Independent Host Factors and Bacterial Genetic Determinants of the Emergence and Dominance of *Escherichia coli* Sequence Type 131 CTX-M-27 in a Community Pediatric Cohort Study

André Birgy,^a Corinne Levy,^{b,c,d} Marie-Hélène Nicolas-Chanoine,^e Aurélie Cointe,^a Claire A. Hobson,^e Mélanie Magnan,^e Stéphane Bechet,^b Philippe Bidet,^a Robert Cohen,^{b,c,d,f} Stéphane Bonacorsi^a

^aIAME, UMR 1137, INSERM, Sorbonne Paris Cité, Service de Microbiologie, Hôpital Robert Debré, AP-HP, Université Paris Diderot, Paris, France

^bAssociation Clinique Thérapeutique Infantile du Val de Marne (ACTIV), Saint Maur des Fossés, France

^cGroupe de Pathologie Infectieuse Pédiatrique (GPIP), Paris, France

^dCentre de Recherche Clinique du Centre Hospitalier Intercommunal de Créteil, Créteil, France

^eIAME, UMR 1137, INSERM, Université Paris Diderot, Sorbonne Paris Cité Paris, France

^fPetits Nourrisson, Service de Néonatalogie, Centre Hospitalier Intercommunal de Créteil, Unité Court Séjour, Créteil, France

ABSTRACT The recent emergence and diffusion in the community of *Escherichia coli* isolates belonging to the multidrug-resistant and CTX-M-27-producing sequence type 131 (ST131) C1-M27 cluster makes this cluster potentially as epidemic as the worldwide *E. coli* ST131 subclade C2 composed of multidrug-resistant isolates producing CTX-M-15. Thirty-five extended-spectrum beta-lactamase (ESBL)-producing ST131 isolates were identified in a cohort of 1,885 French children over a 5-year period. They were sequenced to characterize the ST131 *E. coli* isolates producing CTX-M-27 recently emerging in France. ST131 isolates producing CTX-M-27 ($n = 17$), and particularly those belonging to the C1-M27 cluster ($n = 14$), carried many resistance-encoding genes and predominantly an F1:A2:B20 plasmid type. In multivariate analysis, having been hospitalized since birth (odds ratio [OR], 10.9; 95% confidence interval [CI], 2.4 to 48.8; $P = 0.002$) and being cared for in a day care center (OR, 9.4; 95% CI, 1.5 to 59.0; $P = 0.017$) were independent risk factors for ST131 CTX-M-27 fecal carriage compared with ESBL-producing non-ST131 isolates. No independent risk factor was found when comparing CTX-M-15 ($n = 11$)- and CTX-M-1/14 ($n = 7$)-producing ST131 isolates with ESBL-producing non-ST131 isolates or with non-ESBL-producing isolates. Several factors may contribute to the increase in fecal carriage of CTX-M-27-producing *E. coli* isolates, namely, resistance to multiple antibiotics, capacity of the CTX-M-27 enzyme to hydrolyze both cefotaxime and ceftazidime, carriage of a peculiar F-type plasmid, and/or capacity to colonize children who have been hospitalized since birth or who attend day care centers.

KEYWORDS C1-M27, CTX-M-27, *E. coli*, ESBL, risk factors, ST131, children, fecal carriage

Escherichia coli sequence type 131 (ST131) is an emerging pathogen that was first described in 2008 (1). The spread of CTX-M-15 extended-spectrum β -lactamase (ESBL), the most common ESBL variant in the world (2), is mainly driven by CTX-M-15-producing *E. coli* ST131, explaining the numerous medical researches on this clone.

Moreover, the resistance to extended-spectrum cephalosporins due to CTX-M-15 production is associated with resistance to many other antibiotics in ST131 *E. coli*. As this clone is frequently responsible for extraintestinal infections, its resistance highlights the important challenges in the management of patients (3, 4).

Within ST131, different sublineages have been identified and organized according

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Address correspondence to Stéphane Bonacorsi, stephane.bonacorsi@aphp.fr.

