequence typing (MLST) scheme (http://mlst.ucc.ie/mlst/dbs/Ecoli) were determined as previously described.[4,22] Two ERIC-2 PCR profiles were assumed to be different when they differed from each other by at least one band with high intensity. PFGE patterns, serotypes, fimH allele types, virulence factor genes (n=28) and virotypes were determined as previously described for the isolates of E. coli ST131.[23,24] Briefly, four virotypes have recently been defined and arbitrarily named A, B, C and D on the basis of the presence/absence of four distinctive virulence genes, including afaFM955459 (specific for an ST131 subgroup encoding an Afa/Dr adhesin), iroN (catecholate siderophore receptor), ibeA (invasion of brain endothelium) and sat (secreted autotransporter toxin). The patterns were as follows: virotype A (afaFM955459+ iroN− ibeA− sat+/−), virotype B (afaFM955459− iroN+ ibeA− sat+/−), virotype C (afaFM955459− iroN− ibeA− sat+) and virotype D (afaFM955459− iroN+/− ibeA+ sat+/−). According to the definition of Tenover et al.,[25] two isolates were two strains when their PFGE patterns differed from each other by at least seven bands.

Characterization of the ESBL-carrying plasmids

Plasmids harboured by the ST131 E. coli isolates were rep typed using the PCR-based replicon typing scheme and plasmid sizes were determined using S1-treated genomic DNA followed by PFGE (S1-PFGE).[26] Southern blots were performed on S1-PFGE with the bla_{CTX-M} gene and IncF or IncI1 specific DIG-labelled probes (Roche Applied Science, Mannheim, Germany). IncF plasmids were subjected to replicon sequence typing to determine the FAB formula and IncI1 plasmids were typed by plasmidic MLST.[27,28]

Results

Gut colonization with an ESBL-producing isolate

From January to April 2012, stools were collected from 419 children aged between 3 and 40 months in 25 participating DCCs. The mean number of included children per DCC was 16.7 (range: 8–23). Among these children, 28 (6.7%) attending 16 (64%) of the 25 participating DCCs carried ESBL-producing Enterobacteriaceae in their digestive tract, comprising 27 E. coli and 1 Klebsiella pneumoniae (Table 1). The number of children with a faecal ESBL-producing isolate varied from none to seven in each participating DCC. Only one child was detected with such an isolate in 11 (69%) of the 16 DCCs with colonized children (Table 1). In the remaining five DCCs, there were from two to seven children harbouring a faecal ESBL-producing isolate (Table 1).

ESBL characterization and antibiotic susceptibility

All of the detected ESBLs were CTX-M enzymes, as indicated in Table 1. CTX-M-15 was produced by the K. pneumoniae isolate and 10 (37%) of the 27 E. coli isolates. Among the remaining 17 E. coli isolates, 7 (26%) produced CTX-M-1, 6 (22%) CTX-M-14, 3 (11%) CTX-M-27 and 1 (4%) CTX-M-22. When considering the intermediate susceptible isolates as resistant, 100% of the isolates were resistant to cefotaxime (data not shown), 89% to

Table 1. Antibiotic susceptibility, ESBL type and genetic background of the ESBL-producing isolates

