MAJOR ARTICLE

Emergence and Dissemination of Extended-Spectrum β-Lactamase–Producing *Escherichia coli* in the Community: Lessons from the Study of a Remote and Controlled Population

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Background. Intestinal carriage is a key factor in extended-spectrum β-lactamase (ESBL) infection epidemiology but is difficult to study in open communities. To overcome this problem, we studied a highly stable group of Amerindians for whom we reported an ESBL carriage prevalence of 3.2% in 2001.

Methods. In 2006, ESBL carriage was assessed among 163 healthy volunteer adults. ESBL isolates were identified, and their molecular resistance mechanisms were characterized. Antibiotic use in the year before sampling and the epidemiological characteristics of the population were analyzed. Results were compared to those obtained in 2001.

Results. In 2006, the ESBL carriage prevalence, exclusively comprising *Escherichia coli*, was 8.0%. It mainly consisted of CTX-M–type ESBL. The strains and plasmids carrying ESBL were heterogeneous, but 1 CTX-M-2–producing strain was found in 4.3% of the subjects analyzed. No individual risk factor was identified. However, overall antibiotic use had almost doubled since 2001. A 3-fold increase was noted for β-lactams.

Conclusions. In this population, the frequency of ESBL increased with time because of the appearance of CTX-M ESBL, mimicking what occurs in the developed world. This resulted from the probable repeated introduction of new strains and plasmids and from interindividual dissemination. During the same period, antibiotic use substantially increased.

Antibiotic resistance is a major public health concern worldwide. Many studies have underlined the increasing prevalence of β-lactam–resistant strains, especially for gram-negative bacteria [1]. Most genes encoding β-lactamases are plasmidborne and circulate among bacterial species by horizontal transfer. Among them, extended-spectrum β-lactamase (ESBL) hydrolyse most β-lactams (sparing only cephamycins and carbapenems), leading to a drastic reduction in the antibiotic armamentarium [1]. Recently, a major shift in ESBL epidemiology was observed [2]. Indeed, ESBLs were initially derived from TEM and SHV and were essentially restricted to health care facilities, but over the past 10 years a major increase in the prevalence of ESBL (mainly due to CTX-M–type ESBL) has been observed [1]. The study of intestinal carriage is of the utmost importance for attempts to understand the path of human infections,

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because the colon serves as a reservoir for potentially pathogenic ESBL enterobacteria [3]. Most studies of CTX-M dealing with intestinal carriage have focused on industrialized countries [4–6]; however, according to the limited data available developing countries have also been affected by the increased prevalence of CTX-M–type ESBL-producing Enterobacteriaceae [7, 8]. In addition to CTX-M, the plasmid-encoded cephalosporinase CMY-2, which originated from *Citrobacter freundii*, was also recently observed [9]. However, the epidemiology of ESBL emergence and dissemination in the community is difficult to describe and understand fully, because most populations are exposed to multiple sources of antibiotics and are constantly moving and mixing. By contrast, the population of Trois-Sauts, an isolated Amazonian village where we performed a study in 2001 [7], consists of very stable population and is exposed to a single, well-characterized source of antibiotics. Indeed, exchanges with other populations are rare, and the only source of antibiotics is the health post, where drugs are dispensed by permanent paramedical officers. The drugs are free, and their distribution is precisely recorded. When necessary, villagers are referred to the hospital in Cayenne, the capital city of French Guiana. Medical care is free. The village of Trois-Sauts is located in the southernmost part of the Guianese territory and comprises 4 hamlets spread over a distance of 6 km along the Oyapock River. It is 100 km south of the nearest village (2 days by motorboat). Trois-Sauts is located in a large territory strictly restricted to Amerindians residents with no modern farming or agriculture plants. Villagers share large huts with no modern latrines or hygienic facilities and use a few small areas of the river for drinking, bathing, and disposal of human waste. Nearly all their food is locally produced and consists of crops grown in a traditional manner and meat obtained by fishing or hunting. They do not raise farm animals, except for a few free-ranging chickens. The study we performed there in 2001 showed an ESBL carriage rate of 3.2%, due to a single TEM-52–producing *E. coli* strain. At that time, the annual antibiotic exposure rate was 0.64 treatments per subject per year.

We postulated that this village, because of its unique features, could be used as an example to describe the evolution of ESBL carriage in the community over time.