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to their phylogenetic clade (A, B, and C), *fimH* allele (type 1 fimbriae adhesin-encoding gene), and antibiotic-resistance profile (5).

Clade A is associated with *fimH41*, clade B with *fimH22*, and clade C with *fimH30*. The variations in *fimH* alleles are suspected to reflect the capacity of these clades to colonize different sites (6). Within clade C, which has disseminated worldwide, two subclades have been identified. C1 subclade, also called ST131 H30R, comprises isolates with mutations in the chromosomal *gyrA* and *parC* genes, which confer resistance to fluoroquinolone. The C2 subclade, also called ST131 H30-Rx, groups isolates with the same *gyrA* and *parC* mutations and often the *bla*_{CTX-M-15} gene. These two features are suspected to contribute to the epidemiological success of *E. coli* ST131 (2, 7). C1/H30R and C2/H30-Rx, come from clade B/H22 that is classically cephalosporin and fluoroquinolone susceptible (8, 9). Recently, Matsumura et al. have described the emergence of a distinct cluster (C1-M27) within the C1 subclade (10), belonging to previously reported strains in Asia, in North America, and in Europe (2, 4, 11, 12). These isolates carry the *bla*_{CTX-M-27} gene and possess a similar region to a prophage-like genomic island of *E. coli* PCN033 (10).

Genomic sequence analysis of a number of ST131 isolates has allowed tracing the evolutionary history and the characterization of the main determinants of ST131 (5–8, 13, 14). One of the hypotheses is that plasmids, which use postsegregation killing and addiction systems to persist in strains over time, could play a preponderant role in the evolution of this clone (15). Plasmids belonging to incompatibility (Inc) groups with F replicons (IncF) can acquire genes, including genes encoding antibiotic resistance, and rapidly disseminate. This capacity is suspected to be dependent on the type of IncF plasmid. Indeed, the antibiotic-susceptible isolates of B subclade that is the progenitor of C1 and C2 subclade isolates carry an IncF plasmid with a plasmid multilocus sequence typing (pMLST) type F+:A-:B+ whereas the multidrug-resistant C1 and C2 subclade isolates carry plasmids with pMLST types F+:A+:B+, notably F1:A2:B20 without CTX-M-15, and F+:A+:B-, notably F2:A1:B- with CTX-M-15 (15, 16). Similarly, the C1-M27 isolates, belonging to the C1 subclade, are frequently associated with F1:A2:B20 replicons (12, 17, 18). Although C1-M27 and C1/H30R harboring the *bla*_{CTX-M-14} gene have an identical plasmid type (F1:A2:B20), C1-M27 has emerged in human fecal carriage and has a greater epidemiological impact than CTX-M-14-producing C1/H30R (10).

Genomic analyses highlighted geographical and temporal disparities in the evolution of the subclades. Thus, in the French population, we have recently shown the increasing carriage of CTX-M-27-producing *E. coli* in infants from no CTX-M-27 in 2010 ($n = 399$) and 2011 ($n = 258$) to 2.55% (11/431) in 2015. Within the ST131 clonal group, this corresponds to an increase from no CTX-M-27 in 2010 to 65% in 2015 (4, 11).

In this work, we attempted to search for factors explaining the recent emergence of the C1-M27 cluster. We studied lifestyle- and medical history-related factors of children associated with ST131 carriage, considering the CTX-M variants. We studied a cohort of 1,885 children. We used whole-genome sequencing of the ST131 isolates found in this cohort to study the genetic content of these isolates in terms of antibiotic resistance, virulence-factor-encoding genes, and ST131 evolutionary history over a period of 5 years according to the CTX-M variants produced by these ST131 isolates, namely, CTX-M-15, CTX-M-27, and others, including CTX-M-1 and CTX-M-14.

RESULTS

Sequencing data quality. We used standard metrics to estimate the quality of the *de novo* assemblies (see Table S1 in the supplemental material). An average of 123 contigs was obtained, the mean N_{50} value was 163,294 bp, and the mean coverage was 65×

All the genomic analysis results are presented in Table S1.

