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Carriage of CTX-M-15-Producing *Escherichia coli* Isolates among Children Living in a Remote Village in Senegal▽

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Two out of 20 children with no known antibiotic exposure, living in a very remote Senegalese village, were found to be fecal carriers of a multiresistant *Escherichia coli* clone that produced CTX-M-15. This highlights the current massive spread of extended-spectrum β-lactamases, even in isolated communities.

CTX-M-type β-lactamases have become the most frequently isolated extended-spectrum β-lactamases (ESBL) in *Enterobacteriaceae* (4). There seems to be no limit to their spread through the feces of healthy individuals from urban areas. Thus, they have been frequently isolated in Spain (15), Lebanon (16), Hong Kong (10), Bolivia, and Peru (17, 19) with various prevalences. So far, however, remote-living subjects appear to have been spared, at least in Amazonia (1, 9, 18). Here, we investigated the spread of ESBL in West Africa. Working in Senegal, we searched the most remote and isolated village we could find and assessed the fecal carriage of ESBL-producing *Enterobacteriaceae* in children who had in all probability never taken antibiotics.

Kagnoube, the village in eastern Senegal where the sampling took place, was chosen by local Senegalese investigators because it was very remotely situated (almost unreachable during the rainy season, not served by any concrete road). It comprises about 60 inhabitants living in traditional huts. A shared water pit is used as the source of water, and no river is flowing close. The closest permanent health care facility is 100 km away. The Kagnoube inhabitants reported having taken allopathic drugs only very occasionally. We included 20 healthy children in the study (11 girls and 9 boys aged 1 to 11 years [mean age, 6.9]) with the agreement of their parents, who firmly stated that their children had never taken any Western drug. According to the local legislation and considering the passive nature of the sampling, no approval by an ethical committee was required. The children provided a fresh stool sample, an aliquot of which was immediately inoculated into conservation agar in screw-cap tubes (Bio-Rad, Marne-la-Coquette, France) and sent to France at room temperature for harvesting. There, the presence of *Enterobacteriaceae* resistant to extended-spectrum cephalosporins (ESC) was investigated as follows: (i) in the predominant flora, by testing the antibiotic susceptibility of five *Escherichia coli* strains randomly chosen after inoculation on Drigalski agar using the disc diffusion method, and (ii) in the subdominant flora, by inoculating ChromID ESBL agar plates (bioMérieux, Marcy l'Etoile, France). ESBL production was confirmed by the double-disk synergy test (11). DNA was extracted using a MagNA Pure LC instrument (Roche Molecular Biochemicals, Mannheim, Germany). Clonality was assessed by repetitive extragenic palindromic-PCR as described previously (8). A plasmid transfer assay was attempted by bacterial mating in liquid broth by using rifampin-resistant *E. coli* J53 as the recipient. MICs were determined using Etest strips (AES, Solna, Sweden). When necessary, bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$ (five groups), bla_{VEB}, bla_{PER}, bla_{GES}, $bla_{OXA\text{-}1}$, *aac(6′)-Ib*, *qnrA*, *qnrB*, *qnrS*, IS*Ecp1*, and integrase-encoding *intI*, *intII*, and *intIII* genes were detected by PCR, as previously described (5, 8, 20). Plasmids were extracted with the QIAprep Spin Miniprep kit (Qiagen, Courtaboeuf, France). Phylogenetic groups were determined by triplex PCR (7). The replicon typing of plasmids was performed by multiplex PCR (6, 14). Eventually, a multidrug resistance (MDR) region similar to that described for plasmid pC15-1a (2) was investigated by PCR, using *E. coli* strain TN03 as a positive control (13).

Whereas none of the five randomly chosen *E. coli* isolates per sample (predominant flora) displayed an antibiotic resistance pattern suggestive of ESBL production, stool sample plating on ChromID ESBL agar plates (subdominant flora) yielded one and four cefotaxime-resistant *E. coli* isolates for two children. The five isolates exhibited identical repetitive extragenic palindromic-PCR patterns (data not shown). Thus, one isolate from each of the two children (named KA12 and KA20) was further tested and found to be resistant to coamoxiclav, cefotaxime, ceftazidime, fluoroquinolones, kanamycin, cotrimoxazole, and tetracycline but susceptible to cefoxitin, ertapenem, imipenem, gentamicin, and tigecycline. Genes $bla_{CTX\text{-}M\text{-}15}$ (with insertion sequence IS*Ecp1* immediately upstream), $bla_{TEM\text{-}1}$, $bla_{OXA\text{-}1}$, *aac(6′)-Ib-cr*, and *tet*(A) were present, but *qnr* was not detected. Both strains belonged to phylogenetic group A subgroup A1 (3), were *intI* positive (se-