METHODS

Subjects. From 16 to 23 October 2006, we conducted a point-prevalence study in Trois-Sauts. The way of life was essentially unchanged relative to what it was in 2001 [7]. The village's Wayampi population comprised 525 subjects (388 in 2001). Permanent expatriate French residents in the village (paramedical officers and schoolteachers) still only numbered about 10. Each usually stayed in the village for several years. Also, only a few score of external professional people visited the village in a year.

Villagers were asked to participate in the 2006 study after they had been fully informed about it by the investigators and local authorities. Only adults could be included, because a small financial reward was given for participation and French law does not allow the inclusion of volunteers under 18 years old (the age of legal majority in France) in financially rewarded medical studies. Adult volunteers who were healthy at physical examination signed an informed consent form and were officially included. They answered a standard questionnaire and provided a freshly passed fecal sample. The study was approved by the ad hoc Guadeloupe Ethics Committee (Comité de Protection des Personnes de Guadeloupe, France; no. 06-05).

Bacteriological methods. Fresh fecal samples were inoculated extemporaneously onto Drigalski agar slants in screw-cup tubes, sent to France 2 weeks later, and stored at room temperature. There, the whole culture from each tube was suspended in 1.5 mL of brain-heart infusion broth with 10% glycerol and stored at −80°C. On harvesting, 100-μL aliquots of each broth were cultured on agar plates selective for third-generation cephalosporin-resistant strains (ESBL; AES Chemunex). All members of the Enterobacteriaceae family (defined as oxidase-negative facultative aerobic gram-negative rods) with different morphotypes that grew on these plates were identified at the species level using the API-20E system (bioMérieux) and, if necessary, underwent polymerase chain reaction (PCR) and sequencing of their 16S ribosomal RNA gene [10]. Their antimicrobial susceptibility was determined using the disk-diffusion method, as described elsewhere (http://www.sfm.asso.fr). Phenotypes of resistance were classified into 6 groups, also as described elsewhere [11]. Class A ESBLs were detected using the double-disk synergy test [12]. ESBL strains from the same species that were isolated from different volunteers and that had identical antibiotic susceptibility patterns were differentiated by repetitive extragenic palindromic PCR (rep-PCR) as described elsewhere [13], except that amplification products were separated by 3-h 70-V electrophoresis in 1% agarose gel and stained with SYBR Safe dye (Invitrogen).

Resistance genes, including $bla_{\text{CTX-M}}$ (groups 1, 2, 8, 9, and 25), bla_{SHV}, bla_{TEM}, $bla_{\text{OXA-1}}$, bla_{BES}, bla_{GES}, $bla_{\text{VEB,}}$ bla_{PER}, *qnrA*, *qnrB*, *qnrS*, and *aac(6′)1b*, were amplified with specific primers, as described elsewhere [14]. When class C β-lactamase production in *E. coli* was suspected, $bla_{\text{CMY-2}}$ was detected by PCR [15]. CMY-2 strains were included in the ESBL group for statistical analysis, as suggested elsewhere [16]. bla_{KLU} genes were detected in *Kluyvera* strains by PCR using MA-1 and MA-2 $bla_{\text{CTX-M}}$ universal primers [17] and CTX-M–specific primers [14]. All amplification products were sequenced and submitted to the National Center for Biotechnology Information library (http://blast.ncbi.nlm.nih.gov) for identification. Class 1, 2, and 3 integrons were detected by real-time PCR amplification of

Table 1. Comparison of the Adult Population of Trois-Sauts Included in the Study with the Population Not Included

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int1, *int2*, and *int3* genes, and the inserted gene cassettes were characterized as described elsewhere [18].

The transferability of ESBL genes was assessed by mating with *E. coli* J53^{rif}, as described elsewhere [19]. When mating was negative, transformation into *E. coli* TOP10 (Invitrogen) was attempted by electroporation of whole-plasmid DNA, extracted using a commercial kit (Macherey Nagel). Transformants were selected on Drigalski agar with 1 mg/L cefotaxime. Antimicrobial susceptibility patterns were assessed as described above. The minimum inhibitory concentrations of amoxicillin-clavlanate, cefoxitin, cefotaxime, ceftazidime, imipenem, ertapenem, amikacin, gentamicin, tigecyclin, and ciprofloxacin were assessed on parental strains and transconjugants or transformants by means of E-test strips (AES). Plasmid replicons from parental strains, transconjugants, and transformants were typed by PCR, as described elsewhere [20]. For *E. coli* strains, phylogenetic groups were assigned by triplex PCR [21]. Multilocus sequence typing was performed as described elsewhere [22], and a maximum-likelihood phylogenetic tree was constructed with the PHYML program [23] using sequences from the ESBL strains included in this study, the 72 ECOR collection strains [24], 161 bacteremic strains representative of the genetic diversity of the *E. coli* species [22], and 15 complete *E. coli* genomes [25]. Extraintestinal virulence factor genes (*hly*, *aer*, *papC*, *iroN*, *traT*, *ompT*, *fyuA*, *hra*, and *kpsE*) were detected by PCR, as described elsewhere [26].