Clade, *fimH* allele, and ESBL types. Among the 35 ST131 *E. coli*-colonizing infants, clade typing and *fimH* allele typing showed that 9 (26%) of the isolates belonged to clade A with *fimH41* or single nucleotide variant and 26 (74%) belonged to clade C with

fimH30 or single nucleotide variant. Clade A comprised isolates producing CTX-M-1 ($n = 3$), CTX-M-14 ($n = 2$), CTX-M-15 ($n = 1$), and CTX-M-27 ($n = 3$). Within the C1 subclade, we distinguished the C1-M27 cluster ($n = 14$) and C1-H30R subclade ($n = 2$). All C1-M27 isolates had the *bla*_{CTX-M-27} gene and possessed the insertion site of the structure (M27PP1), including the direct repeat and the prophage-like genomic island of *E. coli* PCN033. The two C1-H30R isolates produced CTX-M-14, and the 10 C2-H30Rx subclades comprised isolates with the *bla*_{CTX-M-15} gene. Overall, the *bla*_{CTX-M-27} gene ($n = 17$) was the most prevalent *bla*_{CTX-M} gene representing 48% of the 35 ST131 isolates (Table 1).

Genes encoding antibiotic resistance and bacteriocin. Repartition of antibiotic-resistance genes according to CTX-M variant and cluster/subclade type are presented in Table 1. Isolates producing CTX-M-27 were more resistant than the others (this difference was not statistically significant with the Kruskal-Wallis test). Resistance score was 5.8, 4.9, and 3 for the isolates producing CTX-M-27, CTX-M-15, and CTX-M-1/M-14, respectively, and their mean number of acquired antibiotic resistance genes was 7.8, 6.2, and 3.5 genes, respectively. Using the *t* test, we analyzed the preferential associations and showed that the GyrAS83L/D87N, the ParCS80I, and the *tet*(A) genes were significantly more frequently associated with the *bla*_{CTX-M-27} gene than with the *bla*_{CTX-M-14}/*bla*_{CTX-M-1} genes (Table 1). Furthermore, the *strA/strB* and *sul2* genes were significantly associated with the *bla*_{CTX-M-27} gene compared with all other *bla*_{CTX-M} variants (Table 1). In contrast, the *bla*_{TEM-1} gene was significantly associated with the *bla*_{CTX-M-1}/*bla*_{CTX-M-14} genes compared with the *bla*_{CTX-M-27} gene, which is never associated with *bla*_{TEM-1}. Although the distribution of the *mphA* gene conferring resistance to macrolides, according to the CTX-M type, was not significant, this gene appeared to be more frequent among the CTX-M-27-producing isolates (70%) than among those producing CTX-M-15 (45%) and CTX-M-1/14 (29%).

Concerning immunity protein, we found 24 isolates (68.5%) with the *imm* gene conferring resistance to colicin Ia, including 6 of the 7 (86%) isolates carrying the *bla*_{CTX-M-1/14} genes, 13 of the 17 (76.5%) isolates carrying the *bla*_{CTX-M-27} gene, and 5 of the 11 (45%) isolates carrying the *bla*_{CTX-M-15} gene. Four isolates (11.5%) carried the *cia* gene encoding colicin Ia among which 3/4 belonged to C1-M27 cluster.

Plasmids and plasmid maintenance systems. Different F-type plasmids were detected. The F1:A2:B20 pMLST was present in 18 isolates, including all C1-M27 ($P < 0.001$) (Table 1).

Among the eight plasmid maintenance systems screened, a homogenous distribution was observed except for the S_{rnCB} system. This system was more frequently detected in the CTX-M-27 isolates than in the CTX-M-15 isolates ($P = 0.008$) or than in the CTX-M-1/14 isolates ($P = 0.03$), and the virulence-associated protein (VagCD) was only detected in isolates carrying the *bla*_{CTX-M-15} gene. Promotion of nucleic acid (*pndAC*) was found in only 4 isolates.