DCC number	Species/child-isolate number	CTX-M type	CAZ	FEP	AMC[b]	LVX[b]	GEN[b]	AMK[b]	SXT[b]	NIT[b]	Resistance score	Phylogenetic group	ERIC-2 PCR profile	ST
1	Ec/C10	1	I	R	R	S	S	S	S	S	1	B2	XII	ST131
2	Ec/C1	1	I	R	I	S	S	S	S	S	1	A	I	ST602
	Ec/C29	1	I	R	I	S	S	S	R	S	2	D	XV	ST350
	Ec/C31	14	I	I	S	R	S	S	S	S	1	B1	VI	ST101
	Ec/C5	27	I	I	S	R	S	S	R	S	2	B2	XI	ST131
5	Ec/C9	14	S	I	I	S	S	S	S	S	1	D	XVI	ST3841
	Kp/C8	15	I	I	R	S	S	S	S	S	1	NA	ND	ND
6	Ec/C21	14	I	R	R	S	R	S	S	R	3	D	XVIII	ST3842
8	Ec/C28	14	I	I	R	R	R	I	R	S	5	B2	IX	ST1193
9	Ec/C12	15	I	I	S	S	S	S	S	S	0	A	IV	ST762
10	Ec/C19	27	R	I	I	R	S	S	S	S	2	B2	XIV	ST131
11	Ec/C30	1	I	R	I	R	S	S	R	S	3	A	III	ST744
12	Ec/C6	14	I	R	S	S	S	S	S	S	0	B2	VIII	ST127
	Ec/C3	15	I	I	R	R	I	R	R	S	5	B2	X	ST131
13	Ec/C26	1	I	R	S	S	S	S	S	S	0	D	XIX	ST3843
18	Ec/C7	15	R	R	S	S	R	S	S	S	1	D	XX	ST3867
19	Ec/C16	22	I	R	I	S	S	S	S	S	1	B2	VII	ST28
20	Ec/C17	1	I	R	I	S	S	S	R	S	2	D	XVII	ST38
22	Ec/C2	14	S	I	I	S	R	S	R	R	4	B1	V	ST58
23	Ec/C13	15	I	I	I	R	S	I	R	S	4	B2	XIII	ST131
	Ec/C14	15	I	S	I	R	S	R	R	S	4	B2	XIII	ST131
	Ec/C15	15	I	S	I	R	S	I	R	S	4	B2	XIII	ST131
	Ec/C22	15	I	S	I	R	R	R	R	S	5	B2	XIII	ST131
	Ec/C23	15	I	I	R	R	R	R	R	S	5	B2	XIII	ST131
	Ec/C24	15	I	S	R	R	R	R	R	S	5	B2	XIII	ST131
	Ec/C25	15	I	I	I	R	S	I	R	S	4	B2	XIII	ST131
24	Ec/C20	27	I	I	I	R	S	S	R	S	3	B2	XIII	ST131
	Ec/C18	1	S	I	I	S	S	S	R	S	2	A	II	ST56

Ec, *E. coli*; Kp, *K. pneumoniae*; CAZ, ceftazidime; FEP, cefepime; AMC, amoxicillin/clavulanic acid; LVX, levofloxacin; GEN, gentamicin; AMK, amikacin; SXT, trimethoprim/sulfamethoxazole; NIT, nitrofurantoin; R, resistant; I, intermediate susceptible; S, susceptible; NA, not available; ND, not determined.
[a]All isolates were susceptible to cefoxitin, imipenem and fosfomycin and resistant to cefotaxime.
[b]Antibiotic molecules used to calculate the antibiotic resistance score.

ceftazidime and 86% to cefepime (Table 1). Regarding molecules other than extended-spectrum cephalosporins, none of the isolates was resistant to cefoxitin, carbapenems and fosfomycin (data not shown), whereas 7% were resistant to nitrofurantoin and 79% to amoxicillin/clavulanic acid (Table 1). The 27 *E. coli* isolates were more often resistant to trimethoprim/sulfamethoxazole (59%) and levofloxacin (52%) than to aminoglycosides (30%–33.3%) (Table 1).

Molecular characterization of the CTX-M-producing *E. coli* isolates

The 27 CTX-M-producing *E. coli* isolates belonged to the main *E. coli* phylogenetic groups: 15 (55.5%) to group B2, 6 (22%) to group D, 4 (15%) to group A and 2 (7.5%) to group B1 (Table 1).

Twenty ERIC-2 PCR profiles were identified among the 27 *E. coli* isolates (Table 1). The isolates from groups A, B1 and D each displayed a unique profile (n = 12) (Table 1). In contrast, an identical ERIC-2 PCR profile (profile XIII) was identified among 8 of the 15 isolates belonging to group B2 (Table 1). Seven of the eight isolates with ERIC-2 PCR profile XIII were detected from the seven colonized children from DCC 23 and the remaining one from one (C20) of the two children found to harbour a faecal ESBL-producing *E. coli* isolate in DCC 24 (Table 1).