Epidemiological data. Demographic data (age, sex, marital status, and number of children), data on lifestyle and the environment (size and location of households, contact with animals, daily activities, and babysitting children ≤5 years old), and data on medical history (current pregnancy, chronic disease, previous hospitalizations and surgery, and oral and parenteral antibiotic intake during the year before sampling) were collected from each volunteer by means of a standard data-collection form. Antibiotic treatments were recorded from the register kept at the health post. The antibiotic treatments of all the villagers not included in the study, as well as their familial status and the location of their household, were also recorded.

Statistical methods. We compared the epidemiological characteristics of ESBL carriers versus noncarriers by means of R software (version 2.6.1; http://www.cran.r-project.org). Univariate analysis with the Pearson χ^2 test and the Fisher exact test was used to compare discrete variables, and the Student *t* test was used for continuous variables. All tests were 2-sided, and the significance level was set at $\alpha = .05$. Because of the large number of explanatory variables tested, the results of the univariate analysis were adjusted using the Holm test for multiple testing [27, 28].

RESULTS

Characteristics of the general population and representativity of the samples tested. The Wayampi community of Trois-Sauts comprised 525 individuals at the time of the present study (a 35.3% increase over 2001), including 238 adults (female-to-male ratio, 1.09; mean age, 34 years). The increase in the size of the population was probably linked to the improvement in health conditions in the village, where every individual benefits from free medical access. One hundred sixty-three adults (65.8%) volunteered to participate (female-to-male ratio, 1.09; mean age, 31.6 years). Fifty of these adults (30.7%) had participated in the previous 2001 study. The characteristics of the study group were not different from those of the rest of the village, except that the volunteering rates differed from one hamlet to another and were highest among the villagers living closest to the health post (Table 1). Ten volunteers (6.1%) had been hospitalized in Cayenne during the year before the study. None of them were positive for ESBL carriage. This percentage was not significantly different from that for the villagers who did not volunteer (5.2% [19/362]).

Antibiotic exposure. Data on quantitative and qualitative antibiotic exposure for the year preceding the 2006 study were available for 512 (97.5%) of 525 villagers. Overall, it reached 1.08 treatments per subject per year (95% confidence interval [CI], 0.94–1.21), contrasting with the rate of 0.64 (95% CI, 0.54–0.75) observed in 2001 ($P < .01$) [7]. Exposure among children was high, at 1.29 treatments per subject per year (95% CI, 1.08–1.50). The corresponding mean exposure rate for the 163 adult volunteers reached 0.90 treatments per subject per year (95% CI, 0.71–1.09) and was not significantly different from the exposure rate for the adult population not included, which was 0.60 treatments per subject per year (95% CI, 0.38–0.83) (Table 1). Antibiotics administered were mainly penicil-

Table 2. Oral and Parenteral Antibiotics Used in Trois-Sauts between October 2005 and October 2006

	No. of treatments		
Antibacterial agents	Among 163 volunteers	Among 68 nonincluded adults	Among 281 children
Penicillins	67	17	215
Cephalosporins	5	1	12
Macrolides	18	11	32
Metronidazole	37	5	73
Ofloxacin	6	1	0
Other[a]	14	6	31
Total	147	41	363

[a] Nitroxoline, doxycycline, or cotrimoxazole.

Table 3. Characteristics of the 8 Extended-Spectrum β-Lactamase (ESBL)–Producing *Escherichia coli* Clones Identified in Trois-Sauts