Virotypes. We determined the virotypes according to the combination of virulence factors described by Mora et al. (19) (Table 1). Virotype A (*nfaE* and *sat*), virotype C (*sat*), and virotype E (*cnf1*, *hlyC*, *papGII*, *papGIII*, and *sat*) were found in 6, 24, and 1 isolates, respectively. We found a combination of virulence factor-encoding genes (including *papGII*, *papGIII*, and *sat*) in two isolates, which was not previously described by Mora et al. Virotype C was more frequently associated with the *bla*_{CTX-M-27} gene than with the *bla*_{CTX-M-15} gene ($P = 0.01$). All C1 subclade (C1-M27 and C1-H30R) isolates displayed only one virotype (virotype C) compared with the virotype heterogeneity observed in subclade C2-H30-Rx and clade A.

Phylogenetic analysis of the 35 *E. coli* ST131 isolates. We used CSI phylogeny (40) to build a phylogenetic tree (Fig. 1) from single nucleotide polymorphisms (SNPs) illustrating the isolates' distribution into two clusters comprising the isolates of *fimH41*/clade A and the isolates of *fimH30*/clade C, respectively. In majority, CTX-M-27-producing isolates were clustered in clade C. Core genome phylogenetic comparisons of all C2-H30-Rx isolates based on alignment with the genome of

TABLE 1 Distribution of genetic features according to CTX-M variants or clades

Genetic feature	No. of isolates (%) according to CTX-M variant and clade											P value of:	
	No. of all isolates (n = 35)	CTX-M-27			CTX-M-15			CTX-M-14/CTX-M-1					
		A (n = 3)	C1-H30R (=C1-M27) (n = 14)	Total (n = 17)	A (n = 1)	C2-H30Rx (n = 10)	Total (n = 11)	A (n = 5)	C1-H30R (n = 2)	Total (n = 7)	CTX-M-27 vs CTX-M-15	CTX-M-27 vs CTX-M14/M1	CTX-M-15 vs CTX-M14/M1
<i>fimH</i> allele and O-type													
<i>fimH</i> 30 or SNV	26		14 (100)	14 (82)		10 (100)	10 (91)		2 (100)	2 (29)			
<i>fimH</i> 41 or SNV	9	3 (100)		3 (18)	1 (100)		1 (9)	5 (100)		5 (71)			
O25b-H4	26		14 (100)	14 (82)		10 (100)	10 (91)		2 (100)	2 (29)			
O16-H5	9	3 (100)		3 (18)	1 (100)		1 (9)	5 (100)		5 (71)			
Resistance and bacteriocin													
Mean resistance score ^a		5.8			4.9			3					
Mean acquired resistance genes		7.8			6.2			3.5					
GyrA-S83L-D87N	26		14 (100)	14 (82)		10 (100)	10 (91)		2 (100)	2 (29)	0.02	0.012	
ParC-S80I-E84V	22		11 (78)	11 (65)		9 (90)	9 (82)		2 (100)	2 (29)		0.05	
ParC-S80I	9		3 (21)	3 (18)		1 (10)	1 (9)				0.01	0.01	
ParE-I529L	35	3 (100)	14 (100)	17 (100)	1 (100)	10 (100)	11 (100)	5 (100)		5 (71)			
GyrA-S83L	3	3 (100)		3 (18)				5 (100)	2 (100)	7 (100)			
<i>mphA</i>	18	2 (67)	10 (71)	12 (70)		5 (50)	5 (45)	1 (20)	1 (50)	2 (29)			
<i>tetA</i>	18	2 (67)	12 (85)	14 (82)		5 (50)	5 (45)	1 (20)		1 (14)	0.003		
<i>tetB</i>	1				1 (100)		1 (9)						
<i>catB3</i> -like	3					3 (30)	3 (27)						
<i>aac(6')/b-cr</i>	3					3 (30)	3 (27)						
<i>bla</i> _{TEM-1}	11				1 (100)	4 (40)	5 (45)	5 (100)	1 (50)	6 (86)	<0.001		
<i>bla</i> _{OXA-1}	3					3 (30)	3 (27)						
<i>bla</i> _{TEM-30}	1		1 (7)	1 (6)									
<i>aadA5/A2</i>	18	2 (67)	10 (71)	12 (70)		5 (50)	5 (45)	1 (20)		1 (14)	0.02		
<i>strA</i>	16	2 (67)	12 (85)	14 (82)		2 (20)	2 (18)	1 (20)		1 (14)	0.001		
<i>strB</i> -like	16	2 (67)	12 (85)	14 (82)		2 (20)	2 (18)	1 (20)		1 (14)	0.001		
<i>aac(3)-IIa/d</i> -like	5					4 (40)	4 (36)		1 (50)	1 (14)			
<i>fosA3</i>	1				1 (100)		1 (9)						
<i>sulI</i>	17	2 (67)	10 (71)	12 (70)		5 (50)	5 (45)	1 (20)		1 (14)			
<i>sul2</i>	16	2 (67)	12 (85)	14 (82)		2 (20)	2 (18)	1 (20)		1 (14)	0.003		
<i>dfra17</i>	17	2 (67)	9 (64)	11 (65)		5 (50)	5 (45)	1 (20)		1 (14)			
<i>sulI/sul2/dfra17</i>	14	2 (67)	9 (64)	11 (65)		2 (20)	2 (18)	1 (20)		1 (14)			
<i>imm</i>	24	1 (33)	12 (85)	13 (76)	1 (100)	4 (40)	5 (45)	5 (100)	1 (50)	6 (86)			
colicin Ia			4 (28)	4 (23)		1 (10)	1 (9)						
Plasmid (pMLST)													
F1:A2:B20	18		14 (100)	14 (82)		1 (10)	1 (9)	2 (40)	1 (50)	3 (43)	<0.001		
F2:A-B10	3	3 (100)		3 (18)									
F2:A1:B-	4					4 (40)	4 (36)						
F10:A1:B-	1					1 (10)	1 (9)						
F36:A-B1	1					1 (10)	1 (9)						
F2:A-B-	1					1 (10)	1 (9)						
F1:A1:B23	1				1 (100)		1 (9)						
F1:A1:B16	1					1 (10)	1 (9)						
F36:A1:B20	1					1 (10)	1 (9)						
F29:A-B10	3							3 (60)	1 (50)	3 (43)			
F2:A2:B20	1						1 (9)			1 (14)			