As indicated in Table 1, the 27 *E. coli* isolates displayed 16 STs. Four (25%) STs, all belonging to group D, were new STs (ST3841, ST3842, ST3843 and ST3867). The four isolates from group A, the two from group B1 and the remaining six from group D, which each displayed a unique ERIC-2 PCR profile, also each displayed a unique ST. In contrast, 12 of the 15 isolates from group B2 displayed ST131.

E. coli ST131 characterization

As indicated in Table 1, the 12 *E. coli* ST131 displayed five different ERIC-2 PCR profiles (X, XI, XII, XIII and XIV).
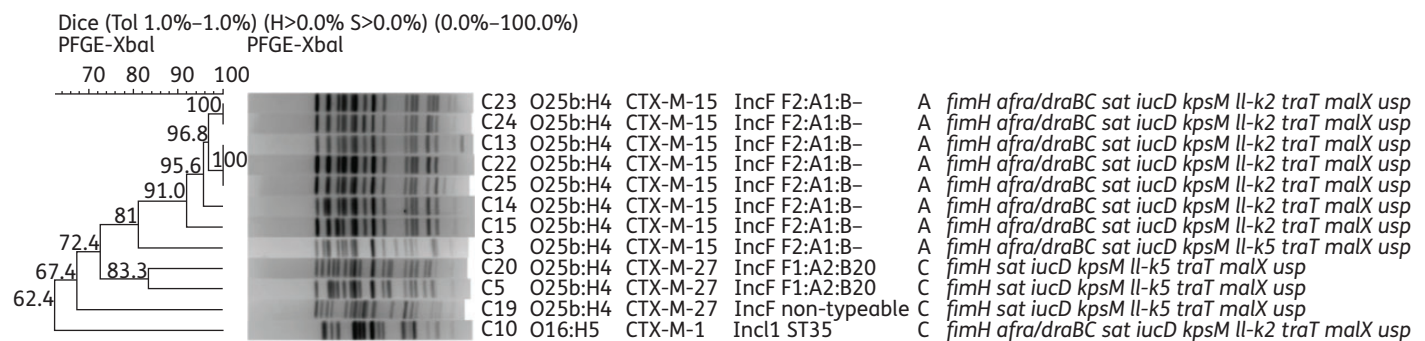
Dice (Tol 1.0%–1.0%) (H>0.0% S>0.0%) (0.0%–100.0%)
PFGE-XbaI   PFGE-XbaI

Figure 1. PFGE of XbaI-digested DNA, serotypes, CTX-M types, ESBL-carrying plasmids, virotypes and virulence traits of the 12 ST131 *E. coli* isolates. The dendrogram was obtained with the UPGMA algorithm based on the Dice similarity coefficient and applying 1% tolerance in the band position. Among the eight ST131 *E. coli* isolates, those harboured by the seven children (C13, C14, C15, C22, C23, C24 and C25) in DCC 23 are not distinguishable from each other, but are distinguishable from the isolate harboured by the child (C20) in DCC 24.

In an attempt to distinguish the eight ST131 isolates with the ERIC-2 PCR profile XIII from each other, PFGE typing was carried out. As indicated in Figure 1, the isolates from the seven children of DCC 23 were indistinguishable from each other, whereas they differed from the isolate from child C20 in DCC 24 by more than seven bands.

All *E. coli* ST131 displayed serotype O25b:H4 and *fimH* allele 30, except for the C10 isolate from DCC 1, which displayed serotype O16:H5 and *fimH* allele 41 (Table 2). Nine of the 28 studied virulence factor-encoding genes (*fimH*, F10 *papA*, *sat*, *iucD*, *chuA*, *kpsM* type II, *traT*, *malX* and *usp*) were detected in all isolates (Table 2). Eight of the 11 isolates with serotype O25:H4 had the *afaFM955459* gene and consequently belonged to virotype A. The remaining three O25:H4 isolates and the single O16:H5 isolate belonged to virotype C as they only had the gene *sat* among the four genes allowing for the virotype differentiation (Table 2).