Clone (no. of isolates)[a]	ESBL type	ID of the strain studied	Phylogenetic group/ subgroup	Virulence genes	TEM-1	Resistance to antibiotics other than β-lactams	Presence of class 1 integron (gene cassette sequencing)	Transfer in *E. coli* recipients[b]	Resistance trait(s) cotransferred with ESBL gene	Plasmid replicon type(s) in recipients
A (1†)	CTX-M-8	S028Ha	A_0	*aer*, *iroN*, *traT*, *hra*, *kpsE*	−	SXT	None	T	None	NT
H (1†)	CMY-2	S028Hc	D_1	*iroN*, *kpsE*	−	NAL, TET	None	C	None	IncI
B (2)	CTX-M-2	S041Ha	B1	*papC*, *iroN*, *traT*, *hra*	+	K, NAL, CIP, SXT, TET	*aadA5*, *dfrA7*	No	NT	NT
C (7)	CTX-M-2	S055Ha	A_1	*iroN*, *traT*	−	SXT, TET	*dfrA7*	C	TMP	IncI, IncFIB
D (1)	SHV-2	S058Ha	A_0	*aer*, *iroN*, *traT*	−	None	None	C	None	IncI
E (1‡)	CTX-M-2	S122Ha	B1	*ompT*, *traT*	+	K, SXT, TET	*dfrA7*	C	K, SSS, TET	IncHI1
F (1‡)	CTX-M-2	S122Hb	D_1	*aer*, *iroN*, *traT*	+	NAL, SXT	*dfrA7*	T	None	NT
G (1)	SHV-2	S141Ha	A_0	*ompT*, *hra*	−	GEN, K, TM, NET, SSS, TET	*aadA1*	C	GEN, K, TM, NET	IncI

NOTE. CIP, ciprofloxacin; GEN, gentamicin; ID, identifier; K, kanamycin; NAL, nalidixic acid; NET, netilmicin; NT, not typeable; SSS, sulfamethoxazole; SXT, cotrimoxazole; TET, tetracycline; TM, tobramycin; TMP, trimethoprim.

[a] The symbols † and ‡ indicate that the same volunteer was carrying 2 different strains.

[b] T indicates transfer by transformation (electroporation), and C indicates transfer by conjugation (mating).

Figure 1. Phylogenetic tree of a panel of 248 *Escherichia coli* strains representing the diversity of the species studied (72 ECOR strains [24], 161 bacteremic strains [22], 15 completely sequenced genomes [25], and the 8 extended-spectrum β-lactamase [ESBL]–producing *E. coli* strains). The tree was reconstructed from multilocus sequence typing concatenated sequences, using the PHYML procedure. *Escherichia fergusonii* was used as an outgroup. Bootstrap values are shown for values >70%. The main phylogenetic groups (A, B1, B2, and D) as well as the 3 additional phylogenetic (groups C, E, and F) are shown [22]. The 8 ESBL-producing strains isolated in Trois-Sauts are boxed.

lins, metronidazole, and macrolides (Table 2). Note that 0.19 β-lactam treatments per subjects per year had been given in 2001 [7], a figure that increased to 0.62 five years later in the global population ($P < .01$). Seventy-five (46.0%) of the volunteers had received a total of 147 treatments that included 18 different antibiotics (data not shown).

Fecal carriage of Enterobacteriaceae resistant to third-generation cephalosporins. Fifty-eight members of the family Enterobacteriaceae were isolated from 45 volunteers by means of ESBL-selective agar plates. Among them, 15 strains, isolated from 13 volunteers, were ESBL *E. coli*. The digestive ESBL carriage rate was 8.0% (95% CI, 3.8%–12.1%). Eleven (6.7%) of these strains, isolated from 11 volunteers, produced CTX-type ESBL. By rep-PCR, they were divided into 8 distinct patterns, which were arbitrarily named A, H, B, C, D, E, F, and G (Table 3). Interestingly, 5 isolates from 5 different volunteers were identified as *Kluyvera georgiana*, a species belonging to Enterobacteriaceae resistance group 6 [11].

Pattern C strains, all of which carried the $bla_{\text{CTX-M-2}}$ gene only,

Table 4. Extended-Spectrum β-Lactamase (ESBL) Carrier Status of the Volunteers, by Sociodemographic, Environmental, and Lifestyle Characteristics

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were present in 7 volunteers (4.3% of the entire study population). Four of the 7 volunteers were 2 married couples living in 2 different hamlets. One of each couple were brother and sister.

Pattern B strains, present in 2 volunteers, and pattern E and F strains, each present in a single volunteer, also carried the $bla_{\text{CTX-M-2}}$ gene but in addition carried the $bla_{\text{TEM-1}}$ gene. Pattern A and H strains, which carried $bla_{\text{CTX-M-8}}$ and $bla_{\text{CMY-2}}$, respectively, were both present in the same volunteer. Strains with patterns D and G carried the $bla_{\text{SHV-2}}$ gene, and each pattern was present in 2 volunteers. In all, the most common ESBL type was CTX-M-2 ($n = 11$), followed by SHV-2 ($n = 2$) and a single isolate of CTX-M-8 and CMY-2. ESBL gene transfer was obtained by conjugation or transformation (Table 3), except for $bla_{\text{CTX-M-2}}$ from pattern B strain.