(Continued on next page)

TABLE 1 (Continued)

No. of isolates (%) according to CTX-M variant and clade													
Genetic feature	No. of all isolates (n = 35)	CTX-M-27		CTX-M-15			CTX-M-14/CTX-M-1			P value of:			
		A (n = 3)	C1-H30R (=C1-M27) (n = 14)	Total (n = 17)	A (n = 1)	C2-H30Rx (n = 10)	Total (n = 11)	A (n = 5)	C1-H30R (n = 2)	Total (n = 7)	CTX-M-27 vs CTX-M-15	CTX-M-27 vs CTX-M14/M1	CTX-M-15 vs CTX-M14/M1
Addiction system													
CcdAB	33	3 (100)	14 (100)	17 (100)	1 (100)	8 (80)	9 (82)	5 (100)	2 (100)	7 (100)			
PemK	35	3 (100)	14 (100)	17 (100)	1 (100)	10 (100)	11 (100)	5 (100)	2 (100)	7 (100)			
SmCB	23		14 (100)	14 (82)	1 (100)	5 (50)	6 (54)	2 (40)	2 (100)	4 (57)	0.008	0.03	
HokSok	23	3 (100)	13 (93)	16 (94)	1 (100)	10 (100)	11 (100)	4 (80)	2 (100)	6 (86)			
PndAC	4		2 (14)	2 (12)		2 (20)	2 (18)						
VagCD	7				1 (100)	6 (60)	7 (63)				<0.001		0.01
Virotype ^b													
C	24	1 (33)	14 (100)	15 (88)		4 (40)	4 (36)	3 (60)	2 (100)	5 (71)	0.01		
A	6	1 (33)		1 (6)		3 (30)	3 (27)	2 (40)		2 (29)			
other	3	1 (33)		1 (6)	1 (100)	2 (20)	3 (27)						
E	1					1 (10)	1 (9)						

^aResistance score corresponds to the number of antibiotic families affected by resistance.^bC, *sat*; A, *nfaE* and *sat*; E, *cnf1*, *hlyC*, *papGII*, *papGIII*, and *sat*.