Eight *E. coli* isolates (C3 and all seven isolates originating from DCC 23, i.e. C13, C14, C15, C22, C23, C24 and C25) presented a CTX-M-15 enzyme carried by a 145 kb IncF F2:A1:B− plasmid (Figure 1). Alternately, the CTX-M-27-carrying plasmids identified in *E. coli* isolates C5 and C20 both belonged to the IncF F1:A2:B20 type (145 kb), whereas the one presented by C19 could not be typed. Finally, the CTX-M-1 enzyme displayed by the C10 isolate was carried by an IncI1 ST35 plasmid (110 kb).

Association of phylogenetic group, CTX-M type and antibiotic resistance score

CTX-M enzymes that strongly hydrolyse ceftazidime (CTX-M-15 and CTX-M-27) were more frequent among group B2 *E. coli* (11/15: 73%) isolates than among group A (1/4: 25%), group B1 (0/2) and group D (1/6: 17%) isolates (Table 1). The mean antibiotic resistance score was higher [3.3 (0–5)] for group B2 isolates than for the isolates of groups A [1.5 (0–3)], B1 [2.5 (1–4)] and D [1.5 (0–3)] (Table 1). Among group B2 isolates, levofloxacin resistance was more frequent among ST131 *E. coli* (11/12) than among non-ST131 *E. coli* isolates (1/3), which displayed resistance frequencies similar to isolates from groups A (1/4) and B1 (1/2). Group D isolates were all susceptible to levofloxacin (Table 1).

Discussion

Increasing dissemination of *E. coli* producing ESBLs, notably CTX-M-type ESBLs, has been described worldwide since 2000.[29] Many studies have reported on the prevalence of such isolates among extraintestinal pathogenic *E. coli*, whereas those reporting on the percentage of individuals carrying these isolates in their digestive tract are less common, particularly among children.[10,12,16,17,19,30]

This is the first study to report faecal carriage of ESBL-producing Enterobacteriaceae among children attending DCCs. Among the 419 children screened in south-eastern France, one was found to harbour a faecal CTX-M-15-producing *K. pneumoniae* isolate and 27 (6.4%) a faecal CTX-M-producing *E. coli* isolate. Löhr *et al.*[31] showed long-term faecal carriage of CTX-M-15-producing *K. pneumoniae* in infants after discharge from neonatal intensive care units where an outbreak of CTX-M-15-producing *K. pneumoniae* had occurred. As a result, detecting faecal CTX-M-15-producing *K. pneumoniae* among children in DCCs is not surprising. However, as expected, the predominant species among the ESBL-producing Enterobacteriaceae isolates was *E. coli*. The percentage of DCC children colonized by CTX-M-producing *E. coli* was relatively high (6.4%) in comparison with the percentage found by Pallecchi *et al.*[16] in Peru and Bolivia (1.7%) and Kaarme *et al.*[17] (2.9%) in Sweden. However, this percentage was similar to that observed (6%) among healthy adults living in the Paris area (France) in 2011[12] and slightly higher than that reported by Birgy *et al.*[19] (4.6%) among French children (one-third attending DCCs) cared for by paediatric practitioners in three different French regions in 2010–11. We found that DCC children were colonized with genetically various ESBL-producing *E. coli* isolates, except for seven children from one DCC. The strain shared by the seven children belonged to clone ST131 and produced CTX-M-15. This clone and this enzyme were dominant among the 27 studied *E. coli* isolates (44% and 37%, respectively). The prevalence of *E. coli* ST131 and CTX-M-15 were higher among the faecal CTX-M-producing *E. coli* isolates from DCC children than among those colonizing other different French populations: mothers and their newborns hospitalized in a Parisian hospital (9.4% *E. coli* ST131 and 18.8% CTX-M-15),[32] and healthy adults living in the Paris area (10% *E. coli* ST131 and 33% CTX-M-15).[12]