Antimicrobial susceptibility of* bla$_{CTX\text{-}M}$-, bla$_{SHV}$-, *and* bla$_{\text{CMY}}$-*carrying* E. coli *isolates. All strains with the same rep-PCR pattern had the same antibiotic susceptibility pattern. However, susceptibility differed among the strains with different rep-PCR patterns. Resistance to cotrimoxazole and tetracycline was present in strains with 5 patterns each (Table 3), and resistance to aminoglycosides was present in strains with patterns B, E, and G (Table 3). Resistance to quinolones was present in pattern H and F strains (nalidixic acid) and in the pattern B strain (ciprofloxacin). Pattern D strains did not exhibit coresistance. Class 1 integrons were present in 5 of 8 patterns. Sequencing of gene cassettes did not show the presence of any ESBL gene (Table 3).

Bacterial genotyping. Strains with rep-PCR patterns A, C, D, and G belonged to phylogenetic group A (patterns A, D, and G belonged to subgroup A_0, and pattern C belonged to subgroup A_1), those with B and E patterns belonged to phylogenetic group B1, and those with H and F patterns belonged to phylogenetic group D (subgroup D_1) (Table 3). Analysis of virulence gene patterns showed that the overall virulence content was small but very diverse among strains; it mainly consisted of genes involved in iron capture (Table 3). No close phylogenic relationship was observed between the strains from each rep-PCR pattern when they were included in an *E. coli* tree constructed from the panel of representative *E. coli* strains (Figure 1).

Replicon typing of the ESBL-harboring plasmids. Analysis of the plasmids obtained after transfer showed that CTX-M-2–carrying plasmids were positive for IncI and IncFIB replicons (pattern C) or for the IncHI1 replicon (pattern E). SHV-2– and CMY-2–carrying plasmids had IncI replicons (patterns C, G, and H). Plasmids from patterns A, B, and F were not typeable (Table 3).

Univariate analysis of the risk factors for ESBL digestive carriage. We found no significant difference between ESBL carriers and noncarriers in terms of sociodemographic data, lifestyle, or medical characteristics, including previous hospitalization in Cayenne and antibiotic exposure during the year before sampling. The only difference was the distribution of carriers among the 4 hamlets (Table 4). However, after application of the Holm adjustment for multiple testing, the *P* value for this difference indicated nonsignificance.

DISCUSSION

The most striking result of our study was that the prevalence of fecal ESBL carriage among the adult Wayampi Amerindian community in Trois-Sauts was as high as 8.0% in 2006, potentially implying that infections that commonly available antibiotics cannot efficiency treat were present. The high rate was mostly due to the appearance of multiple CTX-M–bearing *E. coli* strains. Thus, the carriage rate was almost 3 times higher than the 3.2% that we observed 5 years previously, in 2001 [7]. In addition, at that time the only ESBL enterobacterium isolated was 1 *E. coli* strain bearing TEM-52, which was probably introduced into the village by a patient discharged from the hospital in Cayenne and which subsequently spread to other villagers [7]. This type of ESBL was no longer observed in the present study, in which the appearance of various CTX-M strains was found to be responsible for the large increase in ESBL carriage. This finding illustrates the high diffusion capacity of these ESBL resistance genes in communities once they have been introduced [29]. Such diffusion in Trois-Sauts strikingly mimicked what has been observed in more open settings [30, 31].

The present epidemiological study did not reveal any significant risk factors for individual ESBL carriage in terms of modes of living, demographic characteristics, or medical history, including individual antibiotic use and hospitalizations. Other investigators have also failed to identify good predictors of ESBL carriage in the community [32, 33]. Recent antibiotic exposure has been suggested as a possibility [34], but its predictive value was low [35]. Most of the dissemination of ESBL in Trois-Sauts appeared to be passive, suggesting that cross-transmission occurred. However, it must be stressed that the global population exposure to antibiotics in the village nearly doubled between 2001 and 2006 and that β-lactam use tripled during that period.