FIG 1 Phylogenetic tree of 35 ST131 *E. coli* isolates, collected from 2010 to 2015 in fecal carriage of French children, and 3 reference strains, including EC958, H105, and SE15, to represent the C2-H30Rx subclade, the C1-M27 clade, and the clade A, respectively. The tree is rooted on *E. coli* reference isolate 536. The phylogeny is based on the concatenated alignment of the high-quality SNPs using CSI phylogeny 1.4 tool. Year of isolation, *fimH* allele, belonging to C1-M27 subclade, ESBL variant, chromosomal mutations (GyrA and ParC) conferring resistance to fluoroquinolones, plasmidic pMLST, and virotype are shown using different color symbols.

reference *E. coli* ST131 lineage EC958, revealed an average difference of 442 SNPs. In contrast, the same comparison between isolates within clade A aligned with *E. coli* ST131 reference strain SE15 revealed a SNP average of 282. For all the isolates belonging to C1-M27 cluster and aligned with H105 reference strain, the average SNP difference was of 68 SNPs.

Risk factor analysis. The main results of univariate and multivariate analyses comparing CTX-M-27-producing ST131 isolates with ESBL-producing non-ST131 isolates or non-ESBL-producing isolates are presented in Table S2 in the supplemental material. Briefly, multivariate analysis found two independent risk factors associated with fecal carriage of CTX-M-27-producing ST131 isolates, namely, history of hospitalization (OR, 10.9; 95% CI, 2.4 to 48.8; $P = 0.002$) and being cared for in a day care center (OR, 9.4; 95% CI, 1.5 to 59.0; $P = 0.017$). Multivariate analysis comparing CTX-M-27-producing ST131 isolates with non-ESBL-producing isolates found history of hospitalization as an independent risk factor associated with carriage of CTX-M-27-producing ST131 isolates (OR, 3.5; 95% CI, 1.3 to 9.6; $P = 0.015$).

When CTX-M-15- or CTX-M-1/14-producing ST131 isolates were compared with ESBL-producing non-ST131 isolates and with non-ESBL-producing isolates, no independent risk factor was found.

DISCUSSION

We previously described *E. coli* ST131 carriage in a pediatric community between 2010 and 2015 (4) and observed not only the emergence of the CTX-M-27 enzyme but also its increase in frequency over the study period. CTX-M-27-producing ST131 isolates had been described for the first time in France in 2012 as colonizing children cared for in day care center (12). Since then, emergence of CTX-M-27-producing ST131 isolates has been described in many countries (10, 11, 20–25).

The C1-M27 cluster, which was first observed in our children cohort in 2012, seems to have been selected recently. This is suggested by the following three features: (i) the lower SNP difference between the isolates of this cluster compared with the SNP difference between the isolates of subclade C2 and clade A; (ii) the great homogeneity of the C1-M27 isolates regarding their plasmid content, i.e., IncF plasmid with pMLST F1:A2:B20 as previously described (12, 22, 26); and (iii) the virotype (100% of virotype C).