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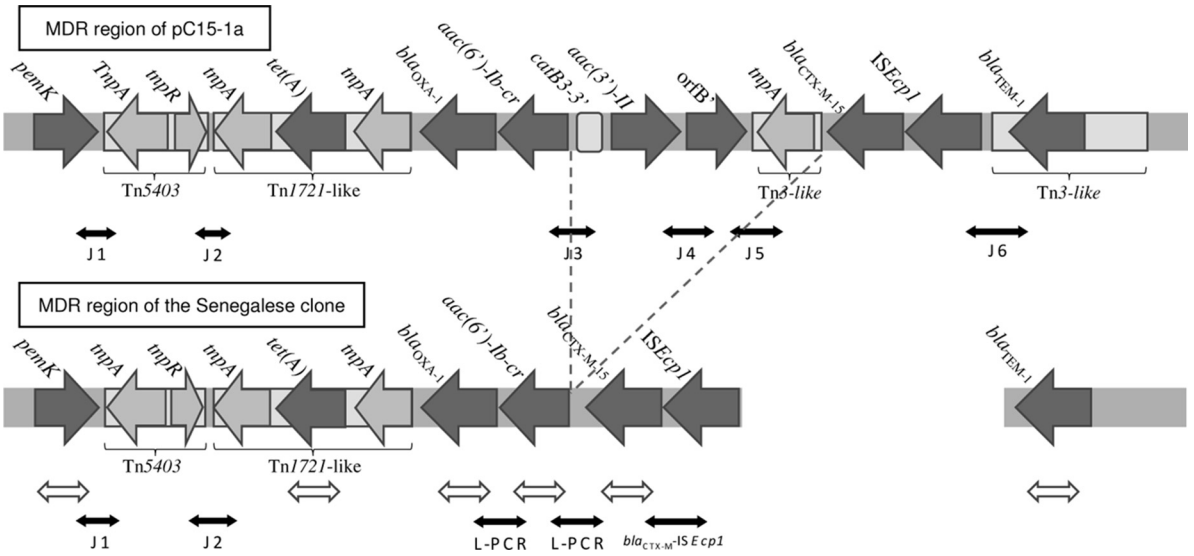

FIG. 1. Schematic representation of the MDR region of pC15-1a (2) (GenBank accession number AY458016) and the MDR region of the Senegalese clone (strains KA12 and KA20) as deduced from PCR experiments. Unfilled double-headed arrows indicate PCRs performed for *pemK*, *tet*(A), bla_{OXA-1}, *aac*(*6′*)*-Ib-cr*, bla_{TEM-1}, and $bla_{CTX-M-15}$. Black double-headed arrows indicate junction PCR assays, conducted as described by Lavollay et al. (13). J1, *pemK-tnpA* (Tn*5403*); J2, *tnpR* (Tn*5403*)*-tnpA* (Tn*1721*-like); J3, *catB3–aac*(*3*)*-II*; J4, *aac*(*3*)*-II–orfB′*; J5, *orfB′-tnpA* (Tn*3*); J6, *IS*Ecp1-bla_{TEM-1}. Junctions between $bla_{CTX-M-15}$ and IS*Ecp1* are also indicated by a black double-headed arrow. Long-PCR (L-PCR) experiments (data not shown) were performed to confirm the location of $bla_{CTX-M-15}$ close to *aac*(*6′*)*-Ib-cr* and bla_{OXA-1}. bla_{TEM-1} could not be located by long PCR (data not shown).

quencing identified cassettes *dfrA17* and *aadA5*), and transferred resistance to ESC, aminoglycosides, co-amoxiclav, tetracycline, and trimethoprim but not to sulfonamides. PCR experiments confirmed the presence of $bla_{CTX-M-15}$, bla_{OXA-1}, bla_{TEM-1}, *aac*(*6′*)*-Ib-cr*, and *tet*(A) in the transconjugants. Although four plasmids were detected in both parental strains, transconjugants had only one carrying an FIA-FIB-FII multireplicon. For both plasmids, PCR-based MDR comparison to pC15-1a revealed the presence of the *pemK*-Tn*5403* and Tn*5403*-Tn*1721*–like junctions but not junctions 3 and 4, which is consistent with the absence of the *aac*(*3*)*-II* gene in our plasmids (Fig. 1). Long-PCR analysis confirmed that $bla_{CTX-M-15}$ was located just upstream from *aac*(*6′*)*-Ib-cr*. No signal was obtained for junctions 5 and 6.

We found that an *E. coli* clone that carries CTX-M-15 and other resistant traits, including OXA-1, TEM-1, AAC(6′)-Ib-cr, and Tet(A), was present in the subdominant fecal flora of two healthy children from a very remote and isolated Senegalese village with very limited access to allopathic medicine. This association of the resistance determinants of the strains detected was very similar to that found in ESBL-producing *E. coli* that circulates worldwide in dense urban areas (2, 12, 13). Pallecchi et al. had observed a rise in CTX-M β-lactamases among children living in Bolivian and Peruvian suburban areas, with fecal carriage rates of 0.1% and 1.7% in 2002 and 2005, respectively (17, 19), highlighting the recent spread of CTX-M genes among healthy children. Interestingly, typeable $bla_{CTX-M-15}$-carrying plasmids from Peru and Bolivia also conveyed *aac*(*6′*)*-Ib-cr* and displayed FIA-FIB-FII replicons (17). Indeed, multireplicon FIA-FIB-FII has already been observed in CTX-M-15-producing strains isolated in France (14). The FII replicon is present on plasmid R100, and pC15-Ia harboring CTX-M-15 was derived from this plasmid through the incorporation of the MDR region (2). Note that even though $bla_{CTX-M-15}$-harboring plasmids from the remote Senegalese village carried also FIA- and FIB-type replicons, their MDR regions were similar to that of plasmid pC15-1a, except that they lacked *aac*(*3*)*-II*. Homologies between the MDR regions of pC15-1a and other CTX-M-15 plasmids have already been observed (2, 13). Here, junction PCR analysis suggested that there were many similarities between the plasmid-borne MDR structures harbored by these strains and those harbored by the TN08, TN36, and EpLA2 strains previously isolated in France (13). This strongly implies that even in the absence of direct antibiotic exposure, the few contacts the inhabitants of Kagnoube had with the outside world and allopathic medicine were enough to allow the CTX-M-15-associated MDR gene machinery to settle and persist in their commensal flora. Thereby, this study stresses the difficulties to be expected in controlling the dissemination of CTX-M-mediated resistance.

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