In Trois-Sauts, ESBL epidemiology exhibited a great diversity of enzymes, genetic supports, and strains, unlike the situation in 2001. The plasmids carrying ESBL genes were indeed highly heterogeneous. As shown in the transformants, the 5 typeable plasmids were from different incompatibility groups, and their antibiotic resistance gene content differed. The phylogenetic background of the ESBL strains was also very diverse. They had no close phylogenic relationships and belonged to multiple *E. coli* phylogenetic groups. Last, their virulence gene content was heterogeneous. Nevertheless, ESBL epidemiology reflected

the South American pattern of ESBL dissemination. Indeed, the most prevalent ESBL was CTX-M-2 (the most prevalent type of CTX-M in South America [36]), which was carried by 4.3% of the population. CTX-M-8 (also quite prevalent in South America [37]) was found in 1 volunteer in Trois-Sauts. Although Trois-Sauts is located in a large, unpopulated area far from any Brazilian or Guianese village, direct or indirect contact does occur intermittently with subjects living in the outside world, and large flows of illegal immigrants from Brazil to French Guiana have been reported [38]. It is therefore highly probable that CTX-M genes were introduced into Trois-Sauts from the outside at various times and then disseminated among the population by cross-transmission, as has been suggested in other settings [39]. This hypothesis is not in opposition with the fact that SHV-2, a natural mutant of SHV-1 [40, 41] that has also been reported in South America [8, 36], was found in 2 volunteers in the present study.

Note that the CMY-2 enzyme was isolated from 1 volunteer from Trois-Sauts at a time when such carriage was still an emerging phenomenon elsewhere [9, 42, 43]. As far as we know, this is the first report of the carriage of a plasmid-encoded cephalosporinase–producing enterobacterium in a remote population. Data on the general diffusion of CMY-type enzymes worldwide are still lacking, but its evolution in Trois-Sauts might provide indications about the burden associated with this enzyme in the years ahead.

Taken together, our results suggest that there have been several importations of ESBL-carrying genetic structures and strains in the village. This oligoclonal mode of dissemination is very different from that observed in 2001. In addition, the presence of the same rep-PCR pattern C strain in 7 volunteers, 4 of whom were closely related, also suggests that household cross-transmission plays an important role in ESBL dissemination. Once again, what happened in Trois-Sauts constitutes a good example of what has been observed previously in household settings where dissemination occurs [30, 31].

It is noteworthy that overall selective antibiotic pressure in Trois-Sauts nearly doubled between these years, reaching 1.08 treatments per subject per year, a value very similar to the 1.11 treatments per subject per year that we calculated from the antibiotic prescription data in metropolitan France [44] and the national population census of French inhabitants (http://www.indices.insee.fr). This increase nearly doubled between these years, particularly for β-lactams.

The last interesting result of our study was the presence of 5 *Kluyvera* strains in 5 volunteers (3.1%). *Kluyvera* species are not usually isolated from healthy humans but are commonly found in environmental soil and water samples [45]. *Kluyvera* species are the most probable origin of CTX-M in *E. coli* [46, 47]. All strains isolated in Trois-Sauts were identified as *K. georgiana,* a species that has even been isolated from nonhospitalized patients in Guyana [48]. All strains isolated in Trois-Sauts carried bla_{KLUY}, which is known to be the progenitor of CTX-M group 9 [48]. However, the possible horizontal transfer of bla_{KLU} genes from *Kluyvera* to *E. coli* was not shown in Trois-Sauts, where all CTX-M genes identified belonged exclusively to groups 2 and 8.

We were unable to describe the dissemination of ESBL among the entire population of Trois-Sauts. However, the group studied seemed to be representative, because more than two-thirds of the adults volunteered and did not differ from the nonvolunteers in any of the characteristics tested. Children, reportedly a reservoir of resistance in the community [49], were regrettably excluded from this 2006 study. This had not been the case in 2001, when no financial reward was proposed. At that time, 50 volunteers (54%) were under 18 years of age; 15 of them were included as adults in 2006. In any case, that no CTX-M was isolated even though many children were included in 2001 strongly suggests that CTX-M strains were indeed not present in Trois-Sauts at that time and that their presence in 2006 was not due to a sampling bias.

Our results show how easily CTX-M genes are able to disseminate in a human community once they are introduced. In the studied population, global selective antibiotic pressure might have helped the process, but personal antibiotic exposure was, strikingly, not an individual risk factor for ESBL colonization, suggesting that dissemination resulted mostly from person-to-person transmission. This suggests that measures of control should focus on reducing overall antibiotic use, in order to reduce intestinal carriage of ESBL genes and its consequences for human health. Our results also suggest that what is currently true for CTX-M genes might also be true for CMY genes in the coming years and that the dissemination of these genes in the community might become another major challenge for antibiotic treatment. The evolution of ESBL in Trois-Sauts might thus provide valuable information for understanding its dissemination in human communities.

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