The switch of CTX-M variants over time leads one to wonder about the reasons for the emergence of certain variants or the selective sweeps of some others. This question remains unsolved. The whole-genome sequencing of our 35 ST131 *E. coli* isolates revealed several striking features. First, isolates expressing the *bla*_{CTX-M-27} gene and particularly those belonging to the C1-M27 cluster tend to be more broadly resistant to antibiotics than the isolates producing other CTX-M variants. Over 70% of the C1-M27 isolates were resistant to fluoroquinolones, macrolides, tetracyclines, aminoglycosides, and sulfonamides. These antibiotic families are rarely used for treating infections in children, except for macrolides, such as azithromycin that is frequently prescribed in infectious diarrhea or respiratory pediatric infections. This use could explain the common presence of the macrolide-resistance-encoding *mphA* gene in the fecal ST131 isolates, notably in the C1-M27 isolates (71%), detected in children. We also observed that the *imm* gene encoding immunity against colicin Ia was common in the C1-M27 isolates (85%). The second striking feature is that the CTX-M-27 enzyme, belonging to CTX-M group 9, mimics the historical CTX-M group 1 variant carried by ST131 *E. coli*, namely CTX-M-15, with regard to the extended-spectrum cephalosporin-resistance phenotype. Indeed, these two enzymes have an excellent hydrolytic trade-off activity on the two extended-spectrum cephalosporins predominantly used in hospitals, namely, cefotaxime and ceftazidime. This is due to the D240G substitution in the two enzymes responsible for a decreased K_m for ceftazidime, probably through the modification of the β 3 strand-residue position during the hydrolytic process (27). Overall, the multidrug resistance, the ability to hydrolyze the extended-spectrum cephalosporins, and the immunity against colicin Ia, displayed by the CTX-M-27-producing isolates, may have contributed to their emergence and their nascent epidemiological success.

An analysis of the association between the children's lifestyle, their medical history, and the carriage of CTX-M variant showed that prior hospitalization (since birth) and being cared for in a day care center are independent risk factors for CTX-M-27-producing ST131 carriage compared with non-ST131 ESBL-producing isolates. These risk factors were found neither for the CTX-M-15 variant nor for the CTX-M-1/CTX-M-14 variants.

It is interesting to note that the first CTX-M-27-producing ST131 isolates described in France in 2012 were found in a pediatric population consisting of children who attended day care centers. This observation and our findings suggest that children could be a special reservoir for this cluster and may participate in its dissemination. This contrasts with the sole risk factor independently associated with CTX-M-15-producing ST131 isolates, i.e., prior residence in long-term care facilities (28). Day care centers and long-term-care facilities are environments where human promiscuity might facilitate the CTX-M-27- and CTX-M-15-producing isolate transmission, respectively. The second

risk factor found in our study, “hospitalization since birth,” refers to hospital, another environment where multidrug-resistant bacteria are easily transmitted between patients due to health care (29). CTX-M-27-producing ST131 isolates are well-known for their transmission and colonization capacity in rehabilitation wards in Israel, illustrating their diffusion capacity (30).

The most plausible hypothesis regarding our success in finding medical history- and lifestyle-related risk factors associated with CTX-M-27-producing ST131 carriage in children lies in the fact that the CTX-M-27 diffusion is recent, especially since we did not find risk factors associated with isolates producing CTX-M-15. CTX-M-15 is the historical CTX-M enzyme carried by *E. coli* ST131. Its global diffusion in the community makes it difficult to find specific risk factors (3, 6, 31).

Despite the small number of *E. coli* bacteria analyzed and sequenced, which is one of the limitations of the study, it appears that hospitalization since birth and attending a day care center allow the diffusion of the ST131 *E. coli* of variant CTX-M-27. However, the reasons for the recent emergence remain unclear and seem to be multifactorial. The C1-M27 cluster isolates and the C2 subclade isolates share two characteristics; first, the production of a CTX-M variant able to hydrolyze efficiently not only cefotaxime, as any CTX-M enzyme, but also ceftazidime; second, the carriage of type F plasmid encoding multiple resistance genes other than the *bla*_{CTX-M} genes. If these two characters have impacted the epidemiological success of the C2 subclade isolates, they might also influence the epidemiological success of the C1-M27 cluster isolates. Even though it is not yet known whether this variant will replace the other existing ones and the extent of its diffusion, we consider this epidemiological finding should be closely monitored in the future.