Table 2. Virulence factor-encoding genes and virotypes displayed by the 12 *E. coli* ST131 isolates

Property/gene	Number (%) of ST131 isolates			
	O25b:H4 CTX-M-15 (n=8)	O25b:H4 CTX-M-27 (n=3)	O16:H5 CTX-M-1 (n=1)	Total (n=12)
Adhesins				
fimH	8	3	1	12 (100)
allele 30	8	3	0	
allele 41	0	0	1	
fimAv$_{MT78}$	0	0	0	0
F10 *papA*	8	3	1	12 (100)
papEF	0	0	0	0
sfa/focDE	0	0	0	0
afa/draBC	8	0	1	9 (75)
afaFM955459[a]	8	0	0	8 (67)
bmaE	0	0	0	0
gafD	0	0	0	0
Toxins				
cnf1	0	0	0	0
cdtB	0	0	0	0
sat[a]	8	3	1	12 (100)
hlyA	0	0	0	0
Siderophores				
iucD	8	3	1	12 (100)
iroN[a]	0	0	0	0
chuA	8	3	1	12 (100)
Capsules				
kpsM II	8	3	1	12 (100)
kpsM II-K2	7	0	1	8 (67)
kpsM II-K5	1	3	0	4 (33)
neuC-K1	0	0	0	0
kpsM III	0	0	0	0
Miscellaneous				
cvaC	0	0	0	0
iss	0	0	0	0
traT	8	3	1	12 (100)
ibeA[a]	0	0	0	0
malX (PAI)	8	3	1	12 (100)
usp	8	3	1	12 (100)
tsh	0	0	0	0
Virotypes				
A	8	0	0	8 (67)
C	0	3	1	4 (33)

[a]Genes allowing for virotype differentiation. PAI, pathogenicity island.

Another difference between mothers/newborns, healthy adults, French children and DCC children was the high proportion of faecal group B2 ESBL-producing *E. coli* in DCC children: 55% versus 15% for mothers/newborns, 14% for healthy adults and 5% for French children. An explanation for these differences could be the dissemination of CTX-M-15-producing *E. coli* ST131, which belonged to group B2, in DCC 23. However, by considering only one child

instead of seven colonized by the same strain of *E. coli* ST131 in DCC 23 and subsequently a total of 21 DCC children with faecal ESBL-producing *E. coli* instead of 27, the percentage of group B2 *E. coli* remains high (43%) and ST131 is still predominant (28.5%). All these findings strongly suggest that DCC children might be a major reservoir of CTX-M-producing *E. coli* ST131 and that infections due to such isolates could become more common in this population. Weissman *et al.*[33] have recently shown that among 10 infections due to CTX-M-15-producing *E. coli* in children hospitalized at Seattle Children's Hospital, 8 were due to *E. coli* ST131. Chandramohan and Revell[7] found that *E. coli* ST131 accounted for 10.2% among the CTX-M-producing *E. coli* causing urinary tract infection in children attending Texas Children's Hospital.

This study is the first to show the presence of *E. coli* ST131 with serotype O16:H5 in France, whereas this type of *E. coli* ST131 has very recently been identified in Japan, Denmark, Australia and Spain.[34–37] In our study, O16:H5 accounted for 8% of the ST131 isolates, whereas it accounted for 1% in Australia, 2.6% in Spain, 4% in Japan and 4.5% in Denmark.[34–37] The 11 O25b:H4 ST131 isolates producing either CTX-M-15 or CTX-M-27 were resistant to fluoroquinolones and belonged to the *fimH*-based *H30* subclone, whereas the single O16:H5 ST131 isolate producing CTX-M-1 was susceptible to fluoroquinolones and belonged to the *fimH*-based *H41* subclone. Recently, Johnson *et al.*[38] showed that the *H30* subclone emerged a decade ago within ST131 and expanded rapidly to become what is now the dominant and most extensively multidrug-resistant lineage of extraintestinal pathogenic *E. coli* worldwide. In the study by Johnson *et al.*,[38] they also identified isolates with *fimH41* that were mostly (80%) susceptible to fluoroquinolones, accounted for 1% of the isolates and were only present between 2007 and 2010. In the study of Matsumura *et al.*,[39] which only reports on serotypes, it was also shown that ST131-O16 isolates were more often (82%) susceptible to fluoroquinolones. Do all O16:H5 isolates belong to *H41* subclone and have they emerged recently among fluoroquinolone-susceptible ST131? Further studies are required to improve our insight into ST131-O16:H5.

According to the virotypes recently defined by Blanco *et al.*,[23] DCC children strains displayed virotypes C and A, the two most prevalent virotypes among the international collection previously published and among the Spanish clinical isolates whose analysis allowed Blanco *et al.*[23] to characterize four virotypes (A, B, C and D). In Spain, virotype A was significantly associated with nursing home residents, whereas virotype D was significantly associated with younger patients and community acquisition.[23] Our results do not seem to coincide with the Spanish epidemiological characteristics. Appropriate studies have to be performed in France to clarify the relationship between virotypes and both clinical features and individuals' lifestyles.

Although the population structure of the faecal ESBL-producing *E. coli* from DCC children was polyclonal (16 STs for 27 *E. coli*), like that of those from healthy adults (18 STs for 21 isolates), we observed that the STs common to DCC children and healthy adult isolates were ST131 (group B2), which is globally described, and ST744.[12,40] However, the majority of the remaining unique STs identified among the children's *E. coli* isolates have already been mentioned several times in the literature: as examples, ST602 producing CTX-M-1, like in our study,[41] ST101 (i) producing carbapenemase (NDM-1) and CTX-M-15 from clinical samples from India,[42] (ii) as foodborne intestinal pathogenic *E. coli* in South Korea,[43] and

(iii) as clinical isolates producing ESBL in Spain,[44] ST58 producing CTX-M-1 (like in our study) in the digestive tract of healthy humans in Tunisia,[9] and producing CTX-M-14 from isolates causing cattle mastitis in France,[45] ST38 in zoo animals, human diarrhoeagenic E. coli, carbapenemase producers in Finland and the UK and in ESBL producers in Canada,[46–51] ST127 assessed by Gibreel et al.[6] as a clone recently emerged in the UK among community-acquired infections, and, ST1193 producing CMY enzymes from a French cystic fibrosis patient.[52]

Interestingly, all CTX-M-15-producing enzymes in ST131 isolates were carried by an identical IncF F2:A1:B− plasmid, which has already been reported in CTX-M-15-producing isolates.[28,53] The IncF F1:A6:B20 plasmid, which carried two out of the three CTX-M-27 enzymes, has also recently been described in CTX-M-27-producing ST131 isolates.[39] Likewise, IncI1 plasmids were reported as carrying ESBL genes in ST131[54] and an IncI1 ST35 plasmid was characterized here, which displayed a CTX-M-1 enzyme. These results suggest that specific IncF or IncI1 plasmid subtypes may participate in the dissemination of specific CTX-M enzymes among the ST131 clone.

In conclusion, to our knowledge, this is the first study to investigate intestinal carriage of ESBL-producing Enterobacteriaceae isolates in children attending DCCs. The majority (96%) of these isolates belonged to E. coli species and all produced CTX-M-type enzymes. Among these E. coli isolates, which colonized 6.4% of DCC children, the worldwide O25b:H4 ST131 H30 subclone producing CTX-M-15 and resistant to fluoroquinolones was both dominant and apparently disseminating in one DCC.[38] This reinforces the necessity of rigorous hygiene measures within DCCs. We identified for the first time in France the O16:H5 ST131 H41 subclone producing CTX-M-1 and susceptible to fluoroquinolones. Whether children might be the reservoir of this subclone recently identified worldwide deserves further investigation.

Transparency declarations
None to declare.

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