MATERIALS AND METHODS

Patients, isolates, and microbiological investigations. As previously described (4), a rectal sample was obtained from 1,885 children between October 2010 and June 2015 by 18 pediatricians from three French regions. Children aged from 6 to 24 months were enrolled during routine checkups with normal findings or when they presented with acute otitis media. We collected data potentially linked to the carriage of antibiotic-resistant bacteria, including antibiotic prescription during the 3 months before enrollment, day care attendance modality, premature birth or caesarean birth, siblings, previous hospitalization (during the previous 6 months and since birth), and travel history in the last 6 months specifying the geographical area visited. The exclusion criteria were antibiotic treatment within the 7 days before enrollment and severe underlying disease. As previously described, clonal group ST131 was found in 25.3% (38/150) of ESBL-producing *E. coli* isolates. Among them, 35, all from different patients, were available for this study.

Whole-genome sequencing. The library preparation and sequencing were carried out as previously described (32). Briefly, DNA was extracted using the MoBio kit (Qiagen). Library preparation was performed using the Nextera kit (Illumina, San Diego, CA, USA). Sequencing was performed either on a MiSeq instrument with 2×300 cycles or on a MiniSeq instrument with 2×150 cycles (Illumina technology) for some isolates that required resequencing for quality reasons ($n = 7$).

Assembly was performed using SPAdes genome assembler (33). We used the Centre for Genomic Epidemiology (CGE) website (<http://www.genomicepidemiology.org>) to characterize the strains (17, 34–36). A resistance score was defined as the number of antibiotic families affected by resistance and the score of acquired antibiotic resistance genes as the total number of acquired resistance genes. Virulence factor genes were identified using the Virulence Finder tool (37), and we also used the NCBI BLAST tool (BLAST+ version 2.2.31) to search for 166 genes (38). Virotypes were determined as described by Mora et al. (19).

Eight plasmid addiction or toxin/antitoxin systems were sought by *in silico* PCR, including PemK-PemI (type II toxin-antitoxin system that is a plasmid emergency maintenance), CcdA-CcdB (coupled cell division locus that is a type II toxin-antitoxin system), RelB-RelE (relaxed control of stable RNA synthesis), SrnB-C (the SrnB-SrnC toxin-antitoxin system that performs a postsegregation killing function using mRNA stability), ParD-ParE (DNA replication), VagC-VagD (virulence-associated protein that is a toxin-antitoxin system), Hok-Sok (host killing that encodes a type I toxin-antitoxin system), and PndA-PndC (promotion of nucleic acid degradation) (39). Finally, the CSI phylogeny tool (40) was used to identify single nucleotide polymorphisms (SNPs) between isolates, and the neighbor-joining tree was built using Mega software version 3.1 using bootstraps calculated on 100 replicates.

Ethics. The study was approved by the Saint-Germain-en-Laye Hospital Ethics Committee (number 10/10/2010-CPP06063). Written informed consents from parents or guardians were obtained.

Statistical analysis. Demographic lifestyle and medical history data were double-entered by using 4D software version 12 and analyzed by using Stata/SE 13.1 (StataCorp., College Station, TX, USA). Microbiological data were analyzed using Fisher exact test or Kruskal-Wallis test for the 35 *E. coli* ST131 isolates. The cohort study was conducted on 1,885 children, and analyses of variables associated with a

carriage of a CTX-M-producing isolate were carried out between each child group colonized with a ST131 isolate producing a specific CTX-M variant (CTX-M-27, CTX-M-15, and CTX-M-1/CTX-M-14) and children colonized with an ESBL-producing non-ST131 isolate ($n = 108$) and children colonized with a non-ESBL-producing *Enterobacteriaceae* isolate ($n = 1,742$). The chi-square test or Fisher's exact test were used in univariate analysis to identify potential variables associated with carriage of a CTX-M-producing isolate and those with a P value of < 0.2 were included into multivariate analysis. Then, we used a logistic regression model, computing odds ratios (ORs), and 95% confidence intervals (95% CIs) to identify independent variables associated with carriage of ST131-producing CTX-M-27, ST131-producing CTX-M-15, and ST131-producing other CTX-M.

Accession numbers. Raw sequences of the 35 isolates were deposited in GenBank under BioProject accession number [PRJNA522367](https://doi.org/10.6026/10.1093/jac/dkm464).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.00382-19>.

SUPPLEMENTAL FILE 1, XLSX file, 0.02 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.02 MB.

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